MBL/WHOI

JOURNAL OF SHELLFISH RESEARCH

VOLUME 8, NUMBER 1 JUNE 1989

The *Journal of Shellfish Research* (formerly *Proceedings of the National Shellfisheries Association*) is the official publication of the National Shellfisheries Association

Editor

Dr. Sandra E. Shumway
Department of Marine Resources
and
Bigelow Laboratory for Ocean Science
West Boothbay Harbor
Maine 04575

EDITORIAL BOARD

Dr. Monica Bricelj
Marine Sciences Research Center
State University of New York
Stony Brook, New York 11794-5000

Dr. Anthony Calabrese
National Marine Fisheries Service
Milford, Connecticut 06460

Dr. Kenneth K. Chew
College of Fisheries
University of Washington
Seattle, Washington 98195

Dr. Charles Epifanio
College of Marine Studies
University of Delaware
Lewes, Delaware 19958

Dr. Paul A. Haefner, Jr.
Rochester Institute of Technology
Rochester, New York 14623

Dr. Robert E. Hillman
Battelle Ocean Sciences
New England Marine Research Laboratory
Duxbury, Massachusetts 02332

Dr. Herbert Hidu
Ira C. Darling Center
University of Maine
Walpole, Maine 04573

Dr. Lew Incze
Bigelow Laboratory for Ocean Sciences
McKown Point
West Boothbay Harbor, Maine 04575

Dr. Louis Leibovitz
Marine Biological Laboratory
Woods Hole, Mass. 02543

Dr. Roger Mann
Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

Dr. Gilbert Pauley
College of Fisheries
University of Washington
Seattle, Washington 98195

Dr. Les Watling
Ira C. Darling Center
University of Maine
Walpole, Maine 04573

Journal of Shellfish Research
Volume 8, Number 1
ISSN: 00775711
June 1989

Some production costs for this volume have been covered by a generous contribution from H. Butler Flower, Frank M. Flower and Sons, Inc., oyster growers from Bayville, New York. Their support is greatly appreciated.

IN MEMORIAM

John W. Ropes

John W. Ropes died at his home in Falmouth, Massachusetts on September 5, 1988. He was 61 years old. Widely recognized for his work in growth and reproductive biology of oceanic bivalves, he was actively publishing in these and associated fields at the time of his death.

John ('Johnny' to his friends) was born May 17, 1927, in Salem, Massachusetts. Much of his boyhood was spent in Jackson, New Hampshire, where he cultivated a life-long interest in natural science and biology. He served in the Navy as a corpsman from 1945–1947, and afterwhich enrolled in Alfred College, in upstate New York. Several of his summers were spent at the Stone Laboratory of Ohio State University, on Lake Erie. It was here that his interest in aquatic biology was nurtured.

Johnny's first professional position was in 1954, with the U.S. Fish and Wildlife Service, in Newburyport, Massachusetts. Over the next decade he and colleagues conducted systematic investigations of the ecology of soft clam and its predator species, including horseshoe crab and green crab (Ropes and Stickney 1965; Ropes 1961; Ropes 1968a). During this period he was also assigned to Fish and Wildlife Service facilities in Kingston, RI, and Boothbay Harbor, ME.

In 1963 he was transferred to Franklin City, VA, and subsequently the following year to Oxford, MD. It was here that he, Dr. Arthur Merrill and others initiated what would be one of the most important studies of the fishery ecology of any bivalve species yet investigated. Their work on the surf clam fishery would form the scientific basis for comprehensive management of the species under the Magnuson act, beginning in 1977. Although not a quantitative biologist, John nevertheless identified the need for a systematic, and intensive program to document the performance, areal extent and biological characteristics of the developing fishery (Ropes 1982). Simultaneous with the fishery sampling program, region-wide fishery independent surveys were undertaken, as were a variety of biological studies, including those directed

to growth, reproduction, behavior, abundance and distribution. His publication record during this period was indeed prodigious, and included work on the reproductive cycle of surf clam (Ropes 1968b), of which he was justifiably most proud. He also found time during this period to complete graduate studies in Marine Sciences at the University of Delaware.

His transfer in 1977 to the National Marine Fisheries Service laboratory in Woods Hole, MA brought with it many new challenges and opportunities. By then the fishery for ocean quahog had been initiated in earnest, but there was little life history information with which to develop rational harvest strategies. A mark-recapture program initiated by John was to provide considerable data on growth and reproduction, and most importantly, conclusively validate through shell microstructure observations, the advanced age and exceedingly slow growth rate of the species (Ropes et al. 1984). Current management programs are based to a great extent on these findings.

John published extensively in NSA journals, and served in several Association posts including Co-Editor of the Proceedings (1970–1971), Officer-at-Large (1976), and Custodian of Records and Publications (1967–1970). He was the recipient of numerous awards, including special recognition by the Oyster Institute of North America, for his work in the surf clam program.

Notwithstanding his professional contributions, it is perhaps Johnny's relationships with young scientists just beginning their careers that will be his greatest legacy. Always quick to share his enormous skills, he cultivated relationships with a variety of young scientists both in government service, and in the academic world. In exchange for sharing his knowledge of technique and approach, he broadened his eclectic interests in marine sciences. John is survived by his wife, Mary. He will be missed.

Dr. Steven A. Murawski
Woods Hole
March, 1989

LITERATURE CITED

Ropes, J. W. 1961. Longevity of the horseshoe crab, *Limulus polyphemus* (L.). *Transactions of the American Fisheries Society* 90:79–80.

Ropes, J. W. 1968a. The feeding habits of the green crab, *Carcinus maenas* (L.). *Fishery Bulletin* 67(2):183–203.

Ropes, J. W. 1968b. Reproductive cycle of the surf clam, *Spisula solidissima*, in offshore New Jersey. *Biological Bulletin* 135(2):349–365.

Ropes, J. W. 1982. The Atlantic coast surf clam fishery, 1965–1974. *Marine Fisheries Review* 44(8)1–14.

Ropes, J. W. & A. P. Stickney. 1965. Reproductive cycle of *Mya arenaria* in New England. *Biological Bulletin* 128(2):315–327.

Ropes, J. W., D. S. Jones, S. A. Murawski, F. M. Serchuk & A. Jerald, Jr. 1984. Documentation of annual growth lines in ocean quahogs, *Arctica islandica* Linne. *Fishery Bulletin* 82(1):1–19.

Journal of Shellfish Research, Vol. 8, No. 1, 1–6, 1989.

BACTERIAL SHELL DISEASE IN CRUSTACEANS: A REVIEW

R. G. GETCHELL
Maine Department of Marine Resources
West Boothbay Harbor, Maine 04575

KEY WORDS: shell disease, chitinoclastic bacteria, lobsters, crabs, shrimp, crustaceans.

Shell disease, the degradation of a crustacean's integument, is actually an external infection where a variety of microorganisms may attack the chitin of the exoskeleton and has been known to affect crustaceans since 1900 (Happich 1900, cited in Rosen 1970). Both chitinoclastic bacteria and fungi have been implicated as causative agents (Rosen 1970). The fungal disease, more often called "burned spot disease", with invasive pathology distinct from the bacterial syndrome will not be discussed in this review (Mann and Pieplow 1938, cited in Rosen 1970).

The simple descriptive term "shell disease" was coined by Hess (1937) to describe exoskeleton lesions on the American lobster, *Homarus americanus*. While several similar terms have been applied to other crustaceans with cuticular lesions, e.g. brown spot disease (Cipriani et al. 1980) and rust disease (Bright et al. 1960), most workers retain the name "shell disease."

The intent of this review is to summarize what is currently known about the following aspects of shell disease: (a) host species, (b) pathology, (c) causative agents, (d) environmental effects, (e) experimental disease reproduction, (f) geographical distribution, (g) disease incidence, (h) preventative measures, and (i) assay methods.

The host species reported to be afflicted with shell disease, in addition to *Homarus americanus*, range from cultured species such as *Macrobrachium rosenbergi* De Man and several penaeid shrimp species to commercially harvested species such as *Callinectes sapidus, Paralithodes camtschatica, H. gammarus, Cancer irroratus, Menippe mercenaria,* and *Chionoecetes tanneri* (See Table 1).

Sections of normal crustacean cuticle contain the various layers that make up the exoskeleton, including from outside to inside: epicuticle, exocuticle, calcified endocuticle, and non-calcified endocuticle (Dennell 1960). In each of the crustaceans infected with shell disease, the exoskeleton lesions typically begin as small dark brown or black pits (Rosen 1970; Fig. 1). Microscopic examination of these lesions usually reveal that the calcified layers of the exoskeleton are eroded. The blackening that is always associated with damage and necrosis of the exoskeleton is due to the production of melanin that has a bacteriostatic, clotting, and localizing function (Unestam and Weiss 1970). The necrotic zone is shallow and tends to spread parallel to the integument rather than into it (Rosen 1967). Many of the lesions eventually merge to form continuous craters (Fig. 2), sometimes accompanied by yellow or orange pigmented material (Young and Pearce 1975; Malloy 1978). In scanning electron microscopy studies, El-Gamal et al. (1986) noted that lesions were covered by a heavy coating of bacterial cells, often embedded in an amorphous mucoid-like material.

In most cases the non-calcified endocuticle remains intact, appearing to form a barrier to the diseased shell (Rosen 1967; Malloy 1978; Roald et al. 1981). However, in some instances, inner tissues have been found to be necrotic beneath the eroded cuticle, perhaps because of factors other than the chitinoclastic bacteria (Gopalan and Young 1975; Young and Pearce 1975; Brock 1983).

Shell disease lesions can affect any surface of the body or appendages, varying in size from tiny to large (Brock 1983). Roald et al. (1981) found the early stages of shell disease on the dorsal surfaces of the chelae and carapace, but deep necrotic lesions were seen on the ventral surface of the large chelae. In later stages, parts of the appendages can be completely eroded (Young and Pearce 1975; Baross et al. 1978). Other parts of the exoskeleton eroded by shell disease include: the chitinous layer of gill filaments (Sawyer and Taylor 1949; Lightner and Lewis 1975; Couch 1978); the ventral abdominal membrane (McLeese 1965); the lateral spines and dactylpodites (Rosen 1967); and the mandibles (Fisher et al. 1976).

Since Hess (1937) isolated a chitinoclastic bacterium from *Homarus americanus* shell lesions, many varieties of chitin digesting bacteria have been implicated as the causative agents of shell disease, most often *Vibrio spp.* Malloy (1978) was able to experimentally infect *H. americanus* with *Vibrio spp.* after abrading the test subjects. Roald et al. (1981) isolated *Vibrio spp.* from infected European lobsters, and Rosemark and Conklin (1983) found most *Vibrio*-infected lobsters were on inadequate diets or were in the advanced stages of shell disease. *Vibrio alginolyticus, V. anguillarum, V. parahaemolyticus,* and *Vibrio spp.* have also been identified as chitin-degrading bacteria from blue crab (*Callinectes sapidus*), penaeid shrimp (*Penaeus spp.*), tanner crab (*Chionoectes tanneri*), dungeness crab (*Cancer magister*), and Malaysian prawn (*Macrobrachium rosen-*

TABLE 1.

A summary of crustaceans reported with shell disease and their locations.

Species	Form of shell disease	Location	Reference
LOBSTERS:			
Homarus americanus	Shell disease, Black gill	Mass.	Estrella, 1984
H. americanus	Shell disease	Calif.	Fisher, 1977
H. americanus	Shell disease	Nova Scotia	Hess, 1937
H. americanus	Shell disease	Nova Scotia	Malloy, 1978
H. americanus	Abdominal membrane lesion	New Brunswick	McLeese, 1965
H. americanus	Gill chitin degradation	Maine	Sawyer & Taylor, 1949
H. americanus	Shell disease	New York Bight	Young & Pearce, 1975
Homarus gammarus	Shell disease	Norway	Roald et al., 1981
CRABS:			
Callinectes sapidus	Shell disease	Chesapeake Bay	Rosen, 1967; Krantz et al., 1969
C. sapidus	Shell disease	Gulf of Mexico	Cook & Lofton, 1973
C. sapidus	Shell disease	South Carolina	Sandifer & Eldridge, 1974
C. sapidus & *Menippe mercenaria*	Shell disease	South Florida	Iversen & Beardsley, 1976
Cancer irroratus	Shell disease	New York Bight	Young & Pearce, 1975
Cancer magister & *Chionoectes tanneri*	Exoskeleton lesions	Oregon	Baross et al., 1978
Paralithodes camtschatica	Shell disease	Alaska	Follet & Grischkowsky, 1981
SHRIMP:			
Penaeus spp.	Exoskeleton lesions	Gulf of Mexico	Cook & Lofton, 1973; Couch, 1978
Penaeus spp.	Cuticular lesions	Gulf of Mexico	Lightner & Lewis, 1975
Penaeus spp.	Brown spot disease	Gulf of Mexico	Cipriani et al., 1980
Penaeus semisulcatus	Shell disease	Kuwait	Tareen, 1982
Crangon setemspinosa	Shell disease	New York Bight	Gopalan & Young, 1975
OTHERS:			
Macrobrachium rosenbergii	Brown spot disease & bacterial necrosis	Hawaii, Florida, Tahiti, & Liberia	Brock, 1983
M. rosenbergii	Shell disease	United Kingdom	Delves-Broughton & Poupard, 1976
M. rosenbergii	Burn spot disease	United Kingdom & Malaysia	El-Gamal et al., 1986

bergii) (Krantz et al. 1969; Cook and Lofton 1973; Lightner and Lewis 1975; Delves-Broughton and Poupard 1976; Baross et al. 1978; Cipriani et al. 1980).

Aeromonas and *Pseudomonas*-like species have been isolated from lesions on penaeid shrimp, Malaysian prawn, and European lobsters (Cook and Lofton 1973; Lightner and Lewis 1975; Delves-Broughton and Poupard 1976; Roald et al. 1981, respectively). An *Aeromonas hydrophila* isolate was identified as a constant inhabitant of exoskeleton lesions of *M. rosenbergii* both from a laboratory facility in Britain and a Malaysian prawn farm (El-Gamal et al. 1986). Several other Gram negative bacillus isolates have also been identified as the probable causative agents of shell disease and these include: *Alteromonas, Flavobacterium*, and *Spirillum* (Cipriani et al. 1980), *Moraxella* and *Photobacterium* (Baross et al. 1978), *Myxobacteria* (Delves-Broughton and Poupard 1976) and *Pasteurella* (Lightner 1983).

Rosen (1970) believed that necrotic pits act as miniature niches where several taxonomic groups, rather than a single taxa, interact to cause the general effect of shell disease. In support of this theory, Brock (1983) lists the causes of brown spot disease in *M. rosenbergii* as bacterial species which produce extracellular lipases, proteases, and chitinases as well as fungi, mechanical trauma, and other damaging events to the epicuticle of the exoskeleton. The epicuticle contains polyphenolic substances which are generally refractive to microbial attack (Dennell 1960). But, Baross et al. (1978) believed that slow microbial degradation of the epicuticle occurs, allowing penetration by chitinoclastic bacteria, and Cipriani et al. (1980) suggested that lipolytic bacteria could initiate shell disease lesions.

Prior cuticular damage has been associated with many of the reports on shell disease. Malloy (1978) was able to reproduce necrosis characteristics of shell disease in lobsters when the integument had been damaged prior to inoculation with chitinoclastic bacteria. He suggested that under stressful conditions such as lobster impoundment, chitin

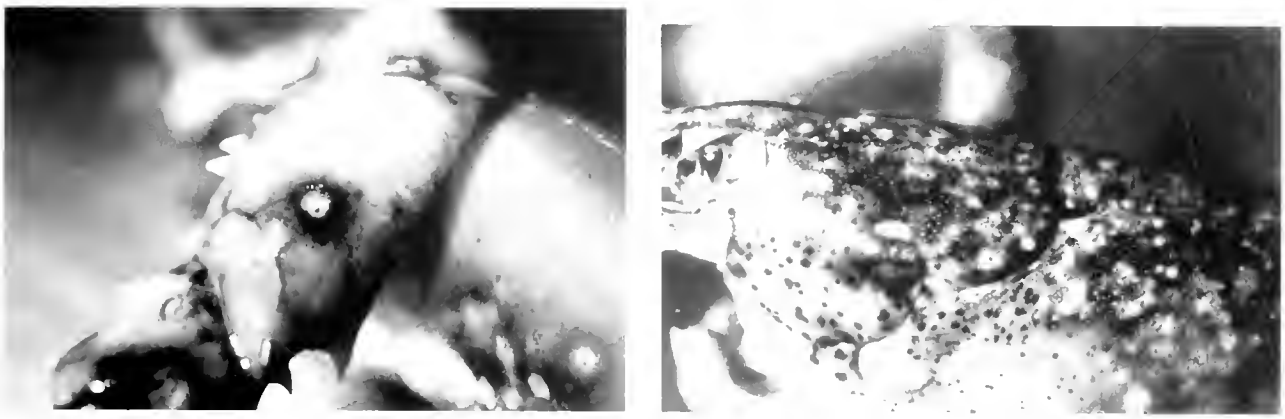


Figure 1. (a) Typical necrotic pits on the cheliped of an adult American lobster (*Homarus americanus*), caught off Boothbay Harbor, Maine in August, 1987, carapace length 83mm; (b) Same specimen with multiple lesions starting to join together on the thorax (Unpublished data).

decomposing bacteria associated with voided waste products and those normally found on lobster exoskeletons might enter punctures and injuries and initiate infections among weakened animals. Cook and Lofton (1973) noted that only mechanically damaged areas on the blue crab shell became necrotic, even though their experimental infection study was inconclusive. Mechanical injuries caused by difficulties during ecdysis, aggressiveness, handling, and high stocking densities have been blamed for predisposing prawns to shell disease (Delves-Broughton and Poupard 1976). Prawns were susceptible to shell lesions within one week by scraping a scalpel across the carapace. Development of lesions in king crabs took two weeks (Bright et al. 1960), and in penaeid shrimp 48–72 hours (Cipriani et al. 1980), but up to three months were necessary in impounded lobsters (Taylor 1948).

An injury-related disease syndrome in hatchery-reared and feral penaeid shrimp has also been reported (Lightner and Lewis 1975; Cipriani et al. 1980). They suggested that the diversity of environments where shell disease is prominent may indicate the presence of several distinct diseases manifesting themselves as one syndrome. Johnson (1983) pointed out that in natural, unstressed environments, chitinoclastic bacteria cause little or no harm; however, in

Figure 2. Expansive necrotic lesions of shell disease covering the lobster's exoskeleton; this lobster was taken from a shipment originating from a pound in Grand Manan, New Brunswick; this female measured 92 mm (563g). Photo courtesy of Jay Krouse, Maine Department of Marine Resources.

captive or cultured animals and ones living in degraded environments, they can be seriously debilitating. Sewage sludge and dredge spoils deposited in large amounts have also been suggested as a cause of shell diseased crabs and lobsters in New York Bight and Oslofjord, Norway (Young and Pearce 1975; Roald et al. 1981). Heavy metal exposure may be one of the predisposing factors for black gill disease of penaeid shrimp (Couch 1978).

Estrella (1984) implicated municipal and industrial wastes in conjunction with environmental conditions which enhanced turbidity and bacterial growth as causing high incidences of black gill and shell disease in *Homarus americanus*. The highest incidences of those closely related diseases were found near Boston Harbor and Buzzards Bay, Massachusetts. The most heavily diseased gills and signs of acute shell disease were observed on specimens collected at sites adjacent to New Bedford's Inner Harbor. Industrial contaminants such as polychlorinated biphenyls (PCB's), heavy metals, and hydrocarbons have been found throughout Buzzards Bay with the highest levels observed in the New Bedford Harbor region (Gilbert et al. 1973; Weaver, 1982, both cited in Estrella 1984). Unfortunately, the lack of historical baseline data on lobster disease incidence off the Massachusetts coast make it difficult to draw accurate conclusions.

High levels of organic matter may provide ideal conditions for chitinoclastic bacterial growth (Gopalan and Pearce 1975). Hood and Meyers (1974) concluded that chitinoclastic bacterial population and its ultimate biomass may be dependent primarily on chitinous substrate input into the estuarine system they monitored. Analysis of blue crab cuticle showed almost half the shell material was in the form of organic carbon. Of that fraction about half was chitin (Boyer and Kator 1985). Highest chitinoclastic bacterial counts corresponded to the period of maximum rate of chitnolysis, which was directly related to production of exoenzymes.

Hood and Meyers (1974) found optimum populations of chitinoclastic bacteria occurred during spring and early summer when median temperatures were above 16.9°C. Shell disease in *Callinectes sapidus* was more prevalent during late fall and winter than during summer (Sandifer and Eldridge 1974), while historically, lobsters that have been impounded through the winter are found infected when removed in the spring (Hess 1937; Taylor 1948; J. Hurst pers. comm.). Malloy (1978) showed that at lower temperatures (2–5°C) lobsters, particularly those with newly formed weaker shells, had higher prevalences of infection. Rosen (1970) describes this seasonal variation, stating that shell disease has been reported in all climatic conditions, from ice covered lakes to semitropical estuaries in summer. He also cites reports of shell disease in all environments where crustaceans occur including bog ponds, lakes, rivers, estuaries, and oceanic littorals. The discovery of infected deep-water crabs by Baross et al. (1978) adds another environment to the list.

The geographical distribution of shell disease reported in the literature further emphasizes the ubiquitous nature of shell disease (See Table 1). In Europe, the causative agents are most often considered to be different species of fungi that attack lobster, crab, and crayfish as well as a number of non-commercial species of craustaceans (Rosen 1970; Stewart 1984).

The level of shell disease in the commercial lobster catch is difficult to assess because the exploitation rate is so high that over 90% of the inshore harvest is comprised of new recruits. Lobstermen, with the exception of those in Maine waters, don't allow many large lobsters to survive in near-shore waters where shell disease incidence is likely to be higher. Since incidence varies with the age/size of the lobster, this complicates the disease assessment. Large lobsters molt less frequently and may be exposed to a causative agent of shell disease for longer periods of time (B. Estrella, pers. comm.). Baross et al. (1978) found that a higher incidence of lesions among female tanner crabs, *Chionoectes tanneri,* was due at least in part to the fact that females of this species cease to molt after their puberty molt. Immature animals, which molt more frequently, are less likely to be seriously affected by shell disease (Fisher et al. 1976).

Estrella (pers. comm.) also stated that Massachusetts lobster dealers do not have major problems with shell disease in their local stocks, possibly due to quick turnover in the marketplace. Unlike Maine, there are very few coastal impoundments in Massachusetts. A number of complaints were received from local dealers regarding their imports during the winters of 1985 and 1986. Problems with unaesthetic appearance, weakness, and elevated mortality for lobsters purchased from coastal pounds in southwestern Nova Scotia and the Jonesport region of Maine were reported.

Control of chitinoclastic bacteria in natural environments is virtually impossible. However, disease problems of captive and cultured crustaceans may be alleviated by increased attention to hygiene, wound avoidance, and proper husbandry (Stewart 1980). The cleanliness of any crustacean holding facility can be improved by removal of exuvia and other wastes and by upgrading water quality through higher flushing rates, filtration, and ultraviolet irradiation. Wound avoidance can be accomplished by careful handling, providing adequate substrate and shelter, reducing density, and shortening the holding time. Proper husbandry practices include many of those listed above as well as providing high quality feed (Rosen 1970; Hood and Meyers 1974; Rosemark and Conklin 1983).

Fisher et al. (1978) emphasizes the importance of selective culling of affected animals to retard the spread of the disease. They also discuss the prophylactic treatment of

lobster larvae by dip treatment in 20 ppm malchite green for 8 min every other day. Juvenile and adult lobsters can withstand higher concentrations of malachite green (Fisher 1977). Protocols for *Macrobrachium* larvae that arrest the bacterial necrosis and have a prophylactic use, are bath treatments with 2 ppm bipenicillin-streptomycin, 0.1 ppm furanace, or 0.65 to 1 ppm erythromycin phosphate (Brock 1983). A combination of malachite green (0.9 ppm) and formalin (22 ppm) was used to treat shell disease in *Penaeus semisulcatus* (de Haan) (Tareen 1982). In advanced cases, however, the infection extended deep into the muscle tissue and these chemicals were ineffective. Finally, Austin and Alderman (1987) state that moderately infected animals may be treated with a bath in 10 ppm oxolinic acid. By 12 h after oxolinic acid treatment, lesions once covered with a deep bacterial mat were almost free of bacteria and those that were seen were completely flattened and plasmolysed (El-Gamal et al. 1986).

Isolation of chitinoclastic bacteria from diseased tissue has been achieved by either complete removal of the lesion and streaking this onto a marine agar plate supplemented with precipitated chitin (Cook and Lofton 1973) or by swabbing the lesion with a sterile loop or swab and inoculating an enrichment broth containing strips of purified chitin (Malloy 1978; Sindermann and Rosenfield 1967). Pure colonies were then obtained by streaking from the broth culture onto chitin agar plates (Cipriani et al. 1980). The formation of clear zones around the bacterial colonies indicated that chitin degradation was occurring (Lear 1963). A recent advance that may allow more rapid diagnosis and treatment is a new assay method that detects chitobiase activity on a filter paper spot test (O'Brien and Colwell 1987).

Only under degraded or crowded conditions does shell disease appear to be highly contagious. Before this disease can be controlled, the practices of commercial interests must change, which include adopting proper prophylactic measures. The contribution of shell disease to the total annual mortality of our crustacean resources is unknown, but given the value of the product, even a limited reduction is of aestethic and economic concern. Protection of crustacean habitat is another step that can be taken. Further delineation of the conditions that allow chitinolytic bacteria to prosper will also be beneficial.

REFERENCES CITED

Austin, B. & D. J. Alderman. 1987. Bacterial shell disease of crustaceans. *Int. Counc. Explor. Sea.* Leaflet No. 31: 4 p.

Baross, J. A., P. A. Tester & R. Y. Morita. 1978. Incidence, microscopy, and etiology of exoskeleton lesions in the tanner crab, *Chionoecetes tanneri*. *J. Fish. Res. Board Can.* 35:1141–1149.

Boyer, J. N. & H. I. Kator. 1985. Method for measuring microbial degradation and mineralization of ^{14}C-labelled chitin obtained from the blue crab, *Callinectes sapidus*. Microb. Ecol. 11:185–192.

Bright, D. B., F. E. Durham & J. W. Knudsen. 1960. King crab investigations of Cook Inlet, Alaska. Unpubl. report cited in Rosen, 1970.

Brock, J. A. 1983. Diseases (infectious and noninfectious), metazoan parasites, predators, and public health considerations in *Macrobrachium* culture and fisheries. In: J. P. McVey (ed.) *Handbook of mariculture, crustacean aquaculture*. CRC Press. Boca Raton, Florida. 1:329–370.

Cipriani, G. R., R. S. Wheeler & R. K. Sizemore. 1980. Characterization of brown spot disease of Gulf Coast shrimp. *J. Invert. Pathol.* 36:255–263.

Cook, D. W. & S. R. Lofton. 1973. Chitinoclastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab. *J. Wildl. Dis.* 9:154–159.

Couch, J. A. 1978. Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and south Atlantic coasts of North America. *Fish. Bull.* 76:1–44.

Delves-Broughton, J. & C. W. Poupard. 1976. Disease problems of prawns in recirculation systems in the U.K. *Aquaculture* 5:201–217.

Dennell, R. 1960. Integument and exoskeleton. In: T. H. Waterman (ed.) *Physiology of crustacea*. Academic Press. New York, New York. 1:449–472.

El-Gamal, A. A., D. J. Alderman, C. J. Rodgers, J. L. Polglase & O. MacIntosh. 1986. A scanning electron microscope study of oxolinic acid treatment of burn spot lesions of *Macrobrachium rosenbergii*. *Aquaculture* 52:157–171.

Estrella, B. T. 1984. Black gill and shell disease in American lobster (*Homarus americanus*) as indicators of pollution in Massachusetts Bay and Buzzards Bay, Massachusetts. *Mass. Div. Mar. Fish.* Pub. No. 14049-19-125-5-85-C.R. 17 pp.

Fisher, W. S. 1977. Shell disease of lobsters. In: C. J. Sindermann (ed.) *Disease diagnosis and control in North American marine aquaculture*. Elsevier Scientific Publishing. Amsterdam, Netherlands. p. 158–162.

Fisher, W. S., R. Rosemark & E. H. Nilson. 1976. The susceptibility of cultured American lobsters to a chitinolytic bacterium. *Proc. World Maricul. Soc.* 7:511–520.

Fisher, W. S., E. H. Nilson, J. F. Steenbergen & D. V. Lightner. 1978. Microbial diseases of cultured lobsters: A review. *Aquaculture* 14:115–140.

Follet, J. E. & R. S. Grischkowsky. 1981. Investigation of shell disease in Alaska king and tanner crabs. *J. Shellfish Res.* 1:132.

Gopalan, U. K. & J. S. Young. 1975. Incidence of shell disease in shrimp in the New York Bight. *Mar. Pollut. Bull.* 6:149–153.

Hess, E. 1937. A shell disease in lobsters (*Homarus americanus*) caused by chitinovorous bacteria. *J. Biol. Board Can.* 3:358–362.

Hood, M. A. & S. P. Meyers. 1974. Distribution of chitinoclastic bacteria in natural estuarine waters and aquarial systems. In: R. S. Amborski, M. A. Hood & R. R. Miller (eds.) *Proceedings of Gulf coast symposium on diseases of aquatic animals*. Lousiana State University. Baton Route, Lousiana. LSU-SG-74-05 p. 115–121.

Iversen, E. S. & G. L. Beardsley. 1976. Shell disease in crustaceans indigenous to South Florida. *Prog. Fish-Cult.* 38:195–196.

Johnson, P. T. 1983. Diseases caused by viruses, rickettsiae, bacteria, and fungi. In: J. P. Provenzano, Jr. (ed.) *The biology of crustacea, pathobiology*. Academic Press. New York, New York. 6:1–78.

Krantz, G. E., R. R. Colwell & E. Lovelace. 1969. *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. *Science* 164:1286–1287.

Lear, D. W. 1963. Occurrence and significance of chitinoclastic bacteria in pelagic waters and zooplankton. In C. Oppenheimer (ed.) *Symposium on marine microbiology*. Charles C. Thomas. Springfield, Illinois. p. 594–610.

Lightner, D. V. 1983. Diseases of cultured penaeid shrimp. In: J. P.

McVey (ed.) *Handbook of mariculture, crustacean aquaculture*. CRC Press. Boca Raton, Florida. 1:289–320.

Lightner, D. V. & D. H. Lewis. 1975. A septicemic bacterial disease of penaeid shrimp. *Mar. Fish. Rev.* 37:25–28.

Malloy, S. C. 1978. Bacteria induced shell disease of lobsters (*Homarus americanus*). *J. Wildl. Dis.* 14:2–10.

McLeese, D. W. 1965. Lesions on the abdominal membrane of lobsters. *J. Fish. Res. Board Can.* 22:639–641.

O'Brien, M. & R. R. Colwell. 1987. A rapid test for chitinase activity that uses 4-methylumbelliferyl-N-acetyl-B-D-glucosaminide. *Appl. Env. Microbiol.* 53:1718–1720.

Roald, S. O., J. Aursjo & T. Hastein. 1981. Occurrence of shell disease in lobsters, *Homarus gammarus* (L.), in the southern part of Oslofjord, Norway. *FiskDir. Skr. HavUnders.* 17:153–160.

Rosemark, R. & D. E. Conklin. 1983. Lobster pathology and treatments. In: J. P. McVey (ed.) *Handbook of mariculture, crustacean aquaculture*. CRC Press. Boca Raton, Florida. 1:371–377.

Rosen, B. 1967. Shell disease of the blue crab, *Callinectes sapidus*. *J. Invert. Pathol.* 9:348–353.

Rosen, B. 1970. Shell disease of aquatic crustaceans. In: S. F. Snieszko (ed.) *A symposium on diseases of fishes and shellfishes*. American Fisheries Society. Washington, D.C. Spec. Pub. No. 5. p. 409–415.

Sandifer, P. A. & P. J. Eldridge. 1974. Observations on the incidence of shell disease in South Carolina blue crabs, *Callinectes sapidus* (Rathbun). In: R. S. Amborski, M. A. Hood & R. R. Millers (eds.) *Proceedings of Gulf coast symposium on diseases of aquatic animals*. Lousiana State University. Baton Rouge, Lousiana. LSU-SG-74-05 p. 161–184.

Sawyer, W. H. & C. C. Taylor. 1949. The effect of shell disease on the gill and chitin of the lobster (*Homarus americanus*). *Maine Dep. Sea Shore Fish. Res. Bull.* 1:10.

Sindermann, C. J. & A. Rosenfield. 1967. Principal diseases of commercially important marine bivalve mollusca and crustacea. *Fish. Bull.* 66:335–385.

Stewart, J. E. 1980. Diseases. In: J. S. Cobb & B. F. Phillips (eds.) *The biology and management of lobsters*. Academic Press. New York, New York. p. 301–342.

Stewart, J. E. 1984. Lobster diseases. *Helgolander Meeresunters.* 37:243–254.

Tareen, I. U. 1982. Control of diseases in the cultured population of penaeid shrimp, *Penaeus semisulcatus* (de Haan). *J. World Maricul. Soc.* 13:157–161.

Taylor, C. C. 1948. Shell disease as a mortality factor in the lobster (*Homarus americanus*). *Maine Dep. Sea Shore Fish. Fish Circ.* 4:1–8.

Unestam, T. & D. W. Weiss. 1970. The host-parasite relationship between freshwater crayfish and the crayfish disease fungus *Aphanomyces astaci:* Responses to infection by a susceptible and a resistant species. *J. Gen. Microbiol.* 60:77–90.

Young, J. S. & J. B. Pearce. 1975. Shell disease in crabs and lobsters from the New York Bight. *Mar. Pollut. Bull.* 6:101–105.

Journal of Shellfish Research, Vol. 8, No. 1, 7–11, 1989.

ALLOMETRIC GROWTH AND ONSET OF MATURITY IN MALE AMERICAN LOBSTERS (*HOMARUS AMERICANUS*): THE CRUSHER PROPODITE INDEX

D. E. AIKEN AND S. L. WADDY
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick, Canada E0G 2X0

ABSTRACT Crusher cheliped propodite volume was obtained from male American lobsters (*Homarus americanus*) from the Bay of Fundy, the Gulf of St. Lawrence and Northumberland Strait to determine relationships between level of allometric growth and size at onset of maturity. Propodite volume (CPV) of the large (crusher) cheliped changed to a higher level of allometric growth when the male lobster reached a mature size. Best-fit linear regressions between CPV and CL for immature and mature males were calculated to estimate size at maturity using Somerton's program MATURE, but this proved to be unnecessary. Simply dividing CPV by the cube of the carapace length yields a Cheliped Propodite Index (CPI) that shows a clear inflection when plotted against carapace length. In addition, CPI values can be used to estimate maturity of individual males: CPI values greater than 22–24 are indicative of a positive influence of maturity on CPV. Because cheliped measurements can be made quickly and without injury to the animal, CPI is an ideal method for assessing maturity in wild American lobsters.

KEY WORDS: *Homarus americanus*, lobster, maturity

INTRODUCTION

Spawning and the presence of eggs are conclusive evidence of sexual maturity in female American lobsters *Homarus americanus*, and the development of secondary sexual characters such as increased abdomen width or engorged abdominal glands can be correlated with ovarian maturation and used to estimate maturity in non-ovigerous females (Aiken and Waddy 1980a, b, 1982, Ennis 1980, Waddy and Aiken 1980).

In male lobsters there is nothing comparable to the presence of eggs that can be conveniently used to establish maturity. Ability to copulate with and inseminate a female lobster is conclusive evidence but this event is too seldom witnessed in the wild to be of practical value. Presence of spermatozoa in the vasa deferentia has been used (Conan et al. 1985), but spermatozoa can be found in the vasa deferentia of male *Homarus* that are too small to mate with and inseminate a female (Aiken and Waddy 1980a, Briggs 1976, Briggs and Mushacke 1979, 1980, Krouse 1973, Templeman 1935, 1944, Van Engel 1980).

Cheliped size is known to undergo changes in allometry as a male lobster matures (Aiken and Waddy 1980a, b, Ennis 1971, 1980, Squires 1970, Templeman 1939, 1944; but cf. Conan et al. 1985), and this change is related to functional maturity, i.e., the ability of a male to inseminate a female (Ennis 1980, Templeman 1934, Waddy and Aiken 1990 and unpublished).

Ennis (1980) used the ratio of crusher cheliped weight to animal weight to demonstrate the change in allometric growth. This method is effective, but not easily used in the field. In earlier reports (Aiken and Waddy 1980a) we described a simple method for estimating cheliped volume and briefly discussed the use of allometric growth in this context (Aiken and Waddy 1980b). Similar principles of allometric growth have been used to estimate maturity in other decapods (e.g. Brown and Powell 1972, Conan and Comeau 1986, Haley 1973, Mashiko 1981, Paulraj et al. 1982, Somerton 1980, Somerton and MacIntosh 1983, Watson 1970).

Our objective here was to apply contemporary methods of allometric growth analysis to male *Homarus americanus* from the southern Gulf of St. Lawrence and the Bay of Fundy, and describe a male "maturity index" that simplifies estimates of onset of maturity in this decapod.

METHODS

Propodite volume of the large ("crusher") cheliped was obtained from male lobsters caught by commercial trap in the Bay of Fundy near Grand Manan (94 males, 61–172 mm CL), the north side (Gulf of St. Lawrence) of Prince Edward Island (715 males, 33–118 mm CL), and Northumberland Strait on the south side of Prince Edward Island (227 males, 64–129 mm CL). In addition, the cheliped propodite volume was obtained for 260 females of 58–230 mm CL from these areas.

The estimate of crusher propodite volume (CPV) was obtained from measurements of length, width and thickness (depth or height) of the crusher propodite (CPV = length × width × thickness). Measurements were to 0.01 cm with a vernier caliper. Propodite length was measured from the rear of the articular condyle to the most anterior point on the propodite. Width was measured across the 'palm' from the top of the ridge beneath the articulation with the dactyl to the outer margin of the propodite, perpendicular to the long axis. Thickness was measured at the midpoint of the propodite "palm" (Fig. 1). Of these three, the measurement of width is subject to the greatest error due to variations in angle of measurement and shape of propodite, but this has a relatively minor impact on the final value.

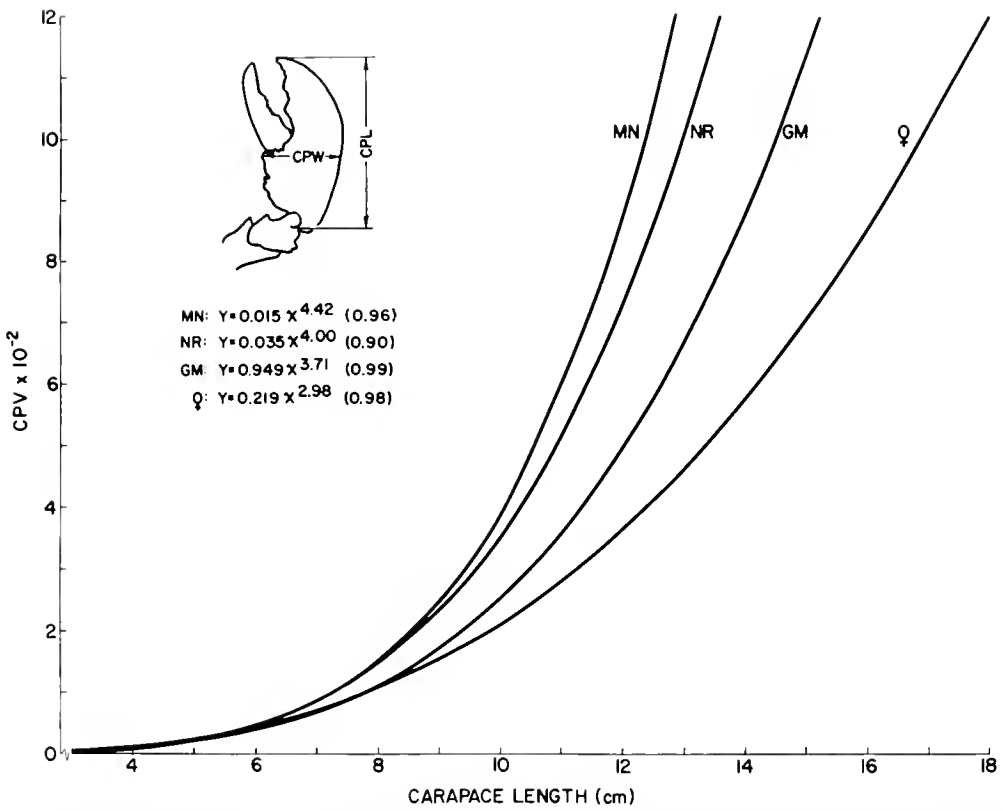


Figure 1. Power curves for male lobsters from Miminegash (MN), North Rustico (NR) and Grand Manan (GM) compared with the female power curve. Points of measurement of crusher propodite length and width are shown at upper left. Depth or thickness is measured at the point occupied by the "CPW" symbol. Correlation coefficients (r) are in brackets after each equation.

RESULTS

Crusher propodite volume (CPV) increased allometrically as a power of the carapace length (CL) according to the growth equation $Y = aX^b$. The level of allometric growth of CPV and CL for females is similar for all areas ($p > 0.01$) and comparable to that of juvenile males from all areas examined. It is described by the equation $Y = 0.22X^{2.98}$ ($r = 0.98$) (Fig. 1).

Values for the constants *a* and *b* were different for males from each geographical area studied, and reflect differences in size at onset of maturity. Males from Northumberland Strait (Miminegash) mature at a slightly smaller size than those from the Gulf of St. Lawrence (North Rustico), and those in turn mature at a smaller size than those from the Bay of Fundy (Grand Manan) (Fig. 1). This parallels what is known about size at maturity of females from these areas (Aiken and Waddy 1986, Campbell and Robinson 1983).

In contemporary analysis of allometric growth, logarithmic transformation is the preferred method for display of morphometric data (Hartnoll 1978, 1982). The CPV data for males from the three areas are displayed this way in Figure 2, along with the common female regression line. Data from the Gulf of St. Lawrence and the Bay of Fundy are the most useful. Those from the Northumberland Strait (Miminegash) contain too few lobsters in the small (immature) category to permit mathematical treatment. For the Gulf of St. Lawrence and Bay of Fundy, best-fit linear regression lines for immature and mature levels of allometry were calculated using the program MATURE developed by Somerton (1980). Intersects and size at 50% maturity are given in Table 1. For the Northumberland Strait sample the mature and immature phases were arbitrarily separated by visual inspection and least squares regression applied to the data on either side.

DISCUSSION

Templeman (1935) recognized that cheliped size is a male secondary sexual character comparable to abdomen width in females, and attempted to demonstrate an inflection between mature and immature phases by plotting cheliped propodite length against total length. This technique was never widely used, and the inflection became even more difficult to demonstrate once carapace length became the standard measure.

Squires (1970) noted a relationship between crusher claw weight and male maturity, and his observations were extended by Ennis (1971, 1980) who correlated CL and crusher cheliped weight and demonstrated an inflection that presumably was related to maturity. Although this is a potentially useful method it has two serious drawbacks as a

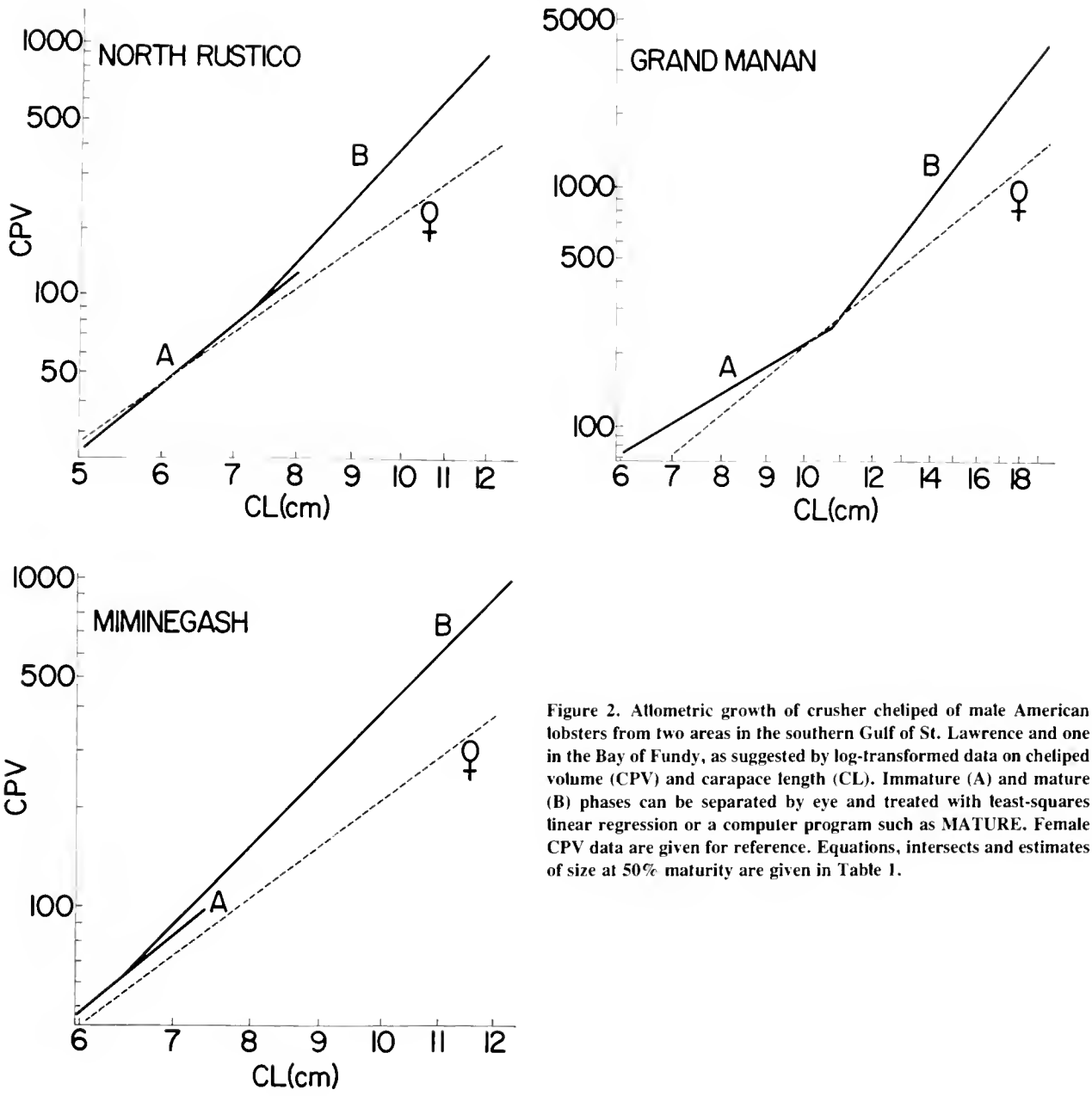


Figure 2. Allometric growth of crusher cheliped of male American lobsters from two areas in the southern Gulf of St. Lawrence and one in the Bay of Fundy, as suggested by log-transformed data on cheliped volume (CPV) and carapace length (CL). Immature (A) and mature (B) phases can be separated by eye and treated with least-squares linear regression or a computer program such as MATURE. Female CPV data are given for reference. Equations, intersects and estimates of size at 50% maturity are given in Table 1.

field technique: a commercially valuable part of the lobster must be removed, and weights of crusher claw and whole animal must occasionally be determined on board small fishing vessels, a technically difficult task.

However, weight is roughly proportional to volume, and cheliped volume can be estimated from the length, width and thickness of the propodite, measurements that can be obtained on board ship without injury to the lobster.

We initially divided this estimate of cheliped volume by carapace length to obtain the so-called Anderson Index (Aiken and Waddy 1980a, b). The Anderson Cheliped Index showed an inflection at the approximate size of maturity (cf. Conan et al. 1985), but it was still necessary to treat the points mathematically to obtain a reasonable estimate of the intersect. For this, log transformation of CPV and CL data is preferred, but this method is inconvenient to use, it provides no information on the maturity of individual males, and it yields criteria that are valid only for lobsters from a specific geographic locality.

To eliminate these difficulties we resurrected the "index" concept. Since the value b is approximately 3 for the power curve of female CPV on CL, $CPV/(CL)^3$ will produce a straight horizontal line when plotted against carapace length. When the CPV is multiplied by 100 for convenience, the value for the constant a is approximately 21–22 for females. Since comparable values are also ob-

TABLE 1.

Equations and calculated intersects (size at onset of sexual maturity) for regression lines of log-transformed crusher propodite volume (CPV) and carapace length (CL), and estimated size at 50% maturity from Somerton's (1980) program MATURE for male lobsters from the southern Gulf of St. Lawrence and Bay of Fundy.

Location		b	a	Carapace Length (cm) Intersect	50% Mature
Miminegash	A[1]:	Ln Y = 3.395	Ln X − 2.189	6.66	*
	B:	Ln Y = 4.267	Ln X − 3.842		
North Rustico	A:	Ln Y = 3.123	Ln X − 1.739	6.64	7.20
	B:	Ln Y = 4.452	Ln X − 4.255		
Grand Manan	A:	Ln Y = 2.195	Ln X + 0.326	10.75	11.60
	B:	Ln Y = 4.523	Ln X − 5.202		

* Insufficient data for program MATURE

[1] A, immature; B, mature

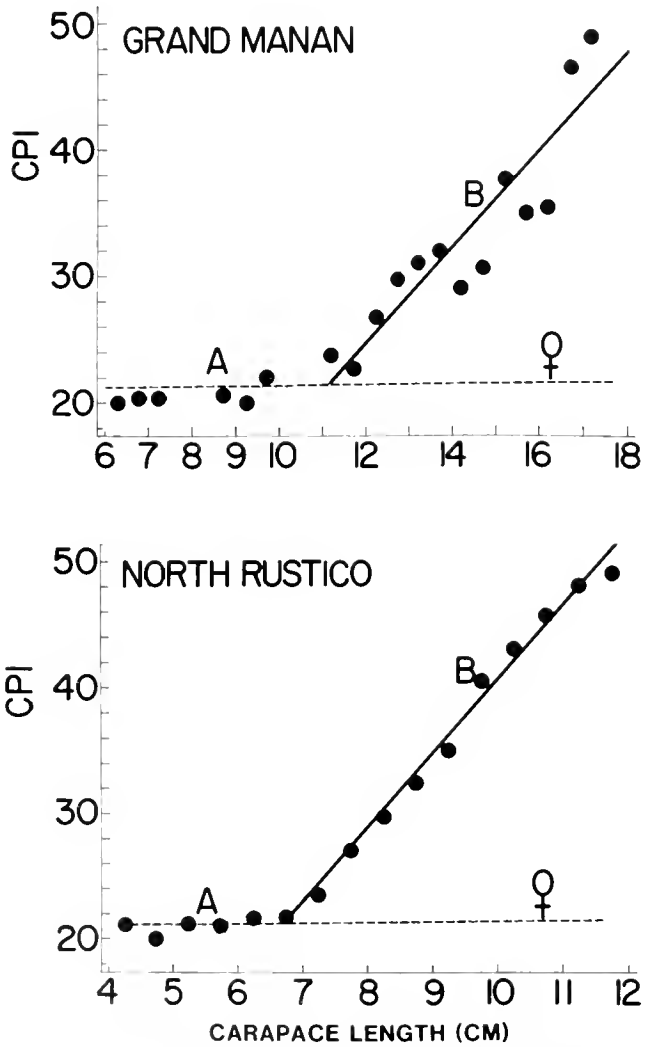


Figure 3. Allometric growth of the crusher cheliped of male American lobsters as indicated by the plot of crusher propodite index (CPI) against carapace length (CL). Intersect on female regression line indicates size at onset of male sexual maturity in the Bay of Fundy (Grand Manan) and Gulf of St. Lawrence (North Rustico). Regression lines calculated by least-squares linear regression (Table 2). Individual points are means of 5 mm CL groups.

tained with immature males, irrespective of size, $100(CPV)/(CL)^3$ may be used as a male maturity index, which we term the CPI, or *Crusher Propodite Index*. CPI values larger than 22–24 are indicative of a higher level of allometric growth and, therefore, of a positive influence of maturity on CPV.

The relationships between CPI and CL for males from North Rustico and Grand Manan are shown in Fig. 3. Plotted points on these graphs are means of measurements within 5 mm CL groups. Inflections are obvious, but if greater precision is required linear regression analysis can be applied and the intersect calculated.

In practical terms only the mature phase need be determined and the inflection (or intersect) estimated from the female baseline. In some areas it can be difficult to obtain sufficient measurements on the full range of immature male sizes to permit calculation of a representative immature regression line. The difference between the immature male and the female lines is generally less than the error introduced by inadequate sampling of immature males.

Calculated intersects of North Rustico and Grand Manan mature males on the female regression line are 67.7 and 110.7 mm CL respectively (Table 2), reasonably close to those (66.4 and 107.5) calculated with log transformed CPV data. The inflection determined from CPI analysis is

TABLE 2.

Equations and calculated intersects of regression lines of crusher propodite index (CPI) and carapace length (CL) for mature male and female lobsters from the southern Gulf of St. Lawrence and Bay of Fundy.

Locatinn	b	a	r	Intersects CL (cm)	CPI
Miminegash	Y = 5.39	X − 13.15	0.71	6.39	24
North Rustico	Y = 5.93	X − 18.81	0.69	6.77	23
Grand Manan	Y = 3.77	X − 20.28	0.73	11.07	22
Females (all areas)	Y = 0.03	X + 21.12			

assumed to represent the onset of maturity in a given stock of lobsters (as opposed to the 50% estimate provided by MATURE).

An important aspect of CPI analysis is that the CPI value at point of inflection is 24 or less, whether the male is 70 mm CL from the southern Gulf or 120 mm from the Bay of Fundy. In other words, it appears to be possible to determine the maturity of an individual male by simply determining his CPI. Studies correlating male CPI with mating capability are currently underway and will be reported subsequently.

Log transformed data are commonly used in maturity assessments based on cheliped allometry. We suggest that the conversion of untransformed data to the CPI described here is more versatile than logarithmic transformation because it is applicable to all male lobsters, irrespective of origin or stock, and can be used to estimate maturity of individuals as well as stocks.

REFERENCES CITED

Aiken, D. E. & S. L. Waddy. 1980a. Maturity and reproduction in the american lobster. *Can. Tech. Rep. Fish. Aquat. Sci.* 932:59–71.

Aiken, D. E. & S. L. Waddy. 1980b. Reproductive Biology. In *The Biology and Management of Lobsters* (J. S. Cobb and B. F. Phillips, eds.). New York: Academic Press. Vol. 1, Chapt. 4, pp. 215–276.

Aiken, D. E. & S. L. Waddy. 1982. Cement gland development, ovary maturation, and reproductive cycles in the American lobster *Homarus americanus*. *J. Crustacean Biol.* 2:315–327.

Aiken, D. E. & S. L. Waddy. 1986. Environmental influence on recruitment of the American lobster, *Homarus americanus:* a perspective. *Can. J. Fish. Aquat. Sci.* 43:2258–2270.

Briggs, P. T. 1976. Aspects of the American lobster in Long Island Sound, New York. NOAA, NMFS, *Commer. Fish. Res. Dev. Act, Job. Completion Rep.*, New York Project No. 3-212-R, 37 p.

Briggs, P. T. & F. M. Mushacke. 1979. The American lobster in western Long Island Sound. *N.Y. Fish Game J.* 26:58–86.

Briggs, P. T. & F. M. Mushacke. 1980. The American lobster and the pot fishery in the inshore waters off the south shore of Long Island, New York. *N.Y. Fish. Game J.* 27:156–178.

Brown, R. B. & G. C. Powell. 1972. Size at maturity in the male Alaskan tanner crab, *Chionoecetes bairdi*, as determined by chela allometry, reproductive tract weights, and size of precopulatory males. *J. Fish. Res. Bd. Canada* 29:423–427.

Campbell, A. & D. G. Robinson. 1983. Reproductive potential of three American lobster (*Homarus americanus*) stocks in the Canadian maritimes. *Can. J. Fish. Aquat. Sci.* 40:1958–1967.

Conan, G. & M. Comeau. 1986. Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio*. *Can. J. Fish. Aquat. Sci.* 43:1710–1719.

Conan, G., M. Comeau & M. Moriyasu. 1985. Functional maturity of the American lobster *Homarus americanus*. *Int. Counc. Explor. Sea* C.M. 1985/K:29.

Ennis, G. P. 1971. Lobster (*Homarus americanus*) fishery and biology in Bonavista Bay, Newfoundland, 1966–1970. *Fish. Res. Board Can. Tech. Rep.* 289:17–46.

Ennis, G. P. 1980. Size-maturity relationships and related observations in Newfoundland populations in the lobster (*Homarus americanus*). *Can. J. Fish. Aquat. Sci.* 37:945–956.

Haley, S. R. 1973. On the use of morphometric data as a guide to reproductive maturity in the ghost crab, *Ocypode ceratophthalmus* (Pallas) (Brachyura, Ocypodidae). *Pac. Sci.* 27:350–362.

Hartnoll, R. G. 1978. The determination of relative growth in Crustacea. *Crustaceana* 34:281–293.

Hartnoll, R. G. 1982. Growth. In *The Biology of Crustacea* (L. G. Abele, ed.). New York: Academic Press. Vol. 2, Chapt. 3, pp. 111–196.

Krouse, J. S. 1973. Maturity, sex ratio and size composition of a natural population of American lobster, *Homarus americanus*, along the Maine coast. *U.S. Dept. Comm., Fish. Bull.* 71:165–173.

Mashiko, K. 1981. Sexual dimorphism of the cheliped in the prawn *Macrobrachium nipponense* (de Haan) and its significance in reproductive behavior. *Zool. Mag.* 90:333–337.

Paulraj, S., P. Mullainadhan & M. H. Ravindranath. 1982. Relative growth before and after sexual maturity in *Ocypode platytarsis* (Milne-Edwards) and *Ocypode cordimana* (Desmarest) (Crustacea: Decapoda). *Int. J. Invert. Reprod.* 5:115–128.

Somerton, D. A. 1980. A computer technique for estimating the size of sexual maturity in crabs. *Can. J. Fish. Aquat. Sci.* 37:1488–1494.

Somerton, D. A. & R. A. MacIntosh. 1983. The size at sexual maturity of blue king crab, *Paralithodes platypus*, in Alaska. *U.S. Dept. Comm., Fish. Bull.* 81:621–628.

Squires, H. J. 1970. Lobster (*Homarus americanus*) fishery and ecology in Port au Port Bay, Newfoundland, 1960–65. *Proc. Nat. Shellfish. Assoc.* 60:22–39.

Templeman, W. 1934. Mating in the American lobster. *Contrib. Canadian Biol. Fish. (NS)* 8:423–432.

Templeman, W. 1935. Local differences in the body proportions of the lobster, *Homarus americanus*. *J. Biol. Board Can.* 1:213–226.

Templeman, W. 1939. Investigation into the life history of the lobster on the west coast of Newfoundland, 1938. *Newfoundland Dept. Nat. Resour. Res. Bull. (Fish)* 16, 98 p.

Templeman, W. 1944. Sexual dimorphism in the lobster (*Homarus americanus*). *J. Fish. Res. Board Can.* 6:228–232.

Van Engel, W. A. 1980. Maturity and fecundity in the American lobster, *Homarus americanus*—review. *Can. Tech. Rep. Fish. Aquat. Sci.* 932:51–58.

Waddy, S. L. & D. E. Aiken. 1980. Abdomen width and carapace length, a maturity index for female lobster, *Homarus americanus*. *Can. Atl. Fish. Sci. Adv. Comm. Res. Doc.* 80/44, 11 p.

Waddy, S. L. & D. E. Aiken. 1990. Controlling mating and insemination in the American lobster, *Homarus americanus*. In Crustacean Sexual Biology (R. Bauer and J. Martin, eds.). New York: Columbia University Press (In Press).

Watson, J. 1970. Maturity, mating and egg laying in the spider crab, *Chionoectes opilio*. *J. Fish. Res. Board Can.* 27:1607–1616.

Journal of Shellfish Research, Vol. 8, No. 1, 13–23, 1989.

GROWTH AND DISTRIBUTION OF SNOW CRAB, *CHIONOECETES OPILIO*, IN THE SOUTHEASTERN GULF OF ST. LAWRENCE

D. A. ROBICHAUD[1], R. F. J. BAILEY[2], AND R. W. ELNER[3]

[1]*Biological Sciences Branch*
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick
Canada, E0G 2X0

[2]*Biological Sciences Branch*
Department of Fisheries and Oceans
Institut Maurice-Lamontagne
P.O. Box 1,000
Mont-Joli, Québec
Canada, G5H 3Z4

[3]*Biological Sciences Branch*
Department of Fisheries and Oceans
P.O. Box 550
Halifax, Nova Scotia
Canada, B3J 2S7

ABSTRACT During 1981–82, 5908 snow crab (*Chionoecetes opilio*) were captured by beam trawling off northwest Cape Breton. Analysis of modes in size frequency distributions indicates that the percentage increase in carapace width (CW) decreases from 55% between instars I and II to 35% between instars IX and X. Based upon the estimated transition rate of individuals between instars, a female would take 3.5 years to reach the average terminal molt size of 69 mm CW and a male 4.5 years to pass the legal minimum size (95 mm CW). Crab density was related more to substrate type than depth. Juveniles (<50 mm CW) had their highest density (15.1 crab/1000 m^2) on mud, although all crab sizes were found on the three substrates (mud, sand, gravel) sampled. The mean density of mature females (17.7 crab/1000 m^2) on mud and sand combined was higher than that for juveniles (9.7 crab/1000 m^2). Juvenile and mature female crabs were patchily distributed within each substrate type. The absence of female crab of 30–50 mm CW and male crab of 30–80 mm CW in the trawl samples was related to later declines in commercial catch rates during 1986 and 1987.

KEY WORDS: snow crab, growth, distribution, recruitment, habitat

INTRODUCTION

Snow crab, *Chionoecetes opilio*, is of commercial importance in the northwest Atlantic, the northern Pacific and the Sea of Japan (Bailey and Elner 1989). In Atlantic Canada, the fishery exploits only males above a legal minimum size of 95 mm carapace width (CW) (Elner and Robichaud 1983). Female snow crab undergo a terminal molt to maturity between 47 and 95 mm CW (Ito 1963, 1967; Watson 1969, 1970). Males attain a terminal molt to morphometric maturity within the range of 52–137 mm CW (O'Halloran 1985; Conan and Comeau 1986). The magnitude of each year's snow crab landings has become increasingly dependent on the annual recruitment of males into the commercial size range (Elner 1982; Elner and Bailey 1986). Currently, there are concerns that male-only crab fisheries, in general, are inherently unstable (*Cancer magister:* Botsford et al. 1983; *Chionoecetes* spp.: Otto et al. 1983, 1984; *Paralithodes camtschatica:* Otto 1986); recruitment patterns for Canadian snow crab stocks are particularly enigmatic (Elner and Bailey 1986; Bailey and Elner 1989). There is critical need for knowledge on the population biology and growth of snow crab in order to forecast recruitment into the fishery and assist managers in stabilizing stocks.

The present study investigates growth and distribution patterns for snow crab off northwest Cape Breton. Through analysis of size frequency distributions it was possible to identify instars and assess growth rates. In addition, density estimates were generated for comparison with previous studies. Missing instars in our samples appear related to subsequent declines in commercial catch rates; we suggest that prediction of annual recruitment into the fishery is possible from assessments of prerecruit abundance, coupled with our findings on growth.

MATERIALS AND METHODS

Sampling was conducted off northwest Cape Breton (Fig. 1) in an area extending from High Capes to Margaree Harbour. The western boundary was parallel to shore at a distance of approximately 30 km. Depth within the study area ranged between 50 and 150 m.

Snow crab collections were made on four research cruises: May 1981 and May, July and September, 1982. The sampling gear was a 3-m wide beam trawl, similar to

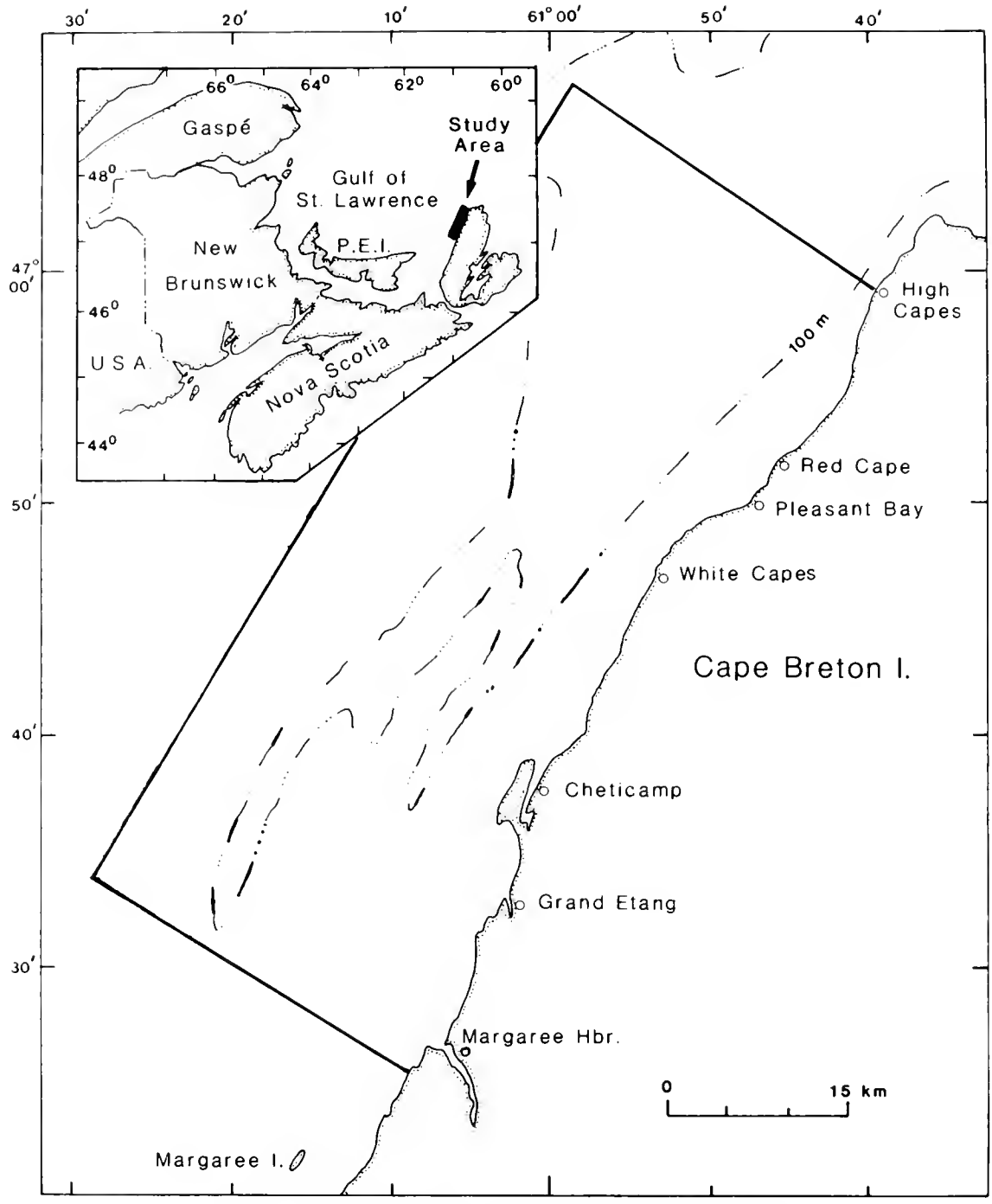


Figure 1. Geographic location of study area in the Gulf of St. Lawrence.

that used by Miller and O'Keefe (1981). The net had a stretch mesh of 64 mm and the cod end was double netting with a stretch mesh of 25 mm. In 1982, 84 sites within the study area were chosen randomly within five 20-m depth strata: 50–70m, 71–90 m, 91–110 m, 111–130 m and 131–150 m (details of the sampling scheme are in Robichaud (1985)). Each tow lasted 15 minutes at a speed of 0.5–2.0 m/s. Position and depth were recorded at the beginning and end of every tow. Onboard the boat, trawl contents were emptied into a sieve (2-cmm metalic mesh size) and washed down with seawater. Snow crab of <50 mm CW were preserved in 4% formaldehyde and taken to the laboratory for further analysis. Larger crab were measured and sexed on the boat. The substrate, sampled with a Van Veen grab, at the completion of each tow, was categorized as either "mud," "sand" or "gravel"; based on the

Buchanan method (Holme and McIntyre 1971), a sediment study by Loring and Nota (1973) in the same area, and criteria described in Robichaud (1985).

At the laboratory, snow crab were measured and sexed with the help of a binocular microscope. Size was determined from the maximum carapace width (CW). Female maturity was assessed by the relative size of the abdomen and the occurrence of eggs (Watson 1970). All males <50 mm CW were classified as immature based on the minimum size of physiological maturity (51 mm CW) given by Watson (1970) and on the minimum size of morphometric maturity (52 mm CW) determined by Conan and Comeau (1986).

Crab densities were calculated from the trawl width and the distance towed and expressed as number of individuals per 1000 m^2. The mean density of crab for a particular criterion (depth stratum, substrate type, season) was estimated by dividing the sum of the number of crab per 1000 m^2 from each tow by the total number of tows for that criterion. The mean size of crabs for a criterion was calculated by combining the sizes of all crabs from each tow and dividing by the total number of crab. Possible differences in mean densities or mean sizes of crabs between criteria were tested by a "one-way" analysis of variance combined with a Duncan test (Duncan 1955) after transforming the data with Log (X + 1). All means given in the text and figures are geometric.

Modal analysis (MacDonald and Pitcher 1979) on the size frequency distributions of the juveniles was used to determine molt or instar classes. For the analysis, each mode in the size frequency profile had to be visually identifiable and contain at least 50 individuals. To increase modal numbers, all size frequencies for juveniles of both sexes collected in 1982 were combined.

After determination of the mean size of each instar, average growth increments were estimated by the Hiatt (1948) method. The same methodology was used by Kurata (1960) and Kon et al. (1968) for snow crab from the Sea of Japan. Growth rates were assessed, by the series of size frequency distributions, by following shifts in instar patterns over time.

Data collected from 27 tows during May 1981 were not included in density and distribution pattern analyses because the cruise plan did not fit the 1982 survey design. However, data from these tows were used in the modal analyses of instar size and growth patterns.

RESULTS

Size Frequency and Growth per Molt

Size frequency histograms over the whole range of snow crab sizes sampled had distinct modes, with the largest mode composed of mature females (50–98 mm CW) (Fig. 2). Although modes were not apparent in the size frequency profiles of males >50 mm CW, modes were evident for crabs of both sexes <50 mm CW. A notable feature in all samples was the virtual absence of crabs within the range 30 to 50 mm CW for females and 30 to 80 mm CW for males (Fig. 2).

From modal analyses of the juvenile size frequencies, five instars were defined (Table 1; Fig. 3) for crabs <50 mm CW. Based on the mean size of the instars and on Hiatt's (1948) method, *C. opilio* growth is described by a straight line equation of the form $Y = AX + B$ where (Y) is the premolt carapace width, (X) is the postmolt carapace width, (A) is the slope of the line or coefficient of growth and (B) is the axis intercept:

Immature males:

$$Y = 1.344\ X + 0.711,\ r = 0.99 \quad (1)$$

Immature females:

$$Y = 1.342\ X + 0.775,\ r = 0.99 \quad (2)$$

Immature males and females combined:

$$Y = 1.351\ X + 0.671,\ r = 0.99 \quad (3)$$

The mean sizes of missing instars (instars I, II, VIII, IX and X, Table 1) were estimated from the regressions and growth per molt was subsequently calculated. The growth increments of juveniles, for both sexes combined, decreased from 55% between instars I and II to 35% between instars IX and X (Table 1).

Size frequency profiles for juveniles, both sexes combined, revealed shifts in instar patterns between months (Fig. 4). During May 1981, instars III, IV, VI and VII were present. During May 1982, in addition to the four instars present during May 1981, instar V was also noticeable. Instar V was practically absent in July 1982. By September 1982, a fresh influx of crabs forming instar II had appeared and crabs forming instars VI and VII had almost all disappeared. Concomitantly, the proportion of crabs in instar III appeared to have decreased, while the proportion of crabs in instars IV and V increased.

Density and Size Distribution in Relation to Substrate and Depth

The mean density of juvenile snow crab was higher ($p < 0.05$) on mud than on gravel and sand (Fig. 5). An exception occurred during May 1982, probably due to the few tows (3) that were made on gravel; there was no significant difference in crab density between gravel and mud bottoms. Juvenile densities were not significantly different ($p > 0.05$) between gravel and sand bottoms for all three sampling periods.

The mean size of juveniles varied with substrate type (Fig. 5). During May 1982, crabs were significantly ($p < 0.05$) smaller on mud (9.3 mm CW) as compared to sand (12.1 mm CW) and gravel (29.4 mm CW). Crab size on gravel in May 1982 was also significantly ($p < 0.05$) larger than on sand. However, few crab were caught on sand and gravel during July and September 1982, and no significant

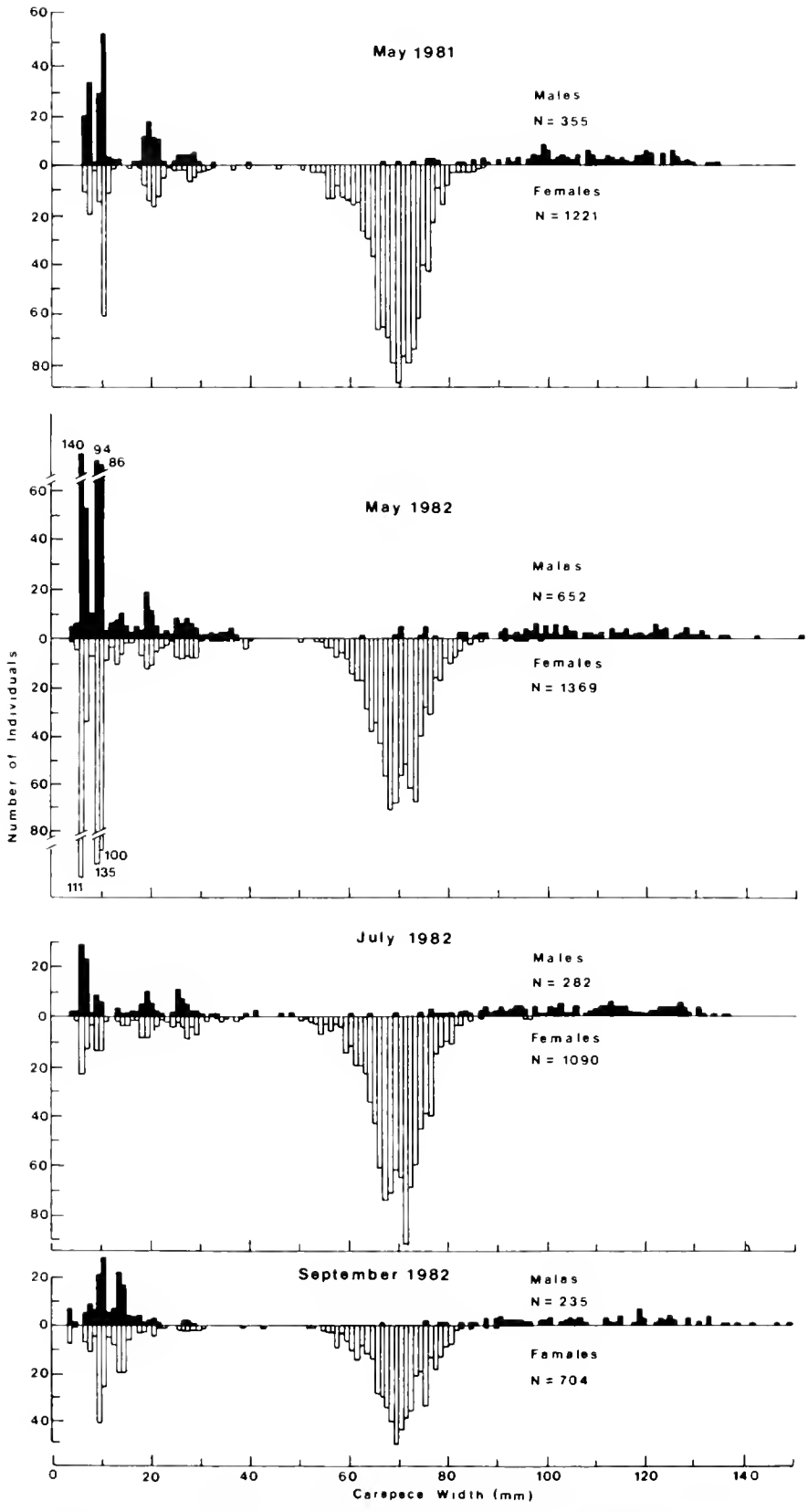


Figure 2. Size frequency histograms for male (top) and female (bottom) *C. opilio* collected by beam trawl in the study area during May 1981 and May, July and September 1982.

TABLE I.

Mean size (mm CW, ± standard error, SE) of snow crab instars from size frequency distributions of juveniles captured during May, July and September 1982. Instar III–VII were determined by polymodal analysis (MacDonald and Pitcher 1979) and the mean sizes of missing instars I, II, VIII, IX and X were extrapolated by the Hiatt (1948) growth equation (Y = AX + B).

Instar	Males (±SE)	Females (±SE)	Males and females combined (±SE)	% increase in CW between instars
I	2.8	2.8	2.9	
II	4.5	4.5	4.5	55%
III	6.8 (±0.4)	6.8 (±0.4)	6.8 (±0.4)	51%
IV	9.9 (±0.6)	9.9 (±0.6)	9.9 (±0.6)	46%
V	13.9 (±1.0)	13.9 (±1.0)	13.9 (±1.0)	40%
VI	19.5 (±1.4)	19.7 (±1.5)	19.6 (±1.5)	41%
VII	26.9 (±1.6)	27.1 (±1.9)	27.1 (±1.7)	38%
VIII	36.9	37.0	37.3	38%
IX	50.3	50.6	51.0	37%
X	68.3	68.7	69.0	35%

differences ($p > 0.05$) were apparent in mean size between substrate types (Fig. 5).

Densities of mature females were significantly ($p < 0.05$) higher on mud than on gravel and sand for all three sampling periods (Fig. 5). There was no significant difference ($p > 0.05$) in mature female densities between sand and gravel for any sampling occasion. Larger mature females tended to be captured on mud and gravel and the smaller females were found on sand, although actual mean size differences were small (Fig. 5). During May and July 1982, the mean sizes of females found on mud and gravel were significantly ($p < 0.05$) larger than for females on sand. There was no significant difference ($p > 0.05$) between the mean size of females on mud and gravel. During September, there was no significant difference ($p > 0.05$) in the mean sizes of mature females between substrate types.

Juvenile and mature female densities and mean sizes varied considerably between depth strata for given substrate types. However, no general trends were obvious (Fig. 6). The only exception occurred on sand where mature females were significantly ($p > 0.05$) larger and more abundant in the 91–110 m depth stratum in comparison to the 50–70 and 71–90 m depth strata.

General trends in the mean size and density of male crabs >50 mm CW were not evident between substrate types or depth strata (Fig. 5, 6).

DISCUSSION

On the basis of our study and other published work, it is possible for the first time to estimate the temporal basis to snow crab life history in the Gulf of St. Lawrence. The eggs of *C. opilio* hatch during April, May and June (Powles 1966; Watson 1969). The larvae develop through one prezoea stage, two zoea stages and a megalops stage (Watson 1969; Ito 1970; Kon 1970). Studies in Chaleur Bay, Gulf of St. Lawrence, have shown the larval duration to be 3–5 months (Lanteigne 1985). In our study, the presence of recently settled crabs in instar I (2.9 mm CW) during September (Fig. 4) concurs with the seasonal disappearance of megalops from the plankton as observed by Lanteigne (1985). During May 1981 and 1982, the virtual absence of crabs from instars I and II (4.5 mm CW) and the relatively large numbers of crabs from instars III (6.8 mm CW) and IV (9.9 mm CW) suggests that, during the winter months, a year's settlement (instars I and II) goes through at least two molts and subsequently reaches instars III and IV by May, approximately 12 months after hatching (Fig. 4). Shifts to larger sizes in the relative strength of modes suggests that crabs from instars III and IV, present during May and July, molt to instars IV and V (13.9 mm CW) by September. Assuming that crabs from instars IV and V go through ecdysis every 6 months (Kon 1970; Watson 1969), they would reach instar VII (27.1 mm CW) by May. Crabs from instar VI and VII present in May are estimated to be at least 2 years of age. Crabs of instar VII in May had virtually all disappeared by September. Assuming that these crabs had molted into instar VIII (37.3 mm CW) during summer and to instar IX (51.0 mm CW) during the following winter, crabs in instar IX would be at least 3 years of age by spring. Ito (1970) assigned a summed duration of 1 year for instars I, II, III and IV, but ignored the larval stages; 16 months for instar V, and approximately 12 months for each stage after and including instar VI. Watson (1969) and Kon (1970) assigned 1 year for the first three instar stages (not including larval stages); 6 months each to instars IV through IX; and 1 year to each after instar IX.

Few crab from instar VIII were present during May and July and none were present in September. Crabs from instar IX were not present in any samples. The absence of snow crab over this same size range in trawl samples from the southwestern Gulf of St. Lawrence in 1980 and 1981 was

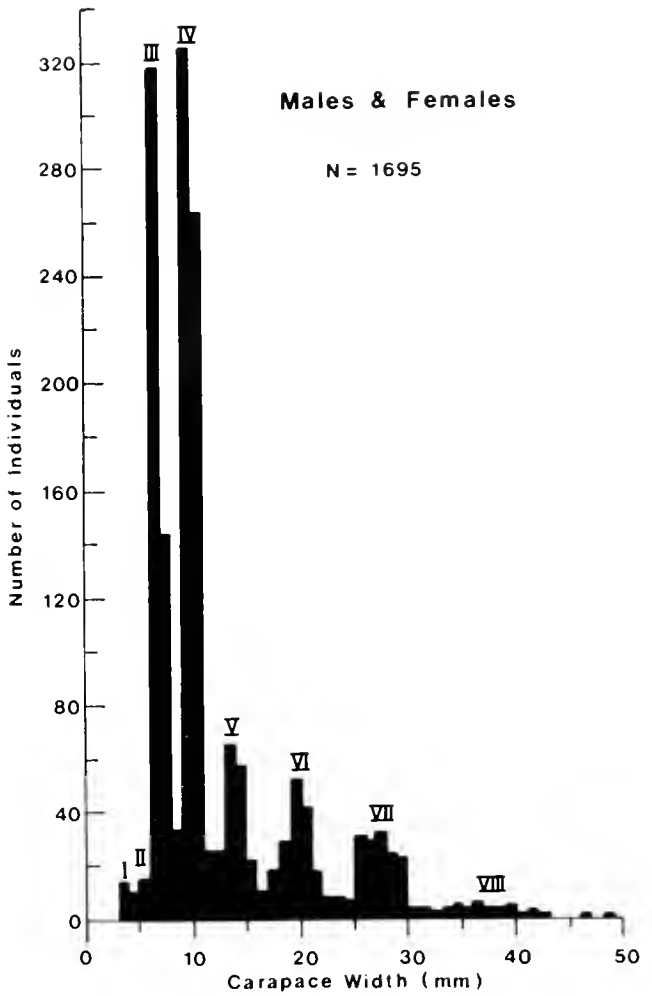


Figure 3. Size frequency histogram for juvenile *C. opilio* (instars I–VIII) collected in the study area during May, July and September 1982.

remarked upon by Coulombe et al. (1985). Coulombe et al. (1985) hypothesized that juveniles >45 mm CW were mainly on gravelly mud, inaccessible to their sampling trawl, and that at physiological maturity they move to deeper muddy substrate where they recruit to the fishery. However, the fact that these crabs were also absent in the stomachs of predatory fish collected concurrently with the present study (Robichaud et al. 1986) suggests that their absence is a real one. If so, our data suggested that there would be a recruitment failure in the fishery in 1985–87, 3–5 years from sampling in 1982. Indeed, assessments in 1986 (Davidson and Comeau 1987) and 1987 (Chiasson et al. 1988) have shown severe declines in commercial catch rates in the western Cape Breton snow crab stocks. In addition, commercial catch rates for the major snow crab stocks in the southwestern Gulf of St. Lawrence dropped dramatically in 1987 (Mallet et al. 1988) and 1988.

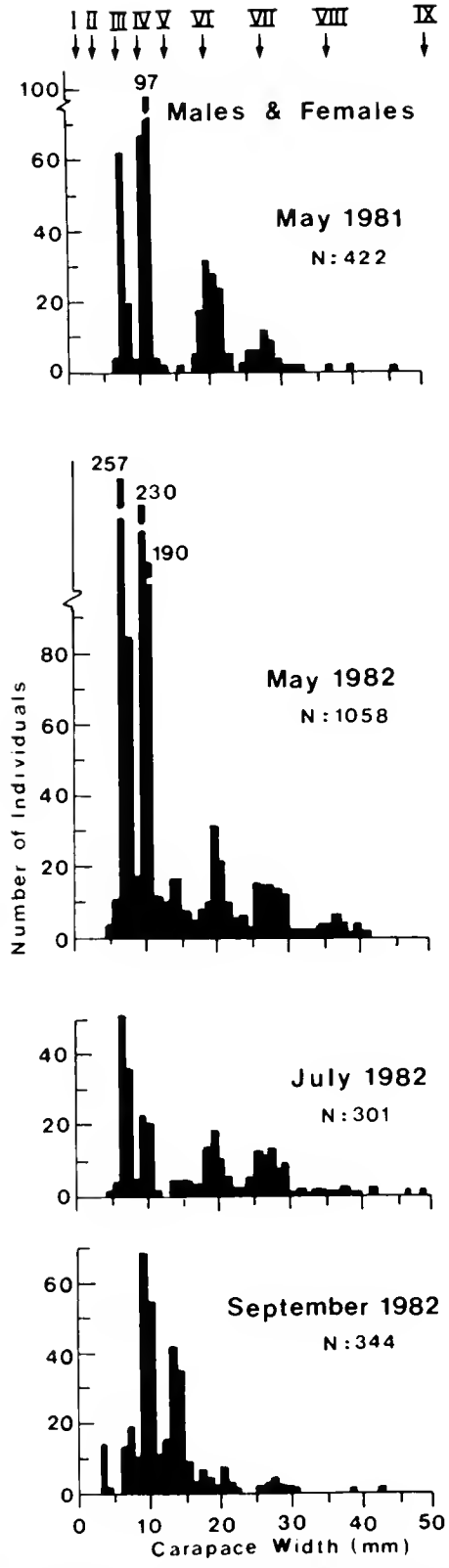


Figure 4. Size frequency histograms for juvenile *C. opilio*, both sexes combined, during 1981–82. Arrows represent estimated positions of instars.

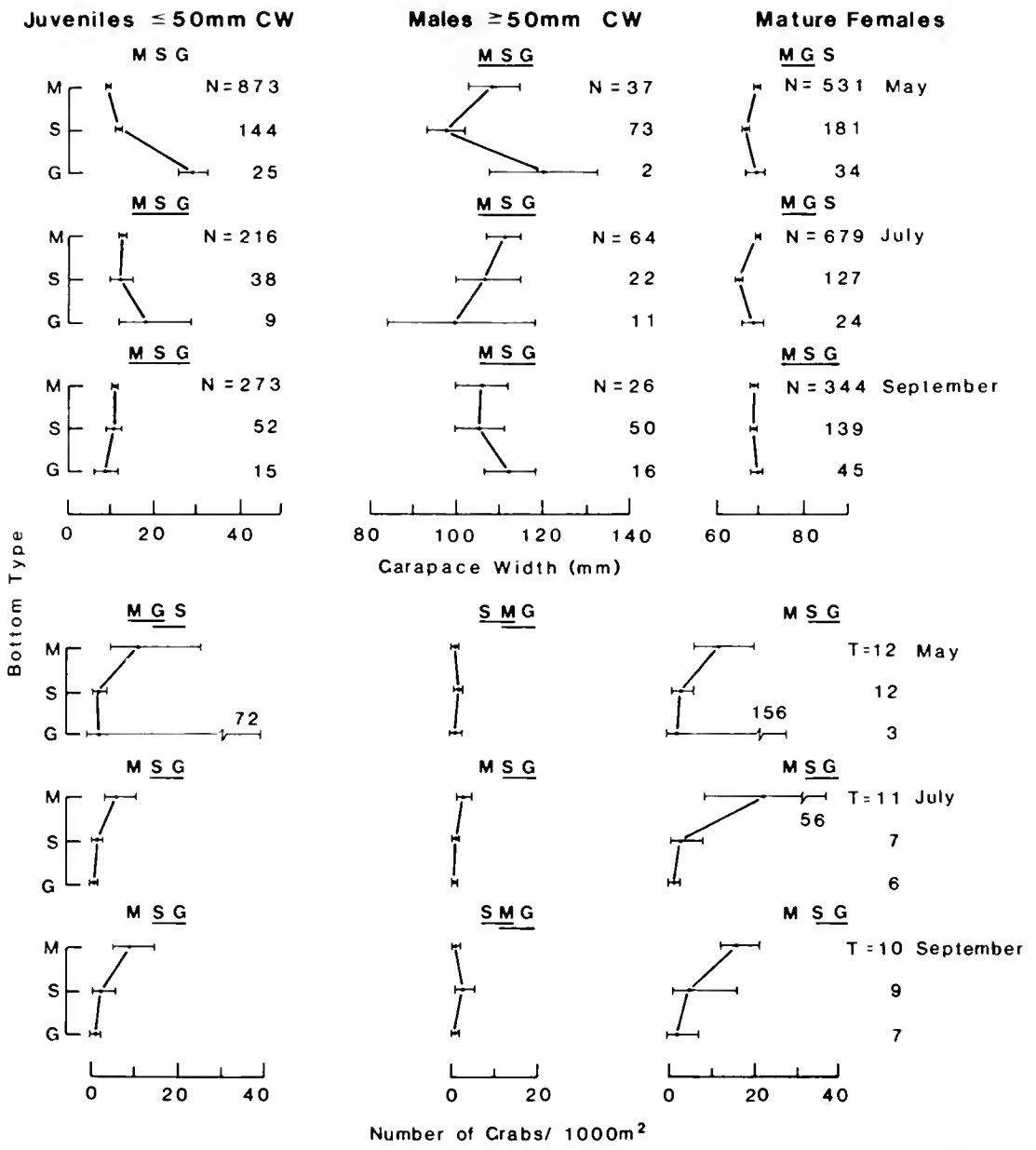


Figure 5. Geometrical mean CW (mm) and mean density (no. crab/1000 m²) (±95% confidence limits) of snow crab in relation to substrate at each sampling month in 1982 (N = number of individuals; T = number of tows; M = mud; S = sand; G = gravel; underlined substrate types are not significantly different at the $p > 0.05$ level).

In our samples, all females larger than 50 mm CW were mature. If female crab follow the growth pattern described above, they could reach maturity during spring (April–June) at instar X (68.7 mm CW), 3.5–4.0 years from larval hatching, assuming a 6–12 month intermolt period between instars IX and X. A large proportion of the males of the same age group as mature females go through one or more additional molts before attaining terminal molt status at morphometric maturity (O'Halloran 1985). Up to 40% of males in the commercial size range (≥95 mm CW) collected by Conan and Comeau (1986) in the Gulf of St. Lawrence were morphometrically immature. Assuming a period of I year between instars X and XI, it is possible that morphometrically immature males in instar XI (92.6 mm CW) could reach a size well into the commercial size range within 4.5 years of hatching. This is considerably faster than the more than 7 years hypothesized by Watson (1969).

Some biases may have been incurred in our study from using a beam trawl. Miller (1975) noted, from observation

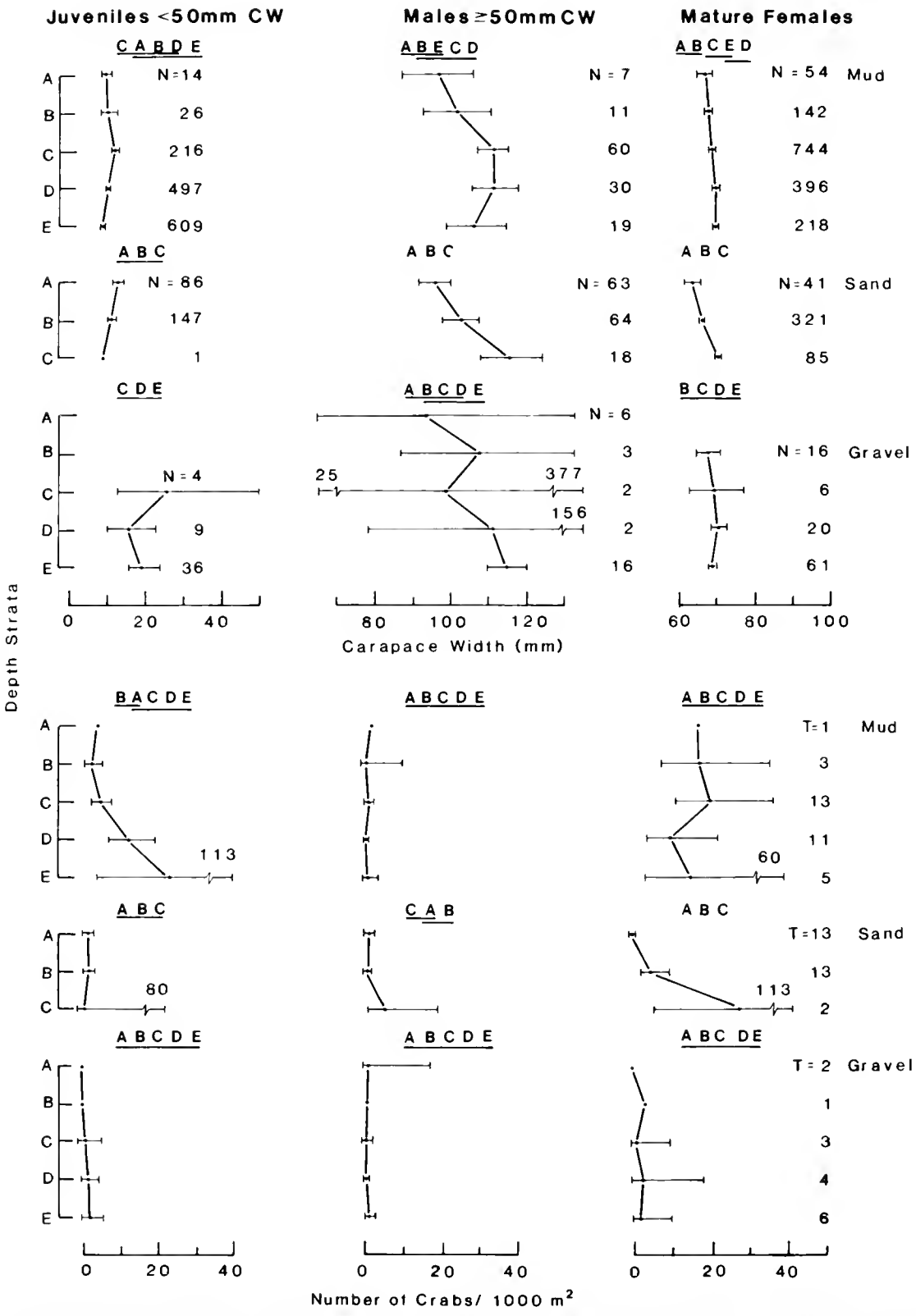


Figure 6. Geometrical mean CW (mm) and mean density (no. crabs/1000 m²) (±95% confidence limits) of snow crab in relation to depth on different substrate types during 1982 (N = number of individuals; T = number of tows; A = 50–70 m; B = 71–90 m; C = 91–110 m; D = 111–130 m; E = 131–150 m; underlined 20 m depth strata are not significantly different at the $p > 0.05$ level).

TABLE 2.

Snow crab density estimates from studies in the Gulf of St. Lawrence and off Newfoundland.

Mean density (no. crab/1000 m²)	Sex	Size	Substrate type	Sampling gear	Location	Reference
1.4	Males	>100 mm CW	Mud & sand	Otter trawl	Chaleur Bay	Powles 1968
2.2	Males	>100 mm CW	Mud & sand	Otter trawl	Shediac trough	Powles 1968
3.5	Males	>100 mm CW	Mud & sand	Bottom photography	Shediac trough	Powles 1968
10.4–18.4	Males	≥40 mm CW	Mud with rock outcrop & sand	Bottom photography	Placentia Bay and Conception Bay, Newfoundland	Miller 1975
11.6	Males	≥70 mm CW	Mud with rock outcrop & sand	Bottom photography	Placentia bay	Miller 1975
3.2	Males	≥70 mm CW	Mud with rock outcrop & sand	Beam trawl	Placentia bay	Miller 1975
12.4	Males	≥70 mm CW	Mud with rock outcrop & sand	Bottom photography	Conception Bay	Miller 1975
2.2	Males	≥70 mm CW	Mud with rock outcrop & sand	Beam trawl	Conception Bay	Miller 1975
8.9	Sexes combined	<30 mm CW	Sand, mud, gravel	Beam trawl	North shore, Gulf of St. Lawrence (June)	Brêthes et al. 1987
28.3	Sexes combined	<30 mm CW	Sand, mud, gravel	Beam trawl	North shore, Gulf of St. Lawrence (July)	Brêthes et al. 1987
47.5	Sexes combined	<30 mm CW	Sand, mud, gravel	Beam trawl	North shore, Gulf of St. Lawrence (September)	Brêthes et al. 1987
12.9	Sexes combined	All sizes	Sand, mud, gravel	T.V. camera sledge	Chaleur Bay	Conan & Maynard 1987
15.1	Sexes combined	<50 mm CW	Mud	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
3.4	Sexes combined	<50 mm CW	Sand	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
1.6	Sexes combined	<50 mm CW	Gravel	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
27.0	Females	≥50 mm CW	Mud	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
6.8	Females	≥50 mm CW	Sand	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
3.2	Females	≥50 mm CW	Gravel	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
2.0	Males	≥50 mm CW	Mud	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
2.4	Males	≥50 mm CW	Sand	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study
0.7	Males	≥50 mm CW	Gravel	Beam trawl	Northwestern Cape Breton (May, July, September)	Present study

with an underwater video camera, that large male crabs were agile enough to avoid a beam trawl and, thus, densities of large males estimated with this type of gear are liable to be artificially low. Similarly, our mesh size was such that some small crabs could have passed right through the net; however, such losses were probably negligible due to rapid occlusion of the mesh with sediment and other benthic organisms. Although all our sampling was con-

ducted during the day, when crabs appear more likely to be buried (Powles 1968; Miller 1975), the trawl dug into all the substrates and would have extracted most crabs. Conan and Maynard (1987) observed 37% of snow crab, predominantly immatures and females, semi-buried in the substrate and hypothesized that such burying behavior could be related to diel activity rhythms. Notwithstanding these potential artifacts, our snow crab density estimates appear in accord with other studies carried out in eastern Canada (Table 2).

Our study indicates that early juvenile snow crab were most dense on mud and can inhibit the same substrate as adults. Brêthes et al. (1987) made similar observations on the basis of beam trawl collections in the southwestern Gulf of St. Lawrence. In contrast, Coulombe et al. (1985), using a beam trawl in the same region, found the highest concentrations of juvenile crab on heterogenous substrate at relatively shallower depths. However, the majority of snow crab captured in the latter study were >45 mm CW compared to the predominantly smaller crab in our study.

The density of snow crab appeared to be more related to substrate type than depth (Fig. 5, 6) as was also observed by Powles (1968) off the Magdalen Islands. Brêthes et al. (1987), Coulombe et al. (1985) and Coulombe (1983) documented changes in crab density with depth which were also strongly correlated to substrate type.

The density of mature females appeared higher on mud than on sand and gravel. While this observation possibly reflects habitat preference behavior, mature females were also significantly larger on mud and gravel (Fig. 5). Although actual size differences were small, they may be attributable to variations in food availability. Poor diet or insufficient food has been reported to reduce the growth increment and lengthen the intermolt period in several crustacean species (Hartnoll 1982), including snow crab (O'Halloran 1985).

Juvenile and mature female snow crab appeared to be patchily distributed within each substrate type. Miller (1975) and Conan and Maynard (1987) also determined, by underwater video cameras, off Newfoundland and in Chaleur Bay, respectively, that the spatial distribution of snow crab is patchy. Aggregated distributions make estimates of biomass difficult (Miller 1975); however, Conan and Maynard (1987) suggest methodology such as kriging for improving the accuracy of the estimates.

While further studies are required to determine if our growth model is applicable to the Gulf of St. Lawrence as a whole, due to possible biotic and abiotic differences between the various crab grounds, it does provide a foundation for predicting annual recruitment into the fishery. Given existing capabilities for identifying morphometrically immature males (O'Halloran 1985), mold prediction (O'Halloran and O'Dor 1988) and assessing pre-recruit abundance (Coulombe et al. 1985; Brêthes et al. 1987; Conan and Maynard 1987) together with our results on growth, short-term estimates of future recruitment strength could be attempted. However, such forecasting would necessarily be crude until information on additional factors, such as natural mortality, movement and the cues for instigating terminal molt status, is available.

The rapid growth rate (4.5 years for males to attain commercial size) may help explain both the resilience and high production of snow crab stocks in the face of intense fishing pressure in the Gulf of St. Lawrence (Beverton and Holt 1959; Elner and Bailey 1986), but does not explain why inter- and intra-stock recruitment patterns have been so variable (Bailey and Elner 1989). A recent physiological study (Foyle 1987) suggests that snow crab growth may be curtailed at low temperatures; recruitment failures or delays in some fisheries could be due to abnormally low bottom temperatures. Other studies have hypothesized that cod predation (Bailey 1982; but see also: Robichaud et al. 1986; Bailey and Elner 1989), larval transport patterns (Davidson et al. 1985) and intra-specific density dependent effects (Waiwood and Elner 1982) influence recruitment. Clearly, actual testing of hypotheses on recruitment mechanisms is now essential.

ACKNOWLEDGMENTS

We are grateful to J. Hurley and B. Best for typing; F. B. Cunningham and P. W. G. McMullon for illustrations; and A. Campbell, G. S. Jamieson, M. Moriyasu and R. Stephenson for helpful discussions on this paper.

REFERENCES

Bailey, R. 1982. Relationship between catches of snow crab, *C. opilio* (O. Fabricius) and abundance of cod (*Gadus morhua* L.) in the southwestern Gulf of St. Lawrence. Proc. Int. Symp. on the genus *Chionoecetes*. Lowell Wakefield Symposia Series. *Alaska Sea Grant Rep.* 82–10:485–497.

Bailey, R. F. J. & R. W. Elner. 1989. Northwest Atlantic snow crab fisheries: lessons in research and management. *In* J. F. Caddy (ed.) *Scientific Approaches to Management of Shellfish Resources*. Chap. 12. 261–280 John Wiley and Sons, New York, NY.

Beverton, R. J. H. & S. J. Holt. 1959 A review of the lifespans and mortality rates of fish in nature, and their relation to growth and other physiological characteristics. *CIBA Foun. Coll. on Ageing* 5:142–180.

Botsford, L. W., R. D. Methot & W. E. Johnston. 1983. Effort dynamics of the northern California Dungeness crab (*Cancer magister*) fishery. *Can. J. Fish. Aquat. Sci.* 40:337–346.

Brêthes, J.-C. F., F. Coulombe, P.-E. Lafleur & R. Bouchard. 1987. Habitat and spatial distribution of early benthic stages of the snow crab *Chionoecetes opilio* O. Fabricius off the north shore of the Gulf of St. Lawrence. *J. Crust. Biol.* 7:667–681.

Chiasson, Y., M. Comeau & M. Moriyasu. 1988. Assessment of the areas 18 and 19, western Cape Breton Island snow crab, *Chionoecetes*

opilio, fisheries, 1987. *Can. Atl. Fish. Sci. Adv. Comm., Res. Doc.* 88/34, 34 p.

Conan, G. Y. & M. Comeau. 1986. Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio*. *Can. J. Fish. Aquat. Sci.* 43:1710–1719.

Conan, G. Y. & D. R. Maynard. 1987. Estimates of snow crab (*Chionoectes opilio*) abundance by underwater television—a method for population studies on benthic fisheries resources. *J. Appl. Ichthyol.* 3:158–165.

Coulombe, F. 1983. Ecologie et distribution d'une population de crabes des neiges, *Chionoecetes opilio*, dans le sud-ouest du Golfe du Saint-Laurent (Baie des Chaleurs). Thèse de maitrise, Université du Québec à Rimouski, Rimouski (Qué.). 123 p.

Coulombe, F., J-C. F. Brêthes, R. Bouchard & G. Desrosiers. 1985. Ségrégation édaphique et bathymétrique chez le crabe des neiges, *Chionoecetes opilio* (O. Fabr.), dans le sud-ouest du Golfe du Saint-Laurent. *Can. J. Fish. Aquat. Sci.* 42:169–180.

Davidson, K. G. & M. Comeau. 1987. An overview of catch, effort and biological trends for the 1986 snow crab, *Chionoecetes opilio*, fisheries in areas 18 and 19, western Cape Breton Island. *Can. Atl. Fish. Sci Adv. Comm, Res. Doc.* 87/53, 35 p.

Davidson, K., J. C. Roff & R. W. Elner. 1985. Morphological, electrophoretic, and fecundity characteristics of Atlantic snow crab, *Chionoecetes opilio*, and implications for fisheries management. *Can. J. Fish. Aquat. Sci.* 42:474–482.

Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics* 11:1–42.

Elner, R. W. 1982. Overview of the snow crab, *Chionoecetes opilio*, fishery in Atlantic Canada. Proc. Int. Symp. on the genus *Chionoecetes*. Lowell Wakefield Symposia Series. *Alaska Sea Grant Rep.* 82–10:3–19.

Elner, R. W. & R. F. J. Bailey. 1986. Differential susceptibility of Atlantic snow crab, *Chionoecetes opilio*, stocks to management, p. 335–346. *In* G. S. Jamieson and N. Bourne [ed.] North Pacific Workshop on Stock Assessment and Management of Invertebrates. *Can. Spec. Publ. Fish. Aquat. Sci.* 92.

Elner, R. W. & D. A. Robichaud. 1983. Observations on the efficacy of the minimum legal size for Atlantic snow crab, *Chionoecetes opilio*. *Can. Atl. Fish. Sci. Adv. Comm., Res. Doc.* 83/63, 26 p.

Foyle, T. P. 1987. Energetics and metabolism in a cold-water crustacean: the snow crab, *Chionoecetes opilio*. Masters Thesis, Dalhousie University, Halifax (N.S.). 84 p.

Hartnoll, R. G. 1982. Growth. Chap. 3, p. 111–196. *In* L. G. Abele (ed.). The Biology of Crustacea. Vol. 2. Academic Press, New York, NY.

Hiatt, R. W. 1948. The biology of the lined shore crab, *Pachygrapsus crassipes* Randall. *Pac. Sci.* 2:135–213.

Holme, N. A. & A. D. McIntyre. 1971. Methods for the study of marine benthos. I.B.P. Handbook 16: 334 p.

Ito, K. 1963. Some observations on the degree of ripeness of the eggs of the snow crab (*Chionoecetes opilio*). *Bull. Jpn. Sea Reg. Fish. Res. Lab.* 11:1–12.

Ito, K. 1967. Ecological studies on the edible crab, *Chionoecetes opilio* O. Fabricius in the Japan Sea. I. When do female crabs first spawn and how do they advance into the following reproductive stage? *Bull. Jpn. Sea Reg. Fish. Res. Lab.* 17:67–84. (Fish. Res. Board Can. Transl. Ser. No. 1103).

Ito, K. 1970. Ecological studies on the edible crab, *Chionoecetes opilio* O. Fabricius in the Japan sea. III. Age and growth as estimated on the basis of the seasonal changes in the carapace width frequencies and the carapace hardness. *Bull. Jpn. Sea Reg. Fish. Res. Lab.* 22:81–116. (Fish. Res. Board Can. Transl. Ser. No. 1512).

Kon, T. 1970. Fisheries biology of the tanner crab. IV. The duration of the planktonic stages estimated by rearing experiments of larvae. *Bull. Jpn. Soc. Sci. Fish.* 36:219–224 (Fish. Res. Board Can. Transl. Ser. No. 1603).

Kon, T., M. Niwa & F. Yamakawa. 1968. Fisheries biology of the tanner crab. II. On the frequency of molting. *Bull. Jpn. Soc. Sci. Fish.* 34:138–142. (Fish. Res. Board Can. Transl. Ser. No. 1129).

Kurata, H. 1960. Increase in size at molting in Crustacea. *Bull. Hokkaido Reg. Fish. Res. Lab.* 22:1–49.

Lanteigne, M. 1985. Distribution spatio-temporelle des larves de crabe appartenant aux genres *Chionoecetes* et *Hyas*, dans la baie des Chaleurs, Canada. Thèse de maitrise, Université de Moncton, Moncton (N.-B.), 161 p.

Loring, D. H. & D. J. G. Nota. 1973. Morphology and sediments of the Gulf of St. Lawrence. *Bull. Fish. Res. Board Can.* 182: 147 p.

MacDonald, P. D. M. & T. J. Pitcher. 1979. Age-groups from size-frequency data: a versatile and efficient method of analysing distribution mixtures. *J. Fish. Res. Board Can.* 36:987–1001.

Mallet, P., Y. Chiasson & M. Moriyasu. 1988. A review of catch, fishing effort and biological trends for the 1987, southwestern Gulf of St. Lawrence snow crab (*Chionoecetes opilio*) fishery. *Can. Atl. Fish. Sci. Adv. Comm, Res. Doc.* 88/32, 39 p.

Miller, R. J. 1975. Density of the commercial spider crab, *Chionoecetes opilio*, and calibration of effective area fished per trap using bottom photography. *J. Fish. Res. Board Can.* 32:761–768.

Miller, R. J. & P. G. O'Keefe. 1981. Seasonal and depth distribution, size and molt cycle of the spider crabs, *Chionoecetes opilio*, *Hyas araneus*, and *Hyas coarctatus* in a Newfoundland bay. *Can. Tech. Rep. Fish. Aquat. Sci.* 1003: 18 p.

O'Halloran, M. J. 1985. Moult cycle changes and the control of moult in male snow crab, *Chionoecetes opilio*. Masters thesis, Dalhousie University, Halifax (N.S.), 183 p.

O'Halloran, M. J. & R. K. O'Dor. 1988. Molt cycle of male snow crabs, *Chionoecetes opilio*, from observations of external features, setal changes and feeding behavior. *J. Crust. Biol.* 8:164–176.

Otto, R. S. 1986. Management and assessment of eastern Bering Sea king crab stocks, p. 83–106. *In* G. S. Jamieson and N. Bourne [ed.] North Pacific Workshop on Stock Assessment and Management of Invertebrates. *Can. Spec. Publ. Fish. Aquat. Sci.* 92.

Otto, R. S., R. A. MacIntosh, T. M. Armetta, W. S. Meyers, J. McBridge & D. A. Somerton. 1983. United States crab research in the eastern Bering Sea during 1982. *Int. North Pac. Fish. Comm. Annu. Rep. 1982:*141–160.

Otto, R. S., R. A. MacIntosh, K. L. Stahl-Johnson & S. J. Wilson. 1984. United States crab research in the eastern Bering Sea during 1983. *Int. North Pac. Fish. Comm. Annu. Rep. 1983:*131–151.

Powles, H. 1966. Observation on the biology of two species of spider crabs, *Chionoecetes opilio* and *Hyas araneus*, in the Gulf of St. Lawrence. *Fish. Res. Board Can. MS Rep.* 884: 27 p.

Powles, H. 1968. Distribution and biology of the spider crab *Chionoecetes opilio* in the Magdalen Shallows, Gulf of St. Lawrence. *Fish. Res. Board Can. MS Rep.* 997: 106 p.

Robichaud, D. A. 1985. Ecologie du crabe des neiges (*Chionoecetes opilio* juveniles au large des côtes nord-ouest du Cap Breton, et ses interactions avec la morue (*Gadus morhua*) et la raie (*Raja radiata*). Thèse de maîtrise, Université de Moncton, Moncton (N.-B.), 168 p.

Robichaud, D. A., R. F. J. Bailey & R. W. Elner. 1986. The predatory behaviour of cod (*Gadus morhua*) and skate (*Raja radiata*) on crab prey species (*Chionoecetes opilio*, *Hyas araneus* and *H. coarctatus*). *Int. Counc. Explor. Sea* C.M. 1986/G:47: 32 p.

Waiwood, K. G. & R. W. Elner. 1982. Cod predation of snow crab (*Chionoecetes opilio*) in the Gulf of St. Lawrence. Proc. Int. Symp. on the genus *Chionoecetes*. Lowell Wakefield Symposia Series. *Alaska Sea Grant Rep.* 82–10:499–520.

Watson, J. 1969. Biological investigations on the spider crab *Chionoecetes opilio*. *Can. Fish. Rep.* 13:23–47.

Watson, J. 1970. Maturity, mating, and egg laying in the spider crab, *Chionoecetes opilio*. *J. Fish. Res. Board Can.* 27:1607–1616.

Journal of Shellfish Research, Vol. 8, No. 1, 25–31, 1989.

PREDATION BY THE OYSTER TOADFISH *OPSANUS TAU* (LINNAEUS) ON BLUE CRABS AND MUD CRABS, PREDATORS OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNAEUS, 1758)[1]

ROBERT BISKER[2], MARY GIBBONS[3], AND MICHAEL CASTAGNA[2]

[2]*Virginia Institute of Marine Science and School of Marine Science The College of William and Mary Wachapreague, VA 23480 (U.S.A.)*

[3]*Virginia Institute of Marine Science and School of Marine Science The College of William and Mary Gloucester Point, VA 23062 (U.S.A.)*

ABSTRACT The oyster toadfish, *Opsanus tau* (Linne), reduces predation by xanthid and portunid crabs on juvenile hard clams, *Mercenaria mercenaria* (Linne), in field cultures. This study examined the influence of size and species on the predator-prey relationship between toadfish and crabs. The mud crabs *Eurypanopeus depressus* (Smith), *Neopanope sayi* (Smith), and *Panopeus herbstii* Milne Edwards of 5–40 mm carapace width and blue crabs *Callinectes sapidus* Rathbun of 77–105 mm carapace width were offered to toadfish of 70–322 mm total length. Toadfish predation rates on mud crabs were higher with increasing toadfish size and lower with increasing crab size. Toadfish injured or killed mud crabs that were one tenth of their total length or approximately one half of their mouth width. Predation of juvenile hard clams by blue crabs was reduced when toadfish were present.

KEY WORDS: predation, toadfish, *Opsanus*, crabs, hard clams, *Mercenaria*

INTRODUCTION

Crabs prey on juvenile hard clams in field culture systems (Eldridge et al. 1976; MacKenzie 1977, 1979; Castagna and Kraeuter 1981; Gibbons and Blogoslawski 1989). Culture techniques used to exclude predators include rafts, trays, cages, and nets (Castagna and Kraeuter 1981; Jory et al. 1984). Biological methods have been used to protect shellfish from predation with varying degrees of success. In particular, the oyster toadfish, *Opsanus tau* (Linne), has been shown to be a biological control of crab predation on juvenile hard clams, *Mercenaria mercenaria* (Linne), cultured in cages with gravel aggregate (Gibbons and Castagna 1985). This laboratory study further investigates the feeding behavior and predation rates by oyster toadfish on four species of crabs that prey on juvenile hard clams.

Opsanus tau is a benthic, non-migratory fish found along the Atlantic coast of the United States (Gudger 1910; Schwartz and Dutcher 1963). It preys mainly on crustaceans, with mud crabs (Decapoda: Xanthidae) and blue crabs (Decapoda: Portunidae) forming the bulk of stomach contents (McDermott 1964; Wilson et al. 1982; Gibbons and Castagna 1985). During the day toadfish ambush prey from their burrows, while at night they stalk prey (Phillips and Swears 1979). The sympatric mud crabs *Eurypanopeus depressus* (Smith), *Neopanope sayi* (Smith), and *Panopeus herbstii* Milne Edwards, and the blue crab, *Callinectes sapidus* Rathbun, are predators of the hard clam, *M. mercenaria*, (Gibbons and Blogoslawski 1989) and live in similar habitats as the oyster toadfish, *O. tau* (Williams 1984). The oyster toadfish preys upon these crabs but not hard clams (Gibbons and Castagna 1985).

The oyster toadfish normally ranges from 180–300 mm total length (TL) with a maximum reported size of 368 mm (TL), (Gudger 1910; Schwartz and Dutcher 1963). Of the three mud crab species examined, the carapace widths (CW) of adult *E. depressus* and *N. sayi* overlap in size and reach 22 mm, while adult *P. herbstii* are larger reaching 62 mm CW (Williams 1984). The larger *C. sapidus* may attain 227 mm CW (Williams 1984). *Callinectes sapidus*, *E. depressus*, and *N. sayi* prey on hard clams up to 33% of the crabs' CW (Carriker 1961; Castagna and Kraeuter 1981; Gibbons 1984). *Panopeus herbstii* is capable of opening significantly larger hard clams, up to 65% of CW (Whetstone and Eversole 1981), because of a large molariform tooth on the dactyl of the master claw. This study examined the influence of toadfish size on predation of mud crabs of various size classes. The effect of toadfish on blue crab predation upon juvenile hard clams was examined in the presence and absence of substrate.

MATERIALS AND METHODS

Nine laboratory experiments were conducted from July to September 1986. Temperature and salinity of ambient seawater were monitored during all experiments. Crabs and toadfish were collected locally and held in flowing seawater. Juvenile hard clams were cultured at the Virginia Institute of Marine Science Wachapreague Laboratory. Toadfish were used only once and were starved for 48 hr

[1]Contribution No. 1525 from Virginia Institute of Marine Science.

prior to each experiment. Experimental chambers (49 × 40 × 26 cm) received ambient seawater at the rate of 2 L/min. Within each chamber a 15 cm high standpipe covered with fiberglass insect screening prevented crab escape. Except where specified, neither structures nor substrate were present in the chambers. Toadfish and crabs from specific size classes were selected randomly for each trial. Experimental treatments and controls were replicated three times. Predation rates of toadfish were recorded as the number of crabs killed/toadfish/24 hr.

Predation by toadfish on mud crabs

Five experiments (I–V) were performed to examine the influence of size on the predator-prey relationship between toadfish and mud crabs. Due to the logistics of obtaining sufficient toadfish and mud crabs, a full factorial design was not carried out. Sizes of toadfish and mud crabs used are shown in Table 1. Toadfish were divided into five size classes based on total length. In addition, mouth width (MW) of each toadfish was measured as the medial distance between the articulation of the articular and quadrate bones on each side of the mouth. Owing to their similar appearance and their sympatric relationship, mud crabs *E. depressus, N. sayi,* and *P. herbstii* were divided into four size classes based on carapace width without consideration of species. Therefore, implications were drawn for mud crabs as a group not single species. A random sample from each size class was selected for estimation of species-size distribution; this distribution was similar to those in other studies (Ryan 1956, McDonald 1982, Williams 1984) (Table 2).

For each experiment treatment chambers held one toadfish and one size class of mud crabs. Control chambers held crabs without toadfish. After 24 hr, predation rates by toadfish and mortality of crabs from confamilial interference were determined. Dead and injured crabs were replaced with live crabs of the same size class and the experiment continued for an additional 24 hr to see if predation may be reduced by satiation. Temperature and salinity ranged from 24–31°C and 30–34 ppt, respectively, during Experiments I–V.

In Experiment I, three toadfish of the smallest size class (70–90 mm TL) were offered the smallest size class of mud crabs (5–10 mm CW). Each toadfish was placed in a treatment chamber with ten crabs. In Experiment II, twelve toadfish of the next larger class (120–140 mm TL) were each offered only one of the four size classes of mud crabs. Ten crabs were used for each size class except the largest class (35–40 mm CW) which was represented by three crabs per replicate. For Experiments III–V, each of the four size classes of mud crabs were offered to twelve oyster toadfish of 170–190, 220–240, and 270–290 mm TL. The largest size class of mud crab was represented by only five crabs per replicate.

Interactions between toadfish, blue crabs, and hard clams

Experiments VI–IX examined the effects of toadfish on predation by blue crabs upon juvenile hard clams with various substrates. Sizes of animals are given in Table 3. Temperature and salinity ranged from 19–26°C and 29–32 ppt, respectively, during the experimental period.

Experiment VI tested the interaction of toadfish with blue crabs in the absence of any substrate. Individual toadfish of 196–320 mm TL were placed in a chamber with one blue crab of 77–96 mm CW. Toadfish and crabs were examined daily for injury or mortality. Blue crabs were exam-

TABLE 1.

Sizes of toadfish and mud crabs used in Experiments I–V.

	Total Length (mm) of Toadfish			Corresponding Mouth Width (mm) of Toadfish
Experiment	Size class	Mean ± SD	N	Mean ± SD
I	70–90	84.3 ± 3.1	3	14.9 ± 0.2
II	120–140	128.4 ± 6.1	12	23.3 ± 1.7
III	170–190	178.0 ± 5.0	12	37.1 ± 2.1
IV	220–240	225.6 ± 5.4	12	47.0 ± 2.3
V	270–290	283.0 ± 6.3	12	65.6 ± 2.9
	Carapace Width (mm) of Mud Crabs			
	Size class	Mean ± SD	N	
	5–10	8.0 ± 1.4	50	
	15–20	18.9 ± 1.4	40	
	25–30	28.0 ± 1.6	40	
	35–40	37.5 ± 1.5	18	

TABLE 2.

Percent size distribution of three species of mud crabs in each size class offered toadfish (N = 10 per class).

Species	Size Class (mm Carapace Width)			
	5–10	15–20	25–30	35–40
Eurypanopeus depressus	20	10	20	
Neopanope sayi	80	50		
Panopeus herbstii		40	80	100

ined for missing appendages and punctures of the carapace. There were nine replicates. The experiment was terminated after 96 h as more than half of the crabs were preyed upon.

Experiment VII tested the effect of toadfish upon predation by blue crabs on juvenile hard clams in the absence of any substrate. Treatments included the presence of single toadfish of 297–318 mm TL, effluent water from toadfish, and the absence of toadfish (control). One blue crab of 67–94 mm CW was placed into each chamber with 30 hard clams of 5.4 mm mean (4.8–6.3 mm) shell height (SH). Mortalities of clams and blue crabs were determined after 24 h and the experiment was terminated because of the high mortality of clams in the control replicate treatments.

The influence of a sand substrate on the toadfish-blue crab-hard clam interactions was examined in Experiment VIII. Treatments included the presence of one toadfish and sand, presence of one toadfish without sand, no toadfish but sand present, and absence of both toadfish and sand (control). Sand was placed in chambers at a depth of 50 mm and hard clams were allowed to burrow into the substrate. Each chamber received one blue crab of 80–96 mm CW and 30 hard clams of 5.8 mm mean (4.7–6.5 mm) SH. After 48 h the mortalities of hard clams and blue crabs were determined.

The addition of a crushed gravel aggregate to sand substrate was tested for influence on the interactions between hard clams, blue crabs, and toadfish in Experiment IX. Treatments included the presence of gravel and toadfish, toadfish without gravel, gravel without toadfish, and absence of both toadfish and gravel (control). All chambers received sand at a depth of 50 mm, 30 hard clams of 5.2 mm mean (4.3–6.3 mm) SH, and one blue crab of 72–105 mm CW. Gravel of 5–15 mm diameter was added at a depth of 25 mm on top of the sand substrate in the chambers. Mortalities of hard clams and blue crabs were determined after 48 hr.

Statistical analyses

Predator-prey size ratios were determined for toadfish by comparing the carapace width of the mud crabs preyed on to the total length of toadfish. Although no predator-prey size relationship was studied in the toadfish-blue crab Experiments (VI–IX), the sizes of those blue crabs preyed upon (injured or killed) by the toadfish were noted and a size comparison was made. Mud crab mortalities between the test and control replicates for Experiments I–V were compared using two-way analysis of variance (ANOVA) after log (x + 1) transformation with toadfish presence and exposure time as variables. Hard clam mortalities for Experiments VII–IX were analysed using one-way ANOVA after log (x + 1) transformation. Differences between numbers of blue crabs preyed on (injured and dead) versus numbers of uninjured blue crabs for Experiments VII–IX were tested with one-way ANOVA after log (x + 1) transformation. Significant differences in treatment means of hard clam mortalities and number of blue crabs preyed on were further analysed using Duncan's new multiple-range test (Steel and Torrie 1960).

RESULTS

Predation rates by oyster toadfish generally increased with decreasing mud crab size and increased with increasing toadfish size (Fig. 1). Mortality of the smallest size class of mud crabs (5–10 mm CW) was significantly higher (d.f. = 1, $p < 0.001$) in the presence of toadfish for toadfish size classes of 70–90 mm TL ($F = 114.6$), 120–140 mm TL ($F = 154.8$), 170–190 mm TL ($F = 162.9$), 220–240 mm TL ($F = 84.0$), and 270–290 mm TL ($F = 43.9$). Mud crabs of 15–20 mm CW had significantly higher mortality in the presence of toadfish size classes of 170–190 mm TL ($F = 227.0$, d.f. = 1, $p < 0.001$), 220–240 mm TL ($F = 169.9$, d.f. = 1, $p < 0.001$), and 270–290 mm TL ($F = 11.9$, d.f. = 1, p =

TABLE 3.

Sizes of toadfish, blue crabs, and hard clams used in Experiments VI–IX.

Experiment	Total Length (mm) of Toadfish		Carapace Width (mm) of Blue Crabs		Shell Height (mm) of Hard Clams	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
VI	252.9 ± 48.1	9	87.2 ± 6.3	9	0	
VII	308.0 ± 12.2	6	80.2 ± 7.7	9	5.4 ± 0.5	30
VIII	310.5 ± 10.9	6	88.8 ± 5.1	12	5.8 ± 0.5	30
IX	308.5 ± 6.0	6	84.5 ± 9.3	12	5.2 ± 0.5	30

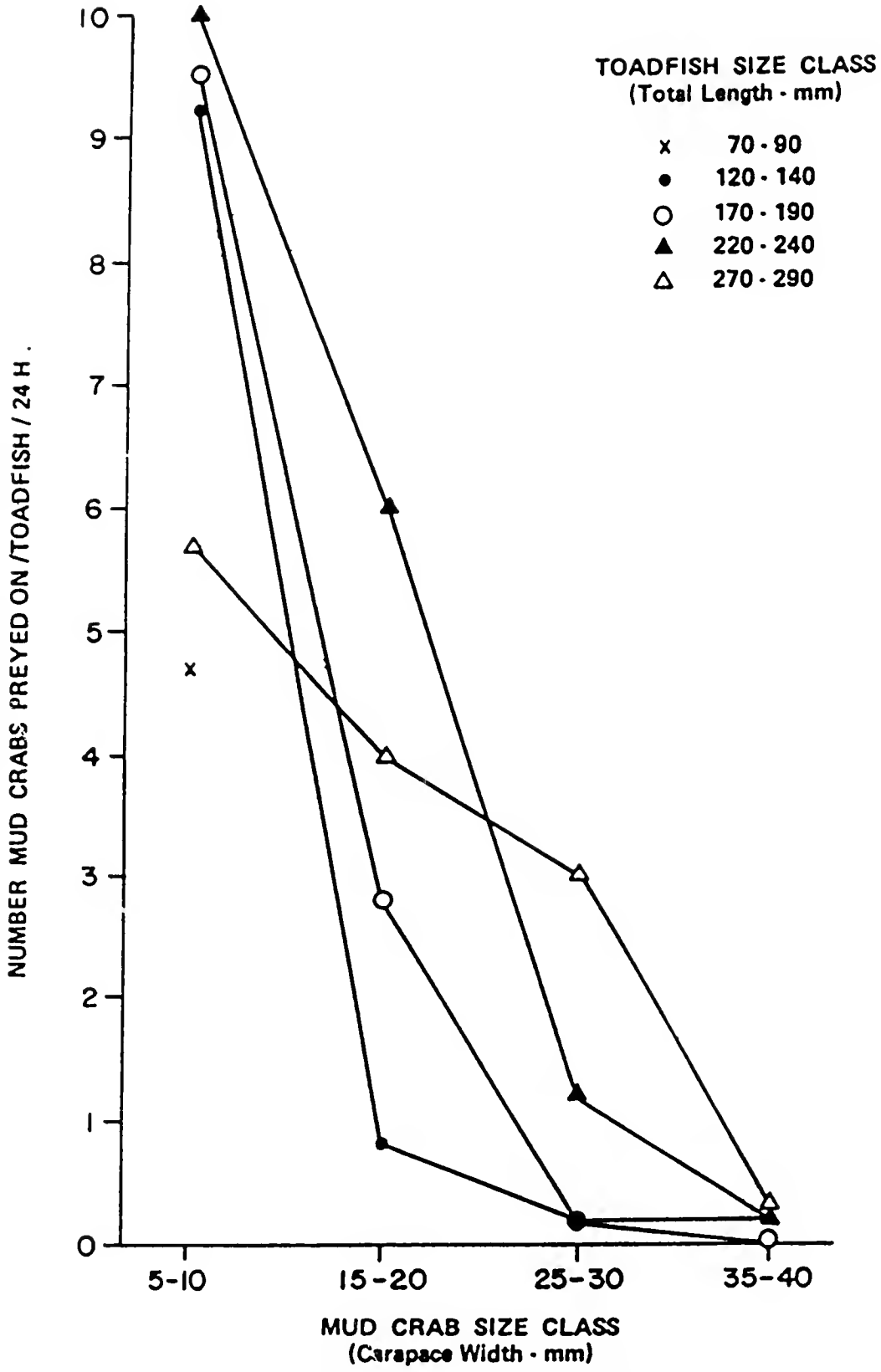


Figure 1. Predation rates by toadfish on mud crabs from Experiments I–V.

0.008). Significant mortalities of mud crabs of 25–30 mm CW occurred in the presence of toadfish of 220–240 mm TL ($F = 11.4$, d.f. = 1, $p = 0.009$) and 270–290 mm TL ($F = 268.6$, d.f. = 1, $p < 0.001$). No significant differences ($p = 0.05$) in predation rates occurred between 24 hr and 48 hr. Toadfish could prey on up to 10 mud crabs/toadfish/24 hr. Although there was no significant predation by toadfish on mud crabs of the largest size class (35–40 mm CW) which was composed of only *P. herbstii*, one crab was eaten by a toadfish of the largest size class (270–290 mm TL).

A predator-prey size ratio of 0.10 (CW/TL) was determined for toadfish preying on mud crabs. When mouth width was considered, toadfish could prey on mud crabs that were almost one half of the mouth width (CW/MW). Mud crabs killed by toadfish were either partially or entirely consumed, or had a punctured carapace. Shell parts from partially digested crabs appeared after 48 hr via regurgitation by toadfish. Mortality of mud crabs in the controls averaged 2.7% per 24 hr for all experiments. Some of this mortality was due to predation and interference behavior by other crabs.

A predator-prey size ratio of 0.32 (CW/TL) was determined for toadfish preying on blue crabs in Experiments VI–IX. Oyster toadfish attacked blue crabs by removing their legs and chelae and puncturing the carapace. Blue crabs were either partially or entirely consumed. Injuries and deaths of blue crabs did not occur until 72 hr after exposure to toadfish in Experiment VI (Table 4), but occurred within 24–48 hr in Experiments VII–IX (Table 5). Toadfish were not injured by blue crabs.

Blue crabs reacted to the presence of toadfish by remaining distant, and some crabs escaped from the experimental chambers (Experiment VI) (Table 4). Blue crabs were returned to the chambers from which they escaped. When sand substrate was available, blue crabs hid by burrowing. Blue crabs did not burrow into the gravel substrate.

No significant differences in blue crab mortalities were found in Experiment VII (Table 5). Blue crab injuries and death in Experiment VIII were significantly higher ($p = 0.05$) in the presence of toadfish with no differences detected between substrate type (Table 5). Experiment IX had significantly higher ($p = 0.05$) blue crab injuries and death in treatments with toadfish and gravel substrate combined than with toadfish present with sand only or treatments without toadfish. Blue crabs did not show any behavioral reaction to toadfish effluent. More injuries and deaths of blue crabs occurred in the presence of toadfish when exposed for 48 hr (Experiments VIII and IX) than 24 hr (Experiment VII). There was no mortality of blue crabs in controls.

In the presence of toadfish, blue crab predation on hard clams was reduced without regard to blue crab condition (i.e. uninjured, injured, or killed). Mortality of hard clams from blue crab predation was significantly lower ($p = 0.05$) in the presence of toadfish than in the presence of toadfish effluent or the absence of toadfish in Experiment VII (Table 5). Experiment VIII tests had significantly lower ($p = 0.05$) clam mortality in the presence of toadfish (Table 5). Toadfish did not consume hard clams.

When considering the gravel on sand substrate in the presence of toadfish in Experiment IX (Table 5), only slightly lower clam mortality occurred than without toadfish. Significantly lower ($p = 0.05$) clam mortality occurred in Experiment IX from treatments having toadfish and gravel on sand, or treatments without toadfish and having gravel on sand, or from treatments having toadfish and without gravel than from treatments without either toadfish or gravel. The sand substrate alone provided no protection for hard clams against blue crabs.

TABLE 4.

The daily condition of blue crabs held with toadfish for 96 hours in Experiment VI.

Elapsed time in hours	Condition of Crab			
	Uninjured	Injured	Dead	Escaped
24	8	0	0	1
48	6	0	0	3
72	6	2	0	1
96	3	1	4	1

DISCUSSION

The oyster toadfish, *O. tau*, preys on mud crabs, *E. depressus*, *N. sayi*, and *P. herbstii*, and the blue crab, *C. sapidus*. In general, toadfish preyed on mud crabs that were no more than one tenth their size (CW/TL). Toadfish had predation rates as high as 10 mud crabs/toadfish/24 hr. Higher predation rates are possible as the toadfish were not fed mud crabs *ad libitum*. Toadfish consumed higher numbers of mud crabs as mud crab size decreased or toadfish size increased (Fig. 1).

The species distribution in mud crab size classes may influence the size effects on predation rates. There was some mortality of mud crabs in chambers without toadfish, probably from interspecific and intraspecific aggression. Mud crabs such as *N. sayi* have ritualized behavioral patterns that reduce aggressive encounters (Swartz 1976).

Under certain conditions, the presence of oyster toadfish reduced predation by blue crabs on juvenile hard clams, owing to the aggressive and predatory behavior of toadfish towards blue crabs. The ability of a blue crab to escape, defend itself, or prey on hard clams was reduced by each encounter with a toadfish. Toadfish pull appendages from the blue crab's body and then kill the crab. Similar be-

TABLE 5.

The influence of toadfish on the predation by blue crabs on hard clams in Experiments VII–IX.

Experiment	Presence of Toadfish	Type of Substrate	Condition of Blue Crab			Number of Clams Eaten (Mean and Range)
			Uninj.	Inj.	Dead	
VII	present	none	1	1	1	0.7 (0–2)
24 h	effluent	none	3	0	0	30.0 (30)
	absent	none	3	0	0	25.0 (15–30)
VIII	present	sand	1	2	0	0.3 (0–1)
48 h	present	none	0	2	1	0.0 (0)
	absent	sand	3	0	0	19.7 (0–30)
	absent	none	3	0	0	30.0 (30)
IX	present	gravel	0	2	1	0.6 (0–1)
48 h	present	sand only	2	0	1	10.0 (0–30)
	absent	gravel	3	0	0	2.3 (1–5)
	absent	sand only	3	0	0	30.0 (30)

havior has been observed in field experiments (Bisker, unpublished data).

Crushed gravel aggregate may act not only to reduce the effectiveness of hard clam predators, but also to enhance the effectiveness of higher-level predators upon hard clam predators. In this study, gravel added to a sand substrate reduced predation by blue crabs on juvenile hard clams, by providing a refuge for hard clams and decreasing the burrowing ability of the blue crab. The inability to burrow reduced the feeding efficiency of the blue crab and increased its risk of exposure to toadfish which resulted in increasing predation on the blue crabs by the toadfish. Predation pressure on juvenile hard clams has been reduced by the addition of crushed gravel aggregate in laboratory studies with mud crabs, *N. sayi,* calico crabs, *Ovalipes ocellatus* (Herbst), and hermit crabs, *Pagurus longicarpus* Say (Gibbons 1984), and field cultures with blue crabs, *C. sapidus,* and other crabs (Castagna and Kraeuter 1981; Arnold 1984).

Field grow-out systems for juvenile hard clams of 6–12 mm SH generally use mesh nettings or cages to exclude predators (Eldridge et al. 1976; Manzi et al. 1980; Castagna and Kraeuter 1981; Walker 1984; Kraeuter and Castagna 1985). Nets with square mesh openings of 11.1 mm or 12.0 mm will not exclude blue crabs, *C. sapidus,* of less than 39.2 mm CW or mud crabs, *P. herbstii,* of 25.2 mm CW, respectively (Bisker and Castagna 1986). Blue crabs of this size may prey on hard clams of 8 mm SH or less while *P. herbstii* can open those clams of 16 mm SH or less. Juvenile crabs are often attracted to grow-out systems for food or refuge, pass through the netted enclosures, and grow to sizes large enough to cause significant mortality on clams (Walker 1984).

The mud crabs *E. depressus* and *P. herbstii* have average densities of 40–50 crabs/m^2 (Dame 1979) but may have mean densities as high as 1000 and 100/m^2, respectively (Bahr 1974). *Neopanope sayi* may reach densities of 54 crabs/m^2 (MacKenzie 1977), while the blue crab, *C. sapidus,* may reach a density of 13 crabs/m^2 (Larson 1974). The abundance, mobility, and high predation rates of these crabs make them serious predators of juvenile hard clams (Eldridge et al. 1976; MacKenzie 1977, 1979).

This study shows that toadfish larger than 220 mm TL can prey on mud crabs less than 30 mm CW and on blue crabs less than 70 mm CW. Hence, toadfish may be used to control crab populations within netted enclosures. In the field, toadfish of 231 mm mean TL reduced crab predation on juvenile hard clams 3 mm in shell length when cultured in cages of 25 mm square mesh with crushed gravel aggregate (Gibbons and Castagna 1985). Toadfish, larger than 170–220 mm TL, may be placed within cages, trays, or beds with crushed gravel aggregate and a mesh of 6–12 mm to control crab predation effectively and reduce manual labor for crab removal. Enclosed grow-out systems that are established in the field prior to planting of hard clams should receive toadfish several days before the addition of clams. The addition of toadfish to field grow-out systems will enable the use of smaller (<8 mm SH) hard clams, and thereby reduce expenses and efforts required to raise clams in these systems.

ACKNOWLEDGMENTS

The authors thank Reade Bonniwell, Nancy Lewis, and Jean Watkinson for valuable assistance. We appreciate Keith Pruitt and Kenneth Terry for supplying toadfish and crabs. Jim Moore helped establish the experiment and maintained animals. Dr. Romuald Lipcius, Dr. Mark Luckenbach, Dr. Roger Mann, Ray Morales, and Dave Stilwell provided editorial assistance.

This work is a result of research sponsored by the Vir-

ginia Institute of Marine Science Sea Grant Program, supported by the Office of Sea Grant, NOAA, under Subcontract 5-29386, project No. R/MG-84-2. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

REFERENCES CITED

Arnold, W. S. 1984. The effects of prey size, predator size, and sediment composition on the rate of predation of the blue crab, *Callinectes sapidus* Rathbun, on the hard clam, *Mercenaria mercenaria* (Linne). *J. Exp. Mar. Biol. Ecol.* 80:207–219.

Bisker, R. S. & M. Castagna. 1986. Predation on single spat oysters *Crassostrea virginica* (Gmelin) by blue crabs *Callinectes sapidus* Rathbun and mud crabs *Panopeus herbstii* Milne-Edwards. *J. Shellfish Res.* 6:37–40.

Bahr, L. M. Jr. 1974. Aspects of the structure and function of the intertidal oyster reef community in Georgia. Univ. of Georgia, Athens, GA 149 p. Ph.D. thesis.

Carriker, M. R. 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria mercenaria*. *J. Elis. Mitch. Sci. Soc.* 77:168–241.

Castagna, M. & J. N. Kraeuter. 1981. Manual for growing the hard clam *Mercenaria*. VIMS Special Report in Applied Marine Science and Ocean Engineering No. 249, 110 pp.

Dame, R. F. 1979. The abundance, diversity and biomass of macrobenthos on North Inlet, South Carolina, intertidal oyster reefs. *Proc. Natl. Shellfish. Assoc.* 69:6–10.

Eldridge, P. J., W. Waltz, R. C. Gracy & H. H. Hunt. 1976. Growth and mortality rates of hatchery seed clams, *Mercenaria mercenaria*, in protected trays in waters of South Carolina. *Proc. Natl. Shellfish. Assoc.* 66:13–20.

Gibbons, M. C. 1984. Aspects of predation by the crabs *Neopanope sayi*, *Ovalipes ocellatus*, and *Pagurus longicarpus* on juvenile hard clams *Mercenaria mercenaria*. State University of New York, Stony Brook, NY. 102 p. Ph. D. thesis.

Gibbons, M. C. & W. J. Blogoslawski. 1989. Predators, Pests, Parasites, and Diseases. J. J. Manzi and M. Castagna (eds.) *Clam Mariculture in North America*, Elsevier Sci. Publ. Co., NY pp. 167–200.

Gibbons, M. C. & M. Castagna. 1985. Biological control of predation by crabs in bottom cultures of hard clams using a combination of crushed stone aggregate, toadfish, and cages. *Aquaculture* 47:101–104.

Gudger, E. W. 1910. Habits and life history of the toadfish (*Opsanus tau*). *Bull. U.S. Bur. Fish.* (1908) 28:1071–1109.

Jory, D. E., M. R. Carriker & E. S. Iverson. 1984. Preventing predation in molluscan mariculture: An overview. *J. World Maricul. Soc.* 15:421–432.

Kraeuter, J. N. & M. Castagna. 1985. The effect of clam size, net size, and poisoned bait treatments on survival of hard clam, *Mercenaria mercenaria*, seed in field plots. *J. World Maricul. Soc.* 16:377–385.

Larson, P. 1974. Quantitative studies of the macrofauna associated with the mesohaline oyster reefs of the James River, Virginia. School of Marine Science, College of William and Mary, Gloucester Point, VA 181 p. Ph. D. thesis.

MacKenzie, C. L. Jr. 1977. Predation on hard clam (*Mercenaria mercenaria*) populations. *Trans. Amer. Fish. Soc.* 106:530–537.

MacKenzie, C. L. 1979. Management for increasing clam abundance. *Mar. Fish. Rev.* 41:10–22.

Manzi, J. J., V. G. Burrell, Jr. & W. Z. Carson. 1980. A mariculture demonstration project for an alternative hard clam fishery in South Carolina: Preliminary results. *Proc. World Maricul. Soc.* 11:79–89.

McDermott, J. J. 1964. Food habits of the toadfish, *Opsanus tau* (L.), in New Jersey waters. *J. Penn. Acad. Sci.* 38:64–71.

McDonald, J. 1982. Divergent life history patterns in the co-occurring intertidal crabs *Panopeus herbstii* and *Eurypanopeus depressus* (Crustacea: Brachyura: Xanthidae). *Mar. Ecol. Prog. Ser.* 8:173–180.

Phillips, R. R. & S. B. Swears. 1979. Social hierarchy, shelter use, and avoidance of predatory toadfish (*Opsanus tau*) by the striped blenny (*Chasmodes bosquianus*). *Anim. Behav.* 27:1113–1121.

Ryan, E. P. 1956. Observations on the life histories and the distribution of the Xanthidae (mud crabs) of Chesapeake Bay. *Am. Midl. Nat.* 56:138–162.

Schwartz, F. J. & B. W. Dutcher. 1963. Age, growth, and food of the oyster toadfish near Solomons, Maryland. *Trans. Am. Fish. Soc.* 92:170–173.

Steel, R. G. D. & J. H. Torrie. 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Co., New York. 481 pp.

Swartz, R. C. 1976. Agonistic and sexual behavior of the xanthid crab, *Neopanope sayi*. *Ches. Sci.* 17:24–34.

Walker, R. L. 1984. Effects of density and sampling time on the growth of the hard clam, *Mercenaria mercenaria*, planted in predator-free cages in coastal Georgia. *The Nautilus* 98:114–119.

Whetstone, J. M. & A. G. Eversole. 1981. Effects of size and temperature on mud crab, *Panopeus herbstii*, predation on hard clams, *Mercenaria mercenaria*. *Estuaries* 4:153–156.

Williams, A. B. 1984. Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, D.C., 550 pp.

Wilson, C. A., J. M. Dean & R. Radtke. 1982. Age, growth rate and feeding habits of the oyster toadfish, *Opsanus tau* (Linnaeus) in South Carolina. *J. Exp. Mar. Biol. Ecol.* 62:251–259.

Journal of Shellfish Research, Vol. 8, No. 1, 33–36, 1989.

BIOLOGICAL CONTROL OF CRAB PREDATION ON HARD CLAMS *MERCENARIA MERCENARIA* (LINNAEUS, 1758) BY THE TOADFISH *OPSANUS TAU* (LINNAEUS) IN TRAY CULTURES[1]

ROBERT BISKER AND MICHAEL CASTAGNA
Virginia Institute of Marine Science and
School of Marine Science
College of William and Mary
Wachapreague, VA 23480 (USA)

ABSTRACT Oyster toadfish *Opsanus tau* (Linne) were tested as biological controls of crab predation on juvenile hard clams *Mercenaria mercenaria* (Linne) in trays with crushed stone aggregate. Clam survival after 34 weeks was 69.5% in the presence of toadfish and 2.3% in trays without toadfish. Toadfish reduced the total number of crabs (mud crabs and blue crabs). Crabs in trays with toadfish present had smaller carapace widths.

KEY WORDS: predation, toadfish, *Opsanus*, crabs, hard clams, *Mercenaria*, tray culture

INTRODUCTION

A major factor limiting production of juvenile hard clams cultured in the field is crab predation (Whetstone and Eversole 1978, Jory et al. 1984, Gibbons and Blogoslawski 1989). Clam growers attempt to exclude predators from field cultured clams by using rafts, trays, cages, and nets (Castagna and Kraeuter 1981, Castagna 1983, Jory et al. 1984). Increasing the chances of high survival rate in clam culture requires the use of seed clams larger than 6 mm shell height (SH) (Kraeuter and Castagna 1985). Large seed is not only more costly than smaller seed, but is often in short supply. The development of a viable method for using smaller seed in field culture is needed.

Walker (1984) suggested that survival of seed less than 18 mm in shell length depended on frequent removal of newly metamorphosed crabs from within cages. Field growout structures often attract or even trap juvenile crabs that pass through netted enclosures and grow to sizes large enough to cause significant mortality on smaller clams. Both mud crabs and blue crabs can prey on clams with SH about one third the carapace width of the crabs and may have feeding rates of 136 and 308 clams/crab/day, respectively (Carriker 1961, Castagna and Kraeuter 1981, Gibbons 1984).

Successful use of small seed clams, *Mercenaria mercenaria* (Linne), (<4 mm SH) in field cultures has been achieved by Gibbons and Castagna (1985). They found that oyster toadfish *Opsanus tau* (Linne) were effective in reducing crab predation on clams planted in the bottom under crushed stone aggregate. Survival after 6 weeks was about 50% in plots containing a single toadfish and 2% without toadfish. Flagg and Malouf (1983) found higher clam survival in uncovered trays that were found to have toadfish living in close proximity. The oyster toadfish, *Opsanus tau*, is a nonmigratory species whose diet consists primarily of crabs (Gudger 1910, Schwartz and Dutcher 1963, McDermott 1964, Wilson et al. 1982). Gibbons and Castagna (1985) found toadfish to be a significant predator of mud crabs (Decapoda: Xanthidae) and the portunid blue crab, *Callinectes sapidus* Rathbun. This study examined the survival of juvenile hard clams as influenced by toadfish presence in trays of small cultured clam seed.

MATERIALS AND METHODS

The experiment was conducted from August 1987 to April 1988 in Bradfords Bay near Wachapreague, VA (U.S.A.). Juvenile hard clams reared at the Wachapreague Laboratory of the Virginia Institute of Marine Science were sieved through a 3 mm mesh screen, caught on a 2 mm mesh screen and divided into 10 groups of 6400 each. A random sample of 100 was photocopied for shell height measurements (hinge to lip) (Haines 1973). Toadfish were collected locally and had total lengths (TL) of 216 ± 15.5 mm (mean ± standard deviation, n = 5). Ten trays, 200 × 100 × 9 cm (inside dimensions) with wood sides were used. The bottoms were fitted with 1.4 mm mesh fiberglass screen over heavier 13 mm mesh plastic screen bottoms. Trays were filled with crushed stone aggregate 2.5 cm deep and covered with 6 mm plastic mesh.

Trays were deployed subtidally (depth at mean low water was approximately 60 cm) on August 19, 1987. Each tray received 6400 clams (3200/m^2) and 5 of the trays received one toadfish each. Trays were sampled on October 6 (48 days) and November 16 (89 days), 1987, and April 19, 1988 (244 days) by taking ten 71.5 mm dia. randomly located core samples in each. The number of live clams per sample was recorded and clams from each tray were photocopied for shell height (SH) measurement. The number and carapace width (CW) of crabs collected in samples were also recorded. On the October sampling, the toadfish was missing from a tray with a torn net. The net was repaired and another toadfish 193 TL was added. Fouling was cleared from the nets at each sampling. At the final sam-

[1]Contribution No. 1526 from Virginia Institute of Marine Science.

pling in April, each tray was thoroughly examined for crabs, which were measured and identified. A final estimate of clam survival was made by determining the total volume of clams and aggregate per tray, taking two random samples of one liter each, and counting the number of live clams per liter.

Prior to statistical testing all data was log transformed which fixed heteroscedatic variances. The number of live clams per core sample was transformed to log (x + 1). The tranformed data were compared in a three-way nested analysis of variance (ANOVA) with trays nested within toadfish treatment and time of sample as factors. Clam shell heights were transformed to log x and compared between sampling times with a one-way ANOVA. Differences in mean shell height were further analysed with the new Duncan's multiple range test (Steel and Torrie 1960). Differences in final shell heights of clams between trays and treatments were compared with a two-way ANOVA. The numbers of mud crabs, blue crabs, and total crabs collected at the end of the study were also log transformed and one-way ANOVA were used to test for differences between treatments. A log transformation was also applied to the carapace widths of mud crabs and blue crabs from the final sampling; one-way ANOVA were used to compare treatments.

RESULTS

Clam survival in the trays as determined by core sampling after 244 days was 69.5% in the presence of toadfish compared to 2.3% when toadfish were absent (Table 1). Using the two-liter subsample method, estimated final clam survival in the presence of toadfish was 69.9% and 2.4% when toadfish were absent. There were significant differences in toadfish presence ($F = 93.0$, d.f. $= 1$, $p < 0.001$) and time of sample ($F = 17.8$, d.f. $= 2$, $p < 0.001$). Differences within treatment trays were not significant ($F = 2.0$, d.f. $= 4$, $p = 0.09$). Shell heights of clams sampled in October, November, and April were not significantly different from each other but were significantly different from initial shell height measurements ($p < 0.05$) (Table 2). Slow clam growth was due to cold water temperatures during the winter and reduced water circulation within trays caused by fouling of nets with red algae, which was removed at October and November samplings. There were no significant differences in shell heights at final sampling due to toadfish presence ($F = 0.00$, d.f. $= 1$), tray ($F = 0.87$, d.f. $= 4$) or toadfish presence–tray interactions ($F = 2.12$, d.f. $= 4$, $p = 0.076$).

Two crab species were found in the trays, the mud crab, *Neopanope sayi* (Smith), and the blue crab, *C. sapidus*. There was no significant difference ($F = 1.3$, d.f. $= 1$, $p = 0.28$) in the mean number of mud crabs found per tray, although the mean number in the trays containing toadfish was lower (15.8/tray) than in trays without toadfish (22.0/tray) (Table 3). There were significantly fewer numbers of blue crabs ($F = 19.3$, d.f. $= 1$, $p = 0.002$) and total crabs ($F = 9.8$, d.f. $= 1$, $p = 0.01$) per tray in the presence of toadfish (Table 3). Significantly smaller carapace widths of mud crabs ($F = 4.2$, d.f. $= 1$, $p = 0.04$) and blue crabs ($F = 9.6$, d.f. $= 1$, $p = 0.003$) were found in those trays with toadfish (Table 4). Blue crabs of 38.4 mm CW and mud crabs of 18.4 mm CW were present in trays after 48 and 89 days, respectively (Table 5).

Toadfish appeared healthy at October and November samplings. The tray missing a toadfish at the October sampling received a new toadfish. This did not appear to affect the results. All toadfish were found dead at the April sampling, which probably may have been caused by exposure to the cold winter surface water temperatures. The light siltation found in all trays allowed for free movement of the toadfish, yet offered no protection from the cold water.

DISCUSSION

Toadfish effectively controlled crab predation on juvenile hard clams starting at 3.6 mm SH for more than 8 months in tray cultures. After almost 7 weeks estimated clam survival was 100% with toadfish present and 46.9% without toadfish. Gibbons and Castagna (1985) found clam survival of 49.2% with toadfish and 1.6% without toadfish using bottom planting in crushed stone aggregate with 25 mm mesh pens instead of trays with 6 mm mesh net covers used in the present study. Further, the toadfish in the pre-

TABLE 1.

Mean number of live hard clams found per core sample with 95% confidence limits (n = 5), and estimated percent survival at October, November, and April sampling periods for trays with toadfish present or absent.

	Toadfish			
	Present		Absent	
Sample date	Mean ± C. L.	% Survival	Mean ± C. L.	% Survival
October	12.9 ± 2.4	100.8	6.0 ± 3.2	46.9
November	6.8 ± 4.9	53.1	1.2 ± 1.2	9.4
April	8.9 ± 2.9	69.5	0.3 ± 0.2	2.3

TABLE 2.

Mean shell heights (SH) in mm with 95% confidence limits (n = 100) for hard clams sampled in August, October, November, and April.

Sample date	SH ± C. L. (mm)
August	3.57 ± 0.02*
October	4.96 ± 0.17
November	5.52 ± 0.19
April	6.14 ± 0.29

* Significantly smaller than rest (p = 0.05).

TABLE 4.

Mean carapace width (CW) in mm with 95% confidence limits for mud crabs and blue crabs found in trays at final sample period in April for trays with toadfish present or absent.

	Toadfish			
	Present		Absent	
	Mean ± C. L.	N	Mean ± C. L.	N
Mud crab	11.7 ± 1.1	79	12.8 ± 0.8	110
Blue crab	19.6 ± 2.6	6	28.5 ± 2.6	40

vious study patrolled half the area of this study. A laboratory study by Bisker et al. (in preparation) reported only a slight decrease in blue crab predation on clams in the presence of toadfish after two days, but used crabs of 84.5 mm CW which were three to four times larger than those found in the field trays. Blue crabs of 84.5 mm CW can pass through nets of 25 mm mesh but not through those of 6 mm mesh, and may prey on juvenile clams at a rate of 307/day (Carriker 1959, Bisker and Castagna 1987). Use of the smaller 6 mm mesh netting eliminated the larger crabs with higher predation rates, therefore enhancing the control of crab predation by the toadfish.

There was a noticeable reduction in survival of those clams in trays with toadfish between the October (100%) and the November (53.1%) samples. This reduced survival may have been caused by an increase in the number of mud crabs and blue crabs large enough to prey on the clams. Water temperatures were still warm enough during this period for active crab predation to occur. Sample error may have contributed to the lower clam survival found in the November sample as the final sampling had 16.4% higher survival.

Labor required for removal of crabs from trays reported by Walker (1984) was not required in our study as toadfish reduced crab numbers and sizes. Toadfish also may reduce crab feeding efficiencies by injuring crabs or by invoking increased defensive behavior in the presence of toadfish. Blue crabs have demonstrated avoidance behavior in the presence of toadfish, and some even crawl out of the water to escape (Bisker et al., in prep.).

TABLE 3.

Mean number of mud crabs and blue crabs found per tray with 95% confidence limits (n = 5) at final sample period in April for trays with toadfish present or absent.

	Toadfish	
	Present	Absent
	Mean ± C. L.	Mean ± C. L.
Mud crabs	15.8 ± 2.7	22.0 ± 10.5
Blue crabs	1.2 ± 1.6	8.0 ± 3.7
Total crabs	17.0 ± 3.8	30.0 ± 12.0

Toadfish reduced the number of blue crabs more effectively than mud crabs. This may be due to the more obvious behavior of the blue crab making it easier to discover. Blue crabs have difficulty burrowing in the crushed stone substrate and are more vulnerable (Bisker et al., in prep.). Toadfish predator-prey size ratios (CW/TL) are 0.10 for mud crabs and 0.32 for blue crabs (Bisker et al., in prep.). All crabs found in the trays were sizes that could be preyed on by the toadfish used.

Neopanope sayi, the mud crab species found in the trays, can devour as many as 134 clams/day and are found as dense as 54 crabs/m^2 (MacKenzie 1977, Gibbons 1984). Blue crabs can eat as many as 307 clams/day but densities are far less, 13 crabs/m^2, perhaps as a result of their antagonistic territorial behavior (Carriker 1954, Larson 1974). Clam mortality in the trays without toadfish averaged 36 clams/day/m^2 for the first 48 days, and averaged about 13 clams/day/m^2 for the entire study. Gibbons and Castagna (1985) found clam mortality rates of about 75 dead clams/day/m^2 in cages without toadfish after 42 days. Crab densities in trays without toadfish were 11 crabs/m^2 for mud crabs and 4 blue crabs/m^2 after 8 months. Crab densities in trays containing toadfish were 7.9/m^2 for mud crabs and 0.6/m^2 for blue crabs. The small density decrease of 3.1 mud crabs/m^2 and 3.4 blue crabs/m^2 in trays with toadfish allowed for 96.8% better clam survival after 8 months.

Mean clam shell heights in the present study increased 1.4 mm after the first 48 days. Gibbons and Castagna (1985) found an increase of 3.5 mm mean shell height after 42 days from clams held on the bottom in cages of 2.5 mm mesh during a similar time of year. The slower clam growth found in the present study was probably caused by reduced water circulation in the trays. The solid wood tray sides and fouling of the 6 mm mesh covers by algae slowed the exchange of water within the trays and thus limited food for the clams.

Although all toadfish were dead at the end of the experiment, they are generally hardy fish and have survived overwintering in other trays that were held in deeper water (per-

TABLE 5.

Carapace widths in mm of mud crabs and blue crabs collected in core samples for each sample period from trays with toadfish present or absent.

	Toadfish			
	Present		Absent	
Sample period	Mud crab	Blue crab	Mud crab	Blue crab
October		7.2		38.4
		9.6		
November	18.4	12.0	4.4	43.2
	6.1			7.9
	5.2			
April	11.7		15.2	22.0
	9.6		15.8	29.8

sonal observation). Toadfish are easy to capture and handle and require little attention during use as a biological control for crabs in clam trays. Results of this study clearly show that use of toadfish is beneficial when used to protect small hard clams less than 10 mm SH, allowing the use of smaller and less expensive clam seed in grow-out systems.

ACKNOWLEDGMENTS

The authors thank Doug Ayres, Reade Bonniwell, Nancy Lewis, and Jean Watkinson for their valuable assistance, and Mary Gibbons, Mark Luckenbach, William Hargis, and Dan Sved for their critical review of this manuscript.

REFERENCES CITED

Bisker, R. S. & M. Castagna. 1987. Predation on single spat oysters *Crassostrea virginica* (Gmelin) by blue crabs *Callinectes sapidus* Rathbun and mud crabs *Panopeus herbstii* Milne-Edwards. *J. Shellfish Res.* 6:37–40.

Bisker, R. S., M. Gibbons, & M. Castagna. Predation by the oyster toadfish *Opsanus tau* (Linne) on blue crab and mud crab predators of the hard clam *Mercenaria mercenaria* (Linne). (in prep.)

Carriker, M. R. 1954. Preliminary studies on the field culture, behavior, and trapping of the larvae of the hard clam, *Venus* (=*Mercenaria*) *mercenaria* L. *Proc. Natl. Shellf. Assn.* 1952:70–73.

Carriker, M. R. 1959. The role of physical and biological factors in the culture of *Crassostrea* and *Mercenaria* in a salt-water pond. *Ecol. Monogr.* 29:219–266.

Carriker, M. R. 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria mercenaria*. *J. Elish. Mitch. Sci. Soc.* 77:168–241.

Castagna, M. 1983. Review of recent bivalve culture methods. *J. World Maricul. Soc.* 14:567–575.

Castagna, M. & J. N. Kraeuter. 1981. Manual for growing the hard clam *Mercenaria*. VIMS Special Report in Applied Marine Science and Ocean Engineering No. 249, 110 pp.

Flagg, P. J. & R. E. Malouf. 1983. Experimental planting of juveniles of the hard clam *Mercenaria mercenaria* (Linne) in the waters of Long Island, New York. *J. Shellfish Res.* 3:19–27.

Gibbons, M. C. 1984. Aspects of predation by the crabs *Neopanope sayi*, *Ovalipes ocellatus*, and *Pagurus longicarpus* on juvenile hard clams *Mercenaria mercenaria*. State University of New York, Stony Brook, NY, Ph.D. Thesis, 102 pp.

Gibbons, M. C. & W. J. Blogoslawski. 1989. Predators, Pests, Parasites, and Diseases. J. J. Manzi and M. Castagna (eds.) *Clam Mariculture in North America*, Elsevier Sci. Publ. Co., New York. pp. 167–200.

Gibbons, M. C. & M. Castagna. 1985. Biological control of predation by crabs in bottom culture of hard clams using a combination of crushed stone aggregate, toadfish, and cages. *Aquaculture* 47:101–104.

Gudger, E. W. 1910. Habits and life history of the toadfish (*Opsanus tau*). *Bull. U.S. Bur. Fish.* (1908) 28:1071–1109.

Haines, K. C. 1973. A rapid technique for recording sizes of juvenile pelecypod molluscs. *Aquaculture* 1:433.

Jory, D. E., M. R. Carriker & E. S. Iverson. 1984. Preventing predation in molluscan mariculture: An overview. *J. World Maricul. Soc.* 15:421–432.

Kraeuter, J. N. & M. Castagna. 1985. The effect of clam size, net size, and poisoned bait treatments on survival of hard clam, *Mercenaria mercenaria*, seed in field plots. *J. World Maricul. Soc.* 16:377–385.

Larson, P. 1974. Quantitative studies of the macrofauna associated with the mesohaline oyster reefs of the James River, Virginia. School of Marine Science, College of William and Mary, Gloucester Point, VA. Ph.D. thesis, 181 pp.

MacKenzie, C. L., Jr. 1977. Predation on hard clam (*Mercenaria mercenaria*) populations. *Trans. Amer. Fish. Soc.* 106:530–537.

McDermott, J. J. 1964. Food habits of the toadfish, *Opsanus tau* (L.), in New Jersey waters. *J. Penn. Acad. Sci.* 38:64–71.

Schwartz, F. J. & B. W. Dutcher. 1963. Age, growth, and food of the oyster toadfish near Solomons, Maryland. *Trans. Am. Fish. Soc.* 92:170–173.

Steel, R. G. D. & J. H. Torrie. 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Co., New York. 481 pp.

Walker, R. L. 1984. Effects of density and sampling time on the growth of the hard clam, *Mercenaria mercenaria*, planted in predator-free cages in coastal Georgia. *The Nautilus* 98:114–119.

Whetstone, J. M. & A. G. Eversole. 1978. Predation on hard clams, *Mercenaria mercenaria*, by mud crabs *Panopeus herbstii*. *Proc. Natl. Shellfish. Assoc.* 68:42–48.

Wilson, C. A., J. M. Dean & R. Radtke. 1982. Age, growth rate and feeding habits of the oyster toadfish, *Opsanus tau* (Linnaeus) in South Carolina. *J. Exp. Mar. Biol. Ecol.* 62:251–259.

Journal of Shellfish Research, Vol. 8, No. 1, 37–42, 1989.

EFFECTS OF SUSPENDED SEDIMENT, HYPOXIA, AND HYPEROXIA ON LARVAL *MERCENARIA MERCENARIA* (LINNAEUS, 1758)

KATHLEEN MCMURRER HUNTINGTON AND DOUGLAS C. MILLER
College of Marine Studies
University of Delaware
Lewes, Delaware 19958

ABSTRACT Recruitment to benthic environments by *Mercenaria mercenaria* (L.) larvae may be influenced by suspended sediment loads and dissolved oxygen concentrations. We tested the effect of these factors on survival and growth of one to four day-old hard clam veligers. A tumbler maintained particles in suspension during 48-hr suspended sediment (0–2200 mg l^{-1}), 24-hr hypoxic (1–6.5 mg l^{-1}; 15–90% saturation at 22°C) and 24-hr hyperoxic (13.7 mg l^{-1}, 180% saturation at 20°C) experiments. Larval survival was not affected by any of the treatments. Growth, however, (as indicated by changes in mean size over time) was negatively affected by 2200 mg l^{-1} sediments and 13.7 mg l^{-1} (180% saturation) dissolved oxygen concentration. Daily afternoon supersaturation, common in eutrophic systems, and infrequent episodes of very high loads of suspended sediments may thus negatively impact *M. mercenaria* growth during the larval stage.

KEY WORDS: suspended sediments, hypoxia, hyperoxia, *M. mercenaria* larvae, survival, growth

INTRODUCTION

Larvae of the northern quahog, *Mercenaria mercenaria*, can experience severe environmental conditions during their 6–20 d planktonic period (Carriker 1961), including high levels of suspended sediment and extremes of dissolved oxygen concentration. Understanding the effect of these factors on survival and growth of larvae can help determine their influence on northern quahog recruitment. Some previous investigations have studied effects of suspended silt, clay, and of hypoxia on survival and growth of *M. mercenaria* larvae. Davis (1960) found that concentrations of silt (particles 4–63 μm in diameter) greater than 1000 mg l^{-1} negatively affected growth but not survival. Clay suspensions (particles <4 μm in diameter) exceeding 500 mg l^{-1} prevented all growth and caused 90% mortality. Morrison (1971) reports that dissolved oxygen levels at or below 4.2 mg l^{-1} (60% saturation at experimental conditions) curtailed larval *M. mercenaria* growth, but larvae were capable of surviving 1 mg l^{-1} conditions for as long as 11 days. Larval tolerance of hyperoxic conditions has not been investigated previously, but growth of juvenile *M. mercenaria* was reduced during 30 d exposure to 111% saturation (Bisker and Castagna 1985).

A number of factors could have caused previous results to overestimate the effect of experimental treatments. Larval concentrations used by Davis (1960) and Morrison (1971) were 100-fold greater than those typical in the field (Carriker 1961), and algal rations were unspecified. The size distribution of particles imposed on the larvae, not just the mass concentration seems to be an important factor (Davis 1960). Geographic variation in tolerance could also complicate the interpretation of experimental results (Loosanoff 1962). Both Davis (1960) and Morrison (1971) used Long Island Sound larvae, and Bisker and Castagna (1985) used juveniles from Virginia. Thus, it may be inappropriate to apply these published results to a variety of field sites which may differ in typical environmental conditions.

Our objective was to determine the effects of levels of suspended sediments and dissolved oxygen typical of Indian River Bay, Delaware, U.S.A. (a local coastal lagoon ecosystem) on survival and growth of *M. mercenaria* larvae from that habitat. During weekly sampling of standard water column and benthic parameters in Indian River Bay during the summers of 1986 and 1987 (Huntington 1988), we found suspended sediment concentrations to range from about 10 to 570 mg l^{-1}, with a 1987 average of about 60 mg l^{-1}. Dissolved oxygen concentration ranged from 1.7 mg l^{-1} (25% saturation) to 12.2 mg l^{-1} (190% saturation), with about 6 mg l^{-1} (approximately 90% saturation) most common during 1987 midmorning sampling. Hypoxic conditions persisted less than 4 hr, but supersaturation lasted almost 12 hr a day. Within this spectrum of environmental conditions, we conducted experiments using larval concentrations and supplied algal food quantities (measured as chlorophyll *a* concentration) typical of summer field values.

MATERIALS AND METHODS

We conducted all experiments on a jar tumbler device (Fig. 1) at 21–24°C, under continuous fluorescent lighting. The tumbler turned 946-ml glass experimental jars filled with glass fiber-filtered seawater (30 ppt) at 3 revolutions per minute to maintain added particles in suspension. Jars were sealed with a Parafilm layer and plastic covers to permit capping without introducing air bubbles and make them both air- and watertight.

Larvae used in our experiments were spawned from adults collected from Indian River Bay. Sibling *M. mercenaria* larvae were maintained at 10–15 larvae ml^{-1} in 40-l aerated cones at room temperature (21–24°C) until

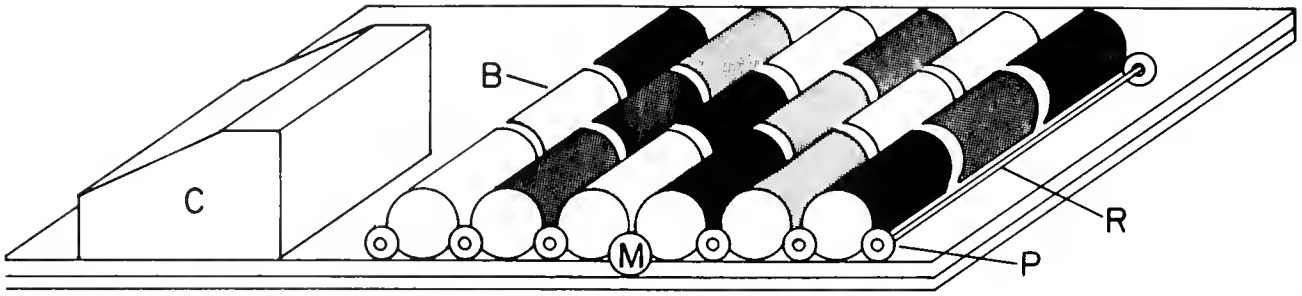


Figure 1. Diagram of plankton tumbler used in suspended sediment and dissolved oxygen experiments. Components labelled are: C, stepper motor power supply and controller; B, jars containing larvae and experimental treatments; M, stepper motor; P, pillow block rod supports; and R, support rods sheathed in vacuum tubing. Also shown by shading is the arrangement of treatments used in one repetition of the suspended sediment experiment. These treatments were blocked such that one of each of the six sediment concentrations occurs in each of the three rows of jars.

use. Larvae were never more than 96 hr, and usually 24 or 48 hr old post-fertilization at start of each experiment. To gain statistical power to detect treatment differences, we repeated experiments several times, using separate groups of siblings. Thus, repetitions of the experimental protocol are not replicates in the statistical sense, and we have allowed for this fact by treating these repetitions as an additional, blocking factor in the anova analysis (see below). *Isochrysis* aff. *galbana* (T-ISO) was available to larvae for food starting 12–18 hr after fertilization. We placed approximately 100 sibling larvae in each 946-ml jar with the appropriate treatment conditions for all the experiments. During experiments lasting more than 24 hr, we daily supplied to each jar sufficient algal culture suspension (10^7 cells per jar, about 20 μg chlorophyll *a* l^{-1}) to approximate summer field chlorophyll *a* concentrations (Huntington 1988).

We used a Yellow Springs Instrument Co. Model 58 oxygen meter and a Model 5739 probe to measure dissolved oxygen concentration in experimental jars. The dissolved oxygen meter was air calibrated to 100% saturation, and dissolved oxygen readings were periodically compared to values determined by the Winkler method (Parsons et al. 1984). We determined algal culture density and the size-frequency distribution of suspended sediment particles with a Coulter Model ZB electronic particle counter.

Suspended Sediment Experiments

We simulated summer field suspended sediment loads by adding to experimental jars known amounts of a natural silt-clay mixture obtained from bottom sediments in Indian River Bay. From occasional summer water samples we found that greater that 95% of the suspended particles were less than 11 μm in diameter (Huntington 1988). The silt-clay suspension used in our experiments was obtained by wet-sieving (on a 63-μm sieve) surface sediment from bottom cores on day of collection, and it was stored at 1°C until use. We determined the treatment levels based on our Indian River Bay field data (Huntington 1988) and previously published work (Biggs 1978). The concentrations used were: 0, 56, 110, 220, 560, and 2200 mg l^{-1}.

Using three jars at each of six concentration levels, we measured dissolved oxygen in all jars, then placed them on the tumbler. Several times (at 12, 24, and 48 hr) after starting the experiment, we measured dissolved oxygen in all jars, and removed one jar of each of the six treatment levels and fixed the larvae in 4% buffered formalin. We transferred larvae to 90% ethanol within 24 hr of fixation. This procedure constituted one repetition of the experiment. In all, we conducted four repetitions of the above procedure.

Mean value length and survival of treatment conditions were determined for all larvae by viewing them in a petri dish under a dissecting microscope. Following death, autolysis causes valves to gape, thus survival was indicated by closed, as opposed to gaping, valves in a preserved larva. Experimental results were expressed as percent survival within the group of larvae recovered from each jar. We measured valve length (to the nearest 10 μm) of each larva (alive or dead at time of preservation) using an ocular micrometer. Although we did not measure larval size at the beginning of each experiment, we assigned treatment combinations to jars at random. Thus any differences in mean size during the experiment will be attributed to treatment differences in larval growth.

We statistically analyzed both survival and mean valve length of live larvae from each jar by exposure time, treatment and repetition (as a random blocking factor) with three-way, mixed model, unbalanced and unreplicated anova (Zar 1984). The unbalanced design was necessitated by lack of data from seven jars of the total 72 (from all four repetitions), since contents leaked or larvae were not recovered. Three or four repetitions were successful for all treatment levels and times except one (i.e., 200 mg l^{-1}, 12 hr combination), for which only two repetitions yielded data. An average of 186 larvae (range: 109–273) were analyzed for each of the 18 treatment combinations. Following the anova, significant results were further analyzed by

Newman-Keuls multiple comparison tests to compare all treatment combinations, and the Dunnett test (Zar 1984) to compare treatments individually with the experimental control.

Dissolved Oxygen Experiments

To adjust dissolved oxygen to hypoxic treatments, we used an air stone to bubble regular laboratory grade nitrogen gas through glass-fiber filtered seawater (30 ppt). At the beginning of the experiments, the dissolved oxygen concentrations (within 0.5 mg l^{-1}) were: 1, 2, 4, and 6.5 mg l^{-1}. At room temperature (22°C) these correspond to approximately 15, 25, 50, and 90% saturation. No suspended sediment was added to the filtered seawater. We placed 16 jars (four each of four hypoxic treatment levels) on the tumbler, and we sampled one jar at each concentration at 2, 4, 6, and 24 hr after starting the experiment. Using a different batch of siblings each time, this procedure was repeated for a total of three repetitions of the experiment. Post-experiment sample processing was as described above, and we were able to run a balanced design, three-way, unreplicated anova. An average of 233 larvae (range: 143–324) were analyzed for each of the 16 treatments consisting of four jars each.

To create hyperoxic conditions, we bubbled Linde extra dry grade oxygen gas through filtered seawater. Before capping jars at the beginning of the experiment, dissolved oxygen concentrations in the control and hyperoxic jars were (within 0.5 mg l^{-1}) 7.6 and 13.7 mg l^{-1}. At room temperature (20°C) these correspond to approximately 95% and 180% saturation. We used five bottles at each concentration: four for survival and growth measurements, and one for microscopic observation of live larvae. We repeated the larval sampling protocol used for hypoxic experiments, except all eight survival and growth bottles were sampled after 24 hr. Though this exposure period is longer than that of supersaturated conditions we measured in the field (typically 12 hr), the longer period allowed for more larval growth, and hence a more sensitive test of hyperoxic effects. These samples were processed as described above. Statistical analysis of survival and mean size for each jar (average 192 larvae per jar, range 130–252) was by one-way replicated anova (Zar 1984) with jars representing true replicates of the two experimental treatments. At the end of the 24-hr experiment, we collected larvae for live observation by gravity filtration on 64 μm Nitex and removed them to a petri dish with squirt bottle spray. We waited about five minutes before microscopic observation to allow the larvae time to regain composure before viewing them.

RESULTS

Suspended Sediment Experiments

Anova analysis revealed a significant difference in larval size ($F_{5,14}$ = 3.35, P = 0.034, Fig. 2), but not survival ($F_{5,14}$ = 0.50, P = 0.77), across the range of suspended sediment concentrations tested. Survival of larvae exceeded 95% for all treatments. None of the two-factor interactions effects were significant in either size or survival anova analysis. Growth was measurable over the 48 hr experi-

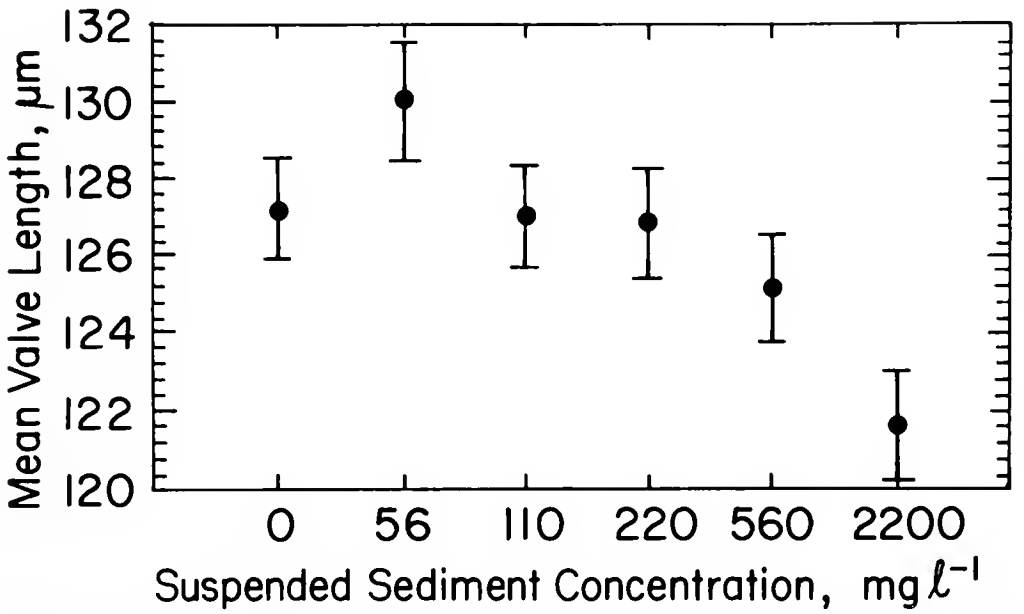


Figure 2. Results of mean larval size in suspended sediment experiments depicted as 95% least significant difference plots for pairwise comparisons at α = 0.05. Dots represent treatment mean larval length. Error bars are based on the error mean square from the anova analysis. Overall anova indicated means significantly differed (P = 0.034). Multiple comparison tests revealed that only 2200 mg l^{-1} differed significantly from other means.

ment, as mean valve length increased about 15% between the 12 hr and 48 hr sampling periods (Table 1). As determined by *a posteriori* Newman-Keuls tests (using an overall experimentwise $\alpha = 0.05$ for these comparisons), the only significantly lower mean among the six concentrations is that at 2200 mg l^{-1}. The Dunnett test also showed the 2200 mg l^{-1} treatment as the only treatment effect differing from that of the control. It is interesting to note the trend in the treatment means (Fig. 2): the mean size at 50 mg l^{-1} exceeds (though not significantly) that of all other treatments and mean larval size decreases with increasing suspended sediment from 50–2200 mg l^{-1}. Dissolved oxygen concentration within suspended sediment experimental jars stayed between 88 and 99% saturation throughout the four 48 hr repetitions of these experiments. We feel this rules out possible confounding effects by differing dissolved oxygen conditions.

Dissolved Oxygen Experiments

Anova analysis of the hypoxic experiments revealed no significant differences in size ($F_{3,6} = 1.28$, $P = 0.36$, Fig. 3A, albeit the downward trend in means with decreasing oxygen concentration is suggestive), or survival ($F_{3,6} = 0.52$, $P = 0.68$) of larvae among the four treatment levels. Again, none of the two-factor interaction effects were significant. The observed rates of growth (about 8.5% per day, Table 1) and survival (in excess of 95%) for all samples are indicative of healthy larvae.

Anova analysis of hyperoxic experiments showed a highly significant difference in mean larval length after 24 hr in hyperoxic conditions ($F_{1,6} = 36.2$, $P = 0.00095$, Fig. 3B). Survival was not significantly different ($F_{1,6} = 0.086$, $P = 0.78$) from that of the control. We observed differences in the swimming behavior between treatment and control groups. Larvae from both groups appeared to have full, golden-colored stomachs, and most were on the bottom of the petri dish during part of the observation time. Actively swimming larvae from control conditions appeared normal, and the ciliated velum was easily visible. In contrast, fewer larvae were active in the hyperoxic group, and those active were more likely to be slowly rotating over the dish bottom, rather than swimming actively through the water as were the control larvae.

DISCUSSION

Hard clam veligers are apparently able to survive and grow for at least 48 hr in conditions spanning the range of sediment loads measured during two summers in Indian River Bay. Only larvae subjected to 48 hr of the highest experimental sediment load (2200 mg l^{-1}) evidenced any negative effects, and then only showed reduced growth, apparently surviving as well as larvae in control conditions. Laboratory evidence (Robinson 1983) indicates *M. mercenaria* larvae employ selective ingestion or absorption of algae in suspensions of latex particles, contrary to evidence for nonselective ingestion of algae in preference to sediment particles (Loosanoff and Davis 1950). This suggests that reduced growth rate in very high sediment loads could be due to clogging of mucus-covered cilia or disruption of feeding by collision with particles in dense suspensions rather than due to ingestion and processing of potentially less nutritionally-valuable sediments. As noted above, the size of 50 mg l^{-1} treatment larvae exceeded (though not significantly) that of the control (0 mg l^{-1}) and all other treatments (Fig. 2). This suggets that low concentrations of suspended silt- and clay-sized sediments enhance growth in larval *M. mercenaria*, as they do for juvenile and adult *Crassostrea virginica* (Ali 1981; Urban and Langdon 1984). This result is surprising in light of previously reported evidence for no effect of silt on shell growth of juvenile *M. mercenaria* (Bricelj et al. 1984, Table 3).

Reduced growth rates of larvae exposed to very high suspended sediment loads would likely decrease the percent larvae surviving to metamorphosis in the field, by prolonging the vulnerable planktonic period (Carriker 1961). While increased turbidity could reduce predation by visual predators (e.g., planktivorous fish), a prolonged planktonic phase would increase the chance for larval death by non-visual predation or hydrodynamic flushing from appropriate environments. Although laboratory results suggest larval survival of high suspended sediment load was not different from that of larvae in a sediment-free environment, in the field we would expect higher larval mortality over the lengthened larval period in highly turbid waters.

The lack of detrimental effect of hypoxic conditions (over 24 hr) was surprising, especially as the three experimental concentrations (1, 2, 4 mg l^{-1}) were all lower than

TABLE 1.

Mean shell length in micrometers of *Mercenaria mercenaria* larvae from suspended sediment and hypoxia experiments.

	Time after start of experiment, hours							
Experiment	2	4	6	12	24	48	F	n_1,n_2
Suspended Sediments	—	—	—	119.0	121.8	136.3	210	2,6
Hypoxia	117.9	118.4	118.8	—	127.9	—	47.3	3,6

Dash indicates no data available. Both F ratios tabled indicate highly significant differences in mean larval size over time ($P \ll 0.001$). Symbols n_1 and n_2 represent numerator and denominator degrees of freedom (respectively) for the F ratio.

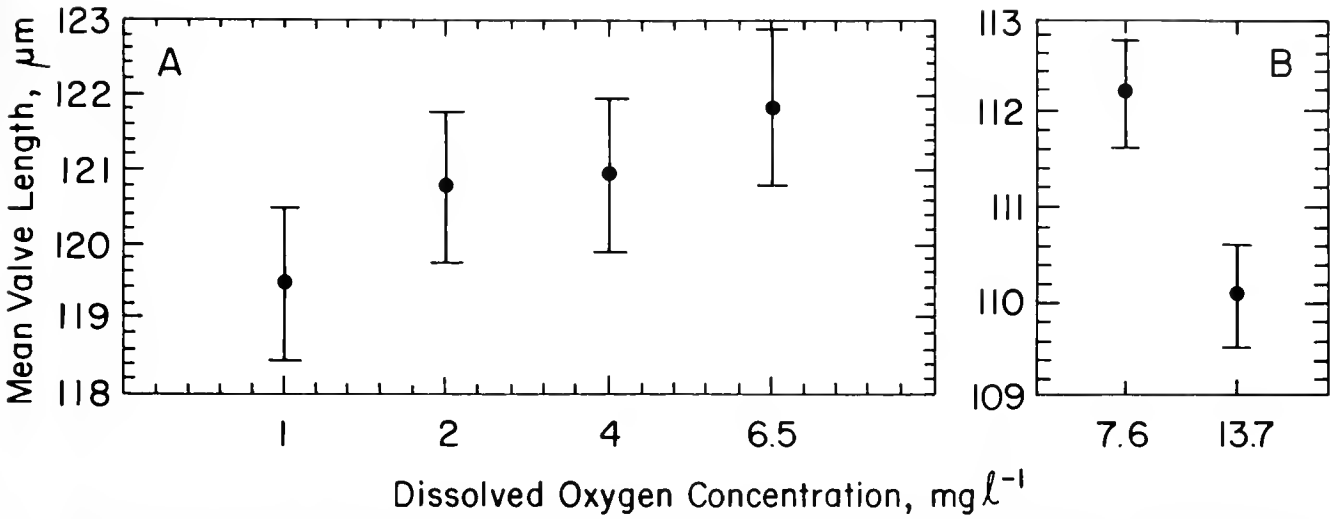


Figure 3. Results of mean larval size in dissolved oxygen experiments depicted as 95% least significant difference plots for pairwise comparisons at $\alpha = 0.05$. Dots represent treatment mean larval length. Error bars are based on the error mean square from the anova analysis. A. Hypoxic treatments: overall anova indicated no significant difference among treatment means. B. Hyperoxic treatments: anova showed treatment means are significantly different at P = 0.00095.

the lower limit Morrison (1971) determined for normal larval growth. Yet, control samples at 6–7 mg l^{-1} did not show significantly better growth or survival by Indian River Bay larvae. We interpret greater survival and growth rates found for Indian River Bay *M. mercenaria* larvae than those Morrison (1971) obtained in hypoxic conditions to be the result of the larval concentration used or geographic variation in larval tolerance (Loosanoff 1962; Walker and Humphrey 1984). Morrison used 15,000 larvae l^{-1} while we chose to use 100 l^{-1} to allow reasonable sample size and to more closely approximate field larval densities. Both concentrations fall within published hatchery or experimental values (Carriker 1961; Robinson 1983), yet rates of survival and growth have been found to be inversely proportional to larval density (Loosanoff et al. 1953). Possibly, greater density reduced growth as food became limiting or waste products built up over the 14 d period of Morrison's (1971) experiments. It is also possible that Long Island Sound *M. mercenaria* stocks are less tolerant of hypoxia than are Indian River Bay *M. mercenaria*.

In our field sampling work in the summer of 1987 (Huntington 1988), we never found dissolved oxygen concentrations below 25% saturation. Pre-dawn dissolved oxygen minima measured were typically about 45% saturation, and persisted for less than four hours before exceeding 90% saturation. Because Indian River Bay veligers successfully survived and grew normally during 24 hr of dissolved oxygen as low as 15% saturation, we expect no influence on hard clam recruitment by hypoxic conditions. In contrast, measured daily afternoon and evening occurrences of supersaturation could decrease hard clam recruitment by increasing the length of the vulnerable larval period (Carriker 1961). We recorded levels of dissolved oxygen as high as 190% saturation, and saturation exceeded that used experimentally on several occasions. Additionally, hyperoxic conditions persisted for almost 12 hr. Although the biochemical mechanism by which hyperoxic conditions cause reduced growth is not known, it could be due to excessive levels of superoxide and other highly-reactive oxygen forms (Fridovich 1977, 1978).

Larvae in Indian River Bay, and in many other eutrophic systems, are exposed to a diurnally-alternating suite of hyperoxic and hypoxic conditions. While 24 hr hypoxic treatment elicited no effect on survival nor growth of veligers, the daily combination of hypoxia and hyperoxia could amplify the reduction of growth rate, hence survival to metamorphosis in field conditions, expected as a result of hyperoxia alone.

In conclusion, growth (as measured by changes in mean larval size over 48 hr), but not survival, of Indian River Bay hard clam veliger larvae was reduced by laboratory exposure to a silt-clay suspension at 2200 mg l^{-1}. We found no significant effect at or below suspended sediment concentrations of 560 mg l^{-1}. Twenty-four hour exposure to hypoxic conditions as low as 1 mg l^{-1} (15% saturation) caused no significant decrease in growth nor survival. Growth rate decreased, however, on exposure to 24 h hyperoxia (13.7 mg l^{-1}, 180% saturation). Daily afternoon supersaturation, common in eutrophic systems such as Indian River Bay, and infrequent episodes of very high loads of suspended sediments can thus negatively impact *M. mercenaria* larval growth.

ACKNOWLEDGMENTS

We thank Mel Carriker, Rick Cole and Jeff Tinsman for help in conducting the experimental and field work. We

also thank Dave and Mug Monte for providing hard clam larvae and broodstock. This work is a result of research sponsored by the State of Delaware Department of Natural Resources and Environmental Control and NOAA Office of Sea Grant, Department of Commerce, under Grant No. NA86AA-D-SG-040 (Project No. SG87 E/G-1). The U.S. Government is authorized to produce and distribute reprints for governmental purposes, notwithstanding any copyright notation that may appear hereon.

REFERENCES CITED

Ali, S. M. 1981. Effects of inorganic particles on growth of the oyster *Crassostrea virginica* (Gmelin). Newark, DE: University of Delaware, College of Marine Studies. 113 pp. M.S. Thesis.

Biggs, R. B. 1978. Coastal Bays. Richard A. Davis, Jr., ed., *Coastal Sedimentary Environments*. NY: Springer-Verlag, pp. 69–96.

Bisker, R. & M. Castagna. 1985. The effect of various levels of air-saturated seawater on *Mercenaria mercenaria* (Linné), *Mulina lateralis* (Say), and *Mya arenaria* Linné, with reference to gas-bubble disease. *J. Shellfish Res.* 5:97–102.

Bricelj, V. M., R. E. Malouf & C. de Quillfeldt. 1984. Growth of juvenile *Mercenaria mercenaria* and the effect of resuspended bottom sediments. *Mar. Biol. (Berl.)* 84:167–173.

Carriker, M. R. 1961. Interrelation of functional morphology, behavior and autecology in early stages of the bivalve *Mercenaria mercenaria*. *J. Elisha Mitchell Sci. Soc.* 77:168–241.

Davis, H.C. 1960. Effects of turbidity-producing materials in sea water on eggs and larvae of the clam (*Venus* (*Mercenaria*) *mercenaria*). *Biol. Bull.* 118:48–54.

Fridovich, I. 1977. Oxygen is toxic! *Bioscience* 27:462–466.

Fridovich, I. 1978. The biology of oxygen radicals. *Science* 201:875–879.

Huntington, K. M. 1988. Influence on suspended sediment and dissolved oxygen concentration on survival and growth of larval *Mercenaria mercenaria* (Linné) (Mollusca: Bivalvia). Newark, DE: University of Delaware, College of Marine Studies. 96 pp. M.S. Thesis.

Lossanoff, V. L. 1962. Effects of turbidity on some larval and adult bivalves. *Gulf Carrib. Fish. Inst.* 14:80–95.

Loosanoff, V. L. & H. C. Davis. 1950. Conditioning *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. *Biol. Bull.* 98:60–65.

Loosanoff, V. L., H. C. Davis & P. E. Chanley. 1953. Effect of overcrowding on rate of growth of clam larvae. *Anat. Rec.* 117:645–646.

Morrison, G. 1971. Dissolved oxygen requirements of embryonic and larval department of the hardshell clam, *Mercenaria mercenaria*. *J. Fish. Res. Bd. Canada* 28:379–381.

Parsons, T. R., Y. Maita & C. M. Lalli. 1984. *A Manual of chemical and biological methods for seawater analysis*. Oxford: Pergamon Press. 173 pp.

Robinson, W. E. 1983. Quantification of ingestion by *Mercenaria mercenaria* (L.) veligers feeding on mixed suspensions of inert material and algae using microspectrofluorometry. *J. Moll. Stud. Suppt.* 12A:167–171.

Urban, E. R., Jr. & C. J. Langdon. 1984. Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. *Aquaculture* 38:277–291.

Walker, R. L. & C. M. Humphrey. 1984. Growth and survival of the northern hard clam *Mercenaria mercenaria* (Linné) from Georgia, Virginia, and Massachusetts in coastal waters of Georgia. *J. Shellfish Res.* 4:125–129.

Zar, J. H. 1984. *Biostatistical Analysis*. Second edition. Englewood Cliffs, NJ: Prentice-Hall. 718 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 43–49, 1989.

THE REPRODUCTIVE CYCLE OF ADULT HARD CLAMS, *MERCENARIA* SPP. IN THE INDIAN RIVER LAGOON, FLORIDA

DONALD M. HESSELMAN[1,*], BRUCE J. BARBER[2], AND NORMAN J. BLAKE[1]

[1]*Department of Marine Science*
University of South Florida
St. Petersburg, Florida 33701

[2]*College of William and Mary*
Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

ABSTRACT The reproductive cycle of hard clams, *Mercenaria* spp. in the Indian River, Florida was investigated over a 16 month period from May 1985 to August 1986. Gametogenesis was assessed by visual observation of histological sections, mean oocyte diameters (obtained from the histological sections), and mean visceral mass index. Gametogenesis was initiated in late summer or early fall and continued throughout the fall and winter. Spawning of gametes was bimodal, with peak periods occurring from September–December and March–June. The summer months of August and September were characterized by a large number of individuals in a resting/spent phase, apparently the result of temperatures in excess of 30°C. Besides having a more prolonged, bimodal spawning period, gametogenesis in clams from the Indian River was less synchronous than in clam populations from more northerly locations. Thus latitudinal trends established between New York and South Carolina were continued to Florida, the southern distributional limit of *M. mercenaria*.

KEY WORDS: hard clam, reproduction, gametogenesis, spawning, Florida

INTRODUCTION

Temperate marine bivalves exhibit considerable intraspecific variability in reproductive cycles which is attributable to phenotypic adaption to environmental variability, genetic divergence, or some combination of both. Local and regional differences in reproductive ecology have been reported for populations of *Mytilus edulis* (Newell et al. 1982), *Argopecten irradians* (Barber and Blake 1983; Bricelj et al. 1987), and *Placopecten magellanicus* (MacDonald and Thompson 1986).

The hard shell calm or quahog, *Mercenaria mercenaria* Linne, a common nearshore inhabitant along the east coast of the United States, exhibits latitudinal variability in the timing of gametogenic events. In Long Island Sound, New York, spawning occurs primarily between August and September (Loosanoff 1937). In Delaware Bay, spawning begins earlier (June) and continues until October, with a peak in August and September (Keck et al. 1975). *M. mercenaria* from Core Sound, North Carolina, also spawn between June and October, with peaks occurring in June and September (Porter 1964). In Clark Sound, South Carolina, the period of spawning begins even earlier (May), with a second peak in October (Eversole et al. 1980; Manzi et al. 1985). In Wassaw Sound, Georgia, Heffernan et al. (1988) found that hard clams from this region exhibit a continuous gametogenic cycle with spring, fall and winter spawning peaks. Thus with decreasing latitude, clam reproduction occurs earlier in the year, and bimodal or polymodal spawning is evident in the protracted spawning periods of the southern populations. This suggests that gametogenesis and spawning occur within an optimal range of water temperature which occurs at different times of the year at different latitudes.

The Indian River lagoon, on the east central coast of Florida, supports an abundant hard clam population which currently is sustaining an intensive fishery. This region is unique because it is near the southern distributional limit of the northern quahog, *M. mercenaria*, along the east coast of the United States (Abbott 1974). The southern quahog, *M. campechiensis* (Gmelin), is also found within the estuary and readily hybridizes with *M. mercenaria* (Dillon and Manzi 1988). Although the reproduction of *M. mercenaria* has been extensively studied, little information exists on the reproduction of adult *M. campechiensis* and no information is available on hard clam populations as far south as the Indian River.

MATERIALS AND METHODS

The Indian River extends approximately 195 km from Oak Hill south to St. Lucie Inlet on the east coast of Florida (Fig. 1). The lagoon is bordered on the east by a series of

*Current address and author to whom all correspondence should be sent: Florida Department of Natural Resources, P.O. Box 1506, Punta Gorda, FL 33950.

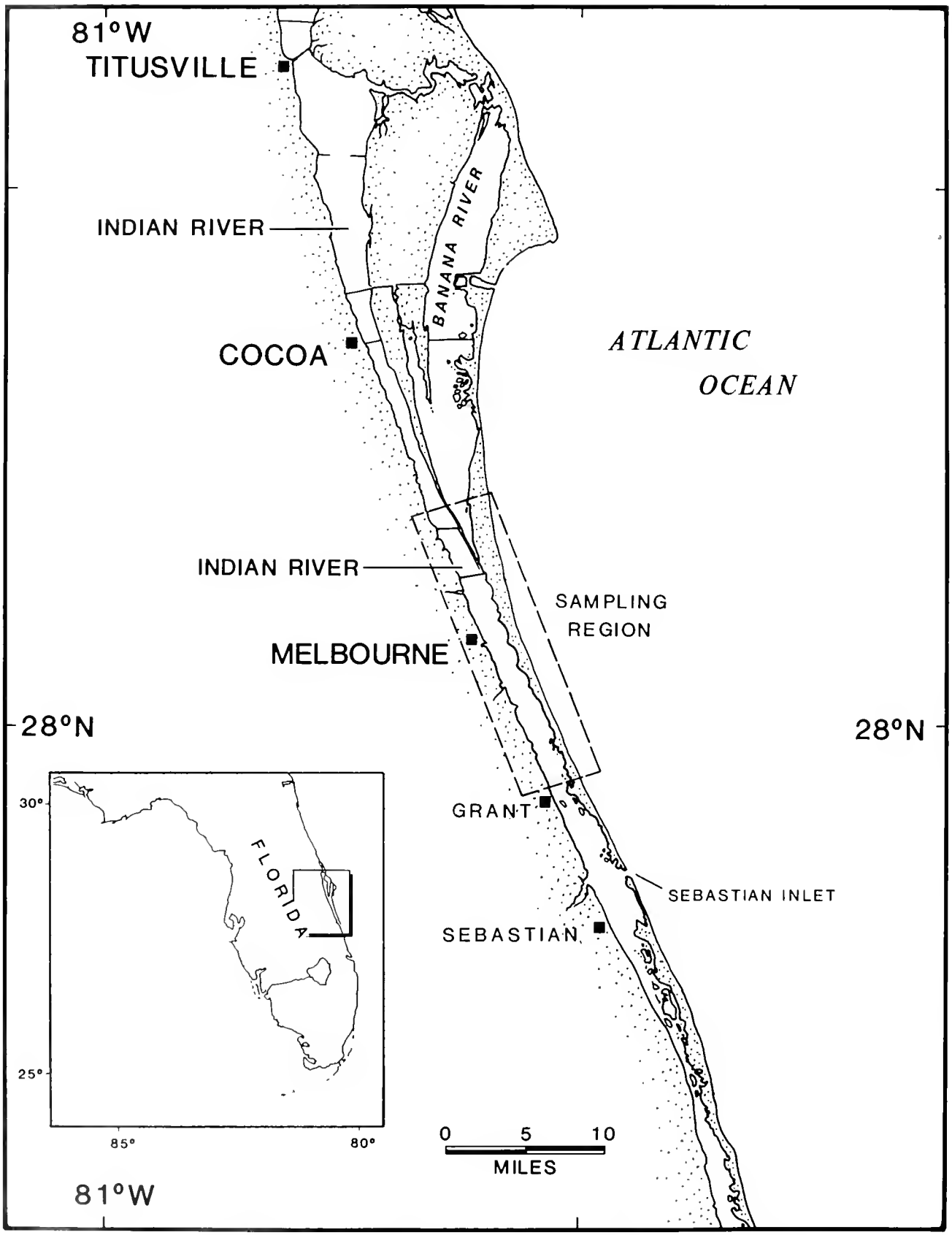


Figure 1. Map of the Indian River lagoon, Florida, showing the location of the sampling region.

barrier islands through which pass five natural and man-made inlets from the Atlantic Ocean. Depth within the lagoon ranges from 0–4 m and averages 1.5 m (White 1986). Sediment is composed of varying percentages of sand, shell, and mud. Water temperatures ranged from 14.5 to 30.5°C (Table 1). Clams inhabiting shallow grass flats (*Thallasia testudinum, Syringodium filiforme,* and *Halodule wrightii*), however, experience a greater range, as temperatures in the summer can exceed 30°C for extended periods. Salinity ranged from 15 to 32.9 ppt and is generally determined by the rainfall-evaporation cycle, but varies according to the proximity of oceanic inlets and sources of freshwater discharge (Barile and Rathjen 1986).

Thirty-two to 96 hard clams were collected at approximately monthly intervals (from May 1985 to August 1986) from a 24 km section of the estuary (Fig. 1). Clams of all sizes were obtained from clammers, or directly using SCUBA. Upon return to the laboratory, the shell height of each individual was measured to the nearest 0.1 mm. The soft tissue was removed, blotted, and weighed to the nearest 0.1 g. The gill, mantle, adductor muscles and foot were then removed, and the wet weight of the visceral mass (containing the gonad) determined. A visceral mass index was calculated from the ratio of visceral mass to total mass. Mean monthly values of the visceral mass index were obtained for a standard size (55.8 mm, average shell height of all clams sampled) clam by regressing $\log_{10}$ transformed shell height on $\log_{10}$ transformed visceral mass index for each sample (Table 2). This was done to ensure that variation in reproductive output as a function of body size did not skew the index.

The visceral mass was placed in Helly's fixative (Luna 1968) for 2–3 hr, removed, cut in 5–7 mm thick sections, and returned to the fixative for another 20–24 hr. Following fixation, the sections were rinsed in tap water for 20 hr, dehydrated, cleared, and embedded in paraplast. Thin sections (7 μm) were stained with Harris's hematoxylin and eosin (Yevich and Barszcz 1977).

Gametogenesis was monitored by visual staging of the thin sections using a modification of the descriptions provided by Loosanoff (1937), Porter (1964), Keck et al. (1975) and Eversole et al. (1980).

Resting/Spent Stage

A complete or almost complete lack of gametes, shrunken follicles and an increase in vesicular connective tissue; residual ova and sperm sometimes seen being phagocytized in the follicles and ducts.

Early Developmental Stage

An increase in follicle wall thickness and early immature gametes proliferating; some oocytes are seen adhering to the follicle walls by a peduncle; lumen of male follicles filled with a loosely arranged mass of spermatocytes; vesicular connective tissue still dominates the interfollicular region of the gonad.

TABLE 1.

Sampled water temperature and salinity from the Indian River lagoon, May 1985 to August 1986.

Sampling Date	Temperature (C)	Salinity (ppt)
5/8/85	26.2	30.0
6/27/85	28.3	32.9
7/31/85	30.2	26.0
9/4/85	29.0	25.8
10/25/85	26.2	21.0
11/22/85	25.0	16.0
12/30/85	14.5	15.0
1/24/86	17.0	18.0
2/38/86	18.5	16.0
3/26/86	20.0	18.2
4/29/86	25.0	16.7
5/29/86	28.0	25.0
7/11/86	30.5	26.5
8/23/86	30.5	24.5

Late Developmental Stage

Follicles rapidly expanding to accommodate the larger and more numerous gametes; in females many oocytes are free in the lumen of the follicles, although the majority remain attached to the follicle wall; a wide range of oocyte sizes results; in males spermatocytes and spermatids are seen in follicles, with about 50% of the follicles containing spermatozoa.

Ripe Stage

Follicles fully expanded and follicle walls thin; lumen of female follicles contain mature ova; mature sperm dominate the lumen of male follicles; germinal ducts have begun to expand and may contain a few mature gametes.

Spawning Stage

Germinal ducts are fully expanded and contain varying numbers of mature gametes; follicles contain fewer mature gametes, although immature gametes are still present (development of gametes proceeds through the initial spawning stage).

Late Spawning Stage

Follicles have begun to contract and are almost empty; some ova and sperm remain in the follicles and germinal ducts; amoebocytes phagocytizing residual gametes.

The gametogenic cycle was also monitored by measuring the maximum diameter of 50 oocytes from up to 10 females from each sample (Barber and Blake 1981; 1983) using an image analysis system (Southern Micro Instruments). Only those oocytes roughly spherical in shape and

TABLE 2.

Size composition of the Indian River hard clam samples, standardized values of the visceral index (VI) and regression variables for obtaining the standardized visceral index. SH = shell height.

Sampling Date	n	Mean Shell Height	Shell Height (Range)	Standardized VI	logVI = a + b*logSH	
					y-intercept	slope
5/8/85	96	52.3	30.0–89.0	0.35	−1.3184	0.4921
6/27/85	48	53.3	31.2–88.1	0.35	−1.2582	0.4602
7/31/85	48	54.4	33.8–90.5	0.39	−0.4374	0.0179
9/4/85	48	54.5	34.1–87.1	0.34	−0.9217	0.2591
10/25/85	36	48.4	35.8–64.6	0.41	−0.6343	0.1408
11/22/85	45	55.7	34.0–96.0	0.40	−0.4263	0.0171
12/30/85	48	57.0	26.7–105.2	0.38	−0.4228	−0.0017
1/24/86	48	56.2	35.5–85.0	0.40	−0.3183	−0.0469
2/28/86	32	64.1	38.5–95.0	0.44	−0.3319	−0.0163
3/26/86	47	59.1	44.7–103.7	0.45	−0.5145	0.0932
4/29/86	48	59.0	34.3–89.8	0.43	−0.3618	−0.0020
5/29/86	48	56.2	35.7–93.1	0.35	0.0159	−0.2715
7/11/86	34	53.5	35.9–81.3	0.37	−1.0954	0.3769
8/23/86	48	57.6	32.5–91.0	0.32	−0.6066	0.0665
Total sample size = 674						
Mean shell height (SH) = 55.8						

showing a nucleus were measured. Mean oocyte diameters were calculated for each date.

RESULTS

Because of the apparent hybridization of *M. mercenaria* and *M. campechiensis*, clams from the Indian River were rarely identifiable to species based on shell morphology (Abbott 1974). Even when differentiation was possible, no differences in reproductive activity were seen. Therefore, all clams were considered to be from the same population.

A total of 674 clams (344 females and 330 males) were examined. The ratio of males to females did not differ significantly from unity ($P < 0.05$, Chi-square). Of the total, 545 were deemed suitable for assessment of gametogenic development. The remainder were afflicted with either gonadal neoplasms (Hesselman et al. 1988) or parasitic trematodes, and were not considered. Clams ranged in size from 26.7 to 105.2 mm in shell height, and were reproductively mature within this range.

Based on visual staging of histological sections, the primary period of male gamete development occurred in June (24%) and July (67%), 1985 and January (86%), 1986 (Fig. 2A). However, in July 1986, all male clams were either spawning or nearing completion of spawning which indicated that initiation of spermatogenesis was delayed in comparison to 1985. Male clams emitted sperm throughout most of the year, with peak periods of spawning (>45% of the sample) from September to December 1985 and February through July 1986. In March 1986 all male clams were actively spawning. The largest percentage of resting/spent males (55%) was observed in August 1986.

Gamete development of female clams followed a similar trend to that of males (Fig. 2B). Oogenesis began in June and July 1985 and continued into February 1986. Development proceeded rapidly throughout the fall of 1985, with numerous oocytes undergoing vitellogenesis and reaching maturity by November when the majority of female clams (88%) were in a spawning or late spawn stage. An additional 45% of the female clams were in the spawning or late spawn stage in December. During the coldest month of January, very little spawning activity was noted. Instead, gametes were undergoing maturation prior to the spring 1986 spawn. Because the fall spawn did not result in total evacuation of the gonads, those oocytes which had not reached maturity in the fall and subsequently not eliminated, appeared to be maintained until the spring. This observation is based on the absence of phagocytosis of the oocytes and on the increasing values of both mean oocyte diameter and mean visceral mass index throughout the winter. The highest proportion of ripe females (29%) occurred in February 1986. The primary spawning period began a month later than male clams and extended from March until July 1986. The largest percentage of resting/spent females was seen in September 1985 (38%) and August 1986 (88%). Analogous to male clams, oogenesis was delayed in 1986 when compared to 1985 and apparently was not initiated until the fall.

The visceral mass index (both male and female clams) substantiated in quantitative terms what was observed histologically (Table 2). The index was maximum in February (0.44), March (0.45), and April (0.43) 1986, with peaks also occurring in July (0.39), October (0.41) and November (0.40), 1985. Minimum values were seen in September 1985 (0.34) and August (0.32) 1986, corresponding

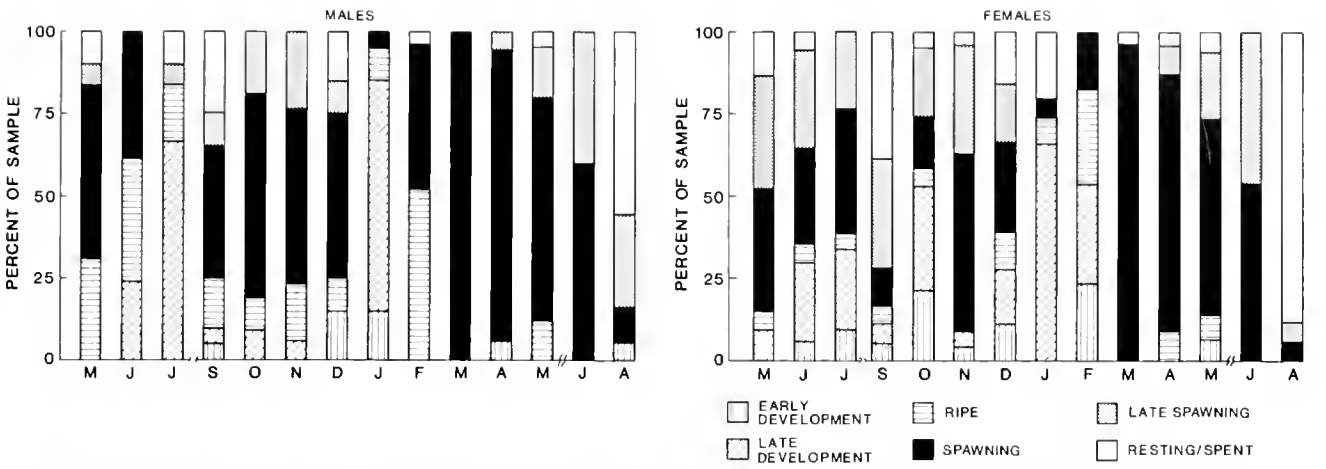


Figure 2. Stages of hard clam gonad development derived from the visual staging of the thin sections, May 1985 to August 1986.

to times at which the greatest proportion of clams in a resting/spent stage were found.

Mean oocyte diameters were reflective of the oogenic cycle (Fig. 3) as described by visual examination of female clams. Maximum diameters (>40 μm) were observed in May 1985 and from February to July 1986 in conjunction with the period of maturation and spawning. Minimum mean diameters, corresponding to resting and early developmental stages, occurred in the late summer and fall. The large percentage of females in a late spawn or resting/spent phase throughout the summer of 1986 prevented oocyte measurements to be obtained from the required 10 females. Individual oocyte diameters ranged from 10.7 μm to 97.7 μm. Although a wide range of oocyte sizes was observed in the germinal ducts, it appeared that ova were mature and ready to spawn at a minimum size of approximately 50 μm. No monthly mean exceeded 45 μm, although some individuals had mean diameters approaching 50 μm. Variation in oocyte diameter was thus the result of variability both between individuals and within individuals.

DISCUSSION

Hard clams from the Indian River, Florida, exhibit a reproductive cycle that differs from those of more northern clam populations examined to date. Spawning is essentially continuous, as clams in a spawning state are found in all months for females and all but one month for males. However, there is a distinct bimodal pattern to spawning activity, with a peak occurring in the spring (February–June) and a lesser peak in the fall (September–December). The largest proportion of resting/spent clams is found between spawning peaks, primarily in late summer. Thus, with decreasing latitude along the eastern coast of the United States, *Mercenaria* spp. has a more protracted period of spawning that becomes distinctly bimodal in Florida. This trend towards bimodal spawning in southern latitudes also has been described in other marine invertebrate species (Giese 1959; Ropes and Stickney 1965).

Temperature has been cited as the major environmental factor regulating reproduction in marine bivalves (Sastry 1979). In general, gametogenesis is initiated and spawning occurs only within fairly narrow, species-specific temperature ranges (Orton 1920; Nelson 1928; Loosanoff and Davis 1952). Thus, differences in the timing of gametogenesis and spawning within a species over a latitudinal range occur because critical temperatures are attained at different times. In *M. mercenaria* spawning is induced at temperatures of 20–25°C throughout its range (see Keck et al. 1975; Manzi et al. 1985 for reviews). In northern locations this temperature is attained only once, and for a fairly short period of time, resulting in a limited period of spawning activity. With decreasing latitude, spawning period becomes more protracted as the time over which the critical temperature occurs is increased. In the Indian River population, the spring spawning period commenced in February as the water temperature exceeded 18.5°C and continued until June when the temperature reached 28.3°C. The fall spawning period (September–December) corresponded to water temperature of 29.0–14.5°C. The December water temperature was due in part to a passing cold front and is considerably below the norm (~20°C) for this time of year. It may be that the clams were induced to continue spawning by the rapid decrease in temperature or alternatively, that spawning had been halted but the gonads retained the appearance of a spawning clam. However, Heffernan et al. (1988) also noted hard clams spawning in Georgia when water temperature was about 15°C.

The late summer period of reproductive inactivity occurred in August and September when water temperature averaged 30°C or greater. Temperature in excess of 30°C is considerably outside the optimal temperature range of 20–28°C and may induce physiological stress which would

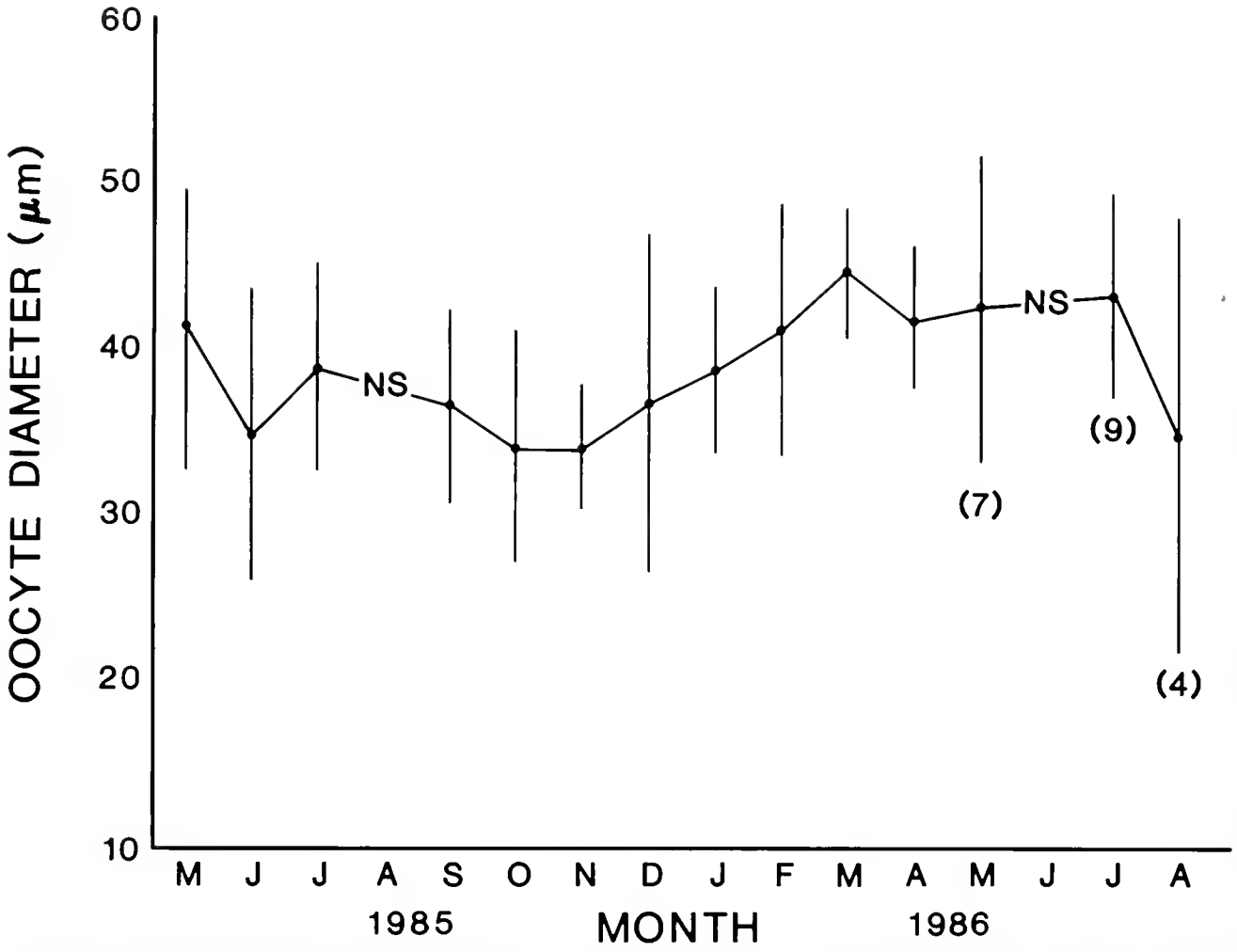


Figure 3. Oocyte diameters of female hard clams, May 1985 to August 1986. ($\bar{x}$ ± 1 s.d.). Numbers in parentheses indicate the number of females analyzed if less than ten.

tend to inhibit gametogenesis. Moreover, the incidence of gonadal neoplasia increased during periods of high water temperature (Hesselman et al. 1988), possibly indicative of stressful environmental conditions.

Even though trends were evident, reproductive activity was quite variable both between and within samples. Variability between samples may have resulted in part from local environmental differences between sites sampled in this study. Such site-specific differences in hard clam reproduction have been noted previously (Ansell et al. 1964; Keck et al. 1975) and may be the result of local differences in temperature, food availability, salinity, or other factors regulating gametogenesis. Variability within samples, which produced large standard deviations in mean visceral mass indices and oocyte diameters in this study, may be even more pronounced in the Florida population since environmental cues are less variable seasonally at this latitude.

Another possible source of variation in this study is the face that *M. mercenaria*, *M. campechiensis*, and their hybrids were sampled simultaneously. No information exists on the reproductive cycle of adult *M. campechiensis*. However, laboratory-spawned, juvenile *M. campechiensis* were found to have a bimodal spawning period in Alligator Harbor, Florida, that was no different from that of similarly reared *M. mercenaria* and *M. mercenaria* × *M. campechiensis* reciprocal hybrids (Dalton and Menzel 1983). No differentiation of the two species or their hybrids was made in this study, so the extent to which genetic differences contributed to reproductive variability is unknown.

This study provides further evidence that hard clam populations along the east coast of the United States show latitudinal variability in the timing of gametogenesis and spawning. The temporal extension of the spawning period of hard clams in southern regions is indicative of a shift toward year-round spawning in the tropics (Giese and Pearse 1974). The trend toward bimodal spawning peaks

may be an adaptation to optimal temperature ranges for reproduction which occur twice a year at southern latitudes. The lack of synchrony in the timing of gametogenic events is considered to be the result of decreased sensitivity to external stimuli (Orton 1920). Outside the range of suitable environmental conditions for gametogenesis and spawning, reproductive success, which ultimately determines the geographical range of a species, is minimized. The large percentage of resting/spent individuals and those having gonadal neoplasia during the months of maximum water temperature are suggestive of some of the ultimate mechanisms by which *M. mercenaria* is limited to further southerly dispersal.

ACKNOWLEDGMENTS

We would like to express thanks to all those who helped in field collections and other aspects of the study, particuarly R. Erdman, M. Moyer, R. Hochberg and A&J Seafood of Melbourne, Florida. We would also like to express our gratitude to Dr. P. Hallock-Muller for use of the image analyzer which was purchased with funds from NSF Grant #BSR-8507343. Thanks are also due to G. Shuert for typing the manuscript and B. Arnold who provided valuable comments on the manuscript. This work was supported by funds from the Gulf and South Atlantic Fisheries Development Foundation, Inc., grant #27-05-31895/18706.

REFERENCES CITED

Abbott, R. T. 1974. *American Seashells*. Van Nostrand and Reinhold Co., New York. 663 pp.

Ansell, A. D., K. F. Lander, J. Coughlan & F. A. Loosmore. 1964. Studies on the hard-shell clam, *Venus mercenaria*, in British Waters. *J. Appl. Ecol.* 1:63–82.

Barber, B. J. & N. J. Blake. 1981. Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamarck) at its southern distributional limit. *J. Exp. Mar. Biol. Ecol.* 52:121–134.

Barber, B. J. & N. J. Blake. 1983. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *J. Exp. Mar. Biol. Ecol.* 66:247–256.

Barile, D. D. & W. Rathjen. Report on Rainfall Event of September and October 1985 and the impact of storm discharge on salinity and the clam population (*Mercenaria mercenaria*) in the Indian River Lagoon. Marine Resources Council, Florida Institute of Technology. November, 1986.

Bricelj, V. M., J. Epp & R. E. Malouf. 1987. Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten irradians*. *Mar. Ecol. Prog. Ser.* 36:123–137.

Dalton, R. & W. Menzel. 1983. Seasonal gonadal development of young laboratory-spawned southern (*Mercenaria campechiensis*) and northern (*Mercenaria mercenaria*) quahogs and their reciprocal hybrids in northwest Florida. *J. Shellfish Res.* 3:11–17.

Dillon, R. T. & J. J. Manzi. 1988. The genetics and shell morphology in a hybrid zone between the hard clams *Mercenaria mercenaria* and *Mercenaria campechiensis*. *Mar. Biol.* 100:217–222.

Eversole, A. G., W. K. Michener & P. J. Eldridge. 1980. Reproductive cycle of *Mercenaria mercenaria* in a South Carolina estuary. *Proc. Nat'l Shellf. Assoc.* 70:22–30.

Giese, A. C. & J. S. Pearse. 1974. Reproduction of Marine Invertebrates. Vol. 1, Acoelomate and Pseudocoelomate Metazoans. Academic Press, New York, pp. 6–49.

Giese, A. C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. *Ann. Rev. Physiol.* 21:547–576.

Heffernan, P. B., R. L. Walker & J. L. Carr. 1988. The reproductive cycle of the hard clam, *Mercenaria mercenaria*, in Wassaw Sound, Georgia. *J. Shellfish Res.* 8:51–61.

Hesselman, D. M., N. J. Blake & E. C. Peters. 1988. Gonadal neoplasms in the hard shell clam, *Mercenaria* spp., from the Indian River, Florida: Occurrence, prevalence and histopathology. *J. Invertebr. Pathol.* 52:436–446.

Keck, R. T., D. Maurer & H. Lind. 1975. A comparative study of the hard clam gonad development cycle. *Biol. Bull.* 148:243–258.

Loosanoff, V. L. 1937. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.). *Biol. Bull.* 72(3), 406–416.

Loosanoff, V. L. & H. C. Davis. 1952. Temperature requirements for maturation of gonads of northern oysters. *Biol. Bull.* 103:80–96.

Luna, L. G. 1968. "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology," 3rd edition, McGraw-Hill, New York. 258 pp.

MacDonald, B. A. & R. J. Thompson. 1986. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus* III. Physiological ecology, the gametogenic cycle and scope for growth. *Mar. Biol.* 93:37–48.

Manzi, J. J., M. Y. Bobo & V. G. Burrell. 1985. Gametogenesis in a population of the hard clam, *Mercenaria mercenaria* (L.), in North Santee Bay, South Carolina. *Veliger* 28(2):186–194.

Nelson, T. C. 1928. Relation of spawning of the oyster to temperature. *Ecology* 9:145–154.

Newell, R. I. E., T. J. Hilbish, R. K. Koehn & C. J. Newell. 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (Bivalvia, Mytilidae) from localities on the east coast of the United States. *Biol. Bull.* 162:299–310.

Orton, J. H. 1920. Sea temperature, breeding and distribution in marine animals. *J. Mar. Biol. Assoc. U.K.* 12:339–366.

Porter, H. J. 1964. Seasonal gonadal changes of adult clams, *Mercenaria mercenaria*, in North Carolina. Proc. Nat'l Shellf. Assoc. 55:35–52.

Ropes, J. W. & A. P. Stickney. 1965. Reproductive cycle of *Mya arenaria* in New England. *Biol. Bull.* 128:315–327.

Sastry, A. N. 1979. Pelecypoda (excluding Osteidae). *In:* Reproduction of Marine Invertebrates, Vol. 4, A. C. Giese and J. S. Pearse (eds.), Academic Press, New York. pp. 113–292.

White, C. 1986. Biological and environmental factors affecting the clamming industry. *In:* An Overview of the Indian River Clamming Industry and the Indian River Lagoon. Florida Sea Grant Extension Program, Technical Paper #44, University of Florida, Gainesville, pp. 45.

Yevich, P. P. & C. A. Barzszcz. 1977. Preparation of Aquatic Animals for Histopathological Examination. U.S. Environmental Protection Agency, Narragansett, R. I. 20 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 51–60, 1989.

GAMETOGENIC CYCLES OF THREE BIVALVES IN WASSAW SOUND, GEORGIA: I. *MERCENARIA MERCENARIA* (LINNAEUS, 1758)

PETER B. HEFFERNAN, RANDAL L. WALKER AND JOHN L. CARR
University of Georgia
Marine Extension Service Shellfish Laboratory
Skidaway Island
P.O. Box 13687
Savannah, Georgia 31416-0687

ABSTRACT The gametogenic cycle of a *Mercenaria mercenaria* (L.) population from Wassaw Sound, Georgia was analyzed from December 1983 to July 1986. Qualitative and quantitative data were compiled monthly from histological preparations, and used in the assessment of reproductive condition. Qualitative data were compiled on staging criteria and gonad indices. Quantitative data were gathered using an image analysis system on gonad area, egg area, egg number, and estimated egg diameter. Continuous gametogenic activity was indicated throughout the study period. Sex ratios were 1:1. A synchronized polymodal breeding pattern was evident, with two or three annual spawning peaks (spring and fall 1984, spring, fall, and winter 1985). Temporal differences in reproductive output were detected, with 1985 levels apparently greater than those of 1984. The benefits of image analysis technology in facilitating a greater appreciation of the gametogenic cycle are discussed.

KEY WORDS: reproductive cycle, gametogenesis, hard clam, *Mercenaria mercenaria*, image analysis

INTRODUCTION

Hard clam, *Mercenaria mercenaria* (L.), landings constituted the second largest molluscan fishery in recent years in Georgia (Dr. S. Stevens, Georgia Department of Natural Resources, personal communication). Together with a growing natural fishery, there has been considerable attention paid of late to the development of a clam mariculture industry in the coastal waters of Georgia (Walker 1983, 1985; Walker and Tenore 1984). At present there are a limited number of clam mariculture operations in production in Georgia.

This study was undertaken to provide information pertaining to the reproductive cycle of native hard clams and to contribute to the further development of their natural and mariculture based fisheries (e.g., Walker et al. 1988). With these data, management guidelines can be established to help maximize annual recruitment into local populations, with a view to improving resource replenishment. By elucidating periods of peak sexual maturity in a local population, we will enable future mariculturists to avail of a possible source of pre-conditioned broodstocks, thus reducing hatchery costs and increasing profits.

Much attention has been paid to the reproductive biology of hard clams along the eastern United States (Loosanoff 1937a, b; Porter 1964; Keck et al. 1975; Eversole et al. 1980; Manzi et al. 1985) and a changing reproductive pattern has been illustrated with decreases in latitude. Eversole et al. (1980) noted that a prolonged breeding season with a synchronized polymodal breeding pattern became more evident the further south the study population was situated. Considering this overlying trend and the differences in latitude, climate and coastal ecology between Georgia and the other areas studied to date, this study was undertaken to elucidate aspects of the gametogenic cycle of Georgia hard clams and to compare the results with those from other areas. Considerable attention has been focused of late on the inconsistency of staging criteria in bivalve reproduction studies (Shaw 1988). Furthermore, in the absence of quantitative data on gamete production, comparative studies are severely restricted (Heffernan et al. 1988; Choi et al. 1988). This prompted the current investigators to employ quantitative techniques (image analysis) as well as standard staging criteria in the current study.

MATERIALS AND METHODS

Sampling and Tissue Processing

Twenty hard clams were collected on a monthly basis (December 1983–July 1986) from a shallow sheltered creek, House Creek, Little Tybee Island, on the northern end of Wassaw Sound, Georgia. Shell length measurements (i.e., largest diameter anterior-posterior) using vernier calipers were taken for all specimens prior to processing for histology. The visceral mass was preserved in a modified formol-alcohol fixative. Prior to histological processing, a standard mid-lateral gonadal tissue sample (*ca.* 1 cm^2) was dissected from each specimen. This tissue portion included elements of the outer epithelial, connective tissue, and gonadal layers and terminated in the digestive area. Tissue samples were dehydrated in an alcohol series, cleared in toluene, and embedded in paraplast. Sections were cut 7–10 μm in thickness using a rotary microtome. Sections were stained with Ehlrichs haematoxylin and counter-stained with Eosin (Bancroft and Stevens 1977).

Qualitative Reproductive Analysis

After a preliminary examination of a wide range of specimens, a 6-stage reproductive staging criteria was adapted (Fig. 1). A random field of gonadal tissue was examined from every specimen and the individual was ascribed to one stage. The staging criteria followed that of Eversole et al. (1980) except for the inclusion of male ripe and female ripe stages.

Males were ascribed to one of three stages: Male Active: Male Ripe and Spawning: Both as described by Eversole et al. (1980); Male Ripe: Ripe males exhibited follicles with densely packed bands of spermatozoa, with little or no empty lumen area. Spermatozoa constituted the majority spermatogenic stage and were encircled by basophilic spermatids and spermatocytes, which closely opposed the narrowed follicle walls. Female Active: Female Ripe and Spawning: Both as described by Eversole et al. (1980); Female Ripe: Densely packed oocytes occupied the majority of the follicle. Most oocytes were free in the follicle lumen and there was little or no empty space within the follicle.

A monthly gonad index (G.I.) (after Kennedy 1977) was computed for the sample. A scoring system with Undifferentiated = 0; and Male or Female Active = 2; Male or Female Ripe = 3; and Male or Female Ripe and Spawning = 1 was adapted. The monthly G.I. for both sexes was determined by multiplying the number of specimens ascribed to each category by the category score, summing all such values and dividing this figure by the total number of males or females analyzed (Fig. 2).

Quantitative Reproductive Analysis

Quantitative analysis of gonad preparations was carried out using on Omnicon image analyzer, housed at the Human and Behavioral Genetics Research Laboratory of Emory University, Atlanta, Georgia. Photomicroscopic images (10 × : 1.25 optivar) were converted to a video signal (field area = 0.639 mm^2). These analog video signals were converted to a binary format using upper and lower grey-level thresholds set by the operator. The image analyzer was capable of carrying out detailed area measurements and statistical analyses on features detected within the grey level thresholds (operator controlled). Four fields per specimen were analyzed to ensure detection of within-specimen variations in gametogenic development.

Females were analyzed for percent gonad, percent of gonad area occupied by oocytes, oocyte number per field and mean oocyte diameter. An operator controlled light pen was used to edit non-gonadal tissue (e.g. intestines and blood vessels) in the evaluation of percent gonad per field. Egg number was manually counted from the omnicon screen and used in the computation of mean oocyte diameter.

Males were analyzed for percent gonad, percent of gonad occupied by spermatogenic stages and for the percent of spermatogenic stages consisting of spermatozoa.

Mean individual values were computed for each data category analyzed by the image analyzer. Mean monthly values were then computed and used in the quantitative assessments of reproduction. Sex ratios were tested against

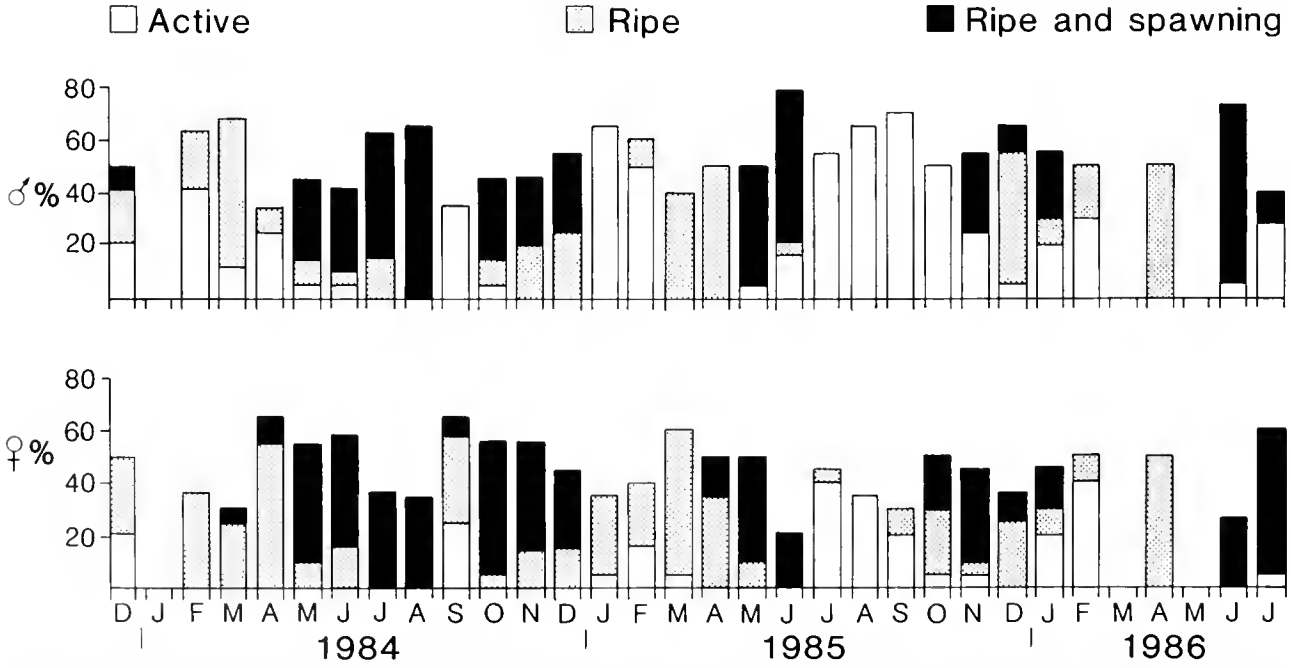


Figure 1. Qualitative data illustrating the sex and developmental stages of hard clams from Wassaw Sound, Georgia (December 1983–July 1986). The length of each shaded area represents the percentage frequency of clams in each developmental stage.

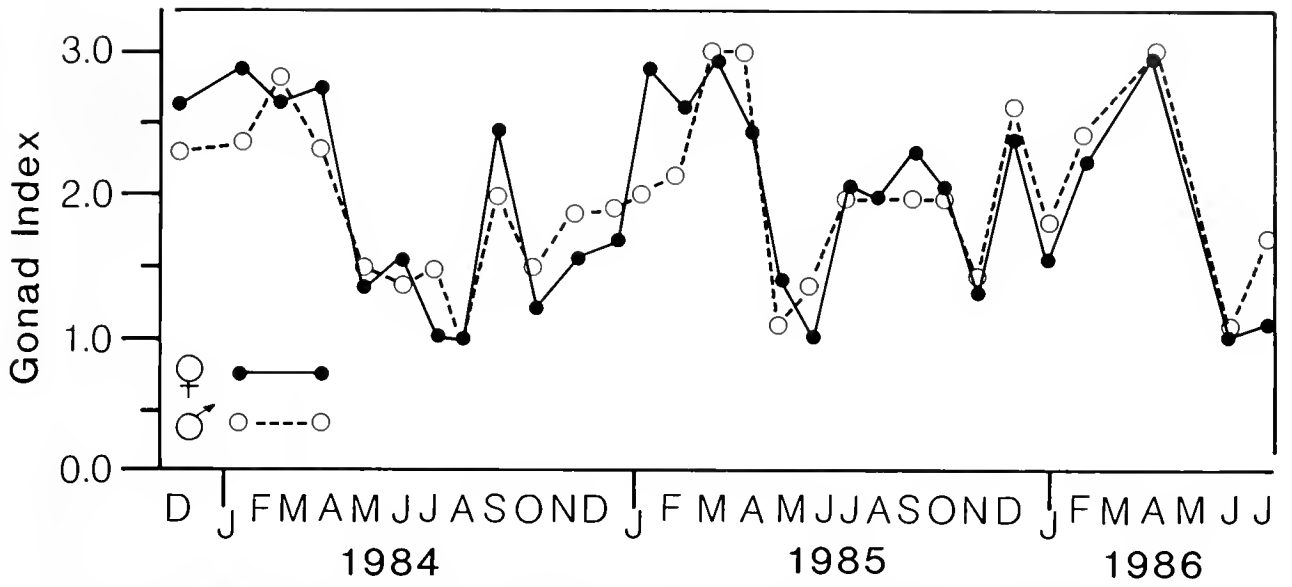


Figure 2. Monthly gonad index values for Wassaw Sound, Georgia hard clams from December 1983 to July 1986.

1:1 ratio with chi-square tests (Steel and Torrie 1960). Statistical analysis (ANOVA and Student t-test, Sokal and Rohlf 1981) was applied to the various quantitative data sets (mean value points) in order to validate (or reject) conclusions drawn from the general patterns observed. Individual percentage data points were arcsine transformed (Zar 1974) prior to statistical analysis. In order to evaluate the expected underestimation of oocyte diameter portrayed by the method of analysis used on the image analyzer (i.e., using all oocytes sectioned to evaluate mean diameter), 30 nucleolated oocytes per female in March 1984 and 1985 were measured microscopically. The mean monthly values for the nucleolated eggs were then compared (t-test) to the mean values for all eggs.

RESULTS

A detailed insight into the gametogenic cycle of the *Mercenaria mercenaria* population in Wassaw Sound was ascertained from the combination of qualitative and quantitative data gathered during the study period (December 1983–July 1986). Monthly qualitative assessments of reproductive condition are illustrated in Figures 1 and 2. From these data, it is apparent that sexually undifferentiated individuals were not encountered during the course of this study. Gametogenic activity was evident throughout the study period in all specimens (Figs. 1 and 2). It appears there were several spawning events during 1984 and 1985.

1984 Spermatogenesis

In 1984 males showed high spawning activity during March (56% ripe)–May (30% ripe and spawning) (Fig. 1). Further qualitative evidence to support this view was present in sharp declines in G.I. values during March–May (1.30) and September–October (0.50) (Fig. 2). The spring spawn continued well into the summer (August G.I. minimum of 1.00) followed by a rapid burst of spermatogenic development into September (G.I. = 2.00). A steady maturation of males was evident following the fall (September–October) spawn of 1984 (Fig. 2). Quantitative data were in agreement with qualitative results for males during 1984. A major spring spawning event was evidenced by statistically significant (Student t-test) changes in all three male parameters (Fig. 3). Gonad area (Fig. 3A) values indicated spawning from February (94.5%) through May (75.3%) with a second burst in June (89%) to August (77.2%). Spermatogenic content levels showed significant declines in February–March (9.3%) and April–May (13.7%) (Fig. 3B). Spermatozoan levels showed a significant drop in the period March–April (27.3%) followed by low level fluctuations through June (Fig. 3C). Quantitative male data showed significant reductions in all male parameters during the fall of 1984 (Fig. 3). While gonad area (Fig. 3A) and spermatogenic content (Fig. 3B) data implied September–October (with drops of 20.1% and 16.9% respectively) was the fall spawning period, spermatozoan percentages (Fig. 3C) indicated spawning starting earlier in August (32.1%)–September (17.1%). Staging criteria, G.I., gonad size, spermatogenic (February through August) and spermatozoan content values all supported the hypothesis that the spring spawn was the largest for males during 1984 (Figs. 1–3).

1984 Oogenesis

Females appeared to have spawned on two occasions during 1984. Major spawning events were indicated by qualitative data for March–May and September–October (Figs. 1, 2). In March 1984, 28.5% of the sample population was made up of ripe females while by May 30% con-

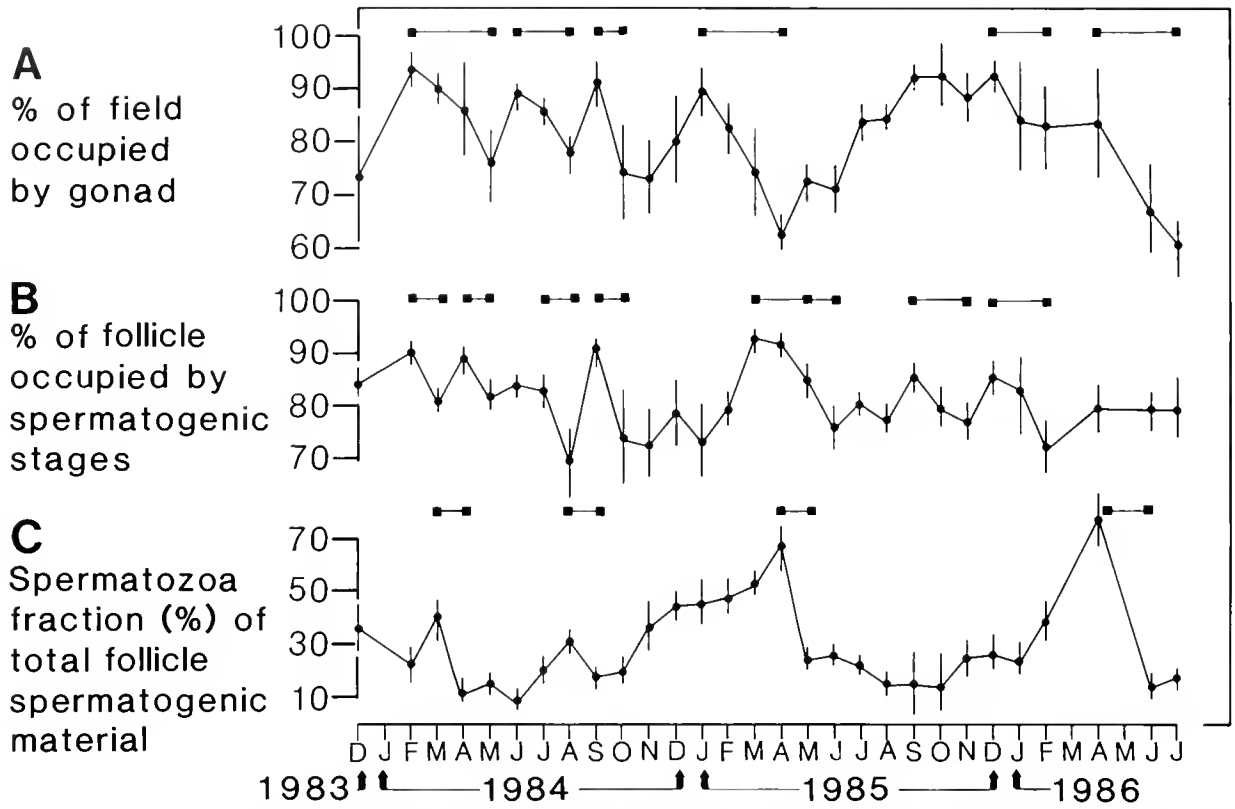


Figure 3. Composite of quantitative data (obtained using image analysis) representing the state of gonad condition for male hard clams in Wassaw Sound, Georgia (December 1983–July 1986). A. Mean percentage of field analyzed occupied by gonad tissue. B. Mean percentage of follicle area occupied by spermatogenic stages. C. Mean percentage of total spermatogenic cell content occupied by spermatozoa. Verticle bars = 2 S.E. about the mean. Horizontal bars signify statistically significant decreases in the various parameters between indicated sampling dates.

sisted of ripe and spawning females (Fig. 1). Similarly, in September 25% of the sample population were "active" females while another 35% were ripe females. This changed sharply by October when 50% of the clams sampled consisted of ripe and spawning females. Sharp declines in female G.I. values from April to May (1.69) and September to October (1.12) also indicated spawning during these periods. G.I. values rose sharply from December 1984 (1.66) to January 1985 (2.85) followed by a minor decline in February (0.28) (Fig. 2). However this was not interpreted as a winter spawning as staging criteria indicates the drop in G.I. values was due to an increase in the proportion of developing (active) females from 5% in January to 16.6% in February (Fig. 1).

Quantitative female data also suggested two major spawning events in 1984 (Fig. 4). Statistically significant declines were observed in gonad area (March–May = 13.8%, September–October = 28.9%, Fig. 4A), oocyte content (March–May = 15.4%, September–October = 9.4%, Fig. 4B) and egg number (March–May = 35, September–October = 34, Fig. 4C) during spring and fall 1984. The estimated mean oocyte diameter data set (Fig. 4D) for 1984 did not indicate any spawning events. Significant changes in mean values were only detected during June (33.1 μm)–July (28.9 μm) and this was not interpreted as an indication of a spawning event. The statistically significant drop in estimated mean oocyte diameter value from December 1984 to January (4.3 μm) 1985 was interpreted as a phase of rapid oocyte development with an abundance of small eggs present in the follicles (Fig. 4D). This agreed with all other quantitative female (Fig. 4A–C) and qualitative (Fig. 2) data which indicated gametogenic development during October 1984–January 1985.

1985 Spermatogenesis

During 1985, qualitative and quantitative data both indicated three spawning events (spring, fall, and winter) took place (Figs. 2–4). Qualitative male data showed a peak maturity level (G.I. = 3.00) during March–April when ripe males constituted 40% of the population (Figs. 1, 2). Major spawning was indicated in April–May when G.I. values fell from 3.00 to 1.10 (Fig. 2). By May 45% of the population consisted of ripe and spawning males (Fig. 1). May–July marked a period of spermatogenic redevelopment while in July–October a plateauing was evident with G.I. values remaining at the 2.00 mark. During October active males made up 50% of the sample population. A sharp decline in G.I. values during October–November

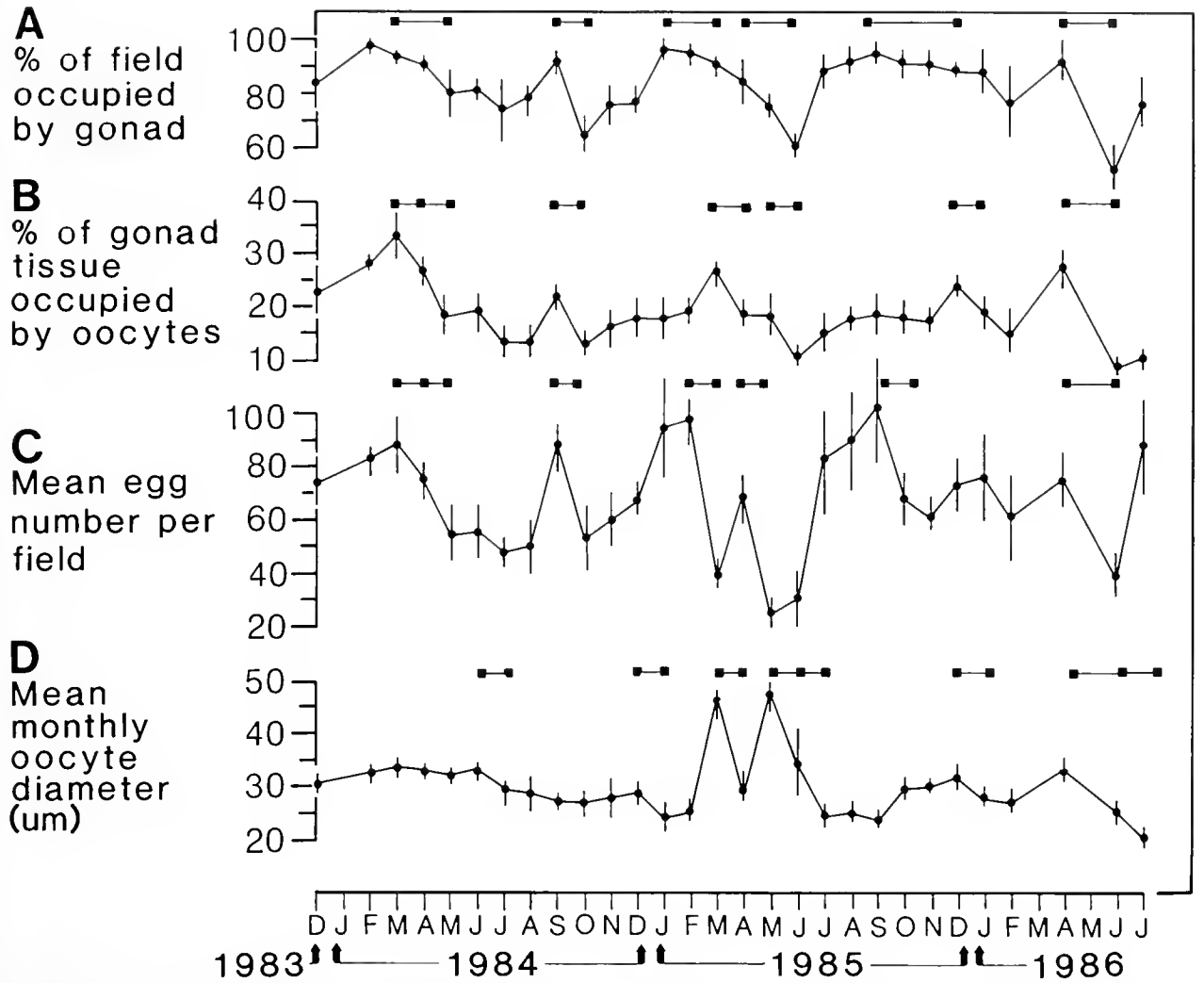


Figure 4. Composite of quantitative data (obtained using image analysis) representing the state of gonad condition for female hard clams in Wassaw Sound, Georgia (December 1983 to July 1986). A. Mean percentage of field analyzed occupied by gonad tissues. B. Mean percentage of gonad tissues occupied by oocytes. C. Mean number of eggs present per field analyzed. D. Mean monthly oocyte diameters. Verticle bars = 2 S.E. about the mean. Horizontal bars signify statistically significant decreases in the various parameters between indicated sampling dates.

(0.55) signified the second spawning of male hard clams in 1985. In November 30% of the population consisted of ripe and spawning males. Rapid spermatogenic development was evident in November–December when the G.I. rose from 1.45 to 2.61 (Fig. 2) and the percentage of ripe males increased to 50% (Fig. 1). The sharp decline in G.I. values in December–January 1986 (0.81) identified a third spawning of males (Fig. 2). By January 1986 ripe and spawning males accounted for 25% of the sample population (Fig. 1).

Quantitative male data for 1985 was strongly supportive of the patterns elucidated by staging criteria and G.I. calculations. A spring spawn was indicated by statistically significant declines in total spermatogenic (April–May = 6.9%, Fig. 3B) and spermatozoan content levels (April–May = 42.2%, Fig. 3C). The significant decline in male gonad area from January to April (28.3%) was surprising in light of the rising trends in both total spermatogenic and spermatozoan levels during the same period (Figs. 3A–C). The only male parameter indicating a statistically significant decline coincidental with the fall spawn as illustrated by qualitative data, was spermatogenic content (Fig. 3B). Spermatogenic percent levels fell from 86.0% in September to 77.4% in November. Male gonad area did however have a declining trend (not significant (NS)) during October–November (Fig. 3A) while spermatozoan levels dropped very slightly in September–October before rising again in October–November. All three male parameters showed declining patterns during December 1985–January 1986, supporting the qualitative data illustrating spawning activity during this period. Gonad area dropped from December (93.4%)–January (87.7%, NS)–February (74.9%,

significant (S)). Spermatogenic content had a similar declining pattern: December (85.7%)–January (83.1%, NS)–February (72.5%, S).

1985 Oogenesis

Female qualitative and quantitative data for 1985 both indicated three spawning events with slight differences from males in the onset of the spring and fall events (Fig. 2). Qualitative data indicated a drop in G.I. from March–June (1.91) (Fig. 2). Staging analyses (Fig. 1) showed that ripe females accounted for 55% of the sample population in March, while ripe and spawning females constituted 40% in May and 21.1% in June. Both data sets indicated female spawning extended from March to June, while the male spawn lasted from April to May (Fig. 2), and possibly into June (Fig. 3B). Quantitative female data also indicated a female spawn during spring 1985. These data suggested the spawn occurred in two major bursts March–April and May–July (Figs. 4B and D). The March–April event was supported by statistically significant declines in oocyte content (7.7%, Fig. 4B) and estimated mean oocyte diameter values (17.2 μm, Fig. 4D), while evidence for a May–June spawning was contained in significant drops in oocyte content (8.4%, Fig. 4B) and size (13.3 μm, Fig. 4D). Gonad area also declined significantly during April–June (24.2%) (see Fig. 4A). Given the lack of collaborating evidence from all other data sets, the decline in gonad area during January–March was not interpreted as an indication of spawning. Egg numbers were observed to decrease significantly during periods of maximum ripeness (e.g. Feb.–March (58.3) and April–May (42.9), Fig. 4C). Females exhibited redevelopment from June (G.I. = 1.00) to September (G.I. = 2.30), by which time two thirds of the females were active and one third were ripe (Figs. 1, 2). Females apparently spawned again from September (G.I. = 2.30) to November (G.I. = 1.33) (Fig. 2). During November 35% of the sample population consisted of ripe and spawning females. Once again the female spawn appeared to commence approximately one month before the male event (Fig. 2). Quantitative data indicative of a fall 1985 spawn were more sparse than for all other events. However, gonad area did decrease significantly from September to December (6.6%) (Fig. 4A) lending support for a spawning, albeit limited. Oocyte area and estimated diameter values (Figs. 4B and D, respectively) illustrated low level declines (NS) and plateauing during this period. Egg number (Fig. 4C) dropped significantly from September to October (34.7), also indicative of spawning activity, before rising again (November–December). A burst of rapid female gametogenic development was indicated from November to December when G.I. values rose from 1.33 to 2.42. During December 25% of the clams sampled consisted of ripe females with another 10% ripe and spawning females (Fig. 1). This percentage of ripe and spawning females rose during December and January 1986 (10% to 15%, Fig. 1) while the G.I. fell sharply (by 0.88, Fig. 2), signifying the third female spawning event for 1985. This interpretation was further supported by significant declines in oocyte content levels (4.9%, Fig. 4B) and estimated oocyte diameters (4.3 μm, Fig. 4D) during December and January. Gonad size data displayed a plateauing trend (Fig. 4A) during this period while egg numbers were apparently increasing (NS).

1986 Spermatogenesis

A major spring spawn was indicated for both sexes during the seven months studied in 1986 (Figs. 1–4). Qualitative male data showed gametogenic development during January–April (Figs. 1, 2). G.I. values rose from 1.80–3.00 (Fig. 2) during this period. In January a large proportion of males were ripe and spawning while in April males were dominated entirely by ripe specimens (Fig. 1). April–June was shown to be a high spawning intensity period, with the G.I. falling sharply (April–June = 1.93) and ripe and spawning males dominating (Figs. 1, 2). Quantitative male data illustrated the same gametogenic pattern, with maturation evident during January–April and then spawning in April–June (Figs. 3A–C). Sharp declines in gonad area April–June (16.6%, NS)–July (23.4%, S) and spermatozoan content April–June (55.0%, S) indicated spawning during this period (Figs. 3A, C). Rising G.I. values (1.07–1.7) and spermatozoan levels (15.4–17.6%, NS) suggested redevelopment among males during June–July (Fig. 2, 3C).

1986 Oogenesis

Oogenesis appeared to follow an almost identical pattern to spermatogenesis during 1986. The proportion of ripe females rose from 10% to 50% of the sample population during January–April, while the G.I. rose from 1.54 to 3.00. This period of maturation (January–April) was followed by intense spawning during April–June, when the G.I. (Fig. 2) fell sharply (2.00) and ripe and spawning females dominated (55.5%) (Fig. 1). All four females quantitative parameters assessed showed statistically significant declines during April–June, indicative of a major spawning crisis (gonad area decline = 41.8%: oocyte content decline = 19.5%; oocyte number decline = 36.1; oocyte diameter decline = 8.3 μm, Fig. 4A–D). A slight rise in G.I. (0.09) (Fig. 2) and increasing trends in female gonad area (Fig. 4A), oocyte content (Fig. 4B), and oocyte number (Fig. 4C) indicated a period of redevelopment during June and July. A continued decrease in estimated mean oocyte diameter during June and July (Fig. 4D) suggested increasing numbers of small developing oocytes, also suggestive of oogenic redevelopment.

Oocyte Diameter

Table 1 illustrates how the mean oocyte diameters, during March 1984 and 1985, computed using the image

TABLE 1.

Comparison between mean monthly oocyte diameters computed using the image analyser, which measured all oocytes sectioned within a field of view, and microscopic measurements of 30 nucleolated oocytes per specimen. S = Significant differences (t-test). The reason image analyzer estimates of the mean diameter value were higher in their underestimation during March 1984 than March 1985 is due to the significantly higher ($p < 0.001$) numbers of equally sized eggs (according to microscopic readings) occupying similar sized (NS, $p > 0.05$) gonads in 1984 and 1985 (see Figure 4). This increased egg number led to a higher compression factor. As a sphere or circle is compressed, the theoretial mean radius value will consequently be reduced, explaining the lower image analyzer estimated mean diameter value in March 1984.

Mercenaria mercenaria mean monthly oocyte diameters (μm).

	Image Analyzer Data (All oocytes present in the field) (±SE)			Microscopic Data (N = 30) (Nucleolated oocytes only) (±SE)		
March 1984	34.8 μm	(±0.91)		53.8 μm	(±2.63)	
	32.2	(±0.77)		48.2	(±3.20)	
	36.3	(±1.18)		59.0	(±2.50)	
	33.1	(±0.41)		54.2	(±3.17)	
	36.1	(±1.13)		59.7	(±2.50)	
Mean	34.5	(±0.81)	Vs.	55.0	(±1.86)	S
March 1985	44.7	(±0.31)		47.3	(±2.51)	
	46.4	(±0.34)		45.8	(±2.72)	
	45.7	(±0.43)		49.7	(±3.54)	
	41.3	(±0.29)		50.0	(±2.26)	
	48.1	(±1.03)		54.2	(±2.09)	
	44.2	(±0.81)		54.5	(±2.18)	
	47.0	(±0.89)		51.2	(±2.58)	
	47.7	(±0.99)		47.3	(±2.09)	
	50.7	(±0.66)		50.3	(±2.16)	
	51.3	(±1.18)		59.5	(±3.79)	
Mean	46.7	(±0.95)	Vs.	51.0	(±1.23)	S

analyzer were, as expected, significantly lower (t-test) than the mean values computed from microscopic measurement of only nucleolated eggs from the same specimens. The discrepancy was considerable in 1984 (20.5 μm) while it was much less in 1985 (4.3 μm). When correlated statistically, the 1984 data showed a correlation coefficient of 0.92 and a relatively steep slope ($y = -27.5 + 2.39x$, where y is measured nucleolated egg diameter and x is omnicon estimated egg diameter). In contrast, the 1985 data showed a lower correlation coefficient of 0.41 and a much lower slope ($y = 25.4 + 0.55x$).

Sex Ratio and Size Data

Statistical analysis (Chi-square test, Steel and Torrie 1960) showed that the sex ratios did not significantly diverge from a 1:1 ratio in any year.

The population studied was dominated by chowder clams (Fig. 5). The vast majority of samples consisted of clams with mean sizes in excess of 75 mm (26 of 29 samples) and all were above 69.5 mm (Fig. 5).

DISCUSSION

It is apparent from the data presented herein that the *Mercenaria mercenaria* population displayed a synchronized polymodal breeding pattern throughout the study period (Figs. 1–4). A considerable degree of flexibility in hard clam gametogenesis was also displayed by the fact that two spawning events occurred in 1984 while three were detected in 1985. In both years the spring spawn was the largest, as evidenced by G.I. values and both male and female quantitative data (Figs. 2–4). The fall spawn of 1984 was considerably larger than its counterpart in 1985 (Figs. 2–4). This may have played a significant role in the occurrence of a third (winter) spawn during 1985, with increased levels of energy reserves available for gametogenesis late in 1985 due to the reduced nature of the fall spawning event. The spring spawn of 1986 was of similar proportions to those of the two previous years studied (Figs. 2–4). Gametogenesis, which was continuous throughout the study period, displayed rapid development and maturation on several occasions (e.g., August–September 1984 and November–December 1985, Figs. 2–4).

Aided with the reported quantitative data (Figs. 3, 4), we are able to test the proportional amounts of gamete production as evidenced by our *per field* sampling procedures from year to year. In the case of the males, spermatozoan levels were significantly higher (t-test) in spring 1985 (April value = 67.1%) than 1984 (March value = 38.95) (Fig. 3C). It can be noticed that total percentage of spermatogenic material dropped between February and March 1984, when spermatozoa reached their maximum value (Fig. 3B, C). In contrast, during 1985 both values continued to rise through to the spermatozoan peak in April (Fig. 3B, C). One can speculate that many of the immmature (spermatogenic) stages present in February 1984 failed to reach final maturity, thus explaining the apparently lower spermatozoan content in 1984. Detected spermatozoan levels for the fall and winter of 1984 and 1985 were not significantly different from one another (Fig. 3C). In a recent report, Gallager and Mann (1986) suggested egg volume as a "more descriptive parameter" than egg diameter for comparative purposes (in their study they were dealing with lipid content levels in oocytes of hard clams and American oysters). We agree with this viewpoint and have incorporated volumetric estimates of oogenesis in our studies of gametogenesis in hard clams and American oysters (Heffernan et al. 1989). When examining female data (Fig. 4), one gets somewhat contradictory indications from 2-dimensional and 3-dimensional evaluations. Calculations based on oocyte area (2-dimensional) data (Fig. 4B) show higher values for March and September in 1984 than 1985, while December levels were greater in 1985 (Table 2). Combining all three months each year, 1985 levels are

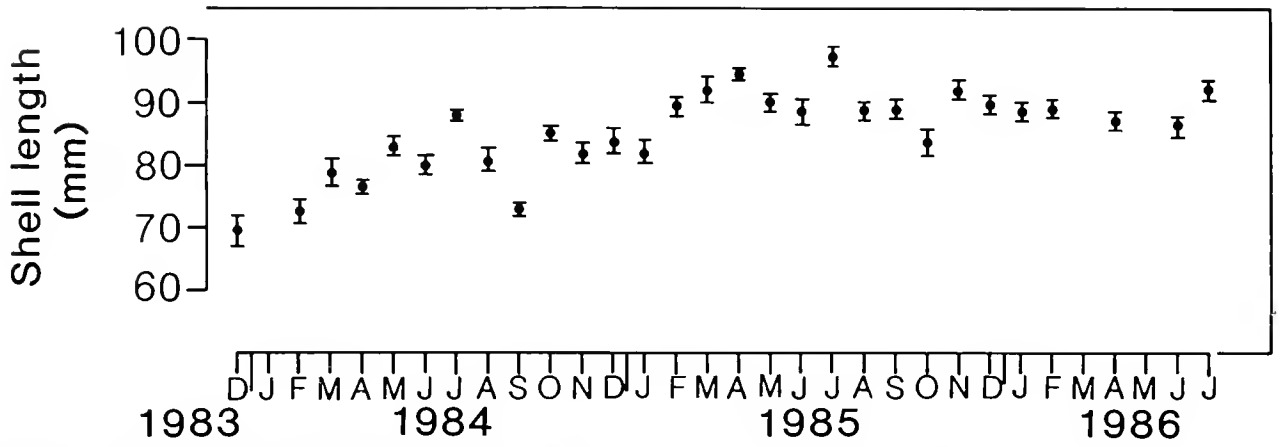


Figure 5. Mean size (with standard errors) of hard clams processed for histological examination of reproductive condition from House Creek, Wassaw Sound, Georgia (December 1983 to July 1986).

shown to have been higher than 1984 (Table 2). However, using a volumetric (3-dimensional) estimate of egg production per field analyzed = $4/3\ \pi\ r^3\ N$ where r = mean egg radius (based on data in Fig. 4D) and N = mean number of eggs/field analyzed (Fig. 4C), we obtain production figures of 1.93 million um^3 eggs/field (March 1984) and 2.76 million um^3 eggs/field (March 1985). While March volumetric values "disagreed" with the 2-dimensional values, those for September (978,857 μm^3 eggs/field, 1984 and 735,618 μm^3 eggs/field, 1985) and December (835,970 μm^3 eggs/field, 1984 and 1,274,997 μm^3 eggs/field, 1985) followed the same pattern. Overall, the three-month combined values for 1985 (4,770,615 μm^3 eggs/field) were greater (1.27 times) than 1984 (3,743,827 μm^3 eggs/field), agreeing with the 2-dimensional data. G.I. values were also higher during the two largest spawns of 1985 (spring and winter) than in 1984 (spring and fall) (Fig. 2). The apparently higher levels of *Mercenaria mercenaria* gamete production in 1985 is in agreement with *Crassostrea virginica* data from the same study site (Heffernan et al. 1989). It is noticeable that hard clams apparently spawned large numbers of relatively smaller eggs in spring 1984 than 1985. While measurements of nucleolated oocytes showed no significant differences in egg size between March 1984 and March 1985 (Table 1), there were significant differences in the image analyzer diameter estimates and egg numbers (Fig. 4C and D, Table 1). Oocyte numbers decreased during periods of maximum maturity (e.g., March and May) in 1985 while they remained high during 1984 (e.g., March). This, coupled with the larger discrepancy between image analyzer and microscopic measurements of oocyte diameter in 1984 than in 1985 (see Table 1) would indicate a production of relatively fewer but more uniformly large eggs in the latter year. A reduction in oocyte numbers during final maturation is a common feature of reproduction in marine invertebrates (Giese and Pearse 1975, 1979). It would be very interesting to see if the differences in reproductive output between 1984 and 1985 are matched by differences in the respective recruitment classes.

It is not possible at this stage to give a reason for the observed differences in gametogenic development between 1984 and 1985. Temperature and salinity data from a somewhat peripheral site illuminate nothing by way of causative influences and one can only speculate on the effects of varying food availability. An interesting feature of the male reproductive cycle in 1986 was the stability in percent of follicle occupied by spermatogenic cells (Fig. 3B), while both spermatozoan content (Fig. 4C) and gonad size (Fig. 4A) decreased. One possible explanation for this phenomenon was a general shrinkage (compression) of follicles following spawning in April.

The application and reliability of image analysis technology to bivalve gametogenic studies is illustrated by the strong agreement of qualitative and quantitative data sets throughout the study period. While the various merits of this system have been documented elsewhere (Heffernan and Walker 1989), it is worth repeating that the quantitative gamete production levels, calculated on a *per field* basis, should be accurate indicators of "whole animal" events given the relatively uniform nature of gonad development in many bivalves (Kennedy and Battle 1964; Keck et al. 1975; Lowe et al. 1982; Wilson and Simons 1985). In the current study, there were instances of 'greater sensitivity'

TABLE 2.

Comparison (ANOVA) of percent of *Mercenaria mercenaria* gonad occupied by oocytes (from Figure 5B) during peak maturity periods in 1984 and 1985.

ANOVA	d.f.	F Ratio	F Probability
Mar. 1984 > Mar. 1985	1	38.3918	0.0001
Sept. 1984 > Sept. 1985	1	4.5770	0.0357
Dec. 1984 < Dec. 1985	1	466.5005	0.0001
1984 < 1985	1	4.260	0.0400

to gametogenic events displayed by quantitative data (e.g., female cycle during spring 1985 when a two phase spawning sequence was detected by quantitative measures (Figs. 4B–D). Qualitative data did not differentiate these events, indicating one continuous spawning burst (Fig. 2). As has been suggested elsewhere (Heffernan and Walker 1989), image analysis systems could be very useful in comparative studies of gonad development among various size classes of the same species. Caution is advised for other workers to ensure the intercomparability of gamete production levels among various size classes. It may prove to be the case that such comparisons may be limited among as yet to be determined size ranges. The 'estimated' mean monthly oocyte diameter values are acknowledged to significantly underestimate egg size (due to the inclusion of tangental oocyte sections in the calculation of the mean). Given their limited usefulness as spawning indicators, as shown in this study, the value of this parameter is questionable. However, if a more accurate value is desired, future workers could achieve it by the use of an on-screen light pen, measuring only nuceolated oocytes (a considerably more time consuming process).

Temperature regimes have been shown by many marine invertebrate researchers to have a profound influence on gametogenesis (e.g., Orton 1920; Nelson 1928; Loosanoff 1937a; Thorson 1950; Loosanoff and Nomejko 1951; Ansell 1961; Porter 1984; Keck et al. 1975; Eversole et al. 1980; Manzi et al. 1985). While a similar influence on clam gametogenesis in Georgia is thought likely we are prevented from examining this due to a lack of hydrographic data from the study site. It appears that local hard clam populations spawned during periods of high algal concentrations (spring, fall and winter) in Wassaw Sound, providing larval stages with abundant food sources. Bishop (1977) showed phytoplankton peaks in Wassaw Sound during these periods in his 1976–77 study. S. Bishop and P. Verity (Skidaway Institute of Oceanography, personal communication) have detected high chlorophyll *a* concentrations during similar periods of 1986–87, with algae cell counts (5–10 μm size range) varying from 2 to 5 $\times$ 10^3 cells/ml (January) to 10^4 cells/ml (September) in the Skidaway River.

Several recent reviews (Eversole et al. 1980; Manzi et al. 1985; and Eversole 1988) have discussed the changing reproductive strategy of *Mercenaria mercenaria* with respect to latitude. Eversole et al (1980), in agreement with the observations of Giese (1959) for a wider range of invertebrates, suggested a prolonged and synchronized polymodal breeding pattern for hard clams in lower latitudes. The current results are in strong agreement with this. Furthermore, this study reveals a more extensive polymodal strategy, with three spawning peaks in one year (1985), among Georgia hard clams. The three 1985 spawns contrast with the findings of Pline (1984) for a tidal-creek population on the south of Wassaw Sound, Georgia. This population exhibited a bimodal cycle, with spring and fall spawning peaks during 1981, similar to our findings during 1984. However, the influence on gametogenesis of the fall/summer 1981 drought and subsequent high salinity stress endured by hard clams in Pline's study remains unknown. Dalton and Menzel (1983) detected winter spawning among young clams in the north west Gulf coast of Florida, but concluded that the cycle was probably bimodal, disregarding the winter spawn due to the unusually high air temperatures during the study period and the presumed inability of larvae to survive the lower water temperatures. Bimodal reproductive cycles have been reported for hard clams in South Carolina (Eversole et al. 1980; Manzi et al. 1985) and North Carolina (Porter 1964), with spring and fall spawning peaks. Unimodal patterns were reported for hard clams in Long Island Sound (Loosanoff 1937b), Delaware Bay (Keck et al. 1975) and Great South Bay, New York (Kassner 1982). Readers are referred to the detailed review of these works by Eversole (1988).

Gametogenesis, as mentioned above, appeared to be continuous and year round in the House Creek population, with no inactive or recuperative specimens observed throughout the course of this study. Pline (1984) had similar findings during his 1981–82 study, discovering only low numbers of recuperative phase specimens from June to August 1981. Recuperative specimens were limited to littlenecks and none were observed in chowders (Pline 1984). In all but three months (December 1983, February and September 1984) during the current study, the mean size of specimens analyzed was in the chowder range (>78 mm) (Fig. 5). Manzi et al. (1985) found similar results from their observations of a chowder-dominated subtidal population in South Carolina. Eversole et al. (1980) found fewer recuperative or inactive forms in older clams than in littlenecks. Other reports indicating a poverty of inactive phase clams include Loosanoff (1937b) (where undifferentiated gonads were not reported for hard clams in Long Island Sound and undifferentiated cells are described along the inner walls of female follicles only immediately after spawning); Keck et al. (1975); and Dalton and Menzel (1983). An even sex ratio among older (chowder) clams has been reported by several workers (Loosanoff 1937a; Eversole et al. 1980; and Dalton and Menzel 1983), and is consistent with the findings of this study.

ACKNOWLEDGMENTS

This work was funded by Georgia Sea Grant/Project number NA84AA-D-00072. The assistance of Dr. A. Falek and Dr. D. Shaffer and their kindness in making the omnicon image analyzer at Emory University so readily available is heartily acknowledged. Mr. D. Jacobi, Mr. J. Griffin and Ms. T. Suddeth are thanked for their contributions to the image analysis work. Dr. D. Gillespie's help with statistics is greatly appreciated. Mr. M. Pline is

thanked for sharing his experiences of the image analyzer. Drs. J. Crenshaw, A. Eversole, D. O'Foighil and Mr. M. Castagna are thanked for their contributions and comments during the drafting of this manuscript. The graphical and clerical assistance of Ms. S. McIntosh, Ms. A. Boyette, and Mrs. J. Haley is appreciated.

LITERATURE CITED

Ansell, A. 1961. Reproduction, growth and mortality of *Venus striatula* (da Costa) in Kanes Bay, Millport. *J. Mar. Biol. Assoc. U.K.* 41:191–215.

Bancroft, J. & A. Stevens. 1977. Theory and practice of histological techniques. Churchill Livingston, Edinburgh, New York, London. 436 p.

Bishop, S. 1977. Effects of ionic copper on natural populations of phytoplankton in continuous culture. Masters Thesis, Georgia Southern College. 97 p.

Choi, K. S., D. Lewis & E. Powell. 1988. An immunologic technique for quantitating oyster eggs. *J. Shellfish Res.* 7(1):209.

Dalton, R. & W. Menzel. 1983. Seasonal gonadal development of young laboratory-spawned southern (*Mercenaria campechiensis*) and northern (*Mercenaria mercenaria*) quahogs and their reciprocal hybrids in northwest Florida. *J. Shellfish Res.* 3(1):11–17.

Eversole, A. 1988. Gametogenesis and spawning in North America clam populations: Implications for culture. J. J. Manzi and M. Castagna (eds.). *Clam Mariculture in North America*. Elsevier, New York. (In Press).

Eversole, A., W. Michener & P. Eldridge. 1980. Reproductive cycle of *Mercenaria mercenaria* in a South Carolina estuary. *Proc. Natl. Shellfish. Assoc.* 70:22–30.

Gallager, S. C. & R. Mann. 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. *Aquaculture* 56:105–121.

Giese, A. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. *Ann. Rev. Physiol.* 21:547–576.

Giese, A. & J. Pearse. 1975. Reproduction of marine invertebrates. Vol. 3. Annelids and Echiurans. Academic Press. New York. 343 p.

Giese, A. & J. Pearse. 1979. Reproduction of marine invertebrates. Vol. 5. Molluscs: Pelecypods and Lesser Classes. Academic Press, New York. 369 p.

Heffernan, P. & R. Walker. 1989. Quantitative image analysis methods for use in studies of bivalve reproduction. *J. Moll. Stud.* 55:135–137.

Heffernan, P., R. Walker & J. Carr. 1988. The reproductive cycle of the hard clam, *Mercenaria mercenaria*, in Wassaw Sound, Georgia. *J. Shellfish Res.* 7(1):208.

Heffernan, P., R. Walker & J. Carr. 1989. Qualitative and quantitative studies on the gametogenic cycles of three marine bivalves in Wassaw Sound,Georgia. II *Crassostrea virginica* (Gmelin). *J. Shellfish Res.* 8:61–70.

Kassner, J. 1982. The gametogenic cycle of the hard clam, *Mercenaria mercenaria*, from different locations in the Great South Bay, New York. Masters Thesis, State University of New York. 72 p.

Keck, R., D. Maurer & H. Lind. 1975. A comparative study of the hard clam gonad developmental cycle. *Biol. Bull.* 148:243–258.

Kennedy, A. & H. Battle. 1964. Cyclic changes in the gonad of the American oyster, *Crassostrea virginica* (Gmelin). *Can. J. Biol.* 42:305–321.

Kennedy, V. 1977. Reproduction in *Mytilus edulis auteanus* and *Aulaconga maoriana* (Mollusca:Bivalvia) from Taylors Mistake, New Zealand. *N. Z. J. Mar. Fresh. Res.* 11(2):253–267.

Loosanoff, V. 1937a. Development of the primary gonad and sexual phase in *Venus mercenaria* Linneaus. *Biol. Bull.* (Woods Hole) 72:389–405.

Loosanoff, V. 1937b. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.). *Biol. Bull.* (Woods Hole) 72:406–416.

Loosanoff, V. & C. Nomejko. 1951. Existence of physiologically different races of oyster, *Crassostrea virginica*. *Biol. Bull.* (Woods Hole) 101:151–156.

Lowe, D., M. Moore & B. Bayne. 1982. Aspects of gametogenesis in the marine mussel *Mytilus edulis* L. *J. Mar. Biol. Assoc. U.K.* 62:133–145.

Manzi, J., M. Bobo & V. Burrell, Jr. 1985. Gametogenesis in a population of the hard clam, *Mercenaria mercenaria* (Linnaeus), in North Santee Bay, South Carolina. *Veliger* 28(2):186–194.

Nelson, T. 1928. On the critical temperatures for the spawning and for ciliary activity in bivalve molluscs. *Science* 67:220–221.

Orton, J. 1920. Sea temperature, breeding and distribution in marine animals. *J. Mar. Biol. Assoc. U.K.* 12:339–366.

Pline, M. 1984. Reproductive cycle and low salinity stress in adult *Mercenaria mercenaria* L. of Wassaw Sound, Georgia. Masters Thesis, Georgia Institute of Technology. 74 p.

Porter, H. 1964. Seasonal gonadal changes of adult clams, *Mercenaria mercenaria* (L.) in North Carolina. *Proc. Natl. Shellfish. Assoc.* 55:35–52.

Shaw, W. 1988. A proposed standardization of stages in the gametogenesis cycles of bivalves. *J. Shellfish Res.* 7(1):132.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry. Freeman, New York. 859 p.

Steel, R. & J. Torrie. 1960. Principals and procedures of statistics. McGraw-Hill Book Co., Inc. New York. 481 p.

Thorson, G. 1950. Reproduction and larval ecology of marine bottom invertebrates. *Biol. Rev. Camb. Philos. Soc.* 25:1–45.

Walker, R. 1983. Feasibility of mariculture of the hard clam, *Mercenaria mercenaria* (Linne') in coastal Georgia. *J. Shellfish Res.* 3:169–174.

Walker, R. 1985. Growth and optimum seeding time for the hard clam, *Mercenaria mercenaria* (L.) in coastal Georgia. *The Nautilus* 99:127–133.

Walker, R. & K. Tenore. 1984. The distribution and production of the hard clam, *Mercenaria mercenaria*, in Wassaw Sound, Georgia. *Estuares* 7(2):19–27.

Walker, R., P. Heffernan & S. Stevens. 1988. Hard clam harvesting season in coastal waters of Georgia. *Malacology Data Net.* (In Press).

Wilson, J. & J. Simons. 1985. Gametogenesis and breeding of *Ostrea edulis* on the west coast of Ireland. *Aquaculture* 46:307–321.

Zar, J. H. 1974. Biostatistical analyses. Prentice Hall. Englewood Cliffs, NJ. 620 p.

Journal of Shellfish Research, Vol. 8, No. 1, 61–70, 1989.

GAMETOGENIC CYCLES OF THREE MARINE BIVALVES IN WASSAW SOUND, GEORGIA II *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

PETER B. HEFFERNAN, RANDAL L. WALKER AND JOHN L. CARR
University of Georgia
Marine Extension Service Shellfish Laboratory
Skidaway Island
P.O. Box 13687
Savannah, Georgia 31416-0687

ABSTRACT Gametogenesis in the American oyster, *Crassostrea virginica*, was studied from December 1983 to July 1986 in Wassaw Sound, Georgia. Qualitative and quantitative data were compiled monthly from histological preparations, and used in the assessment of reproductive condition. Qualitative data were compiled on staging criteria and gonad indices. Quantitative data were gathered using an image analysis system on gonad area, egg area, egg number, and estimated egg diameter. An extended unimodal gametogenic cycle was evident throughout the study. Gametogenesis commenced in October–November with development ceasing during the winter. Rapid maturation of gonads recommenced between February–March. Oysters were generally ripe by April–May and spawning activity was observed from May to October with the major intensity of spawning in the July–September period. Temporal differences were detected in the level of gametogenic development indicating a significantly greater gametogenic output for oysters during 1985 than in 1984. Sex ratios were *ca.* 3 females:1 male.

KEY WORDS: reproductive cycle, gametogenesis, oyster, *Crassostrea virginica*, image analysis

INTRODUCTION

The fishing industry for the American oyster, *Crassostrea virginica* (Gmelin), once flourished in coastal Georgia (Harris 1980), but is virtually nonexistent today. In 1987, only 2,000 Kg of oyster meat valued at $6,800 were landed in Georgia (Gordon Rogers, personal communications, Georgia Department of Natural Resources, Fisheries Statistics Department).

Suggested protocols for future oyster mariculture development in Georgia recommend the collection of natural spat (see Heffernan and Walker 1988). Detailed knowledge of oyster spawning patterns will be a valuable asset in this endeavor. Due to the lack of gametogenic data in coastal Georgia and the importance of *C. virginica* to shellfisheries development in Georgia, we examined the bivalve's gametogenic cycle.

The reproductive cycle of the American oyster has been determined for more northern waters (Coe 1932; Loosanoff 1942, 1965; Kennedy and Battle 1964; Kennedy and Krantz 1982) and for areas in the Gulf of Mexico (Butler 1949; Menzel 1951; Hayes and Menzel 1981). In Georgia, no quantitative study of oyster gametogenesis has been undertaken, but Durant (1968) determined that oysters from populations of Sapelo Island and St. Catherines Sound spawn from May through October with peak spawning occurring during the summer months.

MATERIALS AND METHODS

Sampling and Tissue Processing

An average of 17 (±0.46) oysters (14–20/month except for 8 in March 1986) were collected on a monthly basis (December 1983–July 1986) from a shallow sheltered creek, House Creek, Little Tybee Island, on the northern end of Wassaw Sound, Georgia. We used Vernier calipers to measure shell length (i.e., maximum anterior-posterior distance) for all specimens prior to processing for histology. The visceral mass was preserved in a modified formol-alcohol fixative. Prior to histological processing, a mid-lateral gonadal tissue sample (*ca.* 1 cm^2) was dissected from each specimen. Tissue samples were dehydrated in an alcohol series, cleared in toluene, and embedded in paraplast. Sections were cut 7–10 μm in thickness using a rotary microtome. Sections were stained with Ehlrichs haematoxylin and counterstained with Eosin (Bancroft and Stevens 1977).

Qualitative Reproductive Analysis

After a preliminary examination of a wide range of specimens, a 9-stage reproductive staging criterion was adopted (4 male, 4 female, and an inactive stage, see Fig. 1). A random field of gonadal tissue was examined from every specimen and the individual was ascribed to one stage. Oysters were ascribed: Early Active, Late Active, Spawning or Advanced Spawning and Regressing (as described by Kennedy and Krantz 1982) or Inactive (as described by Kennedy and Battle 1964). A monthly gonad index (G.I.) (after Kennedy 1977) was computed for the population (Fig. 2). We employed a scoring system with Male or Female Late Active = 5; Male or Female Early Active = 4; Male or Female Spawning = 3; Male or Female Advanced Spawning and Regressing = 2; Inactive (undifferentiated) = 1. The monthly G.I. for both sexes was determined by multiplying the number of specimens

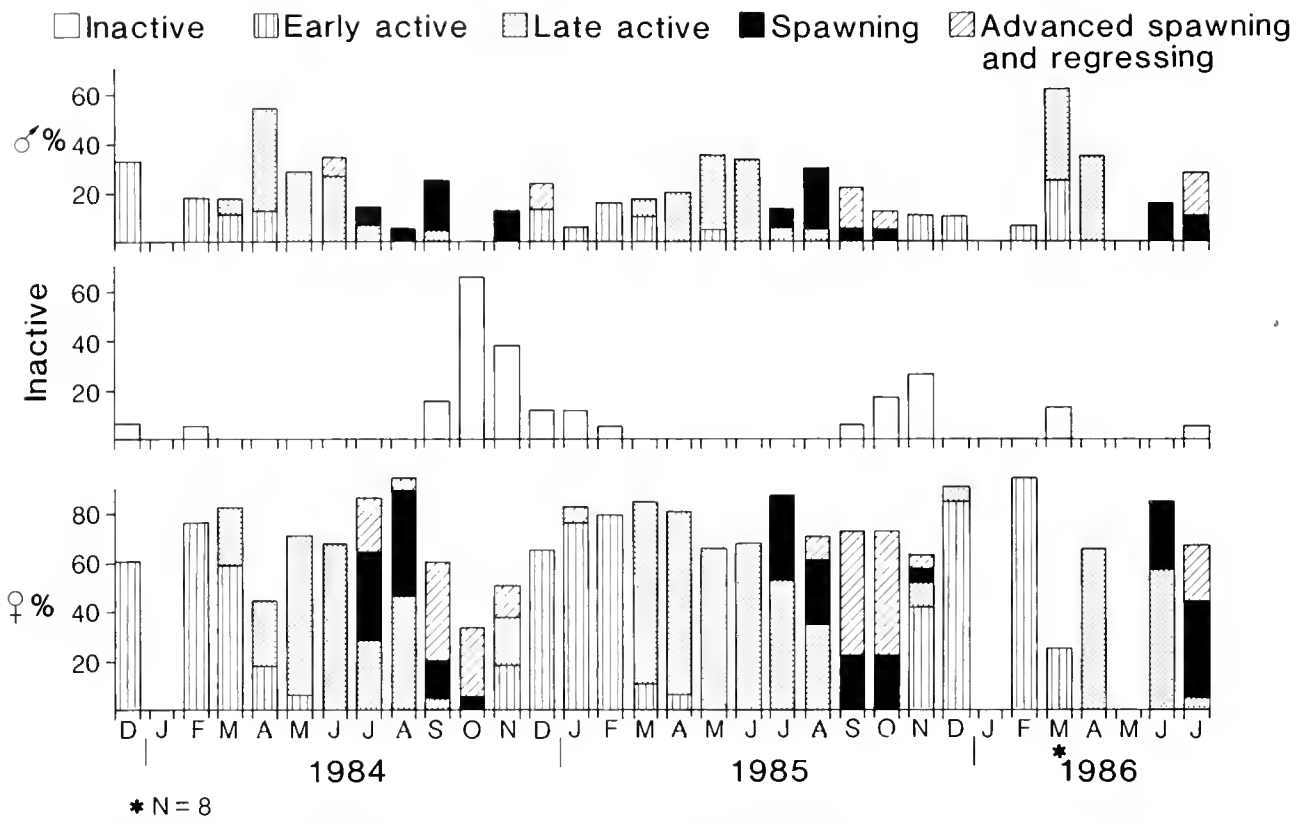


Figure 1. Qualitative data illustrating the sex and developmental stages of American oysters from Wassaw Sound, Georgia (December 1983–July 1986). The length of each area represents the percentage frequency of oysters in each developmental stage.

ascribed to each category by the category score, summing all such values and dividing this figure by the total number of oysters analyzed.

Quantitative Reproductive Analysis

Quantitative image analysis of male and female gonadal material followed the methods outlined in Heffernan et al. (1989). Female data were gathered for percent gonad, percent of gonad area occupied by oocytes, oocyte number per field, and estimated mean oocyte diameter. Similarly, male data were obtained for percent gonad, percent of gonad occupied by spermatogenic stages, and for percent of spermatogenic stages consisting of spermatozoa.

Quantitative analyses of the gametogenic condition of male and female oysters were compiled monthly from February 1984 to July 1986, excepting those periods of early gametogenesis (Males; January–February 1984–86 and April 1985: Females; December 1984–January 1985 and November 1985–February 1986) or during the most advanced stages of spawning and regression among males (August and October 1984 and November–December 1985). During these periods, it was beyond the working capacity of the image analyzer to accurately differentiate between germ cells and follicle wall cells at the magnification level (100×) used throughout the study.

A mean individual value was computed for each category analyzed by the image analyzer. Mean monthly values were then computed and used in the quantitative assessments of reproduction (Figs. 3 and 4). Individual oyster values for all of the quantitative parameters analyzed were compared statistically (t-tests) on a monthly basis during periods of suspected spawning activity (see Table 1). Individual percentage data points were arcsine transformed prior to statistical analysis (Zar 1974). Sex ratios were tested against 1:1 ratio with chi-square tests (Steel and Torrie 1960). In order to evaluate the anticipated underestimation of oocyte diameter portrayed by the method of analysis used on the image analyzer (i.e., using all oocytes present to evaluate mean diameter), 30 nucleolated oocytes per female in May 1984 and June 1985 were measured microscopically. The mean monthly values for the nucleolated eggs were then compared (t-test and linear regression analysis) to the mean values for all eggs.

RESULTS

1984

A unimodal gametogenic cycle was evident among male and female oysters during 1984. Qualitative data indicated there was a period of maturation during March–June when the dominant reproductive stage changed from Early Active (males = 11.8%; females = 58.8%) in March to Late Ac-

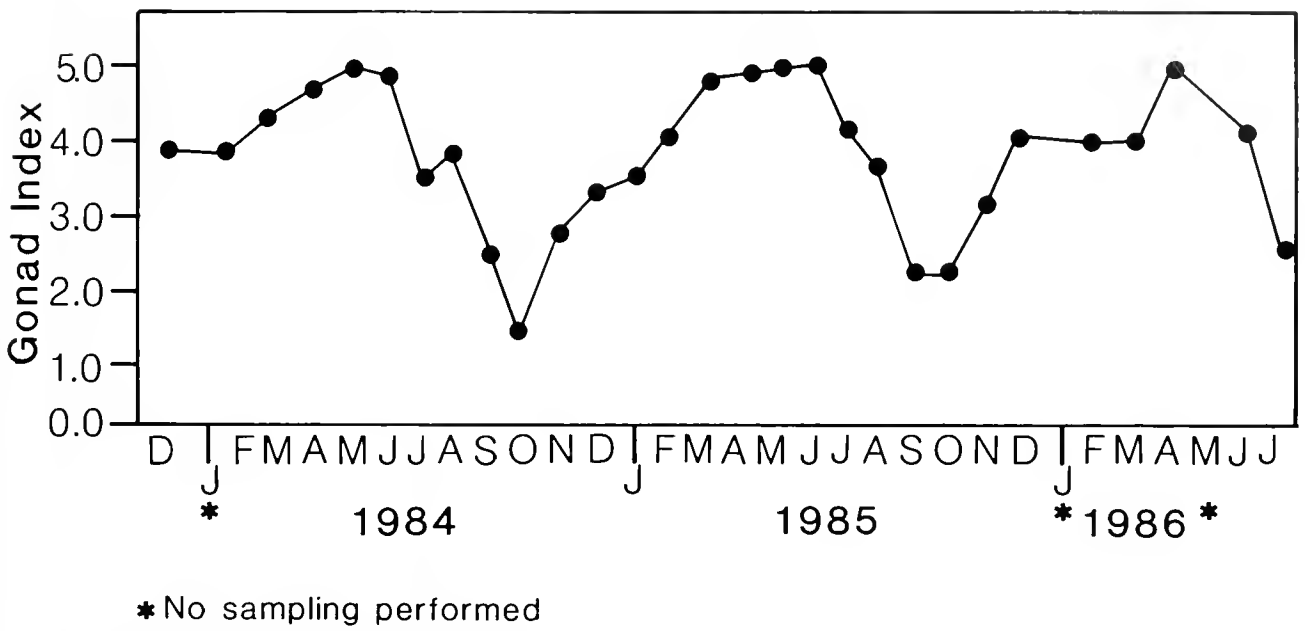


Figure 2. Monthly *Crassostrea virginica* gonad index values for Wassaw Sound, Georgia from December 1983 to July 1986.

tive (males = 27.8%; females = 66.7%) in June (Fig. 1). Gonad Index values rose from 4.29 to 4.83 during the same period (Fig. 2). Quantitative male data also displayed rising trends (maturation) during March–June (Fig. 3). Male gonad size increased from March to April. After a slight drop in May (not significant, NS, $p > 0.05$), it remained high through June (Fig. 3A). Similarly spermatogenic content rose to a peak value in June (Fig. 3B). A significant decline (19.7%) in spermatozoan levels during April–May was the first indication of oyster spawning activity during 1984 (Fig. 3C). Female quantitative data showed a continuous maturation of gonadal material from February through June (Fig. 4). Gonad area, oocyte content, oocyte number, and estimated oocyte diameter all indicate June as the period of peak female maturity during 1984 (Figs. 4A–D).

Qualitative data (Figs. 1 and 2) illustrated spawning activity during June–October. In June, only 5.6% of the population displayed spawning activity while 64.2%, 47.1%, and 75% were either Spawning or Advanced Spawning and Regressing during July, August, and September, respectively (Fig. 1). Gonad Index values reflected a similar pattern with declines from June to July and August to October (Fig. 2). A significant drop in spermatozoan levels during April–May (Fig. 3C), as mentioned above, signified the onset of male spawning during 1984. It would appear from gonad size (Fig. 3A) and spermatozoan content (Fig. 3C) levels that spawning extended from April to July, with April–May and June–July the most intense periods. Females displayed a somewhat later onset of spawning than males. This is indicated by significant declines in gonad area (38.9%), oocyte content (14%), and oocyte numbers (121.6) during July–September (Figs. 4A–C). Estimated oocyte diameter values (Fig. 4D) illustrated little by way of a spawning pattern throughout 1984 (see Discussion). The declines in gonad area, oocyte content, and numbers during April–May 1984 (Figs. 4A–C) were not interpreted as a spawning event because none of these declines were statistically significant (t-test; $p > 0.05$). A noticeable rise (0.30) in G.I. values from July to August was suggestive of some gonad redevelopment, but this was not supported by any male or female quantitative data (Figs. 3–4). October marked a period where inactive (66.7%) and spent individuals (27.8%) dominated the population (Fig. 1).

A new gametogenic cycle was detected by a rise in the percentage of Early Active specimens (all female) from November to December, while the G.I. rose (0.72) in the same period (Figs. 1–2). All three male quantitative parameters indicated a minor 'residual' spawning during November–December (Fig. 3). Given the relatively low gonad size value for November (48%) (Fig. 3A) and the low number of males involved (N = 2), we felt this event represented shedding of residual gametes by male stragglers. Similarly, the relatively large oocytes (26.7 μm) detected in October (Fig. 4D) are thought to represent residual unspawned gametes. The significant decline in estimated oocyte diameter from October (26.7 μm) to November (17.4 μm) was interpreted as oocyte resorption rather than spawning, in light of all other data (Figs. 2, 4A–C). Redevelopment occurred during October–November in female (Figs. 4A–C).

1985

Gametogenic development stalled somewhat during December 1984 to January 1985. Most of the population were

TABLE I.

Comparison between mean monthly oocyte diameters computed using the image analyser, which measured all oocytes (N) sectioned within a field of view, and microscopic measurements of 30 nucleolated oocytes per specimen. S = Significant differences (t−test).

	Crassostrea virginica mean monthly oocyte diameters (um).						
		Image Analyzer Data (All oocytes present in the field) (±SE)			Microscopic Data (N = 30) (Nucleolated oocytes only) (±SE)		
	N						
May 1984	89	20.7 um	(±0.5)		30.8 um	(±1.7)	
	230	16.1	(±0.6)		19.3	(±0.9)	
	129	15.8	(±0.3)		27.2	(±1.5)	
	134	21.3	(±0.7)		34.0	(±1.7)	
	222	25.7	(±2.2)		34.2	(±1.4)	
	89	14.9	(±0.3)		27.5	(±1.4)	
	24	16.6	(±1.0)		30.2	(±1.6)	
	65	14.8	(±0.9)		22.3	(±1.6)	
	133	19.7	(±0.5)		31.5	(±1.8)	
	117	17.7	(±0.4)		24.7	(±1.9)	
Mean	122 (±19)	18.2	(±1.1)	Vs.	28.2	(±1.6)	:S
June 1985	415	22.0	(±1.2)		33.7	(±1.0)	
	429	18.5	(±0.4)		30.5	(±1.2)	
	499	19.9	(±0.3)		37.2	(±1.3)	
	199	25.3	(±0.7)		33.8	(±1.6)	
	170	23.0	(±0.9)		37.0	(±0.9)	
	442	22.2	(±1.2)		38.5	(±1.4)	
	172	19.5	(±0.9)		34.2	(±1.2)	
	207	22.0	(±1.0)		32.3	(±1.4)	
	417	18.8	(±0.3)		32.7	(+1.3)	
	471	18.6	(±0.5)		35.2	(±1.2)	
	432	18.4	(±0.5)		32.5	(±1.2)	
	279	22.1	(±0.8)		38.5	(±1.5)	
	178	19.9	(±0.2)		35.7	(±0.9)	
Mean	332 (±36)	20.8	(±0.6)	Vs.	34.8	(±0.7)	:S

Early Active in December and January (Fig. 1) whereas G.I. values rose slightly from December to January (by 0.11, Fig. 2). By February 1985, 95% of oysters were Early Active and the G.I. rose to 4.05 (Figs. 1, 2). Gonad development and maturation continued from March through June (Figs. 3, 4). In March 79% of the population were Late Active while this figure had risen to 100% by June (Fig. 1). Quantitative male data (Fig. 3) were in close agreement with staging criteria (Fig. 1) and illustrated maturation of males from February through June. Gonad area and spermatogenic content rose sharply (63.7% and 34%, respectively) from February to June (Figs. 3A–B). Spermatozoa, which first appeared in appreciable quantities in March rose to peak levels by June (Fig. 3C). Females were shown to have matured earlier than males in 1985 with peak values for gonad area, oocyte content, and oocyte number in April (Figs. 4A–C). Estimated mean oocyte diameter values (Fig. 4D) plateaued during April–June also indicating female maturity was achieved by April.

Qualitative data indicated June–September as the oyster spawning season for 1985, with G.I. values dropping from June to September (by 2.78, Fig. 2). During July, 40% of the population was spawning while in August this value was 50% with another 10% Advanced Spawning and Regressing (Fig. 1). Statistically significant declines in gonad area (34.2%, Fig. 3A) and spermatozoan content (44.5%, Fig. 3C) during June–August delineated the male spawning period. Spermatogenic content levels also indicated declines during this period (NS, $p > 0.05$; Fig. 3B). There were non-significant ($p > 0.05$) declines in female gametogenic measurements from April to June (Figs. 4A–C). Statistically significant declines were recorded for gonad area (41.5%), oocyte content (26.4%), and egg numbers (192.7) during June–September (see Figs. 4A–C), the presumed major female spawning season for 1985. Estimated oocyte diameter values also declined significantly during this period (May = 21.3 μm, June = 20.8 μm–July = 18.7 μm, see Fig. 4D). September gonad area, oocyte content, and egg number values were also significantly lower than those for April (see Figs. 4A–C).

September–October was a period of relative stagnation, with population structure (Fig. 1) and G.I. (2.22–2.23) varying little. Minimum values for oocyte content (15.4%) and egg numbers (104) indicated a similar pattern of

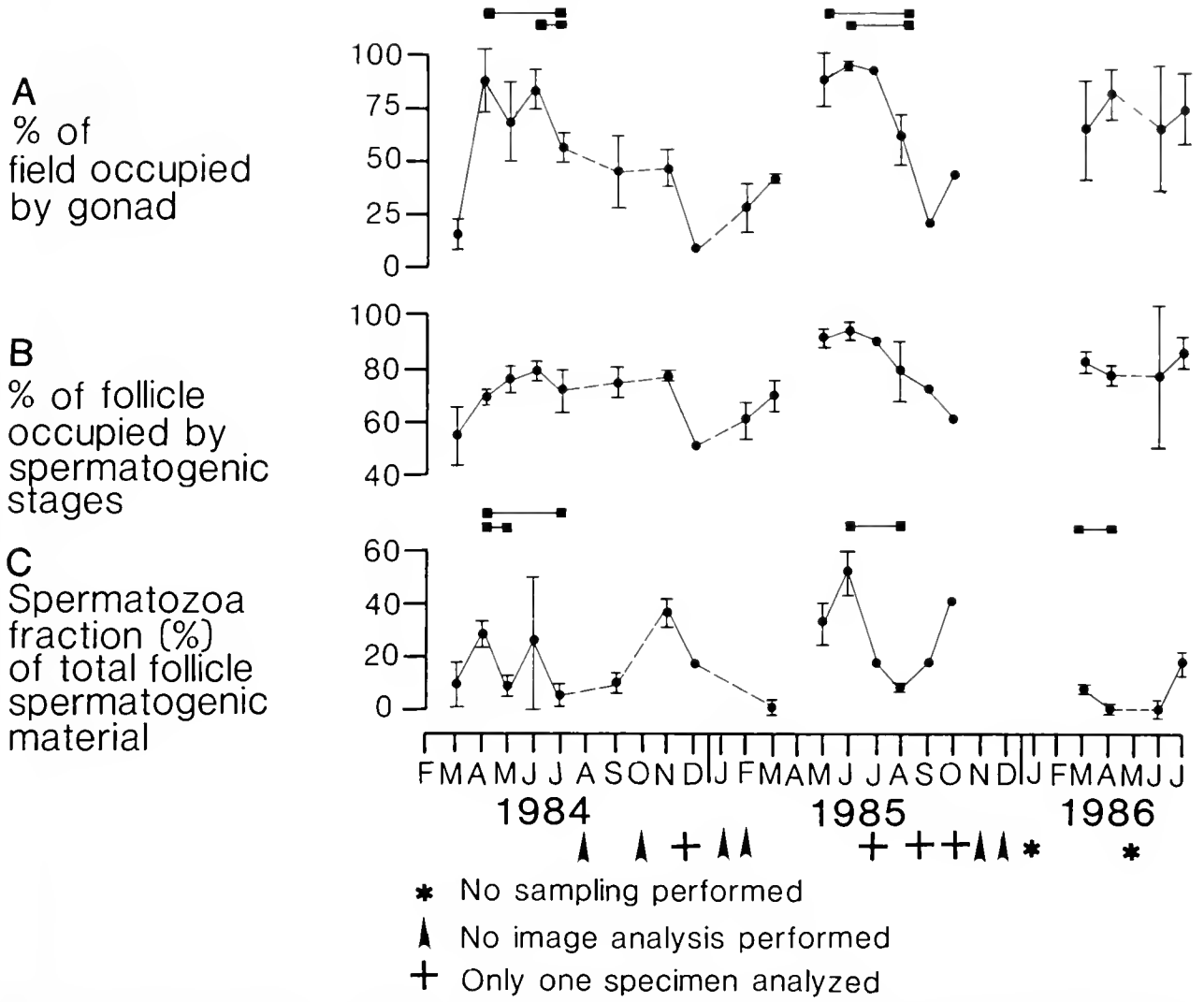


Figure 3. Composite of quantitative data (obtained using image analysis) representing the state of gonad condition for male American oysters in Wassaw Sound, Georgia (December 1983 to July 1986). A. Mean percentage of field analyzed occupied by gonad tissue. B. Mean percentage of follicle area occupied by spermatogenic stages. C. Mean percentage of total spermatogenic cell content occupied by spermatozoa. Vertical bars indicate periods of statistically significant (t-test) declines in the characteristic being measured.

spawned out individuals in October (Figs. 4B and C). Inactive oysters accounted for 16.6% and 26.3% of the population in October and November, respectively (Fig. 1). November–December marked a period of relatively rapid gametogenic redevelopment for oysters (compared to 1984), when the G.I. rose from 3.15 to 4.05. Early Active oysters accounted for 52.6% and 95% of the population in November and December, respectively (Fig. 1).

1986

Another winter cessation of gametogenic development was evident during December 1985–March 1986. Early active specimens continued to dominate during this period (Fig. 1) while the G.I. remained stable at *ca.* 4.00 (Fig. 2). March–April, on the other hand, marked a period of rapid maturation with 100% of oysters Late Active and the G.I. at its maximum level (5.00). While gonad size (Fig. 3A) increased (March to April by 18.1%; NS, $p > 0.05$), spermatogenic cells (Fig. 3B) declined (March to April by 5.8%; NS, $p > 0.05$), as did spermatozoan levels (Fig. 3C) (March to April by 7.5%; S, $p < 0.05$). While the significant decline in spermatozoan content signified spawning, the relatively low spermatozoan levels involved designate it as a minor event. Increased female gonad area (46.3%), oocyte content (13.8%), and oocyte number (87.4) showed maturation during March–April (see Figs. 4A–C). Estimated mean oocyte diameters remained stable (March = 17.6 μm, April = 17.9 μm) during this period (Fig. 4D).

Qualitative data indicated an oyster spawn occurred during April–July 1986. Spawning individuals accounted

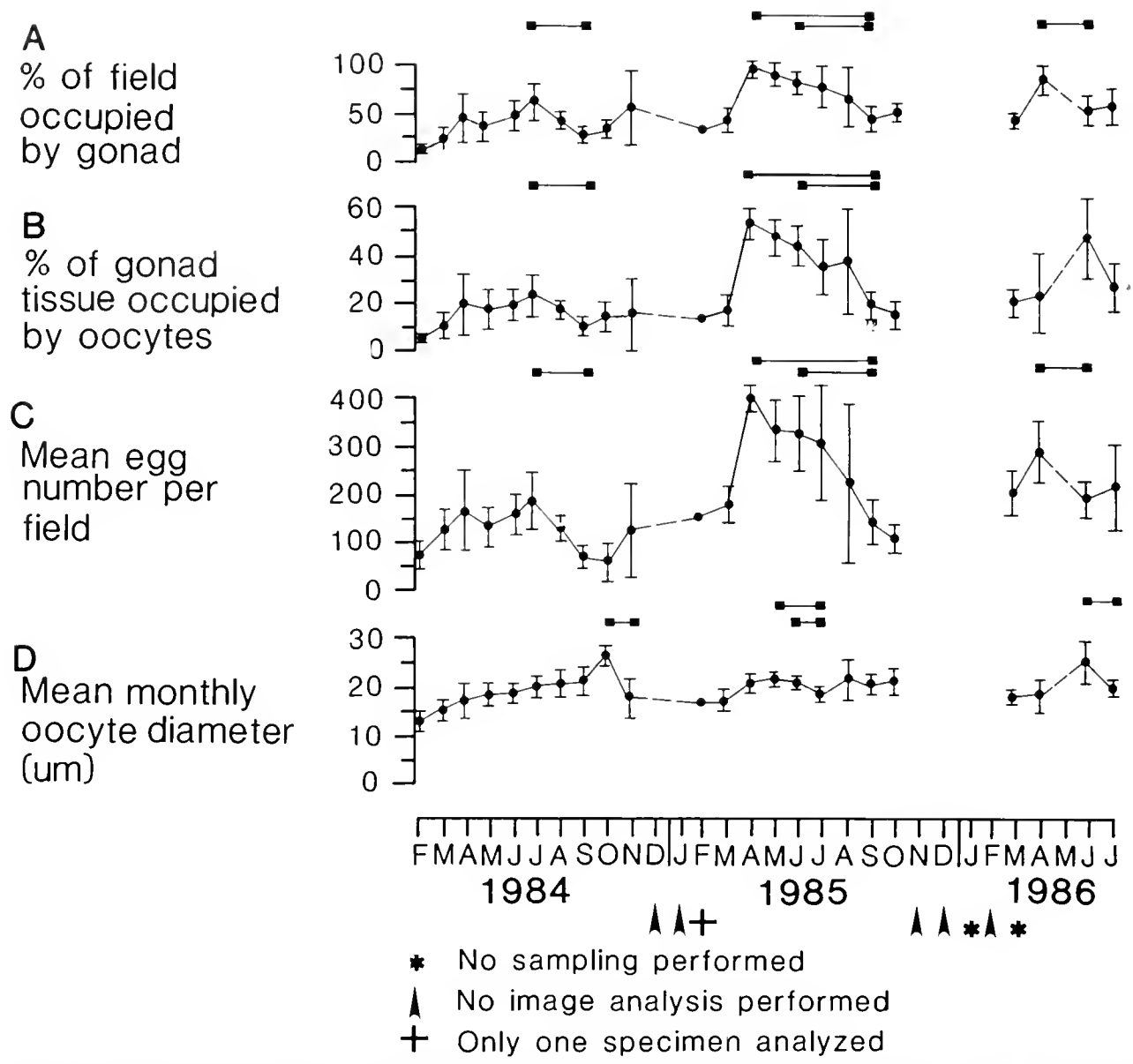


Figure 4. Composite of quantitative data (obtained using image analysis) representing the state of gonad condition for female American oysters in Wassaw Sound, Georgia (December 1983 to July 1986). A. Mean percentage of field analyzed occupied by gonad tissues. B. Mean percentage of gonad tissues occupied by oocytes. C. Mean number of eggs present per field analyzed. D. Mean monthly oocyte diameters. Vertical bars represent two standard errors about the mean. Horizontal bars indicate periods of statistically significant (t-test) declines in the parameter being measured.

for 42.1% of all oysters sampled in June, when 57.9% were Late Active (females). In July, the number of spawning oysters rose to 50% of the population, while another 38.9% consisted of Late Spawning and Regressing specimens (Fig. 1). The G.I. fell from 5.00 (April) to 4.16 (June) and 2.61 (July), indicating June–July as the period of heaviest spawning activity (Fig. 2). Declining trends (all NS, $p > 0.05$) during April–June for all male gametogenic measurements (Figs. 3A–C) indicated a minor male spawning occurred earlier than the female event (with peak activity in May and June). Quantitative female data identified April–June as the period of most intense female spawning. There were significant declines in gonad area (32.8%) and oocyte numbers (98.3) during this period (Fig. 4A and C). The rising trends in oocyte content and estimated mean oocyte diameters for this period were both non-significant ($p > 0.05$) and were thus not considered as contradictory to the evidence for spawning activity (see Figs. 4B and D). Female quantitative data for June–July does not provide any conclusive findings, with non-significant changes in gonad area, oocyte content, and oocyte number values (Figs. 4A–C). However, the significant decline in estimated

mean oocyte diameter (25.5 μm–19.37 μm) during this period was suggestive of some redevelopment as was the apparent increase in egg numbers (Figs. 4C–D). Male quantitative data for June–July would also suggest gametogenic development with increases evident in gonad area (65.8%–75.5%; NS, alpha = 95%), spermatogenic cells (77.6%–88.7%; NS, alpha = 95%), and spermatozoa (1.2%–19.2%; S, alpha = 95%) (see Figs. 3A–C).

Table 1 illustrates how estimated mean oocyte diameters for May 1984 and June 1985 computed using the Omnicon image analyzer were significantly (t-test) lower than those calculated from microscopic measurements (by operator) of nucleolated eggs only. The discrepancy was 9.93 μm in 1984 and 13.98 μm in 1985. A linear regression coefficient was compiled for each year's data. The May 1984 data gave a correlation coefficient = 0.74 [y = 9.18 (±6.2) + 1.04 (±0.3) x], where y is measured nucleolated egg diameter and x is omnicon estimated total egg diameter. The June 1985 data shows a lower correlation coefficient of 0.35 and lower slope [y = 26.3 (±3.8) + 0.4 (±0.3) x].

Female oysters outnumbered males approximately 3 to 1 (size range 5.7–10.6 cm, Fig. 5), with only one functional hermaphrodite (a ripe specimen in May 1984) detected.

DISCUSSION

A detailed insight into the reproductive cycle of oysters in Georgia can be ascertained from the qualitative and quantitative data presented in this report. An extended unimodal gametogenic cycle was evident each year (1984–85). Gametogenesis commenced in November–December, stalled somewhat during the cooler months of December–February/March and recommenced in February/March with maturity attained by April (males 1984, females 1985) through June (females 1984, males 1985). Spawning extended from April through October in 1984, with peak male spawning during April–May and June–July, while females spawned intensely during June–July and August–October. During 1985, spawning lasted from June–September, with intense male activity in June–August and female during June–September. Data for the first seven months of 1986 show spawning of males from March to June and females from April to July.

In addition to the differences in timing of spawning during 1984 and 1985, there were several indications of considerably higher levels of gametogenic development in the latter year. Quantitative female data (Figs. 4A–D), with significantly higher levels of gonad area (2.2x), oocyte content (2.4x), and oocyte numbers (2.2x) (but not egg size), during periods of peak maturity in 1985 as opposed to 1984, clearly illustrate this point (see Table 2 and Fig. 4). Similarly the area occupied by spermatogenic cells was significantly higher (1.2x) among males in June 1985 than June 1984 (see Fig. 3B and Table 2). Further indications of this trend can be obtained from a volumetric estimate (v) of egg production per field analyzed, $v = 4/3\ \pi r^3 N$ where r = mean egg radius and N = mean egg number

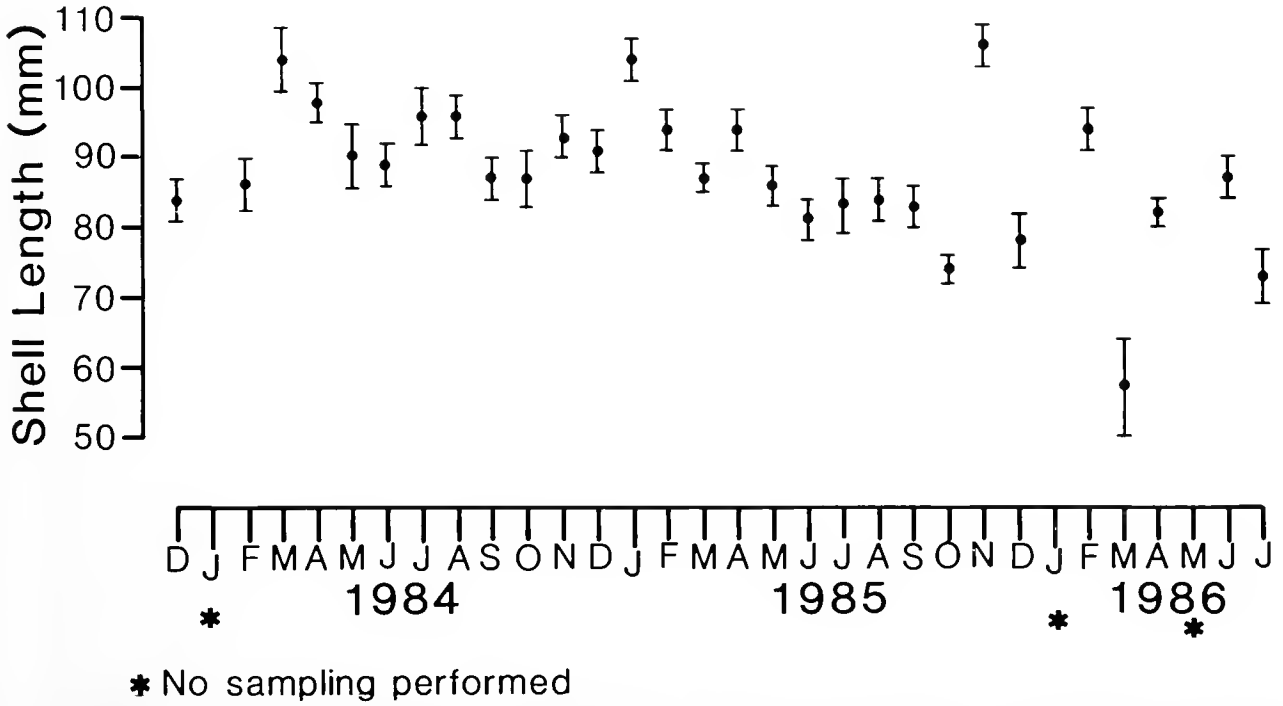


Figure 5. Mean size (with 1 standard error) of *Crassostrea virginica* processed for histological examination of reproductive condition from House Creek, Wassaw Sound, Georgia (December 1983 to July 1986).

per field analyzed. We obtained production figures of 218,993, and 270,121 μm^3 eggs per field analyzed in May and June 1984, respectively, while figures for 1985 show 960,983 and 877,894 μm^3 (3.76 times higher) eggs per field analyzed in May and June, respectively. The magnitude of oocyte production as portrayed by volumetric estimates in 1985 as opposed to 1984 (almost twice that of area comparisons) illustrates how volumetric evaluations are more meaningful than area or diameter values as suggested by Gallager and Mann (1986). This is especially important when studying the nutrient levels stored in oocytes. Ocular micrometer measurements of oocyte diameter (Table 1) were also significantly (t-test) larger at maturity in 1985 than in 1984. Additional indications of higher gametogenic activity levels during 1985 can be obtained from the levels of Inactive oysters, with 1984 levels more than 2 times higher than those of 1985 (see Fig. 1). These observations of significantly higher gametogenic output for oysters during 1985 is similar (although of much greater proportions) to results obtained for hard clams from the same site (Heffernan et al. 1989). Comparisons of gametogenic output based on area measurements and volumetric egg production estimates such as those reported herein should be specific for the various size classes of the species being studied. Several works have shown fecundity levels to be closely related to body size (e.g. Peterson 1983, Vahl 1985, Bricelj et al. 1987, Heffernan and Keegan 1988).

The cause(s) of these temporal differences in reproductive potential is not understood at present. However, Bayne et al. (1978), Newell et al. (1982), Borrero (1987), and Barber et al. (1988b) have suggested that conditions of temperature and/or nutritive stress play a major role in the reproductive cycles of the mussels, *Mytilus edulis* (L.) and *Geukensia demissa* (Dillwyn), and the giant scallop, *Placopecten magellanicus* (Gmelin). Temporal differences in gametogenic production among Georgia oysters and hard clams may be related to varying quantity and quality of available food. However, lacking detailed information on natural phytoplankton populations during this period, this interpretation must remain speculative.

Recent epizootics among Georgia oysters raise the question of the influence of parasitism on the oyster gametogenic cycle. While *Perkinsus marinus* has been identified as the major causitive agent for oyster mortalities in 1986 (Dr. S. A. Stevens, Georgia Department of Natural Resources and Mr. C. A. Farley, Oxford Laboratory, Maryland), *Haplosporidium nelsoni* (MSX) has also been detected. Bayne (1975) suggested that parasitism might reduce fecundity while Barber et al. (1988a) have shown a reduction in fecundity values associated with MSX infection intensity. Ford and Figueras (1988) reported reduced egg size among MSX infected but recovering oysters due to delayed gametogenesis. Given the observed reductions in oyster fecundity, egg size, and the higher Inactive levels in 1984 as opposed to 1985 (this study), the influence of parasitism on Georgia oyster gametogenesis must be contemplated. In an effort to access this, all histological material used in this study is being examined for pathological condition.

Overall, the image analysis system used in this study has been of substantial benefit, especially in quantifying gametogenic production (see above). While the system had its limitations when dealing with early development (a problem also encountered by Barber et al. 1988a using a different image analysis system), it still successfully charted all the major gametogenic events once development was underway. Early development dynamics were adequately assessed using standard qualitative techniques. The limitations of the estimated mean oocyte diameter values obtained from the image analyzer have been critically assessed previously (Heffernan and Walker 1989; Heffernan et al. 1989). Further indications of their "inaccuracy" can be obtained from the fact that estimated diameter means were not significantly different (t-test) at maturity in 1985 as opposed to 1984, while occular micrometer analyses clearly demonstrated significantly larger eggs in 1985 (Table 1). The significantly larger (t-test) number of eggs per field (Fig. 4C) during 1985 (and the resultant compression factor, see Table 1, Heffernan et al 1989) probably led to a depression of the estimated mean value in 1985 (to a level not significantly different from that in 1984). It is worth noting that the temporal differences in gametogenic production levels so clearly demonstrated by the image analysis study (see above) would have been overlooked had

TABLE 2.

Statistical analysis of various *C. virginica* quantitative male and female gametogenic parameters. Individual data points were used in all cases and percentage specimen values were arcsin transformed prior to analysis (T-test).

1984 vs. 1985 Quantitative Analysis *Female Data*	T. stat.	d.f.	alpha
% of Field Occupied by Gonad			
April 1984 vs. April 1985 (44.6%) (99.5%)	6.712	7	2.365
% of Gonad Occupied by Oocytes			
July 1984 vs. April 1985 (22.3%) (53.8%)	6.083	8	2.306
Mean Egg Number Per Field			
July 1984 vs. April 1985 (183.5) (400.5)	18.315	6	2.447
Male Data			
% of Follicle Occupied by Spermatogenic Stages			
June 1984 vs. June 1985 (7[illegible]%) (94.7%)	8.178	10	2.228

we focused solely on conventional staging criteria and gonad indices (see Figs. 1–4).

The general pattern of gametogenesis in Georgia *Crassostrea virginica* reported in this study is in line with the findings of several workers dealing with oysters in the southern United States (see Galtsoff 1964 and Kennedy and Krantz 1982 for literature reviews). Bimodal spawning and setting peaks have been reported in Spring and Fall in the Gulf of Mexico from Florida (Hayes and Menzel 1981), Louisiana (Hopkins et al. 1953), Texas (Hopkins 1931), and Mexico (Rogers and Garcia-Cubas 1981). Similar reproductive patterns have been indicated for South Carolina (McNulty 1953) and North Carolina (Chestnut and Fahy 1952). Spawning activity of oysters has been demonstrated in this study for Georgia from April through September–October (with highest intensity of female activity in July–September) without any distinct Spring–Summer and Fall peaks. However, setting peaks can occur as Galtsoff (1964) indicated, due to either repeated spawning from the same animals or different animals spawning at different times. Bimodal setting patterns have also been reported in more northern waters (Loosanoff 1966 in Long Island Sound; Shaw 1967, in Chesapeake Bay; Kennedy and Battle 1964, Prince Edward Island, Canada), with the onset of gametogenesis generally occurring later in the year as one goes north.

The low incidence of hermaphrodites (1 out of 496:0.2%) detected in this study is common for *C. virginica* (see Kennedy 1983), while considerably lower than that reported for Mexico (2%) by Rogers and Garcia-Cubas (1981). The sex ratios detected for Georgia oysters are in agreement with the findings of several workers which showed a tendency for female domination in oyster populations with increased size and age (e.g., Coe 1943; Nascimento et al. 1980; Kennedy 1983).

ACKNOWLEDGMENTS

This work was funded by Georgia Sea Grant/Project number NA84AA-D-00072. The assistance of Dr. A. Falek and Dr. D. Shaffer and their kindness in making the Omnicon image analyzer at Emory University so readily available is heartily acknowledged. Thanks to Mr. M. Pline, Mr. D. Jacobi, Mr. J. Griffin and Ms. T. Suddeth for their contributions to the Omnicon analysis work. Dr. D. Gillespie and Dr. J. Crenshaw, Jr. helped greatly with statistical and computational matters. We thank Dr. M. Gibbons, Dr. S. Ray, and Dr. A. Eversole for their contributions and comments during the drafting of this manuscript. Mrs. J. Haley typed the manuscript and Ms. A. Boyette and Ms. S. McIntosh prepared the graphics.

LITERATURE CITED

Bancroft, J. & A. Stevens. 1977. Theory and practice of histological techniques. Churchill Livingston, Edinburgh. 436 p.

Barber, B. J., S. E. Ford & H. H. Haskin. 1988a. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy and metabolism. I. Condition index and relative fecundity. *J. Shellfish Res.* 7:25–31.

Barber, B. J., R. Getchell, S. Shumway & D. Schick. 1988b. Reduced fecundity in a deep-water population of the giant scallop *Placopecten magellanicus* in the Gulf of Maine, USA. *Mar. Ecol. Prog. Ser.* 42:207–212.

Bayne, B. L. 1975. Reproduction in bivalve molluscs under environmental stress. Vernberg, F. J. (ed.). *Physiological Ecology of Estuarine Organisms*. University of South Carolina Press, Columbia. SC: 259–277 p.

Bayne, B. L., D. L. Holland, M. N. Moore, D. M. Lowe & J. Widdows. 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *J. Mar. Biol. Assoc. U.K.* 58:825–841.

Borrero, F. J. 1987. Tidal height and gametogenesis: Reproductive variation among populations of *Geukensia demissa*. *Biol. Bull.* 173:160–168.

Bricelj, V. M., J. Epp & R. E. Malouf. 1987. Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten irradians*. *Mar. Ecol. Prog. Ser.* 36:123–137.

Butler, P. A. 1949. Gametogenesis in the oyster under conditions of depressed salinities. *Biol. Bull.* 96:263–269.

Chestnut, A. F. & W. E. Fahy. 1952. Studies on the setting intensities of oysters in Bogue Sound, North Carolina. *Proc. Natl. Shellfish. Assoc.* 43:79–89.

Coe, W. R. 1932. Sexual phases in the American Oyster (*Ostrea virginica*). *Biol. Bull.* 63:419–441.

Coe, W. R. 1943. Sexual differentiation in mollusks I. Pelecypods. *Quar. Rev. Biol.* 18:154–164.

Durant, J. 1968. The effects of temperature and salinity upon the gonadal cycle of *Crassostrea virginica* (Gmelin) in Georgia waters. Feasibility study of methods for improving oyster production in Georgia. Linton, T. L. (ed.). Marine Fish. Divis., Georgia Game and Fish Comm. and University of Georgia. Completion Report 2-10-R, Brunswick, Georgia. 176 p.

Ford, S. E. & A. J. Figueras. 1988. Effects of sublethal infection by the parasite *Haplosporidium nelsoni* (MSX) on gametogenesis, spawning and sex ratios of oysters in Delaware Bay, USA. *J. Aquat. Diseases:* 4:121–133.

Gallager, S. C. & R. Mann. 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. *Aquaculture* 56:105–121.

Galtsoff, P. S. 1964. The American oyster, *Crassostrea virginica* Gmelin. *U.S. Fish and Wildl. Ser. Fish. Bull.* 64:1–480.

Harris, D. C. 1980. Survey of the intertidal and subtidal oyster resources of the Georgia coast. Georgia Department of Natural Resources, Coastal Resources Division, Brunswick, Georgia (Project Number 2-234-R) 44 p.

Hayes, P. F. & R. W. Menzel. 1981. The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Guld of Mexico and its implications for population recruitment. *Biol. Bull.* 160:80–88.

Hefferman, P. & B. F. Keegan. 1988. Quantitative and ultrastructural studies on the reproductive biology of *Pholoe minuta* (Fabricius) with special reference to functional morphology. *Mar. Biol.* 99(2):203–214.

Hefferman, P. B. & R. L. Walker. 1988. Preliminary observations of

oyster pearl net cultivation in coastal Georgia. *Northeast Gulf Sci.* 10(1). 33–43.

Heffernan, P. B. & R. L. Walker. 1988b. Quantitative image analysis methods for use in histological studies of bivalve reproduction. *J. Moll. Stud.* 55:135–137.

Heffernan, P. B., R. L. Walker & J. L. Carr. 1989. Qualitative and quantitative studies on the gametogenic cycles of three marine bivalves in Wassaw Sound, Georgia. *I Mercenaria mercenaria. J. Shellfish Res.* 8:51–60.

Hopkins, A. E. 1931. Factors influencing the spawning and setting of oysters in Galveston Bay, Texas. *Bull. Bur. of Fish.* 47:55–58.

Hopkins, S. H., J. G. Mackin & R. W. Menzel. 1953. The annual cycle of reproduction, growth, and fattening in Louisiana oysters. *Proc. Natl. Shellfish. Assoc.* 44:39–50.

Kennedy, A. V. & H. I. Battle. 1964. Cyclic changes in the gonad of the American oyster, *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 42:305–321.

Kennedy, V. 1977. Reproduction in *Mytilus edulis aoteanus* and *Aulaconmya maoriana* (Mollusca:Bivalvia) from Taylor's Mistake, New Zealand. *N. Z. J. Mar. and Freshw. Res.* 11(2):253–267.

Kennedy, V. S. 1983. Sex ratios in oysters, emphasizing *Crassostrea virginica* from Chesapeake Bay, Maryland. *The Veliger* 25(4):329–338.

Kennedy, V. S. & L. B. Krantz. 1982. Comparative gametogenic and spawning patterns of the oyster, *Crassostrea virginico* (Gmelin), in central Chesapeake Bay. *J. Shellfish Res.* 2:133–140.

Loosanoff, V. L. 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica*, of Long Island Sound. *Biol. Bull.* 83:195–206.

Loosanoff, V. L. 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. *Biol. Bull.* 129:546–561.

Loosanoff, V. L. 1966. Time and intensity of setting of the oyster, *Crassostrea virginica*, in Long Island Sound. *Biol. Bull.* 129:546–561.

McNulty, J. K. 1953. Seasonal and vertical patterns of oyster setting off Wadmalaw Island, SC. *Contribution from Bears Bluff Laboratory.* No. 15, 17 p.

Menzel, R. W. 1951. Early sexual development and growth of the American oyster in Louisiana waters. *Science* 113:719–721.

Nascimento, I. A., M. I. Ramos & A. E. Dos Sastos. 1980. Sex-ratio e ocorrencia de hermaproditismo en *Crassostrea rhizophorae* (Guilding, 1828). Simposio Braseiro de Aquicultura, Academia Brasiteria de Ciencias Anais pp. 385–394.

Newell, R. I., T. J. Hilbish, R. K. Koehn & C. J. Newell. 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* (Bivalvia, Mytilidae) from localities on the east coast of the United States. *Biol. Bull.* 162:299–310.

Peterson, C. H. 1983. A concept of quantitative reproductive senility: application to the hard clam, *Mercenaria mercenaria* (L.). *Oecologia* 58:164–168.

Rogers, P. & A. Garcia-Cubas. 1981. Evolution gonadica a nivel histologico del ostion *Crassostrea virginica* (Gmelin, 1791) del sistema fluvio lagunar Apasta-Pom, Laguna de Terminos, Campeche, Mexico. *An. Inst. Cien. Del Mar y Limnol. Univ. Nacl. Auton. Mexico* 8(1):21–42.

Shaw, W. N. 1967. Seasonal fouling and oyster setting on asbestos plates in Broad Creek, Talbot County, MD, 1963–65. *Ches. Sci.* 8:228–236.

Steel, R. & J. Torrie. 1960. *Principals and procedures of statistics.* McGraw-Hill Book Co., Inc. New York. 481 p.

Vahl, O. 1985. Size specific reproductive effort in *Chlamys islandica:* Reproductive senility or stabilizing selection? Proceedings of the 19th European Marine Biology Symposium. P. E. Gibbs (ed.). Cambridge University Press. Cambridge. 521–527 p.

Zar, J. H. 1974. *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall, Inc. 620 p.

Journal of Shellfish Research, Vol. 8, No. 1, 71–82, 1989.

ANATOMICAL FEATURES IN HISTOLOGICAL SECTIONS OF *CRASSOSTREA VIRGINICA* (GMELIN, 1791) AS AN AID IN MEASUREMENTS OF GONAD AREA FOR REPRODUCTIVE ASSESSMENT[1]

REINALDO MORALES-ALAMO AND ROGER MANN
Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062

ABSTRACT The relationship between gonad area in transverse histological sections of the American oyster *Crassostrea virginica* (Gmelin 1790) and body location from which the section was cut was studied in specimens collected from four stations in the James River, Virginia in 1984 and 1986. Gonad area, expressed as percentage of total body area, increases in an antero-posterior direction; this requires use of sections from the same body location in comparisons between oysters. Approximate body locations, identified according to the anatomy and arrangement of the internal organs in the sections, were grouped into five types with similar gonad area percentages. One of those types is uniquely suitable for identification of a specific body location because it includes an easily recognizable pair of H-shaped structures corresponding to the posterior appendix of the anterior stomach caecum; furthermore, the recommended section type can be readily found on the whole oyster because it is located close to the junction of the gills and the labial palps. Gamete volume fraction (GVF) was positively correlated with percent gonad area (PGA) in most of the section types at three of the stations, suggesting that either measurement may be used to estimate the relative gonadal development in oysters. Differences between collection dates at the fourth station indicated what external factors may disrupt the correlation. It is suggested that gonad area measurements from a series of selected histological sections could be combined with gamete density measurements to estimate total gamete production by an oyster.

KEY WORDS: *Crassostrea virginica*, histological sections, gonad area, anatomy

INTRODUCTION

Gametogenesis in oysters produces an increase in the transverse thickness of the gonad layer located between the mantle and the digestive diverticula (Coe 1932, Galtsoff 1938, Loosanoff 1942). The reverse process ensues as oysters spawn. Measurements of gonad thickness complement estimates of gamete maturation as indicators of the extent to which gametogenesis has progressed in an oyster. Several investigators measured changes in gonad thickness in whole unmounted transverse sections of *Crassostrea virginica* (Gmelin 1790) throughout the reproductive cycle in different years and locations (Loosanoff and Engel 1940, Loosanoff and Nomejko 1951, Hopkins et al. 1953, Loosanoff 1965). Kennedy and Battle (1964) modified those early attempts by measuring the width of the gonad in histological transverse sections of *C. virginica* and relating it to total body width in the section. A transverse section in *C. virginica* is defined here as the plane perpendicular to the antero-posterior axis of the body. The antero-posterior axis passes through the mouth and the adductor muscle or the anus (Jackson 1890, as cited by Yonge 1953).

More recently, other investigators have quantified gonad development in terms of the planar area occupied by gonad tissue in histological sections of oysters and other bivalve molluscs (Table 1). Use of gonad area measurements on histological preparations for comparative purposes requires specification of the location in the animal's body from which the section was taken because gonad area changes with body location (Galtsoff 1964, Loosanoff 1965, Iwantsch 1970, Perdue 1983). Serious difficulties in interpretation of the data can arise if the sections from different animals come from widely separated parts of the body.

Examination of histological transverse sections of *C. virginica* oysters collected in 1984 from the James River, Virginia, indicated that variations in the area occupied by the gonad tissue were related to differences in the anatomical features of the visceral organs. The primary objective of this investigation was to identify the relationship between gonad area and the anatomy and arrangement of organs in transverse sections from different parts of the body. The relationship between gonad area and gamete volume fraction was also investigated.

MATERIALS AND METHODS

Oysters used in this study were collected on July 11 and 12 and August 21, 1984, and on August 12, 1986, from oyster beds in the James River, Virginia, the southernmost tributary of the Chesapeake Bay (Fig. 1). Fifty oysters having a shell height greater than 40 mm were selected at random in 1984 from each of four stations sampled (Nansemond Ridge, Naseway Shoal, Wreck Shoal and Horsehead Rock). Shell height is defined here as the distance between the hinge end of the shell and the opposite end. Transverse cuts were made with a scalpel through the mid-visceral region of each oyster (without prior determination of a precise location for the cuts) to obtain a segment approximately 5–8 mm thick; the segment was then placed in Davidson's AFA fixative and embedded in paraffin after

[1]Contribution No. 1527 from the Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

TABLE 1.

Literature references to use of gonad area, width or thickness in studies of the reproductive development of bivalve molluscs. Thickness refers to measurements made directly on unmounted transverse sections. Width refers to thickness measurements made on mounted transverse sections.

Species	Type of measurement	Citation
Crassostrea virginica	Thickness (R)	Galtsoff (1938)
C. virginica	Thickness	Loosanoff and Engle (1940)
C. virginica	Thickness (R)	Loosanoff (1942)
C. virginica	Thickness	Loosanoff and Engle (1942)
C. virginica	Thickness	Loosanoff and Nomejko (1951)
C. virginica	Thickness	Hopkins et al. (1953)
C. virginica	Width	Kennedy and Battle (1964)
C. virginica	Thickness	Loosanoff (1965)
Mercenaria mercenaria	Area	Porter (1967)
Mytilus edulis	Area	Iwantsch (1970)
M. mercenaria	Area	Keck et al. (1975)
C. virginica	Area	Tinsman et al. (1976)
Mya arenaria	Area	Brousseau (1978)
Crassostrea gigas	Area	Mori (1979)
M. mercenaria	Area	Eversole et al. (1980)
C. gigas	Area	Lannan (1980)
C. gigas	Area	Perdue et al. (1981)
C. gigas	Area	Perdue (1983)
C. gigas	Area	Perdue & Erickson (1984)
C. rivularis	Area	Perdue & Erickson (1984)
O. edulis	Area	Wilson and Simons (1985)
C. gigas	Area	Allen and Downing (1986)
C. gigas	Area	Dinamani (1987)
C. virginica	Area	Barber et al. (1988)

R = Text reference only; no measurements made.

dehydration and clearance through an alcohol:xylene series. Sections 6 μm-thick were cut, mounted and stained with Harris' haematoxylin and eosin. No attempt was made to orient all segments in the same antero-posterior direction before embedding.

Serial sections were prepared from each of 10 oysters collected from the Wreck Shoal bed in August 1986. Five transverse segments were cut from each oyster. Four of the segments, located between the anterior end of the body (corresponding to the shell hinge location) and the pericardial cavity, were of approximately the same width in proportion to the size of the animal. The fifth segment, posterior to the pericardial cavity, was discarded. The cut between the first and second segments at the anterior end of the series was made at the junction of the labial palps and the gills. All segments were placed in the embedding containers with the posterior face up. Several 6-μm sections were cut from each of the segments, starting at the posterior end, usually at intervals of 0.3 or 0.6 mm. Additional sections were cut from some of the segments when needed for clarification of sequential changes in organ arrangement.

Sex of each oyster was recorded and reproductive condition of the gonad was assessed by estimation of the gamete volume fraction (GVF) using point-count volumetry (Chalkey 1943, Weibel et al. 1966, Bayne et al. 1978). Gonad and total body area of individual oysters were determined by projecting the section image (magnified 13 times) to a sheet of paper on a table and tracking separately the outlines of the body and the gonad with an electronic digitizing planimeter. The outline of the body was traced following the outer margin of the mantle and the inner margin of the epibranchial chambers at the posterior end. The outline of the gonad was traced following its outer and inner margins; interstitial spaces between follicles within the gonad were included only to the extent allowed by the precision of the plainmeter's tracking head. The proportion of the total body area occupied by the gonad ($\times 100$) was termed the percent gonad area (PGA).

The conventional characterization of the hinge area in oyster shells as dorsal (Galtsoff 1964, Elston 1980) is disregarded here for specification of the directional relationships in the oyster body. Instead, we adopted the comparative anatomy approach advocated by Stasek (1963) and the attendant definition of the antero-posterior axis in *C. virginica* as the long axis passing through the mouth and the adductor muscle, or alternatively through the anus (Jackson 1890, cited by Yonge 1953; Fig. 2). The axis approximately perpendicular to the antero-posterior axis is then defined as the dorso-ventral axis. In accordance with Stasek's proposal, the labial palps and most of the gills are part of the ventral half of the body and body areas in the opposite half of the body (including the rectum and the

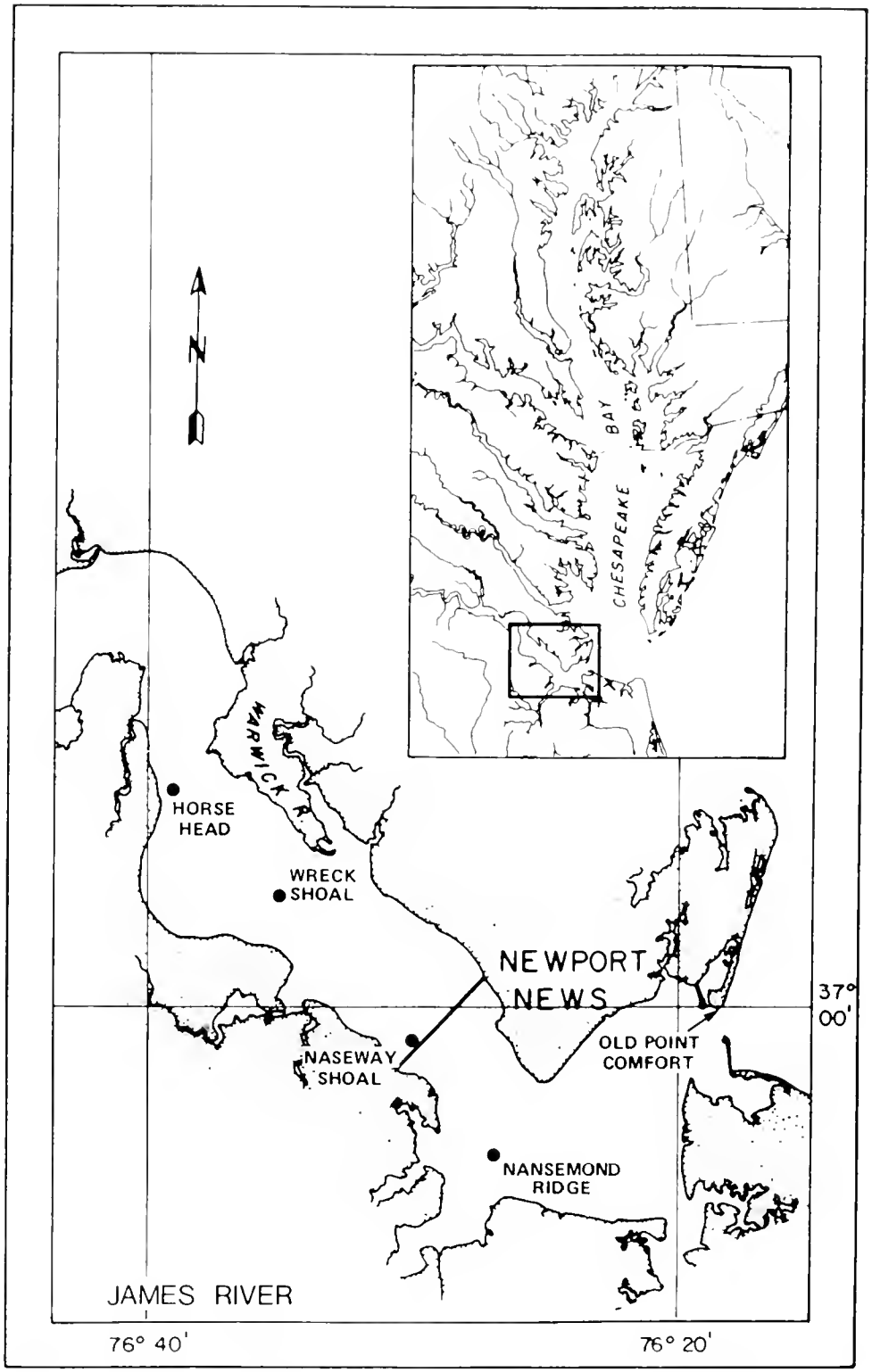


Figure 1. Lower estuary of the James River, Virginia, showing location of the oyster beds from which oysters were collected for this study.

promyal chamber) are part of the dorsal half. Based on these axis definitions, our sections were cut through the transverse plane.

Figures in Shaw and Battle (1957) and Galtsoff (1964) were used to identify visceral organs in the sections. Reference was made most frequently to the morphological descriptions of the stomach given by Shaw and Battle (1957) because they are more detailed than those of Galtsoff (1964). It was difficult to obtain a strict correspondence in details between the histological sections and Fig. 2; there-

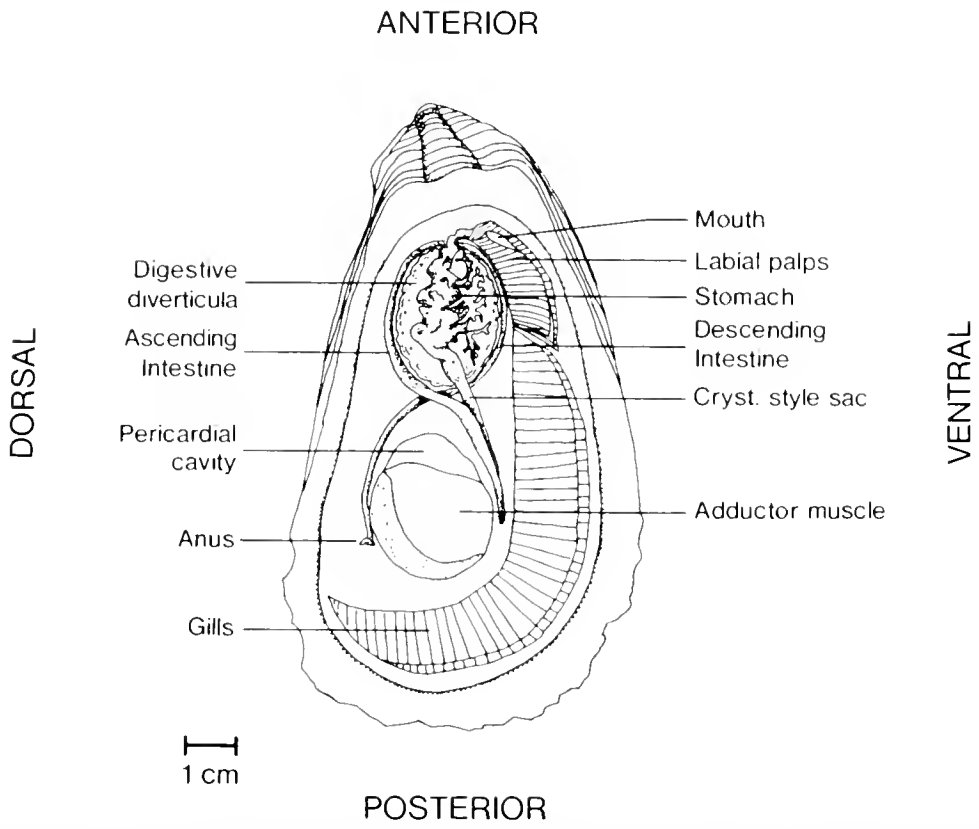


Figure 2. Diagram of the digestive system of *Crassostrea virginica* exposed on the right side by removal of the mantle and surrounding connective tissue after injection of latex (from Galtsoff 1964). Directional body axes based on the comparative anatomy approach proposed by Stasek (1963).

fore, that figure was only used as a general guide to the gross anatomy of the viscera in *C. virginica*.

The serial sections from oysters collected in 1986 were arranged in an antero-posterior direction and grouped into 13 types. The sections of oysters from the July and August 1984 collections were compared with those 13 section types and assigned to the type to which they most closely corresponded. Only sections 2 through 10 were considered essential to this study and were the only ones subjected to analysis. The other sections were included in drawings and descriptions to serve as references. Among types 2 through 10 in the 1986 oysters, adjacent sections with little or no apparent difference in gonad area among them were grouped together as follows: 2–4, 5–6, 7–8, 9 and 10. Mean gonad areas of these groups were compared using one-way ANOVA and Scheffe's multiple contrast test (Zar 1984). Sections from the 1984 collections were also combined into the same type groups for statistical analysis of gonad area and GVF measurements.

Individual plots of GVF against PGA in male and female oysters collected in 1984, separated by date of collection and histological section type, were drawn on the same figure for each station (Figs. 6 and 7). Those figures should not be viewed as a unit but as a composite of up to ten separate sets of data (five section-type groups and two sampling dates). The strength of the relationship between GVF and PGA in these data sets was analyzed using Pearson product-moment correlation analysis (Zar 1984). July and August data were combined for three of the stations; however, the data for the two months were analyzed separately for Horsehead Rock because the plots for female oysters in July were drastically different from those in August.

RESULTS

The anatomic elements used to identify the relative location of a section in the antero-posterior axis of the oyster were the stomach, diverticula, intestinal branches and posterior appendix of the anterior caecum of the stomach (Figs. 2 and 3). The caecum appendix is of major importance in this study. Descriptions of the appendix presented here are based on the work of Shaw and Battle (1957). The appendix appears as a pair of H-shaped structures on the left ventral quadrant in transverse sections of oysters taken near the junction of the gills and the labial palps when the section is viewed in an antero-posterior direction (Fig. 3). The appendix is a compressed band-like structure that coils upon itself one-and-one-quarter turns. The paired H-shaped structures represent the juxtaposed sides of the sectioned coil and their arms are formed by invagination into typhlosoles of the appendix walls. Occasionally, three of the H-

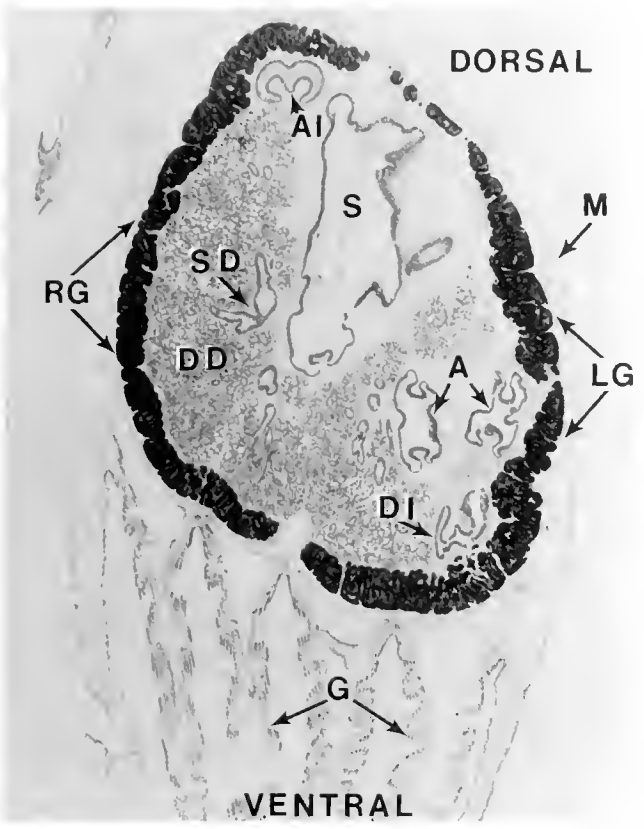


Figure 3. Photograph of anterior face in transverse histological section of an oyster collected at Wreck Shoal, James River, Virginia, in August 1984. A = large appendix of stomach caecum, AI = ascending intestine, DD = digestive diverticula, DI = descending intestine, G = gills, LG = left gonad, M = mantle, RG = right gonad, SD = stomach ducts to digestive diverticula, S = stomach.

shaped structures will be seen in the oyster section when the plane of the cut also passes through the quarter-turn extension of the appendix coil.

Changes in the morphology and arrangement of the visceral organs in *C. virginica,* evident in serial sections extending along the antero-posterior axis between the gills-palps junction and the pericardial cavity, are summarized below and in Fig. 4. The illustrations in Fig. 4 are only basic guideposts representing those changes because variations will be found at intermediate body sites between the sections shown. Those variations, however, will not prevent matching other sections with one of the 13 types given here. Changes in gonad morphology and arrangement are not always mentioned because they vary with stage of gametogenesis and spawning.

Section type 1: Located immediately anterior to or at the junction of the gills and the labial palps. The ventral part of the section is occupied by the palps; however, parts of the gills may also be seen because they are partially overlapped by the palps at the junction. The gonad, when present, does not extend around the ventral end. Stomach appendices appear as wide projections at both the dorsal and ventral ends. The diverticula surround the stomach except at the dorsal end of the section; the area covered by the diverticula on the right side is over twice as great as that on the left side. The ascending branch of the intestine is displaced toward the right dorsally, immediately inward of the gonad, and is separated from the stomach by connective tissue. The descending branch of the intestine is located on the left side of the body at the ventral end, between the diverticula and the gonad.

Section type 2: Located just posterior to, or at, the gills-palps junction. The ventral end is occupied by the gills, although parts of the palps may also be present. The gonad, if present, may extend around the whole body but may also be absent ventrally. The stomach is elongated and the two H-shaped structures are present in a partially distorted form. The diverticula extend around the ventral end but the area occupied on the left side is narrower than in section type 1. Diverticula are absent between the H-shaped structures and between the stomach and its appendix on the left side.

Section type 3: Located posterior to but still close to the palps-gills junction. The ventral part of the section may be occupied exclusively by the gills or may also include parts of the palps. The gonad may or may not extend completely around the ventral end of the section. The stomach is elongated with one major and several smaller ducts leading into the right mass of the diverticula. The two H-shaped structures are separate but may be distorted. The diverticula are almost completely absent from the left side but project slightly leftward between the stomach and the H-shaped structures. There is little change in arrangement of intestine sections.

Section type 4: Located posterior to the palps-gills junction. Very similar to Section 3 except that the gonad extends fully around the ventral end of the section and the ventral end of the body is occupied exclusively by the gills. H-shaped structures are clearly formed. Connection between the stomach and ducts to right side diverticula is absent. Diverticula extend into the left side between the stomach and the H-shaped structures. There is little change in location of the intestine sections.

Section type 5: Stomach appears relatively wider than in previous sections. There is a major duct from stomach to diverticula ventrally and other smaller ducts scattered throughout, particularly on the right side. The H-shaped structures appear fused together, marking the posterior end of the caecum appendix. There is little change in location of the intestine sections. The distance between section types 1 and 5 was approximately 1.2 mm in the paraffin-embedded segment.

Section type 6: Stomach still relatively wide with a major duct to the diverticula extending rightward and ventrad; a partly-connected large duct is directed leftward and anteriad. A few other ducts are visible. The H-shaped structures are no longer present. Diverticula occupy whole

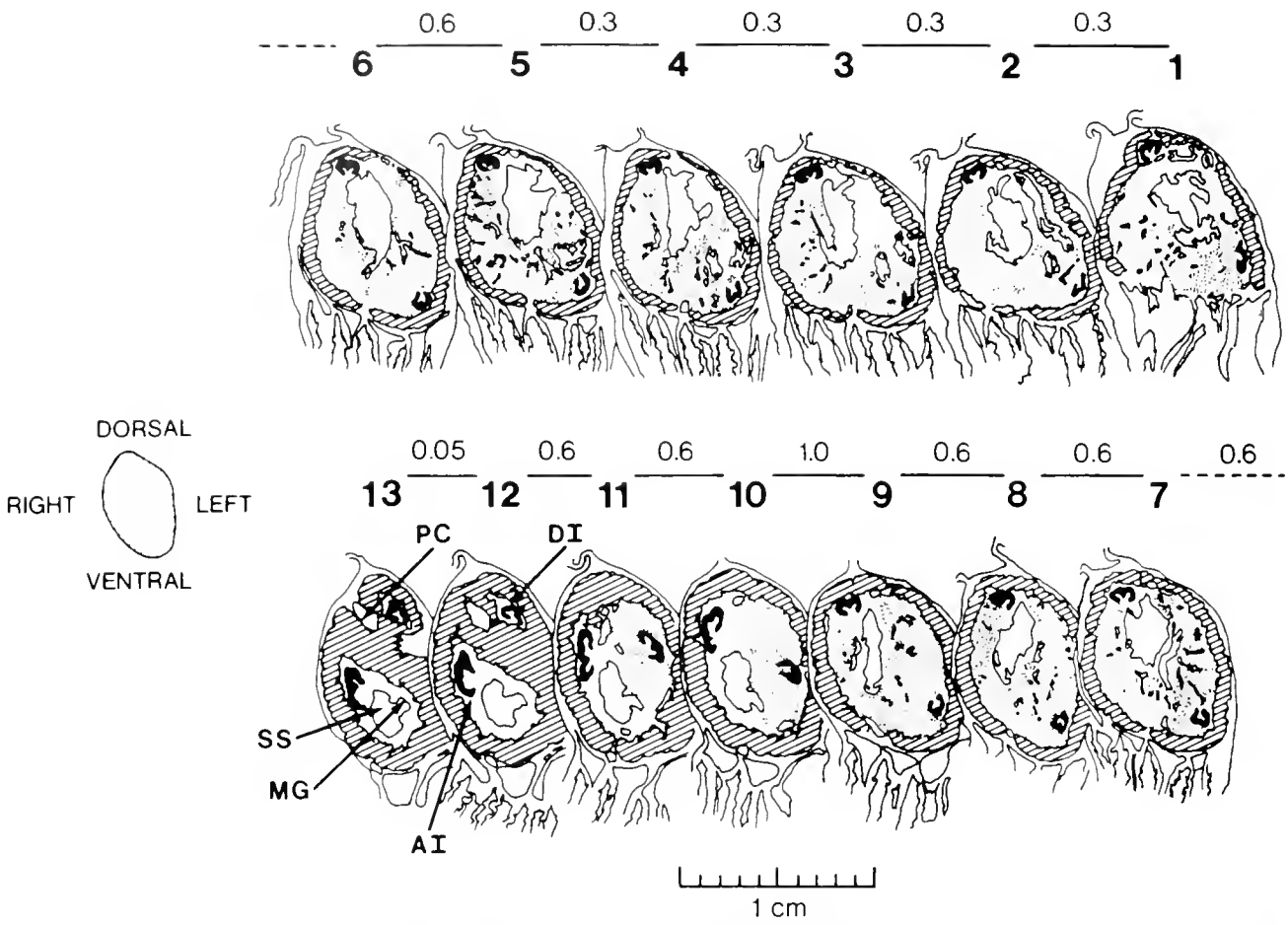


Figure 4. Series of transverse sections (anterior face shown) cut from an oyster between the vicinity of the gills-palps junction (section type 1) and the vicinity of the pericardial cavity (section types 12 and 13). Digits above each section identify section types described in text. Type numbers progress along the antero-posterior axis of the oyster. Distance between sections in the paraffin-embedded segment given, in mm, above line between section type numbers. Organ identification as in Figure 4 and here: MG = mid gut, PC = pericardial cavity, SS = style sac. Oyster collected from Wreck Shoal in August 1986.

right side but are absent from most of the left dorsal quadrant. There is little change in location of the intestine branches.

Section type 7: Stomach is rounded. There is a large detached stomach duct or appendix toward the left side and ventrad; several smaller ducts radiate in the same direction. At this point the central cavity, which corresponded to the stomach in the preceding sections, actually includes part of the mid-gut toward the right side and dorsal end of the body and part of the crystalline style sac toward the left side and dorsal end. These organs can be separated by the morphology of their epithelial lining (Shaw and Battle 1957). From here on we will refer to this cavity as the central cavity. The diverticula are evenly distributed from right to left with the widest part on the ventral end. There is little change in location of the intestine branches.

Section type 8: Similar to section type 7 but central cavity is slightly elongated diagonally. Remnants of major stomach duct or appendix from section type 7 are present. The central cavity includes the style sac at the left and dorsal end and the mid-gut at the right and ventral end. The area occupied by the diverticula on the right side is significantly smaller than in type 7.

Section type 9: Central cavity elongated and narrower than in previous sections and no longer includes the stomach. The dorsal one-third is part of the style sac and the small crescent-shaped projection at the ventral end is part of the mid-gut. The section between the style sac and the mid-gut is bounded by two intestinal typhlosoles (Shaw and Battle 1957). A major disconnected stomach duct to the diverticula appears on the right side and several smaller ducts are scattered throughout the diverticula. The diverticula on the right and left sides are separated from each other; the area occupied on the left side is several times greater than that on the right side.

Section type 10: Most of the central cavity consists of the style sac; the narrow projection leftward is the only part occupied by the mid-gut. The diverticula occupy only the

left side of the body. The descending branch of the intestine is located almost half-way along the left side and the ascending branch appears distorted and is located on the right side.

Section type 11: There is little change in position or morphology of the central cavity. The area occupied by the diverticula on the left side is substantially smaller than in section type 10. The descending branch of the intestine is located half-way along the right side and opposite the ascending branch. This displacement of the intestinal branches in section types 10 and 11 is the result of their being closer to each other in the vicinity of their cross-over point at the posterior end of the diverticula (see Fig. 2).

Section type 12: Central cavity resembles a mushroom on its side. Only the tip of the narrow projection to the left of the section is part of the mid-gut; the sides of the projection are made up of the two intestinal typhlosoles mentioned under section type 9. The wider part of the cavity is the style sac. The descending branch of the intestine is near the dorsal end on the left side. Gonad, when present, separates almost completely the descending branch of the intestine from the pericardial cavity. Ascending branch of intestine still located half-way along the right side. No diverticula present.

Section type 13: Identical to section type 12 except that the descending branch of the intestine is completely surrounded by the gonad (when present) near the dorsal end. The gonads are distinguishable as a small left gonad posteriorly dorsally and a much larger right gonad ventrally, connected by a short neck. The right gonad surrounds the central cavity and the ascending branch of the intestine.

There is a progressive increase in gonad width (and hence in area) in the serial sections in an antero-posterior direction (Fig. 4). PGA means for grouped adjacent section types in three oysters collected in 1986 (Fig. 5) were compared using one-way ANOVA and Scheffe's multiple contrast test after arcsine transformation (Zar 1984). There was no evidence of a difference between section types 2–4 and 5–6 nor between section types 5–6 and 7–8 ($P > 0.05$); however, mean PGA for section types 2–4 was significantly smaller ($P \leq 0.05$) than for section types 7–8 and 10. The mean for section types 7–8 was significantly smaller than the mean for type 10 ($P \leq 0.05$, Fig. 5). Lack of sufficient data for section type 9 prevented adequate comparisons between that type and most others; it was designated a separate type because gonad area appeared intermediate between types 7–8 and 10.

The highest PGA values among the oysters collected in 1984 were recorded in the sections that corresponded to types 9 and 10 regardless of sex, station or collection date (Figs. 6 and 7). Although those two section types were not always associated with the highest PGA values, they accounted for most of the PGA values greater than 30 percent. Less than 25% of the sections in types 7–8 showed

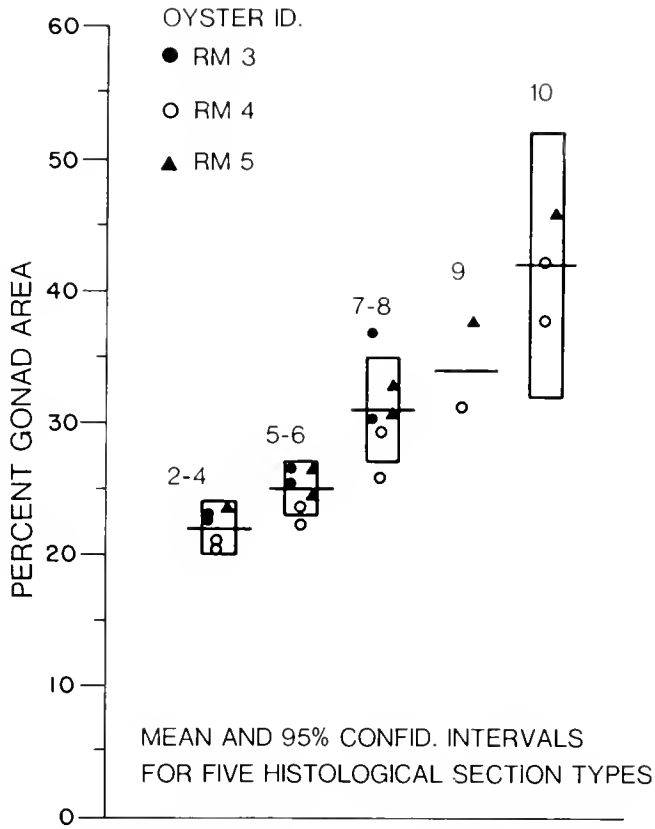


Figure 5. Individual measurements, mean and 95% confidence interval of percent gonad area in serial sections from each of three Wreck Shoal oysters (RM 3–5) collected in August 1986; sections combined into five groups of adjacent types as identified in Figure 5.

PGA values higher than 30. Almost every oyster in types 2–4 and 5–6 had PGA values under 30.

Gonad Volume Fraction and PGA were positively correlated with r values ≥ 0.6 and $P \leq 0.05$ in 20 of 28 tests on the combined data for July and August at three of the four stations (Nansemond Ridge, Naseway Shoal and Wreck Shoal; Table 2). The relationship between GVF and PGA appeared different at Horsehead Rock. Plots of the relationship for female oysters at Horsehead Rock in July were similar to those at the other three stations and high positive correlations were found for three of the five section types (Fig. 6, Table 2). In August, however, the data were radically different; almost all GVF values were higher than 0.8 regardless of the PGA value and only one of four comparisons showed a correlation coefficient higher than 0.6. Most of the male oysters also showed GVF values higher than 0.8 regardless of PGA value at Horsehead Rock in July and August and only two of seven computed r values were higher than 0.6 at $P \leq 0.05$ (Fig. 7, Table 2). Most of the individual component plots in Figs. 6 and 7 appeared to be linear on visual examination while a few appeared to be curvilinear. The data, however, were insufficient for an accurate determination of the nature of the relationship.

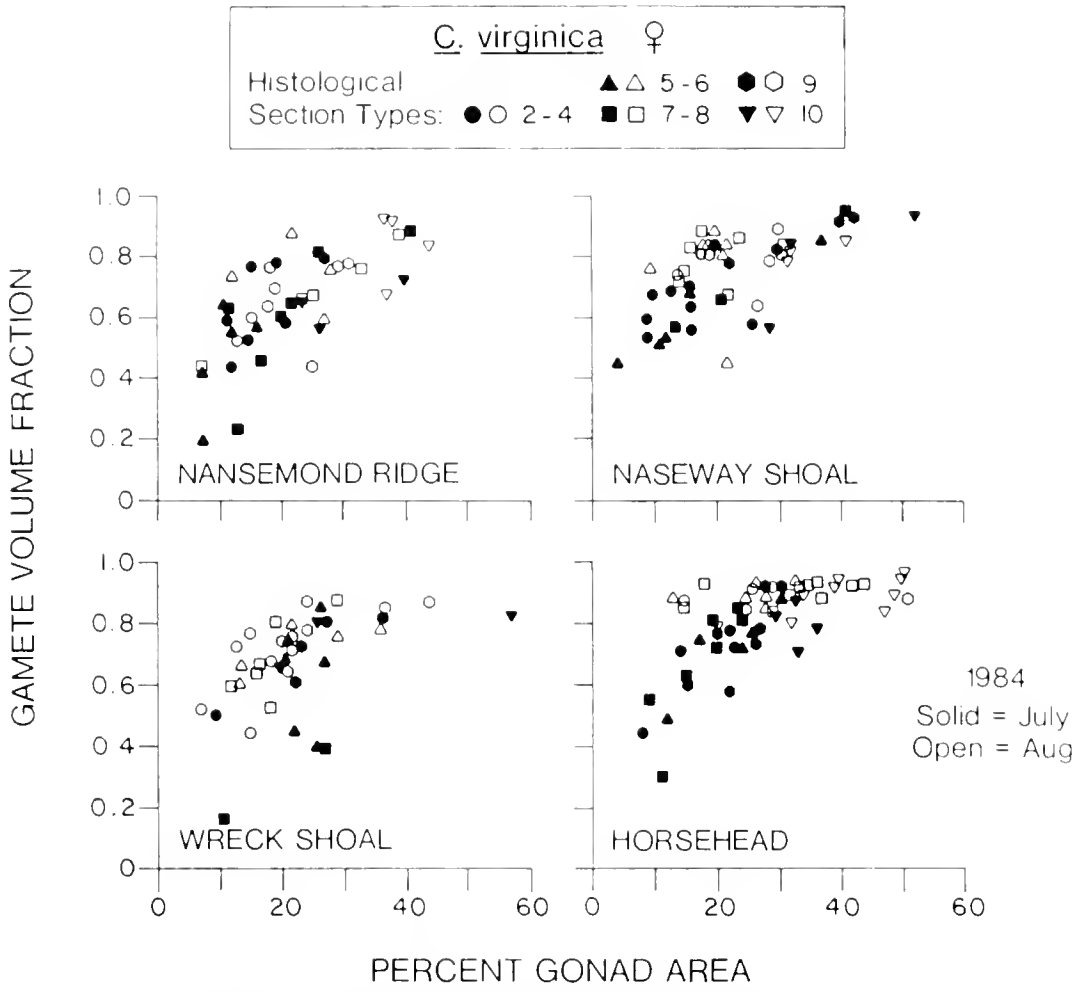


Figure 6. Gamete volume fraction plotted against percent gonad area for individual female oysters collected from four stations in the James River, Virginia, in July and August 1984; sections combined into five groups of adjacent types as identifed in Figure 5. Composite plot for each station consists of up to ten separate sets of data and should not be viewed as a unit.

DISCUSSION

Seasonal reproductive development in bivalve molluscs has been studied primarily in terms of descriptive characterization of gametogenic stages (for example, Kennedy and Battle 1964, Brousseau 1978, 1984, Kennedy and Krantz 1982, Mann 1982, Manzi et al. 1985, Dudgeon and Morton 1983). Quantitative measurements, however, have been used frequently. Such measurements include determination of gamete volume fraction (as in Bayne et al. 1978, Newell et al. 1982, Sundet and Lee 1984, Pipe 1985, Kennedy 1986), gonad area (Table 1), and gamete number and size (as in Keck et al. 1975, Brousseau 1978, Lannan 1980, Barber and Blake 1983, Wilson and Simons 1985, Gustafson et al. 1987). Gravimetric gonad measurements, which follow the increase in weight as the gonad matures and the decrease that occurs on spawning, have also been used but almost exclusively with pectinid species, whose gonad can be separated from the rest of the body (as in Sastry 1966, Ansell 1974, Shafee 1981, MacDonald and Bourne 1987); however, they have also been used a few times for other species (Fox and Coe 1943, Griffiths 1977, Thompson 1979, Bayne and Worrall 1980, Peterson and Fegley 1986).

Quantitative estimates are preferable to descriptive stage characterization because they eliminate the subjectivity and semantic problems associated with the descriptions (Brousseau 1978) and tend to provide ecologically meaningful information. Nevertheless, several investigators have used stage characterizations as supplementary information to quantitative measurements (Keck et al. 1975, Tinsman et al. 1976, Brousseau 1983, Dinamani 1987). Histological examinations that include quantitative measurements of gonadal material should be a part of any study on reproduction of bivalve molluscs because they provide detailed information not available otherwise, as was suggested by Beninger (1987) and MacDonald and Bourne (1987).

The positive correlation between gamete volume fraction and percent gonad area found at three of the four sta-

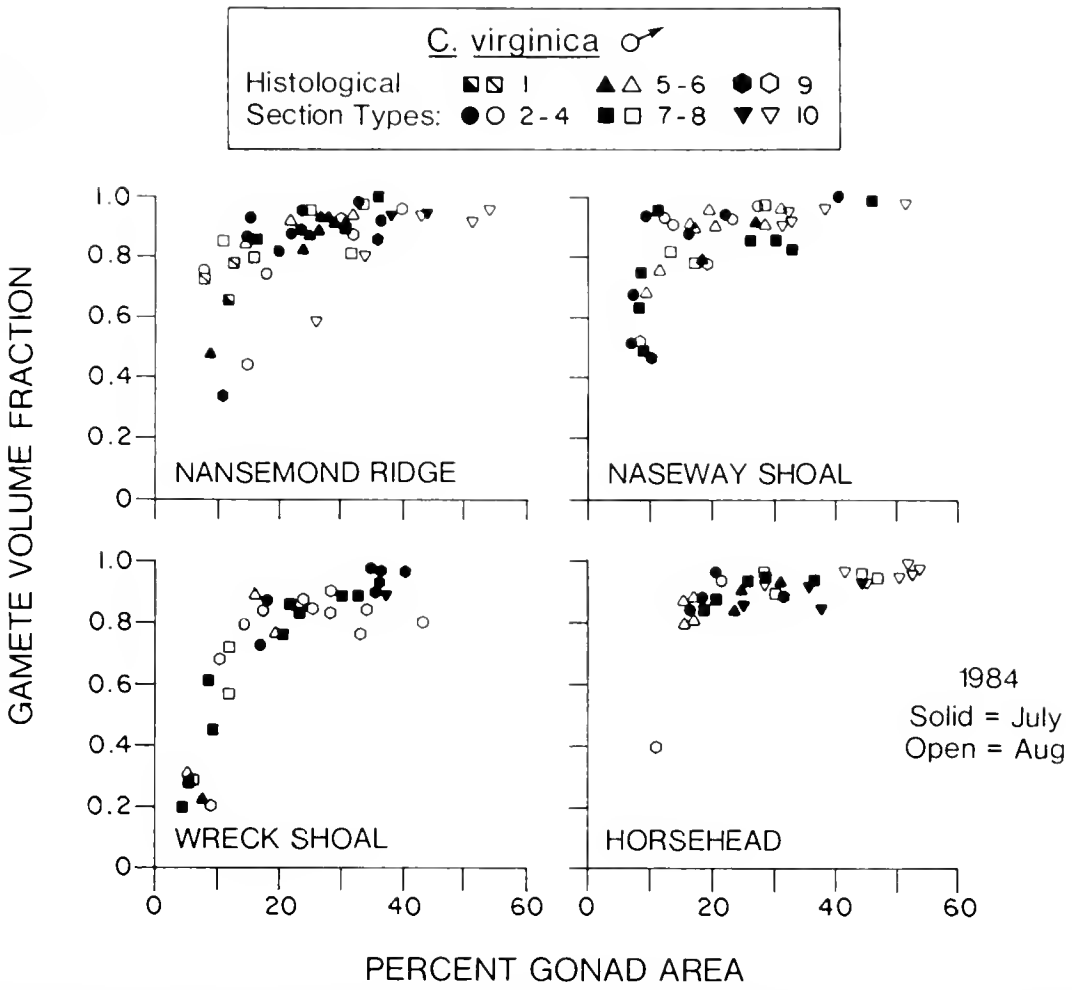


Figure 7. Gamete volume fraction plotted against percent gonad area for individual male oysters collected from four stations in the James River, Virginia, in July and August 1984; sections combined into five groups of adjacent types as identified in Figure 5. Composite plot for each station consists of up to ten separate sets of data and should not be viewed as a unit.

tions on the two collection dates indicates that they are comparable estimates of the same parameter. Apparently, either may be used to monitor progression of reproductive development in bivalves; however, the discrepancies in correlation of GVF and PGA found at Horsehead Rock between females collected in July and August suggest that the relationship between the two may change under some circumstances. These discrepancies may indicate that none of the oysters at Horsehead Rock had spawned at the time of collection in August, possibly due to extremely low water salinities recorded during that time period, but those facts could not be verified. Nevertheless, plots of GVF against PGA may call attention to factors affecting the reproductive biology of oysters and other bivalves.

Gonad volume fraction is a precise indicator of seasonal changes in gametogenic development and incidence of spawning in bivalve populations because it accounts for the continuity in stages of development (Newell et al. 1982). It does not, however, provide a gamete count (Chalkey 1943), nor does it give an estimate of the quantity of gonadal material produced (Hilbish and Zimmerman 1988). Bayne et al. (1982) and Lowe and Pipe (1987), however, combined the volume of the mantle in *Mytilus edulis* with GVF measurements to obtain estimates of total volume of gametes and Hilbish and Zimmerman (1988) estimated the proportional gamete weight of an indiviudal *M. edulis* using body and mantle weights and GVF measurements. Yankson (1986) also used the change in GVF due to spawning as an estimate of fecundity and spawning efficiency in two species of *Cerastoderma*.

Gonad area measurements, on the other hand, may be used to estimate the quantity of gonadal material produced by bivalve molluscs, such as *C. virginica*, whose gonad weight or volume cannot be measured directly. They could be combined with measurements of areal density of gametes on a set of serial sections and integrated into an acceptable estimate of total gamete production. Brousseau (1978) combined visceral displacement volume, oocyte numbers per unit volume, and relative gonad size in serial sections to estimate fecundity in *Mya arenaria*. Tinsman et

TABLE 2.

Correlation coefficient (*r*) and probability values (P) for the relationship between Gamete Volume Fraction and Percent Gonad Area in histological sections from different parts of the body (as indicated by section types) of oysters collected in July and August, 1984, at four stations in the James River, Virginia; July and August data combined for three of the stations. Section types explained in text. n = number of sections.

Station	Section type	Male Oysters			Female Oysters		
		n	r	P	n	r	P
Nansemond Ridge	2–4	7	0.721	0.034	8	0.509	0.099
	5–6	9	0.772	0.007	8	0.753	0.045
	7–8	8	0.666	0.036	12	0.915	0.000
	9	9	0.828	0.003	8	0.195	0.322
	10	7	0.836	0.010	7	0.587	0.083
Naseway Shoal	2–4	10	0.741	0.007	10	0.270	0.225
	5–6	10	0.761	0.005	12	0.580	0.024
	7–8	11	0.618	0.021	10	0.683	0.015
	9	3	0.963	0.087	10	0.774	0.004
	10	5	0.874	0.026	6	0.792	0.030
Wreck Shoal	2–4	4	0.283	0.358	7	0.673	0.049
	5–6	4	0.870	0.065	11	0.315	0.173
	7–8	12	0.929	0.000	8	0.520	0.093
	9	15	0.723	0.001	11	0.784	0.002
	10	1	—	—	2	—	—
Horsehead Rock							
July	2–4	1	—	—	3	0.865	0.167
	5–6	3	0.893	0.148	6	0.906	0.006
	7–8	6	0.856	0.015	7	0.886	0.004
	9	3	0.711	0.248	8	0.896	0.001
	10	4	0.592	0.204	4	−0.417	0.291
August	2–4	1	—	—	2	—	—
	5–6	4	0.443	0.278	6	0.343	0.253
	7–8	4	0.425	0.287	10	0.105	0.386
	9	1	—	—	3	0.097	0.469
	10	7	0.811	0.013	9	0.662	0.026

al. (1976) and Dinamani (1987) computed indices based on gonad area and follicle coverage per unit area for *C. virginica* and *Crassostrea gigas*, respectively. It may also be possible to relate such estimates to whole animal weight to arrive at gravimetric estimates of gonadal production.

Gonad area measurements involving bivalve molluscs may require careful attention to the body location from which histological sections are prepared, depending on the objectives of the study. Body locations have been identified in previous publications involving a variety of bivalve species only in general terms (usually 'the mid-visceral region') and without any explanation of the degree of correspondence between individual transverse sections from different individuals. This presents no serious problem where gametogenesis is uniform throughout the gonad and where the only interest is establishment of the seasonal cycle or spawning incidence; it can, however, lead to serious difficulties in investigations dealing with gonad area comparisons. Perdue (1983) studied the relationship between gonad area and body location in *C. gigas* and concluded that a section from within 8–10 mm of the base of the labial palps would result in similar gonad area measurements. Our observations, however, allow a more precise specification of the section to be used for gonad area measurements in terms of the anatomy and arrangement of the internal organs included in the section.

The description of changes in morphology and arrangement of the internal organs in a series of transverse sections of *C. virginica* presented here permits standardization of the location from which sections are taken in a particular study or in a series of studies with that species. Similar descriptions for other species would be useful in the same manner. Usefulness of the sections and descriptions does not lie in precise identification of body locations but in allowing recognition of the features of specific sections so that only similar sections are used in comparative studies of gonad area. The exact arrangement of organs in the sections illustrated here may not be identical to that in sections prepared by others; variations will occur at body locations intermediate to those shown here and distortions due to shrinkage during fixation are possible. Similarities between other sections and our illustrations, however, will be found readily.

Generation of oyster sections with similar features can

be simplified considerably if efforts are directed toward obtaining sections showing the organ configurations illustrated by section types 2–4. These sections are characterized by the easily recognizable H-shaped structures corresponding to the posterior appendix of the stomach caecum. The possibility exists, however, that some of those sections (especially those very close to the gills-palps junction) may not show a gonad that completely surrounds the visceral mass because part of the ventral side is occupied by the labial palps and they appear to preclude the presence of the gonad at those sites. The presence of a fully circumferential gonad is required for gonad area measurements to represent the maximum obtainable for that section of the body. Therefore, a section similar to type 4, in which the ventral side is occupied exclusively by the gills should be sought.

Section type 4 can be located easily because it is found close to the gills-palps junction, a distinct gross feature of the oyster anatomy. A section similar to type 4 is obtainable by scanning the sequence of sections generated with the microtome; it should be found within 1–2 mm posteriad to the gills-palps junction in the paraffin-embedded oyster segment. The ease with which such a readily identifiable section can be found in *C. virginica* should encourage its use as a standard for studies of reproductive development involving gonad area comparisons in that species.

ACKNOWLEDGMENTS

The cooperation and material assistance of W. J. Hargis, Jr., John E. Olney, Juanita G. Walker, Kay B. Stubblefield, Harold Burrell, W. W. Jenkins and Janet G. Walker is gratefully acknowledged. Manuscript review by our colleagues at VIMS was greatly appreciated. Comments and suggestions by external reviewers contributed greatly to the accuracy and clarity of our manuscript.

LITERATURE CITED

Allen, S. K., Jr. & S. L. Downing. 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings. *J. Exp. Mar. Biol. Ecol.* 102:197–208.

Ansell, A. D. 1974. Seasonal changes in biochemical composition of the bivalve *Chlamys septemradiata* from the Clyde Sea area. *Mar. Biol.* 28:85–99.

Barber, B. J. & N. J. Blake. 1983. Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamarck) at its southern distributional limit. *J. Exp. Mar. Biol. Ecol.* 66:247–256.

Barber, B. J., S. E. Ford & H H. Haskin. 1988. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition index and relative fecundity. *J. Shellfish Res.* 7:25–31.

Bayne B. L., D. L. Holland, M. N. Moore, D. M. Lowe & J. Widdows. 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *J. Mar. Biol. Ass. U.K.* 58:825–841.

Bayne, B. L. & C. M. Worrall. 1980. Growth and production of mussels *Mytilus edulis* from two populations. *Mar. Ecol. Progr. Ser.* 3:317–328.

Bayne, B. L., A. Bubel, P. A. Gabbot, D. R. Livingstone, D. M. Lowe & M. N. Moore. 1982. Glycogen utilisation and gametogenesis in *Mytilus edulis* L. *Mar. Biol. Lett.* 3:89–105.

Beninger, P. G. 1987. A qualitative and quantitative study of the reproductive cycle of the gaint scallop, *Placopecten magellanicus*, in the Bay of Fundy (New Brunswick, Canada). *Can J. Zool.* 65:495–498.

Brousseau, D. J. 1978. Spawning cycle, fecundity and recruitment in a population of soft-shell clam, *Mya arenaria*, from Cape Ann, Massachusetts. *Fish. Bull.* 76:155–166.

Brousseau, D. J. 1983. Aspects of reproduction of the blue mussel, *Mytilus edulis* (Pelecypoda:Mytilidae) in Long Island Sound. *Fish. Bull.* 81:733–739.

Brousseau, D. J. 1984. Reproductive cycle of *Anomia simplex* (Pelecypoda:Anomiidae) from Cape Cod, Massachusetts. *Veliger* 26(4):299–304.

Chalkey, H. W. 1943. Method for the quantitative morphologic analysis of tissues. *J. Natl. Cancer Inst.* 4:47–53.

Coe, W. R. 1932. Development of the gonads and the sequence of the sexual phase in the California oyster (*Ostrea lurida*). *Bull. Scripps Inst. Oceanogr.* Tech. Ser. 3, No. 6, pp. 119–144 + 3 pls.

Dinamani, P. 1987. Gametogenetic patterns in populations of Pacific oyster, *Crassostrea gigas*, in Northland, New Zealand. *Aquaculture.* 64:65–76.

Dudgeon, D. & B. Morton. 1983. The population dynamics and sexual strategy of *Anodonta woodiana* (Bivalvia:Unionacea) in Plover Cove Reservoir, Hong Kong. *J. Zool.* London 201:161–183.

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. *Proc. Natl. Shellfish. Assoc.* 70:65–93.

Eversole, A. G., W. K. Michener & P. J. Eldridge. 1980. Reproductive cycle of *Mercenaria mercenaria* in a South Carolina estuary. *Proc. Natl. Shellfish Assoc.* 70:22–30.

Fox, D. L. & W. R. Coe. 1943. Biology of the California sea mussel (*Mytilus californianus*). II. Nutrition, metabolism, growth and calcium deposition. *J. Exp. Zool.* 93:205–249.

Galtsoff, P. S. 1938. Physiology of reproduction of *Ostrea virginica*. I. Spawning reactions of the female and male. *Biol. Bull.* 74:461–486.

Galtsoff, P. S. 1964. The American Oyster *Crassostrea virginica* Gmelin. *Fish. Bull.* 64:1–480.

Griffiths, R. J. 1977. Reproduction cycles in littoral populations of *Choromytilus meridionalis* (Kr.) and *Aulacomya ater* (Molina) with a quantitative assessment of gamete production in the former. *J. Exp. Mar. Biol. Ecol.* 30:53–71.

Gustafson, R. G., B. D. Gustafson & R. G. B. Reid. 1987. Continuous reproduction in the protobranch bivalve *Solemya reidi* (Cryptodonta:Solemyidae). *Veliger* 29:367–373.

Hilbish, T. J. & K. M. Zimmerman. 1988. Genetic and nutritional control of the gametogenic cycle in *Mytilus edulis*. *Mar. Biol.* 98:223–228.

Hopkins, S. H., J. G. Mackin & R. W. Menzel. 1953. The annual cycle of reproduction, growth and fattening in Louisiana oysters. *Natl. Shellfish Assoc. Conv. Addr.* 1953, pp. 39–50.

Iwantsch, G. F. 1970. The effect of high temperature on the gonadal area of representative cross-sections of *Mytilus edulis* L. Newark DE: University of Delaware. 67 p. Thesis.

Jackson, R. T. 1890. Phylogeny of the Pelecypoda. The Aviculidae and their allies. *Mem. Boston Soc. Nat. Hist.* 4:277–400.

Keck, R. T., D. Maurer & H. Lind. 1975. A comparative study of the hard clam gonad developmental cycle. *Biol. Bull.* 148:243:258.

Kennedy, A. V. & H. I. Battle. 1964. Cyclic changes in the gonad of the American oyster, *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 42:305–321.

Kennedy, V. S. 1986. Expected seasonal presence of *Crassostrea vir-*

ginica (Gmelin) larval populations, emphasizing Chesapeake Bay. *Amer. Malacol. Bull.* Spec. Ed. No. 3:25–29.

Kennedy, V. S. & L. B. Krantz. 1982. Comparative gametogenic and spawning patterns of the oyster *Crassostrea virginica* (Gmelin) in central Chesapeake Bay. *J. Shellfish Res.* 2:133–140.

Lannan, J. G. 1980. Broodstock management of *Crassostrea gigas*. I. Genetic and environmental variation in survival in the larval rearing system. *Aquaculture* 21:323–336.

Loosanoff, V. L. 1942. Seasonal gonadal changes in the adult oyster, *Ostrea virginica*, of Long Island Sound. *Biol. Bull.* 82:195–206.

Loosanoff, V. L. 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. *Biol. Bull.* 129:546–561.

Loosanoff, V. L. & J. B. Engle. 1940. Spawning and setting of oysters in Long Island Sound in 1937 and discussion of the method for predicting the intensity and time of oyster setting. *Bull. U.S. Bur Fish.* 74:217–255.

Loosanoff, V. L. & J. B. Engle. 1942. Accumulation and discharge of spawn by oysters living at different depths. *Biol. Bull.* 82:413–422.

Loosanoff, V. L. & C. A. Nomejko. 1951. Spawning and setting of the American oyster, *C. virginica*, in relation to lunar phases. *Ecology* 32:113–134.

Lowe, D. M. & R. K. Pipe. 1987. Mortality and quantitative aspects of storage cell utilization in mussels, *Mytilus edulis*, following exposure to diesel oil carbohydrates. *Mar. Environm. Res.* 22:243–251.

MacDonald, B. A. & N. F. Bourne. 1987. Growth, reproductive output, and energy partitioning in weathervane scallops, *Patinopecten caurinus*, from British Columbia. *Can. J. Fish. Aquatic. Sci.* 44:152–160.

Mann, R. 1982. The seasonal cycle of gonadal development in *Arctica islandica* from the southern New England shelf. *Fish. Bull.* 80(2): 315–326.

Manzi, J. J., M. Y. Bobo & V. G. Burrell, Jr. 1985. Gametogenesis in a population of the hard clam, *Mercenaria mercenaria* (Linnaeus), in North Santee Bay, South Carolina. *Veliger* 28:186–194.

Mori, K. 1979. Effects of artificial eutrophication on the metabolism of the Japanese oyster *Crassostrea gigas*. *Mar. Biol.* 53:361–369.

Newell, R. I. E., T. J. Hilbish, R. K. Koehn & C. J. Newell. 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (Bivalvia:Mytilidae) from localities on the east coast of the United States. *Biol. Bull.* 162:299–310.

Perdue, J. A. 1983. The relationship between the gametogenic cycle of the Pacific oyster, *C. gigas*, and the summer mortality phenomenon in strains of selectively-bred oysters. Seattle WA: University of Washington, 205 pp. Dissertation.

Perdue, J. A., J. H. Beattie & K. K. Chew. 1981. Some relationships between gametogenic cycle and summer mortality phenomenon in the Pacific oyster (*Crassostrea gigas*) in Washington State. *J. Shellfish Res.* 1:9–16.

Perdue, J. A. & G. Erickson. 1984. A comparison of the gametogenic cycle between the Pacific oyster *Crassostrea gigas* and the Suminoe oyster *Crassostrea rivularis* in Washington State. *Aquaculture*. 37: 231–237.

Peterson, C. H. & S. R. Fegley. 1986. Seasonal allocation of resources to growth of shell, soma, and gonads in *Mercenaria mercenaria*. *Biol. Bull.* 171:597–610.

Pipe, R. K. 1985. Seasonal cycles in and effects of starvation on egg development in *Mytilus edulis*. *Mar. Ecol. Progr. Ser.* 24:121–128.

Porter, H. J. 1967. Seasonal gonadal changes of adult clams, *Mercenaria mercenaria* (L.), in North Carolina. *Proc. Natl. Shellfish. Assoc.* 55:35–51 (1964).

Sastry, A. N. 1966. Temperature effects in reproduction of the bay scallop. *Aequipecten irradians* Lamarck. *Biol. Bull.* 130:118–134.

Shafee, M. S. 1981. Seasonal changes in the biochemical composition and calorific content of the black scallop *Chlamys varia* (L.) from Lanveoc, Bay of Brest. *Oceanol. Acta* 4:331–341.

Shaw, B. L. & H. I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 35:325–347 + 2 pls.

Stasek, C. R. 1963. Orientation and form in the bivalved Mollusca. *J. Morph.* 112:195–214.

Sundet, J. H. & J. B. Lee. 1984. Seasonal variations in gamete development in the Icelandic scallop, *Chlamys islandica*. *J. Mar. Biol. Ass. U.K.* 64:411–416.

Thompson, R. J. 1979. Fecundity and reproductive effort in the blue mussel (*Mytilus edulis*), the sea urchin (*Strongylocentrotus droebachiensis*) and the snow crab (*Chionoecetes opilio*) from populations in Nova Scotia and Newfoundland. *J. Fish. Res. Bd. Canada* 36:955–964.

Tinsman, J. C., S. G. Tinsman & D. Maurer. 1976. Effects of a thermal effluent on the reproduction of the American oyster. Wesch, G. W. & R. W. McFarland, eds., *Thermal Ecology*, Augusta, GA: U.S. Atomic Energy Commission, Technical Information Center, Oak Ridge, Symposium Series (CONF 750423) Vol. 2, pp. 64–72.

Weibel, E. R., G. S. Kistler & W. F. Scherle. 1966. Practical stereological methods for morphometric cytology. *J. Cell. Biol.* 30:23–38.

Wilson, J. H. & J. Simons. 1985. Gametogenesis and breeding of *Ostrea edulis* on the west coast of Ireland. *Aquaculture*. 46:307–321.

Yankson, K. 1986. Reproductive cycles of *Cerastoderma glaucum* (Bruguiere) and *C. edule* (L.) with special reference to the effects of the 1981–82 severe winter. *J. Moll. Stud.* 52:6–14.

Yonge, C. M. 1953. The monomyarian condition in the Lamellibranchia. *Trans. Roy. Soc. Edinb.* 62:443–478.

Zar, J. H. 1984. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs NJ. 718 p.

Journal of Shellfish Research, Vol. 8, No. 1, 83–86, 1989.

CHANGES IN THE GONADAL STATE OF LOUISIANA OYSTERS DURING THEIR AUTUMN SPAWNING SEASON

JULIE D. GAUTHIER AND THOMAS M. SONIAT
Department of Biological Sciences
University of New Orleans
New Orleans, Louisiana 70148

ABSTRACT *Crassostrea virginica* were collected from upper, middle, and lower estuarine sites within four major oyster producing watersheds in Louisiana from September 1987 to November 1987. A significant positive correlation (Kendall Tau correlation = 0.81; $p < 0.0005$) between temperature and average gonad/body (G/B) ratio of oysters was found. The highest G/B ratio was found at a temperature of 28°C and declined rapidly at temperatures below 20°C. A drop in G/B ratio as temperatures declined in October and the synchrony of gametogenic stages (later development, spawning, and advanced spawning-regression) give further evidence for an autumn mass spawning season in Gulf Coast oysters.

KEY WORDS: *Crassostrea virginica*, Louisiana, reproduction, spawning, gametogenesis, temperature

INTRODUCTION

The reproductive cycle of the American oyster (*Crassostrea virginica* (Gmelin) has been more extensively studied in estuaries on the northern Atlantic Coast than those of the Gulf of Mexico. Northern oysters normally exhibit two periods of gametogenic development and one summer spawn. Gonadal development begins in autumn but is cut short due to the onset of low temperatures, which induces dormancy. Gametogenesis is completed in spring with subsequent spawning in summer (Loosanoff 1942). Temperature requirements for gonadal development and spawning of southern oysters (Hopkins 1931) have been reported to be higher than those for oysters living at more northerly latitudes (Loosanoff and Nomejko 1951). Gulf Coast oysters differ from northern oysters in that their autumn gametogenic development is not interrupted by winter dormancy; as long as temperatures remain high, oysters continue gametogenic development resulting in multiple spawns per year (Ingle 1951).

Hopkins et al. (1953) plotted gonadal thickness values of two Louisiana oyster populations collected from February to September. Although gonadal development between the two groups differed slightly throughout the year, spring spawning occurred in synchrony. Hayes and Menzel (1981) observed spawning patterns of both young-of-the-year and older oyster populations from the northeastern Gulf of Mexico. A significant decline in follicle thickness of older oysters occurred in late May and again in late July. A large temperature drop was believed to stimulate the late-summer decline. In the young population, the gonadal thickness did not peak until mid September and steadily declined with declining temperatures throughout October. Speculations were made that, in the absence of a temperature change in late July, mass spawning may not begin until temperatures decreased in the autumn.

In conjunction with a parasitological survey (Gauthier et al., in review), oysters were collected from twelve sites along the Louisiana coast from September to November. Average gonadal thickness at each site along with percent oysters in each gametogenic stage were used to reconstruct the pattern of development and spawning as temperatures decreased with time.

MATERIALS AND METHODS

Ten oysters of moderate size (6–11 cm in "length" or umbo-to-bill distance) were collected from each of upper, middle, and lower estuarine sites of four major oyster producing watersheds in Louisiana (Fig. 1). Each site was sampled only once during the study period (1 September–7 November 1987). Temperature (mercury thermometer) and salinity (Reichert refractometer, Behrens 1985) were recorded at each site.

A 4–5 mm transverse section just posterior to the labial palps was removed and fixed in Davidson's fixative for histological sectioning (Howard and Smith 1983). Gonad/body (G/B) ratios were determined using the method of Kennedy and Battle (1964) which employs ten equidistant measurements of gonadal width relative to body width and is expressed as a percent. The average G/B per site was calculated. Also, gametogeneic stages (as described by Kennedy and Krantz 1982) and the sex of each oyster were determined. Percent oysters in each stage was recorded per site. Kendal Tau correlation coefficients (τ) between G/B ratio and both temperature and salinity were determined using a SAS program installed on a VAX/VMS system at the University of New Orleans' Computer Research Center.

RESULTS

Temperatures dropped from 27 to 26°C in September, 22 to 18°C in October, and 20 to 17°C in November. Salinity varied from 8 ppt to 27 ppt. An attempt was made to collect oysters from as wide a salinity range as possible; however, due to drought conditions that intensified during the study period, salinities at the time of sampling are typi-

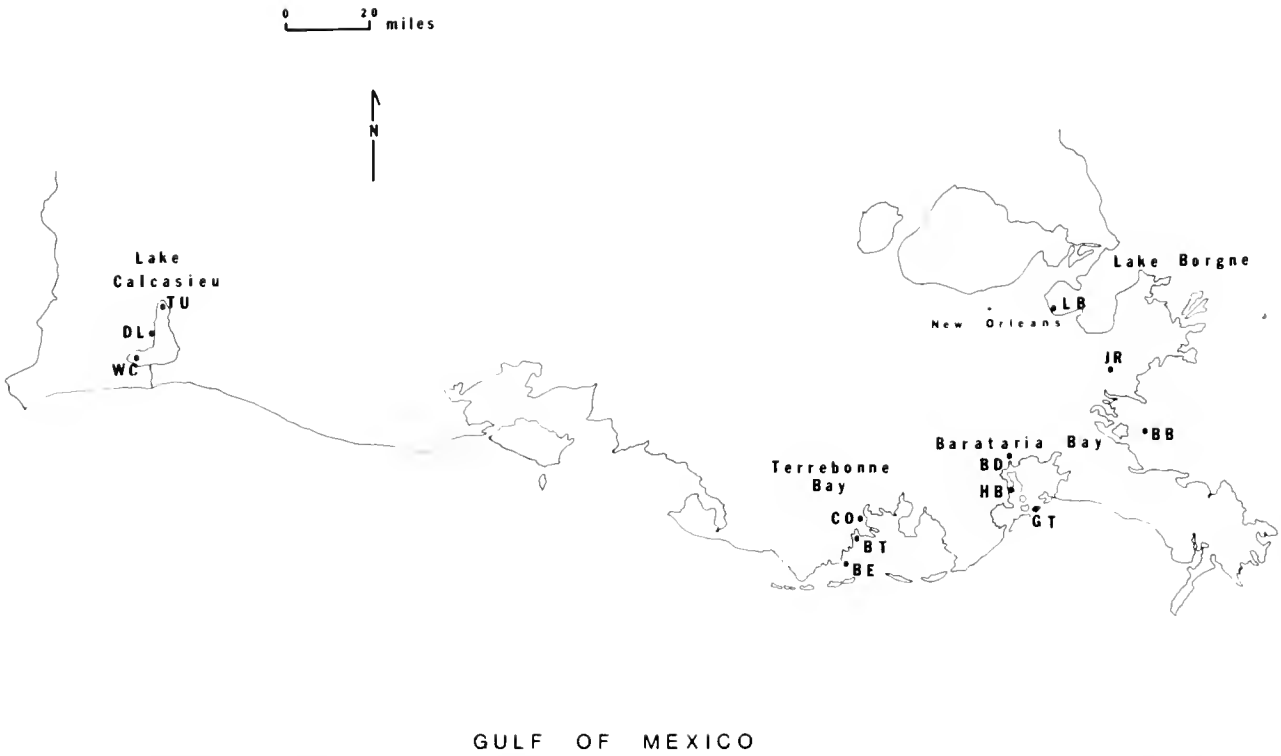


Figure 1. Map of the Lousiana coast showing oyster collecting sites and four major oyster producing watersheds: Lake Calcasieu, Terrebonne Bay, Barataria Bay, and Lake Borgne.

TU = Turner's Bay, DL = Dugas' Landing, WC = West Cove, CO = Cocodrie Harbor, BT = Bay Tambour, BE = Bay St. Elaine, BD = Bayou Denis, HB = Hackberry Bay, GT = Grand Terre, LB = Lake Borgne, JR = Lake Jean Robin, BB = Black Bay.

cally higher than mean conditions. Average G/B ratio and number of oysters in each gametogenic stage are presented for each site in Table 1. G/B values dropped from 28 to 15% in September, 12 to 7% in October, and 3 to 1% in November. Only three gametogenic stages were seen in this study: later development, spawning, and advanced spawning-regression. No oysters were found to be in early development. Most of the oysters collected were female

TABLE 1.

Sex distribution, gonad/body (G/B) ratio, and numbers of oysters in each gametogenic stage at each site. Sites are listed in order of collection along with temperature and salinity at that time.

M = male, F = female, X = unknown, ED = early development, LD = later development, S = spawning, AS/R = advanced spawning-regression.

Site	Date	Temp (*C)	SAL (ppt)	M	F	X	G/B	Gametogenic Stage			
								ED	LD	S	AS/R
LB	9/1/87	27.8	8	0	10	0	28.4	0	6	3	1
BB	9/8/87	28.6	26	2	8	0	28.4	0	8	2	0
CO	9/16/87	23.3	10	3	6	1*	18.0	0	7	2	0
JR	9/27/87	26.6	18	2	8	0	15.4	0	8	2	0
HB	9/29/87	26.0	17	6	4	0	20.4	0	8	1	1
GT	10/6/87	22.0	26	2	7	1*	12.5	0	6	2	1
BD	10/15/87	19.8	15	3	7	0	13.0	0	5	3	2
BT	10/22/87	21.0	25	1	8	1	9.0	0	2	3	5
BE	10/22/87	18.2	27	0	7	3	7.1	0	0	3	7
TU	11/7/87	20.0	22	3	1	6	3.4	0	0	2	8
WC	11/7/87	17.3	25	0	0	10	1.0	0	0	0	10
DL	11/7/87	17.5	23	2	0	8	1.2	0	0	0	10

*unknown due to infestation by *Bucephalus cuculus*

and as advanced spawning-regression proceeded the sex of oysters became indeterminable (Table 1).

The number of oysters in later development was highest in September and declined when temperatures dropped in early October. Spawning oysters became more prevalent throughout October, and by mid November all oysters were in the advanced spawning-regression stage (Fig. 2). The time spent in the spawning state was apparently short, since

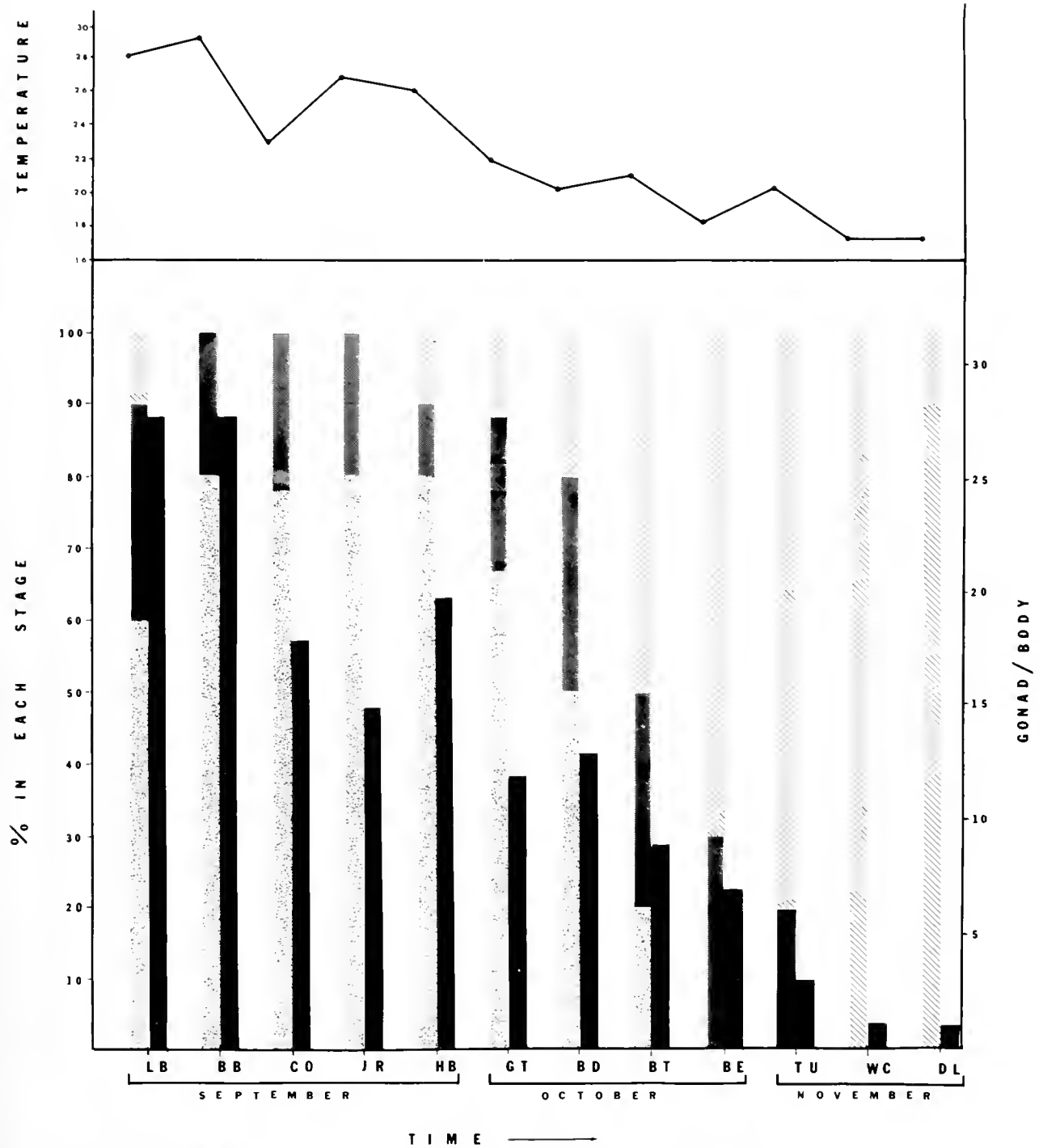


Figure 2. Bar graph and temperature (°C) plot with time showing percent oysters in each gametogenic stage as well as gonad/body (G/B) ratios at each site.

- later development
- spawning
- advanced spawning-regression

most oysters were found in either the later development or advanced spawning-regression stage. A highly significant ($p < 0.0005$) positive correlation ($\tau = 0.81$) was found between the G/B ratio of oysters and temperature. G/B was not significantly correlated with salinity.

DISCUSSION

The reproductive state of bivalves is dependent on a number of endogenous and exogenous factors, most importantly water temperature (Sastry 1975, Hayes and Menzel 1981). In this study, a significant positive correlation was found between G/B ratio and temperature. Analysis of gametogenic stages also revealed temperature-related patterns.

Single populations of oysters were not sampled, and temperature fluctuations at a single site were not determined. However, the composite gametogenic pattern presented in this study is similar to that of a young-of-the-year population discussed by Hayes and Menzel (1981), in which gonadal development was highest in September (water temperature above 28°C) and declined with temperature during October and November (water temperature below 20°C).

The highest G/B ratio was found at a temperature of 28°C and declined rapidly at temperatures below 20°C. Hayes and Menzel (1981) also reported lowest follicle thickness values at about 20°C; developmental-stage data for the young-of-the-year population from the northeastern Gulf revealed active gonads until early November and most spawning during September and October. Comparable results were seen in this study, in which spawning oysters were most prevalent during October. After the temperature dropped to 20°C in early October, most oysters were either spawning or in advanced spawning stages, and the number of oysters in the later development stage consistently decreased.

Southern oysters are reported to have two major spawning seasons per year, an initial spring spawn followed by new gonadal development and a second spawning season in the autumn (Hopkins et al. 1953). Oysters grow more rapidly in higher temperatures (Gunter 1951) and southern oysters apparently accumulate enough gonadal tissue for multiple spawns per year. Temperature seemed to be a significant factor determining the G/B ratio and gametogenic state of Louisiana oysters. Furthermore, this study presents additional evidence for a synchronous autumn mass spawning season of Gulf Coast oysters.

ACKNOWLEDGMENTS

We would like to thank John Markezich for his advice during histological preparations. The following individuals made it possible to obtain oyster samples: John Tesvich, Peter Tesvich, Merrill Perez, Dudley Carver, Jim Cosgrove, Thaweechai Lerjeraprasest, Ann Bull, Ron Dugas, Mark Chatry, and Howard Jones. Thanks to Pennie Brown for typing assistance. Financial support for this project came from the UNO Fisheries Initiative Program.

LITERATURE CITED

Behrens, E. W. 1965. Use of the Golberg refractometer as a salinometer for biological and geological field work. *J. Mar. Res.* 2:165–171.

Gauthier, J. D., T. M. Soniat & J. S. Rogers. A parasitological survey of oysters along the Louisiana coast in relation to salinity. Submitted to *J. World Aquaculture Soc.*

Gunter, G. 1951. The West Indian tree oyster on the Louisiana Coast, and notes on the growth of the three Gulf Coast oysters. *Science* 113:516–517.

Hayes, P. F. & R. W. Menzel. 1981. The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico, and its implications for population recruitment. *Biol. Bull.* 160:80–88.

Hopkins, A. E. 1931. Factors influencing the spawning and setting oysters in Galveston Bay, Texas. *Bull. U.S. Bur. Fish.* 48:345–364.

Hopkins, S. W., J. G. Mackin & R. W. Menzel. 1953. The annual cycle of reproduction, growth and fattening in Louisiana oysters. *Proc. Natl. Shellfish. Assoc.* 44:39–50.

Howard, D. W. & C. S. Smith. 1983. Histological techniques for marine bivalve molluscs. NOAA Technical Memorandum NMFS-F/NEC-25. Oxford, Maryland 97 p.

Ingle, R. M. 1951. Spawning and setting of oysters in relation to seasonal environmental changes. *Bull. Mar. Sci. Gulf Caribb.* 1:111–135.

Kennedy, A. V. & H. I. Battle. 1964. Cyclic changes in the gonad of the American oyster, *Crassostrea virginica* (Gmelin). *Cand. J. Zool.* 42:305–321.

Kennedy, V. S. & L. B. Krantz. 1982. Comparative gametogenic and spawning patterns of the oyster *Crassostrea virginica* (Gmelin) in central Chesapeake Bay. *J. Shellfish. Res.* 2:133–140.

Loosanoff, V. L. 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica* of Long Island Sound. *Biol. Bull.* 83:195–206.

Loosanoff, V. L. & C. A. Nomejko. 1951. Existence of physiologically different races of oysters, *Crassostrea virginica*. *Biol. Bull.* 101:151–156.

Sastry, A. N. 1975. Physiology and ecology of reproduction in marine invertebrates. *In* F. J. Vernberg (ed.). Univ. of So. Carolina, Columbia, S.C. *Physiological Ecology of Estuarine Organisms.* 279–299 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 87–94, 1989.

THE USE OF OYSTER SHELL THICKNESS AND CONDITION INDEX MEASUREMENTS AS PHYSIOLOGICAL INDICATORS OF NO HEAVY METAL POLLUTION AROUND THREE COASTAL MARINAS

JAMES M. MARCUS[1,*] GEOFFREY I. SCOTT[2] AND DEBORAH D. HEIZER[1]

[1]*South Carolina Department of Health and Environmental Control*
P.O. Box 72, Building No. 9
State Park, South Carolina 29147
[2]*School of Public Health*
University of South Carolina
Columbia, South Carolina 29208

ABSTRACT There were no significant differences in shell thickness among and between indigenous populations of oysters (*Crassostrea virginica*) at three recreational marinas in coastal South Carolina or between other estuarine areas of the State. Soft tissue concentrations of heavy metals from the study areas were not elevated relative either to marina proximity or to ambient background monitoring stations. Condition index analyses demonstrated no physiological stress on the oysters. The lack of significant changes in shell thickness and condition index, along with the absence of significantly increased metal levels in tissues and in sediments supports the observation that heavy metals do not appear to be a major pollutant around recreational marinas in coastal South Carolina.

KEY WORDS: oysters, heavy metals, shell thickness, condition index, marinas

INTRODUCTION

Exposure of oysters to heavy metals often results in bioaccumulation of those metals in soft tissues sometimes to high levels, often without any overt evidence of toxic effects (Waldichuk 1974, Mathews et al. 1979). The lack of overt or acute toxicity in exposed oyster populations (e.g., massive die-offs) does not necessarily mean that there have been no low-level, chronic sublethal effects. Numerous laboratory and field studies have examined the potential effects of heavy metal exposure on embryonic and larval stages of bivalves (MacInnes and Calabrese 1979, MacInnes 1980, Zaroogian and Morrison 1981, Watling 1982), on cellular and subcellular responses (George 1982, Simkiss and Mason 1984) and on uptake/detoxication processes (Grieg and Wenzloff 1978, Engel and Brouwer 1982, George et al. 1984, Phelps et al. 1985), with results ranging from no measurable response to demonstration of toxic effects.

While most such studies have focused on toxicant effects on fecundity and embryonic/larval survivability, some authors have suggested that the presence of heavy metals may adversely affect oysters by shell thickening (Waldock and Thain 1983), shell thinning (Frazier 1976) and shell growth inhibition (Cunningham 1976). Bahr and Hillman (1967) and Loosanoff and Nomejko (1955) examined gametogenesis and growth patterns, respectively, in oysters with damaged shells and found no significant negative impacts. Phelps and Hetzel (1987), however, showed that stunted oysters from areas in the Chesapeake Bay had more copper and zinc in their tissues than did normal oysters from an unpolluted area of the Bay.

The purpose of this study was to determine whether recreational marinas added significant loads of heavy metals to their environments and, if so, whether such metal input affected the physiology of indigenous oysters around those marinas as measured by shell thickness and condition index. Usually, concern over inputs from marinas has centered on fecal wastes and their uptake by nearby oysters, given the high potential for human gastrointestinal illness upon consumption. However, marinas also present the potential for pollution of nearby waters by heavy metals and petroleum hydrocarbons. Because of the irregular input of pollutants from marinas, routine detection in the water column is made difficult.

Preferred biological monitors for detection of heavy metals are filter-feeders such as the oyster (Phillips 1977, Cunningham 1979). Uptake of trace metals by and subsequent accumulation in oysters can reflect changes in water-column concentrations of metals. For the study reported here, we chose the American oyster, *Crassostrea virginica* (Gmelin), because of its wide distribution and ecological significance in the estuaries of South Carolina, its economic importance as a commercial and recreational fisheries resource, and its suitability as a sentinel organism for coastal pollution (Farrington 1983).

MATERIALS AND METHODS

The three recreational marinas in southern coastal South Carolina on Hilton Head and Fripp Islands chosen for this study are shown in Fig. 1. These barrier islands contain extensive stands of the smooth cordgrass, *Spartina alterniflora*, and are characterized by an extensive dendritic pattern of many small tidal creeks. The very limited freshwater input to the creeks where the marinas are located and

*Present address: Fluor Daniel, Inc., 50 International Drive, P.O. Box 19019 Greenville, SC, 29602-9019

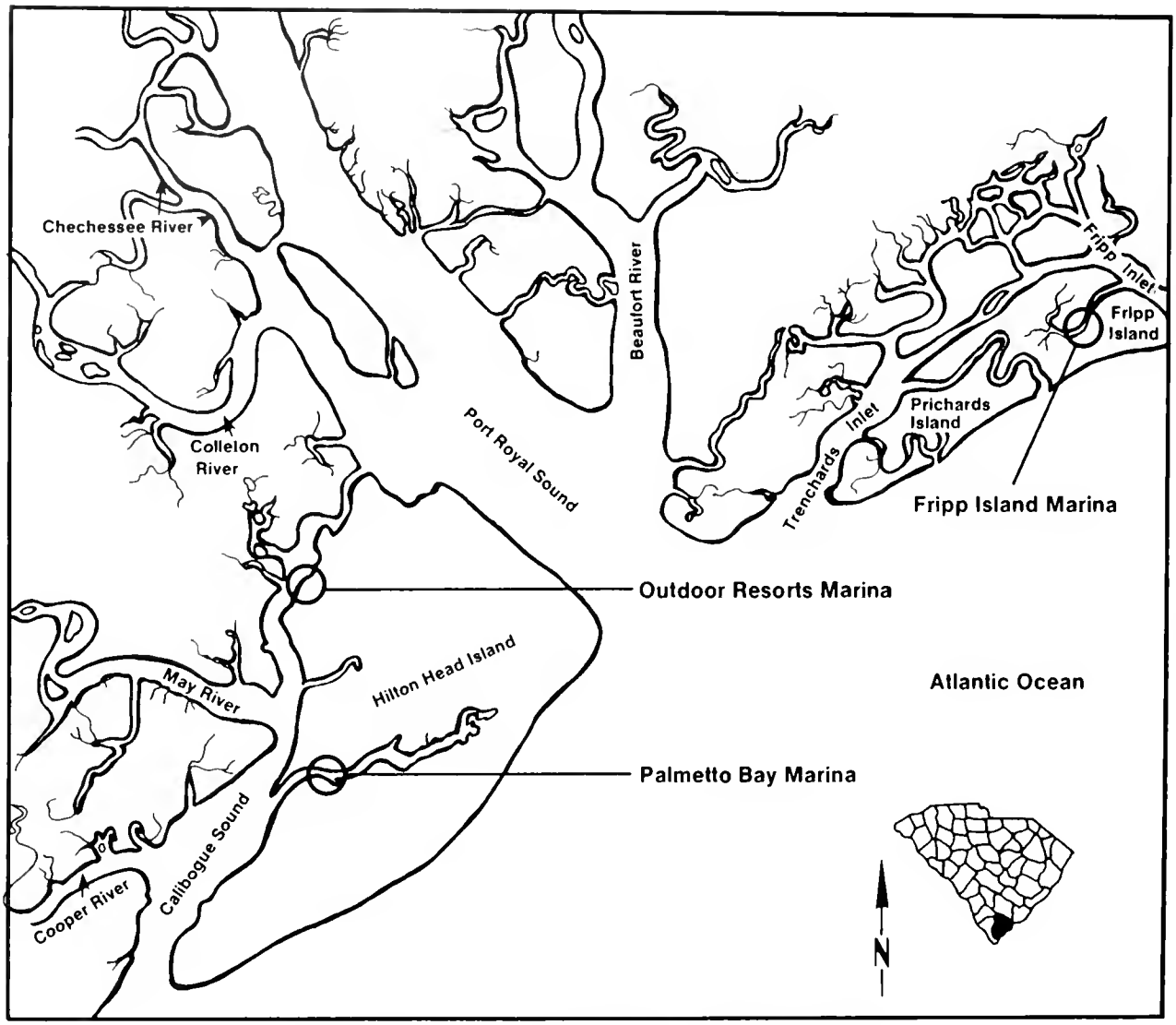


Figure 1. Location of coastal marinas chosen for study in South Carolina.

their close proximity to the Atlantic Ocean result in vertically homogeneous, higher salinity waters (mean of 27 ppt) of two equal flood and ebb tides per day.

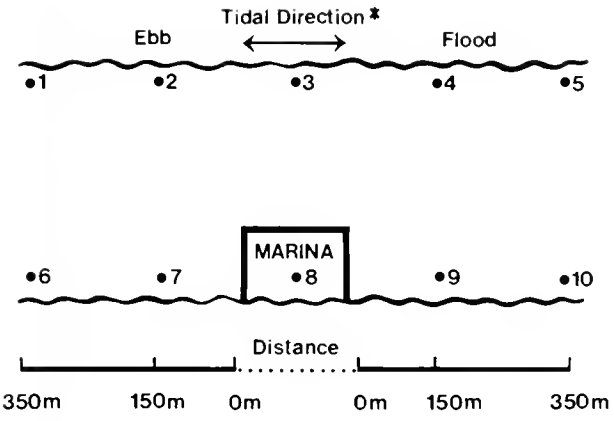
Two of the marinas are large by South Carolina standards: the Palmetto Bay Marina (32 10′N; 80 46′W), which moors approximately 80 boats, and the Outdoor Resorts Marina (32 13′N; 80 46′W), which moors about 70 boats. Both marinas are located on Hilton Head Island in Broad Creek and near Skull Creek, respectively. The Fripp Island Marina (32 20′N; 80 30′W), located on Old House Creek, moors approximately 35 boats and services a small, private resort community. Both the Palmetto Bay and Fripp Island Marinas extend into the main creek channels; the Outdoor Resorts Marina is an excavated basin off of Skull Creek.

A grid of ten stations was placed around each marina, extending approximately 350 m in both ebb- and flood-tide directions for intertidal oyster sampling (Fig. 2). Oysters were manually collected from the mid-intertidal portion of the reefs at all stations where available. Each sample for physiological analysis comprised ten oysters of legally harvestable size (≥7.5 cm in height) per station taken once in the spring and once in the summer. The overall heights for all oysters ranged from 8.5 to 14.2 cm. After all fouling and commensal organisms were removed, condition index (CI) analyses were conducted by the method of Lawrence and Scott (1982), namely, (dry meat wt in g) × 100/(internal cavity vol in cm^3).

Dry shell weight (mg) and height (cm) measurements were obtained for each oyster. Shell area (cm^2) was then computed from shell height (h) by the formula:

$$SA = 2.5\ h^{1.56}$$

as described by Galtsoff (1964). A ratio of shell weight to shell area was then calculated according to Frazier (1976)



*Tidal flow direction reversed from this schematic at Fripp Island Marina.

Figure 2. Schematic diagram of the sampling station grid placed around each marina for collection of oysters.

to arrive at a measure of the weight of the shell per unit area (mg/cm^2), or shell thickness. To determine if this formula was valid for our project, 21 oysters were randomly selected for method evaluation. The perimeter of each right and left valve was traced and the resulting outlines were then planimetered. The planimetered value was related to a known surface area, thus yielding a measured surface area value for each oyster. The area was then computed using Galtsoff's formula and a correlation made of the computed surface area against the measured surface area, which yielded a correlation coefficient of $r = 0.84$ at $p < 0.05$.

Analyses were made at each station for seven different heavy metals in both oysters and sediments from each station. Thirty oysters were collected from each station once per week for three consecutive weeks in the spring and again in the summer and the soft tissues pooled by station

TABLE 1.

Lower detection limits and analytical quality control performance data for total heavy metal analyses.

(a) Oysters

Metal	Lower Limit mg/kg, wet wt	Mean % Spike Recoveries	Mean % CV* for Duplicates
Cadmium	0.2	89.1	1.6
Chromium	1.0	84.9	1.4
Copper	1.0	87.6	0.9
Lead	1.0	87.2	6.1
Mercury	0.25	94.7	3.0
Nickel	1.0	92.5	1.0
Zinc	1.0	97.9	0.8

(b) Sediments

Metal	Lower Limit mg/kg, dry wt	Mean % Spike Recoveries	Mean % CV* for Duplicates
Cadmium	1.0	98.3	1.3
Chromium	5.0	101.0	1.1
Copper	5.0	96.2	1.0
Lead	5.0	94.6	2.0
Mercury	0.25	70.4	4.6
Nickel	5.0	97.1	1.1
Zinc	5.0	90.2	1.0

* CV = coefficient of variation

for analyses. Sediment samples from each station were analyzed once during both seasons. For all metals except mercury, approximately 5 g of oyster tissue taken from the ground and blended composite sample of 30 whole individuals per station were placed in 50 ml acid-washed beakers and transferred to a cold muffle furnace where they were ashed at 450 C for 12–16 hours. After the samples had cooled, 2 ml aqua regia was added to each and then warmed to dissolve the ash. The non-filtered samples were quantitatively transferred to 100 ml volumetric flasks,

TABLE 2.

Mean shell thickness of and mean total heavy metals in oysters from Palmetto Bay (PB), Outdoor Resorts (OR) and Fripp Island (FI) Marinas, South Carolina.

Station	Mean Shell Thickness[a] (mg/cm^2)	Mean Total Metals (mg/kg)	Station	Mean Shell Thickness[a] (mg/cm^2)	Mean Total Metals (mg/kg)	Station	Mean Shell Thickness[a] (mg/cm^2)	Mean Total Metals (mg/kg)
PB-1	438 ± 26.4	273.39	OR-1	444 ± 21.5	402.64	FI-1	354 ± 16.3	310.55
PB-2	362 ± 21.5	446.62	OR-2	384 ± 16.5	416.60	FI-2	292 ± 13.9	245.18
PB-3	505 ± 44.3	398.26	OR-3	372 ± 16.5	367.63	FI-3	366 ± 14.8	308.48
PB-4	368 ± 25.5	401.85	OR-4	*	*	FI-4	*	*
PB-5	282 ± 10.3	384.84	OR-5	427 ± 14.5	376.99	FI-5	328 ± 14.3	368.56
PB-6	378 ± 21.0	312.86	OR-6	436 ± 32.2	411.94	FI-6	380 ± 23.9	324.99
PB-7	406 ± 28.6	398.92	OR-7	*	*	FI-7	339 ± 16.1	334.10
PB-8[b]	364 ± 19.2	471.94	OR-8[b]	370 ± 18.8	541.24	FI-8[b]	315 ± 19.2	337.18
PB-9	388 ± 16.5	458.02	OR-9	432 ± 26.8	530.24	FI-9	366 ± 15.2	281.83
PB-10	404 ± 34.4	479.74	OR-10	342 ± 16.1	419.86	FI-10	336 ± 13.6	256.74

[a] Mean ± one standard error (n = 20 at each station: n = 10 in spring; n = 10 in summer)

[b] Marina location

* Oysters not available

TABLE 3.

Ranges of total heavy metal concentrations in oyster tissues and sediments around three coastal marinas in South Carolina.

Metal	Sample Type	Marina		
		Palmetto Bay	Outdoor Resorts	Fripp Island
Cd	Tissue[a]	0.44–0.59	0.37–0.79	0.43–0.72
	Sediment[b]	all <1.0	all <1.0	all <1.0
Cr	Tissue	<1.0–1.0	<1.0–1.0	<1.0–2.0
	Sediment	8.0–32.0	6.0–29.0	9.0–35.0
Cu	Tissue	13.0–29.0	10.0–46.0	9.6–27.0
	Sediment	<5.0–11.0	<5.0–10.0	<5.0–10.0
Pb	Tissue	<1.0–1.9	<1.0–3.7	<1.0–1.6
	Sediment	7.0–36.0	9.0–44.0	15.0–32.0
Hg	Tissue	all <0.25	all <0.25	all <0.25
	Sediment	all <0.25	all <0.25	all <0.25
Ni	Tissue	<1.0–1.7	<1.0–5.2	<1.0–2.5
	Sediment	<5.0–22.0	<5.0–34.0	8.0–21.0
Zn	Tissue	127–580	206–747	132–423
	Sediment	13.0–49.0	13.0–50.0	25.0–49.0
TOTAL	Tissue	143.69–614.44	219.62–803.94	145.28–457.07
	Sediment	39.25–151.25	39.25–168.25	63.25–148.25

[a] mg/kg, wet weight
[b] mg/kg, dry weight

brought to volume with deionized water, then analyzed by flame atomic absorption spectrophotometry (USEPA 1979) for Cd, Cr, Cu, Pb, Ni and Zn. Routine special caution was taken to avoid zinc contamination from laboratory activities. For the mercury analysis, approximately 0.200 ± 0.002 g was digested and then analyzed using the cold vapor method on an Auto Analyzer System (USEPA 1973b).

Twice during the study, the top 3 cm of sediment within the oyster reef at each station was collected manually using a stainless steel spatula. For all metals except mercury, approximately 5 g of sediment were dried overnight at 103 C on acid-washed watch glasses after which 1.000–1.005 g aliquots of dry sediment were transferred to 100 ml beakers for digestion (USEPA 1973a). After digestion, the samples were aspirated to an Instrumentation Laboratory Model 251 atomic absorption spectrophotometer for analyses (USEPA 1979). Background correction was employed for the cadmium analysis if the cadmium result was higher than the lower detection limit and the sample had an obviously high level of sodium as characterized by a bright yellow flame. For the mercury analysis, another 5 g portion of sediment was dried overnight at 60 C, after which approximately 0.200 + 0.002 g was digested (USEPA 1973b). Mercury was then analyzed using the cold vapor method on an AutoAnalyzer System.

In all digestion procedures, at least 10% of the samples were duplicated and 10% were spiked with a known quan-

TABLE 4.

Ranges of total heavy metal concentrations in oyster tissues and sediments at ambient monitoring sites in lower coastal South Carolina.

Water System	Sample Type	Range of Heavy Metal Concentrations (n = 3; 1984–1986)						
		Cd	Cr	Cu	Pb	Hg	Ni	Zn
Trenchards	Tissue[a]	0.52–0.60	<1.0–1.6	6.8–9.4	<1.0–1.4	all <0.25	<1.0–1.8	120–160
Inlet	Sediment[b]	<1.0–1.2	12–29	<5.0–5.4	<5.0–40	all <0.25	5.0–9.6	17–34
Whale Branch	Tissue	0.90–1.2	all <1.0	8.8—14	all <1.0	all <0.25	all <1.0	500–600
	Sediment	<1.0–1.9	23–46	6.3–8.4	11–81	all <0.25	10–16	44–50
Coosaw River	Tissue	0.64–0.80	<1.0–5.0	10–16	all <1.0	all <0.25	<1.0–12.0	360–580
	Sediment	<1.0–1.7	25–45	<5.0–7.5	9.0–78	all <0.25	9.2–16	40–48
May River	Tissue	0.46–0.60	<1.0–1.4	11–12	<1.0–1.4	all <0.25	<1.0–1.2	all = 220
	Sediment	<1.0–1.9	24–50	8.1–10	8.5–88	all <0.25	13–18	42–52
Broad River	Tissue	0.90–1.6	all <1.0	18–28	all <1.0	all <0.25	all <1.0	300–340
	Sediment	<1.0–1.5	17–37	5.3–9.2	5.0–65	all <0.25	5.8–13	25–39
Savannah	Tissue	all = 0.60	<1.0–1.4	13–16	all <1.0	all <0.25	all <1.0	320–340
River	Sediment	<1.0–2.0	31–54	8.3–13	6.3–91	all <0.25	12–20	45–58

[a] mg/kg, wet weight
[b] mg/kg, dry weight

TABLE 5.

Spearman correlation coefficients between mean total heavy metals in and mean shell thickness of oysters around three coastal marinas in South Carolina.

Marina Treatment	Groupings	Results r	p	n
a. linear metals—linear thickness				
Palmetto Bay	All stations	−0.246	0.295	20
	Left bank stations	−0.311	0.325	10
	Right bank stations	0.098	0.818	10
Outdoor Resorts	All stations	0.089	0.743	16
	Left bank stations	0.297	0.474	8
	Right bank stations	0.075	0.860	8
Fripp Island	All stations	−0.101	0.690	19
	Left bank stations	−0.456	0.185	9
	Right bank stations	0.389	0.341	10
b. linear metals—log10 thickness				
Palmetto Bay	All stations	−0.515	0.128	20
	Left bank stations	−0.200	0.747	10
	Right bank stations	−0.700	0.188	10
Outdoor Resorts	All stations	−0.310	0.456	16
	Left bank stations	0.200	0.800	8
	Right bank stations	0.000	1.000	8
Fripp Island	All stations	0.159	0.683	19
	Left bank stations	0.800	0.200	9
	Right bank stations	−0.564	0.322	10

tity of metals. At least 10% of the samples were repeated during analysis with replicate analyses performed each time after 10 to 12 samples had been processed. A reference sediment standard was digested and analyzed several times with groups of samples during the analysis period to monitor the accuracy of the digestion procedure. The precision and accuracy data for the metals analyses are presented in Table 1.

Statistical analysis for significant differences between stations and station groupings were conducted using the Kruskal-Wallis H test (SAS 1985b, Wilcoxon and Wilcox 1964) at the 95% confidence level. Spearman correlation coefficients (SAS 1985a) were computed to ascertain whether there was a relationship between the shell thickness and the level of heavy metals measured in shellstock. The lower detection limit was used as a discrete value in all statistical computations. This is a conservative statistical method since the mean values reported represent the maximum possible mean levels from the data sets.

RESULTS

Oysters from stations around the three marinas had similar mean shell thicknesses, as those from Palmetto Bay (PB) ranged from 282 to 505 mg/cm^2 while those from Outdoor Resorts (OR) and Fripp Island (FI) ranged from 342 to 444 mg/cm^2 and from 292 to 380 mg/cm^2, respectively (Table 2). The overall mean shell thicknesses ± one standard error were 390 ± 25 mg/cm^2 at PB, 401 ± 20 mg/cm^2 at OR and 342 ± 16 mg/cm^2 at FI. These data did not vary consistently with distance from the marinas throughout each marina area. There were no significant differences between individual stations or between right and left bank groupings at any of the three marinas individually, or between the right and left bank groupings from all marinas collectively. Spring and summer thickness data were pooled at each marina only after it was determined that there were no significant differences between the two seasons.

The mean total metals levels in oyster tissues showed similar levels at PB, OR, and FI Marinas, with ranges of 273.39 to 479.74 mg/kg, 367.63 to 541.24 mg/kg and 245.18 to 368.56 mg/kg, respectively (Table 2). Concentrations of individual metals in tissues and sediments were similar at all three marinas (Table 3), and also similar to ambient background levels from this same geographic area (Table 4; SCDHEC 1988) and to the South Carolina coast in general (Marcus and Mathews 1987). Levels of total metals were also calculated for each station (Table 2). There were no statistically significant differences between stations, however, for either mean specific metals levels or mean total metals levels in oysters from around the marinas. There were also no statistically significant temporal differences in the metal concentrations in oysters at any of the three marinas. The spring and summer metals-in-tissue data sets were combined, therefore, for further statistical analysis.

There were no significant correlations between linear mean total heavy metals (dose) and linear mean shell thickness (response) in tissues at any of the marinas (Table 5a). A log10 transform was made for the thickness data to

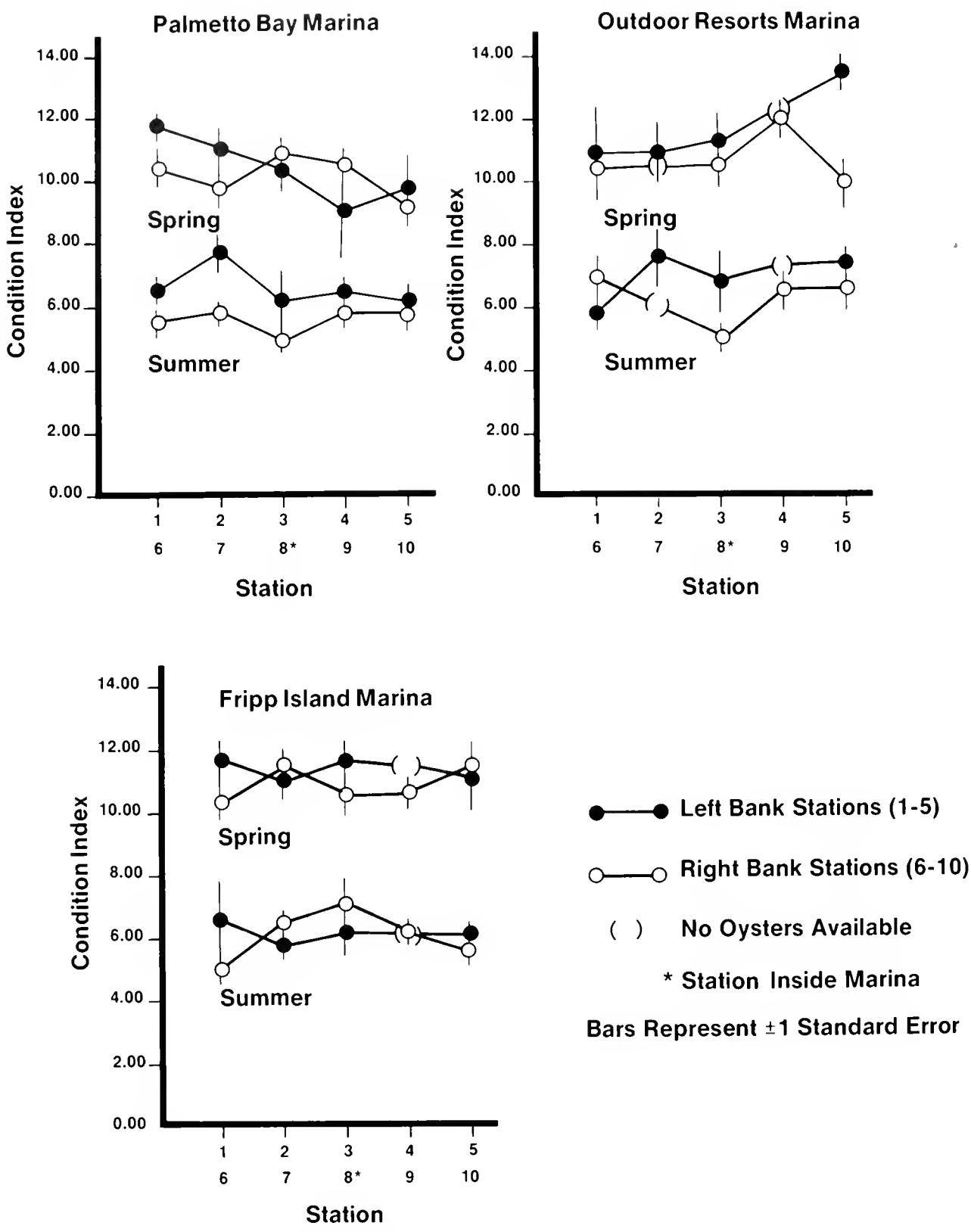


Figure 3. Condition index values from each coastal marina by season.

TABLE 6.

Condition index measurements from oysters around three coastal marinas in South Carolina.

Marina	Sampling Season	Condition Index—Left Bank				Condition Index—Right Bank			
		Mean[a]	SE[b]	n	Range[a]	Mean[a]	SE[b]	n	Range[a]
Palmetto Bay	Spring	10.42	0.43	50	2.59–15.89	10.20	0.27	50	5.06–13.89
	Summer	6.68	0.27	50	3.92–13.69	5.70	0.16	50	3.29–8.16
Outdoor Resorts	Spring	11.67	0.54	40	3.30–19.03	10.85	0.38	40	4.63–16.04
	Summer	6.96	0.41	40	3.40–14.75	6.31	0.31	40	3.18–11.29
Fripp Island	Spring	11.49	0.32	40	5.58–14.84	10.94	0.27	50	6.64–16.36
	Summer	6.19	0.34	40	2.38–16.99	6.23	0.24	50	3.03–12.45

[a] Reported in dimensionless CI units
[b] SE = standard error

ascertain whether a non-linear relationship existed, but as with the linear-linear analysis, there were no significant correlations (Table 5b). Testing against other non-linear models (exponential and asymptotic) yielded similar, non-significant associations.

Condition index analyses revealed no significant physiological differences in oysters either within or between the marinas. Statistically significant temporal differences were observed at each marina, as would be expected because of spawning (Fig. 3 and Table 6). Each marina exhibited CI levels very similar to each other for both seasons. PB oysters had a mean CI of 10.42 and 10.20 for the left and right banks during the spring, respectively, while OR oysters were 11.67 and 10.85 and FI oysters were 11.49 and 10.94. The summer values were lower at all marinas: 6.68 and 5.70 for left and right banks at PB; 6.96 and 6.31 for OR; and, 6.19 and 6.23 for FI. These CI data were important because they demonstrated no physiological differences in oysters both within and between the three marinas.

DISCUSSION

The shell thickness data from indigenous oyster populations around these recreational marinas were similar to shell thicknesses of oysters from other areas of South Carolina. In this study, the mean thicknesses were 390, 401 and 342 mg/cm^2 at PB, OR and FI Marinas, respectively. We have studied oysters from other estuaries in South Carolina, finding similar average thicknesses of oyster shells: Church Creek (means from 322 to 465 mg/cm^2), Stono River (400 to 452 mg/cm^2) and Leadenwah Creek (336 to 475 mg/cm^2). There were no significant differences in shell thicknesses between oysters from around the marinas and those from these other estuaries. Any assumed trace metal input from marinas, therefore, did not affect oyster shell thickness.

The CI analyses showed typical seasonal differences due to spawning between the spring and summer samples. Lee and Pepper (1956) have reported that total solids (e.g., glycogen and lipid) in Southern oysters decreased from 13.5% in the spring to 9.2% in the summer because of spawning, a 31.8% decrease. In this study, the pooled CI values from each marina represented losses of 39.8%, 41.4% and 45.5% at PB, OR and FI, respectively, typical for spawning oyster populations.

There were no suggestions that marina proximity adversely affected the CI values for the indigenous oysters, not even those on the bank common with the marina. Overall, the CI analyses indicated that there had been no impact by the presence of the marinas on the ability of the oysters to maximize the shell cavity by filling it with soft tissue.

An earlier study by Frazier (1976) reported reduced shell thickness in oysters from a control site (267 to 322 mg/cm^2) and a site polluted with trace metals (223 to 277 mg/cm^2). Control shells were 11 to 20% thicker than those from the polluted area. The shell thicknesses measured around the three marinas in this study, however, were clearly greater than those reported by Frazier. This difference in shell thickness data and association with heavy metals contamination was probably due to the difference in metals levels in the different environments. Frazier assayed oysters and sediments for Cd, Cr, Fe, Mn and Zn (μg/g, dry weight). Zinc concentrations were 4100 μg/g at the exposed site and 1700 μg/g at the control site, as compared to concentrations that ranged from 635 to 3235 mg/kg, dry weight around the marinas in this study. Copper was measured at 450 and 60 μg/g at Frazier's exposed and control sites, respectively, whereas around the marinas the levels ranged from 48 to 230 μg/g.

The fundamental difference in contamination between Frazier's sites and the marinas was seen in comparison of sediment data. Cadmium, copper and zinc were 0.7, 123 and 232 μg/g in sediments, respectively, at Frazier's exposed site. Around the marinas, however, sediments had no detectable Cd and maxima of 10 and 50 mg/kg of Cu and Zn, respectively (Marcus et al. 1988). These comparisons between sediment metals burdens indicate a major difference in exposure potential and, therefore, a plausible explanation for the lack of effects on shell thickness of oysters in the marina areas. These comparisons also suggest that recreational marinas such as these, at least in South Carolina, do not serve as major sources of heavy metal input.

The underlying assumptions of this study were two-fold: that marinas could be a real source of heavy metal input to surrounding waters and that such input could significantly alter shell thickness in exposed oyster populations. All data presented herein indicate no change in shell thickness of oysters from around three recreational marinas. The metal levels measured in oysters and sediments from around these marinas were not higher than metal levels found at other similar estuarine areas of South Carolina.

This study has neither confirmed nor disproved prior work indicating thinning (Frazier 1976) and thickening (Waldock and Thain 1983) of oyster shells due to metal exposure. The data presented here indicate that three recreational marinas in South Carolina did not cause adverse changes in either hard tissues (shell thickness) or soft tissues (condition index) of indigenous oysters around those marinas. These observations further support the previous conclusion that heavy metals, unlike polynuclear hydrocarbons (Marcus and Stokes 1985, Marcus et al. 1988), are not a major contribution to aquatic systems from typical coastal recreational marinas in this State.

REFERENCES CITED

Bahr, L. M. & R. E. Hillman. 1967. Effects of repeated shell damage on gametogenesis in the American oyster, *Crassostrea virginica* (Gmelin). *Proc. Natl. Shellfish. Assoc.* 57:59–62.

Cunningham, P. A. 1979. The use of bivalve molluscs in heavy metal pollution research. Vernberg, W. B., Thurberg, F. P., Calabrese, A., Vernberg, F. J. eds. *Marine Pollution: Functional Responses*. New York, NY: Academic Press, 183–221.

Cunningham, P. A. 1976. Inhibition of shell growth in the presence of mercury and subsequent recovery of juvenile oysters. *Proc. Natl. Shellfish Assoc.* 66:1–5.

Engel, D. W. & M. Brouwer. 1982. Detoxification of accumulated trace metals by the American oyster, *Crassostrea virginica:* laboratory vs. environment. Vernberg, W. B., Calabrese, A., Thurberg, F. P., Vernberg, F. J. eds. *Physiological Mechanisms of Marine Pollutant Toxicity*. New York, NY: Academic Press, 89–106.

Farrington, J. W. 1983. Bivalves as sentinels of coastal chemical pollution: the mussel (and oyster) watch. *Oceanus*. 26:18–29.

Frazier, J. M. 1976. The dynamics of metals in the American oyster, *Crassostrea virginica*. II. Environmental effects. *Chesapeake Sci.* 17:188–197.

Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. U.S. Fish and Wildlife Service, *Fish. Bull. 64*. Washington, D.C., 30–32

George, S. G., B. J. Pirie, J. M. Frazier & J. D. Thomson. 1984. Interspecies differences in heavy metal detoxication in oysters. *Mar. Environ. Res.* 14:462–64.

George, S. G. 1982. Subcellular accumulation and detoxication of metals in aquatic animals. Vernberg, W. B., Calabrese, A., Thurberg, F. P., Vernberg, F. J. eds. *Physiological Mechanisms of Marine Pollutant Toxicity*. New York, NY: Academic Press, 3–52.

Grieg, R. A. & D. R. Wenzloff. 1978. Metal accumulation and depuration by the American oyster, *Crassostrea virginica*. *Bull. Environ. Contam. Toxicol.* 120:499–504.

Lawrence, D. R. & G. I. Scott. 1982. The determination and use of condition index of oysters. *Estuaries* 5:23–27.

Lee, C. F. & L. Pepper. 1956. Composition of Southern oysters. U.S. Fish and Wildlife Service. *Commerc. Fish. Rev.* 18:1–6.

Loosanoff, V. L. & C. A. Nomejko. 1955. Growth of oysters with damaged shell-edges. *Biol. Bull.* 108:151–159.

MacInnes, J. R. 1980. Response of embryos of the American oyster, *Crassostrea virginica,* to heavy metal mixtures. *Mar. Environ. Res.* 4:217–227.

MacInnes, J. R. & A. Calabrese. 1979. Combined effects of salinity, temperature, and copper on embryos and early larvae of the American oyster, *Crassostrea verginica*. *Arch. Environ. Contam. Toxicol.* 8:553–562.

Marcus, J. M., G. R. Swearingen, A. D. Williams & D. D. Heizer. 1988. Polynuclear aromatic hydrocarbon and heavy metal concentrations in sediments at coastal South Carolina marinas. *Arch. Environ. Contam. Toxicol.* 17:103–113.

Marcus, J. M. & T. D. Mathews. 1987. Trace metals in South Carolina estuaries. Lindberg, S. E., Hutchinson, T. C. eds. *Heavy metals in the Environment*, Vol. II. Proc. Sixth Inter. Conf., Edinburgh, United Kingdom: CEP Consultants, Ltd., 446–448.

Marcus, J. M. & T. P. Stokes. 1985. Polynuclear aromatic hydrocarbons in oyster tissue and around three coastal marinas. *Bull. Environ. Contam. Toxicol.* 35:835–844.

Mathews, T. D., J. V. Boyne, R. A. Davis & D. R. Simmons. 1979. The distribution of copper and iron in South Carolina oysters. *J. Environ. Sci. Health* A14:683–694.

Phelps, H. L. & E. W. Hetzel. 1987. Oyster size, age, and copper and zinc accumulation. *J. Shellfish Res.* 6:67–70.

Phelps, H. L., D. A. Wright & J. A. Mihursky. 1985. Factors affecting trace metal accumulation by estuarine *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 22:187–197.

Phillips, D. J. H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. *Environ. Pollut.* 13:281–317.

Simkiss, K. & A. Z. Mason. 1984. Cellular responses of molluscan tissues to environmental metals. *Mar. Environ. Res.* 14:103–118.

South Carolina Department of Health and Environmental Control. 1988. Open-file-trend monitoring data. Bureau of Water Pollution Control, Columbia, SC.

Statistical Analysis System. 1985a. SAS user's guide: basics. Cary, North Carolina: SAS Institute, 861–874.

Statistical Analysis System, 1985b. SAS user's guide: statistics. Cary, North Carolina: SAS Institute, 433–506; 607–614.

United States Environmental Protection Agency. 1973a. Tentative digestion of sediments for metals and analysis. Athens, Georgia: Region IV, Surveillance and Analysis Division.

United States Environmental Protection Agency. 1973b. Automated cold vapor AA analysis for total mercury in fish and sediments after digestion. Cincinnati, Ohio: Environmental Monitoring and Support Laboratory.

United States Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. Cincinnati, Ohio: Environmental Monitoring and Support Laboratory.

Waldichuk, M. 1974. Some biological concerns in heavy metals pollution. Vernberg, J. J., Vernberg, W.B. eds. Pollution Physiology of Marine Organisms. New York, NY: Academic Press, 1–57.

Waldock, M. J. & J. E. Thain. 1983. Shell thickening in *Crassostrea gigas:* organotin antifouling or sediment induced? *Mar. Pollut. Bull.* 14:411–415.

Watling, H. R. 1982. Comparative study of the effects of zinc, cadmium, and copper on the larval growth of three species. *Bull. Environ. Contam. Toxicol.* 28:195–201.

Wilcoxon, R. & R. A. Wilcox. 1964. Some rapid approximate statistical procedures. Pearl River, New York: Lederle Laboratories, 1–31.

Zaroogian, G. E. & G. Morrison. 1981. Effect of cadmium body burdens in adult *Crassostrea virginica* on fecudity and viability of larvae. *Bull. Environ. Contam. Toxicol.* 27:344–348.

Journal of Shellfish Research, Vol. 8, No. 1, 95–104, 1989.

ENVIRONMENTAL INFLUENCES ON THE OYSTER INDUSTRY ALONG THE WEST COAST OF FLORIDA

ROBERT L. ALLEN[1] AND R. EUGENE TURNER[2]

[1]*Coastal Fisheries Institute*
Center for Wetland Resources
Louisiana State University
Baton Rouge, Louisiana 70803-7503

[2]*Coastal Ecology Institute*
Center for Wetland Resources
Louisiana State University
Baton Rouge, Louisiana 70803-7503

ABSTRACT This paper analyzes variations in oyster landings along the west coast of Florida (currently $4–$7 million annually) and examines the effects of both natural and man-induced changes in the environment on yields. Fluctuations in yields have been related to natural phenomena such as floods, droughts, and hurricanes, or to human impacts such as overfishing, regulations, and habitat alterations. Although estuarine environmental changes are known to affect oyster production, the relationships between specific critical periods of environmental change and current and future changes in yield have not been well documented. This paper summarizes the various statistical relationships between fishing effort, river flow and air temperature.

Multiple regression models (range of R^2 from 0.42 to 0.91) predicting Florida's oyster yields (west coast only), most of which are for single bay systems, were responsive to spring river discharge and winter air temperature variables. The west coast of Florida has a strong spring harvest that is not severely affected by flooding conditions and that occurs before high temperatures and salinities increase the threat of disease and predation. Spring flooding and summer storms and hurricanes affect the fall and winter oyster harvest. We view annual variations in the biological system as resulting, in part, from climatic factors that the fishing industry responds to and "remembers" the following year. Human interventions also affect the system; management has increased potential yields by depositing substrate suitable for oyster-spat colonization and growth. Thus, these quantified changes in climate, state management, and the fisherman's effort all contribute in interrelated ways to influence variations in annual harvest.

KEY WORDS: Oysters, Florida, environmental influences, Gulf of Mexico, oyster yields

INTRODUCTION

From 1961 to 1978, annual oyster harvests from the west coast of Florida averaged 1.7 million kg of oyster meat or 6.9% of the nation's total harvest. Declining oyster populations in Florida have been blamed on overfishing, natural disaster, water pollution, and under- and overregulation. In a nationwide comprehensive review of the oyster industry, the National Marine Fisheries Service (NMFS) reported that oyster bottoms are shrinking at an annual rate of 0.6%, primarily because of water pollution (U.S. Department of Commerce, NMFS 1977). Additionally, severe flooding, the spread of disease, and the influx of predatory species contribute to high mortalities on the oyster beds. Compared to the industry today, the early industry was not regulated, and many feel that too many regulations now exist (Engle 1963; May 1972; Kennedy and Breisch 1983).

Understanding the influence of climate and environmental conditions on oyster reefs is a major step in understanding and predicting variations in yield. Previous studies along the Gulf of Mexico coast and elsewhere have demonstrated the feasibility of relating changes in yields to variations in climate. Dow (1977) found 24 species of finfish, crustacea, and mollusks (including oysters) to be related in total weight and diversity to sea temperature cycles in the Gulf of Maine. Likewise, Sutcliffe (1972) found strong correlations between the catches of four commercially important fish and river discharge from the St. Lawrence. Studies along the Gulf of Mexico coast have revealed that shrimp, oysters, and blue crabs are influenced by specific environmental conditions (Turner 1978; West 1981).

Computer models describing oyster production have been derived for the central Chesapeake Bay area and for major bays along the Texas coast (Ulanowicz et al. 1980; Texas Department of Water Resources 1981). Strong correlations between spat densities and environmental phenomena were evident in Chesapeake Bay until the 1970s, when the oyster industry became more managed. After reviewing data on oyster yields in the bay, Ulanowicz et al. (1980) expanded their model to include annual seed planting with the natural available stock. Their model successfully predicts between 93% and 98% of the yearly catch from central Chesapeake Bay (Maryland Sea Grant 1980). The Texas model uses freshwater inflow to major oyster-producing bays as the environmental determinant. Its statistical analysis has a coefficient of determination ranging from 0.6 to 0.7 (Texas Department of Water Resources 1981).

We undertook this research to derive useful equations for predicting future oyster yields in the Gulf of Mexico. The west coast of Florida is a good system to analyze for

three reasons: (1) 80% or more of Florida's total oyster harvest is from Apalachicola Bay; (2) environmental forcing factors should be more apparent because of the limited area of the oyster fishery; and (3) the exclusive use of tongs simplifies attempts to estimate effort. To fully explain the relationships between the components in the derived models, we will begin by presenting a brief life history of the oyster, including environmental factors, and a short history of the changes in the oyster industry.

LIFE HISTORY, MANAGEMENT, AND GEAR

Distribution

Oysters are predominantly cosmopolitan coastal organisms found from 64°N to 44°S latitudes. Factors important to the propagation, growth, and survival of the species are bottom type, water circulation, salinity, temperature, food, sedimentation, pollution, competition, disease, and predation (Galtsoff 1964).

Temperature and Salinity

Oysters are poikilothermic-euryhaline and survive temperatures of 0°–90°C and salinities of 5–40 ppt. Although oysters can survive brief periods of adverse conditions by closing their shells, they cannot survive long periods in completely fresh water. Frequent flooding occurs along the Gulf Coast in the major tributaries—the Apalachicola, Alabama, Pearl, Mississippi, Atchafalaya, and Trinity rivers. We use the term *flooding* here to refer to river flooding in which an increased volume of water causes the river to overflow its channels and deliver greater than normal quantities of freshwater inflow to receiving water bodies. This increase in freshwater inflow alters the affected environments; we refer to these alterations as *flood conditions*. In relation to the oyster beds, the most important alteration is the decrease in salinity in the affected Gulf waters due to the increased influx of fresh water. Flood conditions occasionally persist for 30 days or more, and oyster mortality rates can reach as high as 100% in some oyster beds (Galtsoff 1930; Butler 1949, 1952; May 1972). According to Butler (1952), the adult oysters in Mississippi Sound that survived the 1950 flood had delayed gonad development and spawning. During periods of low river discharge and low local rainfall, salinity levels rise, increasing the threat of disease and predation to the oyster reefs (Galtsoff 1964). The saltwater predator *Thais haemosotma* (oyster drill) reportedly may kill 60%–70% of the seed oysters or completely annihilate the crop in some areas (Galtsoff 1964). Higher salinities also tend to weaken the oysters' resistance to disease.

Temperature affects oysters by influencing water transport, feeding, respiration, gonad development, and spawning. Temperatures below 6°C or above 32°C inhibit growth and reproduction. Comparisons of northern and

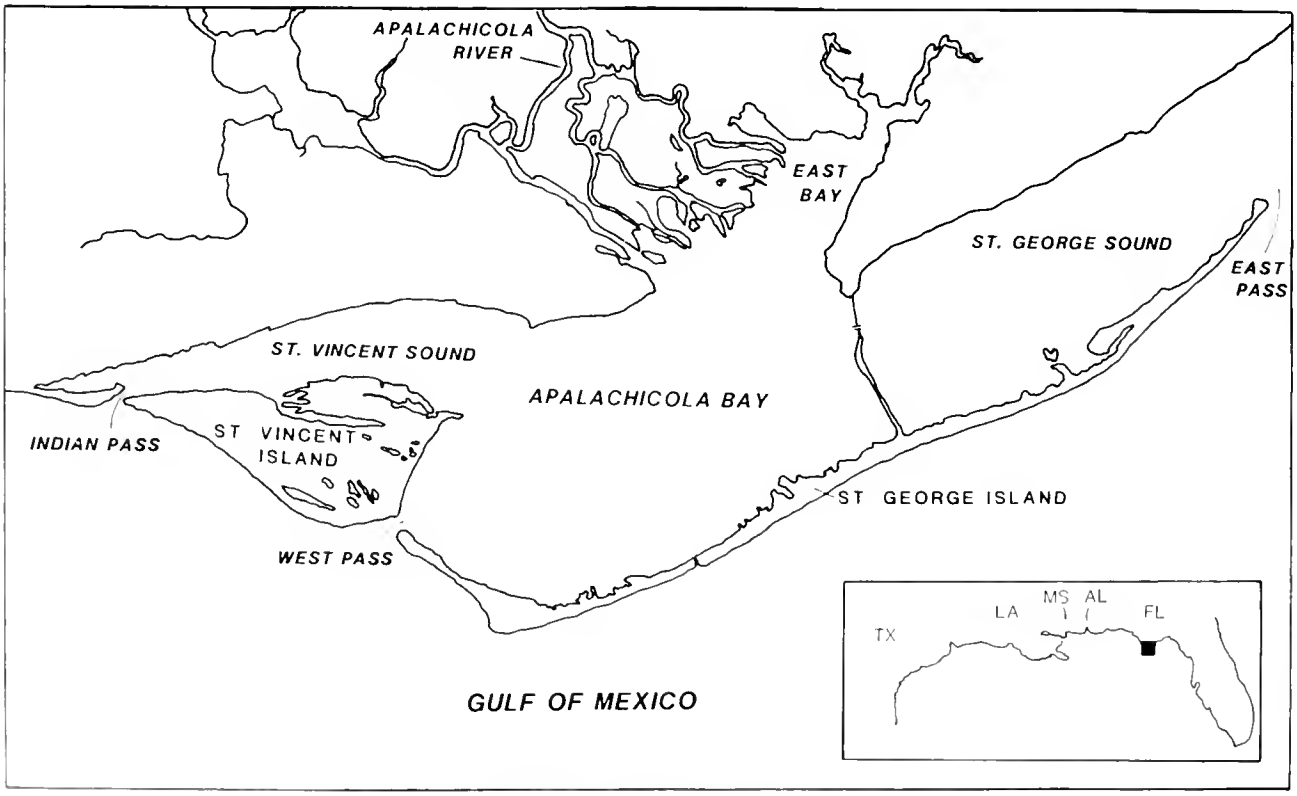


Figure 1. Apalachicola Bay environs.

southern oyster populations reveal the effects of temperature on growth and length of spawning. Oysters in the Gulf of Mexico grow to a marketable size in 2 years and spawn for 7 months annually, whereas oysters in northern latitudes take 4–5 years to reach the same size and spawn for only 2 months. Although spawning was first thought to be triggered by a specific temperature, it is now known to occur in response to a sudden rise in temperature (Butler 1965).

Management

Florida estuaries have a mostly sandy substrate rather than the hard, rocky bottoms and semihard muds that are better suited for oyster beds. To help the industry, Florida, like other Gulf states, has placed old shells, concrete blocks, scrap metal, and other materials on the water bottoms to create new habitat or rehabilitate old oyster grounds. Besides planting materials for the attachment of larvae and spat, Florida harvests undersized oysters from areas designated as seed oyster grounds and then plants them on environmentally favorable public and private beds, where they remain until maturity.

Gear

Oysters are primarily harvested with tongs in Florida. A pair of tongs is an elongated, basketlike apparatus with 2.4- to 3.6-m handles. Tonging is a one- or two-person operation conducted from a small (4.9–5.5 m) flat-bottom skiff. The oysterman uses a scissorlike motion to gather the oysters into the basketlike end of the tongs and lift them into the skiff, where they are culled and sorted. Although Florida fishermen are restricted to tongs, Florida still ranks second behind Louisiana in average catch-per-unit-effort (CPUE) along the northern gulf coast.

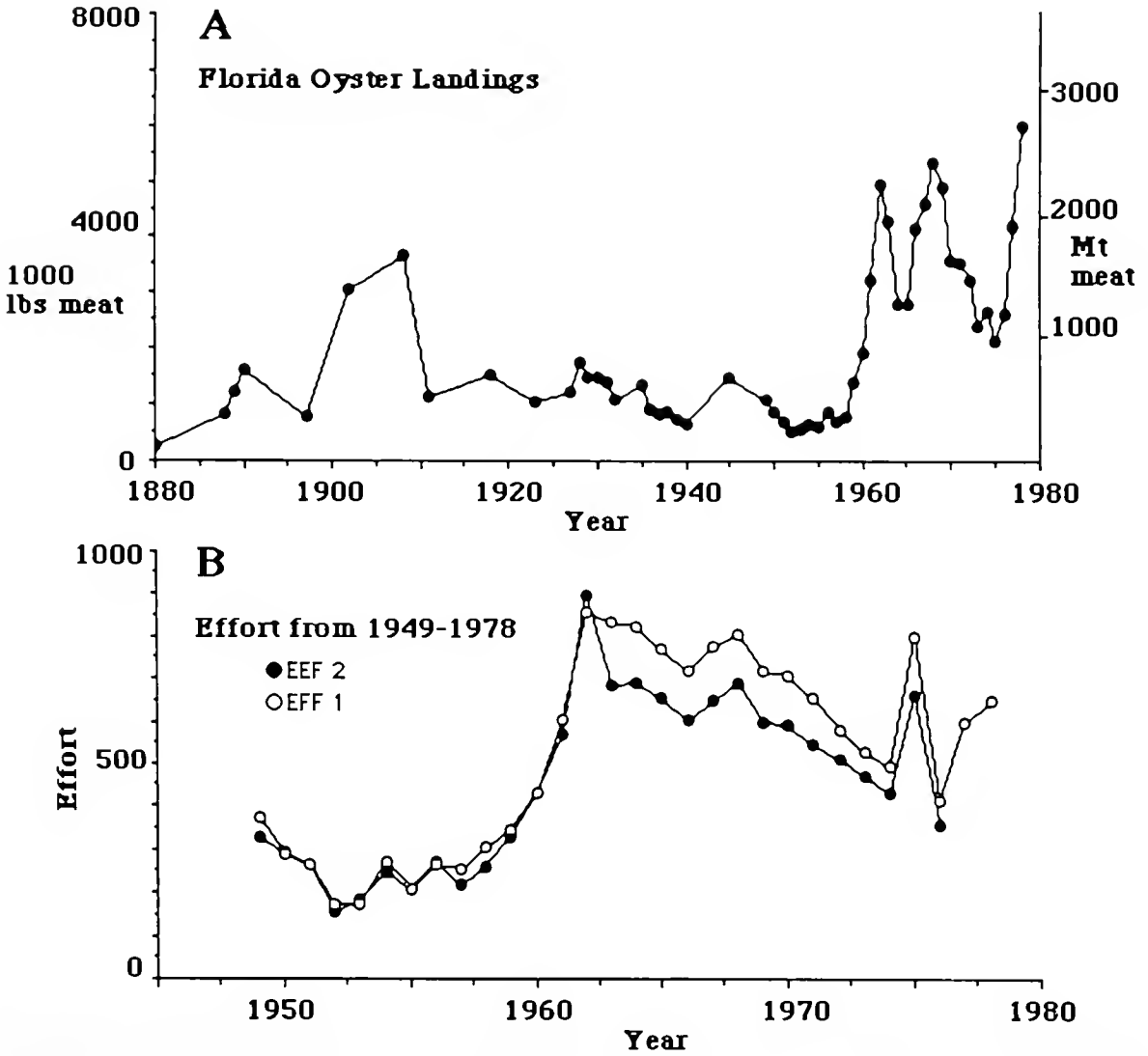


Figure 2. (A) The variation in harvest from 1880 to 1978 and (B) effort from 1949 to 1978 for the two effort terms discussed in the text.

STUDY AREA

The west coast of Florida comprises 843,000 ha of open estuarine water and 369,000 ha of tidal marshland. Over 80% of the state's annual harvest is from a single bay system, Apalachicola Bay, which contains most of the 5,605 ha under oyster cultivation. Apalachicola Bay is a shallow coastal estuary 58 km long and 1.6–22.5 km wide. Its total area of 534 km^2 includes St. George Sound, Apalachicola Bay, St. Vincent Sound, and Indian Pass Lagoon (Fig. 1). The northern end is at the mouth of the large Apalachicola River delta. The Apalachicola River has the highest annual average discharge rate (658 m^3/sec) of any river in Florida (U.S. Department of Commerce, NOAA, and Fla. Department of Environmental Regulation, n.d.) and is formed by the union of the Flint and Chatahootchee rivers, which drain parts of Georgia and Alabama. Discharge rates range from 260 to 5,600 m^3/sec (U.S. Army Corps of Engineers 1978). The seaward terminus is at the St. George and St. Vincent barrier islands.

METHODS

We compiled records of monthly and seasonal river discharge, air temperature, and fishing effort in the numerous bays along the west coast of Florida to find statistically significant relationships affecting yields. Air temperature and river-discharge data are for coastal stations located near the oyster-producing areas. To calculate average monthly temperatures, we used data from the Apalachicola and Carrabelle, Florida, weather stations of the U.S. Weather Bureau (U.S. Department of Commerce, Environmental Science Services Administration, Climatological Data 1948–78). River discharge data were extracted from U.S. Geological Survey water resource data for the Apalachicola River near Blountstown, Florida (U.S. Department of the Interior, U.S. Geological Survey 1948–78).

We obtained data on oyster yields from the *Commercial Fisheries Series* of the U.S. Fish and Wildlife Service (USFWS) and the *Current Fisheries Statistics Annuals* of the NMFS (U.S. Department of the Interior, U.S. Fish and Wildlife Service 1948–69; U.S. Department of Commerce, NMFS 1970–76). These official landing statistics may or may not accurately reflect total yields; however, the methods of collecting data have been fairly consistent for the past 30 years (personal communication, Orville Allen, NMFS, New Orleans). These landing data were used as a relative measure of annual variations in abundance.

A true measure of effort was difficult to obtain from the available data. No information was available on such indices as amount of time actually fished or fuel used. Therefore, we calculated effort two different ways from the operating units listed in the yearly fisheries statistics summaries of the USFWS and NMFS (U.S. Department of the Interior, USFWS 1948–69; U.S. Department of Commerce, NMFS 1968–77). The first effort variable, $E1_{n=yr}$, uses the yearly total number of fishermen harvesting oysters within the state, disregarding that there were fewer tongs than fishermen operating from some vessels, in year "n." The second effort variable, $E2_{n=yr}$, was derived to better explain this apparent fishing-effort discrepancy among vessels and defined as

$$E2_{n=yr} = \frac{FM}{G} * V$$

where FM is the number of fishermen using a gear type, G, on a vessel, V, in year "n" where 0 is the present year. $E2_n$ represents the fishermen's effort in response to the

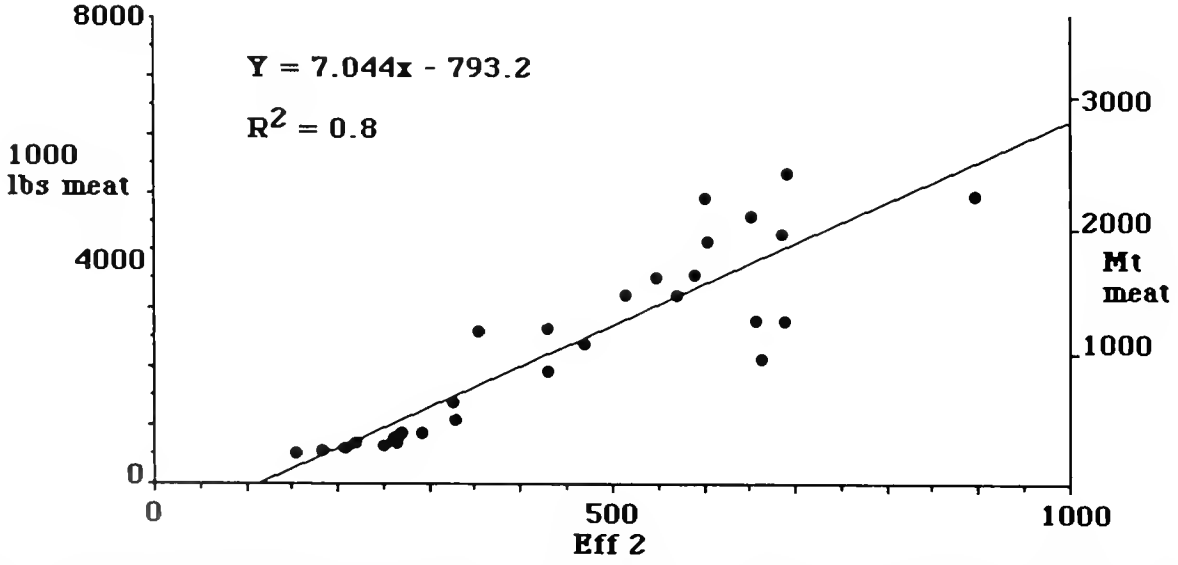


Figure 3. The relationship between the effort term $E2_0$ and harvest from 1949 to 1978. The effort term alone accounts for 80% of the variation. The R^2 for the relationship between $E1_0$ and C_0 is 0.73.

equipment at their disposal. For fishing industries with a one-man/one-year/one-vessel relationship, $E2_n$ will equal $E1_n$. The CPUE for each effort term in year n is the harvest divided by the respective effort term and defined as $CPUEX_{n=yr}$.

Simple and multiple linear regressions and multivariant analysis were performed to test for relationships between fishing effort and environmental factors with harvest. Plots of the residuals were made to test for linearity as well as to determine homogeneity and normality of the data points. Level of significance for all independent model variables discussed here is 0.05.

RESULTS

Oyster production has undergone distinct phases since 1880 (Fig. 2). The reported harvest before 1960 was highest at the turn of the century. Since the 1960s, yields have been much higher than in preceding decades, but variable. The duration of this elevated harvest suggests some long-lasting change that cannot be explained by short-term phenomena such as environmental factors. Effort, as measured by $E1_0$ and $E2_0$, also varied considerably and increased in recent decades (Fig. 2). For this reason, the analysis begins with a discussion of the relationships between effort and harvest, and then introduces a model predicting

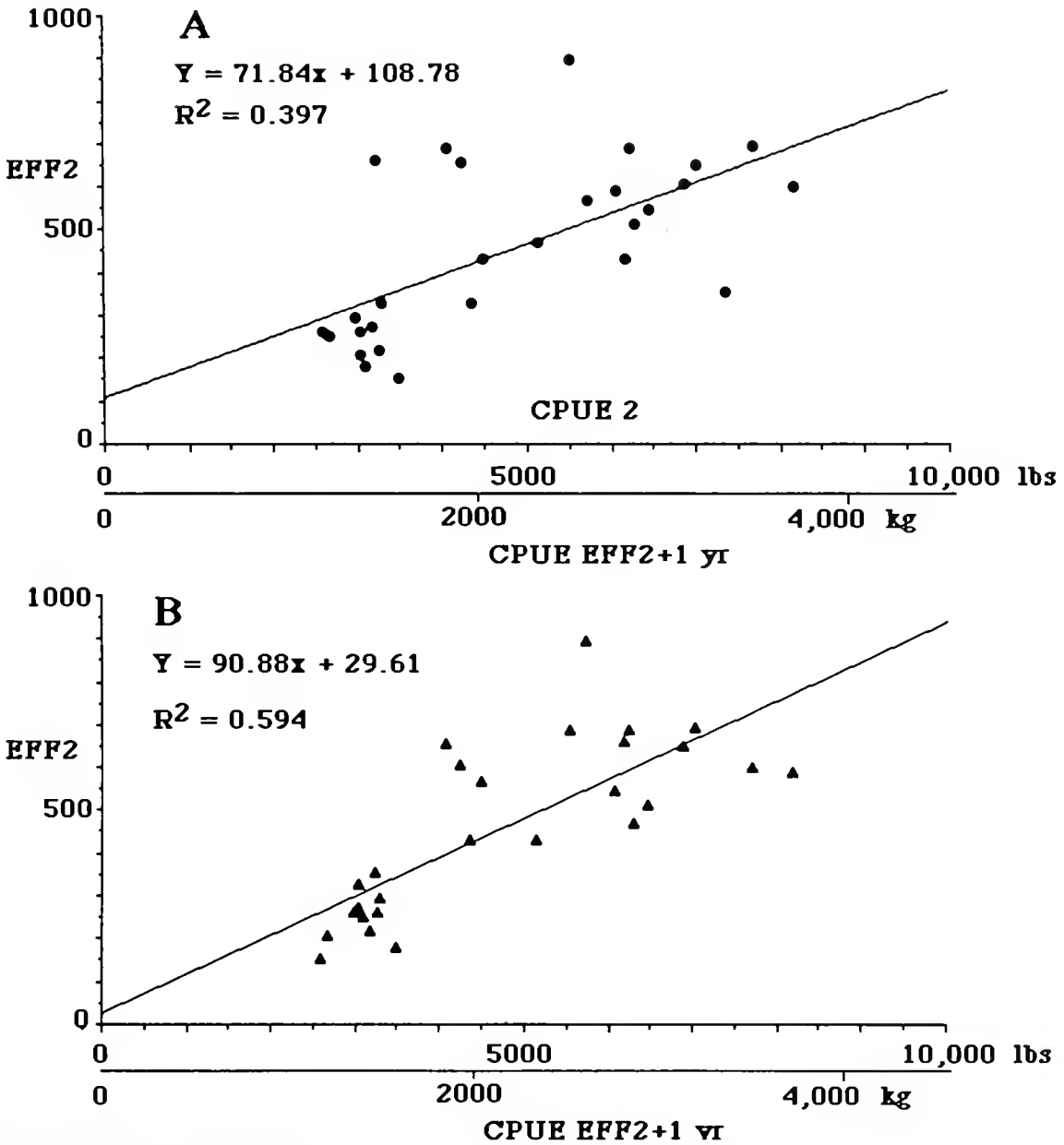


Figure 4. The relationship between the effort term $E2_0$ and CPUE for (A) the present year (y = 0) and (B) the previous year (y = −1).

yields that takes the role of environmental changes into account.

EFFORT

Because tong fishing requires a one-man effort, the Florida oyster industry approximates the one-man/one-gear/one-vessel situation in which the calculated effort term, $E2_0$, approximates the number of fishermen, $E1_0$ (Fig. 2). A simple linear regression of the two effort variables yielded a coefficient of determination (R^2) of 0.966 (n = 27). If the 1962 data set (an outlier) is excluded, then R^2 = 0.999. We conclude from this analysis that $E1_0$ and $E2_0$ are equally useful as relative indices of fishing effort for the Florida oyster fishery.

EFFORT AND HARVEST

The regression analysis of effort (E_0) with harvest (C_0) shows an R^2 of 0.73 for $E1_0$ and 0.80 for $E2_0$ (n = 27; Fig. 3). Harvest has risen directly and linearly with effort. A multiple linear regression between C_0 and $CPUE2_0$ yielded an R^2 of 0.98 (n = 27), indicating a linear relationship between these terms. Knowing the causes for variations in CPUE and between CPUE and effort will therefore help researchers predict future changes in the fisheries.

In this fishery, effort rises linearly with an increased CPUE (Fig. 4A), but the fit of the regression is stronger if the effort term is lagged one year (Fig. 4B; R^2 = 0.59 vs. 0.40), but not two years (R^2 = 0.37). In other words, the estimate of effort is directly responsive to CPUE in the previous season. This result may be an artifact of the manner in which the effort indices are tabulated. The indices may be based more or less on (1) last year's yield or current supply, and/or (2) past or present economic returns. We cannot discern which of these interpretations is more important with these data.

For whatever reason, the amount of fishing effort fluctuates. Thus, variations in the estimate of effort for this year are statistically related to estimates of effort for previous years. A simple linear relationship between next year's fishing effort ($E2_{+1}$) and the present or previous year's effort ($E2_0$) yields an R^2 of 0.67. The prediction of $E2_{+1}$ is improved considerably if the variation in CPUE is considered as well. A multiple-regression model predicting $E2_{+1}$, which includes $E2_0$ and $CPUE_0$, gives an R^2 of 0.79 if all years are included, and 0.82 if 1962 is excluded. From these results, we conclude that effort and CPUE of the present year are directly related to both CPUE and effort for the next year.

A multiple-regression model predicting this year's harvest (C_0) from last year's effort ($E2_{-1}$) and CPUE ($CPUE2_{-1}$) yielded an R^2 of 0.81. Using $CPUE1_{-1}$ and $E2_{-1}$ raised the R^2 to 0.85, and adding a third variable, $E1_{-1}$, increased the R^2 to 0.88 (Table 1).

On the basis of these results, we conclude that effort and harvest are statistically related to a driving force, CPUE, which can itself be estimated and is logically related to future fishery success.

TABLE 1.

Summary multiple-regression statistics for prediction of C_{+1} from E_0 and $CPUE_0$. $P < 0.05$ for all independent variables.

Variables Included	n	R^2	F-test
$E2_0$, $CPUE2_0$	26	0.81	49.7
$CPUE2_0$, Et_0	26	0.85	69.8
$E1_0$, $CPUE1_0$, $E2_0$	26	0.88	54.8

TABLE 2.

Summary multiple-regression statistics for prediction of $CPUE2_{+1}$ from environmental factors and effort. $P < 0.05$ for all independent variables.

Variable Included	n	R^2	F-test
Jan_0 flow (+), Feb_0 flow (−), $Sept_0$ flow (+)	26	0.42	5.59
Jan_0 flow (+), Feb_0 flow (−), $Sept_0$ flow (+), $Winter_0$ temp (−)	26	0.56	6.93
Feb_0 flow (−), $E1_0$ (+)	26	0.69	26.40
Feb_0 flow (−), $Sept_0$ flow (+), $E2_0$ (+)	25	0.71	18.30

A multiple-regression model predicting this year's harvest (C_0) from last year's effort ($E2_{-1}$) and CPUE ($CPUE2_{-1}$) yielded an R^2 of 0.81. Using $CPUE1_{-1}$ and $E2_{-1}$ raised the R^2 to 0.85, and adding a third variable, $E1_{-1}$, increased the R^2 to 0.88 (Table 1).

On the basis of these results, we conclude that effort and harvest are statistically related to a driving force, CPUE, which can itself be estimated and is logically related to future fishery success.

PREDICTING CPUE

Logically, then, CPUE affects future effort and therefore variations in harvest size. We hypothesized that $CPUE_{+1}$ (hence catch) is responsive to factors known to influence oyster mortality and growth, namely temperature and salinity. Several combinations of river flow and temperature proved to be statistically related to future CPUE. The simplest and most meaningful of these relationships are summarized in Table 2. Spring river flow and winter temperature are the most commonly useful terms. The best three-variable model of future CPUE had an R^2 of 0.71 and included February and September river flow and $E2_0$ for independent terms.

TABLE 3.

Summary multiple-regression statistics for prediction of C_{+1} from environmental factors and effort. $P < 0.05$ for all independent variables.

Variable Included	n	R^2	F-test
Feb_0 flow (+), $CPUE2_0$ (+)	25	0.60	17.4
Feb_0 flow (−), $E1_0$ (+)	26	0.81	51.8
Feb_0 flow (−), $CPUE2_0$ (+), Et_0 (+)	25	0.88	51.4
Feb_0 flow (−), $CPUE2_0$ (+), $E1_0$ (+), $E2_0$ (+)	25	0.91	51.9

PREDICTING HARVEST

Future harvests from 1949 to 1978 were best hindcast with a multiple-regression model that included estimates of the last year's effort, February river flow, and CPUE (Table 3). Virtually all of the annual variations in harvest (up to 91%) could be accounted for by this model or similar ones (Table 3).

DISCUSSION

The long periods of gain and decline in the production of the west Florida oyster fishery suggest that factors other than climatic ones, which are relatively stable, significantly influence yields. The annual yields of the early industry rarely dropped below 454,000 kg (1 million pounds). But by the mid-1930s, oyster yields had fallen below the 454,000-kg level as the reefs become depleted of seed stocks and shell supplies. In 1949 oyster stocks and shell-reef materials were so depleted that the Florida Department of Natural Resources started a program of planting cultch on the oyster beds (Whitfield 1973). Before the 1959 oyster season, 23,505 m^3 of shells were planted along the west coast of Florida; of these, over 19,382 m^3 went in Apa-

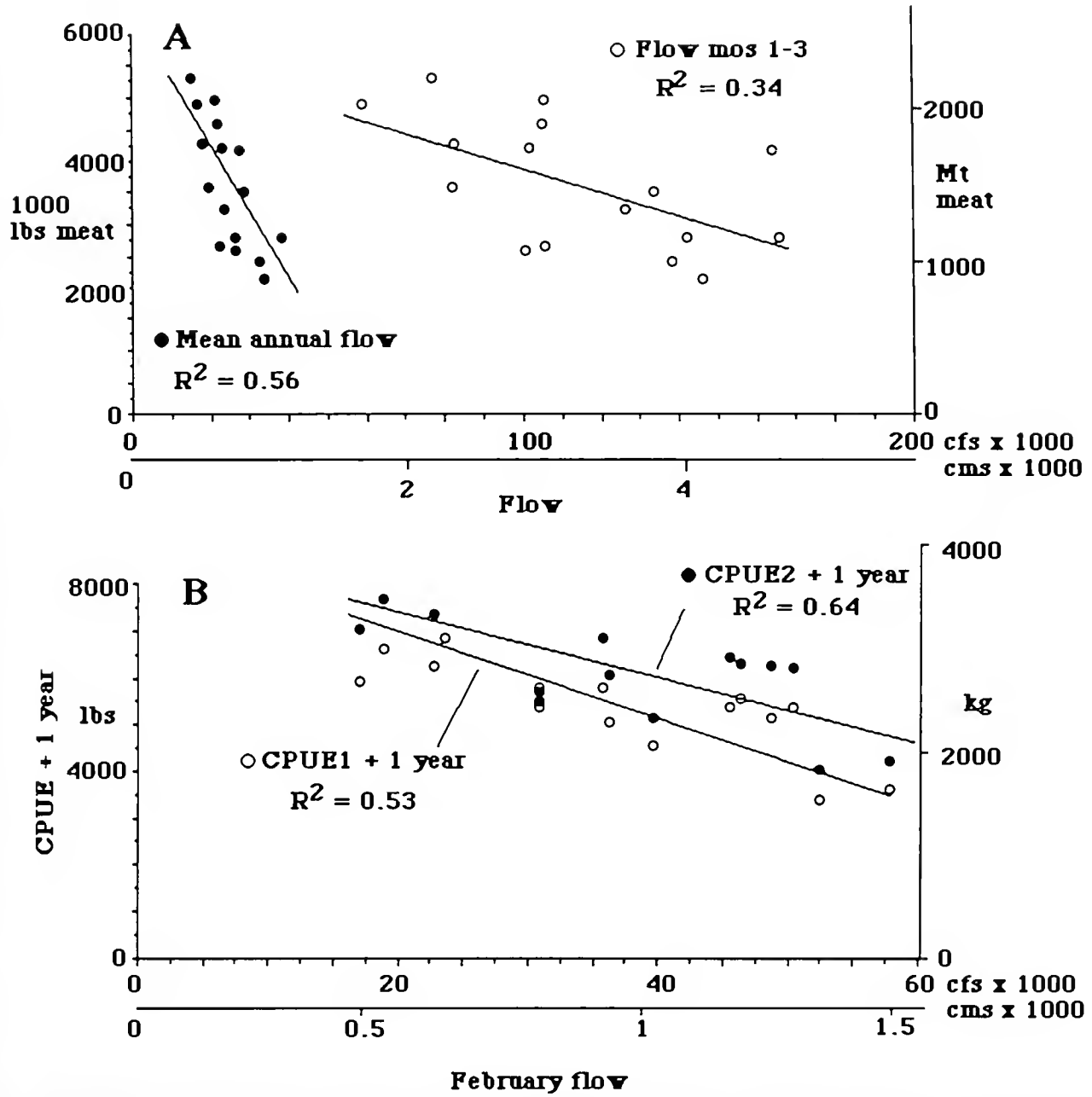


Figure 5. (A) Oyster yields (C_0) and river discharge (yr = 0) from the Apalachicola River from 1962 to 1979. (B) Catch-per-unit-effort (CPUE) + 1 year and flow rates during February (yr = 0) from the Apalachicola River, excluding the 1975 flood year.

lachicola Bay. The increases in harvests during the 1960 and 1961 seasons (Fig. 2) correspond with these efforts. The total harvests for each year from 1960 through 1962 were new state records.

The active management program and increased fishing effort that began in 1949 and intensified during the late 1950s probably caused this increase in yields. Annual yields during the 1950s remained fairly steady, around 349,000 kg. The environmental conditions were relatively stable during the same period, so we did not include river flow and temperature in the regression model. The average river flow of the Apalachicola River during this period was 480 m^3/sec; only one year exceeded the long-term average of 658 m^3/sec.

The relationship between annual oyster yields and annual discharge rates from the Apalachicola River in the years following 1962 is shown in Fig. 5. From 1949 through the late 1950s, these variables exhibited no apparent trend. The depleted state of the oyster beds was probably the underlying cause of the low harvest and the lack of environmental relationship. But once the intensified program began to significantly replenish the diminishing supplies of seed stock and cultch, environmental influences became more evident. Starting in 1962, river flow and harvest were inversely related (Fig. 6). Flood conditions caused yields to decrease; in fact, flooding appears to affect yields over a two-year period, either by increasing mortality or by delaying spawning. Not until the second year after a flood do harvests regain their pre-flood levels. Record floods and poor harvests occurred simultaneously in 1964, 1973, and 1975. Written summaries from the USFWS and NMFS report that declines since 1962 have been related to either weather or a scarcity of marketable-size oysters (U.S. Department of the Interior, USFWS 1964; U.S. Department of Commerce, NMFS 1973, 1975).

In general, patterns of harvest and effort are similar, whereas river flow seems to be inversely related to effort and in some cases lagged by a year or two. Effort decreased in 1963 as a result of rough seas generated by hurricanes near the oyster-producing areas and of a scarcity of legal-size oysters in the fall (U.S. Department of the Interior, USFWS 1963). The extremely large harvest in 1962 of 2.2 million kg of meat may have contributed to the scarcity the following year. Yields continued to decline through the 1964 and 1965 seasons owing to severe flooding conditions. By 1966 effort had decreased in response to the previous two years of poor yields.

Severe flooding again suppressed the yields in 1973 as well as the next year. Since the average growing period for oysters along the Gulf Coast is 18–20 months, harvests were expected to increase during the 1975 season, the second year following the flood. Effort rose by 54% in 1975, probably in response to the recovered reefs. But the 1975 flood reduced yields and devastated the fishing fleet. The number of fishermen increased by over 300 between

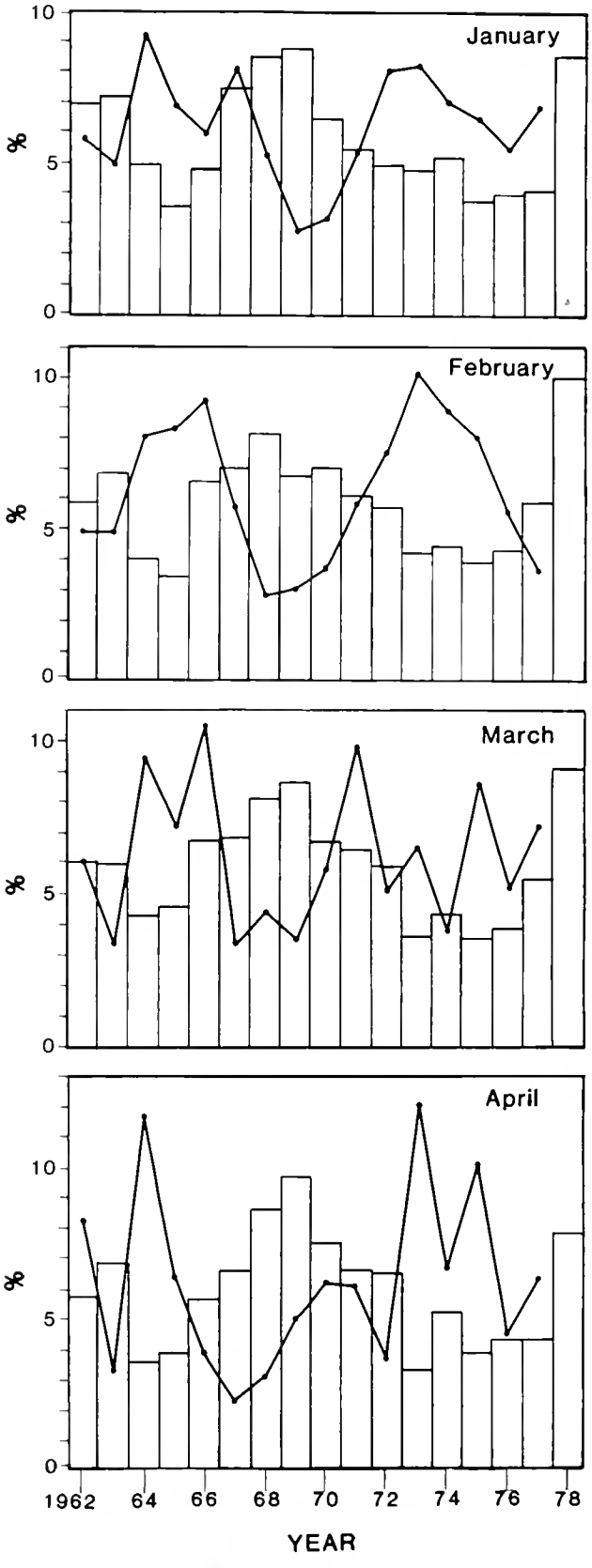


Figure 6. The monthly percentages of catch (bar) and river discharge (line) on the west coast of Florida for January through April, 1962–78.

TABLE 4.

The percentage of oysters harvested in the spring and fall seasons* on the west coast of Florida from 1962 through 1978.

Year	1962	1963	1964	1965	1966	1967	1968	1969	1970
Spring	49.7	61.2	58.2	48.7	50.6	53.1	55.3	62.9	64.7
Fall	50.3	38.8	41.8	51.3	49.4	46.9	44.6	37.1	35.3
Year	**1971**	**1972**	**1973**	**1974**	**1975**	**1976**	**1977**	**1978**	
Spring	61.9	63.6	59.4	61.5	60.5	54.6	44.6	60.3	
Fall	38.1	36.4	40.6	38.5	39.5	45.4	55.4	39.7	

* Data for years in which the fishery closed during the summer months (June–August) were not included. Spring season is from January through May and the fall season from September through December.

1974 and 1975 and decreased by over 380 at the start of the 1976 season. The number of fishermen since then has increased, but at a more cautious rate.

The regression model based on this study indicates that environmental conditions are most critical at the advent of spring spawning. The intense harvesting periods are from September through April. From all indications, the season closes from June through August. In any case, effort is drastically curtailed during this period because of the poor quality of the oysters. Monthly levels of oyster yields increase in September and on through January. As river discharge increases (freshwater inflow, sedimentation, and lower market demand) in the spring, oyster yields start declining in February and continue declining through May (February through September is the critical period for river discharge and temperature in the regression model). Figure 6 shows percentages (January through April) of harvest and river flow for 1962–78. In most cases these two parameters are inversely related. The major events of this period stand out quite clearly: the floods and low harvests of 1964, 1973, and 1975, and the low flow and high yields in the late 1960s.

Little and Quick (1976) reported that the fungus *Perkinsus marinus* (*Labyrinthomyxa marina*) killed 90% of the marketable oysters of Escambia Bay during the first week in September 1971. Escambia Bay is only 256 km west of Apalachicola Bay. This fungus is particularly lethal when hot weather combines with high salinity (Mackin 1951). Annual summaries for Florida in the *Fisheries Statistics of the United States* confirm the scarcity of marketable oysters in the 1971 and 1972 seasons (U.S. Department of Commerce, NMFS 1968–76).

Table 4 lists the percentages of oysters harvested in the spring and fall seasons from 1962 to 1978. Comparing the differences between the two seasons in any given year with past trends makes it more apparent which years had stress conditions. From 1962 to 1967, except for 1963 and 1964, the harvest for each season was roughly 50% of the yearly total. Hurricanes during the summer of 1963 caused turbulent seas that in turn caused fall yields to decrease to only 38.8% of the yearly total. In 1964 flooding caused the difference between the spring and fall yields. The 1965 and 1966 seasons returned to the 50/50 trend. Starting in 1967 and continuing through 1977, however, more of the total harvest was taken in the spring than in the fall season. If oysters were becoming scarce in the late 1960s and early 1970s, it would make sense that the beds would become depleted of marketable-size oysters between the spring and fall fishing seasons. Spring and fall harvest percentages for the 1973 flood year were almost identical to percentages for the 1964 flood year. The fishery did not recover from the 1973 and 1975 floods until 1976, when percentages once again approached the 50/50 level.

The various relationships between fishing effort, river flow, and temperature are summarized in Fig. 7. We view

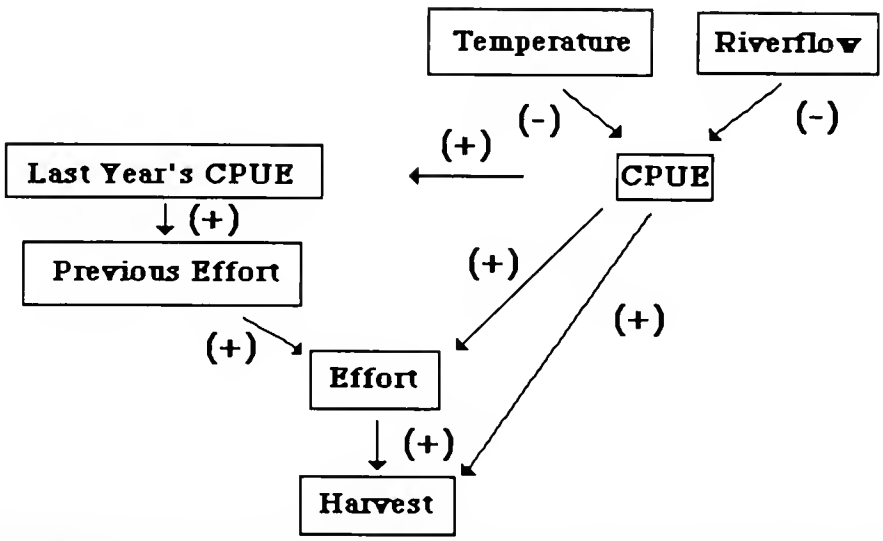


Figure 7. A schematic summary of the dominant relationships between the environmental factors and harvest and effort that result in annual variations in oyster harvest size on the west coast of Florida.

annual variations in the biological system as being driven by climatic factors that the fishing industry responds to and "remembers" the following year. Management has increased potential yields by depositing substrate suitable for oyster-spat colonization and growth. The multiple-regression models predicting Florida's oyster yields were most responsive to spring river discharge and winter temperature variables. Florida has a strong spring harvest that is not severely affected by flooding conditions and that occurs before high salinities and temperatures increase the threat of disease and predation. In areas where the oyster industry is more dispersed along the coastal zone (such as Louisiana and Mississippi), environmental effects are more varied and more influenced by late summer high temperatures and variations in river discharge (Allen and Turner 1984).

ACKNOWLEDGMENTS

The authors thank the following for reviewing earlier drafts of this manuscript: E. R. Allen, D. F. Boesch, and M. C. Hester. This research was initially supported by the Louisiana Sea Grant Program and later by the NOAA Office of Marine Pollution. We particularly thank various National Marine Fisheries Service personnel for sharing their lifelong expertise in fisheries management and data collection. D. A. Fuller, S. P. Risotto, and D. R. West contributed to the data collection and statistical analyses.

LITERATURE CITED

Allen, R. L. & R. E. Turner. 1984. Oyster management and environmental influences in the Gulf of Mexico, 1880–1978. Final report to the National Oceanic and Atmospheric Administration, Office of Marine Pollution Assessment.

Butler, P. A. 1949. An investigation of oyster producing areas in Louisiana and Mississippi damaged by floodwaters in 1945. U.S. Department of the Interior, Fish and Wildlife Service, Special Scientific Report, Fisheries, No. 8. 29 pp.

Butler, P. A. 1952. Effect of floodwater on oysters in Mississippi Sound in 1950. U.S. Department of the Interior, Fish and Wildlife Service, Research Report 31:1–20.

Butler, P. A. 1965. Reaction of some estuarine molluscs to environmental factors. Pages 92–104 *in* C. M. Tarzwell, ed., Biological Problems in Water Pollution: Third Seminar, 1962. Public Health Service Publication 999-WP-25.

Dow, R. L. 1977. Effects of climatic cycles on the relative abundance and availability of commercial marine and estuarine species. Journal du Conseil International Pour L'Exploration de la Mer. 37(3):274–280.

Engle, J. B. 1963. Shellfish advisory service: cooperation with the industry. Pages 79–83 *in* Proceedings of the Gulf and Caribbean Fisheries Institute 16th Annual Session.

Galtsoff, P. S. 1930. Destruction of oyster bottoms in Mobile Bay by the flood of 1924. Pages 741–758, Appendix XI, *in* Henry O'Malley, Report of the U.S. Commissioner of Fisheries for the Fiscal Year 1929, with appendixes. U.S. Department of Commerce, Bureau of Fisheries Doc. 1069.

Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. U.S. Department of the Interior, Fish and Wildlife Service, Fisheries Bulletin 64:1–480.

Kennedy, V. S. & L. L. Breisch. 1983. Sixteen decades of political management of the oyster fishery in Maryland's Chesapeake Bay. *Journal of Environmental Management* 16:153–171.

Little, E. J. & J. A. Quick, Jr. 1976. Ecology, resource rehabilitation, and fungal parasitology of commercial oysters, *Crassostrea virginica* (Gmelin), in Pensacola Estuary, Florida. *Florida Marine Research Publication* 21:1–89.

MacKenzie, C. L. 1977. Development of an aquacultural program for rehabilitation of damaged oyster reefs in Mississippi. *Marine Fisheries Review* 39(8):1–13.

Mackin, J. G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. Bulletin of Marine Science of the Gulf and Caribbean 1(1):72–87.

Maryland Sea Grant. 1980. Oyster formula on the number again. Maryland Sea Grant 3(3):7. University of Maryland, College Park.

May, E. B. 1972. The effect of floodwater on oysters in Mobile Bay. Proceedings of the National Shellfisheries Association 62:67–71.

Sutcliffe, W. H., Jr. 1972. Some relations of land drainage, nutrients, particulate materials, and fish catch in two western Canadian bays. Journal of the Fisheries Research Board of Canada 29(4):357–362.

Texas Department of Water Resources. 1981. Trinity-San Jacinto Estuary: a study of the influence of freshwater inflows. Austin, Tex.

Turner, R. E. 1978. Louisiana's coastal fisheries and changing environmental conditions. Pages 363–370 *in* J. W. Day, Jr., D. D. Culley, Jr., R. E. Turner & A. J. Mumphrey, Jr., eds. Proceedings: Third Coastal Marsh and Estuary Management Symposium. Louisiana State University, Division of Continuing Education, Baton Rouge. 511 pp.

Ulanowicz, R. E., W. C. Caplins & E. A. Dunnington. 1980. The forecasting of oyster harvest in central Chesapeake Bay. Estuarine and Coastal Marine Science 11(1):101–106.

U.S. Army Corps of Engineers, Mobile District. 1978. Coordination report on navigational improvements of the Apalachicola River below Jim Woodruff Dam, Florida. Mobile, Ala.

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. 1968–76. Fisheries Statistics of the United States: Florida. Separate volumes by year. Washington, D.C.

U.S. Department of Commerce, Environmental Science Services Administration, Environmental Data Service. 1948–82. Climatological Data, Annual Summary: Florida. Separate volumes by year. Asheville, N.C.

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. 1970–78. Current Fisheries Statistics Annual Summary: Florida. Separate volumes by year. Washington, D.C.

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. 1977. A Comprehensive Review of the Commercial Oyster Industries in the United States. Washington, D.C.

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, and Florida Department of Environmental Regulation, Bureau of Coastal Zone Management. n.d. Page 33 *in* Final Environmental Impact Statement: Apalachicola River and Bay Estuarine Sanctuary. Washington, D.C.

U.S. Department of the Interior, U.S. Fish and Wildlife Service. 1948–69. Commercial Fisheries Series: Annual Summary. Separate volumes by year. Washington, D.C.

U.S. Department of the Interior, U.S. Geological Survey. 1948–80. Water Resource Data, Surface Water Record. Separate volumes by year. Washington, D.C.

U.S. Department of the Interior, U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries. 1948–67. Fisheries of the United States. Separate volumes by year. Washington, D.C.

West, D. R. 1981. Predictions of blue crab yields for the northern Gulf of Mexico. M.S. thesis. Louisiana State University, Center for Wetland Resources, Baton Rouge. 85 pp.

Whitfield, W. K., Jr. 1973. Construction and rehabilitation of commercial oyster reefs in Florida from 1949 through 1971 with emphasis on economic impact in Franklin county. Florida Department of Natural Resources, Special Scientific Report 38:1–42.

Journal of Shellfish Research, Vol. 8, No. 1, 105–116, 1989.

THE LOCATION AND TOPOGRAPHY OF OYSTER REEFS IN THE RAPPAHANNOCK RIVER ESTUARY, VIRGINIA

JAMES P. WHITCOMB AND DEXTER S. HAVEN
Virginia Institute of Marine Science
School of Marine Science
The College of William and Mary
Gloucester Point, Virginia 23062

ABSTRACT Public oyster grounds in the Rappahannock River, Virginia were charted in 1976 and 1977 using an electronic positioning system to locate oysters, shell, sand, or mud. Hydraulically operated patent tongs were used to sample the bottoms to validate the charts. During this study 17277.6 ha of public bottoms were surveyed; of this total, 3845.3 ha was oyster reef, sand-shell or mud-shell bottoms; the remainder, 13432.3 ha (78%) was sand, mud or buried shell. The location, extent, topography and environment of the oyster producing areas are discussed. Setting of oysters, physiography and productivity were analyzed.

KEY WORDS: substrates slopes physiography, settlement

INTRODUCTION

From 1975 to 1984 the Rappahannock River in Virginia has been the state's leading source of market oysters, producing an average of 146,999 bushels annually (Haven and Whitcomb 1986). Consequently, it is important to understand where oysters occur and the location and extent of bottom substrate types and levels of recruitment. This study utilizes data obtained during a bay-wide investigation of Virginia oyster grounds from 1976–1981 (Haven et al. 1981).[1] Portions of that investigation dealing with the James River and Pocomoke Sound have been published (Haven and Whitcomb 1983; Whitcomb and Haven 1987) and reference may be made to these reports for specific details.

The Rappahannock River starts in the Blue Ridge mountains and flows in a southeasterly direction for 126 km across the piedmont plateau to the "fall line" at Fredericksburg, then 174 km across the coastal plain to enter Chesapeake Bay. It follows a former river valley cut into coastal plain sediments and submergence of the valley during the post-glacial rise of sea level formed the sub-estuary. The 80 km long sub-estuary varies from 2.5 km wide at its mouth to 0.6 km near its saline head (Ellison and Nichols 1970).

A total of 17277.6 ha of river bottom have been included in the public (Baylor Survey) grounds in the Rappahannock River (Figs. 1, 2 and 3). This paper describes the location, extent, topography and environment of the oyster producing areas in the Rappahannock River sub-estuary. Reef geometry is discussed in the middle and lower estuary; oyster recruitment is reported and related to salinities and topography.

Contribution No. 1528 from the Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062.

[1]Funded in part by a grant by the National Marine Fisheries Service through the Virginia Marine Resources Commission (Contract No. 3-265-R-3).

HYDROGRAPHY

Hydrographic observations by the Chesapeake Bay Institute show that water temperature varies seasonally with air temperature from a monthly mean of 4°C in winter to about 28°C in summer, with occasional extremes for short periods (Stroup and Lynn 1963). During late summer when the prevailing temperature is high, oxygen in deeper parts of the river basin is frequently depleted. This condition often kills fish and benthic fauna (McHugh 1967; Officer et al. 1984; and Tuttle et al. 1987). The salinity increases seaward from nearly 0‰ at the head of the sub-estuary to an annual average of 16.5‰ at the mouth. The increase is greatest in the middle (Towles Point to Jones Point) and upper estuary (Figs. 1 and 2); in this gradient zone stratification is most pronounced and salinity fluctuates up to 5‰ daily and 13‰ annually. With seasonal fluctuations of river inflow, the vertical haline stratification alternates from partially mixed to relatively well mixed (Ellison and Nichols 1970).

When river inflow is high, usually in late winter, freshening reduces surface salinity at the mouth to 14‰ and limits salty water to the lower 61 km of the estuary. As in other Chesapeake Bay sub-estuaries mean salinity is typically slightly higher on the north than on the south side of the estuary owning to the influence of the Coriolis force (Pritchard 1952; Ellison and Nichols 1970).

Silty clay is the most widespread type of substratum, but in the lower estuary sand is the principal sediment on the shoals. Also, scouring leaves some sand as lag deposits on bars and in deep holes of the channel floor (Ellison and Nichols 1970).

MATERIALS AND METHODS

This portion of the bay-wide study was completed in 1977. Equipment and survey methods have been reported previously (Haven and Whitcomb 1983; Whitcomb and Haven 1987). The Raydist® electronic positioning grid

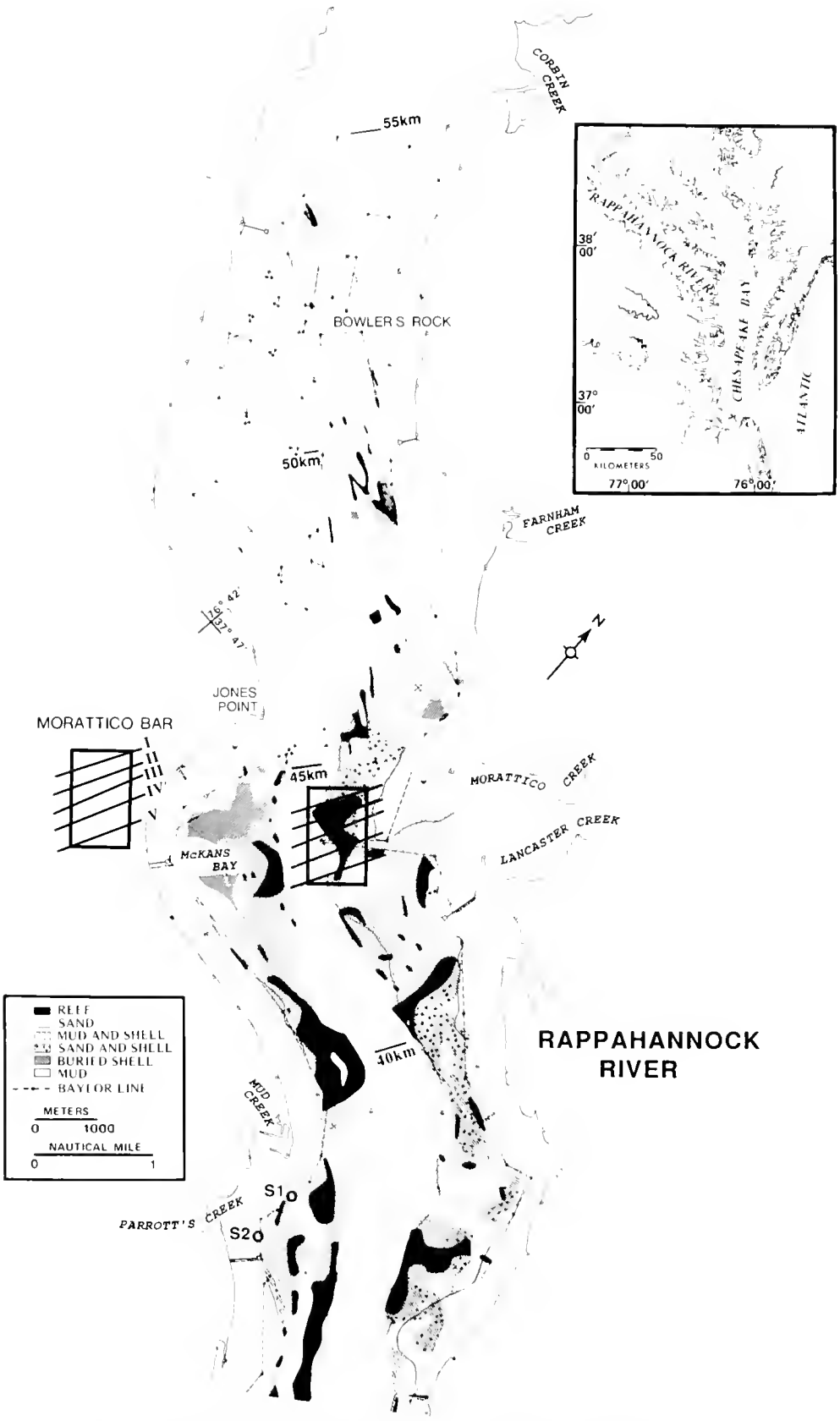


Figure 1. Location of the Rappahannock River (inset, upper right) transects in the Morattico area, oyster reefs and other substrate types. Mud bottoms within the bounds of the Baylor areas, outlined by dashed lines, are unstippled.

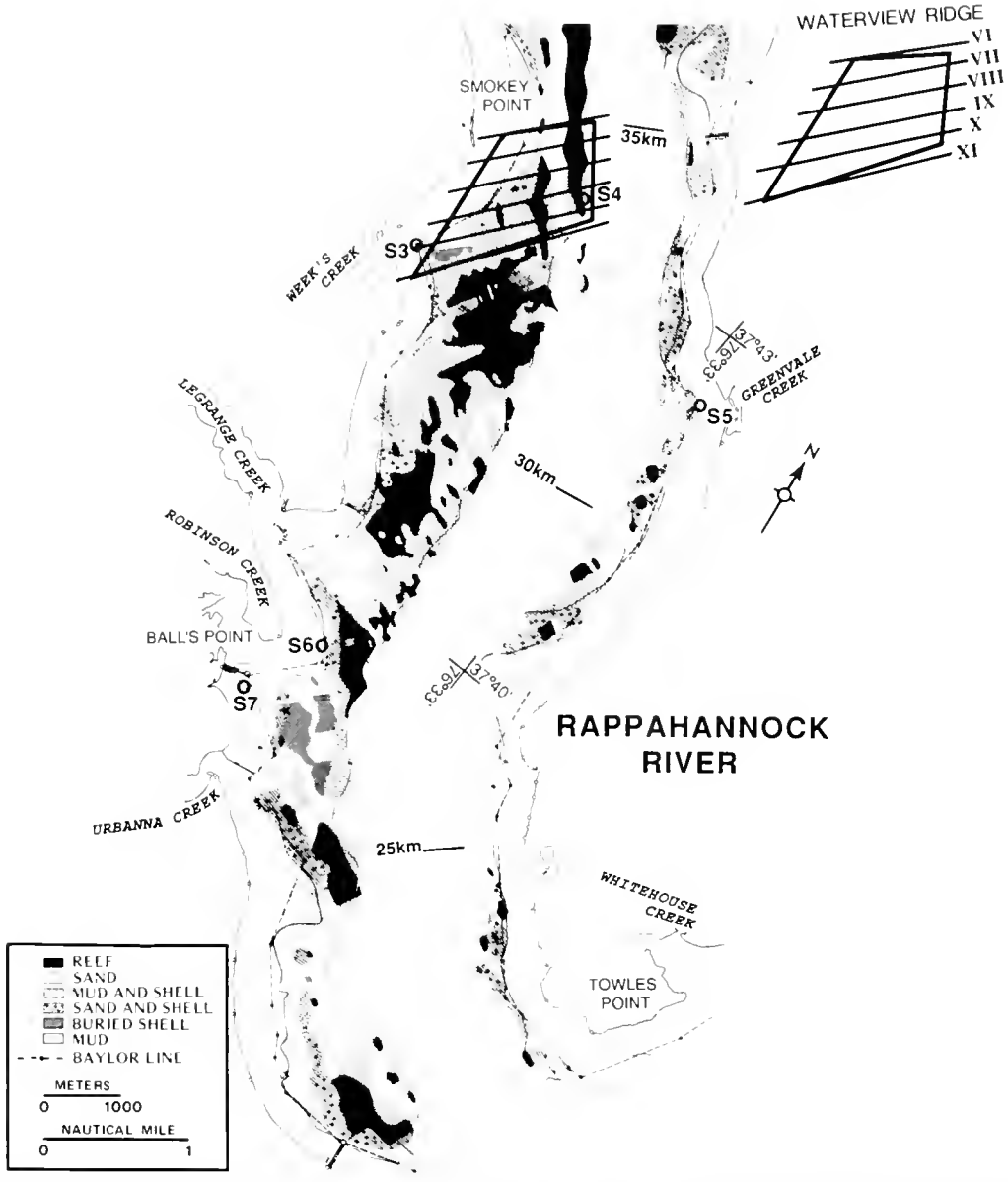


Figure 2. Location of transects in the Waterview Ridge area, oyster reefs and other substrate types. Mud bottoms within the bounds of the Baylor areas, outlined by dashed lines, are unstippled.

system (manufactured by Teledyne Hasting Corp., Hampton, VA) was augmented by an auxiliary system in the Rappahannock River erected by VIMS personnel. The transects were 183 m apart and the stations were 61 m apart where bottom showed little change. Bottom type was determined by probing the bottom with a long pole and this was supplemented by towing an acoustic underwater microphone over the bottom which detected shells. When our survey showed shells were not present the distance between transects was increased to 366 m; and, when substrates and slopes changed rapidly the distances between transects and stations was reduced. Subsequent to the survey, substrate charts of the bottom were constructed as previously described (Haven and Whitcomb 1983; Whitcomb and Haven 1987).

The five types of substrate are as follows:

1. *Oyster Reef:* Firm bottom, probe penetrated 0–5 cm. Shells and oysters were typically abundant. Shells were detected by microphone from 75 to 100% of the time between the probe stations.
2. *Sand-shell:* Firm bottom consisting largely of scattered shells and oysters; probe operator detected the gritty texture of sand. Shells or oysters were detected by the microphone from 25 to 75% of the time.
3. *Mud-Shell:* The probe operator detected a moderately firm crust over a soft bottom. The probe, after pene-

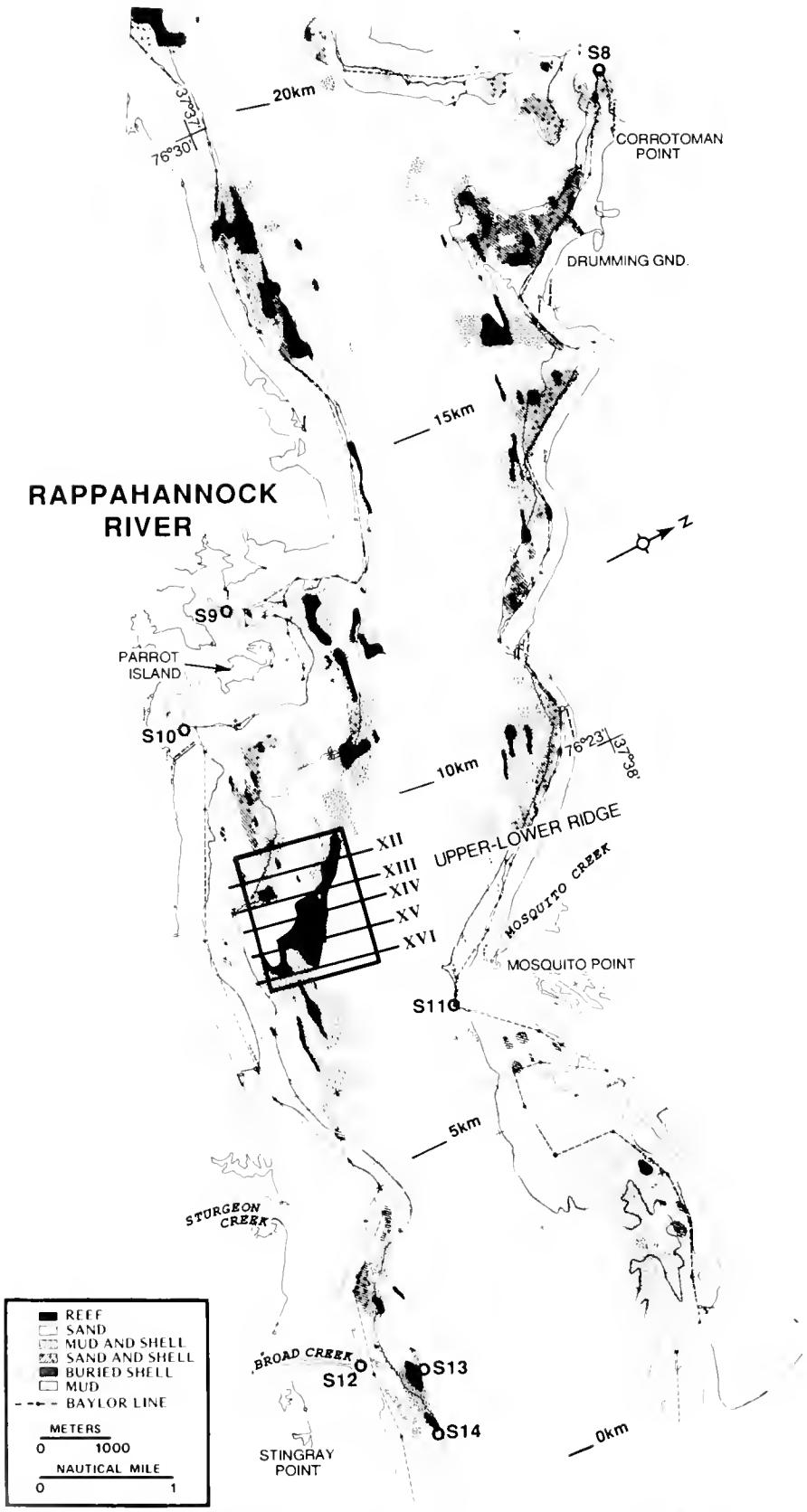


Figure 3. Location of transects in the Upper-Lower Ridge area, oyster reefs and other substrate types. Mud bottoms within the bounds of the Baylor areas, outlined by dashed lines, are unstippled.

trating the crust, could be thrust at least 0.2–0.6 m further into the bottom. Scattered shells and oysters were usually detected by the microphone from 25 to 75% of the time between stations.

4. *Mud:* On these soft bottoms the probe could often be pushed almost one meter into the bottom with little effort. The mud consisted largely of mixtures of silts and clays with some sand. Shells and oysters were usually absent, or very few as determined by microphone.
5. *Sand:* Firm bottoms into which the probe typically did not penetrate more than 2 cm. Few shells or oysters were detected by the probe or underwater microphone. Probe operator detected the gritty texture of sand.

The validity of these five substrate types has been substantiated previously for the James River and Pocomoke Sound areas (Haven and Whitcomb 1983; Whitcomb and Haven 1987). In this study, bottom supporting live oysters and shell was classified as productive; and, bottom supporting surface shell with few or no oysters was classified as potentially productive. Areas lacking shell were classified as barren or non-productive.

After the substrate types were outlined on a map, the area above Smokey Point Light (km 35) was verified by sampling with hydraulically operated patent tongs (Haven and Whitcomb 1983; Whitcomb and Haven 1987). A total of 127 sampling stations were randomly chosen along transects defined by the Raydist® system. Sampling intensity stipulated one sample for each 100 acres (40.5 ha) of oyster reef, 200 acres (80.9 ha) of shell and mud, 200 acres (80.9 ha) of shell and sand, 500 acres (202.4 ha) of mud and 500 acres (202.4 ha) of sand. Each tong grab sampled an area of 0.68 m^2 and penetrated the bottom about 10 cm on oyster reef and 30.5 cm on mud bottoms; each sample consisted of one-half of a Virginia bushel (one Virginia bushel = 0.05 m^3). Data from each grab were recorded as follows: numbers and volume (in U.S. quarts where 1 quart = 0.91 liter) of oysters exclusive of the current year's spat, volume in quarts of shells and fragments, and estimates of the percentage of unburied shell as identified by the presence of fouling organisms. Shell is defined as an entire oyster shell or shell fagments larger than 1 cm^2.

Oyster spatfall was monitored at 14 stations in the Rappahannock River from 1972 to 1980. Weighted strings of 12 oyster shells 5 to 7.5 cm long were suspended (smooth side down) 0.3 to 0.6 m above the bottom for one week periods during the setting season. At weekly intervals numbers of spat on the smooth surface of 10 shells were counted using a dissecting microscope at 15× magnification and the average spat/shell/week was calculated (Table 4). One bushel samples of cultch (bottom material) were dredged from five locations from 1947–1987 each fall after settlement ended and the numbers of spat per bushel were counted (Table 3).

The vertical profile of selected reefs is illustrated in three representative areas using data from transects, 183 m apart, across the reefs (Figs. 4, 5, and 6). The changes in substrate and slopes are shown by plotting type of substrate, depth and horizontal distance to scale. The distances shown in Figs. 1, 2 and 3 are measured from the mouth of the river in kilometers.

RESULTS

Reef Areas

Areas of oyster reef in the upper part of the survey area, as shown by Morattico Bar (km 44) occurred largely on sloping shelves adjacent to the main channel or on the upper portions of the slope leading to the channel. In outline they were often small, isolated, irregular areas, elongated areas parallel to the channel or, occasionally, at right angles to the main axis of the channel (Fig. 1). Depths of the reefs ranged from 1.8 to 5.5 m and, occasionally, as deep as 7.6 m.

Further downriver, from Weeks Creek (km 33) to Towles Point (km 21), oyster reefs have the same general configuration, but they were larger and often interconnected (Fig. 2). Here, because of bottom topography, the reefs largely occurred on the south side of the estuary on the crests of long ridges running parallel to the channel; they were separated by a trough with mud bottom. In general, most oyster reef bottoms occurred between 1.8 and 5.5 m but occasionally were found as deep as 8.8 m.

In the lower part of the estuary, from Towles Point (km 21) to the entrance of the river oyster reef areas were smaller and were more widely separated (Fig. 3); most were on the south side of the estuary. In outline they were similar to those observed elsewhere. A major difference in this section of the estuary is that reefs and areas of mud-shell or sand-shell occurred to depths of 9.1 m. Reefs as deep as these were not observed upriver.

Areas of mud-shell and sand-shell were observed in an irregular pattern throughout the survey area. In general, they surrounded oyster reef areas with mud-shell areas predominating in the deeper waters and sand-shell areas being located in shallower water. Their depth range was the same as the reefs discussed above.

Size of Substrate Types

The surface area of each substrate type in each of the sections of the river presents gradients or trends (Table 1). Mud bottoms comprised the largest component (11,586 ha), or 67.1% of the total area, and the relative size of this bottom type is about the same in each section. In contrast, sand bottoms (1702 ha) constitute only 9.9% of the total area and showed an increasing trend in a downriver direction (1.1 to 21.6%). Most of this increase was in the area below Towles Point (km 21). Mud-shell bottoms totalled 1981 ha, or 11.5% of the total area, and there was a pro-

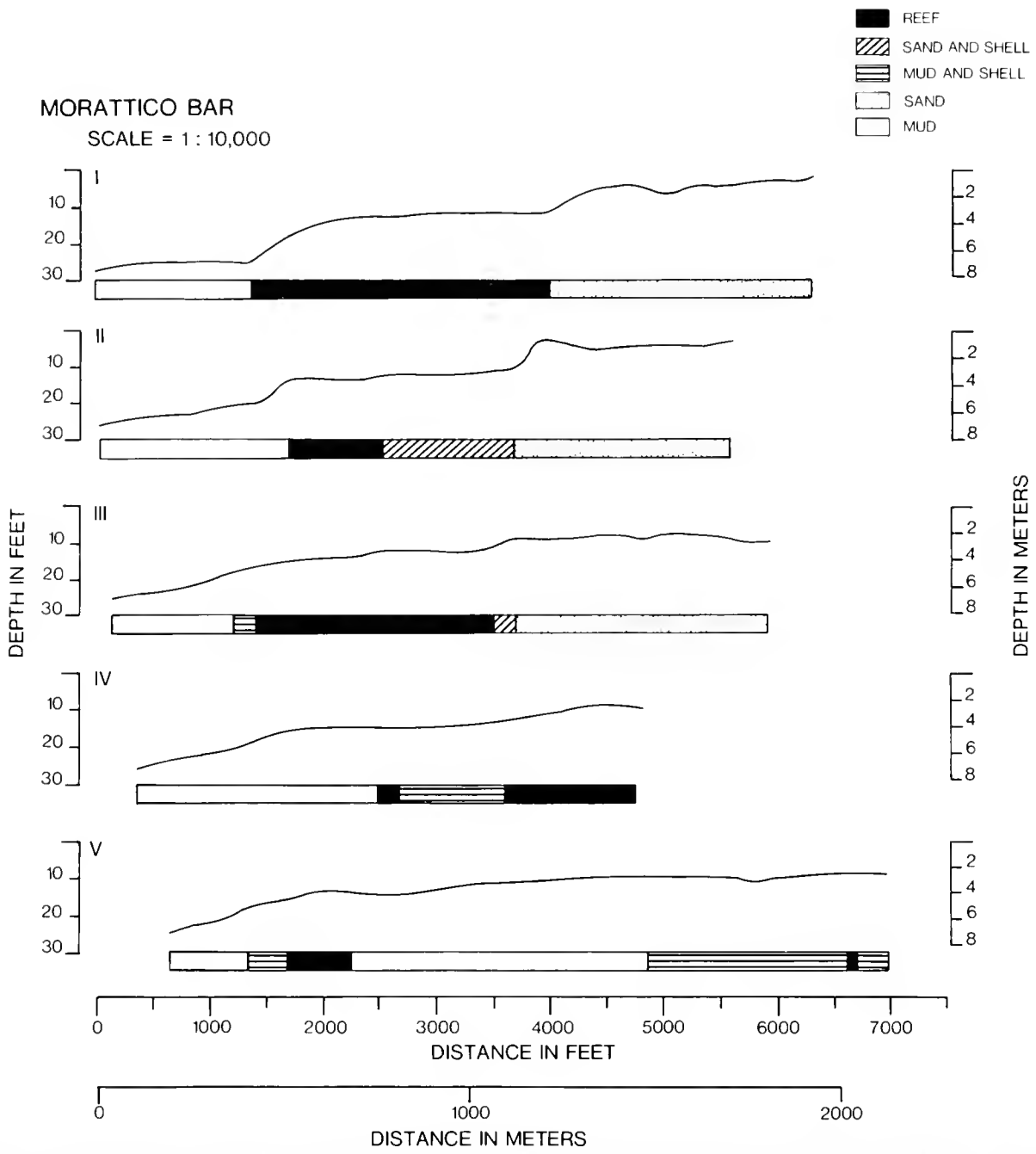


Figure 4. Longitudinal profile of the bottom on Morattico Bar in the Rappahannock River, Virginia, showing substrate, depth and horizontal distances.

gressive decline in relative abundance in the downriver direction (16.9 to 4.9%). Oyster reef areas totalled only 1116 ha, or 6.5% of the total area, and reef areas constituted relatively more of the bottom area in the Morattico Bar (km 44) to Towels Point (km 21) section. Sand-shell areas totalled 748 ha, or 4.3% of the total area, and there was no definite trend or difference in relative abundance in the different sections of the river.

In summary, the public grounds located in the 55 km river from the mouth of the river totalled 42,693.2 acres (17,277.6 ha). Of this, the productive, or potentially productive areas (oyster reef, mud-shell or sand-shell bottoms), totalled 9,501.8 acres (3845.3 ha). Approximately 78% of the public grounds were classified as not productive, or potentially productive.

Oyster and Shell Densities

Sampling with patent tongs to verify substrate types showed a wide variation in number of oysters and amount of shell among the samples in the Rappahannock River (Table 2) similar to the observations in the James River and

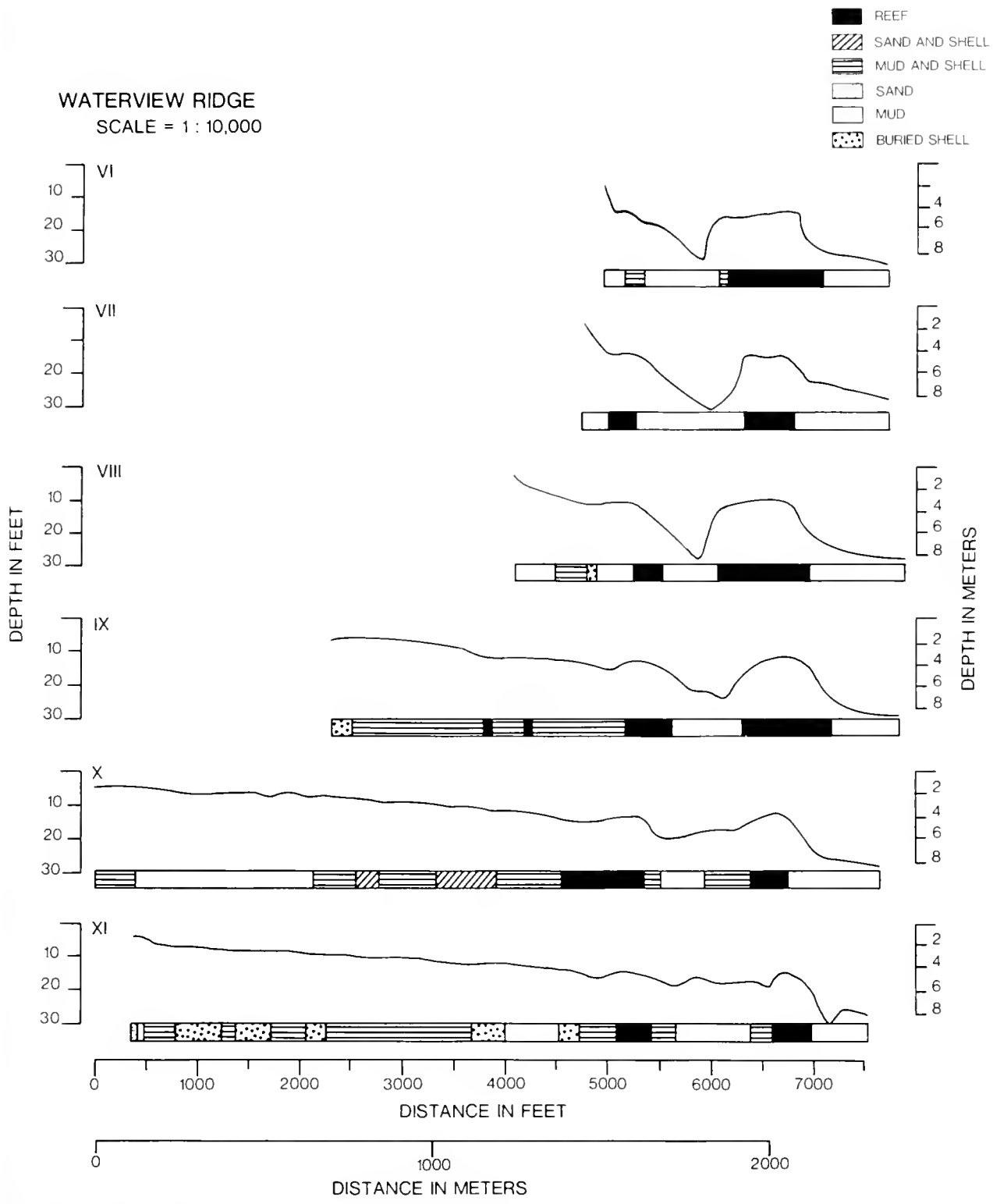


Figure 5. Longitudinal profile of the bottom on Waterview Ridge in the Rappahannock River, Virginia, showing substrate, depth, and horizontal distance.

Pocomoke Sound (Haven and Whitcomb 1983; Whitcomb and Haven 1987). These samples confirmed our observations with the bottom probe and sonic gear as to our classification of bottom types. Bottoms classed as oyster reefs had the highest oyster and shell densities; mud-shell and sand-shell bottoms had lower quantities of oysters and shells. Mud and sand bottoms had few oysters and little shell (Table 2).

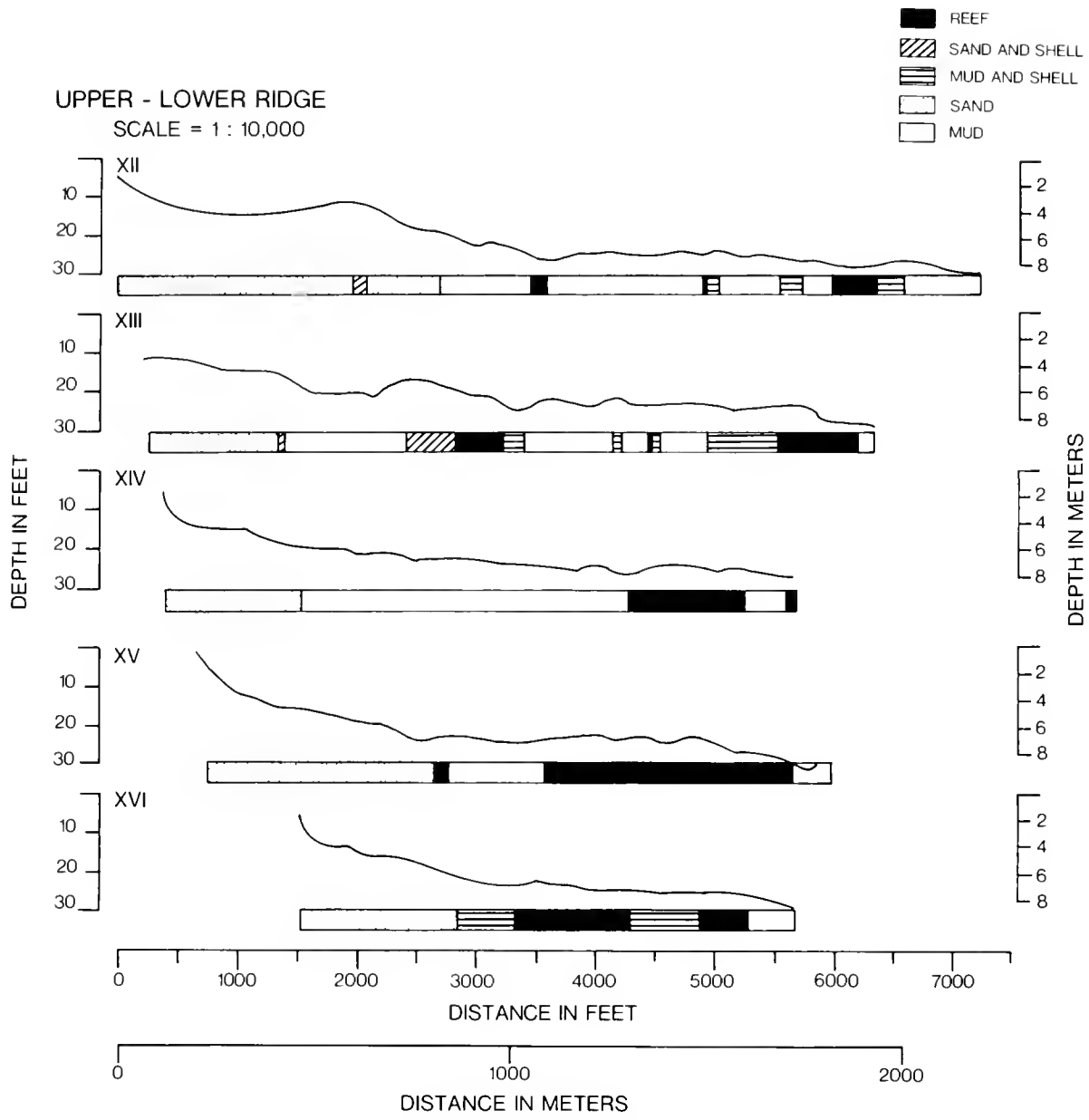


Figure 6. Longitudinal profile of the bottom on Upper-Lower Ridge in the Rappahanock River, Virginia, showing substrate, depth and horizontal distance.

TABLE 1.

Areas of bottom types in hectares and percent of total in each sub-area in the Rappahannock River.

Bottom Type	Total Area (ha)	% of Total Area	Km. From Mouth of River				
			53–46	46–33	33–20	20–7	7–0
Oyster Reef	1116.5	6.5	3.7	12.3	8.8	4.3	1.7
Sand-shell	747.6	4.3	3.0	5.8	5.2	4.9	1.0
Mud-shell	1981.2	11.5	16.9	19.6	11.9	8.6	4.9
Sand	1702.0	9.9	1.1	5.4	4.7	12.0	21.6
Mud	11586.0	67.1	74.4	54.4	68.4	70.1	70.7
Burned Shell	144.3	0.8	0.9	2.4	1.1	<0.1	0
Total Hectares	17277.6 (42692.3 acres)						

TABLE 2.

Number of oysters per m^2, exclusive of 1977 set, and average percent of sample containing single shells and cinder on five bottom types in the Rappahannock River, Va. (July 1977).

Area[1]	No. Samples	Mean Oyster Number · m^{-2}	Average % *Shell-Cinder*
		Oyster Reef	
Km53–Km46	4	3.0	51.0
Km46–Km33	29	3.14	49.6
Km33–Km0	32	4.29	47.0
		Sand-Shell	
Km53–Km46	1	13.43	50.0
Km46–Km33	11	2.31	15.4
Km33–Km0	0	—	—
		Mud-Shell	
Km53–Km46	7	0.82	35.0
Km46–Km33	17	0.79	16.2
Km33–Km0	0	—	—
		Mud	
Km53–Km46	6	0.0	<1.0
Km46–Km33	12	0.0	<1.0
Km33–Km0	0	—	—
		Gravel or Sand	
Km53–Km46	1	0.0	0.0
Km46–Km33	7	0.0	0.0
Km33–Km0	0		—

[1] Distances measured from mouth of river.

Transects

Distribution of substrate types with depth at the Morattico Bar area is shown in Fig. 4 by five transects illustrated on Fig. 1. The profiles show that the oyster reef bottoms generally occurred between 1.8 and 5.5 m and usually form a shelf. Adjacent to the reef on either the offshore or inshore margin was sand-shell or mud-shell substrate. The overall slope of the bottom from the offshore edge of the oyster reef bottom to the inshore end of the transect was 0.05 to 0.13 m (0.18 to 0.44 ft) vertically for each 30.5 m (100 ft) horizontal distance (slopes: 1:556 to 1:227, respectively). However, the reef may be level with adjacent substrate or rise as much as 3.7 m (12 ft) vertically, as on transect I (Fig. 4).

Downriver at Waterview Ridge (km 35) the distribution of bottom types with depth is shown in Fig. 5 by six transects illustrated on Fig. 2. Here, parallel reefs were separated by a muddy slough 4.0–5.5 m (13–18 ft) in depth and there was a deep mud basin 16.7 m (55 ft) in depth offshore. Inshore of the parallel reefs and bottom substrate graded into mud-shell or mud. The overall slope from the offshore edge of the reefs to the inshore end of the transect was 0.04 m to 0.29 m (0.13 to 0.95 ft) vertically for each 30.5 m (100 ft) horizontal distance (slopes: 1:769 to 1:105, respectively). On transect VI the offshore reef was very steep rising 3.4 m (11 ft) in 59.1 m (194 ft) above the mud substrate (slope: 1 to 17.6).

The five transects across Upper-Lower Ridge (km 9) near the mouth of the estuary (Fig. 3) showed a nearly flat oyster reef inshore of a deep basin with depths up to 21.6 m (71 ft) (Fig. 6). The reef was 593 meters in length, varying from 7.0 to 9.1 m in depth, and surrounded by mud or mud-shell substrate. The overall slope of the transects from the offshore edge of the reef to the shore was 0.11 to 0.18 m (0.37 to 0.59 ft) vertically for each 30.5 m (100 ft) horizontal distance (slopes: 1:270 to 1:169, respectively).

Spatfall

The seasonal settlement on dredged bottom shell at five representative locations from 1947 to 1987 showed that annual settlement was very low in the upper river, as confirmed by the shorter term shellstring data. During many years annual settlement was zero, and there were only four periods of exceptional setting intensity; 1949–50, 1953–54, 1962–66 and 1981–83 (Table 3).

The seasonal totals of spat per shell on shellstrings from 1972–80 (Table 4) showed a much higher settlement on shellstrings below Towles Point than in the area upriver. Three of the nine years (1975, 1977 and 1980) were clearly years with above-average potential for settlement on the bottom substrate, as shown by the settlement on shellstrings.

DISCUSSION

The surface configuration of oyster reef, mud-shell and sand-shell areas, and their location in respect to depth and their proximity to channel areas in the Rappahannock River, was similar to that observed in the James River and Pocomoke Sound (Haven and Whitcomb, 1983; Whitcomb and Haven 1987). In outline, the oyster reef areas may be classed as longitudinal, transverse, and irregular (Price 1954; Scott 1968; Bouma 1976; Haven and Whitcomb 1983; Whitcomb and Haven 1987). The distribution of substrate types with depth was also similar to that shown in the James River and Pocomoke Sound. In the Rappahannock River, James River and Pocomoke Sound areas of oyster reef, mud-shell and sand-shell, with one outstanding exception, occurred between 1.8 and 5.5 m contours. The exception was a large reef, 593 m in length, below Parrott Island that extended to 9.1 m in depth called Upper-Lower Ridge (Fig. 3). Bottom samples were not taken from this large reef in this study; however, exploitation by commercial tongers was observed during the 1980–85 period.

Typically, reef areas were located offshore or at the edge of the main channel. Mud-shell bottom often surrounded oyster reefs and they usually terminated the reefs offshore. When present, the mud-shell usually terminated the reefs offshore. When present, the mud-shell usually extends further inshore, as far as the 1.8 m contour. Sand-shell substrates were not as extensive as mud-shell sub-

TABLE 3.

Total seasonal set of *C. virginica* at five representative oyster reefs in the Rappahannock River, Va. 1947–1987. Data show spat/bu of bottom cultch for one Va. bushel.[1]

Year	Bowler's Rock	Morattico Bar	Smokey Point	Hogg House Bar	Drumming Ground
1947	16	0	—	140	166
1948	8	8	0	8	132
1949	12	24	10	12	346
1950	8	24	48	—	184
1951	0	3	2	5	173
1952	—	—	—	—	183
1953	5	8	5	4	90
1954	0	49	216	94	284
1955	—	0	0	18	22
1956	—	4	2	4	8
1957	2	9	53	27	21
1958	0	0	2	0	3
1959	—	0	—	3	118
1960	0	0	0	6	17
1961	0	4	0	0	12
1962	—	2	28	35	156
1963	—	4	29	89	85
1964	15	53	254	82	125
1965	—	52	112	60	227
1966	—	28	42	21	68
1967	—	0	0	0	5
1968	5	4	0	5	29
1969	8	6	8	9	5
1970	0	0	0	0	26
1971	4	2	22	8	142
1972	0	0	0	0	2
1973	1	2	0	2	0
1974	—	0	0	—	—
1975	4	0	0	20	34
1976	2	2	2	0	2
1977	0	0	12	40	270
1978	4	6	4	4	6
1979	0	0	2	4	4
1980	0	0	0	2	16
1981	21	186	202	152	892
1982	0	0	14	6	118
1983	0	0	40	106	24
1984	0	0	0	0	20
1985	22	2	4	4	64
1986	33	72	63	61	7
1987	35	16	11	11	131
$\bar{x}$ Spat/bu.	7	15	31	24	104

[1] Data 1947 to 1966, Andrews, J. D. (Haven et al. 1981).

strates. Occasionally they extended offshore to the 5.5 m contour, but more frequently they were observed in shallower water inshore of the reefs and the mud-shell substrate.

It is obvious that natural recruitment (Table 3) has been typically low, sometimes zero above Towles Point, and low, but occasionally, moderate below Towles Point. This difference in recruitment is not explained by the study. Data on currents, circulation and salinity were not recorded, and concurrent data was not obtained on predators, diseases, etc. It is likely that these factors were involved since other studies show how several variables may interact to bring about differences in settlement in other estuaries (Pritchard 1953; Manning and Whaley 1954; Nelson 1954; Kennedy 1980; Andrews 1982; Krantz and Meritt 1977; and Haven and Fritz 1985). There is a suggestion; however, that water carrying oyster larvae is retained longer below Towles Point. The result of this water retention is a higher potential for settlement below Towles Point (Table 4).

TABLE 4.

Spatfall in the Rappahannock River—1972 thru 1980[1].

Location	Seasonal Total of Weekly Spat/Shell								
	1972	1973	1974	1975	1976	1977	1978	1979	1980
Above Towles Point									
S1—Punch Bowl					0.1	7.7	0	0.9	
S2—Waterview						1.0	0		
S3—Weeks Ck.					0.1	1.7	0		
S4—Smokey Pt.						2.3	0	2.2	0.4
S5—Greenvale Ck.	0	0	1.3	24.9	0.9	1.3	0.2	0.7	9.2
S6—Goose Pt.					0.2	2.9	0.1		
S7—Ball's Pt.					0	1.5	0.4		
Below Towles Point									
S8—Corrotoman Pt.	0	0.4	1.3	15.4	2.7	41.0	1.9	1.9	30.5
S9—Parrott's Rk.							5.6	4.2	1.7
S10—Cedar Bar							0.2		21.0
S11—Mosquito Pt.				18.2	1.5	8.7	8.5	15.6	50.1
S12—Broad Ck. In.		0	0	1.4	11.1				
S13—Broad Ck. Off		0	2.0						
S14—Spike's Rk.					0.8				

[1] VIMS (unpublished).

One aspect of the hydrography that does merit attention is that the deeper waters of the lower Rappahannock often become deficient in DO (dissolved oxygen) during the warmer months (McHugh 1967; Officer et al. 1984; and Tuttle et al. 1987). In spite of the deficiencies of DO in the lower river the Upper-Lower Ridge reefs extends to 9.1 m.

The Chesapeake Bay and its tributaries, including the Rappahannock basin, were flooded with sea water as sea level rose during the Holocene (Nichols 1972). We have speculated that many of the present day oyster reefs in the James River and Pocomoke Sound are the result of the upward growth of these reefs which accompanied that slow rise in sea level (Bouma 1976; Haven and Whitcomb 1983; Whitcomb and Haven 1987). It is probable that the oyster reef areas in the Rappahannock River evolved in the same manner.

In view of their origin, it is evident that the existence or permanence of an oyster reef in the Rappahannock River must depend on the accumulation of oysters and shells to balance the removal by exploitation or natural processes. The fact that recruitment is low or, at times, non-existent in the estuary above Towles Point indicates that this area is susceptible to over fishing.

ACKNOWLEDGMENTS

We are indebted to Mr. Paul Kendall and Mr. Kenneth Walker who assisted in all field studies. We also recognize Mr. Paul Kendall's assistance in tabulating data and entering field data on work sheets. We acknowledge the invaluable and constructive criticisms from Dr. Roger Mann, Dr. Bruce Barber, Mr. Reinaldo Morales and Mr. Dan Sved.

LITERATURE CITED

Andrews, J. D. 1982. The James River public seed oyster area in Virginia. *Va. Inst. Mar. Sci., Spec. Rep. Appl. Mar. Sci. Ocean Eng.* No. 261:1–60.

Bouma, A. H. 1976. Subbottom characteristics of San Antonio Bay. Bouma, A. H., ed; *Shell Dredging and its Influence in Gulf Coast Environments*. Gulf Publishing Co., Houston, TX pp. 132–148.

Ellison, R. L. & M. M. Nichols. 1970. Estuarine foraminifera from the Rappahannock River, Virginia. Contr. from the Cushman Found. for Foraminiferal Res. Vol. XXI, Part 1, Jan. 1970.

Haven, D. S., J. P. Whitcomb & P. Kendall. 1981. The present and potential productivity of the Baylor Grounds in Virginia. Vols. I (167 pp.) and II (154 pp., 52 charts). *Va. Inst. Mar. Sci., Spec. Rep. Appl. Mar. Sci. Ocean Eng.* No. 243.

Haven D. S. & L. W. Fritz. 1985. Setting of the American oyster *Crassostrea virginicia* in the James River, Virginia, USA: temporal and spatial distribution. *Mar. Biol.* 86:271–282.

Haven, D. S. & J. P. Whitcomb. 1983. The origin and extent of oyster reefs in the James River, Virginia. *J. Shellfish Res.* 3(2):141–151.

Haven, D. S. & J. P. Whitcomb. 1986. The public oyster bottoms in Virginia: An overview of their size, location, and productivity. *American Malacological Bulletin, Special Edition* No. 3 (1986):17–23.

Kennedy, V. S. 1980. Comparisons of recent and past patterns of oyster settlement and seasonal fouling in Broad Creek and Tred Avon River, Maryland. *Proc. Natl. Shellfish. Assoc.* 70(1):36–46.

Krantz, G. E. & D. W. Meritt. 1977. An analysis of trends in oyster spat set in the Maryland portion of the Chesapeake Bay. *Proc. Natl. Shellfish. Assoc.* 67:53–59.

Manning, J. H. & H. H Whaley. 1954. Distribution of oyster larvae and spat in relation to some environmental factors in a tidal estuary. *Proc. Natl. Shellfish. Assoc.* 45:56–65.

McHugh, J. L. 1967. Estuarine Nekton: Lauff, G. H., ed; *Estuaries*, Am. Assoc. Adv. Sci., Washington, D.C., (83) pp. 581–620.

Nelson, T. C. 1954. Observations and distribution of oyster larvae. *Proc. Natl. Shellfish. Assoc.* 45:23–28.

Nichols, M. M. 1972. Sediments in the James River, Va. Nelson, B. W., ed; Environmental Framework of Coastal Plain Estuaries. *Geol. Soc. Am. Mem.* 133:169–212.

Officer, C. B., R. B. Biggs, J. L. Taft, L. E. Cronin, M. A. Tyler & W. R. Boynton. 1984. Chesapeake Bay anoxia: origin, development, significance. Science 223:22–27.

Price, W. A. 1954. Oyster reefs of the Gulf of Mexico. Galtsoff, P. S., coordinator, Gulf of Mexico, its origin, waters and marine life. *U.S. Fish Wildl. Serv. Fish. Bull.* 89:491 p.

Pritchard, D. W. 1952. Salinity distribution and circulation in the Chesapeake Bay estuarine system. *J. Mar. Res.* 13:133–144.

Pritchard, D. W. 1953. Distribution of oyster larvae in relation to hydrographic conditions. *Proc. Gulf and Carrib. Fish. Inst.* 5:123–132.

Scott, A. J. 1968. Environmental factors controlling oyster shell deposits. Texas Coast. From *Fourth Forum on Geology of Industrial Minerals*. University of Texas, Austin, TX; 1968 pp. 131–150.

Stroup, E. D. & R. J. Lynn. 1963. Atlas of salinity and temperature distributions in Chesapeake Bay 1952–1961 and seasonal averages 1949–1961. Baltimore, MD: Chesapeake Bay Inst., *Johns Hopkins Univ. Graph. Sum.* Rep. 2. (Ref. 63-1: 410 p.)

Tuttle, J. H., R. B. Jonas & T. C. Malone. 1987. Origin, development and significance of Chesapeake Bay anoxia. Majumdar, S. K., L. W. Hall, Jr. and H. M. Austin, eds. *Contaminant Problems and Management of Living Chesapeake Bay Resources*. The Penna. Acad. of Sci., Easton, Pa. pp. 442–472.

Whitcomb, J. P. & D. S. Haven. 1987. The physiography and extent of public oyster grounds in Pocomoke Sound, Virginia. *J. Shellfish Res.* 6(2):55–65.

Journal of Shellfish Research, Vol. 8, No. 1, 117–123, 1989.

EFFECT OF BIOFILMS OF THE MARINE BACTERIUM *ALTEROMONAS COLWELLIANA* (LST) ON SET OF THE OYSTERS *CRASSOSTREA GIGAS* (THUNBERG, 1793) AND *C. VIRGINICA* (GMELIN, 1791)

RONALD M. WEINER[1,3,*], MARIANNE WALCH[1], MICHAEL P. LABARE[2,3], DALE B. BONAR[1,4] AND RITA R. COLWELL[1,3]

[1]*Center of Marine Biotechnology*
University of Maryland
600 E. Lombard St.
Baltimore, MD 21202
[2]*Marine Estuarine Environmental Sciences Program*
University of Maryland
College Park, MD 20742
[3]*Department of Microbiology*
University of Maryland
College Park, MD 20742
[4]*Department of Zoology*
University of Maryland
College Park, MD 20742

ABSTRACT Biofilms of the periphytic, marine bacterium *Alteromonas colwelliana* were shown to be beneficial to set of *Crassostrea gigas* and *Crassostrea virginica* on glass, polystyrene and mylar surfaces. Oysters set 3–8× more frequently ($p < 0.05$) on filmed surfaces than on control surfaces. A two stage model for oyster set is proposed. Second stage cues are hypothesized to be bacterial exopolysaccharide (EPS) or a molecule bound to EPS. Results suggests that an artificial surface can be made that would be useful in remote set and aquaculture applications.

KEY WORDS: *Alteromonas colwelliana, Crassostrea virginica, Crassostrea gigas*, oyster set, biofilms, exopolysaccharide (EPS), set cues

INTRODUCTION

Microbial Films and Invertebrate Metamorphosis

The formation of pioneer microbial communities on submerged surfaces appears to be beneficial to subsequent attachment and development of many invertebrate larvae (Zobell and Allen 1935, Cole and Knight-Jones 1939, 1949, Wood 1950, Knight-Jones and Crisp 1953, Daniel 1955, Crisp and Ryland 1960, Meadows and Williams 1963, Corpe 1970, Mitchell and Young 1972, Crisp 1974, Kirchman et al. 1982b). A number of investigations have established a general pattern of periphytic succession for colonization of clean surfaces immersed in seawater. Quickly after submersion, surfaces are coated by organic matter (Loeb and Neihof 1975), after which bacteria attach and begin to grow, forming microcolonies within several hours (Zobell 1943, Marshall et al. 1971b, Corpe 1973, DiSalvo and Daniels 1975, Gerchakov et al. 1976, Cundell and Mitchell 1977). Subsequently, diatoms, fungi, protozoans, micro-algae and other microorganisms attach to the surface, forming what is termed the primary slime layer (Skerman 1956, Marshall et al. 1971a, DiSalvo and Daniels 1975, Gerchakov et al. 1976, Jordan and Staley 1976, Cundell and Mitchell 1977). This primary microbial colonization appears to be a prerequisite for the final stage of succession in which larger organisms, viz., invertebrates, attach and grow on the surface.

Periphytic organisms have been implicated in the induction of metamorphosis of invertebrates, including the sea urchin *Lytechinus pictus*, the cnidarians *Hydractinia echinata* and *Cassiopea andromeda*, and the annelid *Janua brasiliensis*. Cameron and Hinegardner (1974) have determined that for *Lytechinus* the responsible factor is a low molecular weight (<5000 Daltons) bacterial byproduct, probably proteinaceous. Muller and his associates (Muller 1973, see Chia and Bickell 1978 for review) found that planula larvae of *Hydractinia* metamorphose in response to an inducer released by a marine pseudomonad at the end of its exponential growth phase. Neumann (1979) reported that *Vibrio sp.* excreted a product that induced metamorphosis of the cnidarian *Cassiopea andromeda*. Lectins were reported to mediate specific bacterial/sponge symbiosis (Muller et al. 1981). Kirchman and coworkers in Mitchell's laboratory have shown that larvae of the marine annelid *Janua brasiliensis* settle on certain microbial films and that certain bacteria may induce metamorphosis (Kirchman et al. 1982a). They suggest that the process is mediated by larval lectins binding to extracellular polysaccharides (EPS) produced by bacteria (Kirchman et al. 1982b, Maki and Mitchell 1986).

*Corresponding author.

Oyster Set on a Single Species Bacterial Film

For more than 40 years bacterial films have been hypothesized to provide attractive settlement surfaces for oyster larvae (Cole and Knight-Jones 1939, 1949). It has been demonstrated that a soluble factor, ammonia, cues larval search behavior, initiating a stage in settlement but that it does not promote larval cementation (Coon et al. 1988, Coon et al. 1989 in manuscript, Walch et al. 1989 in manuscript). L-3,4-dihydroxyphenylalanine (Dopa) also cues swim/search behavior (Coon et al. 1985) but has not been detected *in situ*. One bacterium isolated from oyster hatchery tanks, *Alteromonas colwelliana*, termed LST, was unique among other isolates in promoting extensive set (Weiner et al. 1985). It synthesized two exopolymers, exopolysaccharide (Abu et al. 1986; Labare et al. 1989) and melanin, the endproduct of tyrosinase activity (Labare et al. 1989), intermediates of which are Dopa and trihydroxyphenylalanine (Topa, Dagasan and Weiner 1988).

In this report we demonstrate that for the larvae of *Crassostrea virginica* and *C. gigas*, a biofilm of *A. colwelliana* (Weiner et al. 1985, Weiner et al. 1988) is a major factor in settlement, particularly in the decision to cement down prior to metamorphosis. We propose a two stage hypothesis for larval set, the soluble cue promoting swim/search behavior, and the film promoting crawl/search behavior and cementation. Naturally attractive surfaces such as shell chips (cultch) are in short supply in the Chesapeake Bay area. Thus, *A. colwelliana* and other periphytes become important to the aquaculture industry because they make unattractive surfaces more attractive, offering the promise of controlled artificial setting surfaces.

MATERIALS AND METHODS

Hatchery Facilities and Preparation of Oyster Larvae for Setting Experiments

Competent larvae of the Eastern oyster, *Crassostrea virginica* were obtained from the Maryland Department of Natural Resources oyster hatchery at Deal Island, Maryland. The Pacific Oyster, *C. gigas*, was obtained from Coast Oyster in Quilcine, Washington. Both *C. virginica* and *C. gigas* were maintained and determined to be competent to set as previously described (Coon et al. 1989). Larvae were screened as swimming, eyed, and 220–280 micrometers in diameter. Seting experiments with *C. virginica* larvae were done at the DNR hatchery in 4′ × 8′ × 1′ wooden trays containing course filtered, aerated bay water.

Microscopy: Scanning Electron Microscopy (SEM) and Acridine Orange Direct Counts (AODC) of Biofilms

Specimens to be prepared for scanning electron microscopy were gently rinsed several times in 0.22 micrometer-filtered seawater to remove non-attached microbial cells and adherent debris. Subsequent preparative steps were done as detailed in Maugel et al. (1984). To preserve as natural a condition as possible, a single drop of 4% osmium tetroxide was added to a small volume of water (<1ml.) containing spat. The fluid was quickly removed and replaced with 2.0 ml. of 2.5% PIPES buffered glutaraldehyde (see Maugel et al. 1984), containing NaCl to the desired osmolarity. Following a one hr. fixation, specimens were rinsed several times with PIPES buffer, post-fixed in 1% osmium tetroxide, rinsed in distilled water, and dehydrated with dimethoxypropane. Specimens were dried from liquid CO_2 by the critical point method, mounted on stubs and coated with palladium-gold in a Denton vacuum evaporator. Photographs were taken on Polaroid film on an AMR 1000A scanning electron microscope.

Films were observed and bacteria in the films were counted after rinsing them gently with sterile, filtered seawater and staining them for 3 minutes with 0.1% acridine orange. Films were viewed under epifluorescent microscopy (Zeiss, Axiophot; objective NA 1.3). Films which could not be stained and observed immediately were preserved in 2% formaldehyde at 4°C.

Set of C. gigas *on Glass Coated* A. colwelliana *Biofilms*

To examine the effect of *A. colwelliana* films on set of *C. gigas*, experiments were carried out during two seasons at Coast Oyster Hatchery in Quilcene, Washington. *A. colwelliana* (strain LST-D, a mutant which produces diffusable melanin) was grown in Marine Broth 2216 (Difco) for 24–72 hr at 25°C in 250-ml glass Erlenmeyer flasks. The spent medium was discarded, and the bacterial films on the inside of the flasks were rinsed three times with sterile phosphate buffered saline (20 mM, pH 7.0; PBS). Flasks were stored filled with PBS at 6°C for up to 30 days. No decline in viable counts occurred during this period. Clean, sterile flasks were used as controls. Oysters were set on the films by adding 100 ml seawater containing 3–5 competent, eyed veliger larvae per ml to each flask. The numbers of metamorphosed oysters (spat) set on the flask sides and bottoms were scored after 24 hr, using microscopic observation of cementation and shell growth as criteria for metamorphosis.

Alternatively, films were allowed to form on 3 × 1-in. glass microscope slides which were placed around the inside walls of a growth vessel. These films were rinsed with sterile seawater and exposed to competent oyster larvae as described above. Viable counts and assessments of purity of the bacterial films were done routinely. Slides were examined under phase contrast microscopy (Zeiss Axiophot, objective NA 1.3) and by scanning electron microscopy. Results of these experiments were pooled and grouped according to age of the film.

Set of C. virginica *on Polystyrene Coated by* A. colwelliana *Biofilms*

An experiment to determine the effect of *A. colwelliana* films grown on polystyrene substrata on set of *C. virginica* was conducted at the Maryland Department of Natural Re-

sources oyster hatchery at Deal Island, Maryland. Three strains were used: LST-W, the wild type; LST-D, a hypertyrosinase producer (Dagasan and Weiner, 1988) that synthesizes excess melanin pigment that is not fully polymerized and, therefore, diffusable in agar (Weiner et al. 1988); and LST-V, a mutant that releases excess EPS (Abu et al. 1986).

Each strain was cultivated in polystyrene Petri-plates in broth with gentle agitation, until the stationary phase of growth (72 hrs) for optimal biofilm formation. The media were: Marine Broth 2216 (Difco, Zobell 1941, MB); Brain-Heart Infusion Broth (Difco) + 2.3% NaCl (Difco, BHI); Marine Salts Synthetic Medium (Havenner et al. 1979; Devine and Weiner, Submitted to Microbiologica, AG), containing 125 mM concentrations each of aspartate and glutamate; and Marine Salts Tyrosine Synthetic Medium, which is AG medium amended with 10 mM tyrosine (AGT).

All treatments and controls were done in triplicate. Relative pigment and exopolysaccharide production for each treatment were recorded. The bottom halves of the filmed Petri-dishes were emptied, rinsed twice with sterile seawater and immediately transported to the hatchery. They were placed randomly, face-up in a single layer on the bottom of the setting tray which was filled with course-filtered, aerated bay water. Competent *C. virginica* larvae were evenly distributed into the prepared tank at a density of approximately 40/l and fed regularly during set. After two days, the dishes were rinsed and the number of metamorphosed larvae (spat) was determined, using a dissecting microscope.

Set of C. virginica *on Mylar Coated by* A. colwelliana *Biofilms*

During setting experiments at Deal Island, 4.25 × 11.00 in. sheets of mylar, some containing bacterial films, were tested in the setting trays for their attractiveness to larvae. To prepare the mylar, the sheets were pre-soaked in sterile seawater for one week, then placed into one liter beakers containing approximately 300 ml of *A. colwelliana* LST-D growing in Marine Broth 2216. Control sheets were soaked in sterile marine broth. After 3–5 days growth (23°C), the mylar was rinsed with seawater and air dried. Filmed and control mylar sheets were laid flat on the bottom of the setting trays and exposed to larvae as previously described.

RESULTS

Set of C. gigas *on Glass Coated by* A. colwelliana *Biofilms*

A. colwelliana films were tested on three artificial surfaces, with the finding that each became more conducive to *C. gigas* larval cementation after the film had formed. The biofilms unequivocally promoted enhanced settlement, over uncolonized glass (Table 1). As the films aged, up to 72 hr. when they reached uniformity at several layers of cells in thickness (Fig. 1), they became maximally attractive. Fig. 2 shows a spat developing on such a film. Increased polysaccharide exopolymer (EPS) deposition did not further enhance set. When the cells were grown in glucose, which inhibits tyrosinase activity and consequently Dopa and melanin production (Fitt et al. 1989), films did not cue crawl/search behavior or cementation. Of 1375 larvae tested only 4% set on films of *A. colwelliana* grown in glucose supplemented media (S.E., 1.5; 95% confidence interval for mean, 0.3–8.0).

TABLE 1.

Set of *Crassostrea gigas* on glass coated with *Alteromonas colwelliana* films.

	No. of Replicates	Total Larvae	Mean[a] % Set	95% Confidence
Control	13	2550	9.8 (3.0)	3–16
48-Hour Film	6	875	29.5 (7.3)	11–48
72-Hour Film	12	2890	38.4 (6.3)	25–52

[a] Number in parenthesis is the Std. Error. Cochrans C = Max. Variance/Sum Variances = 0.51, P = 0.053; Bartlett-Box F = 4.993, p = 0.002; Multiple Range Test (Tukey-HSD) Procedure, 0.05 level, denoted groups were significantly different.

Set of C. virginica *on Polystyrene Coated by* A. colwelliana *Biofilms*

Under optimum growth conditions, *A. colwelliana* formed films on the plastic surface as on glass (See Fig. 1). There was 2–3× more set on filmed surfaces than on control polystyrene. ANOVA results supported the observations that certain treatments promoted significant set enhancement ($p < 0.05$). Generally, there was most set when both pigment and EPS were visible (Table 2). These highly attractive surfaces were formed by the wildtype strain cultivated in MB, the hyperpigment producing D strain culti-

Figure 1. Scanning Electron Micrograph of *Alteromonas colwelliana* biofilm from cells cultivated in marine broth for 72 hrs. Note cell elongation (>1.0 micrometer), characteristic of stationary phase when exopolymer production peaks. Bar represents one micrometer.

Figure 2. Scanning electron micrograph of *C. virginica* Set on *A. colwelliana* Biofilm. Note larval metamorphosis to spat (shown) with 24 hours shell growth. Bar represents one micrometer.

vated in MB, and a hyper-EPS producing V strain cultivated in AGT synthetic medium.

Set of C. virginica *on Mylar Coated by* A. colwelliana *Biofilms*

In laboratory studies, an average of 580 larvae set on *Alteromonas colwelliana*-D coated mylar versus 411 on

TABLE 2.

Set of *Crassostrea virginica* on polystyrene coated with films of *Alteromonas colwelliana*, showing effect of strain and culture medium.

Strain[a]	Medium[b]	Visible Pigment[c]	Visible EPS[d]	Mean Set (s.e., n = 3)[e]
LST-W	MB	+	+	251 (199.9)
	BHI	+	+	65 (35.9)
	AG	−	−	68 (64.0)
	AGT	+ + +	−	39 (33.0)
LST-D	MB	+ +	+	167 (122.6)
	BHI	+ +	+	41 (21.2)
	AG	+	−	8 (3.7)
	AGT	+ + +	−	10 (2.8)
LST-V	MB	−	+	26 (7.3)
	BHI	(+)	+	29 (18.5)
	AG	(+)	+	22 (8.2)
	AGT	+ +	+	136 (114.4)
Sterile Unfilmed Control				10 (7.4)
1-Week Seawater-Aged				32 (20.2)

[a] LST-W = wildtype; LST-D = mutant producing diffusable melanin; LST-V = viscous mutant producing excess exopolysaccharide.
[b] MB = Marine Broth 2216; BHI = Brain-Heart Infusion Broth +2.3% NaCl; AG = aspartate/glutamate medium; AGT = aspartate/glutamate medium with added tyrosine (see mat. and met. for added detail).
[c] − = no pigment; (+) = pink or tan; + = light brown; + + = medium brown; + + + = dark brown.
[d] exopolysaccharide indicated by viscosity.
[e] From two-way analysis of variance on logarithmic transformation of the data, strain differences were significant to $p < 0.05$ and medium differences to $p < 0.001$.

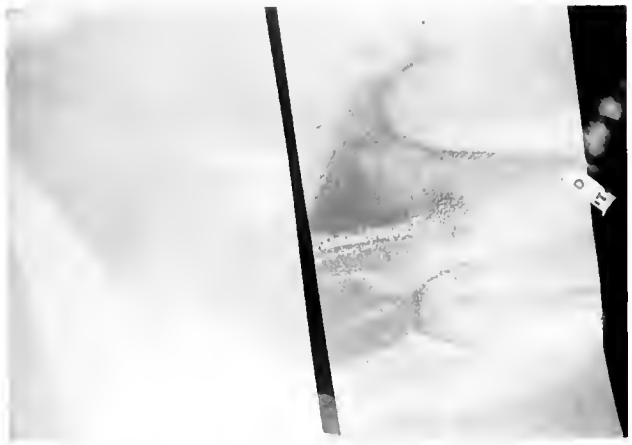


Figure 3. Oyster set on mylar sheets. Sheet at right had 72 hour *A. colwelliana* biofilm. Each dot is a spat. Note the >10X density of set on the mylar sheet containing the biofilm.

controls, an ~50% enhancement. At the Deal Island hatchery, 203 larvae set on *A. colwelliana*-D coated mylar versus 25 on uncoated mylar, an 8-fold increase (Fig. 3). Statistical analyses were not attempted for these experiments because the method of coating the mylar produced biofilms of uneven thickness. However, the areas where the *A. colwelliana* were thickest were readily identified by dark brown pigment. In general, oyster set was clearly greatest in these areas.

DISCUSSION

Oyster set is comprised of two major stages: settlement and metamorphosis (Coon et al. 1989; Fig. 4). In the natural environment, the former is a prerequisite for the latter (Coon et al. 1986). Above the surface of biofilms, ammonia cues swim/search behavior (Coon et al. 1988). While the larvae may sample the substratum, the ammonia cue alone is not sufficient to entice the larvae to remain there. Additional factors are required. A factor(s) in biofilms, and possibly also in cultch, cues crawl/search behavior and cementation, completing settlement according to the two cue model presented in Fig. 4.

The data presented here and elsewhere (Weiner et al. 1985, Walch et al. 1987) conclusively demonstrate that microbial films, specifically those made by *A. colwelliana*, enhance set on glass and other artificial surfaces, making these unattractive surfaces much more attractive. While the observation that microbial films enhance set has been conclusively confirmed, the specific mechanism remains hypothetical. At present, we are testing the theory, using monoclonal antibodies and lectins in blocking experiments, that a specific determinant of the EPS cues crawl/search behavior and cementation.

Alternatively the cuing molecule may be bound to, or entrapped by, the EPS. Candidates are outer membrane proteins, RS layer proteins, and cell membrane fatty acids as in *Phragmatopoma*, for which palmitoleic acid was considered to be a natural inducer of metamorphosis

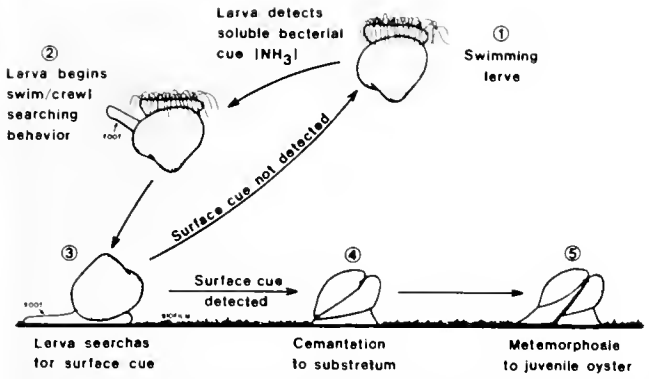


Figure 4. Two cue model of microbial induction of oyster set. Stages 1–3 dipict settlement events, stages 5–6 cementation and metamorphosis.

(Pawlik 1989). Another possible cue is R-type lipopolysaccharide (R-LPS) synthesized by marine bacteria (Sledjeski and Weiner 1988). To test the hypothesis that R-LPS endotoxin on Gram-negative bacteria (e.g., *A. colwelliana*) cues set, R-LPS are being purified and antibodies are being prepared against them for use in cuing/blocking experiments.

Finally, bacterial metabolite(s) that could be beneficial to oyster cementation and metamorphosis include products of tyrosinase activity. Knight Jones (1953) was the first to make a connection between invertebrate larval settlement and tyrosine derivatives, suggesting that quinone-tanned polymeric proteins induced barnacle settlement. Tyrosine derivatives were also implicated in the set of *Crassostrea virginica* (Veitch and Hidu 1971) and later demonstrated to induce both oyster set and metamorphosis (Cooper 1983, Coon 1985). Exogenously applied, soluble Dopa was reported to signal settlement behavior via a dopaminergic-receptor mediated pathway after conversion to dopamine, apparently within the larvae (Coon and Bonar 1987, Bonar et al. 1989). In *A. colwelliana*, two products of tyrosinase activity are Dopa and trihydroxyphenylalanine (Topa, Dagasan and 1988). *A. colwelliana* EPS has been purified and this acidic polysaccharide has been shown to bind these molecules (Labare et al. 1989). Evidence, reported here, for the involvement of Dopa/Topa in enhancing set includes the observations that glucose represses both tyrosinase synthesis and set and that both pigment and EPS appear to be coincident with optimum set on surfaces. In biofilms, however, Dopa and Topa could conceivably function indirectly, enhancing the integrity and longevity of the cue-containing biofilm and altering its physical properties.

In the present study, *A. colwelliana* biofilms, made under various growth conditions, were tested on several surfaces, in the laboratory and in the hatchery, and using two species of *Crassostrea*. There is no reason to suppose that either species would react qualitatively differently to any cue (Coon et al. 1989), although lower percentages of *C. virginica* set, and with less consistancy, using any treatment or surface (see also Coon et al., 1986). It is believed that this fastidiousness of *C. virginia* is manifested in recent inadequate yields from the stressed Chesapeake Bay (Leffler 1988).

Set on experimental biofilms were always compared with set on control surfaces. All surfaces were routinely examined under phase microscopy which revealed that many of the control surfaces were coated with some film from autochthonous flora. While these films were not so nearly well established as the *A. colwelliana* experimental films, they may have promoted (or possibly retarded) some set.

Undoubtedly many factors (e.g., light, surface texture, aeration, oyster density) are involved in oyster behavior and metamorphosis (Knight-Jones 1953, Veitch and Hidu 1971, Bonar et al. 1985, Bonar et al. 1989, Coon et al. 1989), only some of which could be controlled in these experiments, accounting for high set variability. Such variables were minimized in hatchery experiments which were more successful than those in the laboratory. For one, large tanks are required because *A. colwelliana* is aerobic and rapidly reduces dissolved oxygen in microcosms. This was not a problem in the hatchery setting.

This study shows conclusively that microbial films enhance oyster set on artificial surfaces and we have shown that the films of some bacteria are more beneficial to set than others (Weiner et al. 1985). With the decline of the natural industry in Chesapeake Bay and the paucity of cultch, these results could have important implications for the oyster aquaculture industry which is developing on the Chesapeake Bay.

ACKNOWLEDGMENTS

This project was supported by grants P-C-M-82-16054 from the National Science Foundation, S-124-88-008 from the Maryland Department of Natural Resources, #NA88-AAD-00014 from the Maryland Sea Grant College funded by the National Oceanic and Atmospheric Administration, and by University Research Initiative grant #N00014-86K-0696 from the Office of Naval Research.

We are grateful to W. Schafer for help with the experimental design and statistical analysis and to D. Prieur for helpful discussion and collaboration. We acknowledge the assistance of S. Raikin and K. Weiner. We thank Coast Oyster Co. for providing larvae and facilities for some of these studies.

BIBLIOGRAPHY

Abu, G. O., R. M. Weiner, D. B. Bonar & R. R. Colwell. 1986. A capsular viscous exopolymer from the marine bacterium, LST. In Barry, S. & D. R. Houghton (eds.) Proc. of Sixth Int. Biodet. Sympos., Cambrian News LTD. Eng., pp 543–549.

Bonar, D. B., R. M. Weiner, S. L. Coon & R. R. Colwell. 1985. Induction of oyster metamorphosis by bacterial products and biogenic amines. *Bull. Mar. Sci.* 37:763.

Bonar, D. B., S. L. Coon, M. Walch, R. M. Weiner & W. Fitt. 1989.

Control of oyster settlement and metamorphosis by endogenous chemical cues. *Bull. Mar. Sci.* (In press).

Cameron, R. A. & R. T. Hinegardner, 1974. Initiation of metamorphosis in laboratory cultured sea urchins. *Biol. Bull.* 146:335–342.

Chia, F. S. & L. Bickell. 1978. Mechanisms of larval attachment and the induction of settlement and metamorphosis in coelenterates: A review. In Chia, F. S. & M. E. Rice (eds.) *Settlement and Metamorphosis of Marine Invertebrate Larvae,* Elsevier, NY, pp. 1–12.

Cole, H. A. & E. W. Knight-Jones. 1939. Some observations and experiments on the setting behavior of *Ostrea edulis. J. Cons. Perm. Int. Explor. Mer.* 14:86–105.

Cole, H. A. & E. W. Knight-Jones. 1949. The setting behavior of larvae of the European oyster *Ostrea edulis* L. and its influence on methods of cultivation and spat collection. *Fish. Invest. Lond. Ser.* 17:1–39.

Coon, S. L., W. K. Fitt & D. B. Bonar. 1989. Competency and delay of metamorphosis in the Pacific Oyster, *Crassostrea gigas* (Thunberg). *Mar. Biol.* (in press).

Coon, S. L., M. Walch, W. K. Fitt, D. B. Bonar & R. M. Weiner. 1988. Induction of settlement behavior in oyster larvae by ammonia. *Amer. Zool.* 28:70A.

Coon, S. L. & D. B. Bonar. 1987. The role of Dopa and dopamine in oyster settlement behavior. *Amer. Zool.* 27:128A.

Coon, S. L., D. B. Bonar & R. M. Weiner. 1986. Chemical production of cultchless oyster spat using epinephrine and norepinephrine. *Aquaculture* 56:255–262.

Coon, S. L., D. B. Bonar & R. M. Weiner. 1985. Induction of settlement and metamorphosis of the Pacific oyster *Crassostrea gigas* (Thunberg) by L-DOPA and catecholamines. *J. Exp. Mar. Biol. Ecol.* 94:211–221.

Cooper, K. 1983. Potential for application of the chemical Dopa to commercial bivalve setting systems. *J. Shellfish Res.* 3:110–111.

Corpe, W. A. 1970. Attachment of marine bacteria to solid surfaces. In Manley, R. S. (ed.) *Adhesion in Biological Systems,* Academic Press, NY, p. 73.

Corpe, W. A. 1973. Microfouling: the role of primary film forming bacteria. In: Acker, R. F., B. F. Brown, J. R. DePalma & W. P. Iverson (eds). Proc. Third Intern. Cong. Mar. Corrosion Fouling. Northwestern Univ. Press: Evanston, Ill., pp. 598–609.

Crisp, D. J. 1974. Factors influencing the settlement of marine invertebrate larvae. In Grant, P. T. & A. M. Mackie, eds., *Chemoreception in Marine Organisms,* Academic Press, NY, pp. 117–265.

Crisp, D. J. & J. S. Ryland. 1960. Influence of filming and of surface texture on the settlement of marine organisms. *Nature* 185:119.

Cundell, A. M. & R. Mitchell. 1977. Microbial succession on a wooden surface exposed to the sea. *Int. Biodeterior. Bull.* 13:67–73.

Dagasan, L. & R. M. Weiner. 1988. Characterization and *in vitro* translation of an atypical tyrosinase from periphytic marine bacterium. *Abstracts of 3rd. Annu. Amer. Soc. Microbiol. Conf. Biotechnol.* p. 43.

Daniel. A. 1955. The primary film as a factor in settlement of marine foulers. *J. Madras Univ.* 25B:89–200.

Danielsson, A., B. Norkrans & A. Bjornsson. 1977. On bacterial adhesion—the effect of certain enzymes on adhered cells of a marine *Pseudomonas sp. Botan. Mar.* 20:13–17.

DiSalvo, L. H. & G. W. Daniels. 1975. Observations on estuarine microfouling using the scanning electron microscope. *Microbial Ecol.* 2:234–240.

Gerchakov, S. M., D. S. Marsazalek, F. J. Roth & L. R. Udey. 1976. Succession of periphytic microorganisms on metal and glass surfaces in natural seawater. *Proc. Fourth Intern. Cong. Mar. Corrosion Fouling.*

Havenner, J. A., B. A. McCardell & R. M. Weiner. 1979. Development of defined minimal and complete media for the growth of *Hyphomicrobium neptunium. Appl. Environ. Microbiol.* 38:18–23.

Hoagan, T. L. & J. T. Stanley. 1976. Electron microscopic study of succession in the periphyton community of Lake Washington. *Microbial Ecol.* 2:241–251.

Kirchman, D. S., S. Graham, D. Reish & R. Mitchell. 1982a. Bacteria induce settlement and metamorphosis of *Janua (Dexiospira) brasiliensis* Grube (*Polychaeta: spirobidae*). *J. Exp. Mar. Biol. Ecol.* 56:153–163.

Kirchman, D. L., D. S. Graham, D. Reish & R. Mitchell. 1982b. Lectins may mediate in the settlement and metamorphosis of *Janua brasiliensis* (Grube). *Mar. Biol. Lett.* 3:131–142.

Knight-Jones, E. W. 1953. Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *J. Exp. Biol.* 30:584–599.

Knight-Jones, E. W. & D. J. Crisp. 1953. Gregariousness in barnacles in relation to the fouling of ships and to anti-fouling research. *Nature* 171:1109.

Labare, M. P., K. Guthrie and R. M. Weiner. 1989. Characterization of a Polysaccharide Adhesive Exopolymer from *Alteromonas colwelliana. J. Adhes. Sci.* (in press)

Leffler, M. 1988. Neither the best nor worst of times. *Md. Sea Grant Newsletter* 9:3–7.

Loeb, G. I. & R. A. Neihof. 1975. Marine conditioning films. *Adv. in Chem.* 145:319–335.

Maki, J. S. & R. Mitchell. 1986. The function of lectins in interactions among marine bacteria, invertebrates and algae. In Mirelman, D. (ed.) *Microbial Lectins and Agglutinins: Properties and Biological Activity,* John Wiley, NY.

Marshall, K. D., R. Stout & R. Mitchell. 1971a. Selective sorption of bacteria from seawater. *Canad. J. Microbiol.* 17:1413–1416.

Marshall, K. C., R. Stout & R. Mitchell. 1971b. Mechanisms of the initial events in the sorption of marine bacteria to a surface. *J. Gen. Microbiol.* 68:337–348.

Maugel, T. K., D. B. Bonar, W. Creegan & G. B. Small. 1984. Specimen Preparation Techniques for aquatic organisms. In Murphey, J. A. & G. M. Roomans (eds.) *Preparation of Biological Specimens for Scanning Electron Microscopy,* SEM, Inc.: Illinois.

Meadows, T. S. & G. B. Williams. 1963. Settlement of *Spirorbis borealis* Daudin larvae on surfaces bearing films and microorganisms. *Nature* 198:610–611.

Mitchell, R. & L. Young. 1972. The role of microorganisms in marine fouling. Tech. Rept. No. 3, U.S. Office Naval Res. Contract No. N00014-67-A-0298-0026 NR-306-025.

Muller, W. E. G. (1973). Induction of metamorphosis by bacteria and ions in the planulae of *Hydractina echinata:* an approach to the mode of action. *Pub. Seto. Mar. Biol. Lab.* 20:195–205.

Muller, W. E. G., R. K. Zahn, B. Kurelec, C. Lucu, I. Muller & G. Uhlenbruch, 1981. Lectin, a possible basis for symbiosis between bacteria and sponges. *J. Bacteriol.* 145:548–558.

Neumann, R. 1979. Bacterial induction of settlement and metamorphosis in the planula larvae of *Cassiopea andromeda* (Cnidaria:Scyphozoa, Rhizostomeae). *Mar. Ecol. Prog. Ser.* 1:21–28.

Pawlik, J. R. 1989. Drugs in the sea: a critical look at studies of the effects of bioactive compounds on the settlement and metamorphosis of marine invertebrate larvae, with new data on the larval responses of the tube worm *Phragmatopoma lapidosa californica* (Polychaeta: Sabellariidae). *Bull. Mar. Sci.* (in press).

Skerman, T. M. 1956. The nature and development of primary films on surfaces submerged in the sea. *N. Zealand J. Sci. Technol. B* 38:44–57.

Sledjeski, D. D. & R. M. Weiner. 1988. Characterization of *Alteromonas* sp. surface antigens. *Abst. 88th Annu. Meet. Amer. Soc. Microbiol.* p. 224.

Veitch, F. P. & H. Hidu. 1971. Gregarious setting in the American oyster *Crassostrea virginica* Gmelin: 1. Properties of a partially purified "setting factor". *Chesapeake Sci.* 12:173–178.

Walch, M., E. A. Pierce, R. M. Weiner & D. B. Bonar. 1987. Enhanced setting of the eastern oyster on biofilms of specific marine bacteria. *Abst. Annu. Meet. Amer. Soc. for Microbiol.* 87.

Weiner, R. M., V. Coyne, S. F. Raiken & P. Brayton. 1988. *Alteromonas colwelliana*, a new species from oyster water. *Int. J. Syst. Bacteriol.* 38:240–244.

Weiner, R. M., A. M. Segall & R. R. Colwell. 1985. Characterization of a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster). *Appl. Environ. Microbiol.* 49:83–90.

Wood, E. J. F. 1950. The role of bacteria in the early stages of fouling. *Aust. J. Mar. Freshwater Res.* 1:85–91.

Zobell, C. E. 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J. Mar. Res.* 4:42–75.

Zobell, C. E. 1943. The effect of solid surfaces upon bacteria activity. *J. Bacteriol.* 46:93–96.

Zobell, C. E. & E. C. Allen. 1935. The significance of marine bacteria in the fouling of submerged surfaces. *J. Bacteriol.* 29:230–251.

Journal of Shellfish Research, Vol. 8, No. 1, 125–131, 1989.

THE ENERGETIC COST OF *PERKINSUS MARINUS* PARASITISM IN OYSTERS: QUANTIFICATION OF THE THIOGLYCOLLATE METHOD

K.-S. CHOI[1], E. A. WILSON[1], D. H. LEWIS[2], E. N. POWELL[1] AND S. M. RAY[3]

[1]*Department of Oceanography*
Texas A&M University
College Station, TX 77843
[2]*Department of Veterinary Microbiology and Parasitology*
Texas A&M University
College Station, TX 77843
[3]*Department of Marine Biology*
Texas A&M University at Galveston
Galveston, TX 77550

ABSTRACT A quantitative method for counting *Perkinsus marinus* hypnospores is described. Using the method, Mackin's commonly used 0–5-point scale of infection intensity can be shown to be exponential. From general relationships for oysters and protozoa, the impact of *P. marinus* at varying infection intensities on the oyster's energy budget can be estimated. Mortality, decreased growth and decreased fecundity in infected oysters, as described in the literature, can be explained by a reduction in the oyster's available energy by *P. marinus* production and respiration. Because smaller oysters expended proportionally less energy on respiration, the net productivity (growth and reproduction) of larger oysters should be more severely affected by *P. marinus* at a given infection intensity than smaller oysters.

KEY WORDS: *Crassostrea virginica*, oyster, *Perkinsus marinus*, parasitism, energetics

INTRODUCTION

The protozoan parasite, Perkinsus (=*Dermocystidium*) *marinus* is responsible for up to 50% mortality in market-sized oysters in Texas in most years (Hofstetter 1977). Ray (1952) developed a technique for diagnosis of infection with *P. marinus* using fluid thioglycollate medium fortified with antibiotics. After one or two weeks of incubation, the parasite in oyster tissue becomes greatly enlarged in the medium. For determination of the intensity of *P. marinus* infection, Mackin (1962) devised a semiquantitative numerical scale from 0 (uninfected) to 5 (heavily infected) based upon microscopic examination of thioglycollate-cultured and stained suspect tissues (see also Ray 1954).

Although Mackin's method for the determination of infection by *P. marinus* has been used widely and successfully for many years, it suffers from two major drawbacks. First, it is not truly quantitative; the result of the analysis cannot easily be expressed as an amount of parasite material per gram oyster tissue although an estimate can be made of the range in the number of hypnospores present in cultured tissues (Quick and Mackin 1971). Second, since only a small piece of oyster tissue is analyzed, parasites present elsewhere in the oyster may go undetected. In particular, prevalence may be underestimated because very light infections may be misdiagnosed (Quick and Mackin 1971).

Parasitism by *Perkinsus marinus* has been shown to detrimentally affect oysters in a number of ways. Reduced growth, reduced fecundity, and increased mortality often occur in oysters with increased levels of *P. marinus* infection (Menzel and Hopkins 1955a, Mackin 1962, Wilson et al. 1988). The biochemical composition of oysters including lipid, glycogen and amino acid content, is also altered (Soniat and Koenig 1982, White et al. 1988b, Wilson et al. 1988). These results suggest that *P. marinus* exerts a steady drain on the energy resources of the oyster as does MSX in oysters (Newell 1985, Barber et al. 1988a, b) and as do other parasites in other invertebrates (e.g., Anderson 1977, Reaka 1979). Although direct measurement of the energetic drain caused by an endoparasite like *P. marinus* is difficult (e.g., Walkey and Meakins 1970, Zuk 1987), it might be estimated from biomass data. This information has been unavailable, however, because a good estimate of the number of parasites present in infected oysters was unknown.

We developed a technique for extracting *P. marinus* hypnospores from infected oyster tissues cultured in fluid thioglycollate media based upon treating those tissues with 2 M sodium hydroxide (Lewis et al. 1988). This technique provides a preparation of hypnospores free of oyster tissue and other parasitic organisms such as *Nematopsis*. The technique is truly quantitative and objective and so overcomes some of the handicaps involved in the previous thioglycollate method for diagnosis of *P. marinus* infection in oysters. Here we report details of the technique and relate the number of hypnospores per gram of oyster tissue to the semiquantitative assessment of infection intensity based on Mackin's scale. Based on the quantity of hypnospores

present in oyster tissues, the energy lost by the oyster to the parasite can be estimated.

MATERIALS AND METHODS

Market-sized oysters were collected from the Galveston Bay area during January–March 1988. Oyster mantle and gill tissues were excised and incubated in fluid thioglycollate media for two weeks (Ray 1966). At the end of this period, the tissues were cut into smaller pieces, approximately 1 cm^2. Each of these pieces was examined microscopically after treatment with Lugol's solution to determine the intensity of infection by *P. marinus*. Infection intensity was rated on the 0 (uninfected) to 5 (heavily infected) scale of Mackin (1962) as modified and expanded by Craig et al. (1989) (Table 1). Tissues were sorted into tubes of fresh thioglycollate media with respect to their assigned *P. marinus* infection intensity. In this way, all mantle and gill tissues with the same intensity of infection were placed together. The tubes were refrigerated until further analysis.

The analyzed tissues were placed on filter paper to remove excess media and weighed. The weighed tissues were placed into 50 ml tubes and approximately 20 ml of 2 M NaOH per gram cultured oyster tissue was added. The tubes were incubated at 50°C in a water bath for 1 hr. After incubation, each tube was centrifuged at 1600 g for 15 min and the supernatant containing NaOH was removed. The pellet was resuspended in 40 ml of phosphate buffered saline (PBS II) (0.15 M NaCl, pH 7.3), spun at the same speed and time and the supernatant removed. This washing procedure was repeated four times to remove as much of the NaOH as possible. After the final centrifugation, the spores were resuspended in PBS II for analysis.

P. marinus spores can be 'sticky' and sometimes adhere to each other, forming clumps and chains. To obtain a more homogenous mixture of resuspended spores, the spore suspension was stirred on a Vortex mixer. Five 100 μl subsamples were then taken from each tube. To each subsample, 100 μl of Lugol's solution was added. The hypnospores present in two 10 μl subsamples from each tube were counted using an AO Bright-Line Hemacytometer. The total number of *P. marinus* spores in each sample was calculated from the number counted and the dilution factors of the sample. The total number of spores was divided by the wet weight of the tissue to determine the number of *P. marinus* spores per gram wet tissue weight for each level of infection intensity.

RESULTS

The number of hypnospores per gram wet tissue weight yielded a significant exponential regression with Mackin's scale

$$\text{no. spores} \cdot \text{g wet wt}^{-1} = 1409.9\ (10^{0.64296x})$$

($r^2 = 0.91$) where x is a numerical value of Mackin's scale from Table 1 (Fig. 1).

Figure 1 also illustrates the problems associated with Mackin's scale at both extremes of the distribution. First, some negatives (thioglycollate/Ray's method) were found to contain some hypnospores when sufficient tissue was examined. Spore counts vary within any tissue from one location to another (White et al. 1987) (Table 2), hence some very light infections are judged negative by the normal thioglycollate method in which only a small piece of tissue is examined (see also Quick and Mackin 1971). Secondly, the designation of "heavy infection" is an open-ended category covering a wide range of hypnospore numbers and is, then, not directly comparable to the remaining categories of Mackin's scale. Finally, Mackin's is an exponential scale. Hence, infection intensity actually increases by 4×10^5 hypnospores · g wet wt^{-1} between a moderate and light moderately heavy infection (3–4 on Mackin's scale; 1×10^5–5×10^5 hypnospores) but much less, 2.4×10^4 hypnospores · g wet wt^{-1}, between a light and moderate infection (1–2 on Mackin's scale; 6×10^3–3×10^4 hypnospores).

DISCUSSION

Parasites can have a substantial impact on host population dynamics and population stability (See Blower and Roughgarden 1987 for a good marine example) including not only the more dramatic effect of mortality (e.g., Lanciani 1986, Collins 1987) but also more subtle impacts on predation susceptibility (Holmes 1982, Hassell et al. 1982)

TABLE 1.

Level of *Perkinsus* Infection	Assigned Symbols and Numerical Values	
Negative	Neg	0.0
Very Light	VL	0.33
Light	L−, L, L+	0.67, 1.0, 1.33
Light to Moderate	ML−, ML, ML+	1.67, 2.0, 2.33
Moderate	M−, M, M+	2.67, 3.0, 3.33
Moderate to Heavy	MH−, MH, MH+	3.67, 4.0, 4.33
Heavy	H−, H	4.67, 5.0

Modified Mackin's (1962) scale from Craig et al. (1989).

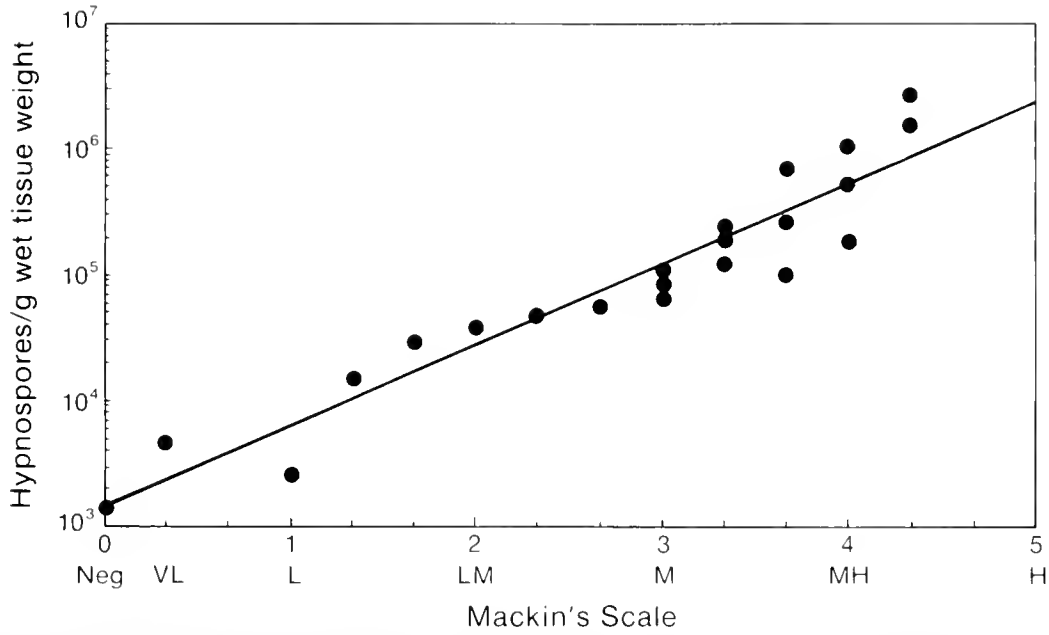


Figure 1. The relationship between Mackin's (1962) scale (x) (see Table 1) and the number of hypnospores per g wet tissue wt. The exponential relationship is no. spores g wet wt^{-1} = 1409.9 ($10^{0.64296x}$).

and fecundity (e.g., Skorping 1985, Kabat 1986) that eventually affect population stability. In many cases, whether or not a parasite has such an effect can be directly related to the metabolic cost of parasitism (Nelson et al. 1986, Hochmuth et al. 1987 and previous references).

The energetics impact of *P. marinus* on oysters can be estimated from the relationship in Fig. 1. For this estimation, we use the energetics model of White et al. (1988a) for oysters and general energetics relationships for protozoa as reviewed by Laybourn-Parry (1987). An estimate of the average diameter of a *P. marinus* cell ranges from 6 μm (range of 3–10 μm; Mackin et al. 1950) to 10 μm (range of 2–20 μm; Ray 1954). We compare calculations based on an average diameter for a *P. marinus* cell in the oyster of 6 μm (Mackin et al. 1950) and 10 μm (Ray 1954) and approximate the volume of a *P. marinus* cell as $V = (4/3)\pi r^3$. From Laybourn-Parry (1987), assuming 1 cm^3 = 1 g wet wt = 0.2 g dry wt and 1 mg dry wt = 20 J, one 6 μm cell contains 4.52×10^{-7} J (2.09×10^{-6} J for a 10 μm cell). Although most *P. marinus* cells are small, a few

TABLE 2.

Mean Infection intensity.

Oyster	Digestive Gland	Mantle	Gill
1	3.2	2.2	2.0
2	3.4	3.1	2.5
3	3.8	2.1	3.6
4	3.7	1.5	3.2
5	3.4	4.0	3.6
6	3.1	2.3	2.3
7	2.1	1.1	1.0
8	3.3	1.7	3.1
9	3.6	1.1	1.0
10	3.0	1.3	1.7
11	M+, M+, MH−	LM+, M−, M−, M−, LM+, M−	M−, M−, M, LM+, LM+
12	M, MH−	LM+, LM+, LM+, LM+, LM+, M	M, MH−, MH−, M+, M+
13	M−, MH−, MH−, MH−, M+	M−, M−, LM, M+, LM+, LM+	M−, MH−, MH−, MH−, M+
14	M, MH−	L+, LM+, M, M−, L+, L	LM, LM+, LM+
15	MH−, M+	M−, LM+, M, LM+, LM+, LM+	M−, M−, LM+, LM, LM+

Above. Mean infection intensity (n ≥ 3 tissue pieces) for digestive gland, mantle and gill using Mackin's scale (Table 1). Below. Individual tissue analyses illustrating the variability within and between tissues.

are large (Ray 1954, Mackin and Boswell 1956); to this extent the following estimates are minimal values.

A parasitic protozoan's energy budget can be approximated as $I = P + R$ where I is ingestion, P, production, and R, respiration. Respiration rate at 20°C for a 6 μm cell was computed from Laybourn-Parry (1987) as 1.00×10^{-9} ml O_2 hr^{-1} and converted using 20.19 J ml^{-1} to 2.02×10^{-8} J hr^{-1}. The values for a 10 μm cell are 3.98×10^{-9} ml hr^{-1} and 8.04×10^{-8} J hr^{-1}. Production/ingestion ratios for protozoa typically range from 0.3–0.8. For sessile predators like *Stentor*, P/I ranges from 0.64–0.85 (Laybourn-Parry 1987). We took a value of 0.78, assuming that the energy expenditure of a sessile predator most closely approximates the case of *P. marinus* and that most ingested material is digestible (an assimilation efficiency near 100%). This is probably a conservative estimate of P/I since Calow (1977) computes the best possible conversion efficiency for a heterotrophic cell as 0.8. Accordingly, we may overestimate actual ingestion somewhat. We then computed I, ingestion, using respiration and the production/ingestion ratio as 9.18×10^{-8} J hr^{-1} 6 μm $cell^{-1}$ (3.65×10^{-7} J hr^{-1} 10 μm $cell^{-1}$) or about 20% of cell caloric value per hr.

These calculations yield a predicted generation time of about 6–7 hr which compares favorably with many amoebae and ciliates (Laybourn-Parry 1987). Ray (1954)

TABLE 3.

Oyster length (mm)	20	40	60	80	100
Oyster weight (g dry wt)	1.12×10^{-2}	1.66×10^{-1}	7.79×10^{-1}	2.33	5.44
Net production (J hr^{-1})	5.29×10^{-1}	2.63	6.01	1.01×10^{1}	1.44×10^{1}
	Estimation for a 6 μm cell				
	P. marinus energy requirements (J hr^{-1} $oyster^{-1}$)				
Infection Level					
Very light infection	1.18×10^{-5}	1.75×10^{-4}	8.20×10^{-4}	2.46×10^{-3}	5.74×10^{-3}
Light infection	3.19×10^{-5}	4.74×10^{-4}	2.22×10^{-3}	6.62×10^{-3}	1.56×10^{-2}
Light-moderate infection	1.40×10^{-4}	2.07×10^{-3}	9.74×10^{-3}	2.91×10^{-2}	6.77×10^{-2}
Moderate infection	6.17×10^{-4}	9.13×10^{-3}	4.30×10^{-2}	1.27×10^{-1}	2.98×10^{-1}
Moderate-heavy infection	2.68×10^{-3}	3.99×10^{-2}	1.87×10^{-1}	5.57×10^{-1}	1.30
Heavy infection	1.19×10^{-2}	1.76×10^{-1}	8.31×10^{-1}	2.49	5.78
	Fraction of the oyster's net productivity used by *P. marinus*				
Very light infection	2.24×10^{-5}	6.66×10^{-5}	1.36×10^{-4}	2.43×10^{-4}	3.98×10^{-4}
Light infection	6.03×10^{-5}	1.80×10^{-4}	3.71×10^{-4}	6.56×10^{-4}	1.08×10^{-3}
Light-moderate infection	2.64×10^{-4}	7.87×10^{-4}	1.62×10^{-3}	2.87×10^{-3}	4.70×10^{-3}
Moderate infection	1.17×10^{-3}	3.47×10^{-3}	7.15×10^{-3}	1.27×10^{-2}	2.07×10^{-2}
Moderate-heavy infection	5.06×10^{-3}	1.52×10^{-2}	3.10×10^{-2}	**5.51×10^{-2}**	**9.05×10^{-2}**
Heavy infection	2.25×10^{-2}	**6.68×10^{-2}**	**1.38×10^{-1}**	**2.47×10^{-1}**	**4.02×10^{-1}**
	Estimation for a 10 μm cell				
	P. marinus energy requirements (J hr^{-1} $oyster^{-1}$)				
Infection Level					
Very light infection	4.70×10^{-5}	6.96×10^{-4}	3.27×10^{-3}	9.73×10^{-3}	2.28×10^{-2}
Light infection	1.27×10^{-4}	1.88×10^{-3}	8.82×10^{-3}	2.64×10^{-2}	6.16×10^{-2}
Light-moderate infection	5.57×10^{-4}	8.26×10^{-3}	3.87×10^{-2}	1.16×10^{-1}	2.70×10^{-1}
Moderate infection	2.44×10^{-3}	3.62×10^{-2}	1.70×10^{-1}	5.10×10^{-1}	1.19
Moderate-heavy infection	1.07×10^{-2}	1.60×10^{-1}	7.53×10^{-1}	2.24	5.24
Heavy infection	4.73×10^{-2}	6.98×10^{-1}	3.29	9.81	2.29×10^{-1}
	Fraction of the oyster's net productivity used by *P. marinus*				
Very light infection	8.84×10^{-5}	2.65×10^{-4}	5.44×10^{-4}	9.67×10^{-4}	1.58×10^{-3}
Light infection	2.40×10^{-4}	7.16×10^{-4}	1.47×10^{-3}	2.61×10^{-3}	4.27×10^{-3}
Light-moderate infection	1.06×10^{-3}	3.14×10^{-3}	6.45×10^{-3}	1.15×10^{-2}	1.88×10^{-2}
Moderate infection	4.62×10^{-3}	1.38×10^{-2}	2.84×10^{-2}	**5.03×10^{-2}**	**8.27×10^{-2}**
Moderate-heavy infection	2.04×10^{-2}	**6.08×10^{-2}**	**1.25×10^{-1}**	**2.22×10^{-1}**	**3.64×10^{-1}**
Heavy infection	**8.91×10^{-2}**	**2.66×10^{-1}**	**5.48×10^{-1}**	**9.69×10^{-1}**	**[1.60]**

Energetics calculations for *Perkinsus marinus*, assuming an average cell diameter of 6 or 10 μm. Data are listed in the following order: First row, oyster length (mm). Second row, oyster weight (g dry wt) (from White et al. 1988a). Third row, net productivity of unparasitized oysters (J hr^{-1}) from White et al. (1988a). Next 6 rows, the energy use (J hr^{-1} $oyster^{-1}$) at 20°C by a 6 μm diameter *P. marinus* cell at given infection intensities and oyster sizes. The number of hypnospores · g wet wt^{-1} $oyster^{-1}$ used for a heavy infection (2.3×10^6 hypnospores · g wet wt^{-1}) is one of many that might be chosen. Next 6 rows, the fraction of oyster net productivity ($A - R = NP = P_g + P_r$) used by *P. marinus* at various infection intensities and oyster sizes calculated as: *P. marinus* requirement (J hr^{-1} $oyster^{-1}$)/oyster net productivity (J hr^{-1} $oyster^{-1}$). Bold = cases where >5% of the oysters' scope for row is used by *P. marinus*. [ ] = cases where the oyster's energy budget is negative—no scope for growth. Final 12 rows, the case for a 10 μm diameter *P. marinus* cell.

observed heavy infections in oysters less than 15–30 days after initial infection. Assuming an oyster size of 2.3 g dry wt, 1 cell as the initial infective element, and no mortality, a heavy infection (e.g., 3.8×10^6 cells g dry wt^{-1}) would take about 22 generations or about 7 days. A reasonable mortality rate could easily double this period of time yielding Ray's (1954) observed results.

In Table 3, we compute the impact of *P. marinus* on oyster energetics using the number of hypnospores per gram for several sizes of oysters at varying infection intensities at 20°C. A Q_{10} of 2.0 (Laybourn-Parry 1987) could be imposed to estimate impact at higher or lower temperatures, assuming that respiration and growth are equivalently affected by changes in temperature. We assume, when computing the impact of *P. marinus* on the oyster's energy budget, that no cell division occurs in the thioglycollate media and that all cells enlarge, so that hypnospore number approximates actual cell number (Ray 1954, Mackin 1962, Perkins and Menzel 1966). Some large multinucleated plasmodia may give rise to more than one hypnospore, but in this case, the parent cell was also much larger than our typical 6–10 μm cell. Hence, estimated volume should be roughly equivalent. Whether or not all cells develop into hypnospores is not clear. In all likelihood, our energetics estimates represent minimal values.

For the oysters' energy budget, we use $A = R + NP$, where A is assimilation, and NP is net productivity: $NP = P_g + P_r$, growth plus reproduction. Table 3 indicates that, at heavy infections in large oysters, *P. marinus* consumes more energy than the oyster has available after meeting its own respiratory needs. That is, the oyster has a negative energy budget. Five percent or more of the energy otherwise available for growth and reproduction is consumed by *P. marinus* in most size classes of oysters with moderately heavy and heavy infections.

In Table 4, we review some of the previous literature on the effect of *P. marinus* on oyster growth and reproduction. In general, significant effects on growth have only been observed in moderately heavily to heavily infected oysters over long study periods where small daily effects can cumulatively be large. The same can be said for reproduction. To the extent a comparison is possible, our energetics estimates of the effect of *P. marinus* on oyster growth and reproduction agree with observation. Although a number of important pathologies are produced by *P. marinus* (Mackin 1951, Stein and Mackin 1955) and similar pathologies can affect the energy budget of other hosts (e.g., Mace and Davis 1972), the effect of *P. marinus* on oyster growth and reproduction at most infection levels can apparently be adequately estimated as a persistent drain on the oyster's available energy due to consumption by the parasite. Of course, any pathological condition might increase this effect, by reducing oyster assimilation for example (Gauthier and Soniat 1988). Hence, Table 3 probably represents a conservative estimate of the impact of *P. marinus*.

Table 3 also indicates that the effect of *P. marinus* at a given infection intensity on growth and reproduction can be expected to be smaller for smaller oysters. The reason is that respiration makes up a lesser fraction of the total energy budget in small oysters (White et al. 1988a), hence the impact of *P. marinus* on net productivity is lower because the initial fraction of energy remaining after respiratory demands are met is greater. Comparable literature data is unavailable, most research on age differences being concerned with the likelihood of infection and the progress of the disease (Andrews and Hewatt 1957, Mackin 1962).

Our calculations assume that oysters feed more or less ad libitum. Mounting evidence suggests food limitation is an important driving force in the oyster's energy budget during some periods of the year (Soniat and Ray 1985, Powell et al. 1987). Because *P. marinus* would not be concomitantly affected, the impact of this parasite might be substantially higher at certain times and population densities than estimated here.

TABLE 4.

Literature accounts of the effect of *P. marinus* on growth and reproduction.

Duration	Mean Infection Level	Effect	Citation
1 mo	4.32	none on growth	White et al. (1988a)
1 mo	4.00	none on growth	White et al. (1988a)
1 mo	1.6–2.2	none on growth	Wilson et al. (1988)
1+ yr	1	none on growth	Menzel & Hopkins (1955b)
1+ yr	5	decreased growth	Menzel & Hopkins (1955b)
5 mo	3	decreased growth	Ray et al. (1953)
5 mo	5	decreased growth	Ray et al. (1953)
1+ yr	0–1.1	none on condition index	Haven (1962)
1+ yr	5	decreased growth	Menzel & Hopkins (1955a)
4 mo	4–5	decreased reproduction	Mackin (1953)
4 mo	2–3	none on reproduction	Mackin (1953)

Tabulation of literature data on the effects of *Perkinsus marinus* infection on oyster growth and reproduction.

As a final caveat, Fig. 1 suggests that hypnospore number may be easily estimated from Mackin's scale. The regression equation was obtained from thioglycollate-incubated tissue examined individually to ensure a uniform infection intensity throughout. Our calculations assume the average infection level for an individual is known. Substantial variation between tissues and between replicate analyses within tissues actually exists in most oysters (Table 2). Replicate analyses would be required to adequately estimate infection intensity for energetics estimates as a function of hypnospore number from Mackin's scale (see also Ray 1966), and heavy infections would, of course, not be estimable at all. The presently described hypnospore separation method permits large tissues to be utilized and more precise estimates of cell number made. Finally, however, we also note that small pieces of tissue are difficult to process quantitatively and the method is time consuming. For simple estimates of prevalence and infection intensity, population monitoring as an example, our data suggest that the thioglycollate method used with Mackin's scale can provide an adequate quantitative estimate of *P. marinus* infection and that this estimate follows an exponential scale.

ACKNOWLEDGMENTS

We thank A. Turpak for collecting the oysters. Thanks to J. Wormuth for typing the manuscript. The research was funded by institutional grant NA85AA-D-FG128 to Texas A&M University by the National Sea Grant Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce to EP, DL and SR. We appreciate this support.

REFERENCES

Anderson, G. 1977. The effects of parasitism on energy flow through laboratory shrimp populations. *Mar. Biol. (Berl.)* 42:239–251.

Andrews, J. D. & W. G. Hewatt. 1957. Oyster mortality studies in Virginia. II. the fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecol. Monogr.* 27:1–25.

Barber, B. J., S. E. Ford & H. H. Haskin. 1988a. Effects of the parasite MXS (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition index and relative fecundity. *J. Shellfish Res.* 7:25–31.

Barber, B. J., S. E. Ford & H. H. Haskin. 1988b. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. II. Tissue biochemical composition. *Comp. Biochem. Physiol. A Comp. Physiol.* 91:603–608.

Blower, S. & J. Roughgarden. 1987. Population dynamics and parasitic castration: a mathematical model. *Am. Nat.* 129:730–754.

Calow, P. 1977. Conversion efficiencies in heterotrophic organisms. *Biol. Rev. Camb. Philos. Soc.* 52:385–409.

Collins, M. R. 1987. Macrohabitat effects on some copepod ectoparasites of striped mullet (*Mugil cephalus* L.). *Bull. Mar. Sci.* 40:376–382.

Craig, A., E. N. Powell, R. R. Fay & J. M. Brooks. 1989. Distribution of *Perkinsus marinus* in Gulf coast oyster populations. *Estuaries.* 12:82–91.

Gauthier, J. D. & T. M. Soniat. 1988. A parasitological survey of oysters (*Crassostrea virginica*) along the Louisiana coast. *J. Shellfish Res.* 7:204–205.

Hassell, M. P., R. C. Anderson, J. E. Cohen, B. Cvjetanović, A. P. Dobson, D. E. Gill, J. C. Holmes, R. M. May, T. McKeown, M. S. Pereira & D. A. J. Tyrrell. 1982. Impact of infectious diseases on host populations group report. In Anderson, R. M. & R. M. May (eds.) *Population Biology of Infectious Diseases,* Springer-Verlag, Berlin, pp. 15–35.

Haven, D. 1962. Seasonal cycle of condition index of oysters in the York and Rappahannock Rivers. *Proc. Natl. Shellfish. Assoc.* 51:42–66.

Hochmuth, R. C., J. L. Hellman, G. Dively & R. F. W. Schroder. 1987. Effect of the ectoparasitic mite *Coccipolipus epilachnae* (Acari: Podapolipidae) on feeding, fecundity, and longevity of soybean-fed adult Mexican bean beetles (Coleoptera: Coccinellidae) at different temperatures. *J. Econ. Entomol.* 80:612–616.

Hofstetter, R. P. 1977. Trends in population levels of the American oyster *Crassostrea virginica* Gmelin on public reefs in Galveston Bay, Texas. *Tex. Park. Wildl. Dept. Tech. Ser.* 24:1–90.

Holmes, J. C. 1982. Impact of infectious disease agents on the population growth and geographical distribution of animals. In Anderson, R. M. & R. M. May (eds.) *Population Biology of Infectious Diseases,* Springer-Verlag, Berlin, pp. 37–51.

Kabat, A. R. 1986. Effects of trematode parasitism on reproductive output of the bivalve *Transennella tantilla. Can. J. Zool.* 64:267–270.

Lanciani, C. A. 1986. Reduced survivorship in *Dasyhelea mutabilis* (Diptera: Ceratopogonidae) parasitized by the watermite *Tyrrellia circularis* (Acariformes: Limnesiidae). *J. Parasitol.* 72:613–614.

Laybourn-Parry, J. 1987. Protozoa. In Pandian, T. J. & F. J. Vernberg (eds.) *Animal Energetics,* Vol. 1, *Protozoa through Insecta.* Academic Press, Inc., New York, pp. 1–25.

Lewis, D. H., K. Choi & E. N. Powell. 1988. Technique for purifying *Perkinsus marinus* hypnospores. *J. Shellfish Res.* 7:204.

Mace, T. F. & C. C. Davis. 1972. Energetics of a host-parasite relationship as illustrated by the leech *Malmiana nuda,* and the shorthorn sculpin *Myoxocephalus scorpius. Oikos* 23:336–343.

Mackin, J. G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. *Bull. Mar. Sci. Gulf Caribb.* 1:72–87.

Mackin, J. G. 1953. Incidence of infection of oysters by *Dermocystidium* in the Barataria Bay area of Louisiana. *Conv. Add. Natl. Shellfish. Assoc.* 1951:22–35.

Mackin, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Mar. Sci. Univ. Tex.* 7:132–229.

Mackin, J. G. & J. L. Boswell. 1956. The life cycle and relationships of *Dermocystidium marinum. Proc. Natl. Shellfish. Assoc.* 46:112–115.

Mackin, J. G., H. M. Owen & A. Collier. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). *Science (Wash. D.C.)* 111:328–329.

Menzel, R. W. & S. H. Hopkins. 1955a. Effects of two parasites on the growth of oysters. *Proc. Natl. Shellfish. Assoc.* 45:184–186.

Menzel, R. W. & S. H. Hopkins. 1955b. The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus. J. Parasitol.* 41:333–342.

Nelson, S. G., M. A. Simmons & A. W. Knight. 1986. The energy burden of the bopyrid parasite *Argeia pauperata* (Crustacea, Isopoda) on the grass shrimp *Crangon franciscorum* (Crustacea, Crangonidae). *Comp. Biochem. Physiol. A Comp. Physiol.* 83:121–124.

Newell, R. I. E. 1985. Physiological effects of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) on the American oyster *Crassostrea virginica* (Gmelin). *J. Shellfish Res.* 5:91–95.

Perkins, F. O. & R. W. Menzel. 1966. Morphological and cultural studies of a motile stage in the life cycle of *Dermocystidium marinum. Proc. Natl. Shellfish. Assoc.* 56:23–30.

Powell, E. N., M. E. White, E. A. Wilson & S. M. Ray. 1987. Small-

scale spatial distribution of oysters (*Crassostrea virginica*) on oyster reefs. *Bull. Mar. Sci.* 41:835–855.

Quick, J. A., Jr. & J. G. Mackin. 1971. Oyster parasitism by *Labyrinthomyxa marina* in Florida. *Fl. Dept. Nat. Resour. Mar. Res. Lab. Prof. Pap. Ser.* 13:1–55.

Ray, S. M. 1952. A culture technique for the diagnosis of infection with *Dermocystidium marinum* Mackin, Owen and Collier in oysters. *Science (Wash. D.C.)* 116:360–361.

Ray, S. M. 1954. Biological studies of *Dermocystidium marinum* a fungus parasite of oysters. *Rice Inst. Pamph. Monogr. Biol.*, Special Issue, 114 p.

Ray, S. M. 1966. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proc. Natl. Shellfish. Assoc.* 54:55–69.

Ray, S., J. G. Mackin & J. L. Boswell. 1953. Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum*. *Bull. Mar. Sci. Gulf Caribb.* 3:6–33.

Reaka, M L. 1979. The effects of an ectoparasitic gastropod, *Caledoniella montrouzieri*, upon molting and reproduction of a stomatopod crustacean, *Gonodactylus viridis*. *Veliger* 21:251–254.

Skorping, A. 1985. Parasite-induced reduction in host survival and fecundity: the effect of the nematode *Elaphostrongylus rangiferi* on the snail intermediate host. *Parasitology* 91:555–562.

Stein, J. E. & J. G. Mackin. 1955. A study of the nature of pigment cells of oysters and the relation of their numbers to the fungus disease caused by *Dermocystidium marinum*. *Tex. J. Sci.* 7:422–429.

Soniat, T. M. & M. L. Koenig. 1982. The effects of parasitism by *Perkinsus marinus* on the free amino acid composition of *Crassostrea virginica* mantle tissue. *J. Shellfish Res.* 2:25–28.

Soniat, T. M. & M. L. Koenig. 1982. The effects of parasitism by *Perkinsus marinus* on the free amino acid composition of *Crassostrea virginica* mantle tissue. *J. Shellfish Res.* 2:25–28.

Soniat, T. M. & S. M. Ray. 1985. Relationships between possible available food and the composition, condition and reproductive state of oysters from Galveston Bay, Texas. *Contrib. Mar. Sci.* 28:109–121.

Walkey, M. & R. H. Meakins. 1970. An attempt to balance the energy budget of a host-parasite system. *J. Fish. Biol.* 2:361–372.

White, M. E., E. N. Powell & S. M. Ray. 1988a. Effect of parasitism by the pyramidellid gastropod *Boonea impressa* on the net productivity of oysters (*Crassostrea virginica*). *Estuarine Coastal Shelf Sci.* 26:359–377.

White, M. E., E. N. Powell, S. M. Ray & E. A Wilson. 1987. Host-to-host transmission of *Perkinsus marinus* in oyster (*Crassostrea virginica*) populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). *J. Shellfish Res.* 6:1–5.

White, M. E., E. N. Powell, S. M. Ray, E. A. Wilson & C. E. Zastrow. 1988b. Metabolic changes induced in oysters (*Crassostrea virginica*) by the parasitism of *Boonea impressa* (Gastropoda: Pyramidellidae). *Comp. Biochem. Physiol. A Comp. Physiol.* 90:279–290.

Wilson, E. A., E. N. Powell & S. M. Ray. 1988. The effect of the ectoparasitic pyramidellid snail, *Boonea impressa*, on the growth and health of oysters, *Crassostrea virginica*, under field conditions. *U.S. Fish. Wildl. Serv. Fish. Bull.* 86:553–566.

Zuk, M. 1987. The effects of gregarine parasites on longevity, weight loss, fecundity and developmental time in the field crickets *Gryllus veletis* and *G. pennsylvanicus*. *Ecol. Entomol.* 12:349–354.

Journal of Shellfish Research, Vol. 8, No. 1, 133–137, 1989.

PRESENCE OF *BONAMIA OSTREAE* AMONG POPULATIONS OF THE EUROPEAN FLAT OYSTER, *OSTREA EDULIS* LINNÉ, IN CALIFORNIA, USA

C. S. FRIEDMAN[1,2], T. MCDOWELL[2], J. M. GROFF[2], J. T. HOLLIBAUGH[3], D. MANZER[4] AND R. P. HEDRICK[2]*

[1]*Bodega Marine Laboratory*
P.O. Box 247
Bodega Bay, California 94923
[2]*Department of Medicine*
School of Veterinary Medicine
University of California
Davis, California 95616
[3]*Tiburon Center*
San Francisco State University
San Francisco, California 94920
[4]*California Department of Fish and Game*
Fish Disease Laboratory
2111 Nimbus Road
Rancho Cordova, California 95670

ABSTRACT European Flat oysters, *Ostrea edulis* Linné, reared in Tomales Bay and the Santa Barbara Channel, California were examined to determine the possible causes of elevated mortality among these stocks. The protozoan parasite, *Bonamia ostreae* was found to be the most significant parasite in these oysters. A rickettsiales-like and a gregarine-like parasite were seen within gill tissues of a flat oyster and one bay mussel, respectively. *Bonamia ostreae* elicited an intense inflammatory reaction in affected flat oysters and is believed to be the cause of the elevated mortality observed in these stocks.

KEY WORDS: European flat oysters, *Ostrea edulis*, protozoan parasite, *Bonamia ostreae*, ricketsiales-like, gregarine-like, bay mussel

INTRODUCTION

Few studies have been conducted to ascertain the status of diseases in shellfish grown along the west coast of North America despite the recent increased demand for shellfish. This, in combination with a decline in natural bivalve mollusc abundance, has encouraged more intensive culture of marine bivalves (Elston and Leibovitz 1980, Chew 1984). Katkansky et al. (1969) examined the feasibility of rearing the European flat oyster, *Ostrea edulis* Linné, in California waters. Flat oysters were planted in Tomales Bay, Drakes Estero, Morro Bay, and Elkhorn Slough in 1963. By 1969 *O. edulis* reared in the latter three sites had suffered stunted growth and up to 100% cumulative mortality. All gapers and up to 57% of the live oysters sampled were found, upon routine histological examination, to be infected with an intrahemocytic microcell. Only the Tomales Bay site appeared to be free of both the microcell and elevated mortality (Katkansky et al. 1969, Katkansky and Warner 1974). In a later study, Elston et al. (1986) surveyed seven locations in Washington state and two sites in California to determine the incidence of the microcell parasite, *Bonamia ostreae*, in flat oysters, *O. edulis*. Bonamiasis was detected in flat oysters cultured in the four Puget Sound, Washington state sites sampled but not among oysters from California. Historically, this disease is associated with up to 80% cumulative mortality within 6 mo after initial introduction of oysters to waters known to contain *B. ostreae* (Poder et al. 1982, Balouet et al. 1983, Elston et al. 1986). Although the two California locations sampled, both in Humbolt Bay were free of the disease, Elston et al. (1986) traced the source of the infected Washington stocks to initial outplanting stock from Elkhorn Slough, California. Katkansky et al. (1969) previously determined that *O. edulis* from Elkhorn Slough were infected with an intrahemocytic "microcell" that Elston et al. (1986) later concluded were *B. ostreae*, the same parasite found in the Washington state oysters.

In response to elevated mortality of flat oysters grown in California, we have conducted an extensive survey of *O. edulis* grown in Tomales Bay and the Santa Barbara channel, two of the principal culture sites in California. The purpose of this study was to identify and enumerate pathogens of flat oysters and to determine the potential impact of such parasites on the commercial oyster industry in California.

MATERIALS AND METHODS

Samples of between 30–56 *O. edulis* were collected from five culture facilities in Tomales Bay between May

*To whom correspondence should be directed.

16–June 16, 1986. Eighteen to 67 flat oysters were collected from the Santa Barbara channel site between May 21–July 28, 1986. In addition, samples of the following species were collected from three Tomales Bay culture sites during April, May and June 1986: bay (blue) mussels, *Mytilus edulis*, Olympia oysters, *O. lurida* and Pacific oysters, *Crassostrea gigas*. All animals were fixed in Davidson's solution (Shaw and Battle 1957) for 48 hr and transferred to 70% ethanol. A 4–5 mm wide section of each fixed oyster including heart tissue was removed ventral to the labial palps, and processed for routine histological examination. Paraffin sections (5 μm) were stained with hematoxylin and eosin and observed and photographed using an Olympus light microscope.

RESULTS

The mortality of certain oyster stocks listed in Table 1 had approached 80% in previous years as reported by the growers. Infected oysters collected in our survey did not show specific signs of disease but some were thin and watery, indicating their poor condition. Light microscopy of stained tissue sections showed massive infiltration of hemocytes into the vesicular connective tissue in certain oysters. These affected areas often surrounded the stomach, digestive diverticulae and to a lesser extent, the gonads (Fig. 1a). Hemocytic infiltration could also be detected within the gills of some individuals. Parasites which measured 2–3 μm in diameter with an eccentric nucleus appeared to be cytozoic within many of the hemocytes in areas of heavy infiltration. Up to 10 parasites could be observed within infected cells (Fig. 1b). *Bonamia ostreae* was found only in the European flat oyster, *O. edulis*. A rickettsiales-like intracellular parasite was observed within the gills of one brooding female *O. edulis* grown in Tomales Bay (TB-5) (Fig. 2a and 2b). There was no apparent host reaction to the intracellular parasite although many cells of the gill epithelium harbored developing bacteria within inclusions.

Gills of one bay mussel from the TB-2 site were infected with a gregarine-like parasite (Fig. 3). There were no parasites detected in any of the *O. lurida* examined during the course of this study.

DISCUSSION

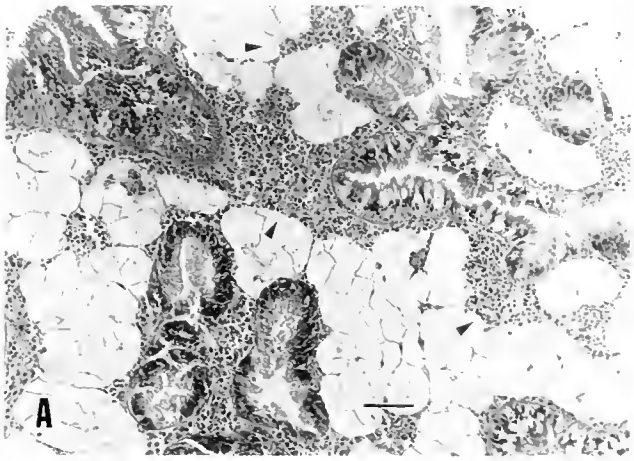
The most prevalent parasite affecting *O. edulis* reared in California coastal waters as determined by this survey was *B. ostreae*. Several species other than the flat oyster harbor intracellular parasites similar to *B. ostreae*. These include: *O. lurida*, the Sydney rock oyster, *Saccostrea commercialis* and *C. gigas* (Farley et al. 1988). In addition, a flat

TABLE 1.

Prevalence of *Bonamia ostreae* among populations of European flat oysters *Ostrea edulis* from Tomales Bay and the Santa Barbara Channel, California

Location	Date	Species	No. Positive / No. Examined	Percentage
TB-1	4-28-86	*M. edulis*	0/60	0
TB-2	5-16-86	*O. edulis*	4/42	9.5
	5-21-86	*O. edulis*	11/56	19.6
	5-30-86	*M. edulis*	0/30	0[1]
	5-30-86	*M. edulis*	0/30	0
	5-30-86	*M. edulis*	0/2	0
	6-16-86	*O. edulis*	1/30	3.3
	6-16-86	*O. edulis*	1/14	7.1
	6-16-86	*O. lurida*	0/8	0
TB-3	5-30-86	*O. edulis*	0/20	0
	6-2-86	*O. edulis*	0/30	0
TB-4	5-30-86	*O. edulis*	0/11	0
	5-30-86	*O. edulis*	0/26	0
	6-2-86	*O. edulis*	0/30	0
TB-5	6-1-86	*O. edulis*	0/41	0[2]
	6-2-86	*O. edulis*	0/24	0
TB-6	6-2-86	*O. edulis*	1/33	3.0
TB-7	6-3-86	*C. gigas*	0/30	0
SB-1	5-21-86	*O. edulis*	0/18	0
		O. edulis	1/12	8.3
		O. edulis	1/14	7.1
	7-28-86	*O. edulis*	2/60	3.0
	7-28-86	*O. edulis*	2/42	4.8
	7-28-86	*O. edulis*	0/67	0

[1] A single animal contained gregarine-like parasites within the gills.
[2] One animal contained rickettsiales-like parasites within the gills.



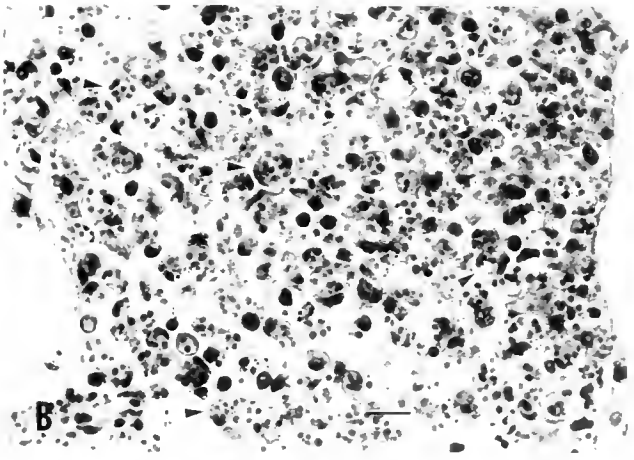
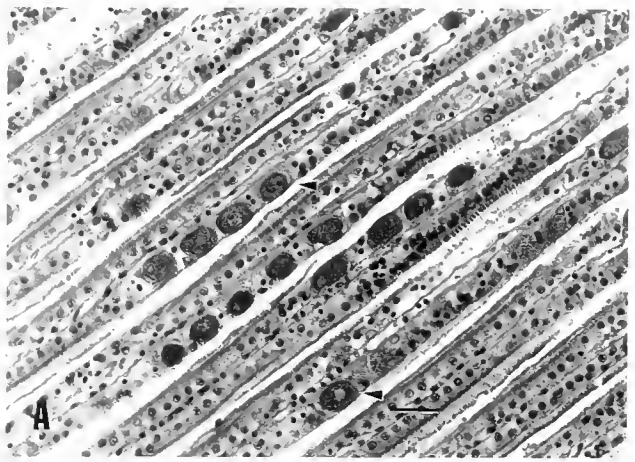


Figure 1. (a) Infiltration of granular haemocytes into the vesicular connective tissues surrounding the digestive diverticulae of a 2 yr old flat oyster (*O. edulis*) infected with *B. ostreae*. H and E stain, Bar = 40 μm. (b) Hemocytes of flat oyster (*O. edulis*) infected with *B. ostreae*. H and E stain, Bar = 10 μm.



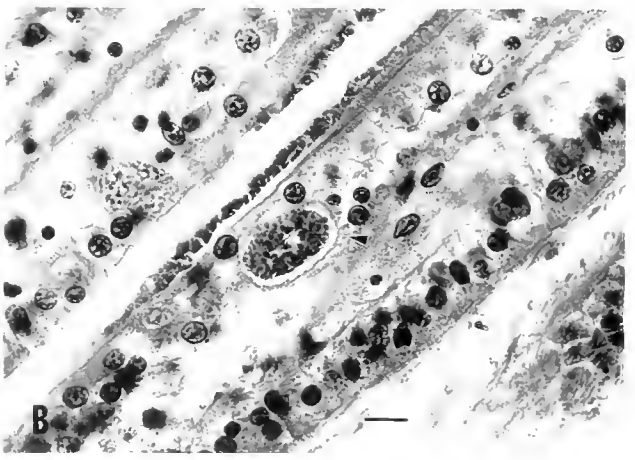


Figure 2. Gills of a female flat oyster (*O. edulis*) containing rickettsiales-like parasites (a) lower magnification (Bar = 30 μm) and (b) higher magnification (Bar = 10 μm). H and E stain.

oyster, *Ostrea lutaria* indigenous to New Zealand, reared in the U.K. in waters known to contain *B. ostreae* infected *O. edulis* have been shown susceptible to bonamiasis (Bucke and Hepper 1987). The diseases caused by "microcell" parasites in these hosts can range from acute to chronic (Farley et al. 1988).

Comparisons of the various "microcells" has been attempted in an effort to resolve their taxonomic relationships (Farley et al. 1988). A new genus (*Microkytos*) has been proposed which contains two of the species, *M. mackini* (g. n. sp. n.) and *M. roughleyi* (g. n. sp. n.), that are the causes of "Denman Island disease" of *C. gigas* and "Australian Winter disease" in *S. commercialis*, respectively (Farley et al. 1988). They can be distinguished from *B. ostreae* by morphological properties, host specificity and tissue tropism (Farley et al. 1988).

Of the various "microcells", *B. ostreae* has been studied in most detail and has been associated with significant losses of the European flat oysters in France (Pinchot et al. 1979, Poder et al. 1982, Balouet et al. 1983) and in Washington state (Elston et al. 1986) and California (Katkansky et al. 1969, Farley et al. 1988) in the United States. In our study, infestations with *B. ostreae* evoked an extensive inflammatory response absent in the few animals harboring rickettsiales-like or gregarine-like parasites. These observations, in conjunction with experimental results of other researchers in Europe and Washington state (Poder et al. 1982, Balouet et al. 1983, Elston et al. 1986), suggest that bonamiasis is a significant cause of *O. edulis* mortality and a hinderance to flat oyster culture in the state of California. In contrast to infection prevalences in other studies, the maximum 20% observed in oysters in our study is relatively low. Katkansky et al. (1969) recorded a higher prevalence of "microcell disease" in flat oysters grown in Morro Bay (29%), Drakes Estero (36%) and Elkhorn Slough (57%). The reason for the low prevalence of bonamiasis in our study compared to that reported by Katkansky et al. (1969) is unknown but may be due, in part, to differences in present culture methods and perhaps development

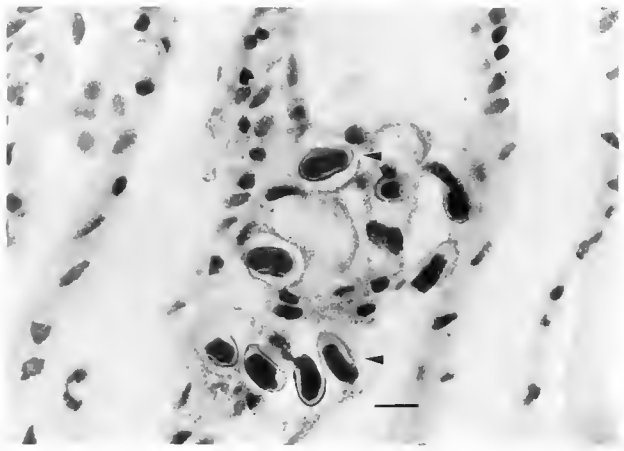


Figure 3. Gregarine-like parasites within the gills of a bay mussel (*M. edulis*). H and E stain, Bar = 10 μm.

of some resistance to the disease. Elston et al. (1987) has shown that certain stocks of European flat oysters are more resistant to bonamiasis than others. The existance of this resistance was demonstrated in experimental studies but the mechanisms involved are unknown. Although the disease can be transmitted directly from one oyster to another, further examination of bay-dwelling animals, including other molluscs, fishes, and worms, could reveal alternative host for *B. ostreae*.We did not detect evidence for such alternative hosts in the bivalve species present in the same waters with flat oysters examined in our study.

Attempts have been made to trace the source of the *B. ostreae* infections in the U.S. and France. Farley et al. (1988) observed "microcells" in *O. edulis* from Milford, Connecticut, the source for the California farms. Elston et al. (1986) further reconstructed the transplantation history of bonamia-infected flat oyster stocks from Elkhorn Slough, California to France, where bonamiasis was first reported in 1979 and subsequently devastated the *O. edulis* culture industry (Elston et al. 1986, Balouet et al. 1983, Poder et al. 1982). The movement of seed from the California brood stock was also believed to have been the source of the *O. edulis* in Washington state (Elston et al. 1986). Although "microcells" observed in *O. lurida*, a native west coast oyster, from Yaquina Bay, Oregon might suggest a west coast source of *B. ostreae* for *Ostrea* spp., these parasites resemble more closely *M. mackini* in their tissue specificity (leydig cells not hemocytes).

Inspections and certifications help but may not prevent the spread of oyster diseases. The oysters shipped to France and Washington state were examined prior to shipment but detection of the parasite in seed is difficult. Perhaps if the oysters had been reared for some period in quarantine and examined a second time the disease would have been detected before they were out planted. Certification combined with controlled rearing of potential imported species of fish and shellfish may be one method to reduce or prevent introductions of exotic diseases to new geographical regions.

In contrast to *B. ostreae*, there was little to no host response to the rickettsiales-like microorganisms in our study. The enlargement of infected host cells is similar to the responses of other bivalve molluscs, such as sea scallops, *Placopecten magellanicus* Gmelin (Gulka and Chang 1984a), Japanese littleneck clam, *Tapes japonica* and Japanese scallop, *Patinopecten yessoensis* (Elston 1986), to similar infestations. In contrast, Gulka and Chang (1984b) reported that blue mussels, *M. edulis*, from Rhode Island were infected with rickettsiales-like microbes which evoked encystment of the parasites. Gulka and Chang (1984a) and Elston (1986) noted that the potential effects of rickettsiales-like infections may not become apparent until the animals are held under stressful conditions, such as intensive culture and certain field environments.

Life stages of several gregarines (Phylum Apicomplexa, Class Sporozoea) have been documented in marine bivalves. Nematosial gregarines use marine pelecypods as intermediate hosts. Oocysts of *Nematopsis schneideri* have been found within the gills of *M. edulis*, *Spisula solida*, *Tapes pullastra*, *Cardium edule*, *Macoma balthica*, and others (Lauckner 1983). The pathogenicity of gregarine infestations in these bivalves and those in our study has not been determined. Parasites similar to the gregarine observed in *O. edulis* in our study have been observed within tissues of approximately 10% of *M. edulis* collected from Bodega Bay and from black abalone (*Haliotis cracheriodii*) and red abalone (*H. rufescens*) from the central and southern California coast (unpublished observations). These parasites were observed within many of the tissues of *M. edulis* and *Haliotis* spp. and may reach these tissues via the circulatory system. Examination of large numbers of gregarine-parasitized bivalves has revealed little or no inflammatory changes associated with these invaders. Further examination is needed to determine the effect of the rickettsiales-like and gregarine-like parasites on mussels and other bivalves to properly assess the significance of these microorganisms to the health of bivalve molluscs and the culture industry.

ACKNOWLEDGMENTS

We would like to thank K. D. Arkush, J. Hyde and D. Ogden for their assistance in sampling oysters. In addition we extend our gratitude to the oysters growers who donated oysters for this survey and to D. Cosgrove for typing this manuscript. Special thanks to the histotechnicians from the University of California, Veterinary Medicine, Teaching Hospital.

REFERENCES

Balouet, G., M. Poder & A. Cahour. 1983. Haemocytic parasitosis: Morphology and pathology of lesions in the French flat oyster, *Ostrea edulis*, L. *Aquaculture* 34:1–14.

Bucke, D. & B. Hepper. 1987. *Bonamia ostreae* infecting *Ostrea lurida* in the U.K. *European Association of Fish Pathologists* 7:79–80.

Chew, K. K. 1984. Recent Advances in the Culture of Molluscs in the Pacific United States and Canada. *Aquaculture* 36:69–81.

Elston, R. A. 1986. Occurrence of branchial rickettsiales-like infections in two bivalve molluscs, *Tapes japonica* and *Patinopecten yessoensis* with comments on their significance. *J. Fish Dis.* 9:69–71.

Elston, R. A., M. L. Kent & M. T. Wilkinson. 1987. Resistance of *Ostrea edulis* to *Bonamia ostreae* infection. *Aquacultue* 64:237–242.

Elston, R. A., C. A. Farley & M. L. Kent. 1986. Occurrence and significance of bonamiasis in European flat oysters, *Ostreae edulis* in North America. *Dis. Aquat. Org.* 2:49–54.

Elston, R. A. & L. Leibovitz. 1980. Pathogenesis of experimental vibriosis in larval American oysters, *Crassostrea virginica*. *Fish. Res. Board Can.* 37(5–8):964–978.

Farley, C. A., P. H. Wolf & R. A. Elston. 1988. A long-term study of "microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g. n.), and two new species, *Mikrocytos mackini* (sp. n.) and *Mikrocytos roughleyi* (sp. n.). *Fishery Bulletin* 86:581–593.

Gulka G & P. W. Chang. 1984a. Pathogenicity and infectivity of a rickettsia-like organism in the sea scallop, *Placopectin magellanicus*. *J. Fish Dis.* 8:309–318.

Gulka, G. & P. W. Chang. 1984b. Host response to rickettsial infection in blue mussel, *Mytilus edulis* L. *J. Fish Dis.* 8:319–323.

Katkansky, S C. & R. W. Warner. 1974. Pacific oyster diseases and mortality studies in California. Mar. Resources Technical Report No. 25, California Department of Fish and Game, Long Beach, pp. 1–51.

Katkansky, S. C., W. A. Dahlstrom & R. W. Warner. 1969. Observations on Survival and Growth of the European Flat Oyster, *Ostrea edulis*, in California. *California Department of Fish and Game* 55(1):69–74.

Lauckner, G. 1983. Diseases of mollusca: Bivalvia to Scaphopoda. In Kinne, O. *Diseases of Marine Animals*, Biologische Anstalt Helgoland Hamburg. Federal Republic of Germany, pp. 542–548.

Pinchot, Y., M. Comps, G. Tige, H. Grizel & M. A. Rabouin. 1979. Recherches sur *Bonamia ostreae* gen. n. sp. n., parasite nouveau de l'huitre plate *Ostrea edulis* L. *Rev. Trav. Inst. Peches Marit.* 43:131–140.

Poder, M., A Cohour & G. Balouet. 1982. Hemocytic parasitosis in European oyster *Ostrea edulis* L.: pathology and contamination. In Payne, C. C., & H. D. Burges (eds.) *Invertebrate Pathology and Microbial Control*, Proc. IIIrd International Colloquium on Invertebrate Pathology, XVth Ann. Meeting for the Society for Invertebrate Pathology, September 6–10, 1982, University of Sussex, Brighton, U.K., pp. 254–257.

Shaw, B. L. & I. Battle. 1957. The gross and microscopic characteristics of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 35:325–347.

Journal of Shellfish Research, Vol. 8, No. 1, 139–149, 1989.

COMMERCIAL PRACTICES AND FISHERY REGULATIONS: THE UNITED STATES NORTHWEST ATLANTIC SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791), FISHERY

JAMES E. KIRKLEY AND WILLIAM D. DUPAUL
Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062

ABSTRACT Fishery regulations are based on discipline specific concepts, empirical analyses, and public comment. Supporting empirical analyses, however, often neglect commercial fishing practices. If supporting analyses do not adequately consider commercial practices, resultant regulations may fail to achieve stated objectives and impose unnecessary costs on industry. This may have been the case for the United States Atlantic sea scallop, *Placopecten magellanicus*, fishery in which empirical analyses determined a target specification of 30 meats per pound, as a maximum average value, would provide significant long-term benefits in terms of yield per recruit and the overall productivity of the resource. Targeted meat count pertained to carefully resected scallops. Industry practices, however, yield different weights and meat counts. These differences suggest that realization of the 30 MPP target count does not require commercially landed scallops to yield, on average, 30 MPP. This paper discusses the relationship between the science, commercial practices, and the determination and enforcement of fishery regulations.

KEY WORDS: commercial practices, *Placopecten magellanicus*, fishery regulation

INTRODUCTION

The sea scallop, *Placopecten magellanicus*, fishery of the United States has been managed and regulated since 1982 (New England Fishery Management Council et al. 1982). The overall objective of the Fishery Management Plan (FMP) is the maximization of the joint social and economic benefits from the harvesting and use of the sea scallop resource. The operational tool for attaining the objective has been to control the size of landed scallops (Smolowitz and Serchuk 1987). Two regulations have been used to control the size of landed scallops. First, vessels which shuck scallops at sea and land scallop meats are subject to a maximum average meat count or number of meats per pound (MPP) restriction. Second, vessels that land scallops in the shell (shell stock) are subject to a minimum shell height restriction.

The current management standards are 30 MPP and a 3.5 in. minimum shell height. A meat is defined as the retained part of the scallop adductor muscle (52 FR 1462 January 14, 1987). Enforcement policy of the Northeast Regional Office of the National Marine Fisheries Service applies a 10% tolerance for prosecuting violations. In 1987, Amendment 2 was implemented by the New England Fisheries Management Council (NEFMC); it specified that the meat count standard would be adjusted upward by 10% from October–January to account for meat weight reductions associated with spawning activity.

Under the current management and enforcement program, a vessel which lands shucked meats is considered to have nonconforming scallops if the average meat count of a sample group exceeds 33 MPP from February through September (36.3 MPP from October–January). Vessels which land shell stock are considered to have nonconforming scallops if 10% or more of the scallops in ten shell stock samples (400 scallops) have shell height smaller than 3.5 in. (52 FR 1462 January 14, 1987).

Smolowitz and Serchuk (1987) indicated several problems with controlling the average meat size being landed. First, meat count and shell size standards do not always yield equivalent results relative to age at first capture. Thus, issues about equitability arise. Second, the meat count standard poses compliance problems because it is difficult to accurately determine meat counts at sea. Third, the observed practice of mixing meats of different sizes does not prevent the exploitation of young or immature scallops.

Naidu (1984, 1987) and DuPaul and Kirkley (1987) suggested additional problems not only with controlling the average meat size but also with inadequate consideration of commercial practices in the determination of the scallop regulations. First, commercial shucking results in a loss in meat yield. Second, varying proportions of scallop meats are landed without the catch component; thus, contributing to additional losses in weight. The biological basis for selecting meat count and shell size standards, however, was based on yield per recruit analyses which utilized data from carefully resected scallops. Performance and enforcement of regulations are based, however, on the sampling of landed meats without due consideration of the loss in yield (Naidu 1987).

There is another problem, however, which both exacerbates and mitigates the problems accompanying losses associated with shucking and landing meats without the catch component. At-sea handling and stowing procedures result in both gains and losses in weight of landed meats compared to freshly harvested and shucked scallops. In partic-

Contribution No. 1531 from the Virginia Institute of Marine Science.

ular, scallops meats stowed for ten or more days may experience losses in weight relative to when bagged; more recently stowed meats likely experience gains in weight (DuPaul and Kirkley 1988, Wilhelm and Jobe 1987).

In contrast, Caddy and Walters (1972) found that dockside counts for iced meats, the most common landed product form, were consistently lower than at-sea or on-deck counts for freshly shucked scallops after 14 days of stowage. Meats took up enough water to reduce their specific gravity and increase their volume. More important with respect to meat count regulations was that Caddy and Walters demonstrated that the on-deck count would be considerably lower on landing (e.g., 40 MPP at sea equaled dockside count of 34 MPP).

The FMP for Atlantic sea scallops suggested that a long-term biological analysis and an economic analysis indicated a target specification of 30 MPP, as a maximum average value, would provide significant long-term benefits in terms of yield-per-recruit. The yield-per-recruit analyses, though, were based on growth relationships that used carefully resected scallops. Shucking and the loss of the catch component result in higher commercial counts than those obtained from carefully resected meats; the fishery may be achieving better yield-per-recruit than landed-meat sampling indicates (Naidu 1987). However, on-deck or at-sea counts could be higher than dockside or landed counts because of on-board handling and stowing procedures; the fishery may be achieving poorer yield-per-recruit than indicated by landed-meat samples.

The management standard and enforcement policy both refer to retained meats. The 30 MPP target in the FMP appears to be in terms of carefully resected scallops. Retained meats are of varying product forms. There are various levels of shucking losses and landed meats with and without the catch component. Gains and losses in weight vary depending on length of time scallops meats are stowed and on-board handling procedures. Thus, the management standard and enforcement policy may be inconsistent with the 30 MPP target of the FMP and unnecessarily 'rigid' or too 'inflexible' with respect to inspection of commercially landed meats. Alternatively, the target specification of 30 MPP may be achieved by landing commercial scallop meats of counts different than 30 MPP.

In this study, differences in the meat weight and meat count of carefully resected and commercially landed meats are examined. Emphasis is placed on differences resulting from shucking, loss of the catch component, and at-sea handling and stowing procedures. It is argued that the losses from commercial practices not only have implications for the management and enforcement program but also for enhancing economic returns to the fishery. Results of the study are primarily confirmatory in nature (Caddy and Walters 1972, Naidu 1984, 1987, Wilhelm and Jobe 1987). However, they are noteworthy because they are based on recently obtained data for the mid-Atlantic scallop fleet.

MATERIALS AND METHODS

As part of a cooperative research program between industry, the National Marine Fisheries Service, the New England Fisheries Management Council, and the Virginia Institute of Marine Science, shell stock samples from mid-Atlantic commercial scallop vessels were regularly obtained between April 1987–May 1988. Samples varied from 1–3 three baskets of unculled scallops, and a basket contained 140–400 scallops. The time of day, area harvested, water depth, water surface temperature, length of tow, and catch of the last tow were recorded by the captain for each sample.

Data routinely obtained from each sample included shell height and adductor muscle weight. Adductor muscles were carefully resected and included the quick and catch component. These data were similar to those used to determine the shell height and meat weight relationship utilized in the yield-per-recruit analyses of the FMP. Additional data collected on 40–120 scallops per sample included the weights of the quick and catch components. These data were used to examine differences associated with the loss of the catch component, which will be discussed later in this paper.

During April and May 1987, weights and shell heights for 67 commercially shucked scallops were obtained and used to calculate commercial counts. Corresponding carefully resected weights were obtained by removing remaining adductor muscle tissue and adding its weight to the commercial weight. These two weights and counts were used in this study to examine losses in yield resulting from commercial shucking.

In contrast to the at-sea method of Naidu (1987), all commercial and corresponding resected weights were obtained from dockside shucking of sea scallops by the captain or crew. This approach posed several problems which limit the accuracy of estimated losses because of shucking. First, there was a tendency by the captain or crew to demonstrate superior shucking capability among peers. Second, dockside conditions permitted more careful shucking than possible at sea; thereby, resulting in higher recovery than normally would be obtained at sea. Third, the 67 scallops were obtained from only 3 vessels with experienced crews; thus, the sample is inadequate for making broad conclusions about shucking related losses by the commercial fleet.

Despite the limitations, the data are useful for obtaining a conservative estimate of losses associated with commercial shucking. Estimates of losses, however, must be viewed as minimum estimates. In practice, losses would be expected to be higher than the estimates of this study.

The data were used to estimate the relationship between meat count of commercially shucked scallops and the meat

count of carefully resected scallops. This permitted examination of possible losses in recovery associated with commercial shucking. In addition, a logit or binary dependent variable model was estimated and used to derive estimates of the probability that meat counts of commercially shucked scallops would exceed 33 MPP (the 30 count standard plus 10% tolerance) given corresponding counts of cleanly shucked or carefully resected scallops. The models were estimated by maximum likelihood procedures available on a micro-computer version of Statistical Software Tools (Dubin and Rivers 1986).

Examination of weight changes owing to at-sea handling and stowing procedures was based on information obtained from 3 commercial trips in August, September, and October 1987. One at-sea sample meat count was made by the vessel captain or first mate for 8–15 bags using a 1 lb. coffee can. Meats are typically packed and stowed in cloth bags. The captain was requested to carefully fill a coffee can until a plastic lid was flush with the top, take a count for 1 bag/day, and mark the bag. Three dockside counts using a coffee can were taken for each marked bag during off-loading. At-sea counts were calculated by dividing the count of the captain by 2.338 lb.; preliminary results of Smolowitz et al. (1988) suggest that a 1 lb. coffee can holds an average 2.338 pounds of meats. Dockside counts per pound were calculated by dividing the number of meats from 3 coffee can counts by the actual weight of the meats. Use of these counts requires the assumption that coffee can samples provide an accurate measure of the meat count/bag.

At-sea counts and weights were compared to the dockside counts and weights to determine possible differences resulting from at-sea handling and stowing procedures. These data were also used to estimate probabilities that dockside counts, conditional on the at-sea count and day of trip, exceeded, equalled, or were less than the at-sea count. Logit models for each of these cases were specified and estimated.

Differences in the weight and meat count between scallops with (muscle-on) and without (muscle-off) the catch component were determined by examining data from 100 trip samples obtained between April 1987–May 1988. Muscle-on and muscle-off weights were obtained for 40–120 scallops/sample for a total of 7,481 observations. Monthly counts by selected shell size intervals were calculated and used to examine the differences in the counts for muscle-on and muscle-off scallops. The meat counts (MPP) were calculated as follows:

$$MPP_i = N_i/weight_i$$

where N_i was the number of monthly observations for the ith shell size interval and weight was the total weight, in pounds, of all scallops in the ith interval.

In addition, shell size, adductor weight, and meat count equivalents were calculated for 1528 scallops for May 1988. These data were used to further examine differences between muscle-on and muscle-off counts. Five models were estimated and used to examine differences and to obtain more information about muscle-on and muscle-off counts.

The relationship between muscle-off (MOF) and muscle-on (MON) counts was estimated with a log-log model:

$$\log_e (MOF) = \alpha + \beta \log_e (MON) + u$$

where u is an error term assumed to be $N(O,\sigma^2)$. This model permitted estimation of muscle-off counts conditional on muscle-on counts.

Two transcendental models were used to examine the relationships between muscle-off and muscle-on counts and shell size. These models were used to estimate the meat counts for scallops of various shell sizes. The two models were of the following form:

$$\log_e (MON) = \alpha + \beta_{11} \log_e (SH) + \beta_{21} SH + u$$
$$\log_2 (MOF) = \alpha + \beta_{12} \log_e (SH) + \beta_{22} SH + u$$

where SH is shell height in mm.

Two logit models were specified and estimated to determine the conditional probabilities that muscle-off counts exceeded the 30 MPP standard and the 33 MPP enforcement statute. The two logit models were as follows:

$$ZMOF30 = \alpha + \beta\ MON$$

where ZMOF30 = 1 (0 otherwise) if MOF > 30 and MON < 30;

$$ZMOF33 = \alpha + \beta\ MON$$

where ZMOF33 = 1 (0 otherwise) if MOF > 33 and MON < 33. The two models were estimated by maximum likelihood procedures available in Statistical Software Tools.

RESULTS

Commercial Shucking

Data limitations prevented a rigorous analysis as done by Naidu (1987). Sixty-seven observations obtained from 3 vessels were believed to be inadequate for making broad based conclusions. Careful dockside shucking resulted in weight losses lower than would be obtained by fishermen at sea. Estimates of losses because of commercial shucking, thus, should be viewed as minimum average losses. Losses in weight between commercially shucked and carefully resected scallops, based on the limited sample, varied between zero and 17.6% (Table 1). The average loss per individual scallop was 5.9%.

In contrast to the results of Naidu (1987), muscle tissue recovery appeared to be higher for larger scallops. The average recovery for scallops larger than 105 mm was over

TABLE 1.

Weight loss and recovery of muscle between carefully resected and commercially shucked sea scallops from the mid-Atlantic region, April thru May 1987.

Shell Height (mm)	Weight loss			Average Recovery
	Minimum	Average	Maximum	
	Percent			
80–85	2.6	6.9	13.5	93.1
86–90	0.0	7.4	17.7	92.6
91–95	0.0	5.1	14.8	96.9
96–100	0.0	5.0	12.4	95.0
101–105	1.3	7.0	16.8	93.0
106–110	0.0	1.1	2.8	99.0
111–120	0.0	2.1	4.2	97.9

TABLE 2.

Estimated meats counts per pound (453.6 grams) for commercially shucked scallops conditional on meat counts for carefully resected scallops, April thru May 1987.

Meat Counts/lb	
Carefully Resected	Commercially Shucked
25.0	26.4
26.0	27.5
27.0	28.6
28.0	29.7
29.0	30.8
30.0	32.0
31.0	33.0
32.0	34.2
33.0	35.4

97%; the average recovery for scallops smaller than 106 mm was below 95%. These results should not be interpreted as contradictions to the results of Naidu in which lower recovery was observed for larger scallops. The sample size of Naidu was considerably larger and the shuckers and experimental conditions were different.

The relationship between the meat counts of commercially shucked (CS) and carefully resected scallops (CR) was estimated by ordinary least squares. The estimated equation and results were as follows:

$$CS = .86\ CR^{1.06} \qquad R^2 = .98$$
$$(2.60)(63.40)$$

where CS and CR are meat counts for commercially shucked and carefully resected scallops; numbers in parentheses are the t-statistics. Estimated meat counts for commercially shucked scallops conditional on carefully resected meat counts are presented in Table 2.

As indicated in Table 2, the average count of commercially shucked scallops was 6.4% higher than the count of carefully resected scallops yielding 25–33 MPP. Differences in the meat count estimated by the regression model increased as the meat count of carefully resected scallops increased; however, this may have been a result of the specified relationship between the two meat counts. Power functions such as the standard weight-length relationship or those in Naidu (1987) and this study tend to underestimate (overestimate) for values of regressors which are lower (higher) than the mean value of the regressor.

Parameter estimates and statistical results for the logit model were as follows:

$$CMPP = -214.6 + 6.9\ CR$$
$$(2.4)\quad (2.6)$$

where CMPP was assigned the value 1 (0 otherwise) if meat counts of commercially shucked scallops exceeded [illegible]. Estimation was accomplished by maximum likelihood procedures and data were limited to observations in which carefully resected counts were less than 33. Estimation of a similar model for 30 MPP was attempted, but convergence of the log-likelihood function could not be achieved. The 33 MPP model correctly predicted 94.4% of the observations.

Probabilities that commercially shucked meats given equivalent carefully resected counts are in Table 3. Results indicate that, on average, meat counts of commercially shucked scallops will be less than 33 MPP (30 MPP standard plus 10% tolerance) for equivalent carefully resected counts <31. The probability that commercial counts >33 MPP is quite high for carefully resected counts of ⩾31.

At-sea Handling and Stowing Procedures

Comparison of at-sea sample counts to dockside sample counts indicated the likelihood of differences (Table 4). The differences in the two counts may be partially due to sampling error or particular at-sea practices. Alternatively, one at-sea and 3 dockside counts may not provide an accurate estimate of the at-sea and dockside counts. These factors should be considered when reviewing the data in Table 4.

Examination of the data in Table 4 indicate a rather consistent time-dependent relationship between the at-sea and dockside counts. In general, the at-sea meat counts for scallops from the beginning of a trip appeared to be lower than the dockside counts; the at-sea counts for scallops harvested and stowed between the sixth and fifteenth day of a trip tended to be higher than the dockside counts.

Probabilities that the dockside count exceeded, equaled, or was less than the at-sea count were estimated by the cumulative logistic distribution function derived from the following logit models:

$$Y = 3.39 - 5.01\ MIX - .38\ DAY + .53\ DUMDAY$$
$$(2.54)\quad (2.40)\qquad (2.72)\qquad (2.54)$$

TABLE 3.

Estimated probabilities of commercially shucked meats exceeding 33 MPP conditional on meat counts of carefully resected scallops.

Carefully Resected Counts	Probability of Exceeding 33 MPP
25.0	0.0
26.0	0.0
27.0	0.0
28.0	0.0
29.0	0.0
30.0	0.0
31.0	.7
32.0	1.0

where Y = 1 (0 otherwise) if dockside count was higher than the at-sea count, MIX is a dummy variable assigned the value 1 (0 otherwise) if the sample consisted of meats of many sizes, DAY was the day of the trip, and DUMDAY equalled the product of the variables DAY and MIX; and

$$Y = \underset{(2.28)}{-3.62} + \underset{(2.89)}{3.84}\ MIX + \underset{(2.65)}{.23}\ DAY - \underset{(2.62)}{.34}\ DUMDAY$$

where Y = 1 (0 otherwise) if the at-sea count was within the mathematical range of observed dockside counts. The model for dockside count being less than at-sea count is equivalent to the model for dockside count exceeding at-sea count but with the signs of the parameters being reversed (− for +). Dummy variables were set to 1 only for the 3rd trip in which the 3 dockside counts and sizes of individual meats widely varied (e.g., 10–80 count meats). The models correctly predicted 76.6 and 76.5% of the observations, respectively.

The estimated cumulative logistic distribution function for the logit distribution was used to estimate the probabilities with minimum mixing of different sized meats (Table 5). Mixing of different sizes of meats is a common practice which has complicated enhancing yield per recruit. However, visual examination of the 3rd sample indicated extreme variability in the size of individual meats. This level of variation was not visually observed in the other two samples or previously observed landings. The probability that the dockside count exceeded (was lower than) the at-sea count was lower (higher) for scallops taken during the end of a trip. The probability that the two counts were equal increased for scallops taken during the latter part of a trip.

Loss of Catch Component

The percentage difference in meat count/month for carefully resected scallops with and without the catch component are presented in Table 6 for 5 arbitrary shell size intervals. Larger differences were observed for all size ranges in October and November 1987; smaller differences were observed for April, May, and August 1987. Temporal and size related variation did not appear to characterize the differences. A statistical examination of the differences, however, was not conducted since the differences would likely be biased because of spatial, temporal, and environmental

TABLE 4.

At-sea and dockside sample counts for three commercial trips in the mid-Atlantic region.

Day of Trip	Trip 1		Trip 2		Trip 3	
	At-Sea[1]	Dock.[2]	At-Sea	Dock.	At-Sea[3]	Dock.[3]
1	24.38	28.69	18.39	22.11	20.10	21.95
2	25.24	28.55	23.52	25.36	28.66	25.04
3	28.23	31.01	25.23	26.86	20.10	21.83
4	20.96	23.62	24.38	29.27	____[4]	____
5	26.52	29.71	29.94	26.69	19.25	22.19
6	27.41	24.78	____	____	____	____
7	27.80	26.23	____	____	____	____
8	25.66	24.63	____	____	____	____
9	25.24	28.98	24.80	24.53	____	____
10	29.51	31.74	26.95	23.70	25.66	28.51
11	21.81	20.89	24.38	23.86	30.80	25.07
12	26.09	25.50	33.36	27.75	____	____
13	21.39	20.09	____	____	____	____
14	27.80	24.63	24.81	24.32	25.66	23.87
15	29.94	28.93	29.08	22.95	20.10	24.86

[1] At-sea count taken for day of trip.

[2] Dock. is the dockside count taken at end of trip.

[3] The meats for trip 3 were extremely variable in size.

[4] ____ indicates no data available.

TABLE 5.

Estimated probabilities of dockside counts exceeding (>) equalling (=), or being less than (<) at-sea counts.[1]

Day of Trip	Estimated Probability		
	Dock. > At-Sea	Dock. = At-Sea	Dock. < At-Sea
1	.95	.03	.05
2	.93	.04	.07
3	.90	.05	.10
4	.85	.06	.15
5	.80	.08	.20
6	.73	.10	.27
7	.65	.12	.35
8	.56	.15	.44
9	.46	.18	.54
10	.37	.22	.63
11	.29	.26	.71
12	.21	.31	.79
13	.16	.36	.84
14	.11	.42	.89
15	.08	.48	.92

[1] Estimates based on minimum mixing of meat sizes; MIX and DUMDAY variables assigned value of zero. Dock. indicates dockside count.

differences (i.e., aggregation bias). It also should be remembered that the difference between 33–33.1 MPP is .3%; enforcement does not statistically test for differences.

There was a clear pattern of size related differences for May 1988; the reasons for this pattern are not known. Over the entire data set of 7,481 observations, the average count for scallops without the catch component was 9.8% higher than the count for scallops with the catch component. This percentage should be considered a maximum value when applied to evaluating commercial counts since meats are landed with and without the catch component.

The estimated double-log model relating muscle-off (MOF) count to muscle-on (MON) count in May 1988 was

$$\underset{(14.81)}{MOF} = \underset{(86.72)}{1.058\ MON^{1.01}} \qquad R^2 = .998$$

where numbers in parentheses are the t-statistics. An examination of the differences between conditional muscle-off counts and observed muscle-on counts indicated larger differences for higher count or smaller size scallops (Table 7). This was consistent with the observed differences in May 1988. This also, however, could be a result of the mathematical form of the model; estimated differences would increase for values of the regressor larger than the mean. Differences were not statistically examined, but it is doubtful that they would be statistically significant.

Estimates of the two transcendental models relating muscle-on and muscle-off counts to shell size (SH) were as follows:

$$\underset{(97.70)}{MON} = \underset{(80.98)}{\exp^{22.91}\ SH^{-4.46}}\ \underset{(35.28)}{\exp^{.0092SH}} \qquad R^2 = .85$$

$$\underset{(96.93)}{MOF} = \underset{(80.16)}{\exp^{23.21}\ SH^{-4.51}}\ \underset{(35.17)}{\exp^{.0094SH}} \qquad R^2 = .85$$

where numbers in parentheses are the t-statistics. Comparison of the estimated parameters indicated similarity between the coefficients. However, a likelihood-ratio-test of the equality of the parameters for the two models failed to accept the null hypothesis that all parameters were equal (chi-squared equaled 98.17).

Examination of expected muscle-on and muscle-off counts conditional on shell size also indicated larger differences for smaller size or higher count scallops (Table 8). The differences were not subjected to statistical validation. Moreover, estimated differences could be exaggerated be-

TABLE 6.

Percentage difference in counts for scallops with and without the catch component, April 1987–May 1988.[1]

Month	Percentage Difference in Mean Count for Selected Shell Size Intervals				
	<89	89–101	102–114	115–126	≥127
April	8.75	8.11	10.76	——[2]	——
May	9.52	10.02	9.60	9.66	——
June	9.14	9.33	9.17	8.67	9.12
August	9.23	8.33	8.37	11.90	8.34
September	10.23	9.68	10.50	9.74	8.97
October	10.56	10.10	10.01	10.37	9.75
November	12.04	10.09	9.66	10.08	10.30
December	9.58	9.43	8.90	9.27	9.71
January	9.78	9.59	9.39	9.88	9.22
February	9.36	9.75	10.31	9.65	9.39
March	9.83	9.46	9.32	9.55	9.80
April	9.81	8.91	9.15	9.06	8.90
May	10.45	9.53	8.89	8.76	8.68

[1] Muscle-off data not available July 1987.
[2] Muscle-off data not available for size range.

TABLE 7.

Estimated muscle-off counts conditional on observed muscle-on counts, May 1988.

Muscle-on Count	Estimated Muscle-off Count	Percentage Difference
25	27.4	9.4
26	28.5	9.5
27	29.6	9.6
28	30.7	9.6
29	31.8	9.6
30	32.9	9.7
31	34.0	9.7
32	35.1	9.8
33	36.2	9.8
34	37.3	9.8
35	38.5	9.9

cause of the functional form. In contrast to the previous models, the meat-count and shell-height model tends to overestimate (underestimate) differences for regressors with values smaller (larger) than the mean.

Estimated counts for muscle-on scallops between 80 and 100 mm, a size range frequently harvested by scallop dredges, were between 60.4–26.9 MPP. Estimated counts for muscle-off scallops of the same size were between 66.7–29.4 MPP. The difference ranged from 10.4% for 80 mm scallops to 9.5% for 100 mm scallops.

Estimates of the two logit models used to estimate the probability of muscle-off scallops exceeding 30 and 33 MPP given muscle-on counts were, respectively:

$$\begin{aligned} \text{ZMOF30} &= -125.3 \;\; +4.6\ \text{MON} \\ &\quad\;\; (5.8) \quad\; (5.8) \\ \text{ZMOF33} &= \;\; -61.0 \;+\; 2.0\ \text{MON} \\ &\quad\;\; (7.9) \quad\; (7.9) \end{aligned}$$

where ZMOF30 and ZMOF33 equal 1 (0 otherwise) if muscle-off counts exceed 30 and 33 MPP. The models correctly predicted 98.3 and 97.7% of the observations for May 1988. All parameters were statistically significant. Estimates of the probabilities are presented in Table 9.

As indicated in Table 9, the probability that muscle-off counts exceeded 30 MPP was quite high for muscle-on counts of 28 MPP; for muscle-on counts higher than 28 MPP, the probability that muscle-off counts exceeded 30 MPP was one. The probability that muscle-off counts exceeded 33 MPP given muscle-on counts <30 was quite low; for muscle-on counts >32, the probability that muscle-off counts exceeded 33 was one. The probability that muscle-off counts exceeded 33 given muscle-on counts of 30.5 and 31 was between .68–.85.

DISCUSSION

Naidu (1987, p. 136) suggested a possible inconsistency between meat weight data used for providing scientific advice and landed meat weights: "Also, the meat count regulations for the scallop fisheries are based on yield-per-recruit analyses, which utilize data from biologically-dissected meats. Fishery performance and enforcement, on the other hand, are based on the sampling of landed meats (i.e., the fishery is achieving better yield than landed meat sampling would indicate)." Naidu also suggested a potential bias in estimating age compositions using commercial meat weight data.

Yield-per-recruit analyses of the United States Northwest Atlantic scallop fishery were based, in part, on weight-length relationships which utilized data from carefully resected scallops. The target specification of 30 MPP in the FMP refers to carefully resected scallops. The management standard of 30 MPP and enforcement procedures, however, apply to landed meats regardless of product form. That is, there is no legal or allowable tolerance for meats which are poorly shucked or do not have a catch component. The 10% enforcement tolerance is applied to com-

TABLE 8.

Conditional estimates of muscle-on and muscle-off counts for selected shell sizes, May 1988.

Shell Size (mm)	Estimated Meat Counts Conditional on Shell Size		Percentage Difference
	Muscle-on	Muscle-off	
80	60.4	66.7	10.4
85	48.3	53.2	10.2
90	39.2	43.1	10.0
95	32.2	35.4	9.7
100	26.9	29.4	9.5
105	22.6	24.7	9.3
110	19.3	21.0	9.2

TABLE 9.

Conditional probabilities of muscle-off counts exceeding 30 and 33 MPP given muscle-on counts, May 1988.

Muscle-on Count	Probability of Exceeding 30 and 33 MPP	
	30 MPP	33 MPP
26.5	0.02	0.00
27.0	0.17	0.00
27.5	0.67	0.01
28.0	0.95	0.01
28.5	1.00	0.04
29.0	1.00	0.09
29.5	1.00	0.22
30.0	1.00	0.44
30.5	1.00	0.68
31.0	1.00	0.85
31.5	1.00	0.94
32.0	1.00	1.00

pensate for at-sea measuring difficulties (Smolowitz and Serchuk 1987). Differences between commercially shucked meats and carefully resected meats suggest an inconsistency between the meat weight data used in the biological analyses in support of the plan and the corresponding meats actually landed by fishermen and subject to enforcement. Alternatively, commercial counts different than the carefully resected target count of 30 MPP may be sufficient for realizing long-term benefits in terms of yield-per-recruit.

In the commercial sector, live scallops are landed on deck. The scallops are then culled by either visual observation or by the criteria that the size of the shell must be at least equal to the palm size of the hand of the individual culling the scallops. Crew members rapidly remove the meats from the shell with a shucking knife; speed and complete removal of the meat are primary concerns. Meats are shucked into small buckets. During a watch or work shift or at the end of a watch, the scallop meats are dumped into a large stainless steel bin, washed with sea water, sampled for meat count by using a coffee can or 1-pint cake frosting container, placed in cloth bags, chilled, and stowed with ice in the hold. At the end of a trip, which varies between 9–18 days, the bags of scallop meats are off-loaded at the dock.

Thus, commercial practices offer several sources through which landed weights and meat counts could vary from carefully resected weights and counts. Naidu (1987) demonstrated losses resulting from shucking; results presented in this study provide documentation of minimum losses resulting from shucking. Naidu (1984) also documented differences between muscle-on and muscle-off weights and counts; this study indicates there are substantial differences between muscle-on and muscle-off counts. Last, results of this study demonstrate that weight and meat counts may vary as a result of at-sea handling and stowing practices; that is, dockside meat counts may be higher or lower than the meat counts obtained during bagging of the scallop meats.

Commercial Shucking

Results in this study suggested that carefully resected scallops yielding 30 MPP in April and May 1987 yielded 32 MPP when commercially shucked. Using Naidu's (1987) results, scallops yielding ~30 MPP would, on average, yield 34 MPP when commercially shucked. Previous results in DuPaul and Kirkley (1987), however, obtained results identical to Naidu's when meat counts were estimated using weight-length relationships. The difference of 2 MPP, although small in value, is quite important in terms of the target specification and the management and enforcement program.

Naidu's results suggest that scallopers would be in violation of the 30 count standard plus 10% tolerance while satisfying the target specification. Results in this study indicate that scallopers would not be in violation while satisfying the target specification. However, both results suggest that the fishery may be achieving better yield-per-recruit than commercially landed meats would indicate. In addition, both results suggest that enforcement of the standard may be overly restrictive with respect to achieving the target specification of 30 MPP.

The loss in yield resulting from shucking also presents a serious economic problem. Data available from the National Marine Fisheries Service indicates that mid-Atlantic scallop dredge vessels between 51–150 gross registered tons landed 108,000 lb. of meat/vessel in 1987; the average revenue per vessel was $432,634. Average losses of 5–10% in weight because of shucking represents an economic loss between $22,770 and $48,070/vessel.

Unfortunately, the potential for increasing yield by alternative shucking procedures is limited by the necessity to shuck a large quantity of scallops over a short period of time. Hiring more experienced labor would increase the yield but may not be feasible because of possible increased cost. Shucking scallops more slowly might increase the yield but may not be feasible because of increased labor requirements. The economic ramifications of more carefully shucking scallops have not been examined. However, the most likely source of improved yields will be more experienced crews.

Differences in the two counts further raises the issue of whether or not the current regulations or commercial practices should be changed. Unfortunately, information necessary to address this issue is limited. Changing the current 10% tolerance would better reflect commercial practices but would also likely result in increased fishing mortality on smaller scallops. Increasing the culling size of scallops would reduce the likelihood of exceeding the 30 MPP standard but would be accompanied, at least in the short run, by reduced landings and revenues. The most likely change would be for industry to change commercial practices. More careful shucking results in substantial economic gain regardless of the type of regulation.

At-sea Handling and Stowing Procedures

At-sea handling and stowing procedures were found to result in both gains and losses. Washing, soaking, and stowing on ice tends to increase the weight and decrease the count. However, the length of time scallops are stowed also affects the weight and count. Weight loss and higher dockside counts were found to characterize scallop meats which were held in the hold between 10–15 days; weight gain and lower dockside counts tended to occur for scallops stowed <10 days.

Improvements in the at-sea handling and stowing procedures are possible. However, studies to determine improved procedures have only recently been initiated. Short-run options for improving the yield include packing bags of less weight and making shorter trips. Thus far, industry ap-

pears to have been receptive only to packing bags of less weight; the benefits of adopting other methods have not been determined.

There appears to be little practical basis for modifying the current regulation or enforcement procedures to account for differences resulting from at-sea handling and stowing procedures. First, mid-Atlantic vessels appear to take longer trips than vessels from other regions, and there is no rational basis, except increased efficiency and quality, to amend the regulation because one sector of the fleet chooses to take longer trips. Increased technical efficiency and improved quality should be pursued, but data necessary for determining the economic benefits are not available. Alternatively, restricting trips to no more than 10–12 days might improve the quality and landed weight of meats, but might result in more frequent trips and higher total annual operating costs. Second, it would be difficult to determine enforcement procedures compatible with weight gain and loss. Last, gains and losses appear to be variable and conditional on season, temperature abuse of the product, and the reproductive cycle. These problems appear to preclude amending the regulations or changing the enforcement procedures to reflect at-sea handling and stowing procedures.

Loss of Catch Component

Fishery researchers derived the 30 MPP target utilizing scientifically dissected muscle-on scallops (Serchuk et al. 1982). Enforcement and the management standard, though, are predicated on commercially landed scallop meats. Thus, the meat count standard and enforcement procedures are inconsistent with the 30 MPP target specification derived by researchers. Simply, fishery researchers determined that a target count of 30 MPP, based on carefully resected scallops with the catch component attached, would make a substantial contribution towards achievement of the objective of the FMP. In actuality, realization of the target count can be accomplished with different commercially landed scallop meat counts.

In the Code of Federal Regulations, Part 650, Atlantic Scallop Fishery, a scallop meat is defined to be the retained part of the scallop adductor muscle (Federal Register 1987). The meat count means the number of scallop meats required to make one pound. Given this definition, the meats to be sampled or inspected appear to be at the discretion of the inspecting officer. In practice, the enforcement officer usually considers any scallop meat which appears to have been cleanly cut regardless of whether or not the catch component is attached.

The enforcement agent takes one pound samples at random from the total amount of scallops in possession. The sample need not be taken dockside but this appears to be the preferred point of inspection. In addition, the person in possession of the scallops may request as many as ten samples be examined as a sample group. A sample group fails to comply with the standard if the averaged meat count for the entire sample group exceeds the standard.

Presently, this occurs if the count exceeds 30 MPP between February and September or 33 between October–January. If the count exceeds 33 MPP between February 1–September 30 or 36.3 MPP between October 1–January 31, the captain or vessel owner is subject to a citation, fine, and forfeiture of catch.

Results presented in the paper indicated that the loss of the catch component in more than 50% of the commercially landed and bagged meats could result in the inadvertent violation of the meat count standard. This would be particularly true if the catch component separated in the bag after at-sea counts were made. The average count for muscle-off scallops over all months and shell sizes was 9.79% higher than the corresponding muscle-on count. Thus, muscle-on scallops yielding counts of 30.06 MPP, which is in excess of the standard but well below the 10% tolerance, could yield muscle-off counts greater than 33 MPP.

Similarly in May 1988, the results indicated a higher probability of being in violation of the 30 MPP standard for muscle-on counts >29 MPP if the scallops were landed without the catch component (Table 10). A muscle-on count of 30.09 MPP yielded, on average, a muscle-off count of 33 MPP.

Improving the commercial yield or count, however, by landing muscle-on scallops appears to be limited. First, not all fisherman are cognizant of the potential losses evident by their discarding the catch component. Second, the loss of the catch component does not always result in reduced dockside weight since the detached catch components are occasionally bagged with the quick component. More important, a large volume of product must be processed over a short period of time; this severely restricts the landing of muscle-on scallops.

A final condition is the frequent at-sea mixing of recently shucked scallops with scallops meats which have already been bagged. Fishermen note the need to do this to stay within the 30 MPP standard. This appears to be a common practice and is believed to result in the separation of the catch and quick components of bagged meats. Quantitative research, however, does not appear to have determined the processing stage primarily responsible for the separation of the two components. Separation of the catch component has been observed during shucking, washing, and subsequent mixing of the meats (Personal observation, DuPaul and Kirkley 1988).

Amending the regulation and enforcement procedures to account for the loss of the catch component appears to be more practical than altering commercial practices. As shown in Tables 6–8, the percentage difference in muscle-on and muscle-off counts appear to be more stable and predictable than differences that are due to shucking and at-sea practices. Moreover, enforcement agents can determine whether or not a scallop meat contains a catch component.

TABLE 10.

Comparison of meat count restriction, meat count violation, and additional ten- and five-percent tolerances in the meat count standard.[1]

Date of Violation	Meat Count at Time of Violation		Meat Count Restriction with Additional Tolerance[3]	
	Legal[2]	Actual	Ten-percent	Five-percent
2/24/87	30.00	34.00	36.00	34.50
6/5/87	30.00	33.47	36.00	34.50
6/6/87	30.00	41.21	36.00	34.50
6/18/87	30.00	34.20	36.00	34.50
6/18/87	30.00	33.60	36.00	34.50
6/22/87	30.00	38.00	36.00	34.50
8/26/87	30.00	34.39	36.00	34.50
10/22/87[4]	30.00	37.90	39.60	37.95
10/23/87	30.00	33.30	39.60	37.95
10/29/87	30.00	33.90	39.60	37.95
11/15/87	30.00	35.60	39.60	37.95
11/20/87	33.00	43.80	39.60	37.95
11/20/87	33.00	40.28	39.60	37.95
11/20/87	33.00	40.30	39.60	37.95

[1] Trips in which violations occurred and penalties were assessed as reported in the January through July issues of Commercial fisheries News.

[2] Legal refers to the count permitted by the regulation. A violation occurs when the average meat count exceeds the legal count plus the 10% tolerance (i.e., 33 and 36.3 MPP). Actual is the count of the trip which resulted in a violation. Meat count with additional tolrance is based on 1988 amendment which increases the standard to 33 between October 1–January 31.

[3] Meat count restriction with additional tolerance equals meat count standard plus current 10% tolerance and additional tolerances of 5–10%.

[4] Seasonal adjustment plus 10% tolerance allowing 36.3 MPP before issuing a citation was in place between November 18 and January 31, 1987. Currently, the adjustment and 10% tolerance applies between October–January.

Based on the stable nature of the change and the ability of enforcement agents to determine the count, it would appear possible to amend the regulation to allow for the loss of the catch component.

The results of this study indicated an average difference of 9.8% between the counts of scallops with and without the catch component; an allowance of 5% to compensate for the loss of the catch component, thus, would not be unreasonable. An enforcement agent should be able to adjust the weight of muscle-off scallops by 5% to obtain the meat count for the scallops being inspected. This type of adjustment would made the regulation more consistent with commercial practices and help industry without increasing juvenile mortality. The 30 MPP standard and target specification would remain unchanged; only enforcement procedures would be changed.

Data on 14 violations that occurred in 1987 are presented in Table 10. It is not known whether or not the published violations included muscle-on or muscle-off scallops, but given that 50% or more of commercially landed scallops contain no catch component, it is likely that the counts included some muscle-off scallop meats. The data are used to illustrate how a change in enforcement policy in the form of increased tolerance to allow for the loss of the catch component might benefit the scallop industry, in terms of reduced violations, without compromising the target specification of 30 MPP.

Of the 14 violations in which penalties were assessed, 57.1% or 8 out of 14 may not have been in violation if the 30 MPP standard allowed a 10% tolerance for the loss of the catch component. The trip of October 22, 1987 may not have been in violation if the seasonal adjustment had been in effect during October 1987 and the standard reflected the loss of the catch component. If only a 5% tolerance had been added to the existing 10% tolerance and seasonal adjustment, 9 of the 14 violations presented in Table 10 may not have occurred.

Although results of this study suggest that it is possible to change enforcement procedures to better reflect commercial practices, a more important issue in need of attention is whether or not an average meat count regulation is fundamentally flawed. Naidu (1984) and Serchuk (1983, 1984) indicated that an average meat count regulation facilitates mixing of small and large meats; thus, the regulations do not have the desired effect of protecting young scallops and enhancing yield per recruit. Results of Shumway and Schick (1987) and DuPaul and Kirkley (1987) demonstrate considerable spatial and temporal variation in meat counts for given shell sizes. This creates an equitability issue in which vessels fishing different areas will have to harvest different size scallops in order to comply with a meat count standard. Alternatively, vessels will deplete those areas having large concentrations of scallops yielding 30 or less MPP.

The above mentioned problems and the results of this study indicate serious problems with the average meat

count regulation and its enforcement. The meat count regulation does not adequately protect young or immature scallops. It poses compliance problems for industry. The meat count regulation does not yield the same results as a minimum shell size relative to age at first capture. Enforcement does not provide adequate consideration of commercial practices. Concluding on a positive note, however, the New England Fisheries Management Council and industry are currently investigating alternative types of regulations which should mitigate the problems addressed in this study.

ACKNOWLEDGMENTS

The authors are indebted to the East Coast Fisheries Association for providing scallop samples and offering constructive criticism of our research. We are especially appreciative of the research support provided by Erling Berg, Brad Brauer, Denny O'Neal, Benny Rose, and William Wells. We would also like to thank Sandra Shumway and Fred Serchuk for reviewing an earlier version of this paper. The views expressed in this paper and any errors are the sole responsibility of the authors.

REFERENCES CITED

Caddy, J. F. & C. Radley-Walters. 1972. Estimating count per pound of scallop meats by volumetric measurement. Fisheries Research Board of Canada, Manuscript Report Series No. 1202. Biological Station, St. Andrews, N.B. 10 p.

Commercial Fisheries News. 1988. Enforcement report. Selected Issues (January, February, March, April, May, June, and July). Stonington, ME.

Dubin, J. A. & R. D. Rivers. 1986. Statistical software tools. Pasadena, CA.: Dubin/Rivers Research. 175 p.

DuPaul, W. D. & J. E. Kirkley. 1987. Progress report No. 2: Industry, NMFS, and VIMS joint-sponsored sea scallop research. Marine Resource Report #87-8. Virginia Institute of Marine Science, VIMS Marine Resource Report #87-8, 26 p.

DuPaul, W. D. & J. E. Kirkley. 1988. At-sea volumetric measures and monitoring meat-count regulations: the sea scallop fishery. Virginia Institute of Marine Science, VIMS Marine Resource Report #88-3, 18 p.

Federal Register. 1987. Code of Federal Regulations, Title 50, Sections 650.2 Definitions. 52 FR 1462, January 14, 1987, 13 p.

Naidu, K. S. 1984. An analysis of the scallop meat count regulation. *CAFSAC Res. Doc.* 84/73:1–18.

Naidu, K. S. 1987. Efficiency of meat recovery from iceland scallops (*Chlamys islandica*) and sea scallops (*Placopecten magellanicus*) in the Canadian offshore fishery. *J. Northw. Atl. Fish. Sci.* 7:131–136.

New England Fisheries Management Council In Consultation with Mid-Atlantic Fishery Management Council and South Atlantic Fishery Management Council. 1982. Fishery Management Plan, Final Environmental Impact Statement, Regulatory Impact Review for Atlantic Sea Scallops, (*Placopecten magellanicus*). Saugus, MA. 142 p.

New England Fisheries Management Council. 1987. Amendment #2 to the Fishery Management Plan for Atlantic Sea Scallops Incorporating an Environmental Assessment and Regulatory Impact Review/Initial Regulatory Flexibility Analysis. September. Saugus, MA. 16 p.

Serchuk, F. M. 1983. Seasonality in sea scallop shell height-meat weight relationships: Review and analysis of temporal and spatial variability and implications for management measures based on meat count. National Marine Fisheries Service, Woods Hole Lab. Ref. Doc. No. 83-85. 30 p.

Serchuk, F. M. 1984. Fishing patterns and management measures regulating size at capture in the Georges Bank sea scallop fishery: A brief historical review. National Marine Fisheries Service, Woods Hole Lab. Ref. Doc. No. 84-11. 10 p.

Serchuk, F. M., P. W. Wood & R. S. Rak. 1982. Review and assessment of the Georges Bank, Mid-Atlantic and Gulf of Maine Atlantic Sea Scallop (*Placopecten magellanicus*) resources. National Marine Fisheries Service (NMFS)/Woods Hole Ref. Doc. No. 82-06. 132 p.

Smolowitz, R. J. & F. M. Serchuk. 1987. Current technical concerns with sea scallop management, pp. 639–644. In Proceedings: The Oceans, An International Workplace. William MacNab and Son, 1172 p.

Smolowitz, R. J., F. M. Serchuk & R. Reidman. 1988. The use of a volumetric measure for determining sea scallop meat count. Unpublished MS. National Marine Fisheries Service, Gloucester Regional Office. Gloucester, Mass. 24 p.

Shumway, S. E. & D. F. Schick. 1987. Variability of growth, meat count and reproductive capacity in *Placopecten magellanicus:* Are current management policies sufficiently flexible? *ICES* C.M. 1987/K.2. 26 p.

Wilhelm, K. A. & B. Jobe. 1987. Weight change of scallop meats during fresh storage. Unpublished MS. National Marine Fisheries Service, Northeast Fisheries Center Gloucester Laboratory. Gloucester, Mass.

Journal of Shellfish Research, Vol. 8, No. 1, 151–172, 1989.

THE USE OF A VOLUMETRIC MEASURE FOR DETERMINING SEA SCALLOP MEAT COUNT

RONALD J. SMOLOWITZ,[1] FREDRIC M. SERCHUK[2] AND ROBERT J. REIDMAN[1]

[1]*Northeast Regional Office*
National Marine Fisheries Service
Gloucester, Massachusetts 01930
[2]*Northeast Fisheries Center*
National Marine Fisheries Service
Woods Hole, Massachusetts 02543

ABSTRACT Laboratory and field experiments were conducted to evaluate whether a volumetric measuring device could reliably provide accurate sea scallop meat count estimates at-sea. A one-pound coffee can was selected as the standard volumetric sampling measure. Calibration experiments revealed that coffee can volumes conform to manufacturing standards although slight differences in total volume exist among some brands. These differences, however, have a negligible effect on scallop meat count measurements. Average meat weight capacity of a coffee can filled with fresh-shucked meats is relatively constant and unaffected by the size mixture of meats within the can. Meat counts determined volumetrically appear to be highly reliable and as precise as counts obtained using weight-based procedures. Differences between at-sea and dockside counts reflect changes in meat condition that occur during handling and storage. A standardized volumetric sampling methodology is proposed along with guidelines for enabling at-sea determination of meat count relative to the current management standard. Assurance that the average meat count in a trip will conform to the management standard is highest when an effort is made to pack each and every bag as closely as possible to the management standard.

KEY WORDS: sea scallops, *Placopecten magellancius*, meat count, volumetric measure

INTRODUCTION

The Atlantic sea scallop, *Placopecten magellanicus*, is the most important molluscan shellfish harvested commercially in the United States. The species is sought for its large adductor muscle (the 'meat') that holds the two valves of the animal together. In 1987, USA landings of sea scallop meats totaled 13,200 metric tons [29.1 million lbs.] valued at $125 million in ex-vessel revenue (Serchuk and Wigley 1988).

Since May 1982, the USA sea scallop fishery has been regulated by the Fishery Management Plan for the Atlantic Sea Scallop Fishery [FMP] developed by the New England Fishery Management Council (one of eight regional fishery management councils established in 1976 by the Magnuson Fishery Conservation and Management Act which extended USA fisheries jurisdiction to 200 n. miles). The overall objective of the FMP is to "maximize over time the joint economic and social benefits from the harvesting and use of the sea scallop resource" (New England Fishery Management Council 1982). Controlling the size of scallops landed was selected as the preferred management strategy, and meat count (# of meats/lb) and shell height standards were enacted to govern the size of scallops permissible in the landings. Current regulations require that shucked scallop meats shall not average >30/lb, while scallops landed in the shell ['shellstock'] must be a minimum of 89 mm in shell height. The meat count measure does not preclude the landing of individual meats too small, by themselves, to meet the standard so long as the average count of the landed meats conforms to the standard. In fact, 'mixing' or 'blending' of small and large meats is a prevalent practice in the fishery. This has enabled harvesters to continue landing large quantities of small meats and still attain the average meat count standard (Serchuk 1983, 1984, Smolowitz and Serchuk 1987).

Concern has been raised by scallop fishermen that gauging meat counts of shucked scallops at sea is both difficult and inexact, even under optimal weather conditions. Many fishermen believe that, without expensive measuring devices to determine meat weights at sea, they cannot be assured that their at-sea meat counts, when checked dockside by enforcement officials using electronic scales, will comply with the legal standard (Smolowitz and Serchuk 1988).

Present dock-side enforcement procedures require that a minimum of 10 one-pound samples be taken to document a violation. The enforcement agent is not required to examine 10 bags [scallops shucked at-sea are normally landed packed in 40-pound bags], although this is a common practice. From each bag selected, a sample of whole meats is drawn using a container that holds more than 1 lb. of meat. For each sample, the number of meats per pound [MPP] is determined by weighing the sample on an electronic scale and dividing the sample weight (expressed in hundredths of lbs.) by the number of meats in the sample. The 'average meat count' for the trip is calculated as the arithmetic mean of the sample counts. A violation of the meat count regulation is issued only if the 'average meat count' exceeds the prevailing standard by >10% (i.e., >33 MPP for the 30 count standard). A penalty schedule, graduated in terms of meat count, is used in assessing the degree of non-compliance (Table 1). For example, if the average count is 35.0

TABLE I.

Penalty schedule for possession of non-conforming Atlantic sea scallops in the USA sea scallop fishery.

Violation (MPP)	Forfeiture (%)	Fine (000's $) 1st Viol.	2nd Viol.	3rd Viol.
30.1–33.0	0%	Verbal Warning		
33.1–34.0	1.0–10.0%	0–10	0–10	0–10
34.1–35.0	14.0–50.0%	0–10	5–15	15–25
35.1–36.0	55.0–100.0%	0–10	5–15	15–25*
36.1–39.0	100%	0–10	5–15	15–25**
>39.0	100%	5–10	10–15	15–25***

Forfeiture Percentages by Meat Count (to nearest 0.1 MPP)

Violation	Forfeiture	Violation	Forfeiture
33.1	1%	35.1	55%
33.2	2%	35.2	60%
33.3	3%	35.3	65%
33.4	4%	35.4	70%
33.5	5%	35.5	75%
33.6	6%	35.6	80%
33.7	7%	35.7	85%
33.8	8%	35.8	90%
33.9	9%	35.9	95%
34.0	10%	36.0	100%
34.1	14%	36.1	100%
34.2	18%	36.2	100%
34.3	22%	36.3	100%
34.4	26%	36.4	100%
34.5	30%	36.5	100%
34.6	34%	36.6	100%
34.7	38%	36.7	100%
34.8	42%	36.8	100%
34.9	46%	36.9	100%
35.0	50%	37.0	100%

* Plus 14 day permit sanction.
** Plus 30 day permit sanction.
*** Plus 60 day permit sanction.

MPP, 50% of the catch is forfeited and a fine of up to $10,000 may be levied.

In spite of seemingly severe penalties for slight infractions of the meat count measure, fishermen generally strive to land scallops as close to the enforcement criterion as possible (i.e., 33 MPP: 30 MPP + 10%). The economic incentive for landing scallops averaging 33 MPP (the enforcement criterion) vs. scallops averaging 30 MPP (the FMP standard) is large; many more scallops (far > + 10%) can be legally harvested at 33 than at 30 MPP. If for every 20-count scallop in the resource there exists a much larger supply of 40-count animals, mixing 100 20-count scallops with 200 40-count scallops will yield 10 lbs of meat averaging 30 MPP. At 33 MPP, 100 20-count scallops can be mixed with 371 40-count animals (86% more 40-count scallops) and yield 14.3 lbs of meat (43% more yield and revenue than at 30 MPP). The latter scenario requires that 40-count scallops be available for each 20-count scallop in the resource, an abundance pattern that often occurs in exploited scallop stocks.

Fishermen currently attempt to gauge meat counts at sea using volumetric procedures. Typically, this involves placing freshly-shucked scallop meats into an empty container (such as a frosting or coffee can), that, when full, is equated to meat count based on personal experience. This process is generally repeated throughout the trip to provide 'real-time' estimates of the meat count of the catch. Despite these efforts, fishermen often find that their at-sea counts differ from those made dockside by enforcement authorities. Moreover, fishermen have commented that these differences do not seem to be consistent or predictable. As such, many fishermen feel that they have been placed unfairly at risk with respect to potential violation of the meat count standard.

Previous studies evaluating volumetric methods of estimating scallop meat counts (Caddy and Radley-Walters 1972, Smolowitz and Serchuk 1987) found that volumetrically-derived counts differed by <10% with weighed meat counts. Comparison of at-sea volumetric counts with dockside counts based on weight (Smolowitz and Serchuk 1987) revealed an average difference of only 5%, with dockside MPP generally lower than at-sea MPP. Differences between at-sea and dockside counts were ascribed to changes in meat condition during storage in the vessel hold and to variations in filling the container when determining the volumetric count.

Although volumetric determination of meat count has been shown to be technically feasible, there is a lack of detailed information on whether a volumetric measuring device can reliably provide accurate meat counts at sea. Only limited data exist on the comparability of calibrated at-sea volumetric counts with meat counts obtained at dockside using electronic scales. In this report, results are provided from laboratory and field experiments conducted to test the hypothesis that a calibrated volumetric measuring device can be successfully used as an accurate alternative to a weight-based measure of meat count. The studies involved the selection, calibration, and field testing of a standard volumetric container to determine meat counts at-sea and dockside. This work was accomplished in partnership with the USA sea scallop industry as part of a Joint Industry-Government Sea Scallop Cooperative Research Program (Smolowitz and Serchuk 1987).

METHODS AND MATERIALS

Selection and Calibration of Volumetric Sampling Device

The choice of a "standard" volumetric device was predicated on 4 factors:

1. Availability of the measuring device to the fishing industry,
2. Expense in purchasing the device,
3. Simplicity and rapidity in using the device,
4. Uniformity of manufacturing specifications of the device.

The desideratum was a volumetric measure that was easily procured, widely available, inexpensive, simple to use, sturdily constructed, and fabricated to uniform specifications. All criteria were met by the one-pound container of coffee available in any supermarket (Fig. 1).

Coffee cans manufactured in the United States are constructed according to voluntary standards of the Can Manufacturers Institute. The one-pound cans are of 3-piece construction, nominal size of 4 1/16″ diameter × 5½″ height (103 × 140 mm), with a capacity of 1,014 ml (±20 ml). The cans, when sold with coffee, come with a plastic lid that can be pressed fit to one end after the can is opened.

To evaluate the uniformity (i.e., quality control) in volumetric capacity among coffee cans of different brands [conceivably manufactured at different facilities], an experiment was conducted in which 17 coffee cans, representing 5 different brands, were tested using distilled water (Table 2). Each can was tared on an electronic laboratory balance (accurate to 0.01 g), and water added to the empty can. The first 1000 ml was added using a 1000 ml graduated cylinder after which a 50 ml graduated cylinder [1 ml gradations] was used to fill the can. Once filled, the weight of the can was recorded. This process was performed twice with each can. During the first time, the can was filled to the point of having a concave meniscus; during the second time, the can was filled to a convex meniscus.

A second series of experiments were conducted to test the constancy of product weight of coffee cans filled with freshly shucked scallop meats. During May 1987–October 1988, 32 shell stock samples (live scallops in the shell) were obtained, on approximately a fortnightly basis, from commercial scallop vessels landing in New Bedford, Massachusetts. Each sample contained about a bushel of unculled scallops from the last tow of the trip. Samples were offloaded on the morning a vessel returned to port and immediately transported to the Woods Hole NMFS Laboratory for processing. In the laboratory, the scallops were shucked in a commercial manner and measurements taken on the shell height and adductor muscle weight [meat weight] of each individual scallop. Data were also recorded

Figure 1. Volumetric sampling devices used in determining sea scallop meat count.

TABLE 2.

Summary of coffee can volumetric calibration experiments.

		Concave Meniscus		Convex Meniscus		Mean Values		
Can #	Brand	Volume (ml)	Net Weight (g)	Volume (ml)	Net Weight (g)	Volume (ml)	Net Weight (g)	Vol./Wt. Ratio
1	Brim	1011	1007.8	1015	1011.5	1013	1009.63	1.0033
2	Chkfulnuts	1013	1010.0	1019	1013.2	1016	1011.60	1.0043
3	Folgers	1017	1013.9	1019	1016.5	1018	1015.20	1.0028
4	Folgers	1017	1015.0	1019	1014.7	1018	1014.85	1.0031
5	Folgers	1017	1017.0	1020	1016.1	1019	1016.55	1.0019
6	Folgers	1010	1007.1	1011	1009.0	1011	1008.08	1.0024
7	Hills	1022	1020.0	1025	1022.5	1024	1021.22	1.0022
8	Hills	1017	1016.5	1018	1020.2	1018	1018.38	0.9991
9	Hills	1013	1015.4	1024	1021.8	1019	1018.62	0.9999
10	Hills	1023	1019.3	1023	1020.0	1023	1019.68	1.0033
11	Hills	1023	1021.2	1025	1023.1	1024	1022.14	1.0018
12	Maxwell	1010	1008.0	1018	1014.7	1014	1011.33	1.0026
13	Maxwell	1015	1013.2	1014	1012.4	1015	1012.82	1.0017
14	Maxwell	1010	1008.6	1016	1014.3	1013	1011.49	1.0015
15	Maxwell	1007	1006.1	1015	1012.4	1011	1009.27	1.0017
16	Maxwell	1008	1006.6	1022	1011.6	1015	1009.10	1.0058
17	Maxwell	1009	1007.2	1016	1013.1	1013	1010.16	1.0023
	Average	1014.2	1012.5	1018.8	1015.7	1016.5	1014.1	1.0023
	Std Dev	5.1905	5.1690	4.0548	4.2971	4.1870	4.5787	0.00153
	Variance	26.941	26.719	16.441	18.465	17.531	20.964	2.33E-06
	Maximum	1023	1021	1025	1023	1024	1022	1.0058
	Minimum	1007	1006	1011	1009	1011	1008	0.9991

on gonad weight and sex for a concurrent study on seasonal variability in scallop meat weight and reproductive condition. After being weighed, the whole shucked meats were placed in a one-pound coffee can. The can was filled with meats until the plastic lid cover would no longer fit to the top of the can without bulging outward (i.e., the lid had to be flat when held at eye level). The number of scallop meats in every full can was recorded as was the total meat weight. Between 1–3 coffee can samples were taken from each bushel of scallops. From the 32 one-bushel samples, 64 individual coffee can weight measurements (comprising 5,291 scallop meats) were obtained.

Evaluation of At-Sea vs. Dockside Meat Count Measurements

Field testing and evaluation of the one-pound coffee can as a volumetric measuring device was conducted using commercial sea scallop vessels from New Bedford, Massachusetts and Hampton Roads, Virginia. At-sea volumetric sampling was performed by scallop fishermen on fourteen commercial trips made between June–December 1987 on 4 different vessels (Table 3).

Typically, the fishing vessels involved in the field work conducted their trips following normal commercial practices. Choice of fishing grounds, fishing procedures, and meat handling activities was left to the discretion of the Captain [as in the norm]. The crew was requested to take a volumetric sample of scallop meats during each day at sea. This entailed filling the one-pound coffee can with scallop meats taken from the washer at the end of one of the watches. The washer, a tank used for holding, mixing, and rinsing scallops prior to bagging, normally holds between 100–400 lbs. of scallop meats by the end of a 6-hour watch [only about 2.3 lbs of meat were generally needed for a volumetric sample]. The number of scallops in each sample was enumerated by the crew and recorded along with a sample number. Samples were consecutively numbered to correspond with day at sea (i.e., the sample taken during the first day was labeled 1, the sample taken during the second day was labeled 2, etc.). A tag with the sample number was placed on one of the 40-lb. linen bags of meats made up from the washer-load of scallops from which the volumetric sample was taken. After labelling, the tagged bag was stored in the vessel hold as in normal practice.

Upon the vessel's return to port, scientific personnel were present to conduct dockside sampling during offloading. From each of the tagged bags, between 1–3 volumetric samples were taken using the same coffee can sampling procedures as used by the crew at sea. In addition, the total tared meat weight of each sample was obtained with an electronic scale. From the fourteen commercial trips,

TABLE 3.

Summary of at-sea and dockside volumetric samples of sea scallop meats, by trip and sampling method. Number of samples are presented in parentheses ( ).

Trip Number	Vessel Name	Landing Date	Fishing Region	Depth (fms)	Average At-Sea Vol. MPP		Average Dock Vol. MPP		Average Dock Wgt. MPP	
					Can	SMD	Can	SMD	Can	SMD
1	Mary Anne	6-04-87	S. of Long Island	26	25.0 (10)	—	26.7 (10)	—	26.3 (10)	—
2	Mary Anne	6-21-87	S. of Long Island	27	27.1 (11)	—	26.0 (22)	—	25.9 (22)	—
3	Mary Anne	7-25-87	S. of Long Island	25	27.1 (7)	—	24.7 (14)	—	25.2 (14)	—
4	Mary Anne	8-11-87	S. of Long Island	24	26.9 (10)	—	24.6* (20)	—	27.4* (20)	—
5	Mary Anne	8-28-87	S. of Long Island	31	26.4 (8)	—	24.0* (16)	—	25.9* (16)	
6	Nordic Pride	9-12-87	Georges Bank	36	29.0 (11)	—	26.7* (22)	—	28.4* (22)	—
7	Mary Anne	9-14-87	Georges Bank	38	25.7 (10)	—	22.7* (20)	—	24.5* (20)	—
8	Carolina Breeze	9-18-87	Mid-Atlantic	30	25.6 (15)	—	26.1 (33)	—	25.9 (33)	—
9	Mary Anne	10-3-87	S. of Long Island	24	26.4 (10)	—	24.7* (20)	—	27.1* (20)	—
10	Carolina Breeze	10-13-87	Mid-Atlantic	30	24.8 (14)	—	24.3 (24)	—	24.2 (24)	—
11	Mary Anne	10-22-87	S. of Long Island	30	26.0 (10)	—	24.7 (20)	26.8 (20)	25.3 (20)	26.6 (20)
12	Mary Anne	11-09-87	S. of Long Island	25	—	24.5 (10)	—	24.4 (20)	—	24.7 (20)
13	Mary Anne	11-27-87	S. of Long Island	29	26.7 (9)	27.3 (9)	26.4 (18)	26.4 (18)	26.4 (18)	26.1 (18)
14	Mary Anne	12-14-87	S. of Long Island	30	30.0 (9)	28.4 (9)	29.0 (7)	29.6 (7)	28.7 (7)	30.1 (7)
	All Trips		Average		26.57	26.77	25.24	26.23	26.10	26.26
			Number of Samples		134	28	246	65	246	65
			Number of Scallops		8339	1706	14227	3881	14227	3881
			Std Dev of MPP Samples		2.738	2.240	2.827	2.979	2.926	3.099
			Variance of MPP Samples		7.498	5.018	7.994	8.872	8.561	9.602
			Max MPP Value of Sample		33.3	29.9	32.3	32.5	32.6	33.0
			Min MPP Value of Sample		18.4	22.4	18.3	18.5	18.3	18.1

* Coffee cans were underfilled.

134 at-sea coffee can volumetric samples (comprising 8,339 scallops) were taken. Dockside, 246 coffee can samples (comprising 14,227 scallops) were obtained from the tagged bags (Table 3). All sample data collected from the field study are summarized, by trip, in Appendix Table 1.

Standard protocol for filling the coffee can evolved during the experiment and was finalized as follows:

> Scallop meats were randomly picked up, a handful at a time, and counted into the coffee can. Only intact meats were used, with bits and pieces of meat discarded. No attempt was made to select meats which possessed both the "quick" and "catch" components of the adductor muscle since the small "catch" component (generally known as the 'sweet meat') is often removed during shucking or separated during washing and handling. Meats were added to the can until the can was slightly overfull. The plastic lid cover was then fitted to the top and pressed on. If the lid bulged out, meats were removed; if not, meats were added. The can was considered filled when, held at eye level with the lid on, no bulge was observed. The decision on whether a can was full was always a question of ±1 meat—with the last meat or two often not randomly chosen.

At sea, using freshly-shucked scallops, it was easier to fill the coffee container by dipping it into the washer, moving the meats into the can, and then fitting the lid as described above. The meats were then counted as they were removed from the can. This procedure could not be used dockside in sampling bags of meats since the coffee can could not be pushed into the packed mass of adhered meats

without causing product damage. Although concerted efforts were made to standardize the filling procedures used by both vessel crews at sea and by personnel dockside, considerable variation in filling practices occurred during the study.

Concern was raised during the developmental phase of this study that the one-pound coffee can might not be an acceptable measuring tool because it could easily be deformed. To address this potential shortcoming, a more rugged volumetric sampler was designed, the 'Scallop Measuring Device' (SMD), and constructed to specifications by the Baadar North American Corporation (Fig. 1). The SMD was fabricated out of stainless steel tubing with a wall thickness of 3 mm. The inside dimensions were 114 mm [height] by 107 mm [diameter], providng a volumetric capacity of 1000 ml. The top lid of the SMD was constructed of 2 mm thick stainless steel, perforated with 6 mm diameter holes, and could be positively seated inside the container at full 1000 ml capacity. Total weight of the SMD was 1854 g.

Field and dockside testing of the SMD was conducted during the last 4 trips of the study. Twenty-eight (28) at-sea samples (comprising 1,706 scallops) and 65 dockside samples (comprising 3,881 scallops) were obtained in the experiment.

Data Analysis

Parametric statistical procedures were used in all data analyses. Mean differences in volumetric capacity between filling methods (concave meniscus vs. convex meniscus) and among brands in the coffee can calibration experiments were evaluated by analysis of variance. Pairwise 'a posteriori' comparisons of mean volumetric capacity between brands of coffee cans was tested using the T′-method (Sokal and Rohlf 1981, p. 245) which employs the studentized augmented range distribution Q′ as a critical value. Determination of constancy of product weight of a filled coffee can of scallop meats was assessed from weight measurements of the 64 individual coffee can samples taken from the one-bushel shell stock samples processed in the laboratory. The 64 samples were considered as independent from one another since the purpose in collecting these data was to assess the aggregate physical properties of a mass of shucked meats, not to estimate any biological parameters of the scallop population as a whole. To evaluate whether the total meat weight of a full coffee can might be influenced by the mix of large and small scallops in a sample, least squares linear regressions were computed regressing:

1. Coffee can meat weight on number of meats per sample,
2. Coffee can meat count (MPP) on number of meats per sample.

Mixing effects were also assessed by regressing the standard deviation of average individual meat weight per sample on:

1. Average individual meat weight per sample,
2. Average meat count (MPP) per sample.

For each relationship, the regression coefficient (i.e., slope) was tested to determine if it differed significantly from zero. Comparability of meat count estimation methods [at-sea volumetric vs. dockside volumetric; at-sea volumetric vs. dockside weight; dockside volumetric vs. dockside weight], using both the coffee can and the SMD, was evaluated by pairwise t-tests of the average meat count values [MPP] obtained from each method.

RESULTS

Calibration of Coffee Can Volumetric Capacity

The average volume of the 17 coffee cans calibrated with distilled water was 1,016.5 ± 2.15 ml [95% confidence limits] (Table 2). Volumetric capacity of individual cans filled to a convex meniscus ranged between 1,011–1,025 ml, while the capacity of cans filled to a concave meniscus varied from 1,007–1,023 ml. As expected, mean convex meniscus volume (1,019 ml) was significantly greater ($P < 0.01$) than mean concave meniscus volume (1,014 ml). Ratios of coffee can volume to net coffee can weight approximated unity [1.0] for all of the cans tested, with little variation in ratios among cans (variance = 2.3×10^{-6}). Both the mean and individual volumetric capacities [using either the convex or concave meniscus filling procedures] of the coffee cans tested in the calibration experiments were well within the manufacturing guidelines (1,014 ± 20 ml) established by the Can Manufacturers Institute. The stability in coffee can volume per weight ratios suggests that any conclusions about volumetric capacity have equal validity with respect to weight.

Analysis of variance revealed that mean volumetric capacity differed significantly ($F = 6.11$, $P < 0.01$) among brands of coffee cans. Volumetric capacity was lowest for Brim (1,013 ml) and highest for Hills Brothers (1,021 ml) (Table 4). Pairwise comparisons between brands indicated no significant difference ($P > 0.05$) in volumetric capacity between Folgers and Hills Brothers coffee cans or between Folgers and Maxwell House cans, but a significant difference ($P < 0.05$) was detected between Hills Brothers and Maxwell House cans. No comparisons could be made with the Brim or Chock Full of Nuts cans since only one can of each brand was used in the experiments.

The significant difference in mean volume between the Hills Brothers and Maxwell House cans was 8.0 ml [0.78%] (Table 4). At 30 and 40 MPP, this corresponds to an average meat count difference between brands of 0.23 (0.78% × 30 MPP) and 0.31 MPP (0.78% × 40 MPP), respectively. For individual cans regardless of brand, the 95% confidence interval for volume is 1016.5 ± 9.2 ml which is ±0.90% (or ±0.27 MPP at 30 MPP).

Determination of Coffee Can Meat Weight Capacity

Based on the 64 coffee can samples of fresh shucked scallop meats processed in the laboratory, the average tared

TABLE 4.

Comparison of coffee can volumetric results, by brand.

Brand	Sample Size (cans)	Mean Volume (ml)	Variance Volume (ml)	95% Confidence Intervals for Mean Volume (ml)	
Brim	1	1013.00	—	—	
Chkfulnuts	1	1016.00	—	—	
Folgers	4	1016.25	14.7500	1010.14	1022.36
Hills	5	1021.30	9.3250	1017.15	1025.09
Maxwell	6	1013.33	2.1667	1011.78	1014.88
Total	17	1016.50	4.1870	1014.35	1018.65

Pairwise Comparisons Among Brands Using T'-Method

Brands Compared	Difference in Means		Test Statistic Q' [0.05]	Test Conclusion
	Absolute	Percent		
Folgers/Hills	5.05	−0.50%	5.57	NS [P > 0.05]
Folgers/Maxwell	2.92	+0.29%	5.57	NS [P > 0.05]
Hills/Maxwell	7.97	+0.78%	4.98	S [P < 0.05]

weight of a one-pound coffee can filled with meats was 1,062 g [2.342 lbs.] (Table 5). Meat weights of full cans ranged between 1,029–1,112 g [2.269–2.451 lbs.], and encompassed sample meat counts from 12.9–53.5 MPP.

The size mixture of scallop meats within a coffee can sample did not affect the average meat weight capacity of a full can. Regression analysis indicated no significant relationship between total coffee can meat weight and number of scallops [P = 0.07] (Table 6, Fig. 2A). Both mean weight per individual meat and sample meat count were significantly correlated (P < 0.001) with individual scallop meat weight variability (Table 6: regressions [d] and [e]) (Fig. 3) indicating that coffee can samples with low meat counts (i.e., high mean weight per meat) had a greater mixture of different-sized scallop meats than did samples with high meat counts.

The relationship between number of meats per full coffee can and coffee can meat count was highly significant [P < 0.001, r = 0.99] (Table 6, Fig. 2B) indicating that the number of meats in a filled can is an accurate predictor of meat count. Since the intercept of the regression was not statistically different from zero (P = 0.18, Table 6), it was possible to estimate meat count from number of meats per can using the calibration equation (Y = 0.427X) obtained by regression through the origin (Table 6: regression [c]).

Because the one-pound coffee can volumetric measure packed equally well regardless of meat size, volumetric sampling data obtained from the at-sea experiments (in which fresh shucked meat weight capacity was expressed as number of meats per coffee can) were converted to MPP by multiplying by 0.427. This facilitated comparison of at-sea volumetric data with data from both dockside volumetric sampling and dockside weight-based sampling.

The average meat weight capacity of a one-pound coffee can filled with scallop meats in dockside condition was significantly lower (P < 0.01) than the capacity of a one-pound can filled with fresh shucked meats (Table 7). Tared mean weight for a full can of dockside meats was 1,039 g (2.291 lbs.) ± 3.5 g (0.008 lbs.) [95% confidence interval], 23 g less [−2.2%] than a full can of fresh shucked scallops. At 30 MPP, the average difference between a volumetric sample of dockside meats and a volumetric sample of fresh shucked meats is −1.53 meats per sample (MPS).

Confidence limits on the meat weight capacity of coffee cans filled with meats in fresh shucked and dockside condition are presented in Table 7. For a single can of fresh product (i.e., shucked at sea), 95% of all cans will hold between 1,029–1,096 g of meat. When measured dockside, 95% of all coffee cans will hold between 997–1,082 g of scallops. One can be 95% certain that the average weight of a can filled with fresh shucked meats will be between 1,058–1,067 g, while the average weight of a coffee can filled with meats in dockside condition will fall between 1,036–1,043 g.

The scallop samples measured dockside in this study were shucked at sea from 1–16 days prior to vessel landing, packed in linen bags placed on ice, and were cold when unloaded in port. At dockside, the meats tended to be drier, stickier, and more rigid than when freshly shucked.

Comparison of At-Sea vs. Dockside Meat Count Measurements

Pairwise evaluation of average meat count values from 8 different sets of at-sea vs. dockside meat count estimation methods (Table 8) indicated no significant difference (P > 0.05) in average MPP between any of the comparisons except in 2 of the 16 tests of at-sea volumetric coffee can MPP vs. dockside volumetric coffee can MPP, and in 2 of the 26 tests of at-sea volumetric coffee can MPP vs. dock-

TABLE 5.

Summary of coffee can samples of freshly shucked scallop meats. Samples obtained from one-bushel shell stock samples collected by commercial scallop vessels landing in New Bedford, Massachusetts in 1987 and 1988.

Trip No.	Sample No.	Date of Landing	Full Can Total Meat Weight (g)	Number of Scallops	Average Weight Meat (g)	Variance Indiv. Meat Weight (g)	Maximum Indiv. Meat		Minimum Indiv. Meat		Average Meat Count (MPP)
							Weight (g)	Count (MPP)	Weight (g)	Count (MPP)	
1	1	5-16-87	1054.0	78	13.51	22.441	30.9	14.7	5.6	81.0	33.6
1	2	5-16-87	1050.0	79	13.29	24.925	33.4	13.6	4.9	92.6	34.4
1	3	5-16-87	1059.0	82	12.91	18.179	30.4	14.9	6.0	75.6	35.1
2	4	10-03-87	1064.4	54	19.71	44.454	29.6	15.3	5.6	81.0	23.0
2	5	10-03-87	1051.7	58	18.13	41.764	31.9	14.2	7.4	61.3	25.0
3	6	11-08-87	1067.5	92	11.60	20.828	24.5	18.5	6.1	74.4	39.1
3	7	11-08-87	1042.4	92	11.33	25.573	29.2	15.5	4.9	92.6	40.0
3	8	11-08-87	1060.6	87	12.19	25.238	30.3	15.0	6.6	68.7	37.2
4	9	11-19-87	1064.0	110	9.67	11.565	21.1	21.5	4.5	100.8	46.9
5	10	11-20-87	1055.6	85	12.42	23.363	31.9	14.2	5.4	84.0	36.5
5	11	11-20-87	1032.4	98	10.53	9.270	21.9	20.7	5.6	81.0	43.1
6	12	11-21-87	1059.7	116	9.14	10.969	25.4	17.9	2.7	168.0	49.7
7	13	11-21-87	1066.2	61	17.48	75.636	47.9	9.5	6.1	74.4	26.0
7	14	11-21-87	1055.7	76	13.89	30.208	35.9	12.6	6.8	66.7	32.7
8	15	11-21-87	1066.9	81	13.17	20.226	27.9	16.3	5.3	85.6	34.4
8	16	11-21-87	1049.9	84	12.50	16.400	30.3	15.0	6.4	70.9	36.3
9	17	11-24-87	1053.3	95	11.09	13.876	26.9	16.9	6.4	70.9	40.9
9	18	11-24-87	1044.9	94	11.12	10.036	31.7	14.3	5.8	78.2	40.8
10	19	11-25-87	1084.0	95	11.41	29.102	36.8	12.3	4.9	92.6	39.8
10	20	11-25-87	1038.8	99	10.49	15.678	21.5	21.1	3.9	116.3	43.2
11	21	11-25-87	1048.8	92	11.40	5.910	17.4	26.1	6.8	66.7	39.8
12	22	11-25-87	1053.5	110	9.58	16.463	27.4	16.6	5.3	85.6	47.4
13	23	11-29-87	1043.5	87	11.99	6.322	19.5	23.3	6.6	68.7	37.8
14	24	12-14-87	1074.2	100	10.74	15.461	23.3	19.5	4.8	94.5	42.2
15	25	1-13-88	1077.7	47	22.93	55.750	39.7	11.4	12.3	36.9	19.8
15	26	1-13-88	1057.7	47	22.50	54.289	38.7	11.7	8.0	56.7	20.2
16	27	2-02-88	1078.9	99	10.90	20.904	27.6	16.4	3.2	141.8	41.6
16	28	2-02-88	1073.4	105	10.22	15.064	20.8	21.8	2.8	162.0	44.4
17	29	2-19-88	1070.3	85	12.59	25.708	25.3	17.9	3.0	151.2	36.0
17	30	2-19-88	1069.8	95	11.26	21.657	29.5	15.4	3.8	119.4	40.3
17	31	2-19-88	1055.4	97	10.88	28.078	35.9	12.6	2.6	174.5	41.7
18	32	3-08-88	1067.4	117	9.12	13.810	21.5	21.1	1.8	252.0	49.7
18	33	3-08-88	1066.9	114	9.36	12.175	23.1	19.6	4.4	103.1	48.5
19	34	3-25-88	1084.9	90	12.05	12.801	22.2	20.4	3.8	119.4	37.6
19	35	3-25-88	1075.0	90	11.94	15.258	24.3	18.7	4.7	96.5	38.0
20	36	4-10-88	1073.7	66	16.27	27.495	29.6	15.3	6.9	65.7	27.9
20	37	4-10-88	1082.0	87	12.44	31.047	32.1	14.1	3.7	122.6	36.5
21	38	5-02-88	1081.7	99	10.93	11.637	28.5	15.9	5.9	76.9	41.5
21	39	5-02-88	1081.7	103	10.50	3.219	16.9	26.8	6.4	70.9	43.2
21	40	5-02-88	1082.1	96	11.27	17.256	32.7	13.9	6.5	69.8	40.2
22	41	5-10-88	1061.6	114	9.31	2.440	14.1	32.2	5.9	76.9	48.7
22	42	5-10-88	1068.6	126	8.48	2.900	15.2	29.8	4.0	113.4	53.5
23	43	5-16-88	1111.7	81	13.72	24.405	32.4	14.0	7.3	62.1	33.0
23	44	5-16-88	1078.7	78	13.83	35.176	39.6	11.5	1.4	324.0	32.8
24	45	6-18-88	1075.1	48	22.40	141.222	55.7	8.1	8.5	53.4	20.3
24	46	6-18-88	1048.1	63	16.64	64.516	61.6	7.4	7.2	63.0	27.3
25	47	6-30-88	1076.1	66	16.30	45.195	37.5	12.1	2.1	216.0	27.8
25	48	6-30-88	1077.7	95	11.34	34.150	26.0	17.4	1.6	283.5	40.0
26	49	7-04-88	1043.7	58	17.99	63.427	55.5	8.2	7.6	59.7	25.2
26	50	7-04-88	1049.9	54	19.44	124.954	57.7	7.9	7.9	57.4	23.3
26	51	7-04-88	1042.6	45	23.17	210.653	63.5	7.1	9.1	49.8	19.6
27	52	7-12-88	1066.9	94	11.35	7.492	21.4	21.2	6.0	75.6	40.0
27	53	7-12-88	1029.3	92	11.19	8.380	22.0	20.6	3.1	146.3	40.5
28	54	7-22-88	1047.4	59	17.75	56.162	52.3	8.7	9.0	50.4	25.6
28	55	7-22-88	1068.9	58	18.43	48.806	40.4	11.2	6.9	65.7	24.6
28	56	7-22-88	1048.3	59	17.77	80.144	59.4	7.6	3.9	116.3	25.5

TABLE 5.

Continued.

Trip No.	Sample No.	Date of Landing	Full Can Total Meat Weight (g)	Number of Scallops	Average Weight Meat (g)	Variance Indiv. Meat Weight (g)	Maximum Indiv. Meat		Minimum Indiv. Meat		Average Meat Count (MPP)
							Weight (g)	Count (MPP)	Weight (g)	Count (MPP)	
29	57	8-08-88	1031.1	70	14.73	31.454	48.6	9.3	8.4	54.0	30.8
29	58	8-08-88	1030.8	73	14.12	22.713	34.0	13.3	7.4	61.3	32.1
30	59	8-26-88	1051.1	30	35.04	166.797	59.4	7.6	12.1	37.5	12.9
30	60	8-26-88	1045.4	32	32.67	306.674	60.2	7.5	10.4	43.6	13.9
31	61	9-13-88	1073.2	66	16.26	41.680	39.4	11.5	8.6	52.7	27.9
31	62	9-13-88	1066.3	72	14.81	30.183	32.2	14.1	8.2	55.3	30.6
32	63	10-18-88	1101.7	98	11.24	37.885	28.9	15.7	4.2	108.0	40.3
32	64	10-18-88	1078.6	118	9.14	24.010	23.3	19.5	3.9	116.3	49.6
	Average		1062.38	82.67							
	No of Samples		64	64							
	Std Dev		16.7407	21.96							
	Variance		280.252	482.3							
	SE of Mean		2.093	2.745							
	Max Value		1111.7	126							
	Min Value		1029.3	30							

side weight coffee can MPP. Overall, the average meat count in dockside volumetric and weight samples was lower than that in samples taken at-sea by 0.63 [−2.1% at 30 MPP] and 0.46 MPP [−1.5% at 30 MPP], respectively. Pariwise comparisons of meat count between various dockside volumetric and weight measurement methods showed no significant differences ($P > 0.05$) in average MPP (Table 8).

To quantify the magnitude of the average difference in MPP between at-sea and dockside measurements that might normally be expected in the USA fishery, a subset of the volumetric sampling data was analyzed consisting of samples from trips lasting between 8–12 days and in which no daily samples were missing. The average at-sea meat count from these trips was 26.72 MPP, equivalent to an average weight per scallop meat of 16.98 g. When these same bags of meat were measured dockside both volumetrically and by weight, average meat counts of 25.71 (17.64 g per meat) and 25.88 MPP (17.53 g per meat) were obtained, respectively. Hence, the average meat count from volumetric samples taken at sea was 3.93% higher than the corresponding dockside volumetric count, and 3.25% higher than the dockside average based on weight. The latter difference represents the net effects of swelling and loss of sweet meats. The volumetric difference, in addition, reflects packing changes effected by variations in meat condition.

The Scallop Measuring Device (SMD) was calibrated using 65 dockside weight samples (Table 3) and had an average meat weight capacity of 1032.4 g (2.276 lbs.) ± 5.1 g [95% confidence interval]. Meat weight capacity of the SMD was not significantly different ($P > 0.05$) from the capacity of a one-pound coffee can filled with dockside meats. Where more than two sets of paired comparisons were able to be performed, no significant differences were detected in the average meat count obtained with the SMD and any of the other at-sea or dockside MPP measurement methods (Table 8).

Reliability of the One-Pound Coffee Can Volumetric Sampling Technique

In addition to testing the hypothesis that a calibrated volumetric measuring device can be used as an accurate alternative for a weight-based measure of meat count, data from the field samples were also used to evaluate the reliability of the sampling process itself. Replicate coffee can samples (from the same bag of meats) were taken in dockside sampling of 11 of the 14 commercial scallop trips. Excluding those dockside samples in which coffee cans were underfilled (from trips 4–7 and 9, see Tables 3 and 7), the data set allowed 10 independent tests of volumetric sampling reliability (Table 8). Fifteen tests of weight-based sampling reliability were performed using all of the replicate data (including the underfilled cans) since both weight and count were known in all cases (Table 8). In none of the tests (volumetric or weight) was a significant difference ($P < 0.05$) in MPP variance detected between replicate samples. For all volumetric samples, the average difference in meat count between replicates was 0.64 MPP, while the average difference in variance between the first and a repeat sample was 0.16 MPP. For weight-based samples, the differences were 0.37 and 0.50 MPP, respectively. Experiment-wide, within bag MPP variance was 2.54 MPP while between bag MPP variance was 7.36 MPP.

TABLE 6.

Regression analyses evaluating the influence of mixing of large and small scallop meats on the average meat weight of a full coffee can volumetric sample.

Parameter	Estimate	Standard Error	T Value	Probability Level
(A) Full Coffee Can Meat Weight (Y) on Number of Meats per Can (X)				
Intercept	1047.95	8.0563	130.08	0.000
Slope	0.1745	0.0942	1.85	0.069
R = 0.229; R-squared = 0.052				
(B) Full Coffee Can Meat Count [MPP] (Y) on Number of Meats per Can (X)				
Intercept	0.3736	0.2771	1.35	0.183
Slope	0.4222	0.0032	130.25	0.000
R = 0.998; R-squared = 0.996				
(C) Full Coffee Can Meat Count [MPP] (Y) on Number of Meats per Can (X) (Regression Through The Origin)				
Slope	0.4265	0.0008	512.84	0.000
R = 0.999; R-squared = 0.999				
(D) Standard Deviation of Weights of Meats in Full Coffee Can (Y) on Average Weight per Meat in Can (X)				
Intercept	−1.63	0.5020	−3.24	0.002
Slope	0.5088	0.0335	15.18	0.000
R = 0.888; R-squared = 0.789				
(E) Standard Deviation of Weights of Meats in Full Coffee Can (Y) on Meat Count (MPP) per Can (X)				
Intecept	14.77	0.8296	17.81	0.000
Slope	−0.2616	0.0228	−11.50	0.000
R = −0.825; R-squared = 0.681				

DISCUSSION

Field and laboratory tests indicate that a one-pound coffee can container can be used as a volumetric measuring device to accurately determine sea scallop meat counts at sea. Although statistically significant differences in volumetric capacity exist among various brands of coffee cans, these differences are small compared to other sources of variability when measuring meat count volumetrically. The resolution of the volumetric technique is roughly 0.5 scallop meats (e.g., the addition or removal of the last meat to obtain a full can divided by the meat weight capacity of a full can). This resolution is about twice as large as the most extreme differences in volume among individual coffee cans irrespective of brand. Average meat weight capacity of one-pound coffee cans filled with fresh-shucked meats is relatively constant and unaffected by mixing of different-sized scallop meats. Nonetheless, if the coffee can technique is adopted in measuring meat counts at sea, it is recommended that fishermen avoid reliance on a single coffee can. By rotating meat count measurements among 3 or more cans, fishermen can reduce the already tiny risk of obtaining a biased meat count which might result from slight differences in individual coffee can volumes. The two key factors in deriving accurate meat counts at sea using the coffee can method are:

1. Proper packing of the measuring device,
2. Taking an adequate number of samples during a trip.

The recommended procedure for taking a volumetric sample at sea is as follows:

1. At the end of the watch, just before bagging, thoroughly mix the meats in the washer.
2. Drain the washer. Then, fill the coffee can with whole meats [discarding bits and pieces], either manually or by carefully scooping with the can, so

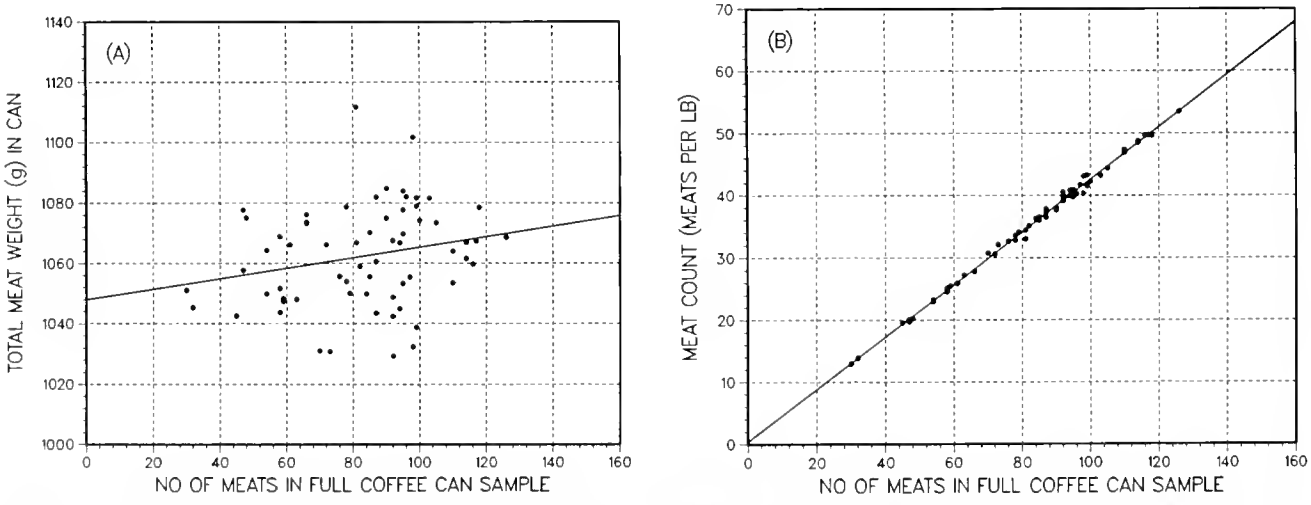


Figure 2. **(A) Regression of total weight of scallop meats in one-pound coffee can volumetric samples on number of meats per sample. (B) Regression of average meat count (MPP) in one-pound coffee can volumetric samples on number of meats per sample.**

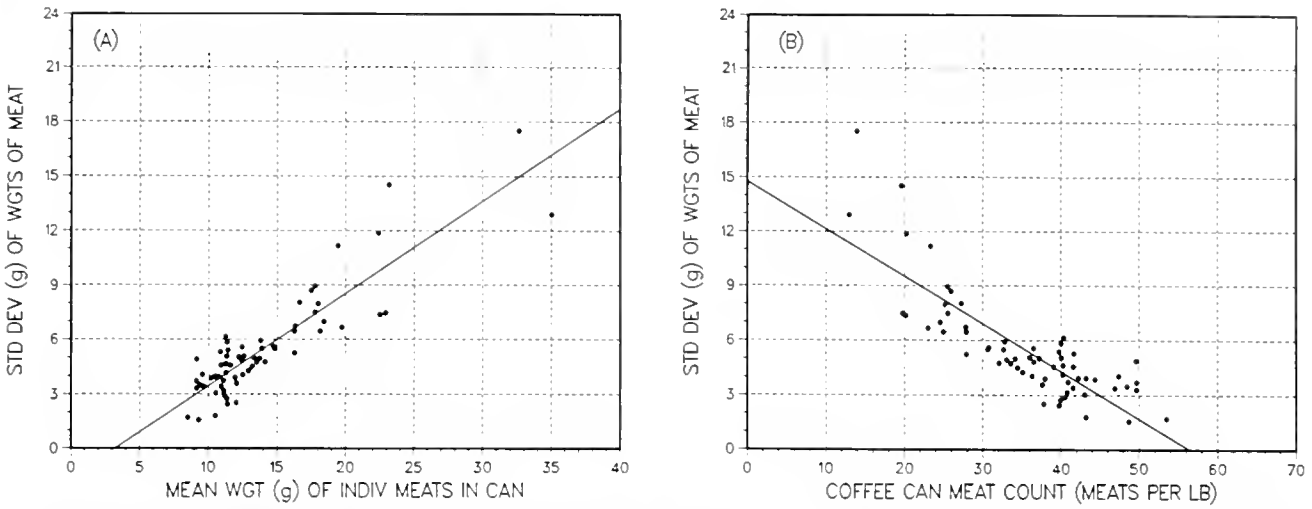


Figure 3. Regressions of standard deviation of weights of individual meats in one-pound coffee can volumetric samples on: (A) Average weight of individual meats in sample; and (B) Average meat count (MPP) per sample.

that the meats overflow the can. Press down on the scallops so a slightly mounded overfill is achieved.

3. Affix the plastic coffee can lid on the can and hold the can at eye level. The cover should bulge out. Remove the cover, eliminate one scallop meat, and repeat the process.
4. When the cover is flat at eye level, the can is properly filled.
5. Count the scallops in the can, and multiply the total by 0.427 to obtain the sample meat count (MPP) [or use Appendix Table 2 which equates the number of meats in a full coffee can to meat count].

The number of coffee can samples to be taken on a trip depends on the level of assurance that is desired in knowing the "true" average meat count for a trip. Based on the data acquired from the 134 at-sea volumetric coffee can samples taken during the field studies (Table 3), a chart of recommended sample sizes was developed to provide guidance for at-sea sampling using the one-pound coffee can technique (Table 9). Sample sizes were calculated using the largest meat count variance observed from a single trip during the study (20.85 MPP per coffee can, *Carolina Breeze*—Appendix Table 1). This approach is conservative in that the number of samples and size of meat count error

TABLE 7.

Summary statistics on meat weight capacity of one-pound coffee can volumetric samples filled with scallop meats in fresh shucked and dockside condition.

	Fresh Shucked Scallop Meats			Dockside Scallop Meats		
	Coffee Can Meat Weight		Meats Per[1] Sample	Coffee Can Meat Weight		Meats Per[1] Sample
Statistic	(g)	(lbs)	(MPS)	(g)	(lbs)	(MPS)
Average	1062.38	2.342	70.26	1039.20	2.291	68.73
Standard Deviation	16.741	0.037	1.11	21.541	0.047	1.42
No. of Samples	64	64		148*	148*	
For a Single Can:						
95% Conf Interval (+/−)	33.45	0.074	2.21	42.57	0.094	2.82
Lower Limit	1028.9	2.268	68.05	996.6	2.197	65.91
Upper Limit	1095.8	2.416	72.48	1081.8	2.385	71.55
For the Average of All Cans:						
95% Conf Interval (+/−)	4.18	0.009	0.28	3.50	0.008	0.23
Lower Limit	1058.2	2.333	69.99	1035.7	2.283	68.50
Upper Limit	1066.6	2.351	70.54	1042.7	2.299	68.96

[1] Assuming 30 meats per pound.

* Only 148 of 246 dockside samples (Table 3) were used. Dockside samples from trips 4–7 and trip 9 were excluded from the analysis since these coffee can samples were not completely filled with meats. In these samples, the plastic coffee can lid cover was not used in determining when a can was full of meats.

TABLE 8.

Comparison of differences in average meat count (MPP) among various at-sea and dockside meat count estimation methods. Comparisons of average differences in meat count variance among methods are also presented.

Measurement Method		Number of Paired Comparisons	Average Difference in MPP	Average Difference in Variance of MPP	No. of Paired Comparisons of Average MPP Significant at $P < 0.05$ (Paired T-tests)			No. of Paired Comparisons of Variance in MPP Significant at $P < 0.05$ (F-Tests)		
Method 1 vs. Method 2					#	%	Average Diff in MPP	#	%	Average Diff in Variance
Sea vs. Sea										
Sea Vol Can	Sea Vol SMD	2	0.76	−0.04	1	50	1.87	0		
Sea vs. Dock										
Sea Vol Can	Dock Vol Can	16	0.63	0.77	2	13	1.98	3	19	−1.47
Sea Vol Can	Dock Wgt Can	26	0.46	−1.51	2	8	1.97	11	42	−3.38
Sea Vol Can	Dock Vol SMD	5	0.21	−6.16	0			4	80	−6.45
Sea Vol Can	Dock Wgt SMD	5	−0.01	−7.18	0			4	80	−7.09
Sea Vol SMD	Dock Vol Can	3	0.17	−7.67	0			2	67	−8.94
Sea Vol SMD	Dock Wgt Can	3	0.21	−8.39	0			3	100	−8.39
Sea Vol SMD	Dock Vol SMD	5	0.29	−1.94	0			1	20	−6.58
Sea Vol SMD	Dock Wgt SMD	5	−0.08	−2.80	0			1	20	−9.13
Dock vs. Dock										
Dock Vol Can	Dock Vol Can Repl	10	−0.64	0.16	0			0		
Dock Vol Can	Dock Wgt Can	36	0.00	−0.10	0			0		
Dock Vol Can	Dock Vol SMD	9	−0.71	1.46	0			0		
Dock Vol Can	Dock Wgt SMD	9	−0.86	0.61	0			0		
Dock Wgt Can	Dock Wgt Can Repl	15	−0.37	−0.50	0			0		
Dock Wgt Can	Dock Wgt SMD	9	−0.61	1.58	0			0		
Dock Vol. SMD	Dock Wgt Can	9	0.46	−2.43	0			0		
Dock Vol SMD	Dock Vol SMD Repl	3	−0.52	−0.92	0			0		
Dock Vol SMD	Dock Wgt SMD	13	−0.27	−0.81	0			0		
Dock Wgt SMD	Dock Wgt SMD Repl	3	−0.59	−0.15	0			0		

Repl = Comparison of replicate samples.

is overestimated and the degree of confidence underestimated. On eleven of the 13 trips in which at-sea coffee can samples were taken in 1987, the trip meat count variance was less than 4.5 MPP. In this regard, the sample size chart reflects more extreme mixing of meats than would generally be expected in the fishery. This is analogous to designing a structure to withstand a 50-yr storm, an infrequent but predictable event.

The following example illustrates how the sample size chart might be used. If one wished to estimate, with 99.9% certainty, the true average meat count of a trip within 3 MPP, 28 samples would need to be taken randomly throughout a trip. If the average meat count determined from the 28 samples was 30.0 MPP, there is only 1 chance in 1000 that the average meat count for all scallops shucked during a trip will exceed 33.0 MPP (i.e., the present enforcement criterion). Only 9 samples would be needed, for the same meat count error (3 MPP), at the 95% confidence level. To estimate the true average trip count within a smaller meat count interval (i.e., <3 MPP), more intensive sampling is required; for example, to be 95% confident of the trip meat count within 1 MPP, 58 coffee can samples would have to be taken. Given the present USA enforcement tolerance of 10% in MPP, a scallop fisherman might take between 16–32 samples per trip to be 99% assured that the average meat count for a trip fell within the tolerance interval. More precision almost always requires more samples, but diminishing returns quickly ensue (i.e., reducing the meat count error from 3 to 2 MPP generally requires doubling the number of samples, while reducing the error from 2 to 1 MPP requires more than triple the number of samples).

The sample size table is only meaningful when an effort is made during a scallop trip to pack each and every bag to comply with the present 30 MPP management standard, not the 33.0 MPP enforcement criterion. Indeed if, after taking 796 samples on a trip the estimated meat count is 32.5 MPP, the probability of this count exceeding 33.0 MPP

TABLE 9.

Number of one-pound coffee can volumetric samples required to be taken during a trip to achieve a given degree of confidence that the average meat count for a trip will not exceed 30 meats per pound (30 MPP) by a given meat count error.

Meat Count Error (MPP)	Degree of Confidence				
	75.0	90.0	95.0	99.0	99.9
0.5	39	137	226	451	796
1.0	10	35	58	116	199
1.5	5	17	27	53	93
2.0	3	10	16	32	54
2.5	3	7	11	22	36
3.0	2	5	9	16	28
3.5	2	5	7	13	22
4.0	2	4	6	11	18
4.5	2	4	5	9	15
5.0	2	3	5	8	13

when sampled by the dockside enforcement procedure is about 50%, not 0.1% (i.e., the probability value listed in Table 9 for deviating from 30 MPP when the meat count error is 0.5 MPP and 796 samples are taken). This seeming discrepancy is a result of the difference in precision between the 796 samples taken at-sea and the 10 enforcement samples taken dockside. The enforcement procedure with its smaller sample size simply cannot determine the average meat count within 0.5 MPP.

The importance of packing each and every bag to the meat count standard can best be explained by example. Suppose that a fisherman could ascertain the meat count with 100% accuracy and packed each bag with 30 meat count scallops. Assuming no changes in meat condition and 100% accuracy in determining the meat count by dockside enforcement procedures, an enforcement check of 10 bags shoreside would yield a 30 MPP trip average. Now let us suppose that the fisherman packed half his bags with just 20 count scallops and the other half with just 40 count scallops and there were 200 bags total. Even though the true trip meat count average is 30 MPP, by only sampling 10 bags dockside there is a 17% probability (based on the binomial distribution) of enforcement officials obtaining an average meat count exceeding 33 MPP for the trip.

The sampling guide indicates that, by taking as few as 3–6 coffee can samples per day (essentially sampling each washer load of scallops to insure that the meat count of the washer complies with the management standard before the meats are bagged), fishermen at-sea can determine with high assurance whether their trip meat count will conform with the legal count when evaluated dockside by enforcement officials.

Precision of Dockside Enforcement Sampling

The within and between bag variances [2.54 and 7.36 MPP, respectively] obtained from the weight-based dockside samples can be used to assess the precision of dockside sampling procedures used by enforcement officials. After adjusting for the difference in sample weight between a full coffee can measured dockside (2.291 lbs.) and the one-pound samples taken during enforcement sampling, the within bag variance of enforcement samples was predicted to be 5.82 MPP with an expected standard error of the mean of 1.70 MPP. Analysis of actual enforcement sampling data* from 6 trips sampled between July–November 1986 [in which replicate samples were taken from each of the bags checked] gave results virtually identical to those expected; within-bag variance was 5.23 MPP with a standard error of the mean of 1.61 MPP. In contrast, the between bag variance from the enforcement samples was 33.36 MPP with a standard error of the mean of 1.83 MPP (determined for a sample size of 10 enforcement samples per trip).

Meat count determinations made either volumetrically, using coffee can samples, or by weight, using electronic scales, thus appear to be highly reliable and of about equal precision. In both methods, within sample variance in MPP is not a significant source of error in estimating the average meat count of a trip.

At-Sea vs. Dockside Meat Count Comparisons

As in previous studies (Caddy and Radley-Walters 1972, Smolowitz and Serchuk 1987), results from the present study indicate that meat counts of scallops measured at sea are usually higher than those obtained when the same batches of scallops are remeasured dockside. Water weight gain by the meats, through absorption of melted fresh water ice during storage in the hold of a vessel, has been identified as a principal factor producing this phenomenon. Caddy and Radley-Walters (1972) reported that the average uptake of water by meats held on ice between 9–14 days was 17%. As a result, scallop meats that measured 40 MPP as fresh-shucked product would measure 34 MPP shoreside. Laboratory experiments on weight changes in scallop meats during fresh storage (Wilhelm and Jobe unpublished MS) revealed that meats stored on ice absorbed water for the first 6 days, reaching a maximum weight gain of 12%. From the seventh day onward, however, meats began losing weight and continued to do so until the study was terminated after 15 days of storage. At that time, the average weight of a meat was 7.5% lower than at the beginning of the experiment. By comparison, scallop meats soaked in fresh water for 3 days showed an average weight gain of 37% but lost weight after soaking and were below their original weight by the eighth day of storage.

Virtually all scallops shucked at sea are stored on ice to preserve product quality. For a scallop trip of 10 fishing days (i.e., about the average trip length in the USA fishery)

*Confidential data from enforcement actions.

assuming equal daily catches, the difference between meat counts taken at sea and those taken dockside would be ~7% based on the laboratory results. An at-sea meat count of 30 MPP would measure 28.0 MPP dockside, while a 35.3 MPP average at sea would measure 33.0 MPP at landing.

Loss of 'sweet meats' during packing and bagging of scallops at sea may explain the apparent disparity in percentage meat weight gain (at-sea vs. dockside) observed in the field samples (3.25–3.93%) vs. that noted in the laboratory. Naidu (1984) found that the 'sweet meat' component of the adductor muscle accounted for 7–9% of the total adductor muscle weight, and that about half (52%) of the scallop meats landed in the St. Pierre [Newfoundland] sea scallop fishery lacked the 'sweet meat'. Separation of the 'sweet meat' can occur during shucking and handling, but more frequently, the 'sweet meat' becomes detached when the scallops were washed prior to bagging (Naidu 1987). If most of the loss of 'sweet meats' occurs during washing, the percentage difference in MPP between at-sea vs. dockside counts will depend on whether the at-sea count was taken before or after the meats were washed. At-sea meat counts determined from volumetric samples taken before washing (with 'sweet meats' intact) will be lower than sample meat counts taken after washing (reflecting some loss of 'sweet meats' and assuming that detached 'sweet meats' are not included in the volumetric sample). The laboratory estimates of the weight gained by meats due to water absorption during storage were based on intact adductor muscles. Hence, laboratory and field results of percentage meat weight gain are only strictly comparable for at-sea samples taken after washing since any reductions in adductor muscle weight from 'sweet meat' loss in at-sea samples taken before washing are not accounted for in the laboratory evaluations.

This issue can be examined from another perspective. If the specific gravity of a fresh-shucked scallop meat (1.064 from Caddy and Radley-Walters 1972) is multiplied by the average weight of distilled water held by a one-pound coffee can (1014.1 g—Table 2), the expected total product weight of a full can of scallop meats would be 1,079 g. The average weight of a full can of fresh-shucked meats obtained from the shellstock samples provided by commercial fishermen in the present study was 1,062 g (Table 7), a 17 g difference or 1.6%. This difference represents imperfect packing of meats (i.e., interstitial spaces between individual meats). The 0.7% disparity between the dockside volumetric and dockside weight sampling estimates of MPP relative to at-sea MPP determinations (+3.93% volumetric vs. +3.25% weight) is thus likely to reflect packing error. As such, the net dockside packing error would be about 2.3% (1.6 + 0.7%).

The net dockside packing error reflects changes in product condition (density and volume) and changes in product integrity (e.g., any loss of 'sweet meats') between sampling at-sea and sampling dockside. The percentage difference in average total weight between one-pound coffee can samples of fresh-shucked and dockside meats taken during the present study was 2.2% (1,062 vs. 1,039 g—Table 7), suggesting that the expected differences in at-sea vs. dockside counts are indeed realized in practice. In essence, dockside samples underestimate the true meat count of scallops packed at sea, thereby providing fishermen with an additional tolerance in complying with the meat count standard.

CONCLUSIONS

This study confirms that a volumetric system for measuring meat counts at-sea can be compatible with the shoreside sampling operations currently in place in the USA scallop fishery. Meat counts determined from volumetric sampling appear to be as accurate and precise as those determined by weight measurements. In this context, one might be tempted to consider replacing the weight-based dockside system of enforcement sampling with a volumetric system. The advantage in doing this would be that at-sea and dockside meat count estimation procedures would be identical, providing some sense of equitability to fishermen in assessing at-sea compliance with the meat count standard. However, to implement a volumetric measuring system dockside would require the development of a legally defensible sampling technique for filling the volumetric container. This problem is more difficult to address dockside than at-sea, due to the condition of the meats at landing, but conceptually it is solvable. While dockside sampling with a volumetric measure might be more time-consuming than by using electronic scales, it could provide more flexibility since enforcement officials would be able to conduct inspections virtually anywhere.

By the same token, the use of electronic scales in dockside enforcement operations is rather straight-forward and provides an accurate and objective means for determining meat counts. There is no 'a priori' reason to switch from a system that has already proven to be legally defensible in court. Moreover, there are many enforcement sytems in society where compliance is evaluated using a different procedure than that used by those being regulated (e.g., radar enforcement of automobile speeding limits vs. speedometer readings used by vehicle operators).

The fundamental issue regarding the ability of fishermen to comply with meat count regulation is more complex than ascertaining the MPP of individual samples or the trip as a whole, regardless of which method of meat count determination is used. The real problem confronting fishermen is the mathematics of optimizing fishing practices (primarily in deciding on which beds to fish) with the time remaining in the trip so that earnings are maximized with a 'legal'

catch. More frequently than not, this optimization procedure involves taking large catches of small scallops (high meat count) to mix with larger-sized meats (low meat count) obtained from less dense beds located in different fishing areas. By its nature, this practice does not lend itself to insuring that all bags placed in the hold are consistent with the average meat count standard (i.e., currently 30 MPP). To comply with the standard, fishermen thus not only have to know how to use an at-sea meat count measuring device (volumetric or otherwise) but also how to pack the catch so that bags with meats of different counts will average out to the legal count when a sub-sample of bags is checked dockside. In general, given the present enforcement sampling regimen, this can only be achieved by packing individual bags as close to the management standard as possible so that between bag variability in meat count is minimized.

ACKNOWLEDGMENTS

This project could not have been possible without the full support of the USA Atlantic sea scallop industry, particularly the Offshore Mariners Association, the East Coast Fisheries Association, and the Seafood Producers Association of New Bedford. We especially thank Captain Martin Manley, the crew of the F/V Mary Anne, NMFS Statistical Reporting Agent Paul Patrick Swain, and the staff at the Marine Advisory Service at the Virginia Institute of Marine Science for their assistance and cooperation throughout the study. Literally, hundreds of individuals participated on various aspects of the project. We offer our appreciation to all of them for their selfless efforts.

We are indebted to Janice Forrester, Ralph Mayo, Tim Smith, and Michael Pennington (Northeast Fisheries Center, Woods Hole) for their constructive reviews of the manuscript.

LITERATURE CITED

Caddy, J. F. & C. Radley-Walters. 1972. Estimating count per pound of scallop meats by volumetric measurement. Fish. Res. Board Can., Manuscript Rept. Ser. No. 1202, Biol. Sta., St. Andrews, New Brunswick, 10 p.

Naidu, K. S. 1984. An analysis of the scallop meat count regulation. Can. Atl. Fish. Sci. Adv. Committee Res. Doc. 84/73, 18 p.

Naidu, K. S. 1987. Efficiency of meat recovery from Iceland scallops (*Chlamys islandica*) and sea scallops (*Placopecten magellanicus*) in the Canadian offshore fishery. *J. Northw. Atl. Fish. Sci.* 7:131–136.

New England Fishery Management Council. 1982. Fishery Management Plan for Atlantic Sea Scallop (*Placopecten magellanicus*) Saugus, MA 149 pp.

Serchuk, F. M. 1983. Seasonality in sea scallop shell height—meat weight relationships: Review and analysis of temporal and spatial variability and implications for management measures based on meat count. Nat. Mar. Fish. Serv., Woods Hole Lab. Ref. Doc. No. 83-35, 30 p.

Serchuk, F. M. 1984. Fishing patterns and management measures regulating size at capture in the Georges Bank sea scallop fishery: A brief historical review. Nat. Mar. Fish. Serv., Woods Hole Lab. Ref. Doc. No. 84-11, 10 p.

Serchuk, F. M. & S. E. Wigley. 1988. Status of sea scallop resources off the northeastern United States, 1988. Nat. Mar. Fish. Serv., Woods Hole Lab. Ref. Doc. No. 88-03, 30 p.

Smolowitz, R. J. & F. M. Serchuk. 1987. Current technical concerns with sea scallop management, p. 639–644. in Proc., Oceans '87—The Oceans, An International Workplace. William MacNab and Son, 1172 p.

Smolowitz, R. J. & F. M. Serchuk. 1988. Developments in sea scallop gear design. in Proc. of the World Symposium on Fishing Gear and Fishing Vessel Design, St. John's, Newfoundland.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry [2nd ed.]. W. H. Freeman and Co., San Francisco, Calif., 859 p.

Wilhelm, K. A. & B. Jobe. MS [unpub]. Weight change of scallop meats during fresh storage. Nat. Mar. Fish. Serv., Gloucester Lab., Gloucester, Massachusetts.

APPENDIX TABLE 1.

Summary of at-sea and dockside volumetric samples of sea scallop meats, by sampling method.

Date of Sample	Measurement Method	Sample #	At-Sea Sample Number								
			1	2	3	4	5	6	7	8	9
6/4/87											
	Sea Vol. Can MPS		62	62	53	56	55	51	62	63	60
	Dock Vol. Can MPS		60	64	50	56	56	68	66	65	66
	Dock Wgt. Can LBS		2.33	2.30	2.36	2.36	2.30	2.35	2.29	2.34	2.31
	Sea Vol. Can MPP		26.5	26.5	22.6	23.9	23.5	21.8	26.5	26.9	25.6
	Dock Vol. Can MPP		26.2	27.9	21.8	24.4	24.4	29.7	28.8	28.4	28.8
	Dock Wgt. Can MPP		25.8	27.8	21.2	23.7	24.3	28.9	28.8	27.8	28.6
6/21/87											
	Sea Vol. Can MPS		63	64	62	65	68	63	67	59	61
	Dock Vol. Can MPS	1	64	60	56	61	55	57	62	65	56
	Dock Vol. Can MPS	2	59	62	57	63	56	62	63	63	61
	Dock Wgt. Can LBS	1	2.30	2.38	2.38	2.30	2.33	2.31	2.25	2.26	2.30
	Dock Wgt. Can LBS	2	2.33	2.34	2.36	2.34	2.32	2.25	2.28	2.30	2.22
	Sea Vol. Can MPP		26.9	27.3	26.5	27.8	29.0	26.9	28.6	25.2	26.0
	Dock Vol. Can MPP	1	27.9	26.2	24.4	26.6	24.0	24.9	27.1	28.4	24.4
	Dock Vol. Can MPP	2	25.8	27.1	24.9	27.5	24.4	27.1	27.5	27.5	26.6
	Dock Wgt. Can MPP	1	27.8	25.2	23.5	26.5	23.6	24.7	27.6	28.8	24.3
	Dock Wgt. Can MPP	2	25.3	26.5	24.2	26.9	24.1	27.6	27.6	27.4	27.5
7/25/87											
	Sea Vol. Can MPS		59	65	65	67	65	65			
	Dock Vol. Can MPS	1	51	62	60	56	61	54			
	Dock Vol. Can MPS	2	60	64	62	54	57	55			
	Dock Wgt. Can LBS	1	2.30	2.30	2.30	2.30	2.30	2.18			
	Dock Wgt. Can LBS	2	2.30	2.30	2.24	2.18	2.24	2.11			
	Sea Vol. Can MPP		25.2	27.8	27.8	28.6	27.8	27.8			
	Dock Vol. Can MPP	1	22.3	27.1	26.2	24.4	26.6	23.6			
	Dock Vol. Can MPP	2	26.2	27.9	27.1	23.6	24.9	24.0			
	Dock Wgt. Can MPP	1	22.2	27.0	26.1	24.3	26.5	24.8			
	Dock Wgt. Can MPP	2	26.1	27.8	27.7	24.8	25.4	26.1			
8/11/87											
	Sea Vol. Can MPS		62	61	64	68	63	62	60	65	62
	Dock Vol. Can MPS	1*	51	67	58	56	57	55	65	60	56
	Dock Vol. Can MPS	2*	44	59	53	60	58	56	57	51	53
	Dock Wgt. Can LBS	1*	2.03	2.09	2.04	1.93	2.04	2.09	2.11	2.04	1.97
	Dock Wgt. Can LBS	2*	2.07	2.15	2.06	2.07	2.01	2.06	2.13	2.02	2.09
	Sea Vol. Can MPP		26.5	26.0	27.3	29.0	26.9	26.5	25.6	27.8	26.5
	Dock Vol. Can MPP	1*	22.3	29.2	25.3	24.4	24.9	24.0	28.4	26.2	24.4
	Dock Vol. Can MPP	2*	19.2	25.8	23.1	26.2	25.3	24.4	24.9	22.3	23.1
	Dock Wgt. Can MPP	1*	25.1	32.1	28.4	29.0	27.9	26.3	30.8	29.4	28.4
	Dock Wgt. Can MPP	2*	21.3	27.4	25.7	29.0	28.9	27.2	26.8	25.2	25.4
8/28/87											
	Sea Vol. Can MPS		64	63	64	59	62	59	61	62	
	Dock Vol. Can MPS	1*	56	54	61	50	55	50	60	60	
	Dock Vol. Can MPS	2*	56	52	55	48	61	53	55	55	
	Dock Wgt. Can LBS	1*	2.23	2.15	2.09	2.14	2.03	2.06	2.18	2.07	
	Dock Wgt. Can LBS	2*	2.25	2.04	2.16	2.28	2.03	2.11	2.11	2.13	
	Sea Vol. Can MPP		27.3	26.9	27.3	25.2	26.5	25.2	26.0	26.5	
	Dock Vol. Can MPP	1*	24.4	23.6	26.6	21.8	24.0	21.8	26.2	26.2	
	Dock Vol. Can MPP	2*	24.4	22.7	24.0	21.0	26.6	23.1	24.0	24.0	
	Dock Wgt. Can MPP	1*	25.1	25.1	29.2	23.4	27.1	24.3	27.5	29.0	
	Dock Wgt. Can MPP	2*	24.9	25.5	25.5	21.1	30.0	25.1	26.1	25.8	
9/12/87											
	Sea Vol. Can MPS		60	73	71	73	68	69	63	70	70
	Dock Vol. Can MPS	1*	51	66	63	65	55	55	56	70	63
	Dock Vol. Can MPS	2*	54	70	56	66	53	66	58	66	63
	Dock Wgt. Can LBS	1*	2.13	2.18	2.12	2.14	2.17	2.21	2.13	2.18	2.18
	Dock Wgt. Can LBS	2*	2.21	2.16	2.11	2.15	2.14	2.24	2.11	2.14	2.15
	Sea Vol. Can MPP		25.6	31.2	30.3	31.2	29.0	29.5	26.9	29.9	29.9
	Dock Vol. Can MPP	1*	22.3	28.8	27.5	28.4	24.0	24.0	24.4	30.6	27.5
	Dock Vol. Can MPP	2*	23.6	30.6	24.4	28.8	23.1	28.8	25.3	28.8	27.5

APPENDIX TABLE 1.

Continued.

Date of Sample	At-Sea Sample Number							Average	Variance	No. of Obs.
	10	11	12	13	14	15	16			
6/4/87										
	62							58.60	19.60	10
	60							61.10	32.99	10
	2.32							2.33	0.0007	10
	26.5							25.02	3.57	10
	26.2							26.67	6.29	10
	25.9							26.28	6.67	10
6/21/87										
	65	62						63.55	6.87	11
	58	55						59.00	13.00	11
	56	59						60.09	7.89	11
	2.22	2.32						2.30	0.0024	11
	2.31	2.18						2.29	0.0031	11
	27.8	26.5						27.13	1.25	11
	25.3	24.0						25.75	2.48	11
	24.4	25.8						26.23	1.50	11
	26.1	23.7						25.62	3.43	11
	24.2	27.1						26.22	2.13	11
7/25/87										
	59							63.57	10.29	7
	50							56.29	23.57	7
	47							57.00	32.67	7
	2.18							2.27	0.0034	7
	2.30							2.24	0.0052	7
	25.2							27.14	1.88	11
	21.8							24.57	4.49	7
	20.5							24.88	6.22	7
	22.9							24.83	3.31	7
	20.4							25.47	6.17	7
8/11/87										
	63							63.00	5.11	10
	56							58.10	22.77	10
	57							54.80	22.62	10
	2.11							2.05	0.0035	10
	2.09							2.07	0.0019	10
	26.9							26.90	0.93	10
	24.4							25.36	4.34	10
	24.9							23.92	4.31	10
	26.5							28.41	4.37	10
	27.3							26.41	4.96	10
8/28/87										
								61.75	3.93	8
								55.75	19.07	8
								54.38	13.70	8
								2.12	0.0046	8
								2.14	0.0080	8
								26.37	0.72	8
								24.33	3.63	8
								23.73	2.61	8
								26.33	4.74	8
								25.49	5.92	8
9/12/87										
	59	70						67.82	24.16	11
	61	60						60.45	32.47	11
	63	65						61.82	31.96	11
	2.11	2.19						2.16	0.0011	11
	2.16	2.13						2.15	0.0015	11
	25.2	29.9						28.96	4.41	11
	26.6	26.2						26.39	6.19	11
	27.5	28.4						26.98	6.09	11

APPENDIX TABLE 1.

Continued.

Date of Sample	Measurement Method	Sample #	At-Sea Sample Number								
			1	2	3	4	5	6	7	8	9
	Dock Wgt. Can MPP	1*	23.9	30.3	29.7	30.4	25.3	24.9	26.3	32.1	28.9
	Dock Wgt. Can MPP	2*	24.4	32.4	26.5	30.7	24.8	29.5	27.5	30.8	29.3
9/14/87											
	Sea Vol. Can MPS		60	64	61	63	57	60	61	63	59
	Dock Vol. Can MPS	1*	56	54	53	57	44	44	58	57	47
	Dock Vol. Can MPS	2*	56	58	54	61	47	44	54	60	47
	Dock Wgt. Can LBS	1*	2.12	2.05	2.11	2.02	2.08	2.16	2.11	2.20	2.14
	Dock Wgt. Can LBS	2*	2.17	2.06	2.07	2.09	2.06	2.14	2.11	2.15	2.18
	Sea Vol. Can MPP		25.6	27.3	26.0	26.9	24.3	25.6	26.0	26.9	25.2
	Dock Vol. Can MPP	1*	24.4	23.6	23.1	24.9	19.2	19.2	25.3	24.9	20.5
	Dock Vol. Can MPP	2*	24.4	25.3	23.6	26.6	20.5	19.2	23.6	26.2	20.5
	Dock Wgt. Can MPP	1*	26.4	26.3	25.1	28.2	21.2	20.4	27.5	25.9	22.0
	Dock Wgt. Can MPP	2*	25.8	28.2	26.1	29.2	22.8	20.6	25.6	27.9	21.6
9/18/87											
	Sea Vol. Can MPS		43	55	59	57	70	62	45	53	58
	Dock Vol. Can MPS	1	54	55	63	65	69				57
	Dock Vol. Can MPS	2	50	64	60	67	64				55
	Dock Vol. Can MPS	3	51	58	65	72	67				56
	Dock Wgt. Can LBS	1	2.36	2.35	2.33	2.32	2.33				2.28
	Dock Wgt. Can LBS	2	2.33	2.27	2.33	2.33	2.34				2.28
	Dock Wgt. Can LBS	3	2.32	2.36	2.32	2.32	2.30				2.29
	Sea Vol. Can MPP		18.4	23.5	25.2	24.3	29.9	26.5	19.2	22.6	24.8
	Dock Vol. Can MPP	1	23.6	24.0	27.5	28.4	30.1				24.9
	Dock Vol. Can MPP	2	21.8	27.9	26.2	29.2	27.9				24.0
	Dock Vol. Can MPP	3	22.3	25.3	28.4	31.4	29.2				24.4
	Dock Wgt. Can MPP	1	22.9	23.4	27.0	28.0	29.6				25.0
	Dock Wgt. Can MPP	2	21.5	28.2	25.8	28.8	27.4				24.1
	Dock Wgt. Can MPP	3	22.0	24.6	28.0	31.0	29.1				24.5
10/3/87											
	Sea Vol. Can MPS		63	66	62	65	63	65	64	56	58
	Dock Vol. Can MPS	1*	61	63	54	57	54	56	55	46	52
	Dock Vol. Can MPS	2*	62	62	58	60	50	57	60	46	54
	Dock Wgt. Can LBS	1*	2.07	2.04	1.97	2.04	2.08	2.09	2.11	2.10	2.10
	Dock Wgt. Can LBS	2*	2.02	2.08	2.09	2.02	2.09	2.07	2.12	2.08	2.09
	Sea Vol. Can MPP		26.9	28.2	26.5	27.8	26.9	27.8	27.3	23.9	24.8
	Dock Vol. Can MPP	1*	26.6	27.5	23.6	24.9	23.6	24.4	24.0	20.1	22.7
	Dock Vol. Can MPP	2*	27.1	27.1	25.3	26.2	21.8	24.9	26.2	20.1	23.6
	Dock Wgt. Can MPP	1*	29.5	30.9	27.4	27.9	26.0	26.8	26.1	21.9	24.8
	Dock Wgt. Can MPP	2*	30.7	29.8	27.8	29.7	23.9	27.5	28.3	22.1	25.8
10/13/87											
	Sea Vol. Can MPS		47	67	47	70	45		69		60
	Dock Vol. Can MPS	1	44	60	45		49				
	Dock Vol. Can MPS	2	53	53	53		55				
	Dock Vol. Can MPS	3	54	59	52		50				
	Dock Wgt. Can LBS	1	2.27	2.29	2.27		2.29				
	Dock Wgt. Can LBS	2	2.28	2.28	2.30		2.31				
	Dock Wgt. Can LBS	3	2.33	2.30	2.30		2.34				
	Sea Vol. Can MPP		20.1	28.6	20.1	29.9	19.2		29.5		25.6
	Dock Vol. Can MPP	1	19.2	26.2	19.6		21.4				
	Dock Vol. Can MPP	2	23.1	23.1	23.1		24.0				
	Dock Vol. Can MPP	3	23.6	25.8	22.7		21.8				
	Dock Wgt. Can MPP	1	19.4	26.2	19.8		21.4				
	Dock Wgt. Can MPP	2	23.2	23.2	23.0		23.8				
	Dock Wgt. Can MPP	3	23.2	25.7	22.6		21.4				
10/22/87											
	Sea Vol. Can MPS		66	61	65	63	61	52	63	64	59
	Dock Vol. Can MPS	1	58	57	60	52	51	42	59	65	51
	Dock Vol. Can MPS	2	65	55	66	52	58	45	62	63	48
	Dock Vol. SMD MPS	1	69	57	70	58	67	45	66	56	55
	Dock Vol. SMD MPS	2	70	65	69	61	54	42	69	62	62

APPENDIX TABLE 1.

Continued.

Date of Sample	At-Sea Sample Number							Average	Variance	No. of Obs.
	10	11	12	13	14	15	16			
	28.9	27.4						28.01	6.88	11
	29.2	30.5						28.69	6.66	11
9/14/87										
	55							60.30	7.79	10
	43							51.30	37.34	10
	44							52.50	42.28	10
	2.18							2.12	0.0032	10
	2.19							2.12	0.0026	10
	23.5							25.75	1.42	10
	18.8							22.39	7.12	10
	19.2							22.92	8.05	10
	19.7							24.27	9.90	10
	20.1							24.78	10.89	10
9/18/87										
	63	57	78	74	58	68		60.00	93.71	15
	55	61	59		57	49		58.55	31.87	11
	55	63	67		62	54		60.09	32.89	11
	54	74	66		50	54		60.64	71.85	11
	2.31	2.29	2.31		2.32	2.29		2.32	0.0006	11
	2.29	2.27	2.30		2.32	2.26		2.30	0.0009	11
	2.32	2.30	2.31		2.31	2.29		2.31	0.0004	11
	26.9	24 3	33.3	31.6	24 8	29.0		25.62	17.09	15
	24.0	26.6	25.8		24.9	21.4		25.55	6.07	11
	24.0	27.5	29.2		27.1	23.6		26.23	6.27	11
	23.6	32.3	28.8		21.8	23.6		26.47	13.69	11
	23.8	26.6	25.5		24 6	21.4		25.26	5.84	11
	24.0	27.8	29.1		26.7	23.9		26.10	6.01	11
	23.3	32.2	28.6		21.6	23.6		26.22	13.62	11
10/3/87										
	56							61.80	14.18	10
	62							56.00	26.22	10
	61							57.00	29.33	10
	2.24							2.08	0.0047	10
	2.21							2.09	0.0028	10
	23.9							26.39	2.58	10
	27.1							24.44	5.00	10
	26.6							24 88	5.59	10
	27.7							26.89	6.17	10
	27.6							27.33	7.26	10
10/13/87										
	60	72	45	72	60	47	52	58.07	114.38	14
	67	52			49	63		53.63	74.27	8
	68	68			55	50		56.88	49.55	8
	62	53			60	61		56.38	21.41	8
	2.31	2.31			2.30	2.34		2.30	0.0005	8
	2.31	2.31			2.30	2.33		2.30	0.0003	8
	2.29	2.28			2.27	2.33		2.31	0.0007	8
	25.6	30.7	19.2	30.7	25.6	20.1	22.2	24.80	20.85	14
	29.2	22.7			21.4	27.5		23.41	14.15	8
	29.7	29.7			24.0	21.8		24.83	9.44	8
	27.1	23.1			26.2	26.6		24.61	4.08	8
	29.0	22.5			21.3	26.9		23.32	12.82	8
	29.4	29.4			23.9	21.5		24.70	9.11	8
	27.1	23.2			26.4	26.2		24.47	4.46	8
10/22/87										
	56							61.00	18.67	10
	63							55.80	46.84	10
	59							57.30	51.57	10
	58							60.10	60.99	10
	63							61.70	70.68	10

APPENDIX TABLE 1.

Continued.

Date of Sample	Measurement Method	Sample #	At-Sea Sample Number								
			1	2	3	4	5	6	7	8	9
	Dock Wgt. Can LBS	1	2.22	2.21	2.20	2.24	2.29	2.29	2.20	2.28	2.23
	Dock Wgt. Can LBS	2	2.27	2.15	2.12	2.24	2.26	2.23	2.22	2.24	2.32
	Dock SMD Wgt. LBS	1	2.23	2.29	2.29	2.27	2.24	2.33	2.31	2.30	2.33
	Dock SMD Wgt. LBS	2	2.30	2.28	2.32	2.25	2.24	2.32	2.27	2.24	2.29
	Sea Vol. Can MPP		28.2	26.0	27.8	26.9	26.0	22.2	26.9	27.3	25.2
	Dock Vol. Can MPP	1	25.3	24.9	26.2	22.7	22.3	18.3	25.8	28.4	22.3
	Dock Vol. Can MPP	2	28.4	24.0	28.8	22.7	25.3	19.6	27.1	27.5	21.0
	Dock Wgt. Can MPP	1	26.1	25.8	27.3	23.2	22.3	18.3	26.8	28.5	22.9
	Dock Wgt. Can MPP	2	28.6	25.6	31.1	23.2	25.7	20.2	27.9	28.1	20.7
	Dock Vol. SMD MPP	1	30.3	25.0	30.8	25.5	29.4	19.8	29.0	24.6	24.2
	Dock Vol. SMD MPP	2	30.8	28.6	30.3	26.8	23.7	18.5	30.3	27.2	27.2
	Dock Wgt. SMD MPP	1	30.9	24.9	30.6	25.6	29.9	19.3	28.6	24.3	23.6
	Dock Wgt. SMD MPP	2	30.4	28.5	29.7	27.1	24.1	18.1	30.4	27.7	27.1
11/9/87											
	Sea Vol. SMD MPS		62	56	61	52	51	56	54	53	59
	Dock Vol. SMD MPS	1	60	53	58	51	54	54	51	54	57
	Dock Vol. SMD MPS	2	62	51	60	56	50	56	54	56	57
	Dock SMD Wgt. LBS	1	2.15	2.24	2.23	2.21	2.30	2.22	2.28	2.32	2.30
	Dock SMD Wgt. LBS	2	2.24	2.26	2.19	2.22	2.26	2.23	2.26	2.21	2.26
	Sea Vol. SMD MPP		27.2	24.6	26.8	22.8	22.4	24.6	23.7	23.3	25.9
	Dock Vol. SMD MPP	1	26.4	23.3	25.5	22.4	23.7	23.7	22.4	23.7	25.0
	Dock Vol. SMD MPP	2	27.2	22.4	26.4	24.6	22.0	24.6	23.7	24.6	25.0
	Dock Wgt. SMD MPP	1	27.9	23.7	26.0	23.1	23.5	24.3	22.4	23.3	24.8
	Dock Wgt. SMD MPP	2	27.7	22.6	27.4	25.2	22.1	25.1	23.9	25.3	25.2
11/27/87											
	Sea Vol. Can MPS		64	65	62	66	64	58	64	62	58
	Sea Vol. SMD MPS		66	67	60	62	62	56	68	61	58
	Dock Vol. Can MPS	1	65	70	54	61	65	50	66	57	48
	Dock Vol. Can MPS	2	66	69	65	68	60	53	70	50	50
	Dock Vol. SMD MPS	1	60	63	55	63	60	57	61	67	49
	Dock Vol. SMD MPS	2	61	65	55	63	66	57	68	58	52
	Dock Wgt. Can LBS	1	2.23	2.29	2.27	2.27	2.28	2.27	2.28	2.23	2.32
	Dock Wgt. Can LBS	2	2.25	2.29	2.33	2.30	2.34	2.29	2.33	2.27	2.29
	Dock SMD Wgt. LBS	1	2.24	2.27	2.30	2.34	2.25	2.35	2.36	2.31	2.30
	Dock SMD Wgt. LBS	2	2.27	2.33	2.31	2.27	2.29	2.28	2.31	2.32	2.31
	Sea Vol. Can MPP		27.3	27.8	26.5	28.2	27.3	24.8	27.3	26.5	24.8
	Sea Vol. SMD MPP		29.0	29.4	26.4	27.2	27.2	24.6	29.9	26.8	25.5
	Dock Vol. Can MPP	1	28.4	30.6	23.6	26.6	28.4	21.8	28.8	24.9	21.0
	Dock Vol. Can MPP	2	28.8	30.1	28.4	29.7	26.2	23.1	30.6	21.8	21.8
	Dock Vol. SMD MPP	1	26.4	27.7	24.2	27.7	26.4	25.0	26.8	29.4	21.5
	Dock Vol. SMD MPP	2	26.8	28.6	24.2	27.7	29.0	25.0	29.9	25.5	22.8
	Dock Wgt. Can MPP	1	29.1	30.6	23.8	26.9	28.5	22.0	28.9	25.6	20.7
	Dock Wgt. Can MPP	2	29.3	30.1	27.9	29.6	25.6	23.1	30.0	22.0	21.8
	Dock Wgt. SMD MPP	1	26.8	27.8	23.9	26.9	26.7	24.3	25.8	29.0	21.3
	Dock Wgt. SMD MPP	2	26.9	27.9	23.8	27.8	28.8	25.0	29.4	25.0	22.5
12/14/87											
	Sea Vol. Can MPS		71	64	72	70	73	75	74	71	62
	Sea Vol. SMD MPS		68	60	66	64	65	67	68	65	59
	Dock Vol. Can MPS			57		73	73	69	67	66	60
	Dock Vol. SMD MPS			54		69	74	68	71	72	64
	Dock Wgt. Can LBS			2.35		2.24	2.33	2.29	2.32	2.31	2.37
	Dock SMD Wgt. LBS			2.31		2.29	2.24	2.24	2.19	2.21	2.21
	Sea Vol. Can MPP		30.3	27.3	30.7	29.9	31.2	32.0	31.6	30.3	26.5
	Sea Vol. SMD MPP		29.9	26.4	29.0	28.1	28.6	29.4	29.9	28.6	25.9
	Dock Vol. Can MPP			24.9		31.9	31.9	30.1	29.2	28.8	26.2
	Dock Vol. SMD MPP			23.7		30.3	32.5	29.9	31.2	31.6	28.1
	Dock Wgt. Can MPP			24.3		32.6	31.3	30.1	28.9	28.6	25.3
	Dock Wgt. SMD MPP			23.4		30.1	33.0	30.4	32.4	32.6	29.0

Coffee cans were underfilled.

APPENDIX TABLE 1.

Continued.

Date of Sample	At-Sea Sample Number							Average	Variance	No. of Obs.
	10	11	12	13	14	15	16			
	2.31							2.25	0.0017	10
	2.32							2.24	0.0041	10
	2.35							2.29	0.0015	10
	2.35							2.29	0.0014	10
	23.9							26.05	3.40	10
	27.5							24.36	8.92	10
	25.8							25.01	9.82	10
	27.3							24.85	9.68	10
	25.4							25.66	12.31	10
	25.5							26.41	11.77	10
	27.7							27.11	13.64	10
	24.7							26.24	13.66	10
	26.8							27.00	13.46	10
11/9/87										
	60							56.40	15.38	10
	59							55.10	10.32	10
	58							56.00	13.56	10
	2.31							2.26	0.0030	10
	2.33							2.25	0.0015	10
	26.4							24.78	2.97	10
	25.9							24.21	1.99	10
	25.5							24.60	2.62	10
	25.5							24.44	2.76	10
	24.9							24.95	3.17	10
11/27/87										
								62.56	8.28	9
								62.22	16.69	9
								59.56	59.28	9
								61.22	67.69	9
								59.44	27.53	9
								60.56	29.28	9
								2.27	0.0008	9
								2.30	0.0009	9
								2.30	0.0018	9
								2.30	0.0005	9
								26.71	1.51	9
								27.34	3.22	9
								26.00	11.29	9
								26.72	12.90	9
								26.12	5.31	9
								26.61	5.65	9
								26.23	11.89	9
								26.62	12.34	9
								25.83	5.40	9
								26.34	5.64	9
12/14/87										
								70.22	19.44	9
								64.67	10.50	9
								66.43	37.29	7
								67.43	45.29	7
								2.32	0.0018	7
								2.24	0.0019	7
								29.98	3.55	9
								28.41	2.03	9
								29.00	7.10	7
								29.63	8.74	7
								28.72	9.22	7
								30.12	11.12	7

APPENDIX TABLE 2.

Conversion chart for a one-pound coffee can volumetric sample. [Fresh shucked meats only].

No. of Meats in Full Can	Meat Count (MPP)	No. of Meats in Full Can	Meat Count (MPP)	No. of Meats in Full Can	Meat Count (MPP)
31	13.2	61	26.0	91	38.9
32	13.7	62	26.5	92	39.3
33	14.1	63	26.9	93	39.7
34	14.5	64	27.3	94	40.1
35	14.9	65	27.8	95	40.6
36	15.4	66	28.2	96	41.0
37	15.8	67	28.6	97	41.4
38	16.2	68	29.0	98	41.8
39	16.7	69	29.5	99	42.3
40	17.1	70	29.9	100	42.7
41	17.5	71	30.3	101	43.1
42	17.9	72	30.7	102	43.6
43	18.4	73	31.2	103	44.0
44	18.8	74	31.6	104	44.4
45	19.2	75	32.0	105	44.8
46	19.6	76	32.5	106	45.3
47	20.1	77	32.9	107	45.7
48	20.5	78	33.3	108	46.1
49	20.9	79	33.7	109	46.5
50	21.3	80	34.2	110	47.0
51	21.8	81	34.6	111	47.4
52	22.2	82	35.0	112	47.8
53	22.6	83	35.4	113	48.2
54	23.1	84	35.9	114	48.7
55	23.5	85	36.3	115	49.1
56	23.9	86	36.7	116	49.5
57	24.3	87	37.1	117	50.0
58	24.8	88	37.6	118	50.4
59	25.2	89	38.0	119	50.8
60	25.6	90	38.4	120	51.2

Journal of Shellfish Research, Vol. 8, No. 1, 173–178, 1989.

EVIDENCE OF A SEMIANNUAL REPRODUCTIVE CYCLE FOR THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791), IN THE MID-ATLANTIC REGION

WILLIAM D. DUPAUL, JAMES E. KIRKLEY AND ANNE C. SCHMITZER
Virginia Institute of Marine Science
School of Marine Science
The College of William and Mary
Gloucester Point, Virginia

ABSTRACT The reproductive cycle of the sea scallop, *Placopecten magellanicus* in the mid-Atlantic region was studied over a 15 month period. One to 15 samples a month were collected from commercial vessels fishing from Long Island to Cape Hatteras in water depths of 37–68 m. Gonad weights were determined for four shell size intervals as an indicator of the reproductive cycle. A sharp decline in mean gonad weights between April–May 1987 and a subsequent increase and decrease in weights between September–November 1987 indicated reproductive processes were occurring on a semiannual cycle. A major spring spawning season was reconfirmed in 1988 by a rapid increase in mean gonad weights between December 1987–January 1988, followed by variable declines in the weights through June. The occurrence of spawning activity for 2 consecutive spring seasons in addition to a fall spawning season suggests that a semiannual reproductive cycle may be a characteristic feature of *P. magellanicus* in the mid-Atlantic region. The ramifications of spring spawning to the mid-Atlantic sea scallop fishery and management policies are addressed.

KEY WORDS: semiannual reproduction, *Placopecten magellanicus*, scallop, gonad weight

INTRODUCTION

In fisheries in which the weight of an individual or part thereof forms the basis for regulation and enforcement, information on the reproductive cycle is necessary. In several bivalve species, including the sea scallop *Placopecten magellanicus* (Gmelin), the adductor muscle decreases in weight during periods of gametogenic development and spawning as its energy reserves are utilized (Ansell 1974, Barber and Blake 1981, Robinson et al. 1981). If regulations and enforcement standards based on weight or count per unit weight fail to consider the changes associated with the reproductive cycle, the objectives of a management plan may not be achieved or industry may experience regulatory compliance problems. The latter problem is of growing concern for the United States sea scallop fishery of the mid-Atlantic region.

Reproduction in bivalves requires that sufficient energy is available for the development of mature viable gametes (Bayne 1976). In *P. magellanicus* this available energy is dependent on accumulated biochemical energy reserves within the organism, food sources in the water column, and water temperature conditions (Sastry 1966, MacDonald and Thompson 1985, 1986, Barber et al. 1988). Since these factors vary with latitude, depth, and hydrographic conditions, differences may exist in the reproductive cycle of the sea scallop as conditions vary along its geographic range from the Gulf of St. Lawrence to Cape Hatteras.

In marine invertebrate species of wide geographic range, variations in reproductive cycles often accompany changes in latitude. Clear latitudinal trends are not always evident. They can be masked by local environmental conditions such as available food and temperature (Giese and Pearse 1974, Sastry 1979). Southern populations, compared to their more northerly counterparts, have been shown to exhibit less synchronization of spawning (Newell et al. 1982), prolonged spawning seasons (Sastry 1979), and reduced fecundity (Serchuk and Rak 1983). The occurrence of semiannual spawning in southern populations as opposed to annual spawning in northern populations of the same species has been reported for *Mercenaria mercenaria* (Porter 1964), *Mya arenaria* (Pfitzenmeyer 1965), and *Spisula solidissima* (Ropes 1968).

Considerable research has been done on the reproductive cycle of sea scallops in the Northwest Atlantic. Most of this research has concentrated on the reproductive cycle of sea scallops on Georges Bank (Posgay and Norman 1958, MacKenzie et al. 1978), Gulf of Maine (Welch 1950, Robinson et al. 1981, Langton et al. 1987), Bay of Fundy (Stevenson 1936, Dickie 1953), and Newfoundland (MacDonald and Thomspon 1986). Research for these areas indicate an annual reproductive cycle, with a single spawning period occurring during the fall when water temperatures range between 8–16°C. Naidu (1970) and Barber et al. (1988), however, noted the possibility of minor spawning during the spring in addition to a major spawning period during the fall off the coasts of Newfoundland and Maine, respectively. MacKenzie et al. (1978), however, did provide information that sea scallops in the mid-Atlantic region may spawn slightly earlier than those in the Northwest Atlantic. These results, based on macroscopic observations of gonad tissue over a 1-week period, indicated that scallops off Long Island and Virginia spawned during July or August.

In comparison, little sea scallop research appears to have been concerned with determining whether or not there are differences in the reproductive cycles for different fishery

Contribution No. 1487 from the Virginia Institute of Marine Science.

resource areas. As a result, the management and regulation of the sea scallop fishery have been based upon the assumption that the primary spawning period for sea scallops of all resource areas is during the fall.

This paper examines the possibility that scallops in the mid-Atlantic region may have a different reproductive cycle than previously documented. The examination of the reproductive cycle is based on an analysis of gonad weights for 4 shell size ranges over a 15-month period.

MATERIALS AND METHODS

Scallops were obtained from commercial fishing vessels participating in the cooperative sea scallop research program involving industry, National Marine Fisheries Services, New England Fisheries Management Council, and Virginia Institute of Marine Science. Data collection began in April 1987 and is to continue through December 1988. Due to the commercial nature of the scallop samples, it was not possible to preselect the catch location or frequency.

Samples ranged from south of Long Island (40°00′N 73°00′W) to north of Cape Hatteras (37°30′N 74°30′W). This is an area ~290 km long running northeast to southwest in water depths of 37–68 m. Most samples were from areas off the coasts of Virginia and Maryland between 38°30′N 74°00′W and 37°30′N 74°30′W. This is an area ~145 km long (Fig. 1).

This study is based on a subsample of scallops obtained from 123 trips between April 1987–June 1988. The number of trips in a given month varied between 1–15. An entire sample usually consisted of 1–2 baskets of unshucked scallops containing between 140–400 scallops/basket. Each basket has an approximate capacity of 1.5 bushels. A subsample of 40–120 scallops was randomly selected from each of these samples.

Each sample was processed within 48 hr of initial collection. At the time of collection, the date and time of the sample, Loran C coordinates, water depth, and surface water temperature were recorded. The adductor muscle and gonad were dissected from each animal. The wet weight of the gonad, with the crystalline style included, was measured to the nearest 0.1 g using an Ohaus Portogram scale. The shell height, the maximum distance between dorsal and ventral margins, was measured to the nearest mm using a standard fish measuring board. A total of 8,002 gonad weight-shell height measurements were compiled for the 15 months of data used in this study.

Time of spawning can be approximated from macroscopic observations of gonad tissue. Changes in gonad weight, however, provide a quantitative indicator of spawning and reproductive development (Giese and Pearse 1974). Standardized gonad weights have been used to determine the reproductive cycle of many bivalve and fish species, and are especially accurate for *P. magellanicus* because the gonad is self-contained and the follicles are retained after spawning; therefore, the majority of weight differences prior to and following spawning can be attributed to the presence or expulsion of gametes as well as their state of maturity (Langton et al. 1987). As gonadal development proceeds, the organ will increase in weight and reach a maximum size just prior to spawning and a minimum size immediately following spawning. Studies which have utilized standardized gonad weight changes or indices with *P. magellanicus* include Thompson (1977), Robinson et al. (1981), Serchuk and Rak (1983), Beninger (1987), Langton et al. (1987), and Barber et al. (1988).

Four 5 mm size intervals for each month were selected for analysis. The selected intervals were 85–89 mm (N = 1,153), 90–94 mm (N = 1,522), 100–104 mm (N = 1,104), 110–114 mm (N = 744), and 85–124 mm (N = 8,002). These intervals were selected because they contained the largest number of observations for each month and were indicative of the size distribution of the commercial harvest. Moreover, the 85–89 and 90–94 mm intervals represent size ranges which generally yield more or less than 30 meats/lb., respectively. The 90–94 mm interval also represents scallops which are believed to have been reproductively active for at least one spawning season (N.E.F.M.C. 1982). Data for both males and females were combined to calculate monthly mean gonad weights for each size interval. Student's t-tests (Snedecor and Cochran 1980) were performed on data from adjacent months for all size groups to determine significant differences.

RESULTS

Mean values of the gonad weights for the four, 5 mm size ranges indicated a consistent pattern over the 15-month period of observations (Table 1, Fig. 2). The weights declined between April–May 1987 and remained low with minor variation between May–September. The weights increased between September–October, followed by a decline in November 1987.

The mean gonad weights for the 4 size groups displayed a less synchronous pattern during the first 6 months of 1988. Although weights for all sizes increased between December 1987–January 1988, mean gonad weights did not reach maximum values during the same month following January. Maximum values for the 110–114 mm size group were observed in February, the 100–104 mm group in January, and the 85–89 and 90–94 mm scallops in March. After March, the mean gonad weights for all size groups declined.

While similar changes in gonad weights were evident in all size groups, results of one-tailed t-tests for inequality of indices between adjacent months were not identical, indicating slight variation in the timing of gonadal development and decline (Table 1). T-tests did not support the null hypothesis of no significant difference between mean gonad weights of adjacent months in the 4 size intervals between April–May 1987, September–October 1987, October–November 1987, December 1987–January 1988, and

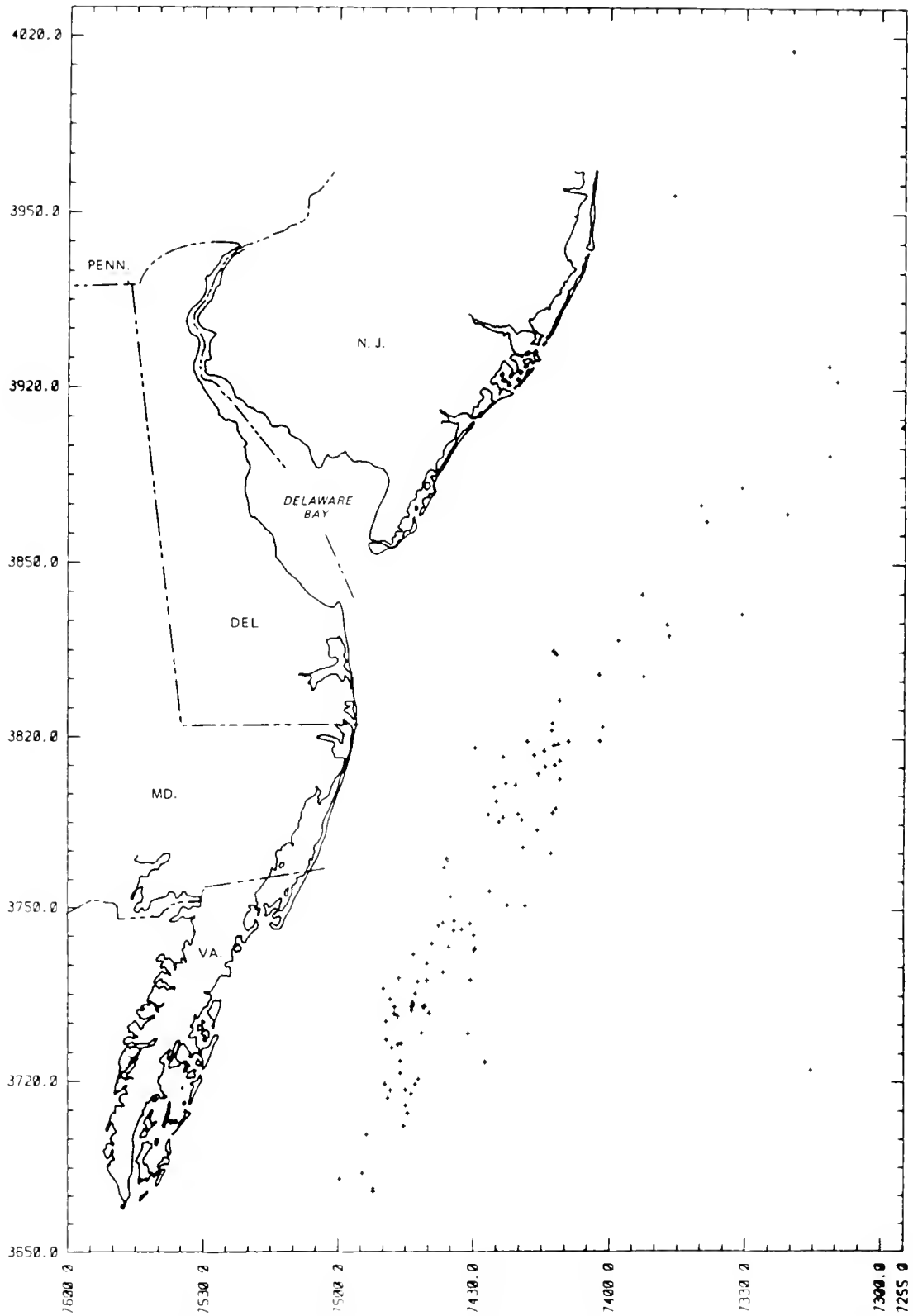


Figure 1. Distribution of *P. magellanicus* samples collected from commercial fishing vessels in the western, mid-Atlantic region (N = 123).

TABLE 1.

Mean gonad weight (X), standard deviation (S), and number of observations per shell size interval (N) for *P. magellanicus*, mid-Atlantic region, April 1987–June 1988.

Month	Shell height (mm) 85–89			90–94			100–104			110–114		
	Gonad weight (grams)											
	X	S	N	X	S	N	X	S	N	X	S	N
Apr. 87	7.6	1.9	25	8.5	1.6	17	12.1	2.9	6	16.4	5.4	4
May	3.0	2.4	34[1]	3.1	1.9	35[1]	5.9	1.8	13[1]	6.7	3.0	11[1]
Jun.	2.4	1.3	22	3.4	1.6	41	4.6	3.0	24	6.9	1.9	2
Jul.	2.0	0.5	6	2.1	0.7	9	3.6	1.9	5		—[2]	
Aug.	2.3	0.7	46	2.4	0.8	80	4.0	1.0	40	5.2	1.1	6
Sep.	2.8	1.1	30[1]	3.0	2.1	58[1]	4.0	1.6	33	6.2	3.6	10
Oct.	5.1	2.9	33[1]	5.4	2.7	52[1]	8.1	3.9	45[1]	12.3	4.5	19[1]
Nov.	2.2	1.8	18[1]	2.9	1.7	35[1]	4.4	2.4	33[1]	7.4	5.1	26[1]
Dec.	2.6	1.1	35	2.8	1.0	33	6.4	2.5	53[1]	7.4	2.1	43
Jan. 88	3.9	0.6	163[1]	5.2	0.2	138[1]	9.7	0.3	138[1]	12.4	0.4	103[1]
Feb.	4.5	2.0	137[1]	5.0	2.1	159	9.2	4.2	54	13.0	5.0	61
Mar.	5.2	1.8	180[1]	6.0	2.0	227[1]	9.4	3.4	128	12.3	3.7	128
Apr.	4.1	1.8	128[1]	5.1	2.5	156[1]	7.3	3.3	156[1]	11.0	5.2	99
May	3.6	1.7	172[1]	4.0	1.9	219[1]	6.3	3.2	188[1]	10.4	5.2	132
Jun.	1.6	0.8	163[1]	2.1	1.0	262[1]	3.6	1.7	239[1]	4.9	2.1	128[1]

[1] Indicates statistically significant difference ($p < 0.05$) between the preceding and referenced month.
[2] No data available for size range.

May–June 1988 ($P < 0.05$). Significant differences in weights were also detected between other adjacent months but not consistently for all size groups and time periods. An increase in gonad weight occurred between August and September 1987 was significantly different for only the 2 smaller size groups. Gonad weights in the 100–104 mm interval significantly increased in weight between November–December 1987. Gonad weights significantly increase for all size ranges between December 1987–January 1988. Significant increases were also evident in the 85–89 mm group from January–March 1988 and in the 90–94 mm group between February–March 1988. While all groups rapidly decreased in weight between May–June 1988, significant decreases in weight also occurred from March–May 1988 in the 3 smaller size groupings.

DISCUSSION

The sharp decline in mean gonad weights betwen April–May 1987 was the first indication of major spawning activity in the spring. Minimal mean gonad weights from June–August reflected a period of quiescence. The majority of the gonads were observed to be undeveloped, translucent and small in size. Slight increases in the mean gonad weights between August–September 1987, significant in the 85–89 mm and 90–94 mm size groups marked the initiation of gonadal development in the fall. The increase to maximum values by October 1987 indicated that most reproductive development was rapid and complete within a one month period. Subsequent post-spawning indices in November were similar to post-spawning indices observed in the preceding summer but of shorter duration. The majority of gonad redevelopment preceding the next spring spawning season occurred rapidly between December 1987–January 1988.

There is evidence of differential gonadal development in various size classes from January–March 1988. Although gonadal development in this second spring season appeared to be protracted over an extended period, subsequent spawning indicated by the sharp decline of mean gonad weights in all size intervals between May–June was similar to that observed between April–May of the previous year. This repeated pattern in spring 1988 confirms the presence of a major spring spawning period.

Because this study period did not cover pre-spawning months for spring 1987, it could not be determined if the protracted state of gonad maturity evident from January–March 1988 also occurred in early spring of the preceding year. A protracted period of gonad development did not precede the fall spawning period. However, when a semiannual reproductive strategy exists, the 2 spawning events occurring within 1 year are often unequal in magnitude and duration (Mason 1958, Ropes 1968, Comely 1974). While variability in initiation, duration, and nature of the reproductive cycle may exist, the occurrence of spawning activity for 2 consecutive spring seasons concurrent with a fall spawn suggests that the semiannual frequency of reproductive development may be a characteristic feature of *P. magellanicus* in the mid-Atlantic region.

Small but significant differences in gonad weight between size groups from January–April 1988 may be attrib-

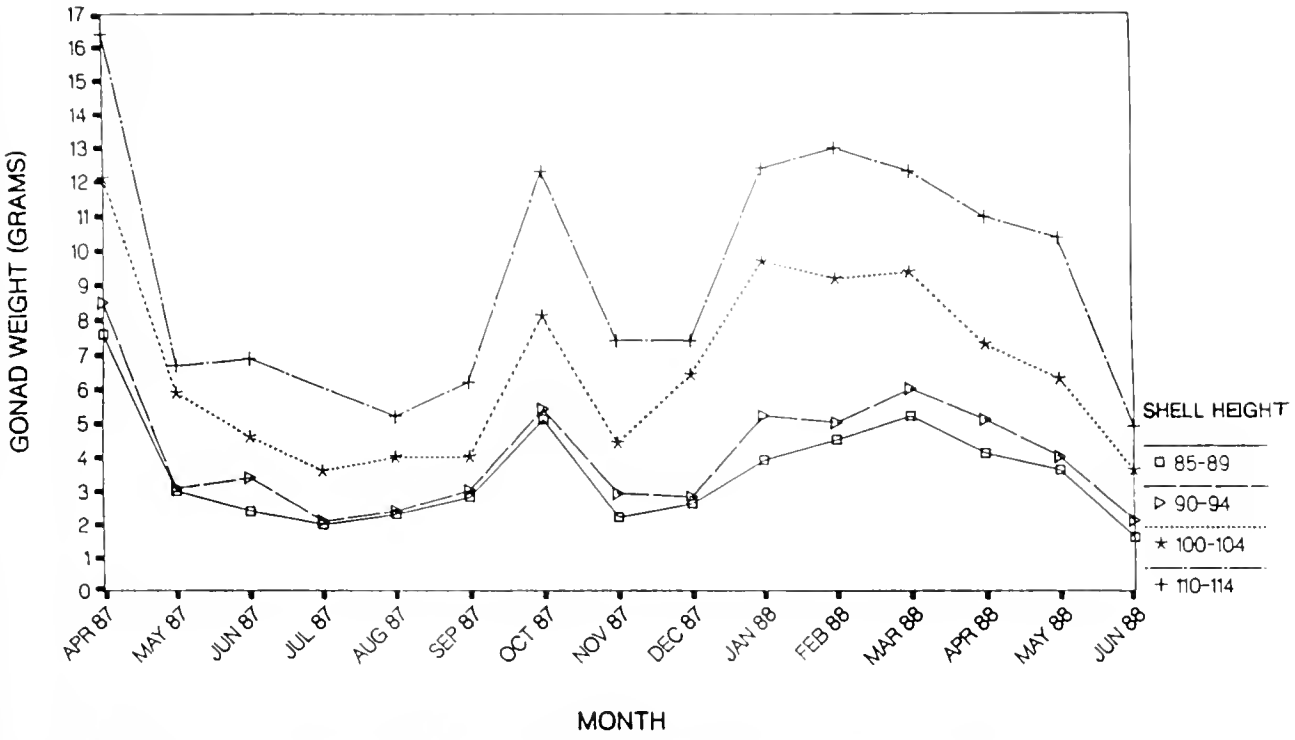


Figure 2. Mean gonad weight (X), standard deviation (S), and number of observations (N)/shell size interval for *P. magellanicus*, mid-Atlantic region, April 1987–June 1987.

uted to aggregation bias as a result of pooling data over a relatively large geographic area and depth range. As a result, the mean gonad weights may reflect area and depth differences as well as monthly differences. Unfortunately, the available data were inadequate for a more precise area and depth analysis. However, despite noted site specific variability in bivalve reproduction, it is important to recognize that the semiannual frequency of reproductive development was observed in all areas sampled. This phenomenon, consistent over a wide geographic range, should be considered a significant factor in the reproductive strategy of the sea scallop.

The significance of the spring spawning to the recruitment processes of the mid-Atlantic sea scallop fishery is uncertain. However the possibility of 2 periods of recruitment warrants further study. In species in which fertilization occurs externally in the surrounding water, synchronicity is critical to the reproductive success of a spawning season and the size of the resultant year class (Langton et al. 1987). The possibility of lysis and resorption of mature oocytes as opposed to the spawning of viable ova is also possible. This phenomenon has been observed in *Argopecten irradians* (Sastry 1966), *Pecten maximus* (Lubet et al. 1987), and *P. magellanicus* (Barber et al. 1988) when spawning was delayed due to low temperatures or lack of food.

Despite the unknown significance of the spring spawning in terms of fishery recruitment, the economic and management ramifications of semiannual spawning may be quite significant. Previous studies have indicated that meat weight for a given shell size changes significantly over the duration of a spawning event (Robinson et al. 1981). Recent management decisions (N.E.F.M.C. 1985, 1987) to allow a seasonal increase to 33 meats/lb. during the months of October–January are an indication that management is sensitive to the problem. Similar implications for a semiannual spawning period represent inequities for harvesters in the mid-Atlantic region when spawning related meat weight losses occur in the spring. Given the indications that there may be significant spatial, seasonal, and interannual variation in sea scallop meat weights, serious consideration must be given to altering the management strategy and enforcement procedures based on meat counter restrictions. Additional management strategies such as gear modifications and effort control measures should be under continuous review given the nature of the fishery. Concurrent with the data presented in this paper, further studies of the reproductive cycle are being undertaken, including histological examination of gonadal tissue, analysis of gonadal index changes associated with seasonal, depth, and spatially related influences, and correlation of adductor meat weight changes to reproductive activity.

ACKNOWLEDGMENTS

The authors would like to extend their sincere thanks to the captains and crews of the scallop vessels from the ports

of Cape May, NJ, Seaford, VA, Wanchese, NC, and Newport News, VA. Without their support, this study would not have been possible. Appreciation is also extended to the members of the East Coast Fisheries Association. Ms. Nancy Chartier of Virginia Institute of Marine Science, Department of Advisory Services, prepared the graphical presentation of monthly gonadal indices. Funds for this study were provided by the Virginia Institute of Marine Science and the Virginia Sea Grant College Program, N.O.A.A., Dept. of Commerce, under contract NA80AA-D-00021.

REFERENCES CITED

Ansell, A. D. 1974. Seasonal changes in biochemical composition of the bivalve *Chlamys septemradiata* from the Clyde Sea area. *Mar. Biol.* 25:85–89.

Barber, B. J. & N. J. Blake. 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians* (concentricus). *J. Exp. Mar. Biol. Ecol.* 52:121–134.

Barber, B. J., R. Getchell, S. E. Shumway & D. Schick. 1988. Reduced fecundity in a deep-water population of the giant scallop *Placopecten magellanicus* (Gmelin) in the Gulf of Maine, U.S.A. *Mar. Ecol. Prog. Ser.* 42:207–212.

Bayne, B. L. 1976. Aspects of reproduction in bivalve molluscs. In Wiley, M. (ed.) Estuarine processes. Vol. 1. Academic Press, N.Y. 506 pp.

Beninger, P. G. 1987. A qualitative and quantitative study of the reproductive cycle of the giant scallop, *Placopecten magellanicus*, in the Bay of Fundy (New Brunswick, Canada). *Can. J. Zool.* 65:495–498.

Comely, C. A. 1974. Seasonal variations in the flesh weights and biochemical content of the scallop *Pecten maximus* L. in the Clyde Sea area. *J. Cons. Int. Explor. Mer.* 35:281–295.

DeVlaming, V., G. Grossman & F. Chapman. 1982. On the use of the gonosomatic index. *Comp. Biochem. Physiol.* 73A:31–39.

Dickie, L. M. 1953. Fluctuations in abundance of the giant scallop, *Placopecten magellanicus* (Gmelin), in the Digby area of the Bay of Fundy. *Fish. Res. Bd. Can.*, M.S.S. rept. Biol. Sta. no. 526.

Giese, A. C. & J. S. Pearse. 1974. Introduction: General Principles. In: Reproduction of Marine Invertebrates. Vol. 1. (A. C. Giese and J. S. Pearse eds.) Academic Press, NY 545 pp.

Langton, R. W., W. E. Robinson & D. Schick. 1987. Fecundity and reproductive effort of sea scallops *Placopecten magellanicus* from the Gulf of Maine. *Mar. Ecol. Prog. Ser.* 37:19–25.

Lubet, P. E., J. Besnard, R. Faveris & I. Robbins. 1987. Physiologie de la reproduction de la Coquille Saint-Jacques (*Pecten maximus* L.). *Oceanis* 13:265–290.

MacDonald, B. A. & R. J. Thompson. 1985. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. II. Reproductive output and total production. *Mar. Ecol. Prog. Ser.* 25:295–303.

MacDonald, B. A. & R. J. Thompson. 1986. Influence of temperature and food availability on the energetics of the giant scallop *Placopecten magellanicus*. III. Physiological ecology, the gametogenic cycle and scope of growth. *Mar. Biol.* 93:37–48.

MacKenzie, C. L. Jr., A. S. Merrill & F. M. Serchuk. 1978. Sea scallop resources off the notheastern United States coast, 1975. *Mar. Fish. Rev.* 40:19–23.

Maddala, G. S. 1977. *Econometrics*. McGraw-Hill Book Company, New York, N.Y. 516 pp.

Mason, J. 1958. The breeding of the scallop *Pecten maximus* (L.) in Maine waters. *J. Mar. Biol. Assoc. U.K.* 37:653–671.

Naidu, K. S. 1970. Reproduction and breeding cycle of the giant scallop *Placopecten magellanicus* (Gmelin) in Port au Port Bay, Newfoundland. *Can. J. Zool.* 48:1003–1012.

Newell, R. I. E., T. I. Hilbish, R. K. Koehn & C. J. Newell. 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (Bivalvia, Mytilidae) from localities on the east coast of the United States. *Biol. Bull.* 162:299–310.

New England Fishery Management Council. 1982. Fishery management plan. Final environmental impact statement. Regulatory impact review for Atlantic sea scallops (*Placopecten magellanicus*). 149 pp.

New England Fishery Management Council. 1985. Amendment #1 to the fishery management plan for Atlantic sea scallops. Incorporating an environmental assessment and regulatory impact review/initial regulatory flexibility analysis. 30 pp.

New England Fishery Management Council. 1987. Amendment #2 to the fishery management plan for Atlantic sea scallops. Incorporating an environmental assessment and regulatory impact review/initial regulatory flexibility analysis. 14 pp.

Pfitzenmeyer, H. T. 1965. Annual cycle of gametogenesis of the soft-shelled clam, *Mya arenaria*, at Solomons, Maryland. *Ches. Sci.* 6:52–59.

Porter, H. J. 1964. Seasonal gonad changes in adult clams, *Mercenaria mercenaria* (L.) in North Carolina. *Proc. Natl. Shellfish. Assoc.* 55:35–52.

Posgay, J. A. & K. D. Norman. 1958. An observation on the spawning of the sea scallop, *Placopecten magellanicus* (Gmelin), on Georges Bank. *Limnol. Oceanog.* 3:142.

Robinson, W. E., W. E. Wehling, M. P. Morse & G. S. McCleod. 1981. Seasonal changes in soft body component indices and energy reserves in the Atlantic deep-sea scallop, *Placopecten magellanicus*. *Fish. Bull.* 79:449–458.

Ropes, J. W. 1968. Reproductive cycle of the surf clam, *Spisula solidissima*, in offshore New Jersey. *Biol. Bull.* 135:349–365.

Sastry, A. N. 1966. Temperature effects in reproduction of the bay scallop, *Aequipecten irradians* (Lamark). *Biol. Bull.* 130:118–134.

Sastry, A. N. 1979. Pelecypoda (excluding Ostreidae). In: Reproduction of marine invertebrates. (A. C. Giese & J. S. Pearse eds.) Academic Press, NY, 292 pp.

Serchuk, F. M. & R. S. Rak. 1983. Biological characteristics of offshore Gulf of Maine sea scallop populations: size, distributions, shell height-meat weight relationships and relative fecundity patterns. *Nat. Mar. Fish. Serv.*, Woods Hole Lab., Ref. Doc. No. 83-07.

Snedecor, G. W. & W. G. Cochran. 1980. *Statistical Methods*. Seventh edition. Ames, Iowa: Iowa State University Press, 507 pp.

Stevenson, J. A. 1936. The Canadian scallop; its fishery, life-history, and some environmental relationships. M.A. Thesis. University of West Ontario. London, Ontario, 164 pp.

Thompson, R. J. 1977. Blood chemistry, biochemical composition, and the annual reproductive cycle in the giant scallop, *Placopecten magellanicus*, from southeast Newfoundland. *J. Fish. Res. Bd. Can.* 34:2104–2116.

Welch, W. R. 1950. Growth and spawning characteristics of the sea scallop, *Placopecten magellanicus* (Gmelin), in Maine waters. M.A. Thesis. University of Maine, Orono, 95 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 179–186, 1989.

GROWTH OF THE PURPLE-HINGE ROCK SCALLOP, *CRASSADOMA GIGANTEA* GRAY, 1825 UNDER NATURAL CONDITIONS AND THOSE ASSOCIATED WITH SUSPENDED CULTURE

B. A. MACDONALD[1] AND N. F. BOURNE[2]
[1]*Ocean Sciences Centre*
Marine Sciences Research Laboratory
Memorial University of Newfoundland
St. John's, Newfoundland, Canada A1C 5S7
[2]*Department of Fisheries and Oceans*
Fisheries Research Branch
Pacific Biological Station
Nanaimo, British Columbia, Canada V9R 5K6

ABSTRACT Natural growth rates, the production of shell and body tissue were determined for *Crassadoma gigantea* (formerly *Hinnites*) and compared to similar estimates for rock scallops (<4 years) grown in suspended culture. Cultured scallops exhibited faster shell and tissue growth reaching a mean shell height of ~80 mm in 3 years while 4 years were required to reach a similar size when grown on the bottom. Growth rates of juveniles raised in the laboratory and suspended in the water column (5, 10, and 15 m) were well correlated with indices of ambient food quality, such as the organic fraction and energy values of total seston. Growth rates of these juveniles were promising but more information on nursery and growout technology, including methods to reduce biological fouling is required to predict the mariculture potential of this species in British Columbia.

KEY WORDS: rock scallop, growth, production, aquaculture

INTRODUCTION

The purple-hinge rock scallop *Crassadoma gigantea* (Gray) (formerly *Hinnites*, Bernard 1986) is usually found attached to hard substrates from the intertidal zone to water depths of 60 m along the Pacific coast from Baja, California to southern Alaska (Abbott 1974, Bernard 1983a). Despite a wide spatial distribution very little biological information exists for this species. A commercial fishery does not exist for rock scallops in British Columbia because of patchy spatial distribution, difficulties in harvesting and management concerns of overharvesting in some locations. However, recreational diving fisheries exist and this species is considered potentially valuable for mariculture operations (Leighton and Phleger 1977). Rock scallops are unique among pectinids because they have abandoned their free-swimming adult lifestyle and permanently cement to rocky substrates at approximately 20–30 mm in shell height (Yonge 1951). Settling and attachment behaviour may have advantages in culture operations because it enables spatial distribution of the scallops to be regulated and optimized (Leighton 1977).

The technique of suspending filter feeding bivalves in the water column has become a popular commercial tool for enhancing their growth rate and reducing effort spent on locating and collecting them. Evaluating the mariculture potential of a benthic species such as the rock scallop requires information on their growth and survival while being suspended in the local environment. Such studies will also provide data on the most appropriate water depths for hanging scallops in order to optimize the relationship between growth, survivorship and reduced biological fouling. Whether or not scallop production is enhanced sufficiently using husbandry techniques to warrant the additional costs associated with suspended culture requires the measurement of growth under natural conditions on the bottom (Ventilla 1982). Unlike other pectinid species population growth rates under natural conditions have not been reported for rock scallops because irregular shell surfaces prevent age from being estimated for individuals using traditional shell ring analysis.

Annual growth lines on the calcareous portion of the ligament have been used to estimate age in other pectinids thus potentially providing the opportunity for determining age of individual rock scallops and describing population growth rates (Merrill et al. 1966, Johannessen 1973). While shell growth patterns have been used extensively for estimating age of bivalves there have been few attempts to validate techniques for determining age (for review see Lutz and Rhoads 1980). There are inconsistencies in the formation of some of these internal (Crabtree et al. 1980, Hughes and Clausen 1980) and external (Gruffydd 1981) shell growth lines in bivalves grown under similar conditions as well as problems with their detection in identical specimens (Crabtree et al. 1980). Ideally some indication of accuracy and precision should be ascertained for any method that is used to estimate age in order to obtain some degree of confidence in assigning age to individual specimens. The use of known age specimens, often from culture operations has assisted in verifying the annual nature of these growth lines.

Preliminary studies by Leighton and Phleger (1977) indicated that rock scallops grown under suspended culture

conditions in California waters attained a marketable size of 120 mm in shell height in 2–3 years. Relatively slow natural growth rates, poor tolerance to low salinity and a major energy investment in shell material were suggested as factors that might inhibit *Crassadoma gigantea* from supporting a commercial operation in British Columbia (Bernard 1983b). The objective of this study was to compare the growth rates of rock scallops held in suspended culture to individuals grown on the bottom. This information would be used to assist in evaluating their mariculture potential in British Columbia.

MATERIALS AND METHODS

Information on growth rates of *Crassadoma gigantea* in British Columbia include studies on the following 3 groups of scallops:

(a) *Natural population* represented by individuals ranging in shell height from 19–175 mm. Scallops were collected from water depths of 5–20 m along the coast of Vancouver Island by SCUBA divers on approximately a monthly basis in 1985;

(b) *Natural spat* obtained from collectors in 1983, 1984 or 1985 and reared in suspended pearl and lantern nets (5–10 m) at Redonda Sea Farms, Refuge Cove (Fig. 1). Samples of known age individuals (1–3 years) were obtained in July 1986 and used to verify the existence of annual growth lines on the calcareous portion of the ligament.

(c) *Juveniles* raised in the laboratory (see Thompson et al. 1987 for details of rearing techniques) were suspended in Departure Bay at the Pacific Biological Station over a 7-month period (February–August 1985) to gain information on potential problems or advantages associated with rearing this species at various depths in the water column (5–15 m).

Natural Growth Rates

Shell height, defined as the maximum distance between dorsal (hinge) and ventral margin (Seed 1980) was recorded to the nearest 0.1 mm for all scallops using vernier calipers. Weights of the shell and soft tissue were determined separately after drying at 80°C for 48 hr. Interpretation of annual ridges on the ligament enabled age to be estimated in individuals from the natural population. The von Bertalanffy function was fitted to the data from the natural population using the following equation;

$$H_t = H_\infty [1 - e^{-K(t-t\varnothing)}]$$

where H_t = shell height at time t; H_∞ = mean asymptotic shell height (mm); K = Brody growth coefficient; and $t\varnothing$ = a parameter representing time when shell height equals 0.

Variable Growth Related to Water Depth

Juvenile scallops, raised in the laboratory and ranging in shell height from 23–31 mm, were separated into 4 groups of 30 using a random numbers table. Three of the 4 groups were placed in pearl nets and then suspended at either 5, 10, or 15 m water depth on a single line in Departure Bay, British Columbia in early February 1985 (Fig. 1). In an attempt to simulate more natural growth conditions associated with the sessile lifestyle of this bivalve, individuals from the fourth group were cemented to unpolished ceramic tiles with a drop of fast drying epoxy resin placed near the umbo. When attaching scallops to the substrate, care was taken not to hinder opening and closure of the valves and to provide adequate space to facilitate unrestricted growth. Ceramic tiles were secured to the bottom of the pearl net to prevent movement and suspended at 10 m depth.

Pearl nets were retrieved and individual shell heights measured approximately every 6 weeks. Before returning scallops to their appropriate depths all fouling organisms were removed from the pearl nets. Numbers of dead scallops were recorded in each net and percent mortalities calculated by expressing the number of dead at the end of the sampling interval (6 weeks) as a proportion of the number alive at the beginning of the interval. In August samples (n = 6) were haphazardly removed from each group to determine the weight of soft tissue to the nearest 1.0 mg after drying at 80°C for 24 hr.

Seawater temperatures were measured and duplicate water samples collected every 6 weeks from the experimental depths using a Niskin water sampler. Total particulate material in the water was concentrated by filtering seawater (2.0–4.01) through ashed preweighed Whatman GF/F filters (5.5 cm diameter). Filters were washed with a few mls of isotonic ammonium formate before drying at 80°C and reweighing. Energy content of the particulate material representing the potential food for these suspension feeding bivalves was measured by wet oxidation techniques (Newell 1982).

Estimating Production

Production of shell material (Ps) and soft tissue (Pg) were calculated for each age class of cultured and naturally grown rock scallops. Production of gametes (Pr) was not calculated separately because in young individuals (<3 yr) Pr represents <10% of total production which has also been observed for another long-lived pectinid species, *Placopecten magellanicus* (MacDonald 1986). Pg was calculated from increments in total tissue weight between consecutive year classes [W(t + 1) − Wt] and converted to energy units where lg dry weight = 24.5 kJ (Thompson 1977). Ps was estimated from annual increments in shell weight multiplied by the organic content of rock scallop

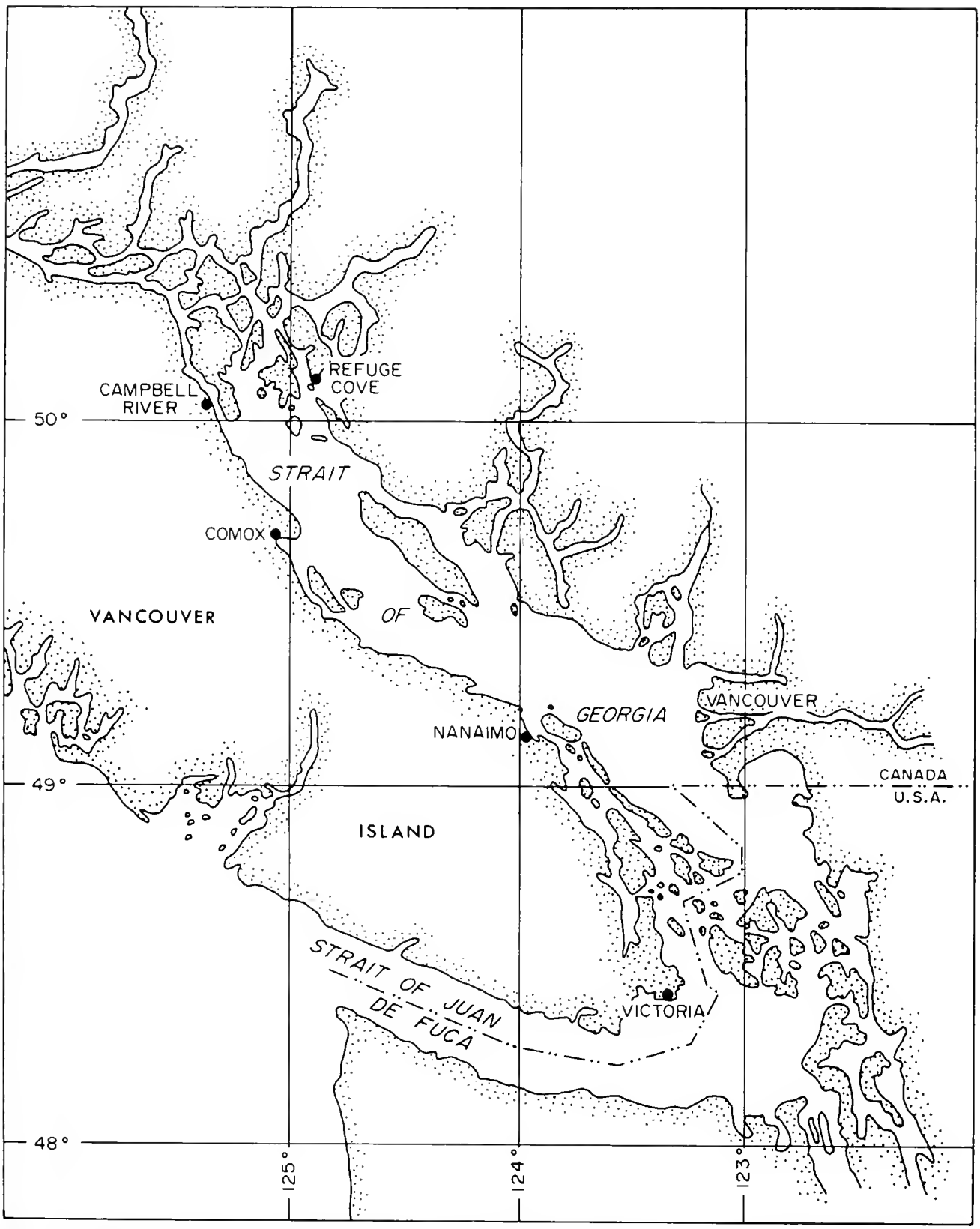


Figure 1. Locations of experimental growth sites for juveniles in Departure Bay, Nanaimo and grow-out site at Redonda Sea Farms in Refuge Cove.

shell (weight loss on ignition after 18 hr at 450°C) where lg organic matter in shell = 21.2 kJ (Hughes 1970). There was no significant difference in the organic content of shells of young cultured or wild scallops (ANCOVA $F = 0.34$, $df = 2, 18$, $P < 0.84$), so a mean value of 1.9% was used to calculate Ps for both groups.

RESULTS

Growth and Production; Natural vs. Suspended

Shell growth rate for a natural population of *Crassadoma gigantea*, consisting of individuals up to 20 years of age were described using the von Bertalanffy function (Fig. 2). Scallops grown under conditions associated with suspended culture had greater shell heights, shell weights and soft tissue weight at a given age than the natural scallop population grown on the bottom (Fig. 3, Table 1). Despite greater Ps for each age class of cultured scallops (mainly due to greater shell height), they had thinner shells per given height than their counterparts grown on the bottom (ANCOVA $F = 5.59$, $df = 2, 50$, $P > 0.001$). Statistical results were based on comparisons of individuals within the size range 30–80 mm, from both groups. The production of shell and soft tissue increased steadily with age (<5 yr) while turnover ratios (P/B) declined. P/B ratios were similar for cultured and wild scallops despite lower total production in the latter.

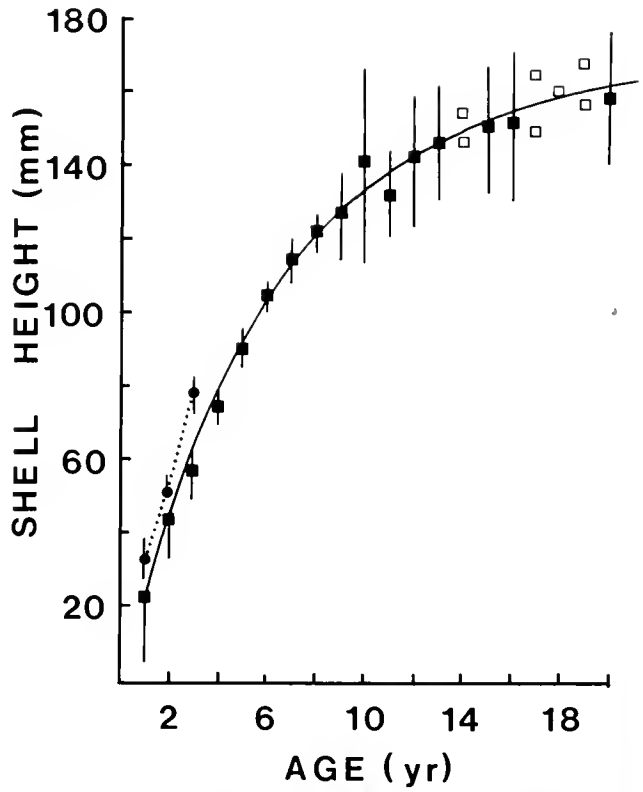


Figure 2. Mean shell heights (±95% confidence intervals) for several age classes of a natural population of *Crassadoma gigantea* (■———■) fitted to a von Bertalanffy equation ($K = 0.17$, $H_\infty = 167$ mm, $t_0 = 0.27$) and 3 age classes of cultured scallops (● · · · ●). Solid symbols represent means based on at least 3 individuals whereas open symbols (□) indicate a single data point.

Growth of Juveniles Held at Various Water Depths

Environmental Conditions

In Departure Bay water temperatures were higher at the shallowest experimental depths except during February to April when the water column was homogeneous (Fig. 4a). Particulate organic matter (POM) expressed as a percentage of total particulate matter (TPM) was also consistently greater in shallower water (Fig. 4b). As the water depth increased the energy content of available particulates decreased, especially in June, and became generally less variable temporally (Fig. 4c). More detailed seasonal environmental data from Stephens' (1966) study of particulate matter in Departure Bay revealed a similar pattern of lower variability and reduced phytoplankton abundance at greater depths (Fig. 4d). Stephens' (1966) study provided information on the spring bloom in May and confirmed that phytoplankton was relatively more abundant in shallower water of Departure Bay during the summer months.

Growth and Mortality

Changes in mean shell height for each of the 4 groups of rock scallops are shown in Fig. 5a. At the beginning of the experiment (February and March), corresponding to periods of identical water temperatures and relatively similar seston characteristics, there were no apparent differences in shell height between the 4 groups. Despite the similar water temperatures being experienced by all groups in May, differences in shell height emerged presumably related to food availability. Scallops from the shallowest depths were smallest and those attached to substrate, largest. By the end of the experiment scallops grown at 5 m unexpectedly displayed the smallest shell heights and while they were not statistically shorter than scallops grown at 15 m, they were significantly smaller than individuals grown at 10 m. Attached scallops displayed greater shell growth than their counterparts at 10 m. (ANCOVA, Waller-Duncan K ratio t tests $F = 44.2$, $df = 3,77$, $p < 0.001$).

There were no significant differences in body tissue weight between groups by the end of the experiment in August. Equivalent or greater body weights for scallops attached to the substrate indicated that resources were not diverted from somatic production in order to provide energy for additional shell production.

Higher mortality was observed for all groups early in the experiment during cool winter/spring months but was almost nonexistent during warmer months after the spring bloom. With the exception of attached scallops displaying high mortality in April, possibly associated with handling or attachment stresses, mortality was greatest in the appar-

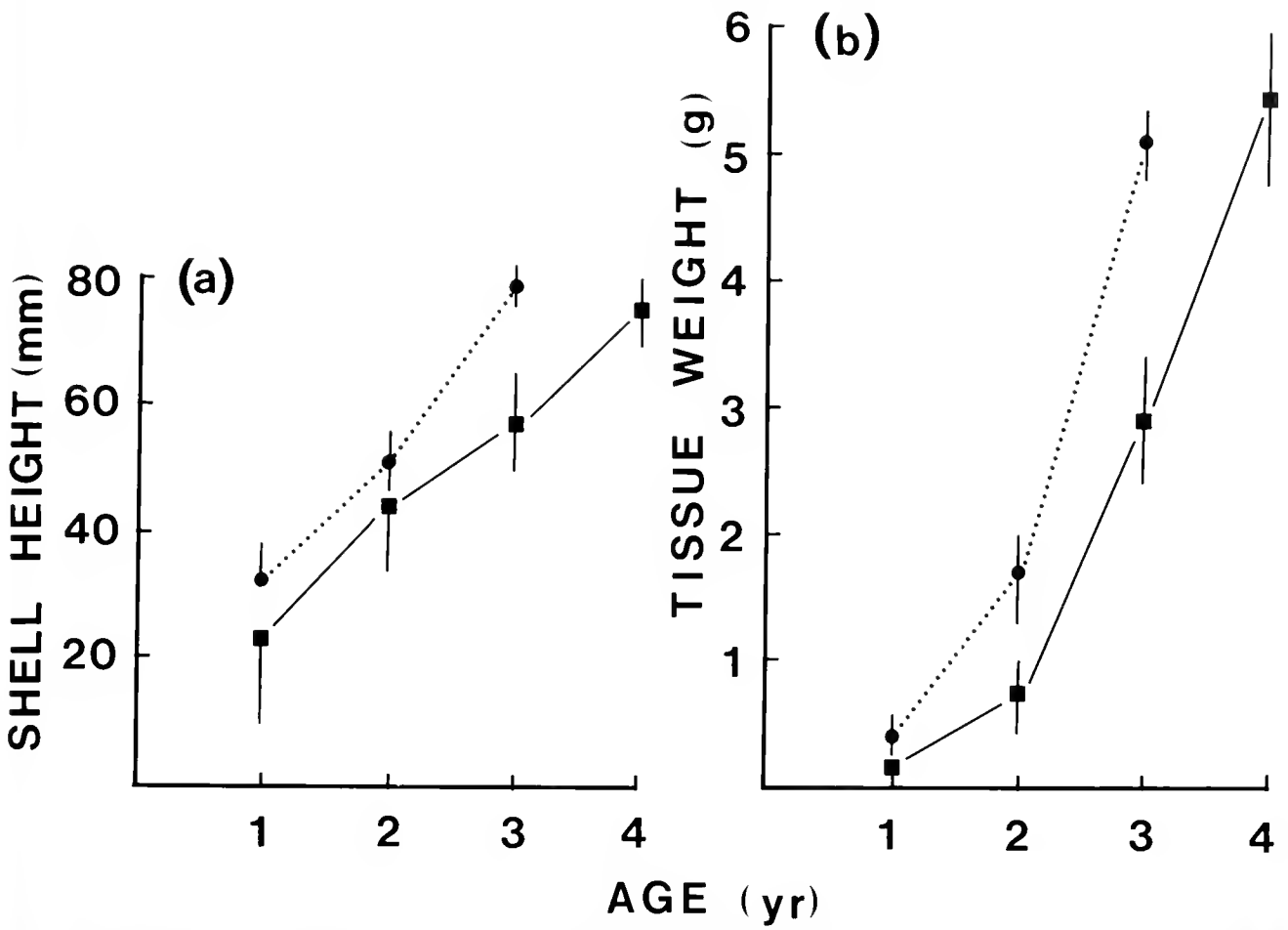


Figure 3. Age-specific estimates for (a) mean shell height and (b) total dry tissue weight (±95% confidence intervals) for *Crassadoma gigantea* grown on the bottom (■———■) and in suspended culture (● · · · ●).

ently less favorable deeper water (Fig. 5b). Mortality was not observed among scallops held at 10 m corresponding to the experimental depth generally providing the best growth rates.

DISCUSSION

The natural rate of growth for *Crassadoma gigantea* has not previously been described, apparently due to the lack of annual shell growth rings traditionally used for age deter-

TABLE I.

Estimates of mean shell height and weight plus energy equivalents for total tissue biomass (B; kJ), shell production (Ps; kJ yr^{-1}), tissue production (Pg; kJ yr^{-1}), and total production = Ps + Pg (Pt; kJ yr^{-1}) and turnover ratios (Pt/B) for individuals in each age class of natural and cultured *Crassadoma gigantea*.

Age (yr.)	Shell Ht. (mm)	Shell Wt. (g)	B (kJ)	Ps (kJ)	Pg (kJ)	Pt (kJ)	Pt/B
Suspended							
1	31.8	1.72	9.31	0.69	9.31	10.00	1.07
2	51.0	9.84	41.40	3.26	32.10	35.36	0.85
3	78.6	55.43	124.95	18.28	83.55	101.83	0.82
Natural							
1	22.8	0.93	3.43	0.37	3.43	3.80	1.11
2	44.2	4.18	17.64	1.30	14.21	15.51	0.88
3	56.9	23.19	70.90	7.62	53.26	60.88	0.86
4	75.1	55.20	133.28	12.83	62.38	75.21	0.56
5	90.7	105.60	240.92	20.21	107.64	127.85	0.53

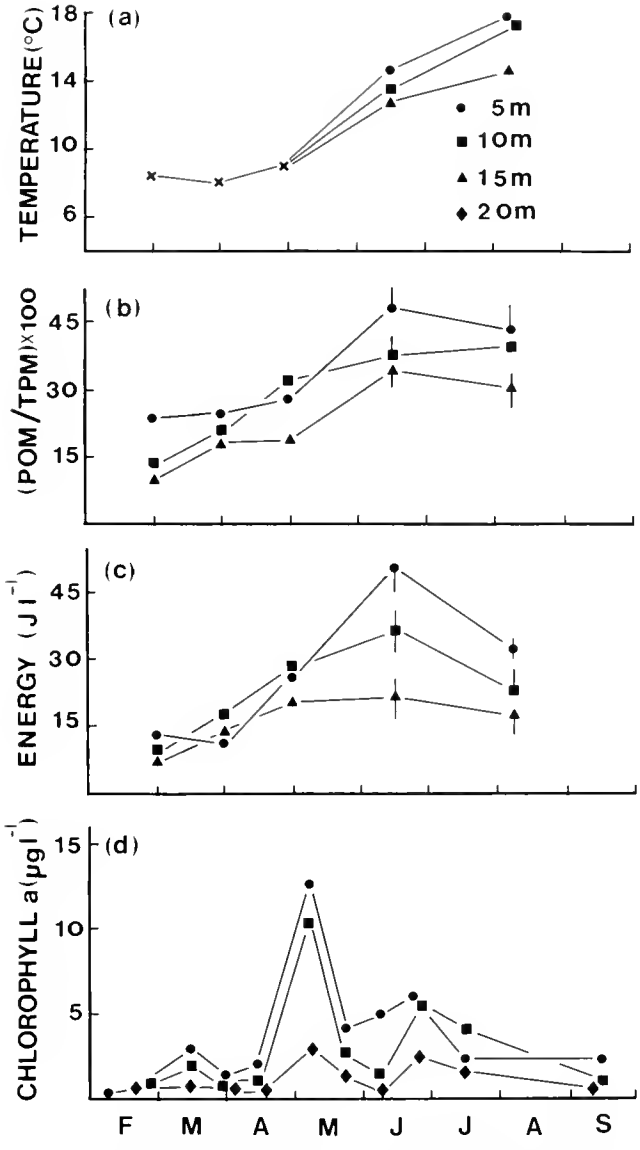


Figure 4. Seasonal cycles of (a) sea water temperature, (b) particulate organic matter (POM) expressed as a percentage of total particulate material (TPM) and (c) energy content of particulate matter at 3 water depths in Departure Bay in 1985. Chlorophyll *a* estimates (d) from Stephens' (1966) seasonal study of particulate material at similar sample depths in Departure Bay. Symbols represent means of duplicate analysis and where applicable, ranges of estimates.

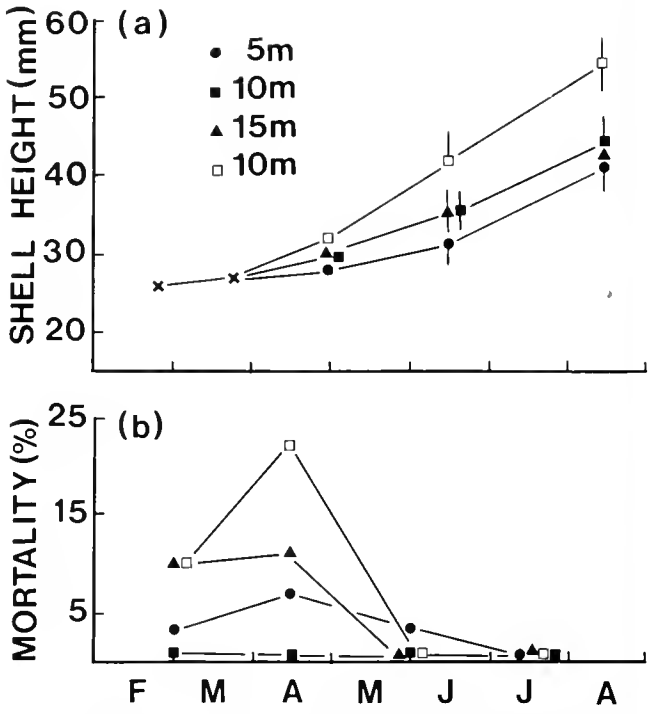


Figure 5. Monthly changes in (a) mean shell heights (±95% confidence intervals) and (b) mortality estimates for groups of *Crassadoma gigantea* suspended at three depths (5 m = ●; 10 m = ■; 15 m = ▲) in Departure Bay (attached scallops 10 m = □).

mination in pectinids. *Placopecten magellanicus* is one of the few species of scallop where external rings on the shell (Stevenson and Dickie 1954) and increments on the calcified portion of the ligament (Merrill et al. 1966) have been verified as techniques for determining individual age. While neither method is ideal, increments on the ligament more accurately reveal the true age and give more consistent results than counting external annuli on the shell of *P. magellanicus* (MacDonald 1984). Johannessen (1973) reached a similar conclusion when evaluating methods for estimating age in *Chlamys islandica*. Increments on the ligament of *C. gigantea* were confirmed to be annual using cultured specimens of known age. This enables their individual ages to be estimated and population growth rate to be described using the von Bertalanffy ($K = 0.17$, $H_\infty = 167$ mm) model for comparative purposes. Growth rates are much slower than those reported for another large species of pectinid from British Columbia *Patinopecten caurinus* ($K = 0.39$, $H_\infty = 157$ mm, MacDonald and Bourne 1987) but are similar to *P. magellanicus* on the east coast of North America (Schick et al. 1987 for summary).

The tissue production and growth in shell height shown by these rock scallops after 4 years of natural growth can be attained in 3 years in suspended culture. Similar improvements in the growth rates of scallops suspended in the water column have been reported for *Placopecten magellanicus* on the east coast (MacDonald 1986). *Patinopecten yessoensis* from Japan reach marketable size in 2 years in hanging culture (suspension) but it requires 3 years when grown on the bottom (Ventilla 1982). Despite greater costs associated with hanging culture better survivorship and higher production/unit of time make this method more efficient economically than rearing scallops on the bottom (Ventilla 1982).

It has been suggested by Foster-Smith (1975), Widdows et al. (1979) and Vahl (1980) among others, that increases in the more variable inorganic fraction (PIM) may dilute the useful organic fraction (POM) of seston thereby inhibiting feeding, energy acquisition and productivity in filter feeding bivalves. Therefore, measurement of the organic

fraction alone is not necessarily a good indicator of suitable conditions for growth. As a result, the relationship between PIM and POM has received a great deal of attention including recent studies assessing the influence of environmental factors on the growth of bivalves suspended in the water column. Studies by Wallace and Reinsnes (1985) confirmed the negative impact of PIM on *Chlamys islandica*, but, experiments on *Mytilus edulis* have revealed that growth may also be well correlated with particulate organic carbon (Page and Hubbard 1987) and chlorophyll distribution (Anders and Lopez 1988).

While the relationship between PIM and POM is an important factor to consider in bivalve feeding and growth studies, it does not provide the necessary information on biochemical composition, nutritional value or the overall quality of the organic fraction (Bayne et al. 1987). For example, in Fig. 4b and c the mean organic fraction of the seston at 5 m in early April was 24% (equivalent to 12 J l^{-1}), whereas at 10 m the seston consisted of only 21% organics but equivalent to 18 J l^{-1}, i.e., 3% more organics but less energy. This variability in food quality may partially explain why growth rates were greater at 10 m than 5 m during the first half of the experiment despite apparently better or equivalent conditions at 5 m. The relationship between PIM and POM may be of critical importance depending upon the species being considered for mariculture and whether they possess mechanisms to cope with high silt loads. The likelihood of experiencing seasonal detrimental levels of PIM should also be considered prior to selecting grow out sites.

However, variability in food quality does not explain why growth is slower at 5 m than at 15 m where potential food in terms of both quantity and quality appeared to be consistently poorer in the deeper water. Leighton (1979) also reported slower growth in his shallowest experimental group of *Crassadoma gigantea* which he attributed to competition for food and space by fouling organisms such as barnacles, bryozoans, hydroids and colonial tunicates. Wallace and Reinsnes (1985) reported fouling of *Chlamys islandica* on their shallowest enclosures (2, 12 m) but it was not severe enough to reduce growth below rates observed for the deepest group of scallops (40 m). Fouled mesh on the nets may have been partially responsible for slower growth at 5 m because the intensity of fouling on the enclosures in Department Bay decreased as water depth increased. Fouling has also been shown to have an adverse effect on growth and survival of *Argopecten irradians* (Duggan 1973).

Unless alternate methods of confinement and natural attachment of rock scallops as proposed by Phleger and Leighton (1980) offer clear advantages in growth rate or survivorship there is little point in providing substrate for attachment of this species. While scallops attached to a substrate produced more shell than free scallops from the same water depth there were no differences in weight of the soft tissue. Monical (1980) also reported similar tissue weights between free and attached rock scallops. This additional shell material was apparently produced without detracting from energy available for soft tissue production. This information plus the results of other studies by Rodhouse et al. (1984) and MacDonald (1986) demonstrating that suspended bivalves have thinner shells than individuals grown on the bottom should reduce concerns (i.e., major investment in shell) expressed by Bernard (1983b) on the mariculture potential of this species.

In California *Crassadoma gigantea* reaches a shell height of 120 mm in 2 or 3 years (Leighton and Phleger 1977). Natural rock scallop spat from British Columbia grew to a mean shell height of 80 mm in 3 years with some individuals obtaining heights of 90–100 mm. Juveniles raised in the laboratory grew rapidly from 25–52 mm during 5 summer months in Departure Bay (Fig. 5). Growth to a shell height of 100 mm within 2 years is desirable for commercial operations and may be possible through improved nursery technology that would produce 20–30 mm juveniles 3 months after spawning. Other alternatives include the possibility of marketing small whole rock scallops, as presently done with 2 local species of small scallops *Chlamys hastata* and *C. rubida* that are harvested in a small fishery in British Columbia (Bourne and Harbo 1987). Further research is needed to improve grow-out techniques. In order to evaluate fully the mariculture potential of this species additional research should include rigorous selection of sites that provide an optimal balance between water depths offering rapid growth and acceptable levels of fouling.

ACKNOWLEDGMENTS

We thank N. Gibbons of Redonda Sea Farms for giving us access to his unpublished data on growth rates of cultured scallops. Samples of natural scallops were provided by Canadian Benthic, Bamfield, British Columbia. C. Hodgson and W. Carosfeld assisted with field and laboratory measurements. While conducting this project at the Pacific Biological Station in Nanaimo, the senior author was supported by a Canadian Government Visiting Postdoctoral Fellowship.

REFERENCES CITED

Abbott, R. T. 1974. American Seashells: The Marine Mollusca of the Atlantic and Pacific Coasts of North America (2nd ed.) New York, NY: Van Nostrand Reinhold Co.

Anders, M. L. & G. Lopez. 1988. The effect of seston on the growth of bivalves at three depths in Long Island Sound. *J. Shell. Res.* 7:146–147.

Bayne, B. L., A. J. S. Hawkins & E. Navarro. 1987. Feeding and digestion by the mussel *Mytilus edulis* L., (Bivalvia: Mollusca) in mixtures

of silt and algal cells at low concentrations. *J. Exp. Mar. Biol. Ecol.* 111:1–22.

Bernard, F. R. 1983a. Catalogue of the living Bivalvia of the eastern Pacific Ocean: Bering Strait to Cape Horn. *Can. Spec. Publ. Fish. Aquat. Sci.* 61:102.

Bernard, F. R. 1983b. Physiology and the mariculture of some northeastern Pacific bivalve molluscs. *Can. Spec. Publ. Fish. Aquat. Sci.* 63:24.

Bernard, F. R. 1986. *Crassadoma* gen. nov. for "*Hinnites*" *giganteus* (Gray, 1825) from the Northeastern Pacific Ocean (Bivalvia: Pectinidae). *Venus, The Japanese Journal of Malacology* 45(1):70–74.

Bourne, N. & R. Harbo. 1987. Size limits for pink and spiny scallops. In Harbo, R. M., & G. S. Jamieson (eds.) *Status of invertebrate fisheries off the Pacific coast of Canada (1985/86) Can. Tech. Rept. Fish.* and *Aquat. Sci.* 1576, pp. 113–122.

Crabtree, D. M., C. D. Clausen & A. A. Roth. 1980. Consistency in growth line counts in bivalve specimens. *Palaegeogr. Paleoclimatol. Palaeoecol.* 29:323–340.

Duggan, W. F. 1973. Growth and survival of the bay scallop, *Argopecten irradians*, at various locations in the water column and at various densities. *Proc. Natl. Shellfish Assoc.* 63:68–71.

Foster-Smith, R. L. 1975. The effect of concentration and inert material on the assimilation of algae by three bivalves. *J. Mar. Biol. Ass. U.K.* 55:411–418.

Gruffydd, Ll. D. 1981. Observations on the rate of production of external ridges on the shell of *Pecten maximus* in the laboratory. *J. Mar. Biol. Ass. U.K.* 61:401–411.

Hughes, R. N. 1970. An energy budget for a tidal-flat population of the bivalve *Scrobicularia plana* (da Costa). *J. Anim. Ecol.* 39:357–379

Hughes, W. W. & C. D. Clausen. 1980. Variability in the formation and detection of growth increments in bivalve shells. *Paleobiology* 6:503–511.

Johannessen, O. H. 1973. Age determination in *Chlamys islandica* (O. F. Muller), *Astarte* 6:15–20.

Leighton, D. L. 1977. Experimental rearing of the purple-hinge scallop, *Hinnites multirugosus* held at several depths off La Jolla, California. *Mar. Biol.* 51:229–232.

Leighton, D. L. & C. F. Phleger. 1977. The purple-hinge rock scallop, a new candidate for marine aquaculture. *Proc. World Maricul. Soc.* 8:457–469.

Lutz, R. A. & D. C. Rhoads. 1980. Growth patterns within the molluscan shell. An overview. In Rhoads, D. C. & R. A. Lutz (eds.) *Skeletal Growth of Aquatic Organisms*, Plenum Press, New York, pp. 203–254.

MacDonald, B. A. 1984. The partitioning of energy between growth and reproduction in the giant scallop, *Placopecten magellanicus* (Gmelin). Ph.D. thesis, Memorial University of Newfoundland, 202 p.

MacDonald, B. A. 1986. Production and resource partitioning in the giant scallop *Placopecten magellanicus* grown on the bottom and in suspended culture. *Mar. Ecol. Prog. Ser.* 34:79–86.

MacDonald, B. A. & N. F. Bourne. 1987. Growth, reproductive output, and energy partitioning in weathervane scallops, *Patinopecten caurinus*, from British Columbia. *Can. J. Fish. Aquat. Sci.* 44:152–160.

Merrill, A. S., J. A. Posgay & F. E. Nichy. 1966. Annual marks on shell and ligament of sea scallop *Placopecten magellanicus Fish. Bull.* U.S. 65:299–311.

Monical, J. B. 1980. Comparative studies on growth of the purple-hinge rock scallop *Hinnites multirugosus* (Gale) in the marine waters of Southern California. *Proc. Natl. Shellfish Assoc.* 70:14–21.

Newell, R. I. E. 1982. An evaluation of the wet oxidation technique for use in determining the energy content of seston samples. *Can. J. Fish. Aquat. Sci.* 39:1383–1388.

Page, H. M. & D. M. Hubbard. 1987. Temporal and spatial patterns of growth in mussels *Mytilus edulis* on an offshore platform: relationships to water temperature and food availability. *J. Exp. Mar. Biol. Ecol.* 111:159–179.

Phleger, C. F. & D. L. Leighton. 1977. Aquaculture of the purple-hinge rock scallop. University of California, Sea Grant College Program, Institute of Marine Resources, Annual Report, 1975–76, IMR Ref. 77-104, pp 108–111. (Copies available from the Sea Grant College Program Office, Scripps Institute of Oceanography, La Jolla, California 92037, USA).

Rodhouse, P. G., C. M. Roden, M. P. Hensey & T. H. Ryan. 1984. Resource allocation in *Mytilus edulis* on the shore and in suspended culture. *Mar. Biol.* 84:27–34.

Seed, R. 1980. Shell growth and form in the Bivalvia. In Rhoads, D. C. & R. A. Lutz (eds.) *Skeletal Growth of Aquatic Organisms*, Plenum Press, New York, NY, pp. 23–68.

Schick, D. F., S. E. Shumway & M. Hunter. 1987. A comparison of growth rate between shallow water and deep water populations of the scallops *Placopecten magellanicus* (Gmelin, 1791) in the Gulf of Maine. *Amer. Malacolog. Bull.* 6:1–8.

Stephens, K. 1966. Seasonal changes in the vertical distribution of particulate and soluble material in Departure Bay, B.C. Can, MS Rep. *Fish. Aquat. Sci.* 904:32.

Stevenson, J. A. & L. M. Dickie. 1954. Annual growth rings and rate of growth of the giant scallop, *Placopecten magellanicus* (Gmelin) in the Digby area of the Bay of Fundy. *J. Fish. Res. Bd Can.* 11:660–671.

Thompson, D., N. Bourne & C. Manson. 1987. Scallop breeding studies. In Jamieson, G. S. (ed.) *1983 and 1984 Invertebrate Management Advice*, Pacific Region, Can. MS Rep. *Fish. Aquat. Sci.* 1848:97–107.

Thompson, R. J. 1977. Blood chemistry, biochemical composition and annual reproductive cycle in the giant scallop, *Placopecten magellanicus*, from southeast Newfoundland. *J. Fish. Res. Bd Can.* 34:2104–2116.

Vahl, O. 1980. Seasonal variations in seston and the growth rate of the Iceland scallop, *Chlamys islandica* (O. F. Muller) from Balsfjord, 70°N. *J. Exp. Mar. Biol. Ecol.* 48:195–204.

Ventilla, R. F. 1982. The scallop industry in Japan. *Adv. Mar. Biol.* 20:309–382.

Wallace, J. C. & T. G. Reinsnes 1985. The significance of various environmental parameters for growth of the Iceland scallop, *Chlamys islandica* (Pectinidae), in hanging culture. *Aquaculture* 44:229–242.

Widdows, J., P. Fieth and C. M. Worrall 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol.* 50:195–207.

Yonge, C. M. 1951. Studies on the Pacific coast mollusks. III. Observations on *Hinnites multirugosus* (Gale). Univ. Calif. Publ. Zool. 55:409–420.

Journal of Shellfish Research, Vol. 8, No. 1, 187–196, 1989.

THE EFFECTS OF NATURAL SESTON PARTICLE SIZE AND TYPE ON FEEDING RATES, FEEDING SELECTIVITY AND FOOD RESOURCE AVAILABILITY FOR THE MUSSEL *MYTILUS EDULIS* LINNAEUS, 1758 AT BOTTOM CULTURE SITES IN MAINE

CARTER R. NEWELL[1], SANDRA E. SHUMWAY[2,3], T. L. CUCCI[3] AND R. SELVIN[3]

[1]*Great Eastern Mussel Farms*
Tenants Harbor, Maine 04860
[2]*Maine Department of Marine Resources*
W. Boothbay Harbor, Maine 04575
[3]*Bigelow Laboratory for Ocean Science*
W. Boothbay Harbor, Maine 04575

ABSTRACT Particle selection, both as a function of size and organic content, by the mussel (*Mytilus edulis*) was investigated using flow cytometric techniques. Feeding of mussels from several discrete locations was monitored using natural particle assemblages from the respective areas as food sources. Particles were analyzed for their fluorescing intensities as well as particle size (spherical diameter) and samples were preserved for bacterial counts. Results indicated that clearance rates by mussels were approximately 40% higher on phytoplankton (particles with chlorophyll fluorescence) than on nonfluorescent particles on 5 of the 6 days sampled. On day 6 there was evidence that high levels of nonfluorescent particles inhibited the ability of mussels to feed selectively. The implications of a feeding selectivity threshold on mussel energy acquisition are discussed.

Results are compared to water samples taken directly above a commercial lease site in which food quality was lower directly over the mussel bed than that measured higher in the water column.

Prefiltering the water for analysis on a single aperture resulted in the reduction of microscopic counts by as much as 75% and much less when smaller diatoms were dominant. The presence of chain-forming phytoplankton species resulted in an underestimation of cell numbers when counted using the flow cytometer. Over two-thirds of the algal species identified from gut contents were benthic in origin. Analyses of gut contents indicate that large particles (up to 110 μm) may form a significant portion of the diet of *M. edulis*.

KEY WORDS: mussels, *Mytilus edulis*, particle selection, diet, feeding

INTRODUCTION

The feeding behavior of mussels (*Mytilus edulis*) in response to natural particle assemblages (seston) is of interest for the selection of mussel farm sites and in the calculation of carrying capacities for bottom culture sites. Variations in the concentration of phytoplankton cells and silt particles may be correlated with position in the estuary, tidal stage, storm events or location at the mussel farm site. Responses both in the initiation of feeding at low particle concentration and the behavior of a feeding selectivity response have important implications for the feeding and growth of seeded mussels.

The effects of food (seston) quantity and quality on the physiology and growth of suspension-feeding bivalve molluscs have been the subject of numerous investigations (see Bayne and Newell 1983, Winter 1978, Bayne et al. 1987 for reviews), but few have measured feeding rates using natural particle suspensions (Bayne and Widdows 1978, Thompson 1984, Widdows et al. 1984, Lucas et al. 1987). While the quality and quantity of sestonic food has been shown to affect growth (Stromgren and Cary 1984) and feeding physiology (Kiorboe et al. 1980, Widdows et al. 1979, Bayne et al. 1987) of *M. edulis*, experiments have analyzed feeding behavior in response to particle size and concentration using a Coulter Counter, in which particles are distinguished only in terms of spherical equivalence in size. Only recently have experiments considered particle type using flow cytometric techniques (Shumway et al. 1985, Cucci et al. 1985). While these experiments have indicated selective feeding by some species using mixed algal suspensions, none have used natural suspensions consisting of algal cells and inorganic particles.

Investigations of selective feeding by bivalves in mixtures of algae and silt have demonstrated feeding selectivity at food concentrations above the pseudofeces threshold (Kiorboe et al. 1980; Kiorboe et al., 1981; Kiorboe and Mohlenberg, 1981; Newell and Jordan, 1983), but none have examined whether *M. edulis* can enhance its energy gain below the pseudofeces threshold at low concentrations of seston (under 4–5 mg/l, Widdows et al. 1979) by feeding selectively on algal cells.

Reported here are experiments which examine mussel feeding behavior (clearance rate) as a function of particle type (fluorescing particles, phytoplankton vs. non-fluorescing particles) and particle size using flow cytometry and seawater pumped from above three mussel cultivation sites. Data are compared with field measurements of seston quality at a commercial lease site, and the results of the flow cytometry experiments are compared with settling chamber counts of phytoplankton in filtered and unfiltered samples.

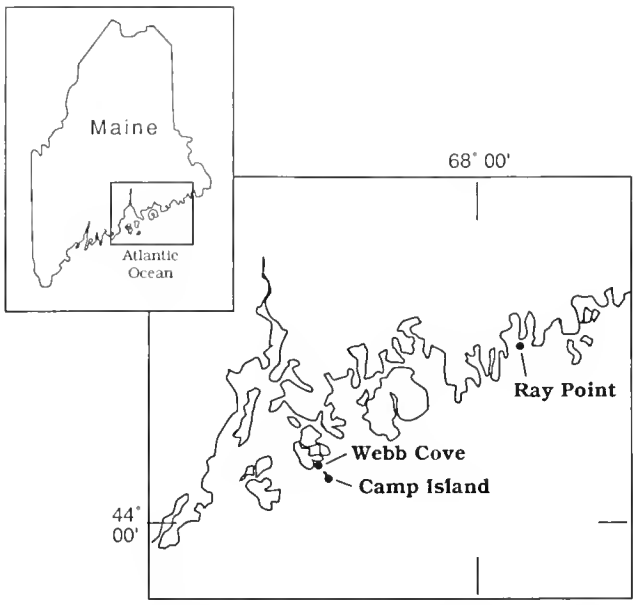


Figure 1. Sampling locations for mussels and water used in feeding experiments. All sites are commercial mussel bottom culture leases.

METHODS

A series of laboratory feeding experiments were designed to assess possible particle selection in the mussel *M. edulis*. The first set of experiments used mussels that were collected from coastal Maine waters (seeded bottom culture leases) at Webb Cove, Camp Island Cove and Ray Point (Fig. 1) one day prior to their use in the experiments and held in filtered seawater (0.7 μm) at ambient temperatures (14–15°C). This allowed the animals to purge previously ingested material from their guts. On the day of each experiment, water samples were pumped from 0.5 m off the bottom at each respective site, using a Rule 450 GPH centrifugal pump and 15 m of 13 mm interior diameter Tygon tubing. The water was prefiltered through 53 μm Nitex screening, kept refrigerated in the dark in large carboys and flown to the laboratory facilities in Boothbay Harbor, Maine. For each lease site, duplicate experiments were performed using 6 animals/experiment. Outer sites were located in deeper water near the seaward portions of the lease sites, and inner sites were closer to shore. Individual mussels were placed in gently aerated beakers containing 2l of seawater from their respective collection sites. Control vessels were left without animals to correct for changes in cell concentrations during the experiment. Experiments lasted 1 hr after which water samples were collected for flow cytometric analyses.

Horizontal variability in food availability over a subtidal mussel bed was assessed over a 600 m transect into Webb Cove. Stations 1, 3, 5, 7, 9 and 11 were pumped from 0.5 m below the surface while stations 2, 4, 6, 8, and 10 were pumped 0.5 m above the bottom (Fig. 2). Water samples were again refrigerated in the dark and flown immediately to the laboratory at Boothbay Harbor and analyzed for particle size, concentration and chlorophyll fluorescence using the FACS analyzer (see below).

Flow Cytometry (FCM)

Water samples were analyzed using a FACS Analyzer flow cytometer (Becton Dickinson, Mountainview, CA). The instrument was set up to analyze for chlorophyll a fluorescence (>665 nm) having an excitation light source of 426 (±20 nm) from a mercury arc lamp. The analyzer is able to simultaneously measure cell volume (equivocal spherical diameter, Coulter Counter principle) and chlorophyll fluorescence. As a result, the phytoplankton component can be easily distinguished from the total particulates of seawater. A total of 10,000 particles were analyzed for

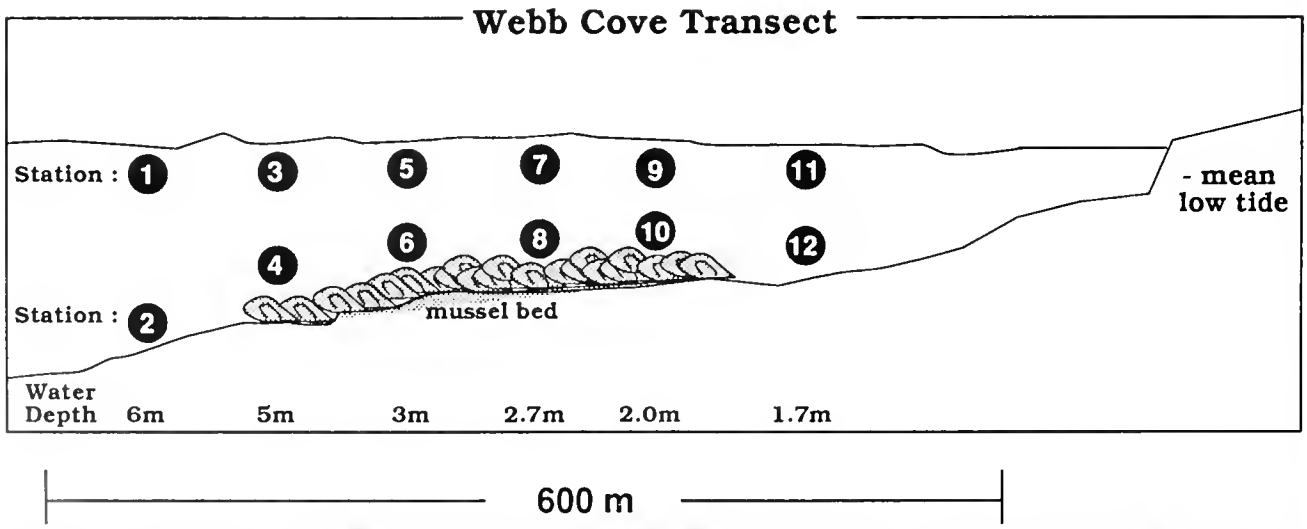


Figure 2. Transect on flood tide over seeded mussel lease area. Mussel density averaged 20–20 L m^{-2} with approximately 30% of the bottom covered with seed. Odd numbered stations were 0.5 m below the surface, even numbered stations were 0.5 m off the bottom.

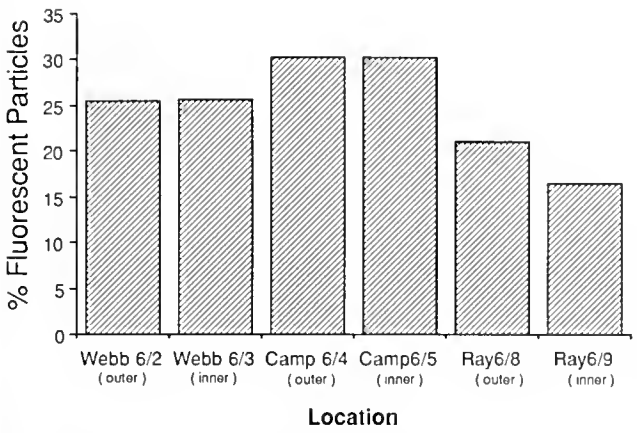


Figure 3. Food quality (% of particles with chlorophyll fluorescence) in control samples. Values are means of triplicate samples for all dates except June 2 where there was only one control.

each sample within the size range of 2.5–35 μm in diameter (using a 75 μm orifice and a current of 0.71 mA). Particles over 35 μm in diameter but under 53 μm were analyzed as fluorescing and non-fluorescing but were off scale for actual volume determination. The volume of sample analyzed was determined gravimetrically whereby the difference in weight (mg) of the sample from pre- and post-analysis was the total volume analyzed in ml. Particle densities were subsequently calculated.

Flow cytometric analyses were done on the water in each of the control and experimental vessels before the addition of the animals to the vessels (time 0 and after 60 min. Clearance rates were calculated based on the number of cells removed from suspension during the experiments using the Coughlan method (Coughlan 1969). The data were converted to clearance rates of a standard animal of 1 g dry flesh weight, using the equation:

$$\text{feeding rate} = ax^b \quad \text{where } b = 0.66$$

(Møhlenberg and Riisgard 1979)

Data were collected for total number of particles (both chlorophyll and non-chlorophyll), size fractionation of the particles present at each location and those cleared by the individual mussels. In addition, initial bacterial concentrations were determined. Settling chamber counts of cells and cell identifications were also made on filtered subsamples and unfiltered water from each of the sampling stations.

Gut Content Analysis

Mussels were collected from cages held subtidally at the laboratory in order to determine the types of particles ingested by the mussels. Gut content analyses were made immediately after collection as described previously (Shumway et al. 1987). Mussels from the collection sites used in the feeding experiments could not be used for gut content studies due to the time involved in returning the samples to the laboratory. Preliminary attempts to use these animals indicated that digestion was too rapid and after only 1 hr very few cells were identifiable from the guts.

RESULTS

The flow cytometer (FACS analyzer) allowed for the simultaneous analysis of both the size (particle volume) and fluorescence characteristics of prefiltered natural particle assemblages from 3 different lease sites on 6 separate occassions. Food quality, as percent of particles with chlorophyll fluorescence in control beakers, are presented in Fig. 3 and Table 1. Mean concentration of non-fluorescent particles reached a high of 14,200 ml-1 on June 9, and had a low of 7,139 ml-1 on June 4. Concentration of fluorescent particles (phytoplankton) had a maximum value of 4,639 ml-1 on June 2 and a low of 2,392 ml-1 on June 8. Food quality, as percent fluorescent particles, was highest at Camp Island (over 33%) on June 4 and 5 and lowest at Ray Point on June 9 (16.4%).

The effects of prefiltering water (53 μm mesh) on the numbers of phytoplankton cells were examined on 4 occasions. Settling chamber counts indicate a total reduction of cell numbers by prefiltering as high as 54.1% (Ray Point, 6/9/87) and a greater reduction (up to 74.4%) for larger cells than for total cells (Table 2). The experimental diets therefore underestimated true food resource availability at the mussel farm sites. The data also indicate that for studies of natural seston using the flow cytometer, prefiltering water samples should be done with caution. When smaller diatoms were dominant (e.g., *Chaetoceros gracilis*), there

TABLE 1.

Initial particle concentrations and food quality of experimental treatments. All samples are means of 3 controls except Webb Outer, 6/2/89, and are in numbers/ml.

Location	Date	Total Particles	Fluorescent Particles	Non-fluorescent Particles	Quality % Fluorescent
Webb Outer	6/2/87	16292	4639	11653	28.5
Webb Inner	6/3/87	10279	2830	7449	27.5
Camp Outer	6/4/87	10769	3630	7139	33.7
Camp Inner	6/5/87	10821	3629	7192	33.5
Ray Outer	6/8/87	11429	2392	9038	20.9
Ray Inner	6/9/87	16982	2782	14200	16.4

TABLE 2.

Effects of prefiltering water (53 μm mesh) on cell concentration in natural seston samples for flow cytometric analysis. Concentration is in million cells · l^{-1}.

	Total Cells			Cells over 15 μm		
Date	Unfiltered	Filtered	% Less	Unfiltered	Filtered	% Less
6/9 SFC	14.588	13.014	10.8	10.256	26.280	74.4
6/9 BOT	26.562	12.183	54.1	1.218	0.522	57.2
6/5 BOT	14.385	14.417	0	2.345	1.824	22.3
6/4 BOT	14.382	11.348	21.1	2.108	1.011	49.9

were less pronounced effects of prefiltering. Further, gut content analyses indicate that large particles (up to 110 μm) form a significant portion of the diet.

The cell concentrations estimated by the flow cytometer were compared with settling chamber counts and it was found that cell concentrations were generally underestimated by an average of 17.7% (Table 3). This is probably due to the fact that phytoplankton chains would be counted as one particle and heterotrophic flagellates would not be counted as fluorescent particles by the flow cytometer.

Particle counts from the flow cytometer allowed for separate determinations of clearance rates by the mussels as a function of:

1. Total particles (comparable to a standard Coulter Counter).
2. Fluorescent particles (phytoplankton).
3. Non-fluorescent particles (sediment particles, microheterotrophs).

Examination of the data suggests certain trends in feeding rates when examined with respect to particle type. Clearance rates for animals of 1 g standard weight were about 40% higher on the fluorescent particles (phytoplankton) than on the non-fluorescent particles on five of the six days (Table 4, Fig. 4). Clearance rates based on total cells, however, did not reveal large differences between groups.

On June 2, total particle concentration was similar to June 9 (about 16,000 · ml^{-1}) but higher food quality on June 2 (28.5% fluorescing cells) resulted in enhanced filtration rates on phytoplankton vs. non-fluorescent particles (4.45 vs. 2.2 $l \cdot h^{-1}$). On June 9, lower food quality (16.4% fluorescing cells) resulted in lower filtration rates and no evidence of particle selection for phytoplankton cells vs. non-chlorophyll particles (1.69 vs. 1.76 $l \cdot h^{-1}$).

In order to determine whether particle selection was size specific, water samples of control beakers and mussel beakers were examined for the percent of particles cleared in each size group (3–5 μm, 5–8 μm, 8–10 μm, 10–15 μm) for each of the experiments (Fig. 5). Data are not presented for particles of larger diameter due to their low frequency of occurrence in the samples. Mussels cleared higher percentages of fluorescent particles than non-fluorescing particles regardless of cell size except on June 9. It therefore appears that at least in the size range studied, feeding selectivity is not size specific.

ANOVA RESULTS

Analyses of variance were performed on clearance rates with day, lease area (Webb Cove, Camp Island and Ray Point) and location (inner lease or outer lease) as classes. Clearance rates of total particles were significantly affected by lease area ($P < 0.02$), with rates significantly higher at Webb Cove than at Camp Island or Ray Point (3.08, 2.16 and 1.99 $l \cdot h^{-1}$, respectively. However, rates on total particles were not significantly different by day or location. Clearance rates on fluorescent particles varied significantly with day with rates on June 3 higher than those on June 9 ($P < 0.0001$). Rates on fluorescent particles also varied with lease, with rates significantly higher at Webb Cove than at Camp Island or Ray Point ($P < .0001$). Clearance rates on non-fluorescent particles did not significantly vary with day. Therefore, it is concluded that higher clearance rates on total particles observed by the mussels at Webb Cove were due to their enhanced rates on phytoplankton rather than changes in their feeding rates on non-fluorescent particles.

The ratio of feeding rates on phytoplankton to rates on non-fluorescent particles (selectivity coefficient) was the lowest on June 9, correlated with the lowest clearance rates of all the experiments (1.69 $l \cdot h^{-1} \cdot g^{-1}$).

TABLE 3.

Comparison between flow cytometer (FCM) and settling chamber counts of phytoplankton cells. All samples taken 6/10/87 along field transect into Webb Cove, and were prefiltered with a 53 μm plankton net.

FCM Cells ml-l	Settling Chamber Cells ml	Percent Difference
3508	5210	−32.7
3853	5303	−27.3
4228	4095	+3.2
3328	5518	−39.7
2499	3029	−17.5
2515	2839	−11.4
1293	1275	+1.4
	Mean	+17.7%

TABLE 4.

Clearance rates of mussels with respect to particle type for each day. Values are means with standard deviations in parentheses.

Date	Location	N	Dry Wt. (g)	Total Particles $l\ h^{-1}\ g^{-1}$	Fluorescent Particles $l\ h^{-1}\ g^{-1}$	Non-fluorescent Particles $l\ h^{-1}\ g^{-1}$
6/2/87	Webb Cv. Outer	4	0.65 (.07)	2.70 (.88)	4.45 (.11)	2.20 (.77)
6/3/87	Webb Cv. Inner	6	0.41 (.09)	3.33 (.79)	5.09 (.90)	2.96 (.79)
6/4/87	Camp Is. Outer	6	0.54 (.08)	2.19 (.87)	3.13 (1.22)	1.83 (.83)
6/5/87	Camp Is. Inner	5	0.47 (.10)	2.14 (1.01)	3.06 (1.38)	1.96 (.92)
6/8/87	Ray Pt. Outer	5	0.73 (.12)	2.34 (1.05)	3.34 (0.72)	2.14 (1.10)
6/9/87	Ray Pt. Inner	6	1.07 (.26)	1.69 (.93)	1.76 (1.09)	1.66 (.93)

In order to determine on which days clearance rates were significantly enhanced on phytoplankton vs. non-fluorescent particles, paired sample one-tailed t-tests were performed for each experiment. Clearance rates were significantly higher on phytoplankton on June 2 and 3 (Webb Cove) and June 8 (Ray Point) ($P < 0.05$). On June 4 and 5 (Camp Island), low clearance rates in one individual on each day (0.63 $l \cdot h^{-1}$, June 4 and 0.76 $l \cdot h^{-1}$, June 5) contributed to high variance and non-significance in the t-tests on those two days.

A transect of water samples pumped along a water depth gradient into a commercial lease site (even numbers 0.5 m off the bottom, odd numbers 0.5 m from the surface, Fig. 2) revealed a trend toward reduced numbers of fluorescent particles (phytoplankton) on the lease, especially in the bottom waters (stations 8, 10 and 12, Table 5, Fig. 6). Food quality decreased in bottom waters (19–23% fluorescent particles) vs. surface waters (24–27% fluorescent particles) at all farm stations except the inner one (under 2 m depth) in which food quality was consistently low (16% fluorescent particles). The effects of mussels on food availability at selected sites (5 m depth and 2 m depth) are summarized in Table 6. A reduction of about ⅓ of food available to the mussels was observed along a horizontal gradient into the lease. However, the possibilities of stratification and other factors known to influence phytoplankton gradients should be considered in the interpretation of this data.

In the only other study on the food habits in *M. edulis* Field (1911) examined the digestive tracts of 50 individuals. He identified 29 species of diatoms and 9 species of protozoa. His results are presented in Table 7 with minor corrections and species sizes added. His analyses actually included 29 diatoms, 6 dinoflagellates, 1 silicoflagellate and 2 tintinnids. With only 3 exceptions (noted on Table 7) all scientific names are still valid. He also noted that detritus made up the bulk of gut contents. In the present study, detritus and bacteria also formed a major portion of the gut contents along with unidentified pennate diatoms. This is not surprising as the role of detritus as a major food source for bottom dwelling invertebrates has long been recognized. Blegvad (1914) listed all lamellibranchs as 'true detritus eaters' (see also Field 1922).

Analysis of gut contents is difficult and the loss of the more fragile cells and quickly digested species makes a complete analysis almost impossible. The variety of intact and identifiable cells found in the gut does, however, suggest that the technique is useful. Our results and those of Field (1911) (Table 7 and 8) indicate that *M. edulis* feeds commonly on large particles. This result may be misleading in that the smaller cells are more readily digested and not identifiable in the guts. In a laboratory experiment, mussels were fed on a pure culture of the cryptomonad *Chroomonas salina* (Wislouch Butcher clone 3C) and the gut contents examined immediately afterwards and at short intervals for the presence of whole cells. After only one hour, no cryptomonads were resolvable under microscopic examination. It is likely that other easily digested species including those which serve as prime food sources are overlooked through gut analyses. Examination of the algal species present in the water column at the time of samplings indicate that the mussels were feeding on all particle

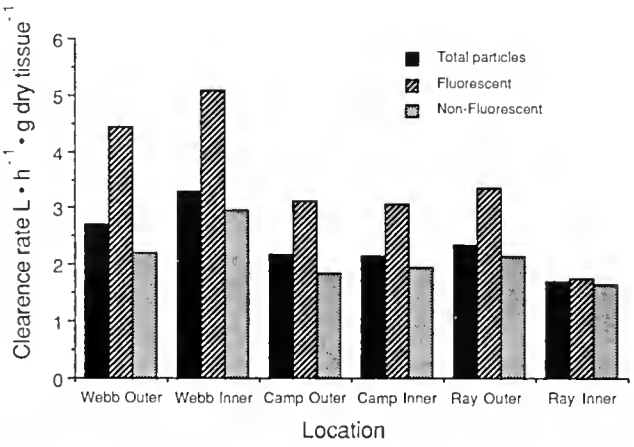


Figure 4. Clearance rates of mussels (L h^{-1}) with respect to particle type (total particles, fluorescent particles, nonfluorescent particles) for each sample location. Sample dates (1987) are the same as in Fig. 3.

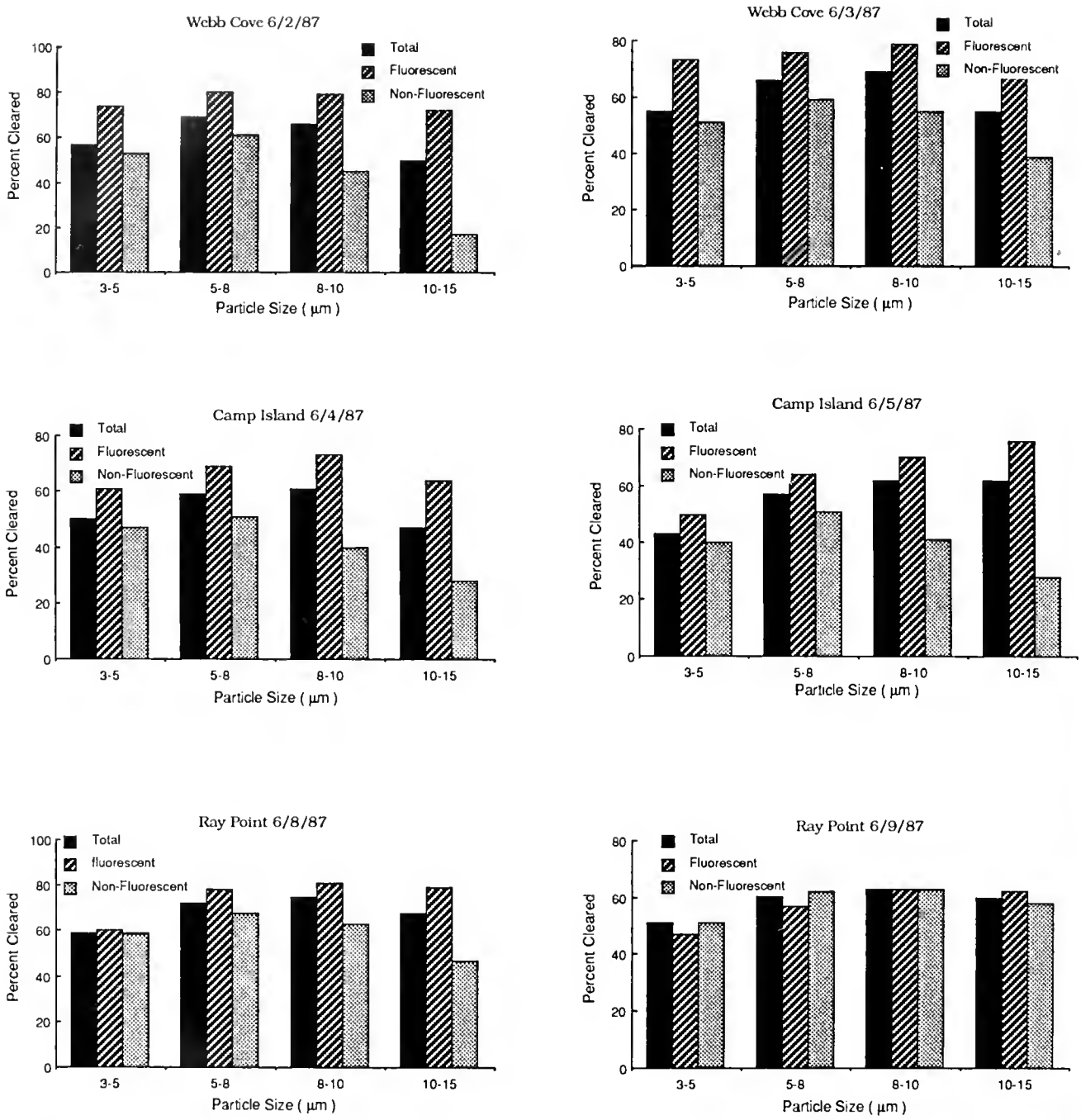


Figure 5. Percent of particles cleared during 1 hr feeding experiments with respect to particle type and size (equivalent spherical diameter). Values are means of 6 replicates except June 5, 8 (5 replicates) and June 2 (4 replicates).

types present. There was no obvious seasonal variation in food items present with the exception of *Dinophysis* spp. which were generally absent during winter months (November–March). While the mussels obviously take advantage of the phytoplankton in the water column, it is evident that they also depend, to a large extent, on resuspended bottom sediment rich in detrital matter and benthic algal species. Over ⅔ of the algal species identified by Field and in the present study are benthic in nature.

DISCUSSION

The use of the flow cytometer has allowed us to examine mussel feeding behavior with respect to particle type, and has revealed significant differences in mussel clearance

TABLE 5.

Large-scale horizontal variability in food availability along a 600 m transect into a seeded mussel lease, June 10, 1987. All water samples prefiltered with a 53 μm mesh. Concentration is particles ml^{-1}.

Station	Water Depth (m)	Total Particles	Fluorescent Particles	Non-fluorescent Particles	% Fluorescent	Bacteria $\times 10^6 l^{-1}$
1	6	11330	3113	8217	27	1256
2	6	13550	3634	9916	27	1518
3	5	13154	3508	9646	27	1523
4	5	17283	3853	13430	22	1468
5	3	15962	4228	11734	26	1548
6	3	15595	3614	11981	23	1654
7	2.7	13727	3328	10399	24	1841
8	2.7	12473	2645	9828	21	1806
9	2	9690	2499	7191	26	1957
10	2	13482	2515	10967	19	1619
11	1.7	7910	1293	6617	16	1897
12	1.7	8303	1352	6951	16	1443

rates on phytoplankton vs non-fluorescent particles, independent of particle size. Other workers (Lucas et al. 1987) found that mussels clear particles at similar rates in size ranges of 3–30 μm, but due to instrument limitations, feeding rates could not be distinguished with respect to particle type. They found a maximum resource yield, estimated by the C/N ratio, in natural particles in the size range of 5–25 μm diameter.

Our data indicates that a threshold for feeding selectivity occurs: when food quality estimated as percent fluorescent particles decreased below 20% (June 9), the mussels lost their ability to selectively filter out phytoplankton from mixed particle assemblages. Selective feeding would have the net result of leaving in suspension non-fluorescent inorganic particles with a decrease in food quality over the mussel bed. The field transect supports the results of the feeding experiments, with reduced food quality above the mussel bed at the commercial lease site. The consequences of feeding selectivity, and a possible threshold for this to occur, are that mussels on the outer edge of a lease site may have a higher food resource and higher feeding rate than those further inside the lease. Reductions in food quality may lead to a depression of feeding rates, a loss of the ability to select algae from silt particles, and slower growth of seeded mussels at inner lease sites.

Other workers (Famme and Kofoed 1983, Newell and Thompson 1985, Bayne and Widdows 1978) observed reduced feeding rates and particle retention in mussels during the spawning period, possibly due to a "shunt flow" of water bypassing the filaments of the gill demibranches. Since the experiments were performed in June, which is the normal spawning period for Maine mussels, it is possible that low clearance rates and the absence of a feeding selectivity response on June 9 were correlated with spawning condition in those mussels. Mussel mean dry flesh weight was 1.07 g on June 9 vs. 0.41 g on June 3, the days which were significantly different in clearance rates on fluorescent particles. Since mussel reproductive effort increases with mussel size (Bayne 1976), there is a chance that mussels on June 9 were reproductively active. No release of gametes

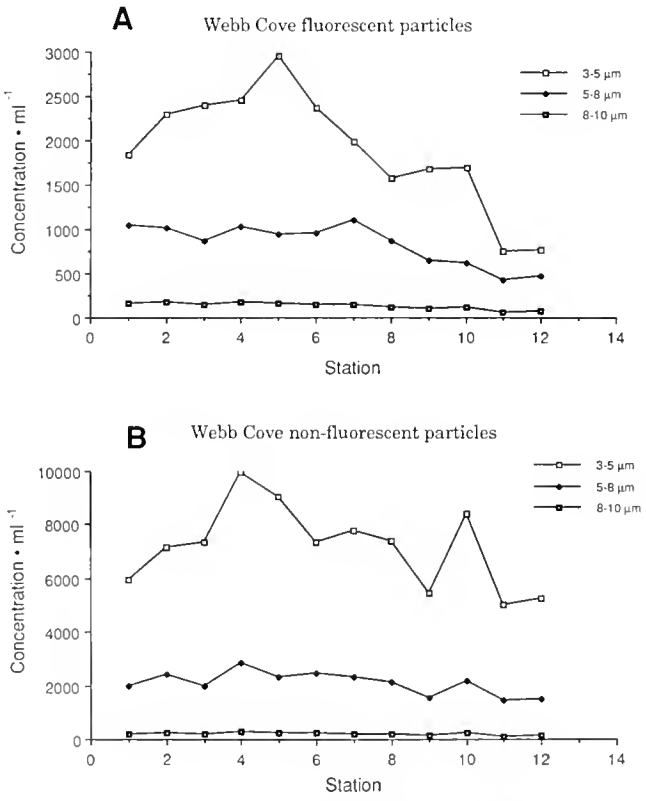


Figure 6. Large-scale horizontal variability in food availability along a 600 m transect into a seeded lease area, June 10, 1987. See text, Fig. 2 and Table 5 for details. Concentration of particles with respect to particle size for phytoplankton (A) and nonfluorescent particles (B).

TABLE 6.

Outer vs. inner cove sites: effects of mussels on food availability. Sample stations are the same as Fig. 1, all samples taken on June 10, 1987.

Station	Quality (% Fluorescent)	FCM No. Cells ml^{-1}	Mean	Settling Chamber (cells ml^{-1})	Mean	$\mu g\ Cl^{-1}$	Mean	μgNl^{-1}	Mean
3	27	3508	3681	5210	5257	462	445	57.3	57.0
4	22	3853		5303		428		56.7	
9	26	2499	2507	3029	2934	296	302	26.7	32.4
10	19	2515		2839		307		38.1	
Percent Difference			31.9		44.2		32.2		21.5

TABLE 7.

Organisms identified in gut contents of the mussel, *Mytilus edulis*, by Field (1911).

Species	Size (μm)	Habitat	Occurrence
Bacillariophyceae			
Actinoptychus undulatus	20–86	B	common
Amphipora lepidoptera	(240–280) × (32–36)	B	very common
Amphora proteus	40–60	B	frequent
Biddulphia favus	86–100; pervalver axis 40–50	P	frequent
Coscinodiscus excentricus	40–140; mostly 100	P	frequent
Grammatophora marina	60–70 × (10–12)	B	frequent
Hyaladoiscus subtilis	40–115	B	very common
Melosira sculpa	chain	B	very common
Navicula didyma	(50–90) × (17–36)	B	common
Navicula lyra	(70–120) × (27–40)	B	occasional
Navicula lanceolata	36–44	B	frequent
Navicula splendida var. *puella*	(70–160) × (24–40)	B	occasional
Nitzschia sigma	(200–240) × (10–12)	B	common
Nitzschia sigma var. *rigida*	(120–180) × (7–8)	B	common
Nitzschia sigma var. *sigmatella*	450 μ long	B	common
Pleurosigma affine	(140–220) × (28–36)	B	frequent
Pleurosigma angulatum	(128–280) × (32–36)	B	frequent
Pleurosigma balticum	(236–500) × (28–32)	B	common
Pleurosigma decorum	(220–260) × (24–28)	B	common
Pleurosigma elongatum	(130–380) × (24–30)	B	common
Pleurosigma naviculaceum	(80–100) × (15–20)	B	very common
Rhabdonema adriaticum	(40–100) × (10–15)	B	frequent
Rhabdonema arcuatum	(30–70) × (12–15) axis (50–250)	B	frequent
Rhizoselenia setigera	(8–25) length up to 300	P	very common
Stephanopyxis appendiculata varturris	40–90 μ long	P	occasional
Surirella ovalis var. ovata[1]	length 45–80	B	common
Synedra gallionii	(165–300) × (10–13)	B	very common
Tabellaria fenestrata	17–21	B	frequent
Dinophyceae			
Distephanus speculum	20–60	P	common
Exuviaella lima[2]	(32–50) × (20–28)	P	very common
Exuviaella marina[2]	(32–50) × (20–28)	P	common
Glenodinium compressa	24–64	P	common
Peridinium[3] *divergens*	(80–84) × 56	P	common
Prorocentrum micans	(35–70) × (20–50)	P	very common
OTHER			
Ceratium fusus	(200–300) × (15–30)		frequent
Tintinnopsis beroidea			very common
Tintinnopsis davidoffi			common

[1] now considered two species
[2] genus now *Prorocentrum*
[3] genus now *Protoperidinium*

TABLE 8.

Organisms identified in gut contents of the mussel, *Mytilus edulis*.

Species	Size (μm)	Habitat[a]	Occurrence
Bacillariophyceae			
Achnanthes longipes	60	B	occasional
Amphipora sp.	80	B	occasional
Amphora spp.	40	B	occasional
Coscinodiscus sp.	85	B/P	occasional
Eucampia zoodiacus	100 (chain)	P	occasional
Leptocylindrus sp.	30–45	P	occasional
Licmophora sp.	20–56	B	common
Melosira sulcata	30–40 (chain)	B	common
Navicula spp.	24–250	B	very common
Nitzschia closterium	70–100	B	common
Nitzschia seriata	100	B	occasional
Nitzschia spp.	10–100	B	very common
Pleurosigma sp.	110	B	common
Skeletonema costatum	15–35 (chain)	P	occasional
Surirella sp.	10–25	B	common
Thalassiosira spp.	15–25	P	very common
Thalassiosira gravida	20	P	occasional
Thalassiosira rotula	60–78 (chain)	P	common
Thalassiothrix nitzschioides	40–70	B	common
unidentified pennates	20–50	B	very common
Dinophyceae			
Dinophysis sp.	30	P	occasional
Dinophysis acuminata	50–55	P	occasional
Dinophysis acuta	50–65	P	occasional
Dinophysis norvegica	50–65	P	occasional
Dinophysis rotundata	35–50	P	occasional
Gonyaulax spinifera	25	P	occasional
Heterocapsa sp.	30	P	occasional
Prorocentrum micans	55	P	very common
Protogonyaulax tamarensis	35	P	common
heterotrophic dinoflagellate	50	P	common
autotrophic *Peridinium*	30–35	P	occasional
dinoflagellate cysts	35–40	B	occasional
Other			
silicoflagellate strew			common
Dictyoca	10–15	P	occasional
Distephanus	30–45	P	occasional
zooplankton strew			common
detritus			very common
bacteria			very common
motile flagellates	3–15		occasional
motile ciliates	75–110		common

[a] B = Benthic; P = pelagic

was observed during the feeding experiments. Mussel tissues were not examined histologically.

By prefiltering the water, a small aperture could be used on the flow cytometer providing resolution of clearance rates at small size scales. Problems with prefiltering include the removal of large diatoms and for this reason, the field transect data should be interpreted with caution. Since the flow cytometer counted heterotrophic flagellates as non-fluorescent particles, the experiments underestimated feeding rates on other potentially nutritious particles. However, settling chamber counts made on sub-samples of water from each experiment revealed a dominance by autotrophic diatoms, especially *Chaetoceros debile*, *Skeletonema costatum*, *Nitzschia pungens*, *Leptocylindrus minimus*, *C. compressus*, *Thalassiosira decipiens*, *C. perpusillus*, *C. gracilis*, and *Dinobryum* sp. common. In all samples, diatoms were an order of magnitude more abundant than *Cryptomonas* sp. and micro-flagellates for cells under 15 μm when examined at low power (560×). These data are in general agreement with the gut analyses which indicated a preponderance of diatoms.

At low concentrations of total seston, Bayne et al.

(1987) found that the quality of a mussel's diet is best expressed as organic matter/unit volume of particles, and that scope for growth was a function of dietary quality over the short term. Our data supports those conclusions, with an additional mechanism, the selective feeding on phytoplankton over non-flourescent particles, operating to maximize energy gain during periods of relatively high food quality at low seston concentrations. At low food quality, mussels may compensate for the absence of feeding selectivity by increasing gut fullness and absorption efficiency (Bayne et al. 1987).

It is unclear how the mussels can select algal cells over inorganic particles of equivalent spherical diameter, in the absence of pseudofeces production. Previous workers have concentrated on the role of the labial palps in this regard (Kiorboe et al. 1980, Kiorboe and Mohlenberg 1981, Newell and Jordan 1983). If selection is taking place on the gills, it may be correlated with factors such as cell shape, electrical charge, or chemical cues such as algal ectocrines (Ward and Targett 1988). If chemical cues are involved, the absence of feeding selectivity at low food quality may be due to the dilution of chemical cues below a certain threshold for a feeding selectivity response. The threshold for feeding selectivity will be examined in future experiments.

The present study clearly shows a feeding selectivity response in *M. edulis*, and a threshold at which this occurs. Siting of mussel bottom culture leases should therefore consider both the quantity and quality of sestonic food available to the mussels. Sites adjacent to intertidal mudflats subject to wind wave-induced resuspension of inorganic particles may be suboptimal for mussel feeding and growth. Sites with high proportions of algal cells (over 20% fluorescent particles as estimated with the flow cytometer) appear to be the most promising. The role of larger phytoplankton cells in the nutrition of mussels should also be considered in future experiments.

ACKNOWLEDGMENTS

This research was supported by the National Science Foundation SBIR Award ISI8660201. Thanks are due to Dick Larrabee, Bruce Davis and Jack Hamblem for field support at their mussel farm sites. We also thank R. F. H. Freeman and V. M. Bricelj for comments on the manuscript.

REFERENCES

Bayne, B. L. 1976. Aspects of reproduction in bivalve molluscs. In Wiley, M. (ed.) *Estuarine Processes, Vol. 1, Uses, stresses and adaptation to the estuary*. Academic Press, NY, pp. 432–448.

Bayne, B. L., A. J. S. Hawkins & E. Navarro. 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. *J. Exp. Mar. Biol. Ecol.* 111:1–22.

Bayne, B. L. & R. C. Newell. 1983. Physiological energetics of marine molluscs. In Wilbur, K. M. (ed.) *The Mollusca, Vol. IV*. Academic Press, NY, pp. 407–515.

Bayne, B. L. & J. Widdows. 1978. The physiological ecology of two populations of *Mytilus edulis* L. *Oecologia* 37:137–162.

Blegvad, H. 1914. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. Report, Danish Biological Station to the Board of Agriculture, Vol. XXII, 1914 (1915), p. 41–78.

Coughlan, J. 1969. The estimation of filtration rate from the clearance of suspensions. *Mar. Biol.* 2:356–358.

Cucci, T. L., S. E. Shumway, R. C. Newell, R. Selvin, R. R. L. Guillard & C. M. Yentsch. 1985. Flow cytometry: a new method for characterization of differential ingestion, digestion and egestion by suspension feeders. *Mar. Ecol. Prog. Ser.* 24:201–204.

Famme, P. & L. H. Kofoed. 1983. Shunt water flow through the mantle cavity in *Mytilus edulis* L. and its influence on particle retention. *Mar. Biol. Lett.* 4:207–218.

Field, I. A. 1911. The food value of sea mussels. Bull. U.S. Bur. Fish. Vol. XXIX pp. 85–128.

Field, I. A. 1922. Biology and economic value of the sea mussel, Mytilus edulis. Bull. U.S. Bur. Fish. XXXVIII, 127–259.

Kiørboe, T. & F. Møhlenberg. 1981. Particle selection in suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 5:291–296.

Kiørboe, T., F. Møhlenberg & O. Nohr. 1980. Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. *Ophelia* 18:193–205.

Kiørboe, T., F. Møhlenberg & O. Nohr. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis*. *Mar. Biol.* 61:283–288.

Lucas, M. I., R. C. Newell, S. E. Shumway, L. J. Seiderer & R. Bally. 1987. Particle clearance and yield in relation to bacterioplankton and suspended particle availability in estuarine and open coast populations of the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 36:215–224.

Møhlenberg, F. & H. U. Riisgard. 1979. Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. *Mar. Biol.* 54:143–147.

Newell, R. I. E. & S. J. Jordan. 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* 13:47–53.

Newell, R. I. E. & R. J. Thompson. 1984. Reduced clearance rates associated with spawning in the mussel, *Mytilus edulis* L. (Bivalvia: *Mytilidae*). Mar. Biol. Lett. 5:21–33.

Shumway, S. E., T. L. Cucci, R. C. Newell & C. M. Yentsch. 1985. Particle selection, ingestion and absorption in filter-feeding bivalves. *J. Exp. Mar. Biol. Ecol.* 91:77–92.

Shumway, S. E., R. Selvin & D. F. Schick. 1987. Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): A qualitative study. J. Shellf. Res. 6:89–95.

Stromgren, T. & C. Cary. 1984. Growth in length of *Mytilus edulis* fed on different algal diets. *J. Exp. Mar. Biol. Ecol.* 76:23–34.

Thompson, R. J. 1984. The reproductive cycle and physiological ecology of the mussel *Mytilus edulis* in a sub-artic, non-estuarine environment. *Mar. Biol.* 79:277–288.

Widdows, J., P. Fieth & C. M. Worrall. 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol.* 50:195–207.

Widdows, J., P. Donkin, P. M. Salkeld, J. J. Cleary, D. M. Lowe, S. V. Evans & P. E. Thompson. 1984. Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). *Mar. Ecol. Prog. Ser.* 17:33–47.

Winter, J. E. 1978. A review of the knowledge of suspension feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture* 13:1–33.

Journal of Shellfish Research, Vol. 8, No. 1, 197–212, 1989.

EXPERIMENTAL SHORE LEVEL TRANSPLANTATION OF THE NEW ZEALAND COCKLE *CHIONE STUTCHBURYI*

S. J. DOBBINSON, M. F. BARKER* AND J. B. JILLETT
Portobello Marine Laboratory
and Department of Zoology,
University of Otago,
Box 56
Dunedin, New Zealand

ABSTRACT The little neck clam or cockle, *Chione stutchburyi* (Wood 1828) is abundant on enclosed soft shores throughout New Zealand. In the present study in Otago Harbour on the south east of the South Island, cockles were transplanted at four different densities (0.25, 0.5, 1.0 and 1.5 × ambient density as determined from an initial field survey) from low, mid and high shore levels to each of the other shore levels. Experimental treatments were maintained for 12 months. Animals kept at their original levels acted as controls for transplant experiments. Growth was monitored monthly, survival was determined after 6 months and at the end of the experiment. Condition was also determined at the end of the experiment. The results of the initial field survey indicated that lower on the shore *Chione stutchburyi* are more numerous and larger than at higher shore levels. Mid and high shore cockles transplanted to lower shore levels showed a significant increase in size compared to controls. Larger low shore cockles showed no significant change in length at these low shore levels. Density treatments had no significant effect on growth. Mortality increased with height on the shore. High shore origin *Chione stutchburyi* suffered the least mortality but mortality of low and mid shore origin cockles increased significantly when they were transplanted to higher levels. Condition was measured by a condition index and also change in mean wet and dry weights. Density was not significant as an independent factor in measurements of condition. There was, however, a significant increase in tissue of mid and high shore origin cockles when transplanted to low shore levels while condition of large, low shore origin cockles decreased at higher shore levels. Enhanced growth and condition and lowered mortality of cockles at lower shore levels is probably due to two factors. Firstly, increased submergence time at low shore levels increases time available for feeding and secondly, lower shore bivalve populations deplete phytoplankton in water reaching upper shore populations. Harvesting of low shore cockles and transplanting small individuals from upper to lower shore levels are suggested as possible management strategies.

KEY WORDS: *Chione;* cockle; transplantation; growth; mortality; condition

INTRODUCTION

Bivalves of the family Veneridae and Cardidae are abundant in many soft shore habitats around the world, e.g. species of *Mercenaria, Chione, Venus* and *Cardium*. Many of these species are commercially exploited which has stimulated a large number of studies on their population dynamics.

Manipulative cage experiments have produced much of the recent information on growth, competitive interactions and predation of bivalves. These confirm that intraspecific competition for space and food resources is likely to be involved in controlling the abundance and size distribution of bivalves (Brock 1980, Peterson 1982a). Predation, when intense, will affect these populations especially if predators, such as flatfish or crabs, feed on juveniles (Hylleberg et al. 1978, Brock 1980, Peterson 1983a, Sanchez-Salazar et al. 1987).

In New Zealand the commercial exploitation of soft-shore bivalves has been small, nevertheless such bivalves have historically played an important role in recreational fishing. One of the most abundant species on enclosed soft shores is the cockle *Chione stutchburyi* (Wood 1828). It has been the subject of several studies on growth, recruitment, distribution, ecology and general biology. Larcombe (1971) compared its distribution around the country. He found that most populations consist of adult *Chione stutchburyi* of mean size approaching their maximum lengths. His studies indicated that growth rates and potential production of *Chione stutchburyi* declined with increasing elevation above low water and with distance from the entrance of the harbour where larger volumes of water are replaced per tide. Larcombe (1971) attributed these relationships to two factors, both dependent on the feeding mechanisms of *Chione stutchburyi:* (i) that shore height differences determined the duration of submergence of the *Chione stutchburyi* limiting the time available for these suspension feeders to obtain organic matter, and (ii) that the water flowing over large populations of suspension feeders would become depleted of organic matter.

Biological interactions of *Chione stutchburyi* have been investigated in the Avon-Heathcote Estuary (Stephenson 1981), and in the Ohiwa Harbour (Blackwell 1984). Blackwell studied two different regions of the Ohiwa Harbour *Chione stutchburyi* populations, a channel and a more estuarine region. Patterns of favourable growth and recruitment in areas already highly populated with adult *Chione stutchburyi* were evident from frequency distribution data of the natural population, including estimates of individual growth rates and his experiments manipulating density and micro elevation (shore height). Both Stephenson and Blackwell's density manipulations resulted in apparent in-

*To whom requests for reprints should be sent.

traspecific competition limiting growth, recruitment and increasing mortality at higher density levels.

Surveys of Otago Harbour and Blueskin Bay intertidal flats (Wildish 1984, Voice 1975, Witman 1974) indicate that at this latitude (45°S), the influence of tidal height may play a major role in determining growth, survival and potential production of the populations. However, there has been little work on the role of population density in influencing these factors in this region. The present study investigates the effects of tidal height and intraspecific competition on the growth and survival of *Chione stutchburyi* in Otago Harbour. Manipulative experiments were designed to transplant *Chione stutchburyi* at different densities to different shore levels to determine the effect on growth and survival at increasing height above chart datum.

MATERIALS AND METHODS

Study Area

Otago Harbour (latitude 45° 47–53′ S; longitude 170° 44–30′ E) is an inlet formed between the Otago Peninsula and the main coastline of the south east of the South Island, New Zealand. The harbour waters range in salinity from 31.53–34.79‰ (Robertson 1973). Lower salinities are produced on mixing with fresh water from the Leith River at the head of the harbour. Seasonally temperatures vary between monthly means of 16°C in summer and 6.4°C in winter. It is likely that temperatures on the shallow intertidal flats are similar to those measured by Roper (1979) in the shallows of Papanui Inlet. These tend to be warmer in summer (20–25°C), and colder in winter (minimum 3°C), than the main channel temperatures.

The Harwood intertidal sand flats where experiments were conducted are located toward the entrance of the Otago Harbour on the Otago Peninsula. Water reaches this area from both a subsidiary channel of Lower Portobello Bay, and from the main shipping channel crossing the expansive intertidal flats to the north of the site (Fig. 1). Tides are semi-diurnal and have a mean range of 1.7 m from MHW to MLW at spring tides.

The beach slopes gently and is exposed for several hundred metres at low tide. The fauna of these intertidal flats resembles that of many enclosed and protected soft shores in New Zealand, as described by Morton and Miller (1973). The infauna is dominated by a variety of deposit and suspension feeding animals. The dominant bivalves of this beach are *Chione stutchburyi* and *Tellina liliana*. Other local beaches are equally well populated by *Chione stutchburyi*. Harwood was chosen for its relatively uniform topography and gradual slope to the main channel, these providing a more homogeneous site for the experiments having less site effects than areas with tidal pools and raised banks. Difficult parking along the road and permanent residents adjacent to the area were thought to be sufficient to deter vandalism of experimental cages.

Initial Field Survey

A field survey of the *Chione stutchburyi* populations at Harwood was carried out at the beginning of the study in mid April 1984. This was to establish whether typical gradients in size and biomass occurred with change in tidal height on these sand flats. Samples of *Chione stutchburyi* were collected from 0.25 m^2 quadrates of sediment at different shore levels from 5 transects spaced approximately 80 m apart along the shore (transect A–E as marked on Fig. 1). All infauna was removed to a depth of 10 cm with a spade, then sieved through a 2 mm mesh circular framed sieve. Samples were taken from low water up to the upper edge of the sand flat. Sampling was not continued to the high water mark as the top of the intertidal includes a stone wall that supports the road. The number of cockles from each sample, their shell length and the biomass per sample was determined after each sample of *Chione stutchburyi* was scraped clean of algae and debris.

Experimental Design

The results of the initial field survey indicated that both size and numerical density/shore level gradient was a general feature of the whole of the surveyed area. However, the southern region of this area was chosen for the detailed experiments as an embayment at the northern end of the surveyed area may have biased treatments along the shore. On the 12th May 1984 on a low spring tide, experimental manipulations of cockles commenced at three shore levels immediately to the south of transect E as shown on Figure 1: low shore at 0.0 m above chart datum (CD), a mid shore level at 0.42 m above CD and a high shore level at 0.65 m above CD. *Chione stutchburyi* of sizes 44–48 mm, 21–24 mm and 17–20 mm (mode sizes from low to high water as determined from the initial survey, see Fig. 3) were used for transplant and density manipulations of low, mid and high shore cockles. Cockles from all three original shore levels were transplanted to each other shore level. The *Chione stutchburyi* remaining at their original elevations provided controls for transplant experiments. These were maintained at four different density levels dependent on the original ambient density (×) at each shore level. Thus 0.25, 0.5, 1.0 and 1.5 (×) density levels were used. Each treatment had three replicates. A fully crossed multifactorial analysis of variance (ANOVA) was used in data analysis. The twelve different treatments with three replicates at each shore level required 108 enclosures (here after termed cages). The cages consisted of strips of galvanised mesh welded to form circular fences of 0.5 m^{-2} area and 20 cm in height. Neighbouring cages were separated by 10 cm spaces and were positioned by pushing them vertically into the sediment. Gradual sinking of the cages occurred and was corrected periodically to the original level. Over summer months large pieces of free floating algae which had formed during spring growth frequently became trapped within the mesh of the cages. This was periodically removed from the cage edges and sediment surface.

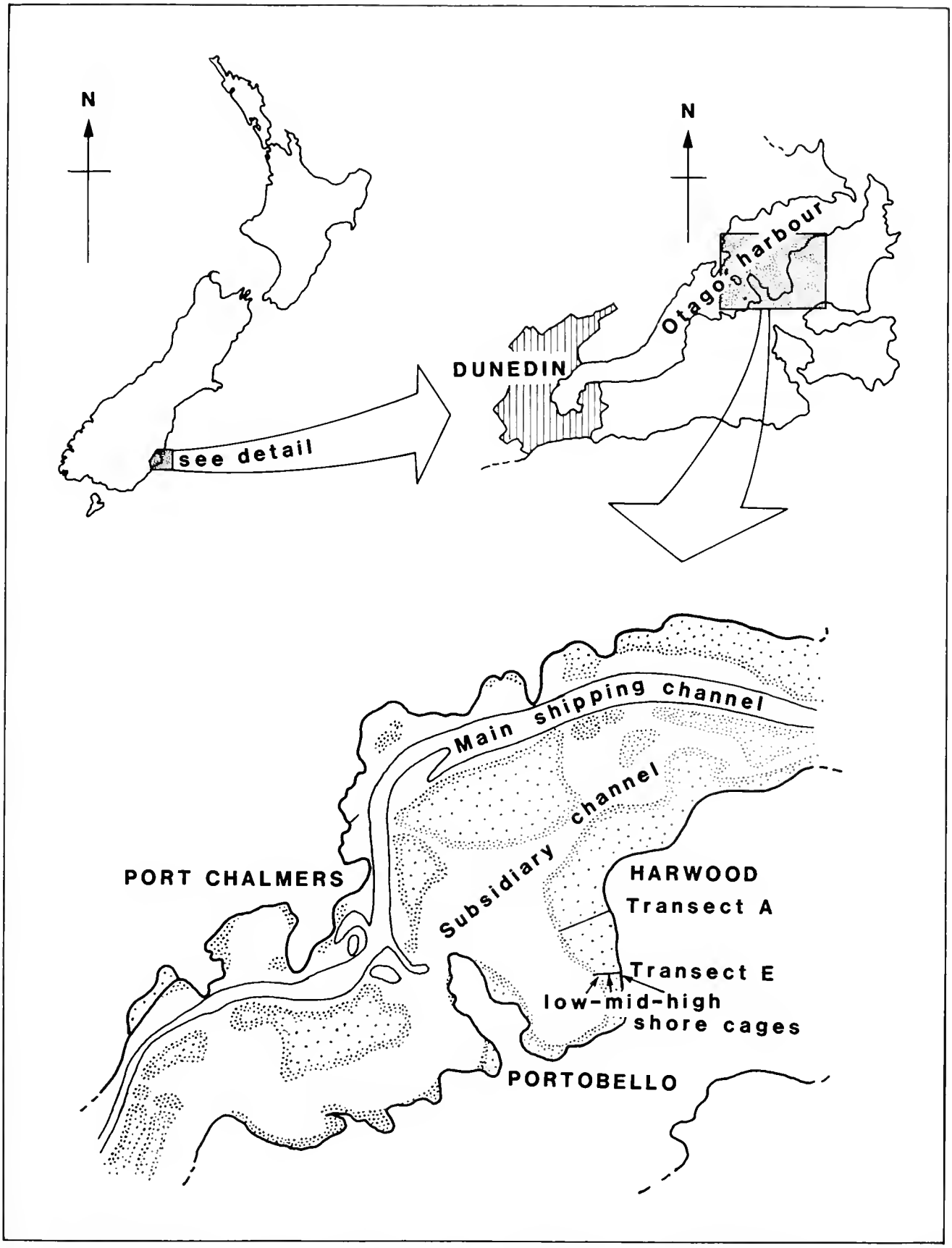


Figure 1. The study site at Harwood within Otago Harbour and its location on the southwest coast of New Zealand. The positions of transects used for the initial field survey and experimental cages are shown.

In setting up the experiments the sediment and infauna were removed to a depth of 10 cm or until a relic shell bed was reached. Residual cockles and shells were located and removed using the finger ploughing technique described by Peterson (1982a). The sediment was returned to the cages by washing it through a 2 mm sieve.

Chione stutchburyi used in experimental treatments were collected from strips of the sand flats at the same levels as the cages. Shell length of all experimental cockles was measured using a measuring board marked at 44–48, 21–24 and 17–20 mm intervals. The cockles selected were individually wiped to remove excess water and sediment before a quarter of a valve per cockle was covered with red enamel modelling paint and left to dry. Cockles remained out of seawater for an average of 6 hours (including the collection time). The cockles for the experimental treatments were kept for a maximum of 4–6 days in the laboratory in oxygenated seawater tanks until they were ready to be returned to the study site. They were then removed and counted before being placed in the appropriate cage. The selection, marking and placement procedure was carried out over the winter months and by 16th September 1984 the experimental treatments were complete with a total of 30,861 caged *Chione stutchburyi*.

Growth

Monthly sampling of shell lengths of experimental treatments began at the end of September 1984 and continued over the 1984–85 summer until the end of the growth season in May 1985. It took up to 6 days each month to sample each of the 108 cages. Twenty five cockles randomly selected from each cage were taken back to the laboratory in labelled plastic pottles. The shell length of each *Chione stutchburyi* in the sample was measured to the nearest 0.1 mm and recorded. They were returned to the correct pottle and placed in seawater to be returned to their original cage the next day.

Survival

Survival counts were carried out in November 1984 and May 1985. The sediment within each cage was removed by digging and then checking by the finger ploughing technique for remaining *Chione stutchburyi* and the sediment sieved back into the cages by washing through the 2 mm sieve. All live marked cockles, after counting and recording, were returned to cages. Broken and empty shells were retained in labelled plastic bags and then recorded. Immigrant cockles were counted and recorded then returned to the area outside the cages. In May 1985, at the completion of the experiment all migrants, live cockles and empty shells were collected in labelled bags. The cockles were taken to the laboratory and either counted on arrival or stored in flowing seawater to be counted at a later date.

The percentage mortality per cage was calculated from the number of whole marked shells and the number of single valves/2 collected during growth sampling and from the November survival counts and the final May survival count. The mortality rate was based on the percentage of dead to live marked shells retrieved. This method does not assume that all marked *Chione stutchburyi* lost from the cages were dead. Brousseau (1978) also based her mortality rates only on the bivalves recovered.

Condition

Cockles collected at the end of the experiment were left for a minimum of 24 hours in running seawater in the laboratory. Larcombe (1971) and Blackwell (1984) state that 24 hours is sufficient to clear any sediment remaining in the digestive tracts of *Chione stutchburyi*, or at least to standardize any proportion of sediment in the population. A random sample of 10 cockles from each cage was taken and their shell lengths measured to the nearest 0.1 mm. Algae or sediment present was removed from each cockle in the sample and excess water removed. The total weight of the sample was measured and the total volume determined by displacement in a measuring cylinder (see later). The cockles were opened by steaming, the cooked flesh and shell were then immersed in cold fresh water to prevent shrinkage. The flesh was carefully removed from each shell, excess water blotted away and the flesh volume and flesh weight of the sample were determined. The flesh was dried to a constant weight at 100°C and the weight recorded. The empty shells were blotted dry and the shell weight of the sample was recorded and the total shell volume of the sample was determined. All weights were recorded on an electronic balance to the nearest 0.01 gm. Volumes were determined by measuring the water displaced by addition of the cockle shell or tissue to two displacement jars with volumes of 132.6 and 726.1 mm of the type described by Baird (1958) and Franklin (1971). This allowed calculation of the displaced volume to the nearest 0.1 mm.

The mean values of these measurements were used to calculate the condition index for each sample of cockles based on the flesh to internal shell cavity ratio, described by Baird (1958). Condition indices plus the wet and dry flesh weight and mortality data for the different samples was analysed by three factor ANOVA while the growth data was analysed by a four factor ANOVA. The means were compared using a Student-Newman-Kuels (S-N-K) test.

Sedimentology

At the completion of the experiment in May 1985, before the cages were dismantled, sediment samples were taken from inside and outside of the cages at each end and at the middle of each of the rows of cages at each shore level. Grain size analysis was carried out by a similar

method to that described by Folk (1968). The data was analysed graphically. Sorting, skewness, kurtosis and mean and mode grain sizes were recorded.

Surveying

Elevation of the cages was measured using a surveyor's level and theodolite. A theoretical submergence rate for each shore level was calculated from tidal predictions in the N.Z. Nautical Almanac.

RESULTS

Initial Survey

The initial survey of the natural population showed that *Chione stutchburyi* were more numerous toward the lower shore (Table 1). The lower shore quadrats also contained a wider size range of *Chione stutchburyi,* frequently with bimodal peaks of mainly large individuals and a few smaller individuals of less than 20 mm. Further up the shore *Chione stutchburyi* were less numerous and of a smaller size. Based on the criterion of Larcombe (1971), individuals capable of reproduction are those greater than 20 mm in length. Individuals of this size were found predominantly below low water neap tides on this intertidal sand flat.

Increase in length towards the lower shore is also reflected in an increase in biomass down the shore towards the main channel (Fig. 2). The numeric density of *Chione stutchburyi* increased towards the lower shore but often reached a peak at the mid tide level (this was consistent within the chosen study area). The mean size ($gm \cdot 0.25m^{-2}$) of cockles increased towards the lower shore in all 5 transects with one minor exception at a single station at 200 m in transect B (Fig. 2B). The increased potential growth towards low spring tides is a nonlinear relationship, some distortion may be attributed to the embayment. Transect A is most affected (Fig. 2A). This transect is exposed for longer periods at each tidal cycle and may also be influenced by a drainage channel that traverses the lower end of this transect. The shell length range was from 5–51 mm, the maximum length being recorded from a quadrate at the low shore cage level. The southern end was chosen as the main experimental site because, as mentioned earlier, it avoided the embayment feature and displayed the

TABLE 1.

Density and length frequency of *Chione stutchburyi* collected from 0.25 m² quadrats sampled along 5 transects (A to E, see figure 1).

Site	Density No. · 0.25 m^{-2}	Length (mm)	Frequency per shell length class (mm) 0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45+	Ht. above C.D. (m)
A1	10	20.0	0	0	0	4	6	0	0	0	0	0	1.25
A2	84	16.9	0	0	11	63	10	0	0	0	0	0	0.91
A3	180	17.1	0	5	28	113	34	0	0	0	0	0	0.52
A4	397	20.2	0	21	11	97	222	44	2	0	0	0	0.32
A5	266	27.8	0	0	15	20	6	101	118	4	2	0	0.21
A6	267	30.7	0	4	24	19	4	5	81	122	7	1	0.13
B1	24	18.2	0	0	0	21	3	0	0	0	0	0	1.20
B2	85	16.5	0	0	13	70	2	0	0	0	0	0	1.04
B3	150	15.7	0	1	46	94	9	0	0	0	0	0	0.88
B4	237	16.6	0	25	26	137	48	1	0	0	0	0	0.72
B5	179	17.7	0	1	21	112	45	0	0	0	0	0	0.56
B6	0												0.40
C1	28	20.3	0	0	1	8	19	0	0	0	0	0	1.15
C2	85	16.3	0	0	10	70	5	0	0	0	0	0	0.89
C3	148	15.7	0	2	44	92	10	0	0	0	0	0	0.64
C4	167	20.8	0	2	7	34	110	14	0	0	0	0	0.38
C5	279	26.1	0	0	25	25	17	120	86	4	0	0	0.17
D1	87	18.7	0	0	12	82	3	0	0	0	0	0	1.10
D2	137	15.6	0	1	42	91	3	0	0	0	0	0	0.98
D3	187	16.1	0	0	43	129	14	1	0	0	0	0	0.83
D4	169	18.7	0	0	6	95	67	1	0	0	0	0	0.67
D5	208	20.8	0	3	11	38	144	12	0	0	0	0	0.51
D6	387	24.6	0	6	25	18	84	226	28	0	0	0	0.35
E1	43	17.4	0	0	5	28	10	0	0	0	0	0	1.05
E2	138	19.4	0	1	5	66	64	0	2	0	0	0	0.89
E3	273	21.6	0	0	23	31	168	49	2	0	0	0	0.73
E4	292	29.2	0	0	12	18	2	78	159	22	1	0	0.57

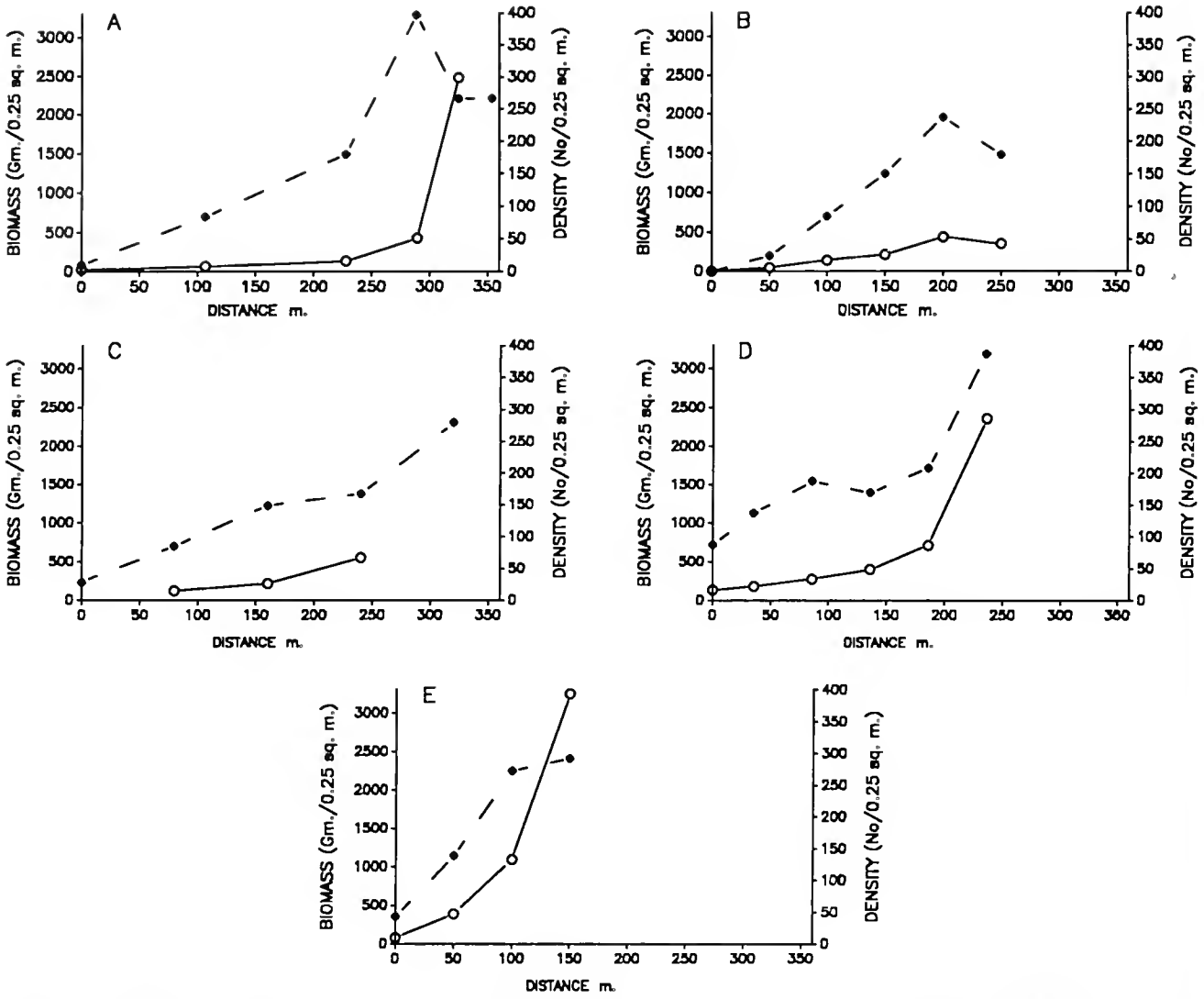


Figure 2. Biomass (open circles, solid line) and density (filled circles dotted line) in grams and number 0.25m^{-2} respectively for *Chione stutchburyi* from the 5 transects sampled in the initial field survey. Transect A is at the northern end of the shore; transect E at the southern end. The seawall at the upper end of the shore is at 0 meters and 350 meters is approximately low water spring tide at the point where the greatest area of shore is exposed.

broad scale size gradients consistent with the rest of the surveyed area (Fig. 3 shows the size frequency of cockles found at low, mid and high shore levels at this southern site). Maximum densities were also recorded at the south east end of the surveyed area at transect E. Densities within the surveyed populations ranged from 0–15,588 m^{-2} and biomass from 0–12.98 kg m^{-2}.

Growth

Shell length data for the three shore levels and four density treatments, collected from September 1984 to May 1985, was analysed in a 4 factor ANOVA (Table 2). To normalize the raw data, a log e transformation was required. The transformed data then conformed to skewness and kurtosis tests. Analysis of variance is robust and operates well despite heterogeneity of variances when sample numbers are equal. Comparison of means using the SNK test was carried out where the null hypothesis, that all the means are equal, was rejected ($p < 0.05$). Bartlett's test was not used and is regarded as being subject to failure in use with non-normal data (Zar 1974).

The results of the time × shorelevel × origin interaction (Fig. 4) indicate that a significant difference ($p < 0.001$) in mean shell length occurs at different origins and shore levels of *Chione stutchburyi*. The larger low shore origin cockles had no significant change ($p < 0.05$, SNK test) in shell length over the experimental period (Fig. 4A, Table 2). The smaller mid and high shore cockles showed significant increase ($p < 0.05$, SNK test) at the low shore level during the first five or six months of the sampling

(Fig. 4B & C and Fig. 5) but by late autumn this growth began to decline. It is possible the increase in standard deviation with increase in length may have produced an artificial peak in shell length by March–April. Similar patterns of increase in shell length are supported by the shore level × origin × density (Fig. 6) and time × density × shore level SNK tests (Table 2). These two interactions show the density treatments had no significant effect on the shell length growth. It should also be noted that significant growth did not occur for mid and high shore cockles at ambient densities. The only significant change in shell length ($p < 0.05$) is at ½ (×) ambient density, (see density SNK test Table 2). This increase was not sustained at ¼ (×) ambient density.

These results indicate that significant increase in shell length only occurred at the low shore level and was not influenced by the density treatments. Growth curves (Fig. 7) were thus fitted by the least squares method, only from the untransformed data collected from ambient density cages placed at the low shore level. The parameters for each fitted curve are found in Table 3. Various growth equations have been developed to describe the rate of growth in fish populations. The growth rates shown are derived from relatively few *Chione stutchburyi* (n = 25), and cover a very narrow size range. The least squares method was considered more accurate for this data than the Walford plots which require larger proportions of the size range of the populations to estimate the parameters of the Von Bertalanffy curve.

The large low shore origin cockles growth curve of best fit is a polynomial curve of negative slope (Fig. 7A, for parameters of these equations see Table 3), resulting from a lack of increase in mean shell length over the experimental period. It has a rate of $r = 0.999$, $Y = 46.26 - 0.09136\ r^{kx}$.

Mid and high shore origin growth curves are similar and have growth rates within two standard deviations of each other, 0.827 and 0.762 respectively (Fig. 7B & C), distinct from the rate of growth of the low shore origin cockles. The equation for mid shore origin cockles is $Y = 27.932 - 10.685\ e^{-kx}$. The equation for the high shore origin curve is $Y = 27.785 - 6.874\ e^{-kx}$.

From these equations the maximum growth for the season may be estimated from parameter A (parameter B here represents the decrease in growth rate with season as well as the decrease in growth rate with increase in length). This was 6mm for the mid shore origin and 10mm for the high shore origin *Chione stutchburyi* transplanted to the low shore level cages.

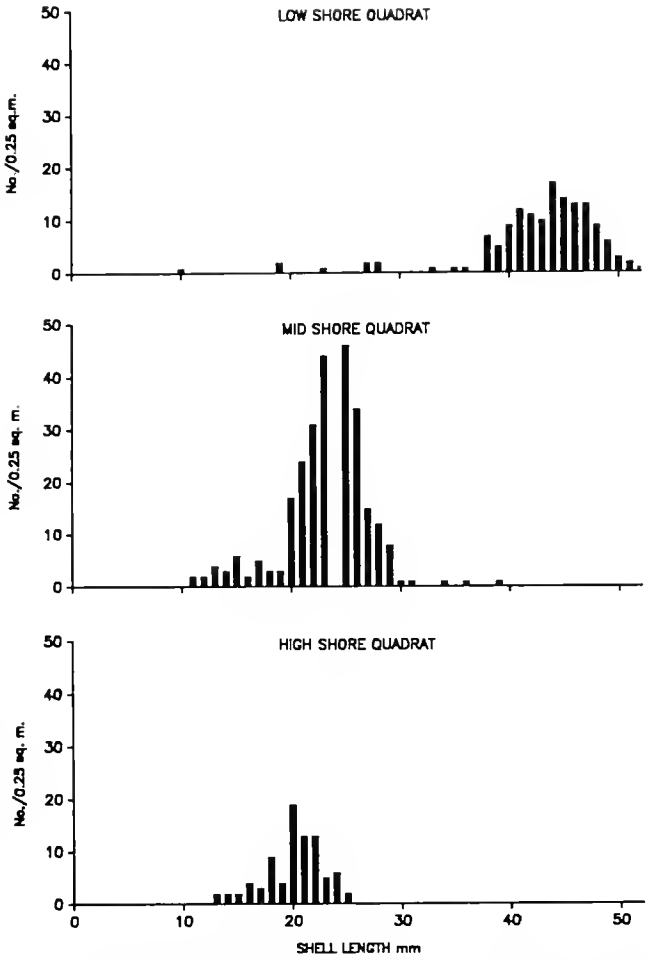


Figure 3. Length frequency histograms from 0.25 m^{-2} quadrats taken at the southern end of the cage experiments at low, mid and high score levels, 0 m, 0.42 m and 0.65 m above chart datum respectively.

Mortality

Percentage mortality for the shore level and density treatments was transformed by using an arc sine function after multiplication by 100. This transformed data was analysed in a three factor ANOVA (Table 4).

The effects of both the shore level and origin factors are significant ($p < 0.001$). Mortality increases with height above chart datum (see Fig. 8A). High shore origin *Chione stutchburyi* suffer the least mortality and the SNK tests (Table 4) indicate they are least affected by shore level treatments. Mortality of the low and mid shore origin *Chione stutchburyi* increased at the mid and high shore level cages. Mortality of these cockles at the low shore levels was significantly smaller and similar to mortality levels of high shore origin *Chione stutchburyi*.

The ANOVA and SNK tests (Table IV, Fig. 8B) indicate that the density treatments had no significant effect ($p < 0.05$) on the mortality of *Chione stutchburyi*. It is possible that with further replication the increase in mortality at the maximum density level at the high shore level might have become significant.

TABLE 2.

Analysis of variance and S-N-K tests on log e transformed shell length data.

Treatments
Times (T): T1 = Sept., T2 = Dec., T3 = Jan., T4 = Feb., T5 = March, T6 = April, T7 = May
Shore level (S): low, mid, high
Origin (O): low, mid, high
Density (D): ×¼, ×½, ×1, +½.

Analysis of Variance

Source	s.s.	d.f.	m.s.	F	P Value
T	0.2177	6	0.0353	196.7	<0.001
S	1.8968	2	0.9484	5287.48	<0.001
O	91.4694	2	45.7347	254981.55	<0.001
D	0.0105	3	0.0035	19.45	<0.001
TS	0.3690	12	0.0308	171.42	<0.001
TO	0.1704	12	0.0142	79.18	<0.001
TD	0.0048	18	0.0003	1.47	N.S.
SO	1.0044	4	0.2511	1399.95	<0.001
SD	0.0130	6	0.0022	12.07	<0.001
OD	0.0005	6	0.0001	0.42	N.S.
TSO	0.2387	24	0.0100	55.45	<0.001
TSD	0.0107	36	0.0003	1.66	<0.01
TOD	0.0063	36	0.0002	0.98	N.S.
SOD	0.0102	12	0.0009	4.72	<0.001
TSOD	0.0101	72	0.0001	0.78	N.S.
Error	0.0753	4200	0.0002		
Total	95.5018	6710	0.1423		

Result of S-N-K tests after ANOVA of shell length means.
Time: T1 < T2 < T3 < T4 < T5 = T7 < T6
Shore Level: mid < high < low
Origin: high < mid < low
Density: ½ = ¼ = 1 < ½

Condition

Changes in the flesh condition of *Chione stutchburyi* at the different density and shore level treatments were compared using three different measures of condition, condition index, change in mean wet weight and change in mean dry weight.

Mean condition index, measuring the percentage of tissue volume to mantle cavity volume, was transformed to a log e function then analysed in a three factor ANOVA (Table 5, Fig. 9A).

The density treatments had no significant effect ($p < 0.05$) on the condition indices of *Chione stutchburyi*. The condition index increased in mid and high shore cockles transplanted to the low shore level cages. The tissue of the high shore origin cockles increased to occupy significantly more space in the mantle cavity than the flesh of mid and low shore origin *Chione stutchburyi* (see Fig. 9A). This may be related to either differences in allometry at different sizes of *Chione stutchburyi* and/or possibly, shell growth lagged behind the rapid increases in tissue growth of the transplanted high shore origin cockles.

Mean wet weight was transformed with a log e function then analysed in a three factor ANOVA (Table 6).

The shore level × origin interaction (Table 6, Fig. 9B) showed that there was a significant ($p < 0.001$) increase in the tissue of mid shore origin as well as of high shore origin *Chione stutchburyi* at the low shore level cages. Low shore origin cockles have significantly ($p < 0.05$) more tissue than mid and high shore origin *Chione stutchburyi* at all three shore levels. These larger cockles were adversely affected by the transplant treatments, those transplanted to the mid and high shore levels had a significantly ($p < 0.05$) lower wet weight of tissue than the low shore level control cages. Density is not significant as an independent factor (Table 6) but both the shore level × density (Fig. 9C) and origin × density interactions were significant in the ANOVA. The shore level × density SNK test (Table 6) finds the *Chione stutchburyi* at the low shore level cages at maximum density, (½×) had significantly ($p < 0.05$) lower tissue wet weights than at lower densities (see Fig. 9C). The origin × density SNK test shows no density treatment effect. [This is the only interaction in the analysis for which the SNK test results for the raw data and the transformed data were different. The log e mean tissue weights of each original of *Chione stutchburyi* being significantly different ($p < 0.05$, SNK test) but the difference in

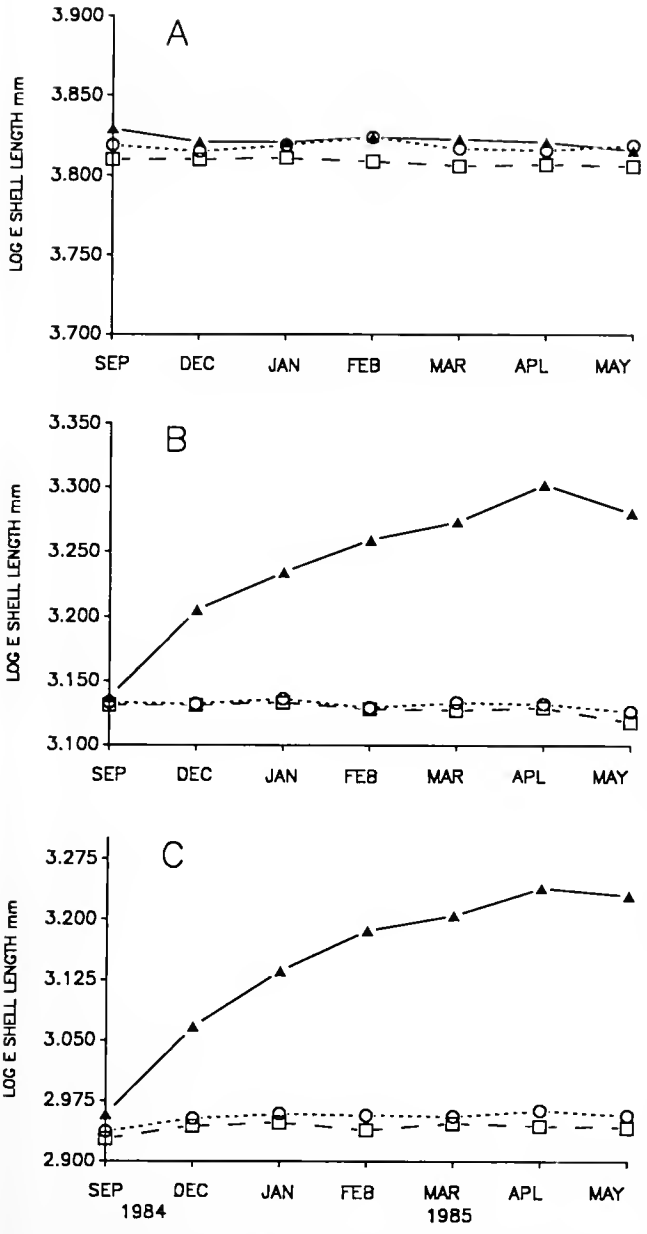


Figure 4. Average shell length of the (A) low shore origin, (B) mid shore origin and (C) high shore origin *Chione stutchburyi* at ambient densities at each shore level during the experimental period. Filled triangles and solid lines and are low shore, open squares and long dashed lines mid shore and open circles and dotted lines high shore levels.

raw data means for mid and high shore origin cockles were not significant.]

Mean dry weight data was transformed to a log e function then analysed in a three factor ANOVA (Table 7). The density treatment again had no significant effect ($p < 0.05$) on condition as measured by tissue dry weight. Fig. 9B shore level × origin, shows the increase in tissue dry weight of mid and high shore origin *Chione stutchburyi* transplanted to the low shore level cages. Mean tissue dry weight of low shore origin *Chione stutchburyi* decreased on transplanting to the higher shore levels.

Sediment Analysis

Grain size analyses of the sediment inside and outside the cages at each shore level showed that the sediment consisted of well to very well sorted fine sand. Skewness toward the coarser fraction occurred due to the presence of small shell fragments. Sorting and skewness values appear to be independent of mean grain size. It is concluded that no change in the sediment deposition occurred within the cages during the course of the experiment either as a cage effect or as the result of sieving.

DISCUSSION

Various methods are available for the assessment of shell length increments within a population of bivalves, e.g.: monitoring the length of marked individuals (Orton 1926), measuring the shell length of successive growth rings (Orton 1926, Kristensen 1957, Brock 1980, Richardson 1980, Lewis et al. 1982), following the shift in the position of peaks with time in a length frequency analysis (Tegelberg & Magoon 1969) or measuring daily shell growth increments for measurement of short term growth (Richardson 1979). In the present study the large number of *Chione stutchburyi* per cage made it difficult to find a particular cockle at any given time. It was therefore necessary to follow growth of samples from each cage population of marked cockles, rather than individually identified cockles. Inclusion of replicate treatments in the experimental design meant population growth rather than growth of individuals provided the variance. Peterson (1982b) showed that the presence of larger clams has a greater impact on resources than smaller clams. He included both smaller and larger cockles in cage treatments in an experiment set up in Mugu Lagoon and followed the increase in growth of individuals. Both his results and those of Kristensen (1957) suggest that the growth rate of smaller individuals were reduced more than larger individuals where food was limited. Measurements of both size groups in density treatments used in the present study would have been desirable but due to the large numbers of individuals involved in the experiment identical length frequencies for each treatment would have been almost impossible to obtain from each of the shore levels.

Significant increments in mean shell length were recorded for the smaller and high shore origin *Chione stutchburyi* at the low shore level only. The larger low shore origin *Chione stutchburyi* showed no significant increase in shell length at any of the monitored shore levels. This suggests that the sizes chosen for transplantation had already approached an asymptotic maximum at each shore level. Any effects on shell length of density treatments is masked by the lack of growth within the time period of the experi-

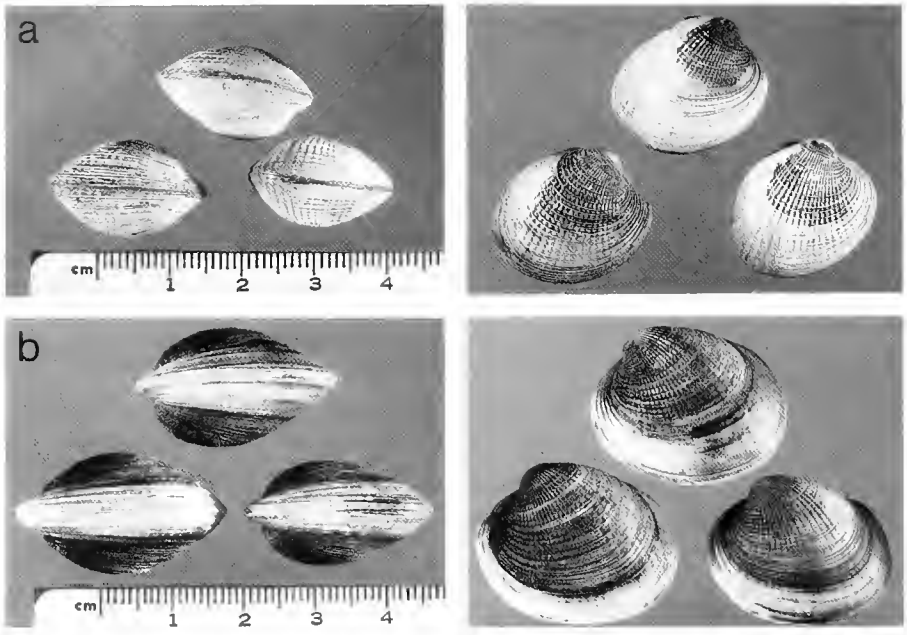


Figure 5. Photograph of mid shore origin *Chione stutchburyi* collected at the end of the experimental period showing A individuals remaining at the mid shore level and B individuals transplanted to the low shore level. Note the white area around the margins of the valves denoting new growth.

ment at the higher shore levels and for the larger slow growing *Chione stutchburyi*. Such size dependent effects could have been avoided by using smaller cockles for the low shore origin treatments. The disadvantage of choosing a particular size class in this experiment is evident from the diminished information available from the slower growing large individuals. The growth equations fitted to data from the low shore level cages describe the growth between September 1984 and May 1985. Thus the parameter "A" for the equations, rather than giving an estimate of the maximum length for the populations, estimates the final length for that season. It appears that these lengths had already been reached at an annual growth of approximately 6 mm for the mid shore origin and 10 mm for the high shore origin *Chione stutchburyi*. This compares with annual growth of 2–4 mm in cockles from Waitati and Papanui inlets (Wildish 1984). Use of the von Bertalanffy growth equation was not possible due to the narrow range of sizes (Brousseau 1979). The least squares regression equations are of similar form and perhaps describe the rate of growth better than equations derived from Ford Walford plots which require a wider size range to improve their accuracy.

Measurement of tissue condition was carried out at the completion of the experiments. This means that the changing proportions of somatic and gonad mass were not examined and the values are expected to have been different seasonally. Spawning in *Chione stutchburyi* occurs in late autumn (Stephenson 1981) when the dry weight of gonad represents approximately 40–50% of the standing crop (Larcombe 1971).

The method of calculating condition indices developed by Baird (1958) relies on the accurate measurement of tissue and shell volumes. The methods used in the present study were time consuming and to obtain reasonable accuracy the mean volume of the sample was measured rather than measuring volumes of the parameters of individuals separately.

All three of the condition measurements used show the tissue volume and weight of all the experimental cockles had a maximum at the low shore level. The condition index results indicate only minimal changes in the tissue to mantle cavity ratio occurred for low and mid shore origin *Chione stutchburyi*. Tissue condition index increases of high shore origin cockles at the low shore level indicate that growth of tissue was at a higher rate than shell growth. Measurement of mean tissue wet weight show a significantly lower condition occurred at the low shore level with the maximum density treatments.

The major disadvantage of the type of cages used was the large number of unmarked *Chione stutchburyi* that entered the cages. The probable mode of entry was by current and wave action moving the cockles along the sediment surface into and out of the cages over the top of the mesh. This is supported by the large numbers of immigrant cockles found in the mid shore cages and marked *Chione stutchburyi* collected outside the cages. Individuals in the surrounding population at this level are much smaller in size than at the low shore level, thus these are more likely to be moved by the bottom currents and turbulence than the larger *Chione stutchburyi*. The population at the high shore

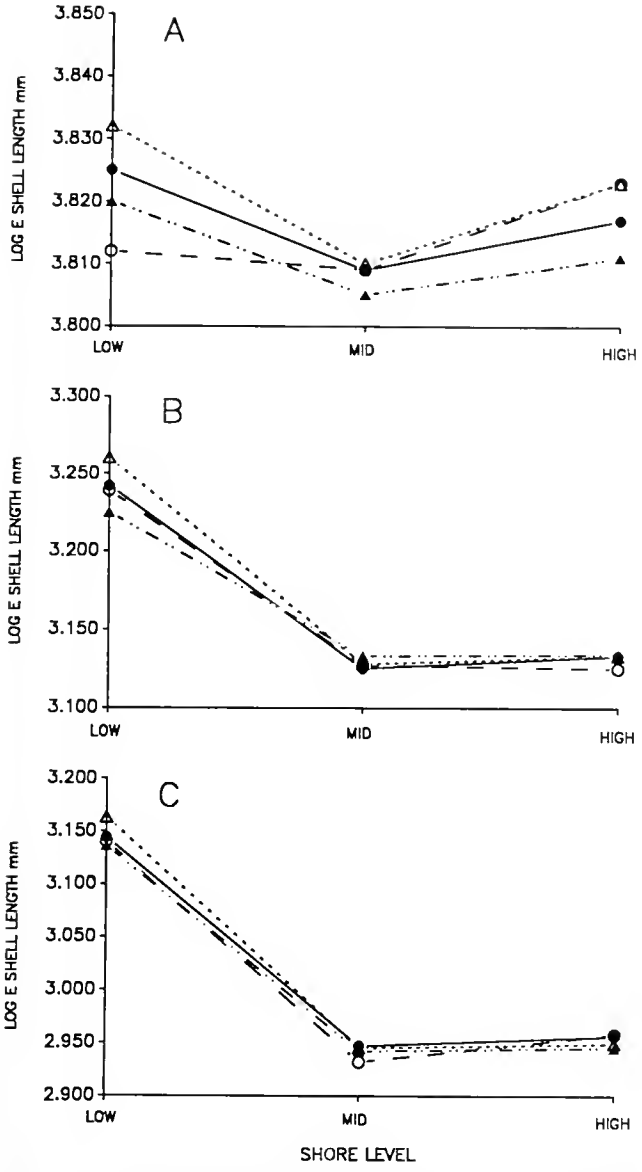


Figure 6. Average shell length of the (A) low shore origin, (B) mid shore origin and (C) high shore origin *Chione stutchburyi* for the four density levels at the low, mid and high shore level cages. Open circles and long dashed lines, open triangles and dotted lines, filled circles and solid lines and filled triangles and dashed and dotted lines are for ¼, ½, 1 and ½ × ambient density respectively.

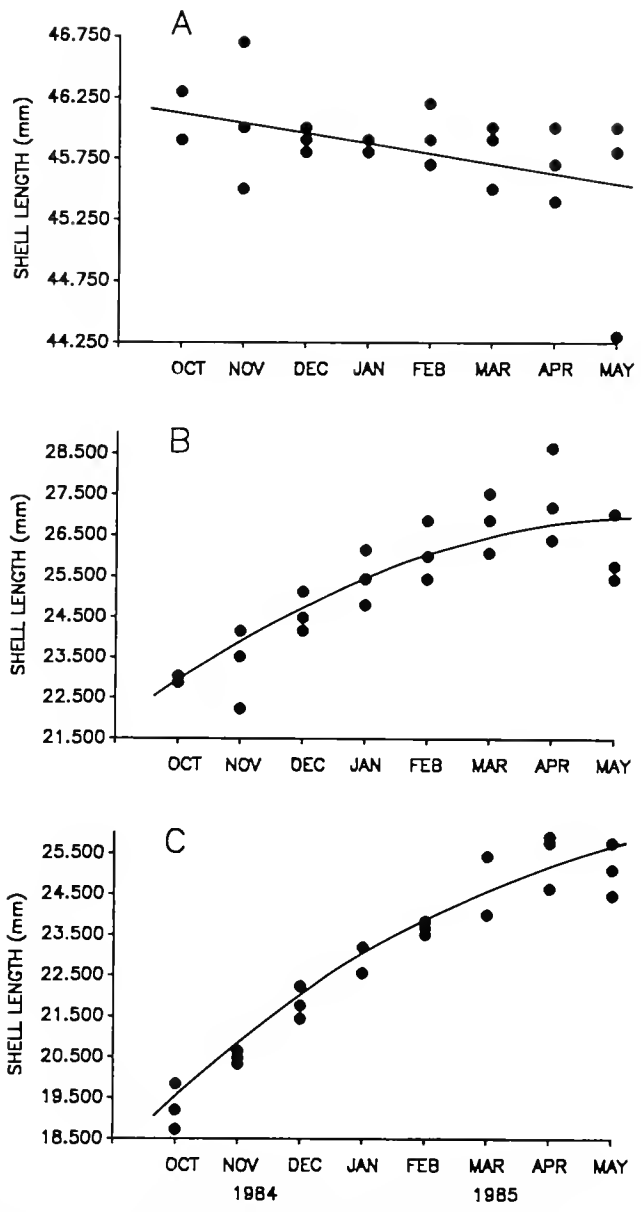


Figure 7. Growth curves fitted to the mean shell length of (A) low (B) mid and (C) high shore origin *Chione stutchburyi* sampled from ambient density treatments at the low shore cages over the 1984–85 summer period.

level are of similar size but are less numerous and fewer immigrants were recorded at this level. An alternative explanation is that high tag loss occurred at the mid shore cages. However, this seems unlikely as even on worn marked cockles some paint was nearly always visible and it is improbable that paint loss would be size dependent or that this level was more prone to wave action than the cages at the other shore levels.

The presence of immigrants in the cages would affect the density treatments and estimates of mortality. Cages with very high numbers of immigrants were omitted from the analysis and the cell filled by a missing data calculation based on the average of the remaining replicates for that particular treatment. Thus in estimates of mortality we tried to avoid inaccuracies that might have resulted from assuming that all the experimental cockles remained in the cages or that all those lost from the cages were dead.

Increased mortality rates were recorded for larger *Chione stutchburyi* at the mid and high shore level cages. This is more likely the result of starvation with decreased submergence time at the mid and high shore level cages rather than increased physiological stress associated with

TABLE 3.

Parameters for fitting growth curves to shell length data from low shore cages at density (x) for low, mid and high shore origin *Chione stutchburyi*. "A" is the maximum length L, "B" the reduction in rates of growth due to increased size of individuals (i.e. always negative), "R" is the rate of growth and "K" is a constant.

Low Shore Origin
N = 24, s.s 3.2305, d f. 21, r.m.s. 0.1538

	mean	polynomial coefficients
R	0.9997	
B	355.5430	46.2609
A	−309.2821	−0.0914
K	0.0003	

B and A are out of bounds parameters

Mid Shore Origin
N = 24, s.s. 6.2048, d.f. 21, r.m.s. 0.2955

	mean	S.E.	correlation		
R	0.8273	0.0457	1.0000		
B	−10.6852	1.0594	−0.8502	1.0000	
A	27.9315	1.4267	0.9801	−0.9295	1.0000
K	0.1896	0.0553			

High Shore Origin
N = 24, s.s 13.6303, d.f. 21, r.m.s. 0.6491

	mean	S.E.	correlation		
R	0.7621	0.0963	1.0000		
B	−6.8742	0.7993	−0.2442	1.0000	
A	27.7848	1.0957	0.9563	−0.4770	1.0000
K	0.2717	0.1263			

increased fluctuations in temperature and salinity with longer periods of exposure. Gillmor (1982) suggests that most intertidal bivalve species are physiologically adapted to withstand the fluctuating temperatures and salinities within their distribution limits. Larcombe (1971) found no differences in growth rate that were related to latitudinal differences in temperature or salinities above 25‰, further evidence that the increase in mortality is probably due to a lack of food, rather than the increased physical stresses of the longer exposure periods at the higher shore levels. Starvation is also indicated by the lower tissue dry weights recorded for low shore origin *Chione stutchburyi* after transplantation to mid and high shore levels. Although not significant, there was an increase in mortality recorded in high shore origin *Chione stutchburyi* transplanted to the low shore level cages. These cages were subject to smothering by the green alga *Ulva lactuca* and this may have caused some increase in mortality in the cages affected due to reduction of water flow and deoxygenation. Similar mortality occurred by algal fouling in a sublittoral cage experiment described by Arntz (1977). Some bias in the mortality estimates can be expected from omitting the crushed shell material, especially if small cockles are more likely to be crushed by predators such as crabs. Sanchez-Salazar (1987) found that 97% of the cockles *Cerastoderma edule* taken by crabs were <15mm in length. An improved method of assessing such material would be useful in these types of experiments.

In the present study predation of *Chione stutchburyi* within cages was also attributed to crabs, especially *Cancer novaezelandae* (of which one large individual was found in one of the low shore cages) and resident birds, whelks and possibly other species of crabs. Sanchez-Salazar (1987) found crab predation to be more intense on small cockles lower on the shore while at higher levels oystercatchers take large cockles. In the present study the intensity of predation by different species is not known, however it would have been interesting to assess the level of predation that occurred over the experimental period. This could have been achieved by maintaining additional cages with mesh lids to exclude predators of various sizes. Use of this type of cage would however have increased the likelihood of introducing additional cage effects into the experimental design.

Heavy predation on smaller cockles may explain the observed recruitment patterns of the initial field survey. Recruitment at Harwood appears to coincide with the higher densities of adult *Chione stutchburyi* found on the lower shore. These observations are also supported by Blackwell (1984) at Ohiwa Harbour where recruitment was positively correlated with high density adult beds. Wear (1984) also found survival of smaller thin shelled bivalves was enhanced when placed in densely packed adult beds. Larval settlement may be reduced by the presence of adults (Kris-

TABLE 4.

Analysis of Variance and S-N-K tests of arc sine × 100 transformed mean percentage mortality data from shore level transplants and density treatments.

Treatments
Shore level (S): low, mid, high
Origin (O): low, mid, high
Density (D): ×¼, ×½, ×1, ×½

Analysis of Variance

Source	s.s.	d.f.	m.s.	F	P Value
S	0.4163	2	0.2082	29.06	<0.001
O	0.8727	2	0.4363	60.91	<0.001
D	0.0572	3	0.0191	2.66	N.S.
SO	0.4614	4	0.1154	16.10	<0.001
SD	0.1039	6	0.0173	2.42	<0.05
OD	0.0137	6	0.0023	0.32	N.S.
SOD	0.1003	12	0.0084	1.17	N.S.
Error	0.4299	60	0.0072		
Total	2.4553	95	0.0259		

Result of S.N.K. tests after ANOVA of pereentage mortality means.
Shore level: low < mid = high
Origin: high < mid = low
Density: N.S.

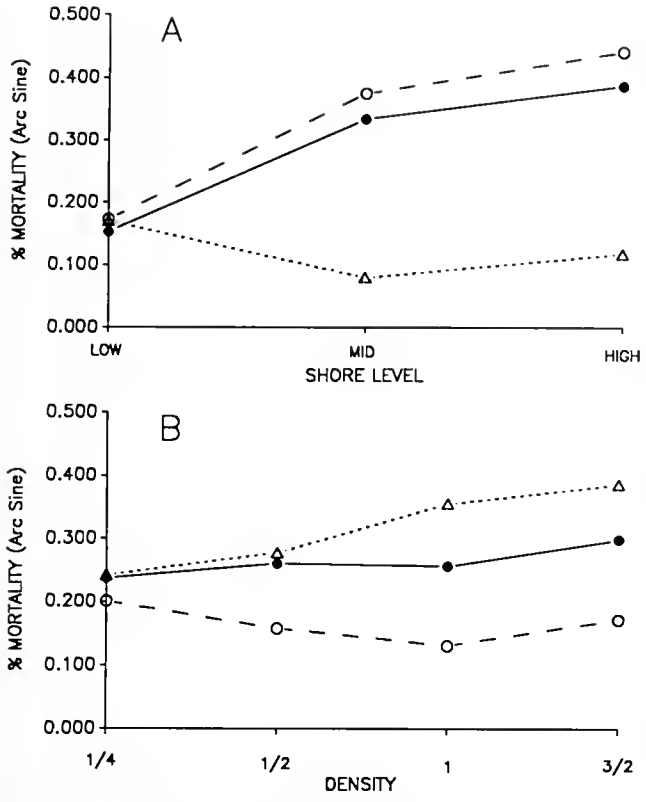


Figure 8. Mean percentage mortality, derived from the fraction of dead shells recovered over the experimental period for low, (open circles and dashed lines) mid (filled circles and solid line) and high (open triangles and dotted line) shore origin *Chione stutchburyi* at (A) the three cage shore levels and (B) the four density treatments.

tensen 1957), but later enhanced survival of juveniles and/or migration to dense adult beds seems likely.

The results of the present study clearly show that shore level has a major effect on the growth and survival of *Chione stutchburyi*, with enhanced growth and survival towards the low water spring tide level on this tidal flat. The results suggest that the shore level size gradients of *Chione stutchburyi* are related to differences in growth rates rather than size selective mortality or active predation of a particular size group, as has been suggested by Vermeij (1972) and seems to occur in populations of *Cerastoderma edule* at Traeth Melynog in North Wales (Sanchez-Salazar 1987).

Two mechanisms have been proposed to explain the enhanced growth at the low shore:

1. That submergence time produces growth rates dependent on the time available for feeding (Mason 1968, Kristensen 1957, Richardson et al. 1980).
2. That the water passing over the low shore populations is depleted of phytoplankton before reaching the high shore populations (Kristensen 1957 and Larcombe 1971).

It is possible that both of these play some part in explaining the higher growth rates at lower tide levels found in the present study. Rapid renewal of plankton rich water from the lower Portobello Bay channel would however tend to minimise phytoplankton depletion by low shore populations so submergence time is likely to be the more limiting factor.

Beentjes (1984) found that there were no differences in the feeding rates of *Chione stutchburyi* collected from high and low shore stations in Otago Harbour. This would imply that with a period of longer submergence low shore cockles could filter proportionally more than high shore animals. Larcombe (1971) estimates the upper limit of distribution of *Chione stutchburyi* to be at levels with a minimum submergence period of 3½ hours per tide. The minimum submergence period before growth is affected in *Cardium edule* is 5 hours per tide, estimated by Kristensen (1957) from length frequency distributions at different shore heights in the Wadden Sea. In areas where the upper shore was exposed for less than 4 hr/tide there was no tidal effect on growth. He found the largest reduction in growth where the upper shore is exposed for nine hours or more.

Various studies (Hancock 1965, Larcombe 1971, Hylleberg et al. 1980, Peterson 1982b and Blackwell 1984), report reduced growth in cockle species occurs with increased intraspecific density. In the present study the density effects can be regarded as minimal, for the only significant effect was a reduction in tissue wet weight at the maximum density for the low shore cages. It would have been necessary to monitor this condition more frequently over the experimental period to determine whether the reduced tissue growth was due to cage effects or not. The increase in mortality at the higher shore levels indicates

TABLE 5.

Analysis of variance and S-N-K tests on log e transformed mean condition index data for the shore level and density treatments.

Treatments
Shore level (S): low, mid, high
Origin (O): low, mid, high
Density (D): ×¼, ×½, ×1, ×3/2.

Analysis of Variance

Source	s.s.	d.f.	m.s.	F	P Value
S	1.0389	2	0.5195	17.08	<0.001
O	0.6334	2	0.3167	10.41	<0.001
D	0.0516	3	0.0172	0.56	N.S.
SO	0.5024	4	0.1256	4.13	<0.01
SD	0.1035	6	0.0173	0.57	N.S.
OD	0.2973	6	0.0496	1.63	N.S.
SOD	0.6800	12	0.0567	1.86	N.S.
Error	1.8252	60	0.0304		
Total	5.1324	95	0.0540		

Result of S.N.K. tests after ANOVA of condition index means.
Shore level: mid = high < low
Origin: low = mid < high
Density: N.S.

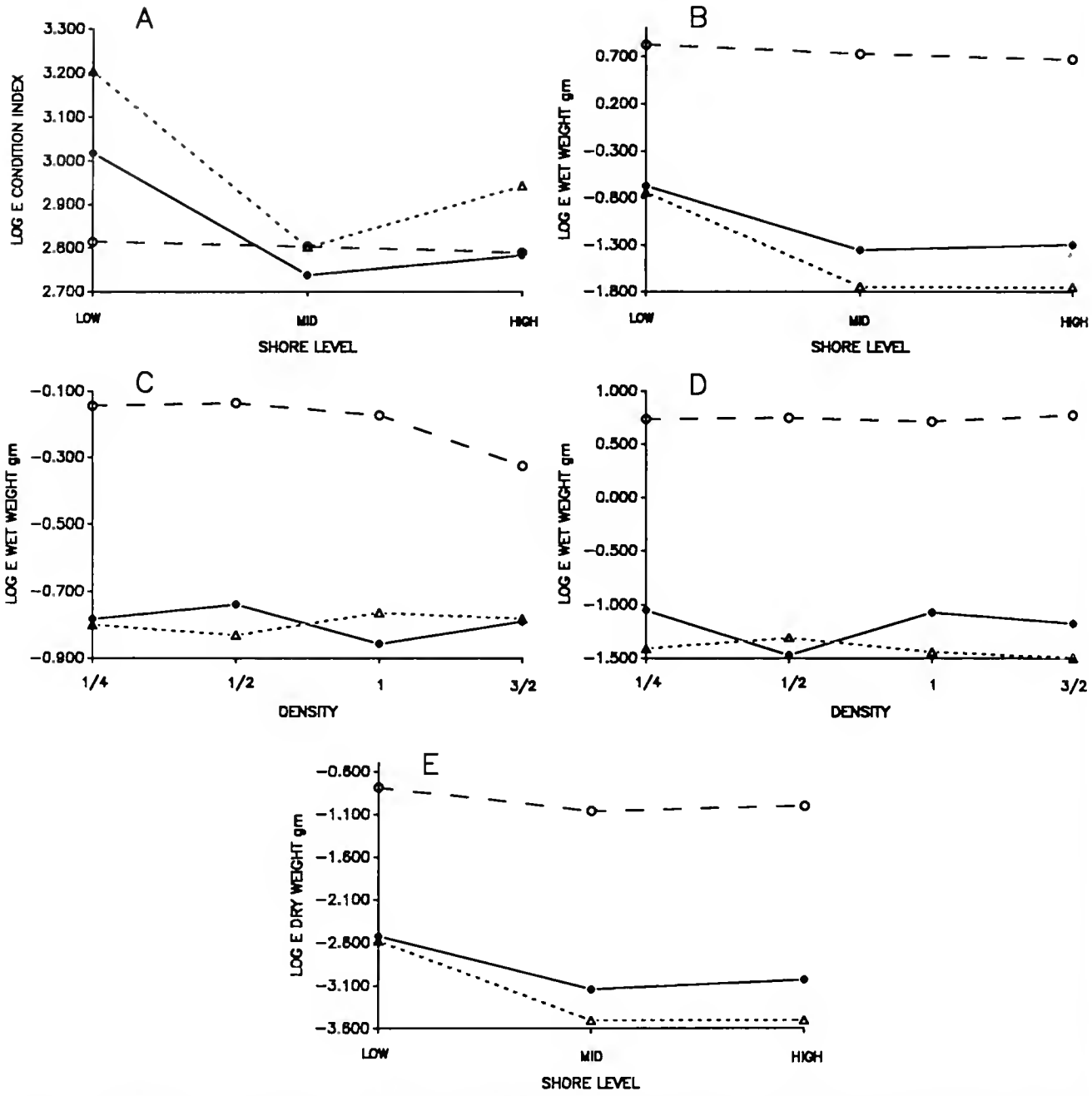


Figure 9. (A) Mean condition indices and (B) mean tissue wet weight of low, mid and high shore origin *Chione stutchburyi* at each shore level. (C) Mean tissue wet weight for each shore level at the four density treatments and (D) mean tissue wet weight for low, mid and high shore origin cockles at the four density treatments. (E) Mean tissue dry weights of low, mid and high shore origin cockles at each shore level. Low shore is open circles and dashed line, mid shore closed circles and solid line and high shore open triangles and dotted line.

feeding is impaired at upper levels. If intraspecific competition for food resources had occurred, density related mortality would have been expected at these levels despite the lack of growth at the mid and high shore level. Brock (1980) recorded no density dependent growth of cockles for his sublittoral cage experiments and surmised that the high densities may have been offset by rapid water renewal. In the present study the enhanced growth of *Chione stutchburyi* at the low shore cages in transplant treatments may have resulted in part from rapid renewal of plankton rich water from the Lower Portobello Bay channel.

Blackwell (1984) transplanted *Chione stutchburyi* to low and mid shore level cages in the Ohiwa Harbour. Low shore cages were placed on a low tide shell bank at 0.2 m and high shore cages at 1.2 m above C.D. at a channel location. A significant reduction in growth, tissue condition

TABLE 6.

Analysis of variance and S-N-K tests on log e transformed mean tissue wet weight data for shore level and density treatments.

Treatments
Shore level (S): low, mid, high
Origin (O): low, mid, high
Density (D): ×¼, ×½, ×1, ×3/2

Analysis of Variance

Source	s.s.	d.f.	m.s.	F	P Value
S	8.5659	2	4.2829	280.09	<0.001
O	97.9464	2	48.9732	3202.69	<0.001
D	0.0676	3	0.0225	1.47	N.S.
SO	3.2118	4	0.8029	52.51	<0.001
SD	0.2299	6	0.0383	2.51	<0.05
OD	0.2165	6	0.0361	2.36	<0.05
SOD	0.2508	12	0.0209	1.37	N.S.
Error	1.0704	70	0.0153		
Total	111.5592	105	1.0625		

Result of S.N.K. tests after ANOVA of tissue wet weight means.
Shore level: high = mid < low
Origin: high = mid < low
Density: N.S.

TABLE 7.

Analysis of variance and S-N-K tests on log e transformed mean tissue dry weight data for shore level and density treatments.

Treatments
Shore level (S): low, mid, high
Origin (O): low, mid, high
Density (D): ×¼, ×½, ×1, ×3/2

Analysis of Variance

Source	s.s.	d.f.	m.s.	F	P Value
S	8.1236	2	4.0618	477.84	<0.001
O	107.2127	2	53.6063	6306.41	<0.001
D	0.0337	3	0.0112	1.32	N.S.
SO	1.9201	4	0.4800	56.47	<0.001
SD	0.0840	6	0.0140	1.65	N.S.
OD	0.0307	6	0.0051	0.6013	N.S.
SOD	0.0394	12	0.0033	0.3867	N.S.
Error	0.5865	69	0.0085		
Total	118.0307	104	1.1349		

Result of S.N.K. tests after ANOVA of tissue dry weight means.
Shore level: mid = high < low
Origin: high < mid < low
Density: N.S.

and survival occurred at the low shore cages. Blackwell's field survey of this area indicated the low shore supported fewer adults than the mid shore region. He attributed these differences to intraspecific competition at these levels. The broad scale shore level trends in his study were overridden by localized variability in growth. Blackwell used much smaller cage sizes than those of the present study and was able to produce maximum density treatments of $3200/m^{-2}$. The maximum densities for the present study were $2000/m^{-2}$ for the mid shore origin cockles. It is possible that intraspecific competition begins at some range between these two densities. As *Chione stutchburyi* already covered twice the surface area of the cages at this density, it is reasonable to expect that intraspecific competition at higher levels of density will be due to both space and food resource competition, since Peterson (1982b) recorded reduced growth of *Chione undatella* and *Protathaca staminea* at densities covering less than eleven percent of the surface area of his cages.

The advantage of using factorial analysis of variance for the study was to compare directly the difference in magnitudes of the density effects and shore level effects. On tidal sand flats the height on the shore is more important in determining the demographic features of the *Chione stutchburyi* population than intraspecific competition. The results also show that the large *Chione stutchburyi* of four to five centimeters periodically found on the upper shore are likely the result of these cockles attaching to seaweed and drifting from the lower shore as described by Larcombe (1971). Further study on the habits of filter feeding populations and their importance in the depletion of phytoplankton in shallow estuarine and intertidal communities is required to deduce the relative importance of submergence duration and depletion of food in determining intraspecific shore level gradients. In Otago Harbour and the surrounding estuaries, submergence length is important in determining the size distribution gradients of *Chione stutchburyi* within the intertidal zone.

The potential for developing the *Chione stutchburyi* fishery in the Otago region is indicated by the present study. Improved yields may be attained by removing the larger individuals found towards the lower shore. In years of poor recruitment, or in areas where adult densities are low on the lower shore, smaller individuals from the upper shore could be transplanted to these areas and faster growth rates, larger tissue weights and enhanced survival of these transplanted cockles could be expected.

ACKNOWLEDGMENT

We are grateful to MAFish for a grant in support of this research.

REFERENCES

Arntz, W. E. 1977. Results and problems of an "unsuccessful" benthos cage predation experiment (Western Baltic). pp 31–34 In B. F. Keegan, P. O. Ceidigh & P. J. S. Boaden (eds.) *Biology of Benthic Organisms*. 11th European Marine Biology Symposium, Galway 1976. Pergamon Press, New York 1976.

Baird, R. H. 1958. Measurement of condition in mussels and oysters. *J. Con. Int. Explor. Mer*. 23:249–257.

Beentjes, M. 1984. Aspects of feeding in *Chione stutchburyi* with emphasis on differences between shore heights. Unpubl. B.Sc. Hons. Dissertation. University of Otago, Dunedin, New Zealand.

Blackwell, R. 1984. Aspects of the population biology of *Chione stutchburyi* in Ohiwa Harbour, Bay of Plenty. Unpubl. Ph.D thesis. University of Auckland, Auckland, New Zealand.

Brock, V. 1980. Notes on relations between density, settling, and growth of two sympatric cockles, *Cardium edule* (L) and *C. glaucum* (Brugiere). *Ophelia*, Suppl. 1:241–248.

Brousseau, D. J. 1978. Population dynamics of the soft-shell clam *Mya arenaria*. *Mar. Biol.* 50:63–71.

Brousseau, D. J. 1979. Analysis of growth rate in *Mya arenaria* using the von Bertalanffy equation. *Mar. Biol.* 51:221–227.

Folk, R. L. 1968. Petrology of sedimentary rocks. Hemphill, Austin, Texas, 170 pp.

Gillmor, R. B. 1982. Assessment of intertidal growth and capacity adaptations in suspension-feeding bivalves. *Mar. Biol.* 68:277–286.

Grace, R. V. 1966. The bottom communities of the entrance to the Whangateau Harbour. *Tane* 12:63–70.

Hancock, D. A. 1969. An experiment with overcrowded cockles *Cardium edule*. Marine Biological Association of India. Symposium Series 3 Part III. (Proceedings of the Symposium on Mollusca, Cochin, 1968) pp 396–402 & Plates 1–4.

Hancock, D. A. & A. Franklin. 1972. Seasonal changes in the condition of the edible cockle (*Cardium edule* L.). *J. Appl. Ecol.* 9:567–579.

Hulberg, L. W. & J. S. Oliver. 1980. Caging manipulation in marine soft-bottom communities: Importance of animal interactions or sedimentary habitat modifications. *Can. J. Fish. Aquat. Sci.* 37:1130–1139.

Hylleberg, J., V. Brock, & F. Jorgensen. 1978. Production of sublittoral cockles, *Cardium edule* L. with emphasis on predation by flounders and sea stars. *Nat. Jutl.* 20:183–191.

Kristensen, I. 1957. Differences in density and growth in a cockle population in the Dutch Wadden Sea. *Arch. Neerl. Zool.* 12:315–453.

Larcombe, M. F. 1971. The ecology, population dynamics, and energetics of some soft shore molluscs. Unpubl. Ph.D. thesis, University of Auckland, Auckland, New Zealand.

Levinton, J. S. 1972. Stability and trophic structure in deposit-feeding and suspension-feeding communities. *Am. Nat.* 106:472–486.

Lewis, J. B., S. Saleh, H. M. Reiswig, & C. M. Lalli. 1982. Growth, production and biomass of the burrowing protobranch mollusc *Yoldia limatula* in the Bideford River, Prince Edward Island, Canada. *Mar. Biol.* 70:173–179.

Mason, J. 1968. Experimental transplanting of cockles *Cordium edule* Linaeus. *Proceedings of the Symposium on Mollusca—Part III.* pp. 824–831.

Morton, J. E. & M. C. Miller. 1973. *The New Zealand Seashore*. Collins, Auckland, second edition, 653 pp.

Orton, J. H. 1926. On the rate of growth of *Cardium edule*. Part 1. Experimental observations. *J. Mar. Biol. Assoc. U.K.* 14:239–279.

Peterson, C. H. 1982a. Clam predation by whelks (*Busycon* spp): Experimental tests of the importance of prey size, prey density, and sea grass cover. *Marine Biology* 66:159–170.

Peterson, C. H. 1982b. The importance of predation and intra- and interspecific competition in the population biology of two infaunal suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella*. *Ecol. Monogr.* 52:437–475.

Peterson, C. H. 1983. Interactions between two infaunal bivalves, *Chione undatella* (Sowerby) and *Protothaca staminea* (Conrad), and two potential enemies, *Crepidula onyx* (Sowerby) and *Cancer anthonyi* (Rathburn). *J. Exp. Mar. Biol. Ecol.* 68:145–158.

Powell, A. W. B. 1979. *New Zealand Mollusca: marine, land and freshwater shells*. Collins, Auckland, 500 pp.

Rainer, S. F. 1969. Marine benthic ecology in Otago; the macrofauna of deposit substances in the Otago Harbour and Blueskin Bay. Unpubl. Ph.D thesis, University of Otago, Dunedin, New Zealand.

Rainer, S. F. 1981. Soft-bottom communities in Otago Harbour and Blueskin Bay, New Zealand. *New Zealand Oceanographic Institute Memoir*, Vol. 80.

Ralph, P. M. & J. C. Yaldwin. 1956. Seafloor animals from the region of Portobello Marine Biological Station, Otago Harbour. *Tuatara* 6:57–85.

Richardson, C. A., D. J. Crisp & N. W. Runham. 1980. Factors influencing shell growth in *Cerastoderma edule*. *Proc. R. Soc. London Ser. B* 210:513–531.

Richardson, J. R., A. E. Aldridge & W. de L. Main. 1979. Distribution of the New Zealand cockle, *Chione stutchburyi*, at Pauatahanui Inlet. *N.Z.O.I. Field Report* No. 14.

Roper, D. S. 1979. The role of sheltered inlets in the lives of locally occurring flatfish. Unpubl. M.Sc. thesis. University of Otago, Dunedin, New Zealand.

Sanchez-Salazar, M. E., C. L. Griffiths & R. Seed. 1987. The interactive roles of predation and tidal elevation in structuring populations of the edible cockle, *Cerastoderma edule*. *Estuarine Coastal Self Sci.* 25:245–260

Stanton, D. J., B. B. Bohool & C. Beasley. 1977. Intertidal zone of Delaware Inlet, Nelson, New Zealand. *N. Z. J. Mar. Freshwater Res.* 11:577–587.

Stephenson, R. L. 1981. Aspects of the energetics of the cockle *Chione* (Austrovenus) *stutchburyi* in the Avon-Heathcote estuary, Christchurch, New Zealand. Unpubl. Ph.D. thesis, University of Canterbury, Christchurch, New Zealand.

Tegelberg, H. C. & C. D. Magoon. 1969. Growth, survival and some effects of a dense razor clam set in Washington. *Proceedings of the National Shellfisheries Association* 59:126–135.

Vermeij, G. J. 1972. Intraspecific shore-level size gradients in intertidal molluscs. *Ecology* 53:693–700.

Virnstein, R. W. 1977. The importance of predation by crabs and fishes on benthic infauna in Chesapeake Bay. *Ecology* 58:1199–1217.

Voice, A. J. 1975. The invertebrate ecology and the *Chione stutchburyi* population on the intertidal flats at Aramoana, Otago Harbour, New Zealand. Unpubl. B.Sc. Hons Dissertation, University of Otago, Dunedin, New Zealand.

Wear, R. G. 1984. Paddle crabs are probable predators. *Catch* 22:11–13.

Wildish, K. 1984. Further analysis of results from the survey of *Chione stutchburyi* (the New Zealand cockle) populations at Papanui and Waitati inlets. Unpubl. report. In *The Cockle Resource in the Otago Region*.

Witman, J. D. 1974. Sedimentological and ecological studies of the Blueskin Bay Estuary. Unpubl. thesis, University of Otago, Dunedin, New Zealand.

Woodin, S. A. 1974. Polychaete abundance patterns in a marine soft-sediment environment: the implications of biological interactions. *Ecol. Monogr.* 44:171–187.

Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, N.J., 620 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 213–218, 1989.

VARIATIONS IN SHELL AND RADULA MORPHOLOGIES OF KNOBBED WHELKS*

BRUCE A. ANDERSON,[1] ARNOLD G. EVERSOLE,[2] AND WILLIAM D. ANDERSON[3]

[1]*Natural Resources Department*
Southhamptom Town Hall
Southhampton, NY 11968
[2]*Department of Aquaculture, Fisheries and Wildlife*
Clemson University
Clemson, SC 29634-0362
[3]*South Carolina Wildlife and Marine Resources Department*
P.O. Box 12559
Charleston, SC 29412

ABSTRACT Knobbed whelks (*Busycon carica*, Gmelin) collected from 3 offshore fishing locations along the South Atlantic Bight were analyzed for variation in shell and radula morphology. Initial analysis indicated that female whelks were significantly ($P \leq 0.05$) larger than male whelks. Significant differences in shell morphology occurred between male and female whelks of the same shell length (SL). Shell morphology of female whelks was significantly different among the 3 fished locations. Among fishing locations, relative spire height and spine length exhibited the most consistent differences. Radula dentition and 3 of 5 denticle measurements varied significantly with SL. Analysis of covariance using SL as a covariate indicated that significant differences in 7 measures of radula morphology occurred among the fishing locations. The 2 locations separated by the greatest distance exhibited the most distinct differences in shell and radula morphology.

KEY WORDS: biometrics, *Busycon*, knobbed whelk, local populations, radula, sexual dimorphism, shell morphology

INTRODUCTION

The knobbed whelk, *Busycon carcia* (Gmelin), is a large marine snail commercially harvested along the South Atlantic Bight. Female knobbed whelks produce strings of egg capsules which are firmly anchored to bottom sediments. The snails develop through trochophore and veliger larval stages while still encapsulated, and emerge with shell structure and locomotory behavior similar to that of adults (Magalhaes 1948, Pulley 1959). Data collected from mark-recapture studies indicate that movement in adult whelks is limited (Anderson et al. 1985). Consequently, longshore movement among populations is restricted.

Commercial fishermen report that whelks appear to be concentrated in particular localities along the South Atlantic Bight, and almost all of the whelks processed in South Carolina come from 3 or 4 separate fishing locations (Anderson et al. 1985). Pulley (1959) suggested that the species is composed of numerous distinct populations and that gene flow between adjacent populations may be low or negligible. Hollister (1958) observed a high degree of variation in shell morphology of *B. carica* collected from geographically isolated populations. Shell morphology and other phenotypic differences between populations could be maintained if gene flow is sufficiently restricted.

The whelk industry in South Carolina has increased dramatically since its beginning in 1978, and with increased fishing activity, there has been some concern that these limited fishing locations may become overexploited. To develop an effective management strategy, knowledge of population structure in these localities is an important consideration. The objective of this study was to determine if the knobbed whelks fished commercially come from discrete populations. Shell and radula morphometric characters were used to detect variations among whelks harvested from 3 widely separated fishing locations.

MATERIALS AND METHODS

Commercial catches of knobbed whelks from 3 separate offshore (1–3 km) fishing locations were sampled during the spring fishing season of 1984. Fishing locations were near McClellanville and Charleston in South Carolina and St. Katherine's Island in Georgia. McClellanville is ~80 km north of Charleston and 250 km north of St. Katherine's Island, and Charleston and St. Katherine's Island are ~170 km apart.

Shells were measured to the nearest 1.0 mm following the conventions of Magalhaes (1948) and included shell length (SL), aperture length (AL), shell width including spines (SWs), and shell width excluding spines (SW) (Fig. 1a), and spire height (SpH) (Fig. 1b). These linear shell measurements were used to calculate the following ratios: relative shell width [(SW/SL) × 100]; relative spire length [(SL − AL/SL) × 100]; relative spine length [(SWs − SW/SL) × 100]; and relative spire height [(SpH/SL) × 100].

Whelks (n = 1223) were extracted from their shells and

*Technical Contribution No. 2893 of the South Carolina Agricultural Experiment Station, Clemson University.

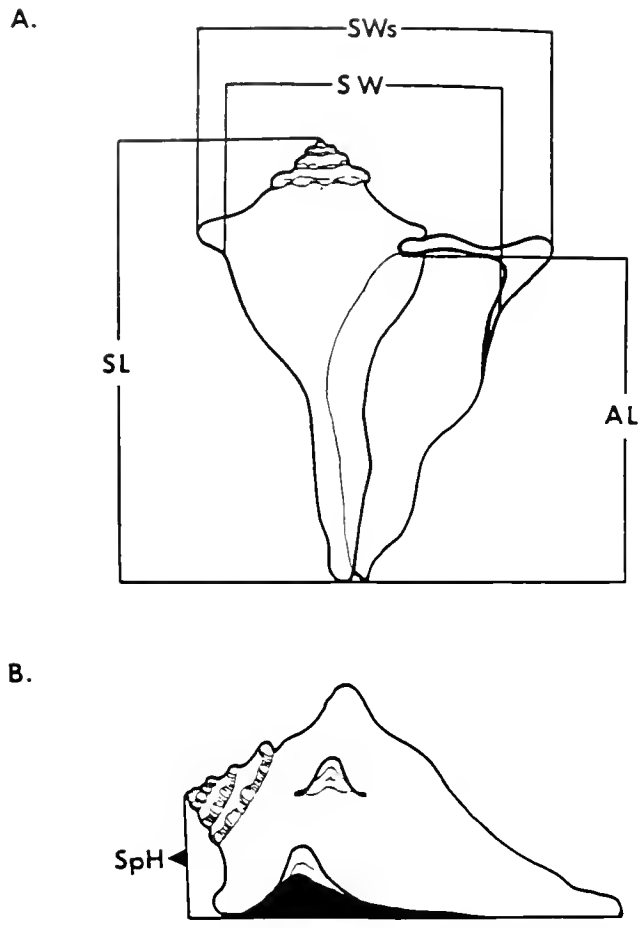


Figure 1. Shell of *Busycon carica:* A is the ventral view of the shell showing shell length (SL), aperture length (AL), shell width including spines (SWs), and shell width excluding spines (SW); B is the lateral view of the shell showing spire height (SpH).

sex determined by the presence or absence of a penis. Shells damaged (6–8) during the extraction process were deleted from the data base. The radula was removed from each whelk and stored in 50% ethyl alcohol. Shells were then thoroughly cleaned and oven dried to a constant weight (0.01 g). Relative shell weight was obtained by dividing shell weight (ShWt) by SL and multiplying by 100. Presence or absence of a tumid ridge, which refers to a conspicuous buildup of shell on the siphonal canal (Abbott 1974), was also noted for each whelk.

Radulas from 50 randomly-selected knobbed whelks obtained from each of the 3 fished locations were cleaned and softened in 10 M potassium hydroxide. Central portions of radulas were dehydrated with 100% ethyl alcohol, cleared in alpha terpineol and mounted in Klearmount on a glass slide.

Dimensions of 6 lateral teeth (3 on each side) were measured (to nearest 0.01 mm) using a compound microscope with an ocular micrometer. Care was taken to avoid measuring the worn teeth on the anterior half of the radula ribbon as well as obviously damaged teeth. Terminology and dimensions of denticles (Berrie 1959) included (Fig. 2): maximum length of the tooth (A); height of exocone (B); distance from tip of exocone to junction of the nearest mesocone (C); distance from junction of the most distal mesocone to junction of the most proximal mesocone (D); distance from tip of endocone to junction of the nearest mesocone (E); and height of the endocone (F). Ratios computed were: relative exocone height [(B/A) × 100]; relative exocone distance [(B/C) × 100]; relative mesocone distance [(D/A) × 100]; relative endocone distance [(E/A) × 100]; and relative endocone height [(F/A) × 100]. Numbers of mesocones and medial cusps were also recorded.

Analysis of covariance was performed on all shell and radula parameters in order to determine their dependence upon SL using the General Linear Model (GLM) of Statistical Analysis Systems (SAS Institute Inc. 1982). Shell and radula parameters which were significantly related ($P \leq 0.05$) to SL were corrected using SL as a covariate. This procedure permits a comparison between whelks of the same mean SL. Comparisons of the corrected means were made between sexes and among fishing locations using linear contrasts. Arcsin transformations of the percentages possessing a tumid ridge were made before statistical analysis.

RESULTS

Relative spire height, spire length, and spine length and shell weight were significantly ($P \leq 0.05$) related to SL. Relative shell width was the only shell ratio which was not observed to vary significantly with SL. The shells of larger whelks more commonly had tumid ridges. All radula ratios except relative exocone distance were significantly ($P \leq 0.05$) related to SL. In addition, larger (SL) whelks ap-

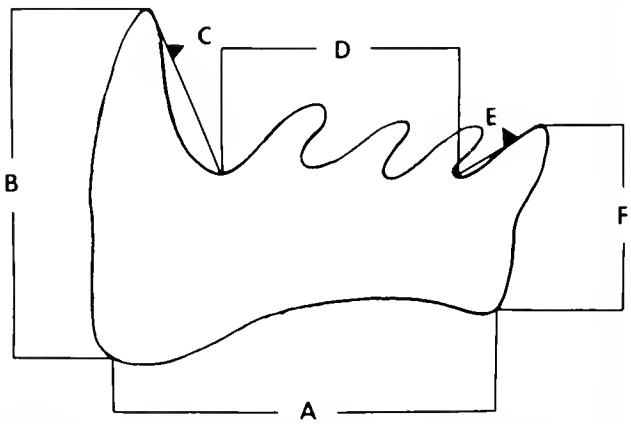


Figure 2. Lateral tooth from the radula of *Busycon carica* showing maximum length of the tooth (A), height of exocone (B), distance from tip of exocone to junction of the nearest mesocone (C), distance from most distal mesocone to junction of the most proximal mesocone (D), junction of the distance from tip of endocone to junction of the nearest mesocone (E), and height of the endocone (F).

peared to have had fewer mean numbers of mesocones and medial cusps.

Female knobbed whelks outnumbered male whelks ~4 to 1 in the samples. Male whelks (n = 251) averaged 113.9 mm SL (SE = 8.2) and ranged 86–137 mm while female whelks (n = 966) averaged 130.6 mm SL (SE = 8.7) and ranged from 95–203 mm. Significant differences ($P \leq 0.05$) were detected between sexes in the means of all shell ratios after correcting for SL differences (Table 1). Female whelks the same SL as males had significantly ($P \leq 0.05$) larger shell widths, spine lengths, spire heights and shell weights while males had significantly ($P \leq 0.05$) longer spires (Table 1).

Shell measurements corrected for SL for female whelks from the 3 fishing locations are summarized in Table 2. Statistical analysis was restricted to female whelks because of their disproportionate numbers in samples and in the commercial catch (Weinheimer 1982, Anderson et al. 1985). Significant differences ($P \leq 0.05$) were detected among all fishing locations in the mean percentage of female whelks with a tumid ridge, relative spire height and spine length. Mean relative spine lengths of female whelks from Charleston were intermediate to those from McClellanville and St. Katherine's Island. In addition, female whelks from McClellanville and St. Katherine's Island, the 2 collection sites farthest apart, were also significantly different ($P \leq 0.05$) in mean relative spire length and shell weight. It should also be noted that the range of shell ratio values for each fishing location overlapped (Table 2).

The number of mesocones within a row of lateral teeth and the number of medial cusps were constant for each individual whelk (n = 150). Significant differences ($P \leq 0.05$) occurred between McClellanville and St. Katherine's Island samples in the mean number of mesocones and medial cusps (Table 3). Only relative exocone distance varied sigificantly ($P \leq 0.05$) among all 3 fishing locations. However, whelks from McClellanville and St. Katherine's Island differed statistically ($P \leq 0.05$) in all the other radula ratios. Considerable overlap was also observed in the range of these values among the fishing locations (Table 3).

DISCUSSION

Female *B. carica* had a wider SL range and occurred more frequently in larger SL class intervals than males in collections from waters near Beaufort, NC (Magalhaes 1948), Charleston, SC (Weinheimer 1982) and the 3 sites sampled during the study. Statistical analysis from the present study agrees with the results of Weinheimer (1982) that the SL of female whelk samples is significantly larger than male whelks. Differential growth, survival and protandric hermaphroditism are among the more commonly suggested reasons for sexual dimorphism in prosobranch gastropods (Webber 1977). We have observed that tagged female *B. carica* grow faster than tagged male knobbed whelks (Eversole and Anderson 1988) and Kraeuter et al. (1989) have preliminary evidence that *B. carica* exhibits protandric hermaphroditism; however, no information is available on differential survival in this species.

Subtle differences in shell morphology between sexes occur in some prosobranch gastropods. Male *Lunatia lewisi* (Gould) have heavier shells than females the same tissue weight (Bernard 1968). Hallers-Tjabbes (1979) found a difference between sexes in the shape of the aperture in *Buccinum undatum* L. Sexual dimorphism in SW has been reported in the genera *Nucella* and *Strombus* with females having proportionally greater SW than males of similar SL (Webber 1977 and references within). Shells of female *B. carica* have wider apertures than males. Although the reasons for differences in shell morphology are not fully understood, the wider aperture of a female whelk would accommodate a larger foot and should provide a greater surface area for stability (Kitching et al. 1966) and oviposi-

TABLE 1.

Least square means*, standard errors and ranges of shell morphologies using SL as a covariate for male and female *Busycon carica*.

	Male		Female	
Shell Morphologies	**N**	**$\bar{x}$ ± SE (range)**	**N**	**$\bar{x}$ ± SE (range)**
Relative Shell Width (SW/SL × 100)	251	55.6 ± 0.2 (48.8–65.6)	966	57.0 ± 0.1* (50.4–73.3)
Relative Spine Length (SWs-SW/SL × 100)	251	6.4 ± 0.3 (3.8–16.1)	966	8.3 ± 0.1* (0.0–21.8)
Relative Spire Length (SL-AL/SL × 100)	251	13.9 ± 0.2 (8.6–22.4)	966	13.2 ± 0.1* (5.6–37.8)
Relative Spire Height (SpH/SL × 100)	251	23.6 ± 0.1 (21.0–35.4)	966	24.4 ± 0.1* (17.7–55.0)
Relative Shell Weight (ShWt/SL × 100)	251	97.8 ± 1.4 (34.0–151.0)	964	104.5 ± 0.7* (53.3–289.9)
Tumidity (%)	255	22.0 ± 3.0	968	23.6 ± 1.3

* Means in the same row followed by an asterisk (*) are significantly different ($P \leq 0.05$) using linear contrasts.

TABLE 2.

Least square means*, standard errors and ranges of shell morphologies using SL as a covariate for female *Busycon carica*.

Shell Morphologies	McClellanville N	McClellanville $\bar{x} \pm SE$ (range)	Charleston N	Charleston $\bar{x} \pm SE$ (range)	St. Katherine's Island N	St. Katherine's Island $\bar{x} \pm SE$ (range)
Relative Shell Width (SW/SL × 100)	332	57.0 ± 0.1[a] (50.4–73.3)	351	56.9 ± 0.2[a] (51.3–64.2)	283	57.2 ± 0.2[a] (51.4–65.3)
Relative Spine Length (SWs-SW/SL × 100)	332	7.1 ± 0.2[a] (0.0–15.9)	351	8.1 ± 0.2[b] (0.6–21.7)	283	9.7 ± 0.2[c] (2.1–21.8)
Relative Spire Length (SL-AL/SL × 100)	332	14.1 ± 0.1[a] (7.5–37.8)	351	12.7 ± 0.1[b] (5.6–20.0)	283	12.8 ± 0.1[b] (6.9–20.0)
Relative Spire Height (SpH/SL × 100)	332	23.9 ± 0.1[a] (20.5–32.4)	351	24.9 ± 0.1[b] (17.7–55.0)	283	24.6 ± 0.1[c] (20.6–29.0)
Relative Shell Weight (ShWt/SL × 100)	332	109.8 ± 1.1[a] (53.3–264.0)	349	101.3 ± 1.2[b] (53.9–289.9)	283	101.1 ± 1.2[b] (55.4–235.9)
Tumidity (%)	334	30.9 ± 2.2[a]	351	2.4 ± 2.4[b]	283	37.4 ± 2.3[c]

* Means in the same row followed by the same letters are not significantly different ($P \leq 0.05$) from each other using linear contrasts.

tioning. Meat yield (i.e., head-foot portion) was also found to be greater from female knobbed whelks than males (Anderson et al. 1985). As noted by Aldridge et al. (1986), it is often assumed that greater reproductive expenditures and/or large biomass of female molluscs are compensated for by greater kinetic expenditures in males, but the bioenergetics of sexual dimorphism are rarely so simple.

Shell shape and sculpture has conventionally been used in the classification of gastropods (Thompson 1942, Raup 1966, Vermeij 1971, Hallers-Tjabbes 1979, Dillon and Davis 1980, Harasewych 1981). Differences in shell shape among populations of gastropods may reflect genetic differences. Relative spire height is synonymous to the angle of elevation which Vermeij (1971) used to describe species of gastropods, and relative spine length is a measure of the extent of knobbiness which Abbott (1974) used to classify whelks. Relative spire height and spine length appeared to be the most consistent features different among samples of harvested knobbed whelks. Other features such as relative shell weight appeared to be less useful in distinguishing samples, possibly because whelk shells are often abraded by burrowing and broken during feeding (Carriker 1951). Also, detection of a tumid ridge becomes more subjective in the less pronounced cases.

Taxonomists commonly use radula dentition and morphology as identification criteria for gastropods below the

TABLE 3.

Least square means*, standard errors and ranges of radula morphologies using SL as a covariate for male and female *Busycon carica*. Six medial and lateral teeth/individual (n = 150) were analyzed for variation in radula morphology.

Radula Morphologies	McClellanville $\bar{x} \pm SE$ (range)	Charleston $\bar{x} \pm SE$ (range)	St. Katherine's Island $\bar{x} \pm SE$ (range)
Relative Exocone Height (B/A × 100)	96.2 ± 0.3[a] (82.9–107.2)	96.7 ± 0.3[a] (85.9–107.1)	95.0 ± 0.3[b] (84.2–108.6)
Relative Exocone Distance (B/C × 100)	179.5 ± 0.7[a] (147.7–218.4)	187.9 ± 0.8[b] (163.6–228.6)	184.0 ± 0.7[c] (153.2–241.4)
Relative Mesocone Distance (D/A × 100)	40.7 ± 0.3[a] (25.3–52.8)	40.4 ± 0.3[a] (28.4–54.6)	39.9 ± 0.3[b] (22.1–59.7)
Relative Endocone Distance (E/F × 100)	63.4 ± 0.4[b] (43.8–84.4)	64.0 ± 0.6[a] (47.2–84.8)	65.1 ± 0.4[b] (43.2–83.7)
Relative Endocone Height (F/A × 100)	60.6 ± 0.2[a] (50.6–71.0)	60.8 ± 0.2[a] (53.5–68.8)	59.4 ± 0.2[b] (50.0–67.2)
Number of Mesocones	5.12 ± 0.05[a] (4–8)	5.06 ± 0.05[ab] (4–6)	4.96 ± 0.05[b] (4–7)
Number of Medial Cusps	3.34 ± 0.03[a] (2–5)	3.23 ± 0.03[b] (2–5)	3.25 ± 0.03[b] (1–4)

Means in the same row followed by the same letters are not significantly different ($P \leq 0.05$) from each other using linear contrasts.

species level (Howe 1930, Berrie 1959, Abbott 1974, Hunter 1975). Identification of races within certain species is possible because radula dentition and morphology is believed to be under strict genetic control (Berrie 1959) and not affected by environmental factors (Russell-Hunter 1978).

Shell size does appear to be an important variable to consider when comparing radula dentition and morphology among some gastropod populations (Howe 1930, Katsigianis and Harman 1973). Number of cusps decreased and tooth shape changed with increased SL in *B. carica*. Significant differences, however, were observed in radula dentition and morphology even after correcting for SL differences among the knobbed whelks collected from 3 different fishing areas.

Clinal variations in shell morphologies have been reported for *B. carica* collected from 5 widely separated locations along the species range (Edwards 1988). She found that spire height decreased and spinosity (spine length) and shell weight increased in a north–south direction and hypothesized that these observed variations were related to a north–south gradient in sediment type and of increased predation. Our results do not agree with these observations (i.e., spire height increased and shell weight decreased in a north–south direction), possibly because the substrates at the 3 offshore collection sites in this study were similar (Edwards 1988 and references within). To further identify or disclaim agents by which selection is acting (e.g., increased predation in this relatively uniform marine province) will require more detailed information about offshore populations of knobbed whelks than we possess at this time. Of course, selection in stocks of *B. carica* could also be following the pattern which has been termed anacoluthical (McMahon and Russell-Hunter 1977, Russell-Hunter 1983) in which different adaptations have proceeded in discontinuous series.

Berrie (1959) maintained that the shape of radula teeth are under rigid genetic control and that small differences in radula shape are unlikely to have any adaptive significance. Obviously, variation in such features could serve as a naturally occurring genetic "marker" (Hunter 1975). Statistical comparisons of radula dentition and shape indicate significant differences among the 3 whelk collections, but no general clinal pattern was apparent in the data (Table 3). Also, we are not aware of any examples in the literature of clinal variation in radula shape. Furthermore, both Berrie (1959) and Hunter (1975) stress the lack of geographic clines in radula shape in their lymnaeid populations. If we assume that gene flow is sufficiently low among these 3 collection sites as would be expected with an organism like a whelk with limited longshore movement (Pulley 1959); and that the character exhibiting variation (i.e., radula shape) has little selective value (Berrie 1959), then we may also conclude that these collection sites represent discrete populations of whelks. It is possible that a contiguous distribution of whelks exists along the South Atlantic Bight, but observations from many exploratory cruises and discussions with commercial fishermen indicate that this is not the case. Evidence of discrete populations of knobbed whelks presents the fishery biologist with the prospect for more efficient management of the resource on an individual locality basis rather than a regional strategy.

ACKNOWLEDGMENTS

Financial support was provided by the South Carolina Agricultural Experiment Station, South Carolina Sea Grant Consortium and National Marine Fisheries Service. We thank the many commercial fishermen for sharing information and specimens and Larry W. Grimes for statistical assistance. Authors also wish to thank Dr. W. D. Russell-Hunter, Dr. Joseph R. Tomasso and 2 anonymous reviewers for critically reading an earlier draft of this manuscript and offering many helpful suggestions.

REFERENCES CITED

Abbott, R. T. 1974. *American Seashells*, 2nd Ed. Van Nostrand Reinhold Co., New York. 663 pp.

Aldridge, D. W., W. D. Russell-Hunter & D. E. Buckley. 1986. Age-related differential catabolism in the snail, *Viviparus georgianus*, and its significance in the bioenergetics of sexual dimorphism. *Can. J. Zool.* 64:340–346.

Anderson, W. D., A. G. Eversole, B. A. Anderson & K. B. Van Sant. 1985. A biological evaluation of the knobbed whelk fishery in South Carolina. NMFS Compl. Rep. No. 2-392-R. S.C. Wildl. Mar. Resour. Dept., Charleston. 72 pp.

Bernard, F. R. 1968. Sexual dimorphism in *Polinices lewisi* (Naticidae). *Nautilus* 82:1–3.

Berrie, A. D. 1959. Variation in the radula of the freshwater snail *Lymnaea peregra* (Muller) from northwestern Europe. *Ark. Zool.* 12:391–404.

Carriker, M. R. 1951. Observations on the penetration of tightly closing bivalves by *Busycon* and other predators. *Ecology* 31(1):73–83.

Dillon, R. T. & G. M. Davis. 1980. The *Goniobasis* of southern Virginia and northwestern North Carolina: Genetic and shell morphometric relationships. *Malacologia* 20:83–98.

Edwards, A. L. 1988. Latitudinal clines in shell morphologies of *Busycon carica* (Gmelin 1791). *J. Shellfish Res.* 7:461–466.

Eversole, A. G. & W. D. Anderson. 1988. Growth and movement of offshore populations of *Busycon carica* and *B. canaliculatum* evidenced by mark and recapture results. *J. Shellfish Res.* 7:157.

Hallers-Tjabbes, C. C. ten. 1979. Sexual dimorphism in *Buccinum undatum* L. *Malacologia* 18:13–17.

Harasewych, M. T. 1981. Mathematical modeling of the shells of higher prosobranches. *Bull. Am. Malacol. Union* 4:6–16.

Hollister, S. C. 1959. A review of the genus *Busycon* and its allies. *Paleontogr. Am.* 4:49–126.

Howe, S. W. 1930. A study in the variations in the radula of a snail with particular reference to the size of the median teeth. *Nautilus* 44:53–66.

Hunter, R. D. 1975. Variation in populations of *Lymnaea palustris* in upstate New York. *Am. Mid. Nat.* 94(2):404–420.

Katsiglanis, T. S. & W. N. Harman. 1973. Variation in radular teeth of *Helisoma anceps* (Mencke). *Nautilus* 87(1):5–7.

Kitching, J. A., L. Muntz & F. J. Ebling. 1966. The ecology of Lough Ine XV. The ecological significance of shell and body forms in *Nucella*. *J. Anim. Ecol.* 35:113–126.

Kraeuter, J. N., M. Castagna & R. S. Bisker. 1989. Growth rate estimates for *Busycon carica* in Virginia. *J. Shellfish Res.* 8:(In Press).

Magalhaes, H. 1948. An ecological study of snails of the genus *Busycon* at Beaufort, N.C. *Ecol. Monogr.* 18:377–409.

McMahon, R. F. & W. D. Russell-Hunter. 1977. Temperature relations of aerial and aquatic respiration in six littorial snails in relation to their vertical zonation. *Biol. Bull.* 152:182–198.

Pulley, T. E. 1959. *Busycon perversum* (Linne) and some related species. *Rice Univ. Instit. Pamph.* 46:70–89.

Raup, D. M. 1966. Geometric analysis of shell coiling: General problems. *J. Paleontol.* 40(5):1178–1190.

Russell-Hunter, W. D. 1978. Ecology of freshwater pulmonates. In Fretter V. & J. Peake (eds.) *Pulmonates, Volume 2A*. Academic Press, NY, pp. 335–383.

Russell-Hunter, W. D. 1983. Overview: Planetary distribution of and ecological constraints upon the Mollusca. In Wilbur, K. M. (ed.) *The Mollusca, Volume 6*. Academic Press, NY, pp. 1–27.

SAS Institute Inc. 1982. *SAS User's Guide: Statistics 1982 Ed.* SAS Institute Inc., Cary, NC. 584 pp.

Thompson, D. A. 1942. *On Growth and Form*. Cambridge Univ. Press, NY. 1116 pp.

Vermeij, G. J. 1971. Gastropod evolution and morphological diversity in relation to shell geometry. *J. Zool. (Lond.)* 163:15–23.

Webber, H. H. 1977. Gastropoda: Prosobranchia. In: Giese, A. C. & J. S. Pearse (eds.) *Reproduction of Marine Invertebrates. Volume 4. Gastropoda*. Academic Press, NY, pp 1–97.

Weinheimer, D. A. 1982. Aspects of the biology of *Busycon carica* (Gmelin, 1791) in waters off South Carolina, with emphasis on reproductive periodicity. M.S. Thesis, College of Charleston, SC. 92 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 219–225, 1989

GROWTH RATE ESTIMATES FOR *BUSYCON CARICA* (GMELIN, 1791) IN VIRGINIA

JOHN N. KRAEUTER[1], MICHAEL CASTAGNA[2] AND ROBERT BISKER[2]

[1]*Rutgers University*
NJAES and Fisheries and Aquaculture Technology Extension Center
Shellfish Research Laboratory
P.O. Box 687
Port Norris, NJ 08349
[2]*Eastern Shore Laboratory*
Virginia Institute of Marine Science
College of William and Mary
Wachapreague, VA 23480

ABSTRACT Although whelks are a conspicuous predator on intertidal flats, limited data are available on their growth rates. We have attempted to use 3 independent measures of growth to arrive at estimated growth rates for juvenile and mature whelks. Average length growth rate for the first 10 yrs of life in laboratory growth studies was 14.4 mm/yr. Opercular studies for field collected individuals indicate that individuals 12–14 yrs old averaged 6.5 mm increase in length/year. Growth of mark-recaptured individuals indicated growth of mature whelks was episodic with indefinite periods of no or negative growth (due to shell chipping) interspersed with periods of rapid growth.

KEY WORDS: *Busycon carica*, growth, whelks

INTRODUCTION

There is little published literature on individual growth rates of busyconine whelks or conchs. The work of Magalhaes (1948) near Beaufort, North Carolina is the only published study to have measured growth of *Busycon carica* in the field, and Sisson (1972), working in Narragansett Bay, Rhode Island, appears to be the only study of the growth of *Busycon canaliculata*. Ecological information and size data are available for *Busycon canaliculata* primarily in relation to the trap fishery on Cape Cod, Massachusetts (Shaw 1960, Davis and Matthiessen 1978) and in Rhode Island (Sisson 1972). The general ecology of the whelks is best known from Magalhaes (1948) and Peterson (1982) in North Carolina and the efforts of Menzel and Nichy (1958), Paine (1962, 1963) and subsequent studies by Kent (1983a, b) in Alligator Harbor, Florida.

Techniques of age determination in gastropods have not received the attention that has been devoted to pelecypods. Kubo and Kondo (1953) appear to have been the first to provide evidence that opercular marks can be utilized for age determination. Santarelli and Gros (1985) have shown that the age of *Buccinum undatum* can be determined from opercular striae and Sire and Bonnet (1984) were able to show that daily growth lines could be found in the operulum of *Turbo setosus*.

This study presents data on growth of *Busycon carica* in Virginia. The study used 3 separate techniques to estimate growth rate: mark-recapture, opercular growth marks and laboratory rearing. The growth estimates from these are compared to those of Magalhaes (1948).

METHODS

The study site was an intertidal flat ~0.8 km in length on Cedar Island on the north side of Wachapreague Inlet, Virginia. Sediment composition of the flat graded from sand to sand mixed with mud as it extended northward from Wachapreague inlet to the inlet of a marsh creek (Brandywine channel). The substrate inside the marsh creek became increasingly muddy and searches for whelks within this habitat were limited to the creek mouth delta. Spring tidal range at Wachapreague Inlet is 1.4 m and salinities are typically 28–32‰.

Three methods were used to investigate growth in *Busycon carica:*

1. Measurement of individuals marked and recaptured in the field,
2. Examination of growth lines on the operculum,
3. Measurement of laboratory reared individuals.

Measurements were made of length and width in field studies, but only length was recorded for laboratory studies. Length is the maximum distance between the tip of the spire and the tip of the siphonal canal. Width was determined by placing the organism on a fish measuring board (Wildlife Supply Co.) with the aperture down and with the body whorl and distal end of the siphonal canal aligned against the zero end. The width was recorded as the greatest distance to the opposing side of the body whorl as indicated by the sliding blade. Spines were included in the width measurement. Laboratory reared animals were measured at first with venier calipers and, when large enough, with a measuring board. Individuals collected from the

field were measured with the standard fish measuring board. The precision of this measurement was determined by taking 5 length and width measurements on each of 16 individual whelks and determining the mean and standard deviation.

The first method of investigating growth in *B. carica* was developed as an integral part of a mark-recapture study. Individuals were sequentially numbered, measured, sexed and released (Table 1). Recaptures were made at multiple times once an individual was released. If multiple recaptures were made measurements were repeated, but only the last recapture was used to calculate growth rate. The seasonal, but episodic, nature of growth in this species (and the fact that fewer and fewer recaptures are made after 1 yr in the field) makes data for the last recapture the best estimate of growth. Data have been divided into 2 separate categories based on the marking technique. For the first 2 yrs (Old series OS) shells were scrubbed with a wire wheel attached to an industrial grinder, dried and marked on the body whorl opposite the aperture with a felt tip marker. After the second yr it became apparent that recoveries for growth studies would take longer than 2 yrs and that felt tip marks would not last more than 2 seasons. The same procedures were used to clean the shell for the second marks (New series NS), but after drying a numbered adhesive backed tag was placed on the shoulder of the shell on the side opposite the aperture. Epoxy resin was placed over the tag followed by a piece of fine mesh fiberglass cloth and another coat of epoxy resin. This procedure not only secured the tag, but made it resistant to abrasion, allowed fouling to be scraped off and yet permitted the number to be read. No tag loss was documented with this method. Tags were found up to 6.9 yrs after tagging.

The second method, aging by marks on the operculum, was an attempt to derive an independent measure of individual growth. Opercula from known age laboratory reared individuals were sectioned lengthwise and polished. Sections were scanned by 3 separate judges for darkened "growth checks" suggestive of annually-formed marks using either a dissecting microscope or, in the case of smaller individuals, a compound scope. Mean ages for the whelks were computed and compared with the known laboratory age. Similar data were obtained by one individual scoring each sectioned operculum at least 3 times. A later modification involved embedding opercula in plastic resin before cutting and polishing. Because the laboratory growth studies were conducted in sand substrate with an abundant (but of limited diversity) source of food and in ambient flowing seawater we presumed that the major opercular marks were due to growth changes from temperature shifts and that these were equivalent to marks observed on opercula of field collected specimens.

To examine the relationship between various soft parts and presumed age of whelks, field collections were made during the summer of 1980, the shell of each was measured, the soft parts were wet weighed and the opercula removed. The opercula were sectioned and at least 3 age estimates were made for each operculum.

For the third growth estimate we used culture techniques. *B. carica* were hatched from egg cases that had been collected in the winter of 1977. The newly hatched individuals from several egg cases were reared inside a polypropylene bag filter that received flowing ambient Wachapreague Channel seawater. This was done because numerous attempts to keep newly hatched whelks in aquaria or on substrates such as sand or mud failed because the animals continually climbed out of the substrate to the highest point and desiccated. When the animals reached ~20 mm shell length they were transferred to running seawater trays with sand substrate from the intertidal flat and supplied with live clams (initially *Mulinia lateralis* and later *Mercenaria mercenaria* or *Mya arenaria*). Length measurements were made on individuals removed from the substrate in June, August, September, October and November during the first year and 1–3 times/yr in subsequent years. Samples of these individuals formed the basis for confirming the annual ring formation within the operculum. Individuals were not sexed prior to the spring of 1986.

TABLE 1.

Numbers of conchs *Busycon carica* marked and released for individual growth rate studies.

Mark-Release Period	Number Released
Old Series	
Fall 1975	250
Spring–Summer 1976	250
Fall 1976	60
New Series	
Spring 1977	302
Fall 1977	342
Spring 1978	308
Fall 1978	305
Spring 1979	213
Fall 1979	342
Spring 1980	299

RESULTS

Error due to measurement was determined by repetitive measurement of the same individual. The mean and 95% confidence limits for 16 individuals that were measured 5 times each were: Length (mean ± a S.E. of 0.0697 mm) and width (mean ± a S.E. of 0.0771 mm). Estimates from mark recapture data yielded highly variable results and a high percentage of individuals exhibited negative growth rates. It is important to note that laboratory and opercular growth estimates reflect the annual growth cycle while, because most of the individuals recaptured were in the field

for less than a year, most of the mark-recapture data did not. Laboratory studies indicated that most growth occurred between May–October (Fig. 1). Growth rates estimated from mark-recapture data were normalized to daily growth by dividing the measured growth by the number of days the particular animal had been in the field (or since the last measurement in the laboratory). Yearly growth rates were calculated from these daily estimates and are based on a 183 day growth period each year. All data for growth are first given for length and then for width (indicated by a preceding w). If only one set of data is presented, it is for length.

The OS recaptured whelks were out for an average of 209 days, and 40 (38%) (w 35) of the 105 recaptured exhibited negative growth. Of the 184 epoxy tagged NS whelks recaptured, 80 (w 79), or 43% exhibited negative growth. These NS individuals were out for an average of 279 days.

Average growth of NS whelks was 1.8 ± 1.1 mm/yr (w 1.1 ± 0.7 mm/yr) (all confidence intervals are reported as mean ± standard error at t = 0.05) while OS grew at 0.7 ± 0.9 mm/yr (w 0.3 ± 0.6 mm/yr). If negative growth rates are assumed to be 0 the rates become 3.2 ± 1.0 mm/yr (w 2.1 ± 0.6 mm/yr) and 1.9 ± 0.7 mm/yr (w 1.1 ± 0.3 mm/yr) for the NS and OS, respectively. If the data are reconfigured to contain only those individuals that were in the field for more than 365 days, the average growth rate becomes 1.9 ± 1.5 mm/yr (w 1.6 ± 0.9 mm/yr), and for same data set, if negative growth is considered as 0 the average growth becomes 2.3 ± 1.4 mm/yr (w 1.7 ± 0.8 mm/yr).

The smallest individual tagged and recaptured was out for 132 days and grew from 138–151 mm (0.098 mm/d) or 17.9 mm/yr (w (a separate individual) 73–84 mm in 602 days (0.018 mm/d or 3.3 mm/yr). Most whelks marked and released were larger than 170 mm (w 90 mm). The largest growth increment measured was 59 mm (w 34 mm) for an individual that was recaptured after 498 days (0.118 mm/d or 21.6 mm/yr) (w 0.068 mm/d or 12.5 mm/yr). The fastest growth rate was by an individual 197 mm long (w separate individual 93 mm) that grew to 220 mm (w 106 mm) in 104 days (0.22 mm/d or 40.4 mm/yr) (w 0.125 mm/d or 22.5 mm/yr). The longest time between release and recapture was 2534 days and that individual (initial 189 mm (w 97 mm) final 187 mm (w 95 mm)) had a negative growth rate of 0.14 mm/yr for both length and width.

Based on our combined tag studies an average growth rate for whelks by size class is provided in Table 2. Estimates for individuals smaller than 150 mm are suspect because of the few numbers represented. An average growth rate estimate obtained by combining all seven individuals smaller than 160 mm is 9.5 mm/yr or 0.052 mm/d.

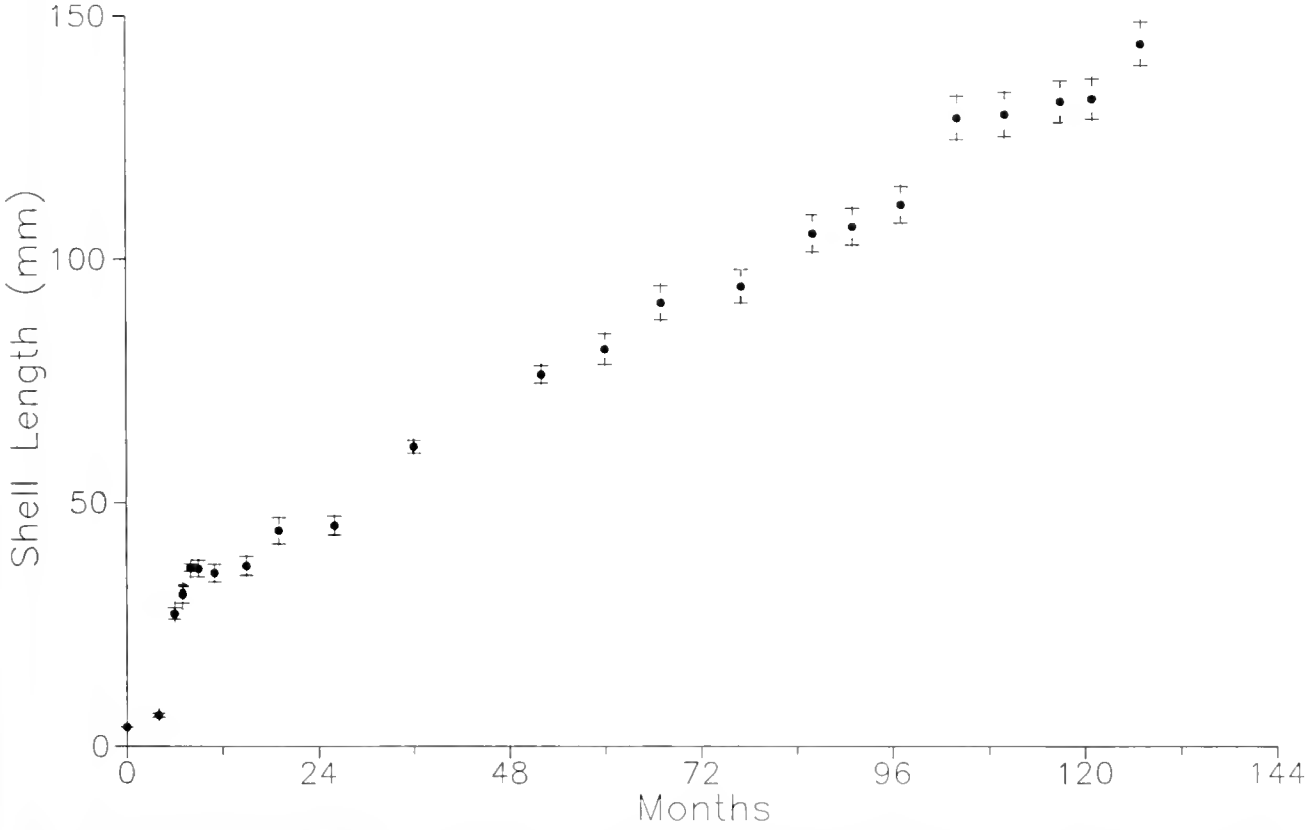


Figure 1. Growth of laboratory reared *Busycon carica*. All data are mean shell length ± 95% confidence limits. The zero point on the monthly axis indicates hatching in late February.

Growth rates for males were not calculated because too few were obtained to make accurate estimates. The second column in Table 2 provides data for only female whelks. Most males were in the 180–209 mm size classes and these appreciably reduced the growth rate estimates for those classes. Males did not alter estimates of yearly growth rate for size classes above 200–209 mm.

Opercular aging has received less work than analogous techniques for pelecypods. Initial estimates based on opercula of 3.6 yr old laboratory reared individuals indicated they were 5 yrs old or that the actual age was overestimated by 1.4 yrs. Refinement of the technique by careful attention to be sure that the operculum was cut at the proper angle relative to the origin, with other specimens the following year (actual age 4.3 yrs) yielded an estimated age of 4.8 yrs. This technique was further refined by embedding opercula from 3 individual 6+ and 3 individuals 7+ yrs old in liquid plastic (Clark 1980). These embedded opercula were then sliced, polished and microscopic examination of growth bands yielded average ages of 6 and 7.2 yrs respectively (Fig. 2).

Growth rate estimates from opercula sections of field collected individuals are given in Table 3. There were few males (15) in these collections and with the exception of a whelk in the 18–20 yr old group, no age group contained males smaller than the smallest female. The males were included within the data sets because there was no obvious separation by size. Due to the potential for error with the opercular technique, the irregular nature of whelk growth, what appears to be significant shell loss due to damage, and the relatively few specimens in each age category we have chosen to lump the data into 3 yr intervals (Table 3). The growth rate estimate for these whelks was obtained by averaging the means and was 2.1 mm/yr. Length-at-age, width-at-age and wet meat weight-at-age are given for the same individuals Figs. 3, 4 and 5, respectively. Least squares linear regressions provided the best fit to the data based on the largest R^2 (SAS 1987). Particular note should be made of the low meat weight relative to shell size for the 2 oldest individuals.

TABLE 2.

Growth rate estimates for *Busycon carica* as determined by tag studies at Wachapreague, Virginia. The data incorporate negative growth rates tabulated as 0 before computations. Males were not considered separately because too few were marked and recaptured. Yearly rates are the daily rate multiplied by 183 days to reflect growth during the 6 month warmer season.

	Male and Female whelks			Female whelks only		
Size Class mm	N	Daily mm	Yearly mm	N	Daily mm	Yearly mm
240–249	16	0.0038	0.7	16	0.0038	0.7
230–239	46	0.0047	0.9	45	0.0049	0.9
220–229	67	0.0123	2.3	66	0.0126	2.3
210–219	46	0.0107	2.0	46	0.0107	2.0
200–209	25	0.0085	1.6	18	0.0118	2.2
190–199	19	0.0197	3.6	17	0.0230	4.2
180–189	21	0.0236	4.3	16	0.0301	5.5
170–179	25	0.0134	2.5	21	0.0151	2.8
160–169	16	0.0550	10.1	16	0.0550	10.1
150–159	2	0.0904	16.5	2	0.0904	16.5
140–149	4	0.0205	3.8	3	0.0104	1.9
130–139	1	0.0983	18.0	1	0.0983	18.0

The growth curve for laboratory reared animals is given in Fig. 1. The lack of growth during the cooler months is particularly evident during the first year when data were taken more frequently. The first year mean growth from 4–36.5 mm was the greatest change recorded. Average size after 10 yrs of growth was 144 mm. Data were based on a minimum of 25 individuals measured for each data point until 1985 when the numbers were reduced to 20. These same individuals have been the basis for the sexual

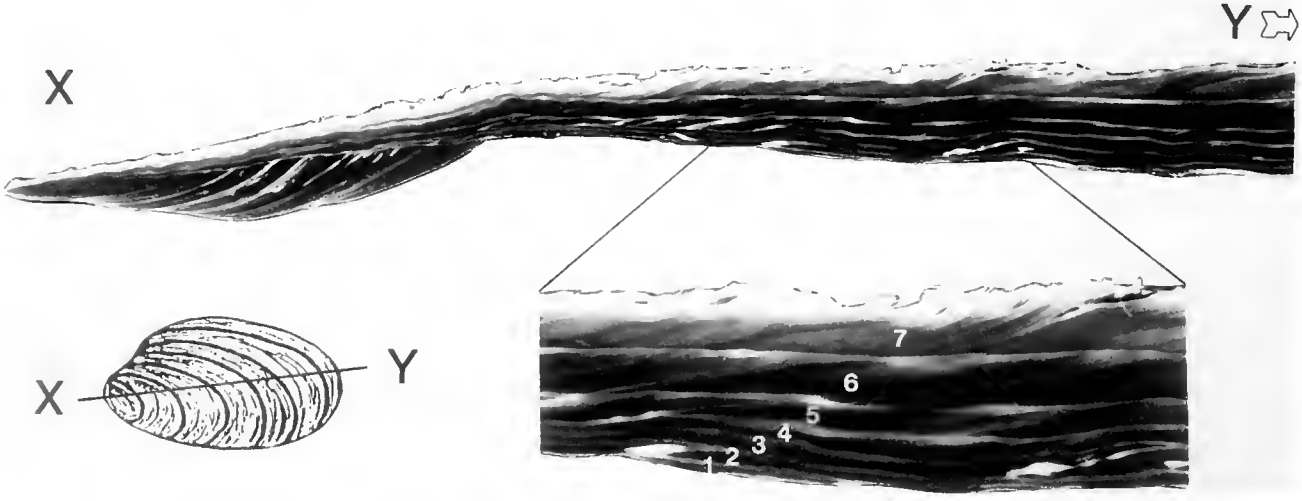


Figure 2. Cross section of a *Busycon carica* operculum from a laboratory reared individual indicating the yearly growth increments.

TABLE 3.

Growth rate estimates of *Busycon carica* in Virginia based on age estimated from sectioned opercula. Growth increment is the average growth for the three years between the age midpoints.

Estimated Opercular Age (yrs)	Mean Size (mm)	Midpoint Age (yrs)	Years	Growth Increment (mm)	Yearly Mean Growth (mm)
21–23	225.5	22	3	4.4	1.5
18–20	221.1	19	3	9.3	3.1
15–17	211.8	16	3	16.3	5.4
12–14	195.5	13	3	19.4	6.5
9–11	176.1	10			

maturation studies (see below) from April 1985–October 1987.

Of the 289 individuals that were tagged, sexed and recovered no sex reversals were noted. Males were 7.8% of the 295 OS whelks that were sexed and 9.0% of the 1859 NS individuals. Sexual differences were not checked in the hatchery reared individuals until they were 9 yrs old (mean size 130 mm). At that time all 20 individuals had either well developed (9) (based on size) or a small (11) penis. By October 1987 the same individuals were sexed and found to be males with a well developed (10) or a moderately developed (10) penis. No laboratory reared whelks have been identified as females to date.

DISCUSSION

Magalhaes (1948) reported that newly hatched *B. carica* were ~4 mm long. This is the same as for those hatched for the present study. She was able to maintain the newly hatched individuals for less than a month and one individual added 1.5 mm in 22 days. There do no appear to be any other published estimates of growth for newly hatched *Busycon*.

Growth rate for individuals maintained in our laboratory averaged 13.2 mm/yr (Fig. 1). The greatest growth rate was during the first year when the animals grew from 4 mm at hatching to an average of 36.5 mm. The first year's growth is probably at or near maximal rate for mid-Atlantic climatic conditions. Growth rate in subsequent years may be slightly slower than that of organisms held under optimum conditions. We did not attempt to provide a mixed diet of pelecypods for the juveniles, and we do not know of any information on the nutritional requirements of whelks. The lack of proper diet or many other culture factors may have reduced growth. The species provided to them were pelecypods, of proper size, that we were culturing for other studies. Growth, during the last year reported, averaged 11.9 mm between December 1986–October 27, 1987. Little or no growth occurred between the first of November and the end of the following March. Data from other opercular or shell ring studies on other gastropods also indicates that most growth is during the warmer months (Santarelli and Gros 1985, Williamson and Kendall 1981, Wefer and Killingley 1980). We are continuing these studies to further examine growth rate and to determine the time of development of egg laying females.

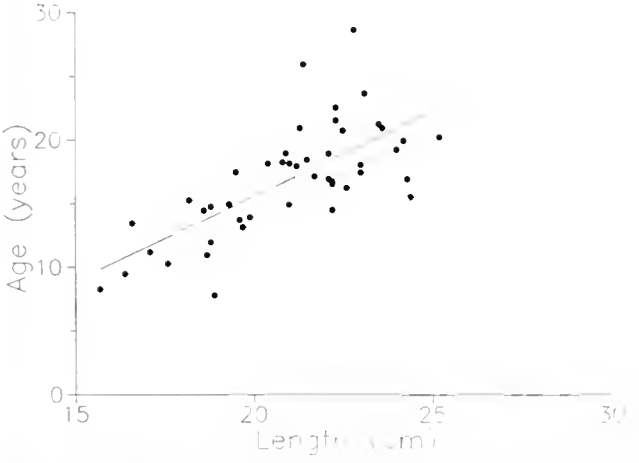


Figure 3. Length at age relationship for *Busycon carica* in Virginia based on opercular age data. Least squares regression provided the best fit for the data. Y(age) = −10.99 + 1.33x (n = 45, r^2 = 0.50, Probability = 0.0001).

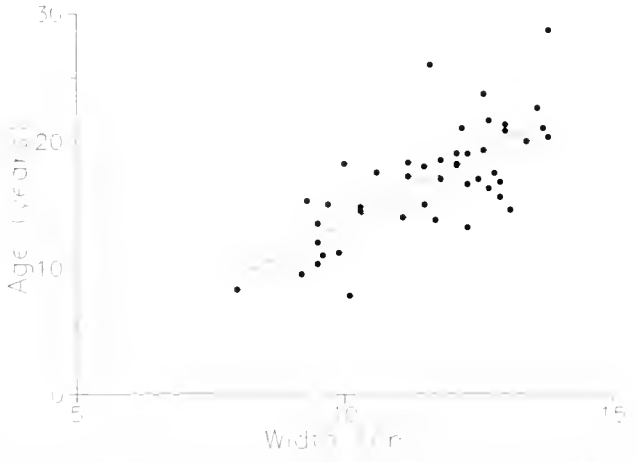


Figure 4. Width at age relationship for *Busycon carica* in Virginia based on opercular age data. Least squares regression provided the best fit for the data. Y(age) = −7.96 + 2.15x (n = 45, r^2 = 0.52, Probability = 0.0001).

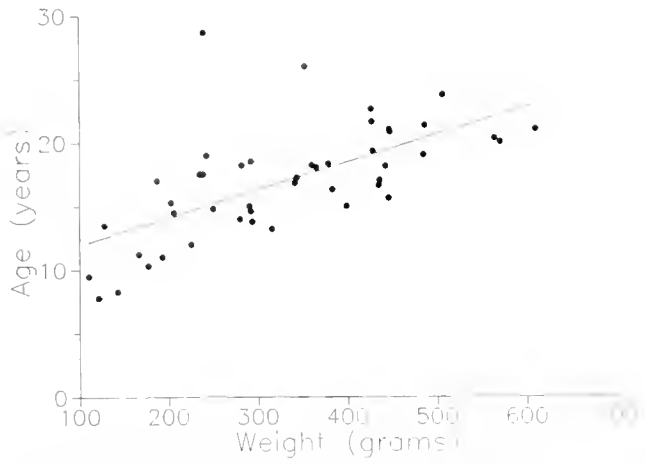


Figure 5. Wet weight at age relationship for *Busycon carica* in Virginia based on opercular age data. Least squares regression provided the best fit for the data. Y(age) = 9.79 + 0.022x (n = 45, r^2 = 0.40, Probability = 0.0001).

All reports of efforts to tag *Busycon* and later recover them for growth studies note the highly irregular growth rate and that few if any small whelks were found. Magalhaes (1948) found that "even immature specimens showed no change in size in a year, or two, in the field under apparently favorable conditions". She also found that individuals in the field for up to 810 days had not grown. Davis and Matthiessen (1978) reported their data in terms of width of the body whorl and thus their results cannot be directly related to the growth rates from the present study. We often found negative growth, but many of the smaller incremental losses may be due to measurement error. Some of the negative growth rates may be due to breakage of the shell during feeding. *B. carica* and other heavy shelled busyconine whelks use their shell in feeding and often chip the outer edge of the body whorl (Colton 1908, Warren 1916, Magalhaes 1948, Carriker 1951, Menzel and Nichy 1958, Paine 1962, Peterson 1982). Predators may also chip the shell margins of whelks (Magalhaes 1948).

The data provided by Magalhaes (1948, Table 15) are limited to 19 individuals that grew during her study. These 19 individuals were in the field for an average of 438 days and grew 12.3 mm or about 0.028 mm/d (10.2 mm/yr for a 365 day year). No mention was made of the numbers of recaptures that had no or negative growth. During our studies 43% of the whelks had either negative or no measurable growth for the period. Magalhaes (1948) reported 48% with no growth in her Table 12, but it is uncertain whether these are from the same samples as those reported in the growth studies. The average growth of North Carolina whelks with 8 specimens of 0 growth added to the total (similar to the 43% of our whelks) would have been 0.020 mm/d or 7.2 mm/yr. If these data are recalculated to our estimated 183 day growing season the annual growth would be 3.6 mm/yr. This figure is within the standard error (t = 0.05) of 3.2 ± 1.0 mm/yr for similarly corrected Virginia data.

We feel that the use of data with correction for negative growth provides the best estimate of increase in size for mature *Busycon carica*. There is no significant difference between the average growth for NS whelks in the field for an average of 279 days (3.2 ± 1.0 mm/yr) and the 2.3 ± 1.4 mm/yr estimated if the data set is restricted to those that were recaptured after at least 365 days. Because yearly rates are based on 183 days we feel they are comparable with the rates derived from opercular and laboratory studies at the same latitude. It is uncertain how this relates to sites farther north or south, but data provided by Magalhaes (1948) seem to indicate slightly more rapid growth in North Carolina.

Opercular aging studies were fewer in number and probably had the greatest source of error. The section that is cut across the operculum is critical, and because of the asymmetrical growth pattern it is relatively easy to miscount early growth rings. Embedding, slicing and polishing opercula allows better resolution of these early growth rings. Yearly mean growth (Table 3), based on opercular sections, decreased with age and if the mean of the samples is computed the growth rate for larger whelks is estimated to be 2.1 mm/yr, not appreciably different than the 2.3–3.2 mm/yr estimated from tag studies. Ten year old laboratory reared individuals averaged 144 mm while those estimated from opercular studies (midpoint 10 yrs old) averaged 176 mm. The difference could be attributed to either stunting due to laboratory rearing or to errors in the opercular technique. Mean yearly growth of laboratory reared individuals between ages 9 and 10 was 11.3 mm while the opercular method estimated those 12–14 years old were growing 6.5 mm/yr.

The greatest growth rate was the first year of life (32.5 mm/yr) and during the following 9 years rates varied from 22.4–3.4 mm/yr (average of 14.4 mm/yr for the first 10 yrs based on lab studies or 17.6 mm/yr based on opercular age). Growth beyond this point slows to ~3–4 mm/yr by the time the organism is 190 mm long or ~18–20 yrs old and is further reduced beyond 23 cm total length.

It is important to emphasize that, although we have provided estimates of yearly growth based on daily average growth rates, growth of mature whelks appears to be episodic. Our mark recapture studies and those of Magalhaes (1948) both had large numbers of whelks that did not grow for long periods of time. This lack of growth plus the loss of shell due to chipping resulted in significant numbers of mature individuals with negative growth rates.

ACKNOWLEDGMENTS

The authors would like to thank all the individuals of the VIMS Eastern Shore Laboratory that have contributed to

this effort over the past years. Particular acknowledgment is given to Lowell Fritz (Rutgers) for his help with embedding and opercular sectioning techniques and James Moore (VIMS) for his assistance with marking and in the field. This is contribution number D 32001-4-88 from the New Jersey Agriculture Experiment Station, Cook College and 1509 from Virginia Institute of Marine Science. Supported by New Jersey state funds.

LITERATURE CITED

Carriker, M. R. 1951. Observations on the penetration of tightly closing bivavles by *Busycon* and other predators. *Ecology* 32:73–83.

Clark, G. R. II 1980. Study of molluscan shell structure and growth lines using thin sections. In Rhodes, D. C. & R. A. Lutz (eds.) Skeletal growth of aquatic organisms, Plenum Press, NY, pp. 603–606.

Colton, H. S. 1908. How *Fulgur* and *Sycotypus* eat oysters, mussels and clams. *Proc. Acad. Nat. Sci. Philadelphia* 60:3–10.

Davis, J. P. & G. C. Matthiessen. 1978. Investigation on the Whelk fishery and resource of Southern New England. Report to National Marine Fisheries Service (Contract No. 03-7-043-35161) from Marine Research Inc. Falmouth, Massachusetts. 49 pp.

Kent, B. W. 1983a. Natural history observations on the busyconine whelks *Busycon contrarium* (Conrad) and *Busycotypus spiratum* (Lamarck). *J. Moll. Stud* 49:37–42.

Kent, B. W. 1983b. Patterns of coexistence in busyconine whelks. *J Exp. Mar. Biol. Ecol.* 66:257–283.

Kubo, I. & K. Kondo. 1953. Age determination of the *Babalonia japonica* (Reeve) an edible marine gastropod, basing on the operculum. *J. Tokyo Univ. Fish.* 39:199–207.

Magalhaes, H. 1948. An ecological study of snails of the Genus *Busycon* at Beaufort, North Carolina. *Ecological Monographs* 18:379–409.

Menzel, R. W. & F. E. Nichy. 1958. Studies of the distribution and feeding habits of some oyster predators in Alligator Harbor, Florida. *Bull. Mar. Sci. Gulf and Caribbean* 8:125–145.

Paine, R. T. 1962. Ecological diversification in sympatric gastropods of the genus *Busycon*. *Evolution* 16:515–523.

Paine, R. T. 1963. Trophic relationships of 8 sympatric predatory gastropods. *Ecology* 44(1):63–73.

Peterson, C. H. 1982. Clam predation by whelks (*Busycon* spp.): Experimental tests of the importance of prey size, prey density, and seagrass cover. *Mar. Biol.* 66:159–170.

Santarelli, L. & P. Gros. 1985. Determination de l'age et de la croissance de *Buccinum undatum* L. (Gasteropoda : Prosobranchia) a l'aide des isotopes stables de la coquille et de l'ornementation operculaire. *Oceanol. Acta* 8:221–229.

SAS Institute Inc. 1987. SAS/STAT guide for personal computers. SAS Institute Inc. Cary, North Carolina 1029 pp.

Shaw, W. N. 1960. Observations on habits and a method of trapping channeled whelks near Chatham, Massachusetts. US Fish and Wildlife Service, Special Scientific Report—Fisheries No. 325. 6 pp.

Sire, J. Y. & P. Bonnet. 1984. Croissance et structure de l'opercule calcifie du gasteropode polynesien *Turbo setosus* (Prosobranchia: Turbinidae) : determination de l'age individuel. *Mar. Biol.* 79:75–87.

Sisson, R. T. 1972. Biological and commercial fisheries related research on the channeled whelk *Busycon canaliculatum* (Linne) in Narragansett Bay, Rhode Island. Masters Thesis, University of Rhode Island.

Warren, S. 1916. The feeding habits of *Busycon*. *Nautilus* 30:66–68.

Wefer, G. & J. S. Killingley. 1980. Growth histories of strombid snails from Bermuda recorded in their O-18 and C-13 profiles. *Mar. Biol.* 60:129–135.

Williamson, P. & M. A. Kendall. 1981. Population age structure and growth of the trochid *Monodonta lineata* determined from shell rings. *J. Mar. Biol. Assoc. U.K.* 61:1011–1026.

Journal of Shellfish Research, Vol. 8, No. 1, 227–232, 1989.

GENETIC VARIABILITY AND GENE FLOW IN POPULATIONS OF *CRASSOSTREA VIRGINICA* (GMELIN) FROM THE NORTHERN GULF OF MEXICO

JAMES M. GRADY, THOMAS M. SONIAT, AND JAMES S. ROGERS
Department of Biological Sciences
University of New Orleans
New Orleans, Louisiana 70148

ABSTRACT Allelic variation at 23 presumptive gene loci was assessed among 10 populations of *Crassostrea virginica* from the northern Gulf of Mexico, extending from Biloxi Bay, Mississippi, westward to Galveston Bay, Texas, using standard procedures of starch gel electrophoresis. Ten of the 23 loci surveyed proved to be monomorphic across all population samples. The percentage of polymorphic loci among populations ranged from 30.4–39.1 with a mean of 34.8. Allelic variation at 6 gene loci, Aat-1, Icdh-1, Icdh-2, Mdh-1, Mdh-2, and Iddh, was limited to the occurrence of 1 or a few rare alleles usually in the heterozygous condition. Allelic diversity was consistently highest among all populations at 5 loci, Gpi, Lap-1, Mpi, Pgdh, and Pgm-1. Estimates of average heterozygosity among populations appeared to be slightly lower than reported for eastern Gulf coastal and Atlantic populations of this species. Gene flow among populations, as measured by the average number of migrant individuals/generation (Nm), was quite high (Nm = 7.25). The allelic frequency differences observed did not represent a discernible geographic pattern; differentiation due to local selective pressures is a more likely explanation.

KEY WORDS: oysters, *Crassostrea virginica*, genetics, Gulf of Mexico

INTRODUCTION

Genetic differentiation among populations of the American oyster, *Crassostrea virginica* (Gmelin), as determined through allozyme electrophoresis, appears to be quite limited (Buroker 1977, 1983, 1984, Groue and Lester 1982). This low level of genetic divergence among populations of a species with an extensive geographical range generally has been attributed to the nature and duration of the larval stage of this species (Buroker 1984, Rose 1984). *C. virginica* exhibits a planktonic larval stage that persists for 14–21 days during which time individuals are subjected to active and passive transport (Galtsoff 1964, Wood and Hargis 1971). Under these conditions zygotic dispersal and, consequently, gene flow among populations is significant (Buroker 1984).

While revealing a high degree of genetic similarity among populations of American oysters across broad geographical regions, previous studies of genetic variation in *C. virginica* have presented evidence of microgeographic genetic differentiation (Buroker 1983, Rose 1984). Genetic variation in these instances was not clinal in nature but was correlated with local environmental variability, e.g., salinity, temperature. As a consequence, genetic divergence was viewed as the result primarily of differential selectional regimes.

The present study was designed to survey genetic variability based on allozyme electrophoresis among central Gulf Coastal populations of *C. virginica* with a special emphasis on coastal Louisiana populations. Emphasis was placed on this region due to the highly variable nature of the extensive wetlands that comprise the coast of Louisiana and the significant commercial production of oysters within this region. The purpose of the study was thus to evaluate genetic variation across this relatively small geographic region, to determine levels of gene flow among populations, and to investigate the relationship between allelic variation and variation among salinity regimes.

MATERIALS AND METHODS

Ten populations of oysters were sampled from the main oyster-producing watersheds along the mid northern Gulf of Mexico from Galveston Bay, Texas to the Mississippi Sound (Fig. 1). Thirty oysters, from 6.0–12.5 cm in length, were sampled from each population. Adductor muscle tissues were excised and frozen at −70°C.

Tissues were homogenized in equivalent volumes of 0.25 M sucrose with 0.001 M dithioerythritol. Homogenates were centrifuged at 5,000 × g for 20 min at 4°C. Supernatant fractions were subjected to electrophoretic separation in 12% starch gels. Enzyme systems examined and electrophoretic conditions used to achieve separation are listed in Table 1. Histochemical staining procedures were modified from Harris and Hopkinson (1976). Individual alleles were assigned alphabetical designations based on relative motility.

The BIOSYS package of computer routines devised by D. L. Swofford and R. B. Selander was employed for calculating allele frequencies, heterozygosities, genetic distance and F-statistics, for performing chi-square tests of deviation from Hardy-Weinberg equilibrium and of allele frequency variation among populations, and for constructing phenograms and a Wagner tree of genetic distance relationships among populations. F. J. Rohlf's MINT program was used for constructing a shortest connected network and a principal coordinates ordination of the populations. Rogers' (1972) genetic distance was employed for

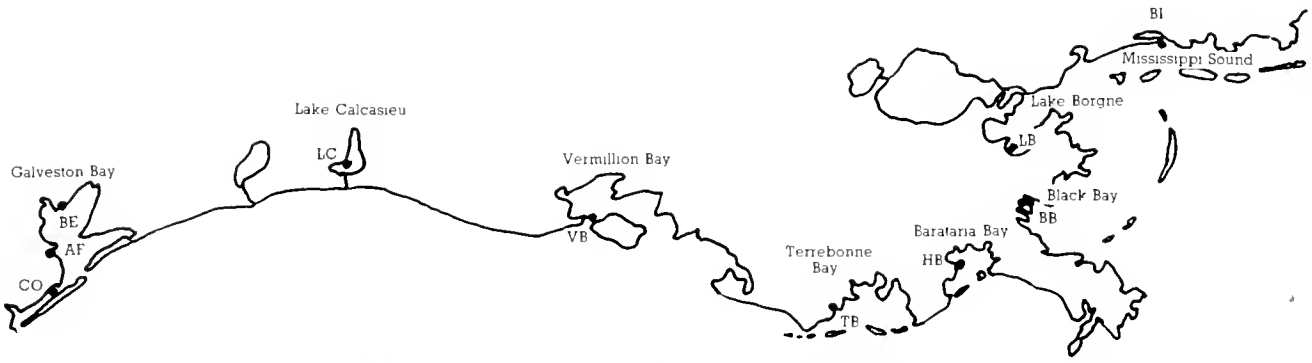


Figure 1. Map of the northern Gulf of Mexico from Galveston Bay to Mississippi Sound. Sample stations include Confederate reef (CO), April Fool reef (AF), Beasley's reef (BE), Lake Calcasieu (LC), Vermillion Bay (VB), Terrebonne Bay (TB), Hackberry Bay (HB), Black Bay (BB), Lake Borgne (LB), and Biloxi Bay (BI).

constructing the Wagner tree, shortest connected network and principal coordinates since it is a metric distance and thus more suitable for these sorts of analyses than distances that are not metrics. UPGMA phenograms were constructed with Rogers' distance and Nei's (1972) distance. Pearson product-moment correlations of salinity with the frequencies of 22 alleles that exhibited appreciable variability were calculated with the CORR procedure of SAS. All calculations were performed on the VAX cluster at the University of New Orleans Computer Research Center.

RESULTS

Of the 23 loci tested, 13 were polymorphic (Table 1) for the populations examined. Significant genotypic frequency deviations from Hardy-Weinberg (HDYWBG) expectations were observed in a number of populations (Table 2). The Lap-1, Pgm and Mpi loci showed deviations from HDYWBG expectations in the greatest number of populations, whereas Gpi, Pep-1, Pep-2, Aat-1 and Mdh-2 each showed HDYWBG deviation at a single site. In all but 1 of 17 cases heterozygotes were less frequent than expected.

TABLE 1.

Enzyme systems, electrophoretic conditions and presumptive gene loci examined.

Enzyme	E.C. Number	Locus Designation	Electrophoretic Conditions
Aspartate aminotransferase	2.6.1.1	Aat-1*	A,D
Aminopeptidase	3.4.11.1	Lap-1*	A,B
Fructose-bisphosphate aldolase	4.1.2.13	Ald	C
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6pdh	C
Glucose-6-phosphate isomerase	5.3.1.9	Gpi*	A,B
Glutamate dehydrogenase	1.4.1.2	Gtdh	D
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	Ga3pdh	C
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh	A,C
Hexokinase	2.7.1.1	Hk	D
L-Iditol dehydrogenase	1.1.1.14	Iddh*	B
Isocitrate dehydrogenase	1.1.1.42	Icdh-1*	A,C
		Icdh-2*	A,C
Malate dehydrogenase	1.1.1.37	Mdh-1*	A
		Mdh-2*	A
NADP-dependent malate dehydrogenase	1.1.1.40	Me	A,B
Mannose-6-phosphate isomerase	5.3.1.8	Mpi*	A,B
Peptidase (glycyl-L-leucine)	3.4.13.11	Pep-1*	A,B
(phenylalanyl-L-proline)		Pep-2*	A,B
Phosphoglucomutase	5.4.2.2	Pgm*	A,D
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh*	A,D
Superoxide dismutase	1.15.1.1	Sod-1	B,D
		Sod-2	B,D
Xanthine dehydrogenase	1.2.1.37	Xdh	B

Electrophoretic buffers are as follows: A—Tris-citrate, pH 7.5 (Stein et al. 1985); B—Tris-citrate, LiOH, boric acid, EDTA, pH 8.1 (Buroker 1978), C—NaPhosphate, pH 6.5 (Buroker 1978); D—Tris-borate, EDTA pH 9.0. *polymorphic loci in which the frequency of the most common allelle < 0.99.

TABLE 2.

Fixation index (F) values for populations and loci at which there are statistically significant deviations from Hardy-Weinberg expectations. Positive values indicate deficiencies of heterozygotes.

	Population							
Locus	CO	AF	BE	VB	TB	BB	LB	BI
Aat-1	—	—	1.00	—	—	—	—	—
Gpi	—	−0.12	—	—	—	—	—	—
Lap-1	—	0.45	0.47	0.43	—	—	—	—
Mpi	0.94	0.60	—	—	0.41	0.71	0.70	0.59
Mdh-2	—	—	—	—	0.65	—	—	—
Pep-1	—	—	0.47	—	—	—	—	—
Pep-2	—	—	—	—	0.15	—	—	—
Pgm	0.45	—	—	0.39	0.61	—	—	—

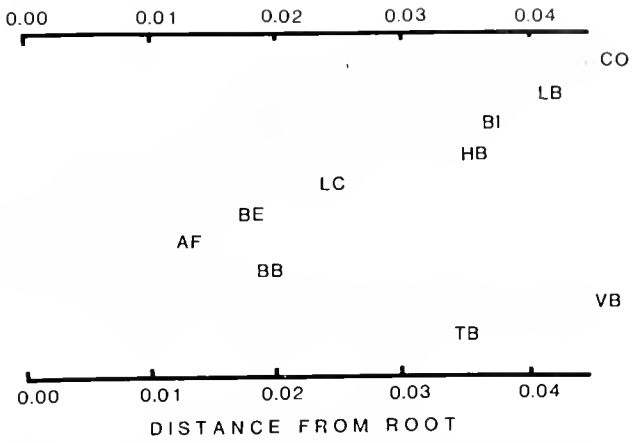


Figure 2. A distance Wagner tree of Rogers distance values, showing the affinities of the different populations. Sample site names are given in Fig. 1.

Chi-square tests of allelic frequency differences across all populations showed significant differences at the Iddh, Mdh-2, Pgdh, Lap-1, Pgm and Mpi loci.

To determine if variations in allelic frequencies formed a geographical pattern, sample sites were grouped into 3 regions, Galveston Bay, east of Galveston Bay and west of the Mississippi River, and east of the river. Wright's (1978) F-statistics (Table 3) indicate that the observed allelic variation is not explained by this simple geographical pattern since the F for regions-to-total is essentially zero. Further lack of a geographical pattern is apparent from the Wagner tree of Rogers' (1972) genetic distances (Fig. 2). For example, CO oysters (the westernmost site) were more similar to LB oysters (the easternmost Louisiana site) than to other oyster populations from Galveston Bay (AF and BE). AF and BE populations were more similar to a group which included BB and LC oysters. VB and TB oysters were similar to each other and formed a distinctive group. The UPGMA phenograms (not shown) of Rogers' and Nei's genetic distances also demonstrate an absence of regional grouping. Overall, the Rogers' genetic distances between populations were small, ranging from 0.024 (AF and BE) to 0.062 (VB and HB).

Estimating gene flow among the oyster populations by the "private alleles" method of Slatkin (1985) and Barton and Slatkin (1986) produces a value for Nm, the average number of migrant individuals/population/generation, of 7.25. This is quite high, since any Nm > 1 is sufficient to prevent a population that is receiving migrants from diverging from the population(s) that is the source of the migrants.

Rogers (1972) genetic distances were used to construct a principal coordinates ordination and a shortest connected network for the populations (Fig. 3). Similarities among the Galveston Bay sites are more apparent using this method (as compared to the previous phenogram) in that a connected network is formed by populations from CO, BE, and AF. CO, VB and a group consisting of LC, BI and HB occupy extreme positions on the principal coordinates graph.

Estimates of mean heterozygosity (Table 4) for the 10 Gulf of Mexico populations ranged from 0.085 (CO) to 0.135 (HB). These values are lower than those reported by Buroker (1983) for the Gulf of Mexico, which ranged from 0.200–0.254. Observed or direct count values were always lower than those expected by HDYWBG assumptions—in some cases significantly so (e.g., CO and LB).

No significant correlation was found between the frequency of a Lap-1 allele and salinity (Fig. 4). Confederate reef (CO) is an outlier in the correlation; however, if CO is removed from the analysis the correlation is stronger, but not significant at the 0.05 level. (Use of long-term salinity data did not improve the correlation.) CO was the highest salinity reef (30 ppt at the time of sampling), whereas VB, which had the lowest Lap-1^d allele frequency, had the lowest salinity (4 ppt). Of 21 other allele frequencies that exhibited appreciable variation, only one, Idh-2^b, was significantly correlated with salinity ($r = -0.67$). However, since it is expected that one in 20 independent correlation coefficients will appear significant at the 0.05 level by chance alone, it is likely that this instance is a spurious correlation.

TABLE 3.

A hierarchical analysis of allelic frequency differentiation of localities (sample sites or populations) versus regions (Galveston Bay, east of Galveston Bay and west of the Mississippi River, and east of the river) versus total population using the method of Wright (1978).

Comparison		Variance	
X	Y	Component	F_{XY}
Locality	Regions	0.05981	0.017
Locality	Total	0.05349	0.015
Region	Total	−0.00632	−0.002

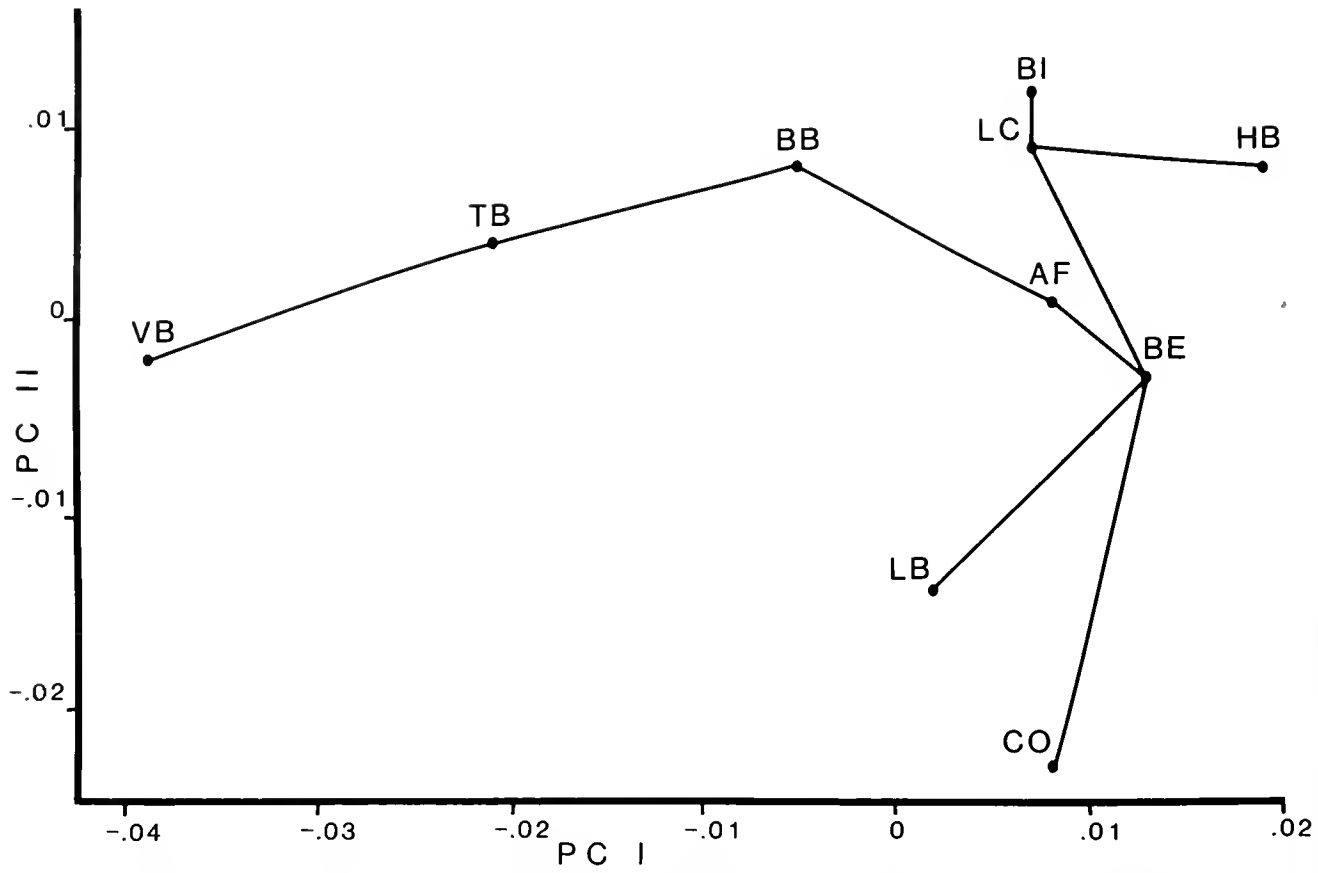


Figure 3. A principle coordinates ordination with the shortest connected network between populations. Sample site names are given in Fig. 1.

DISCUSSION

The low levels of genetic divergence among the 10 populations studied are probably due to substantial gene flow among populations. Certainly a lengthy planktonic larval stage, as seen in *C. virginica*, would contribute to zygotic dispersal ultimately leading to genetic homogeneity across populations, especially in situations where the effective population size is relatively large and significant isolating mechanisms are lacking. One might expect the Mississippi river to be a barrier to gene flow, since northerly-flowing ocean currents diverge at the river delta (Leipper 1954), this was not the case. It should be noted that oystermen transfer large numbers of oysters from seed grounds east of the Mississippi river to bedding (growout) grounds west of the river (Dugas et al. 1983). In the present study statistically significant variations in individual allelic frequencies have been demonstrated even in the presence of high levels of gene flow. This finding supports the notion of Ehrlich and Raven (1969) that populations which freely exchange genes may differentiate and that this differentiation may be due to different selection regimes within the species' range.

The allelic frequency differences observed did not represent a discernible geographical pattern; differentiation due to local selective pressures is a more likely explanation. Salinity would appear to be one important environmental factor acting to influence genetic variation based on both the variable salinities encountered in the present study and the previous demonstration of a relationship between salinity and allelic frequencies at the Lap-1 locus among populations of *Mytilus edulis* (Linnaeus) (Koehn 1983). However, allelic composition at the Lap-1 locus among the study populations was not significantly correlated with salinity. A variety of other environmental and ecological factors including parasitism can be hypothesized as local selective agents influencing allelic frequencies. Buroker (1983), for example, suggested *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) or MSX disease as a possible environmental selective force.

Significant deficiencies of heterozygotes have been discovered in several other electrophoretic surveys of molluscs (Berger 1983). Possible explanations include inbreeding, immigration from neighboring populations with substantially different allele frequencies, and the presence of null or inactive alleles. Foltz (1986) produced evidence from laboratory crosses that oysters carry null alleles at Mpi and Lap loci, 2 of the loci that most frequently exhibited deficiencies in our study. In some cases in our study the deficiencies are so great that one would expect, assuming the

TABLE 4.

Mean sample size and number of alleles/locus, percent polymorphic loci and mean heterozygosities for the various populations. Mean heterozygosity is given as direct counts and as Hardy-Weinberg (HDYWBG) expectations. Standard errors are in parentheses.

Population	Mean Sample Size/Locus	Mean No. of Alleles/Locus	Percentage of Loci Polymorphic	Mean Heterozygosity	
				Direct-Count	HDYWBG Expected
1. Confederate Reef	30.0	2.3	34.8	0.085	0.138
	(0.0)	(0.4)		(0.029)	(0.045)
2. Black Bay	30.0	2.4	39.1	0.125	0.156
	(0.0)	(0.4)		(0.043)	(0.051)
3. Beasley's Reef	29.9	2.1	39.1	0.103	0.145
	(0.1)	(0.4)		(0.036)	(0.048)
4. Hackberry Bay	29.7	2.2	39.1	0.135	0.164
	(0.2)	(0.3)		(0.042)	(0.052)
5. Lack Calcalsieu	29.9	2.3	30.4	0.123	0.159
	(0.1)	(0.4)		(0.040)	(0.053)
6. Biloxi	29.8	2.1	30.4	0.125	0.152
	(0.1)	(0.3)		(0.042)	(0.052)
7. April Fool Reef	29.8	2.3	30.4	0.114	0.150
	(0.1)	(0.4)		(0.040)	(0.051)
8. Lake Borgne	30.0	2.0	34.8	0.093	0.128
	(0.0)	(0.3)		(0.032)	(0.044)
9. Vermillion Bay	29.9	2.0	30.4	0.113	0.150
	(0.1)	(0.3)		(0.039)	(0.050)
10. Terreborne Bay	30.0	2.3	39.1	0.112	0.156
	(0.0)	(0.3)		(0.036)	(0.049)

null allele hypothesis, to find null homozygotes with no activity for the enzyme. Although a few oysters in our samples could not be scored for certain enzymes, these did not always occur in populations with significant deficiencies of heterozygotes. Vice versa, several populations that exhibited significant deficiencies of heterozygotes yielded no evidence of null homozygotes. Foltz (1986) noted that other studies of marine bivalves have produced similar results. He pointed out that assuming that null homozygotes are lethal or semilethal would require the additional assumption of high mutation rates or strong positive selection for the null heterozygotes.

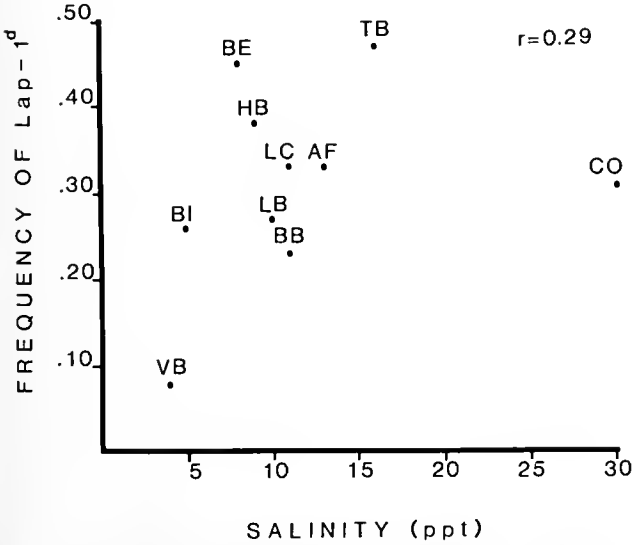


Figure 4. Correlation between the frequency of the Lap-1^d allele and water salinity. Sample site names are given in Fig. 1.

We have unpublished data on the incidence of *Perkinsus marinus* (Mackin, Owen and Collier), a known cause of oyster mortality, from all the oysters analyzed in this study (see also Soniat and Gauthier in press). Chi-square tests revealed no significant relationships between the frequency of individual alleles and intensity of infection. Heterozygosity of individual oysters was calculated, and a Kendall tau beta (τ) correlation was calculated to test the relationship between heterozygosity and infection intensity. Infection levels tended to be lower in oysters that exhibited higher levels of heterozygosity ($\tau = -0.087$), yet the relationship was not significant at the $P \leq 0.05$ level ($P = 0.077$). Thus, although allelic frequency differences exist which may be best explained by local selective pressures, the nature of these selective agents are still unknown.

ACKNOWLEDGMENTS

We are grateful to Roy Ary, Marc Breaux, Julie Gauthier, Rick Howell, Livingston Lanclos, Martin Mayer, Al Segura, Lorene Smith, Andy Terpak, and Susan Vreeland for their assistance in the collection of oysters. Thanks to Janet Forbes for typing the manuscript. The project was primarily funded by the University of New Orleans Fisheries Initiative Program.

REFERENCES CITED

Barton, N. H. & M. Slatkin. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* 50:409–415.

Berger, E. M. 1983. Population genetics of marine gastropods and bivalves. In Russell-Hunter, W. D. (ed.) *The Mollusca,* Academic Press, Inc., NY, pp. 563–596.

Buroker, N. E. 1978. Behavioral and population genetic studies of oyster species of the family Ostreidae. Ph.D. diss. Univ. Washington. Seattle, Wash. 230 p.

Buroker, N. E. 1983. Genetic differentiation and population structure of the American oyster *Crassostrea virginica* (Gmelin) in Chesapeake Bay. *J. Shellfish Res.* 3:153–167.

Buroker, N. E. 1984. Gene flow in mainland and insular populations of *Crassostrea* (Mollusca). *Biol. Bull.* 166:550–557.

Dugas, R. J., R. V. Pausina & M. Voison. 1983. The Louisiana oyster industry, 1980. In Chew, K. K. (ed.) *Proceedings of the North American Oyster Workshop,* LSU. Division of Continuing Education, Baton Rouge, pp. 101–111.

Ehrlich, P. R. & P. H. Raven. 1969 Differentiation of populations. *Science* 165:1228–1232.

Foltz, D. W. 1986. Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. *Evolution* 40:869–870.

Galtsoff, P. S. 1964. The American oyster, *Crassostrea virginica* (Gmelin). *Fish. Bull. Fish Wildl. Serv. U.S.* 64:1–480.

Groue, K. J. & L. J. Lester. 1982. A morphological and genetic analysis of geographical variation among oysters in the Gulf of Mexico. *Veliger* 24:331–335.

Harris, H. & D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publ. Co. Amsterdam.

Koehn, R. K. 1983. Biochemical genetics and adaptation in molluses. In Hochachka, P. W. (ed.) *The Mollusca* Academic Press, NY, pp. 305–330.

Leipper, D. F. 1954. Physical oceanography of the Gulf of Mexico. *Fish. Bull. Fish Wildl. Serv. U.S.* 89:119–137.

Nei, M. 1972. Genetic distance between populations. *Amer. Natur.* 106:283–292.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. *Stud. Genet. VIII, Univ. Texas Publ.* 7213:145–153.

Rose, R. L. 1984. Genetic variation in the oyster, *Crassostrea virginica* (Gmelin), in relation to environmental variation. *Estuaries* 7:128–132.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39:53–65.

Soniat, T. M. & J. D. Gauthier. In press. The prevalence and intensity of *Perkinsus marinus* from the mid northern Gulf of Mexico, with comments on the relationship of the parasite to temperature and salinity. *Tulane Studies Zool. Bot.*

Stein, D. W., J. S. Rogers & R. C. Cashner. 1985. Biochemical systematics of the *Notropis roseipinnis* complex (Cyprinidae: Subgenus *Lythrurus*). *Copeia* 1985:154–163.

Wood, L. & W. J. Hargis, Jr. 1971. Transport of bivalve larvae in a tidal estuary. In Crisp, D. J. (ed.) *Fourth European Malacological Symposium,* Cambridge Univ. Press, Cambridge, pp. 29–44.

Wright, S. 1978. Evolution and the genetics of populations, vol. 4; Variability within and among natural population. Univ. Chicago Press, Chicago, 580 p.

Journal of Shellfish Research, Vol. 8, No. 1, 233–239, 1989.

AN ANALYSIS OF TEN STATE AQUACULTURE LEASING SYSTEMS: ISSUES AND STRATEGIES

M. RICHARD DeVOE AND ANDREW S. MOUNT
South Carolina Sea Grant Consortium
287 Meeting Street
Charleston, South Carolina 29401

ABSTRACT The institutional framework established for aquaculture was examined, with particular attention to leasing of public resources in coastal states. Related laws and regulations in 10 states were analyzed to determine the types of lease programs available, minimum and maximum size and duration, degree of exclusivity, and fee and bond structures. Initial phone interviews were conducted with agency personnel in 23 coastal states; officials of 10 selected states were questioned further to qualitatively determine the effectiveness in implementation of leasing programs. Of the 23 coastal states contacted, 19 have so-called "traditional" shellfish lease programs, while only 12 have adopted "contemporary" aquaculture leasing mechanisms. Provisions of leasing programs vary greatly among states, but as a whole, few programs actually meet the needs of the culture industry. Many issues, including concerns over public access, use conflicts and lack of suitable sites, must be resolved before the extensive results of United States aquaculture research and development programs can be used by the private sector in domestic waters.

KEY WORDS: aquaculture, leases, regulation, United States, South Carolina

INTRODUCTION

Aquaculture, the culture of aquatic animals or plants under either natural or artificial conditions, has been embraced by many states as a means to supplement seafood supplies and create economic opportunities. This interest arises from three basic beliefs:

1. Worldwide overfishing and water pollution will reduce harvests from traditional fisheries industries,
2. Waters along the coast, both estuarine and inland, represent the best areas which can be manipulated adequately for aquaculture operations,
3. Aquaculture, like agriculture, is an enterprise where production can be controlled at every level, thus assuring a high quality product (Buck and Dodge 1984).

The potential of aquaculture in South Carolina is great. The state has a vast array of natural resources conducive to the industry and an excellent climate for the culture of cold- and warm-water species (Joint Legislative Committee on Aquaculture in press). Aquaculture is now practiced in 42 of South Carolina's 46 counties, mostly in private upland ponds or impoundments.

Aquaculture uses a variety of systems, including ponds, raceways, silos, circular pools, closed (water recycling) systems, cages and net pens, and sea ranching. For shellfish, rafts, cages, nets, and longlines on submerged bottoms, suspended in the water column, or in on-land facilities are used (Joint Subcommittee on Aquaculture 1983). These systems cannot be randomly placed in any aquatic environment. The establishment of an aquaculture operation requires the following:

1. High Water Quality Locations: An aquaculture operation must be located in an area where the availability and maintenance of high quality waters is assured. The culturist must have guarantees that current and future uses of adjacent waters will not reduce the quality of waters where species are cultured.
2. Access to the Aquaculture Site: In choosing a site, the aquaculturist must consider an array of environmental and operational factors. Aquaculture requires both an aquatic environment and an adjacent on-land base of operation. Selection of a site by a prospective culturist will be affected by the types of aquatic and upland property rights provided.
3. Exclusive Fishing and Culturing Rights: In many states throughout the country, common property rights to state waters are granted to the public for navigation, recreation and fishing. However, many aquaculture methods practiced now and proposed for the future require some degree of "exclusivity of use" of the water column and/or submerged lands. Balancing exclusive use with guaranteed rights of the public will continue to require serious consideration.
4. Financial Investment: The establishment of an aquaculture operation requires a significant financial investment. The availability of venture capital, public and private sector loans, or other funds will depend in great part on the anticipated stability of the operation, which includes vested property rights. Without these rights, investors may be few.

The success of an aquaculture operation therefore depends upon the ability of the culturist to exercise control over the site through ownership, lease or other form of conveyance. However, for aquaculture operations that require the use of public resources, many states lack the necessary institutional and regulatory structure to balance the needs of aquaculture with those of the users of public resources. One approach several states have used is to establish aquaculture leasing programs, which convey property rights to

submerged lands and, in some cases, the water column, offering some degree of exclusivity to aquaculture sites.

The call for establishing a statewide aquaculture leasing program in South Carolina has been made in the state's Strategic Plan for Aquaculture Development (in press) and the Report of the Governor's Economic Development Task Force (1988). In anticipation of the need to offer for consideration alternative approaches of aquaculture lease programs, the authors initiated a study to acquire, analyze and present information on leasing programs in a select number of coastal states.

The reader should recognize that the study results reported here are preliminary. The goal of this initial effort was not to conduct a statistical survey, but to gather information for initial review. Plans are now being made to follow this analysis with a formal examination, building on these results.

Additionally, a number of formal studies over the last 10 years (Owen 1978, Clay et al. 1981, Wildsmith 1982, Riggio 1985, Kundell 1988, and others) have examined the legal and institutional framework for leasing submerged lands and superjacent waters for aquaculture. However, these authors focused their analyses on leasing policies and regulatory programs as written, and have suggested model leasing programs based on those analyses. The work described here focuses, in part, on how state leasing programs are working in practice.

Finally, two terms used throughout this paper require definition. "Shellfish Leases" are defined as those traditional leases that have been granted by states to shellfishermen for harvest and cultivation of existing shellfish grounds. "Contemporary Aquaculture Leases" are provided to culturists involved in more intensive forms of aquaculture, such as raft, net pen and cage culture. These terms are used to assess the response of states to the current needs of the aquaculture industry.

APPROACH

Initial contacts were made with officials in all 23 coastal states to determine the number of types (traditional and contemporary) of aquaculture leasing programs currently in place. Ten states were selected for the preliminary analysis: Maine, Rhode Island, New York, New Jersey, Delaware, Maryland, Virginia, Florida, California and Washington. Their selection was based on our desire to include states that collectively represented a wide geographical range and broad set of regulatory programs.

The preliminary analysis included an examination of current leasing legislation and regulations, which focused on five features:

1. Scope of culture lease,
2. Type of lease,
3. Lease size and duration,
4. Exclusivity of lease (measured as the degree of protection offered to culturists against pollution, theft and trespassing), and
5. Lease fees and bonds.

This information was gathered through the examination of codified state laws and regulations.

A series of telephone interviews was held in 1988 with officials of the 10 states to gather data on the effectiveness of their aquaculture leasing programs. Information requested included a brief historical review of aquaculture leasing in the state, the number of applications submitted and approved over the last five years, the number of existing leases (traditional vs. contemporary) along with total acreage leased, on-going legislative and regulatory efforts, and opinions on the programs in general. Information obtained from this review was compared to results of the analyses of legislation and regulations to determine the "effectiveness" of existing leasing programs.

STATUTORY AQUACULTURE LEASING PROGRAMS

Scope of Culture Leases

Culture leases were classified into four groups: freshwater, marine, bottom (including submerged bottom and intertidal bottom) and water column (or "three-dimensional") culture, which could include the surface in some situations (Table 1). The leasing programs of all 10 states cover marine and bottom areas. Only four of the states—Maine, Rhode Island, Florida, and Washington—formally proscribe water column leasing. Water column leasing exists in Maryland and California although it is not officially promoted. Freshwater leases appear to be available in Florida; however, the demand is not great.

Type of Lease

The 10 leasing programs were also classified from a historical perspective (Table 1). Every state with the exception of Maine, Rhode Island and California maintain traditional shellfish leasing programs today (although Rhode Island did at one time grant shellfish leases in Narragansett Bay). Even though a majority of states throughout the country are promoting the development of the aquaculture industry, only five of the 10 surveyed have adopted leasing programs for more contemporary forms of aquaculture.

Residency requirements vary from state to state (Table 1). New York, New Jersey, Maryland and Virginia will not lease submerged lands to nonresidents.

Lease Size and Duration

There is appreciable variation among state aquaculture leasing programs regarding acreage and duration (term) of the lease (Table 2). These differences depend upon the respective policies of each state towards aquaculture, but are primarily determined by the amount of acreage available for leasing.

TABLE 1.

Types of aquaculture leases available in 10 coastal states.[1]

State	Fresh	Marine	Bottom	Column	Shellfish	Culture	Resident
ME	N	Y	Y	Y	Y	Y	N
RI	N	Y	Y	Y	N	Y	N
NY	N	Y	Y	N	Y	N	Y
NJ	N	Y	Y	N	Y	N	Y
DE	N	Y	Y	N	Y	?	N
MD	N	Y	Y	Y?	Y	N	Y
VA	N	Y	Y	N	Y	N	Y
FL	Y?	Y	Y	Y	Y	Y	N
CA	N	Y	Y	?	N	Y	N
WA	N	Y	Y	Y	Y	Y	N

[1] Note: Y = Yes, Y? = Probable, ? = Unknown, N = No.

Maine, Rhode Island, Florida and Washington have implemented contemporary aquaculture lease programs. The negotiated size of each lease is based upon the amount of acreage available and the demonstrated abilities of the culturist to utilize the area to its maximum potential. Only Rhode Island negotiates the terms of leases, based primarily on the experience and capabilities of the culturist.

In New York and Delaware, no less than 50 acres can be leased; this presents a barrier to operators who wish to start out small and build upon initial successes (D. Davies, Long Island Regional Planning Board, per. comm.). Maximum acreages are set for most states and vary widely. Of particular note is the state of Virginia, where up to 5,000 acres in the Chesapeake Bay can be leased at a time.

Finally, the terms of a lease vary, ranging from one year with annual renewals in New Jersey and Delaware to 25 years with 25-year renewals in California.

Exclusivity of Lease

Exclusivity offers the culturist protection against pollution, theft, and trespassing in the area leased. As Table 3 illustrates, the leasing program of the state of Washington provides explicit protections against all three. Five states include provisions that concern water quality in their leases. For example, Maryland decreases the lease rent if water quality decreases in an area.

Most states include theft provisions in their leases; Virginia is an exception. In Florida, the requirements are not clear. The degree of protection against trespassing depends on how a state interprets its public access policies. Maryland and Virginia provide riparian owners with first rights to adjacent acreage; California and Rhode Island expressly forbid limitations on access to public waters, although California does make exceptions. In Maine, Delaware, Florida and Washington, existing regulations expressly forbid trespassing on leased properties. In Maine, conditions established through a public hearing process encourage the greatest multiple compatible use of an area to be leased.

Lease Fees and Bonds

Costs associated with the lease of public areas are determined through a combination of rental fees, royalty payments and performance bonds (Table 3). Rental fees can be "variable," "fixed" or "negotiated." Examples of variable fees can be found in Rhode Island, where an applicant must pay $75 to lease ½ acre or less, $150 to lease ½–1 acre, and $100 for each additional acre. In Maryland, the annual rental is fixed each year by the Department of Natural Resources, and in California, the rental can, at times, be determined through a bidding process (in competitive situations). Fixed fees are common in states such as Delaware where an acre of state bottom can be leased annually for $0.75 by residents, and $1.50 by non-residents. In Florida, fees are negotiated during the application process.

Royalty payments vary from state to state. In California, for example, there is a fee of $0.02/packed gallon of oysters. In Florida, on the other hand, the basic rental

TABLE 2.

Size and duration of leases available in 10 coastal states.

State	Size (acres) Minimum	Size (acres) Maximum	Duration (yrs)
ME	0	150	10 (Renewable)
RI	Variable	Variable	Variable
NY	50	???	10 (Renewable?)
NJ	0	2	1 (Renewable)
	5	200	1 (Renewable)
DE	50	100	1 (Renewable)
MD	1	30	20 (Renewable)
	5	500	20 (Renewable)
VA	1	3,000	10 (Renewable)
	1	5,000	10 (Renewable)
FL	Variable	Variable	10 (Renewable)
CA	Variable	Variable	25 (Renewable)
			20 (for Kelp)
WA	1	40+	5 (Renewable)

TABLE 3.

Exclusivity of leases and fee and bond requirements on leases available in 10 coastal states.

State	Exclusivity			Fees and Bonds		
	Water Quality	Theft	Trepass	Rents	Royalties	Bonds
ME	Yes	Yes	Yes	Fixed	None	Yes
RI	Yes	Yes	Public	Variable	None	None
NY	None	Yes	None	Variable	None	Yes
NJ	None	Yes	None	Fixed	None	None
DE	None	Yes	Yes	Fixed	None	None
MD	Yes	Yes	Riparian	Variable	None	None
VA	None	None	Riparian	Variable	None	None
FL	Yes	?[1]	Yes	Negotiable	Yes	Yes
CA	None	Yes	Public	Variable	Yes	None
WA	Yes	Yes	Yes	Variable	Yes	Yes

[1] ? = Unknown.

charge is supplemented by royalties determined after the productivity of the operation has been established. Criteria used to determine productivity in Florida are "probable rates of productivity" and "marketability and value of the product."

Where performance bonds are used, they generally ensure maximum productivity of the area and/or adequate cleanup of the site once the operation is terminated. Four (Maine, New York, Florida and Washington) of the 10 states require the payment of performance bonds. Maine, for example, requires the payment of a $500 bond for the bottom culture of shellfish or mussels, and $5,000 for suspended shellfish culture or the pen culture of finfish.

AQUACULTURE LEASING IN PRACTICE

Of the 23 coastal states contacted initially, 19 were found to have traditional shellfish leasing programs in place, while only 12 had developed and implemented leasing programs which address the needs of contemporary aquaculture.

Major results of a series of phone interviews with regulatory officials in the 10-state analysis are presented in Table 4. A brief review of the responses is provided below.

Maine

Maine's aquaculture leasing program has only been in existence since 1971, and is recognized as one of the more progressive programs in the country. It depends heavily upon an elaborate public hearing process, which is held for each application. Decisions are based on the merits of the application and pre-set regulatory standards, including riparian owner ingress and egress, navigation, fishing, other aquaculture uses, existing ecosystem support, source of organisms to be cultured, and interference with public facilities. The state has issued 66 aquaculture leases totaling 1,003 acres.

Rhode Island

Rhode Island has a long and sometimes tumultuous history of traditional oyster culture in Narragansett. The vision of Rhode Island oystermen protecting their claims to the Bay bottoms with rifles and shotguns is still a vivid memory for some natives. Today, however, no traditional leases are held. Instead, seven contemporary aquaculture leases have been let for clam and mussel culture. No applications for aquaculture leases have been submitted to the state Coastal Resources Management Council in over two years; pressures from commercial shellfishermen have created a negative political climate for aquaculture development.

New York

No traditional shellfish leases have been issued since the 1930's, although the regulatory program remains "on the

TABLE 4.

Acreage under lease in 10 coastal states.

State	Shellfish	Aquaculture	Remarks[1]
ME	0	1,003	66 leases
RI	0	67	Seven leases
NY	Private	15	Assignments issued
NJ	31,000	?	Seven hatcheries
DE	7,000	0	Problems with MSX
MD	10,300	0	Problems with MSX
VA	104,000	0	Problems with MSX
FL	2,076	10	No more traditional shellfish leases issued
CA	0	2,203	Moratorium on contemporary aquaculture in place
WA	3,000	423	Variety of species cultured

[1] See text for further explanation.

books." Nevertheless, private owners of submerged bottoms in Long Island Sound still hold large acreages; one company alone owns 13,000 acres of Great South Bay.

Although the state does not have a formal leasing program for contemporary aquaculture, it has granted "assignments" to three persons on the eastern end of Long Island, each five acres in size and renewed on an annual basis. However, the assignments do not convey any vested property rights to the area. Also, several municipalities have been given the authority to issue leases, and at least two oyster mariculture companies hold town leases, which terminate at the end of this century. There is little interest by culturists to obtain leases due to extreme pressures brought about by conflicts among users for limited water resources.

New Jersey

New Jersey has issued 1,100 traditional shellfish leases covering 31,000 acres of Delaware Bay and Atlantic coast bottoms; these leases are for clams and oysters only. The leases provide title to the shellfish resource on the bottom, not the bottom itself. Additionally, a number of inquiries have been made to the N.J. Department of Environmental Protection regarding water column leasing.

The state has also issued leases for one oyster and six clam hatcheries (acreage unknown); most are located along the Atlantic coast. More than 35 applications submitted to obtain leases are waiting for processing; the state does not have enough staff to survey the properties. Therefore, unlike New York, interest in aquatic farming remains high, although a formal regulatory program for contemporary aquaculture has yet to be established.

Delaware

There are more than 7,000 acres under lease for traditional oyster culture operations in Delaware; its leasing program is not designed to accommodate contemporary aquaculture. The oyster pathogen, *Haplosporidium nelsoni* (MSX) has created serious problems resulting in very low harvests, but leaseholders are maintaining their leases for "better times" ahead. According to officials with the Wetlands Section of the Delaware Department of Natural Resources and Environmental Control, no significant interest in leases for aquaculture exists.

Maryland

Maryland leases ~10,300 acres to 939 leaseholders for traditional oyster culture. Unfortunately, the MSX problem has seriously impacted the industry here as well. Because of pressures brought to bear by Maryland watermen, no more acreage is available for lease; however, there is interest by the public in culturing other species in public waters. The state appears ready to venture into this arena, as demonstrated by the recent passage of aquaculture legislation by the state General Assembly and signed by the Governor. The state of Maryland requires the payment of fees for culture leases; however, no fees are assessed for marinas and other uses of public lands. This appears to be a common practice in most states.

Virginia

The situation in Virginia is similar to that of Delaware and Maryland. Over 104,000 acres are leased by the state to 7,215 persons for traditional oyster culture, a practice that began in 1894. Again MSX is a problem; there is little interest in investing in seed or shell until the disease problem is resolved. An additional 1,300 leases, covering 8,600 acres, have been granted to riparian owners and others. However, contemporary aquaculture has yet to take hold in Virginia.

Florida

Traditional shellfish leasing was first encoded into laws in 1913 in Florida. More than 2,000 acres are leased to 161 persons, although as of June 1988, no more traditional shellfish leases will be let by the state. New legislation, first signed into law in 1969 and amended in 1975, 1984, and 1988, gives authority to the Florida Department of Natural Resources to provide leases for contemporary aquaculture. However, only two leases totaling 10 acres have been issued through December 1988, although large tracts of leasable area are available in the state. In 1987, 20 lease applications were submitted to the state, with 12 of those pending final decisions. According to state officials, interest in aquaculture is high, due in part to state support.

California

California has a very progressive regulatory program for contemporary aquaculture. Aquaculture policies and regulations are clearly written and comprehensive, covering all aspects of the industry. The state has let 25 leases totaling 2,203 acres; species cultured include abalone, kelp, oysters, rock scallops, clams, and mussels. Competition for the limited acreage available for lease has become a problem since criteria have yet to be developed to allow for auction of lease properties. The state has placed a moratorium on the issuance of additional kelp leases until bid specifications can be developed and implemented.

Washington

The state of Washington manages leasing programs for traditional and contemporary aquaculture enterprises. Traditional shellfish culture started in the 1870s in the state; today, over 3,000 acres are leased by 180 persons for oysters and clams. The first fish pens for salmon showed up in waters of the state in 1971. The economic feasibility of salmon net-pen culture was demonstrated and by 1983 a "stampede" for water column leasing reached its peak. In 1980, floating culture for scallops and mussels emerged. Today, 40 "floating" operations have been provided

leases. Twenty leases are for salmon culture and 20 for mussels, totaling over 400 acres. An additional 23 acres are leased for *Nori* (seaweed) culture. All floating culture operations are located within sounds and harbors.

An average of 20 lease applications per year have been made over the last five years. There are 80 pending applications; 30 renewals and 50 new requests. Interest in aquaculture in public waters is increasing every year in Washington state.

DISCUSSION

Many states, through policy statements and legislation, have called for the accelerated development of the aquaculture industry.

The cultivation of many promising aquaculture species will require the use of public lands and waters. High water quality environments, access to both high grounds and adjacent waters, and exclusivity of use are factors which aquaculturists must consider as they seek appropriate sites. Contemporary regulatory mechanisms to accommodate these needs as well as technological advances in aquaculture must be established. Our analysis suggests that many states have yet to do so and, for those that have, the programs are now undergoing clarification and refinement. We have identified 5 criteria, common to all states, that must be considered if a leasing program is to be successful in attracting aquaculture to a state:

1. Scope—A leasing program should provide for bottom and water column leasing in saltwater areas. Freshwater bodies should be available for lease, where appropriate.
2. Size and duration—A lease term should provide the culturist with enough time to start and establish the operation and, at the same time, provide the state with enough flexibility to reassign or terminate leases for just cause. A term of 10 years (renewable every five years) provides an appropriate balance. The size of each lease should be negotiated based upon the amount of acreage available and the capabilities of the culturist.
3. Exclusivity—Areas available for lease must have adequate protections against diminished water quality, theft, and trespassing.
4. Costs—It is important that the culturist know the costs of doing business in a state before making investments in time and dollars. State leasing programs which establish lease fees, bonds, and royalties *after* initial start-up could upset the culturist's financial planning.
5. Residency—Leasing programs that include residency requirements could be counterproductive to a state like South Carolina, which is trying to lure entrepreneurs and large companies into the state to establish aquaculture operations.

These five criteria could serve as a starting point for states interested in developing a legal and regulatory framework to accommodate the aquaculture industry. Wildsmith (1982) pointed out: "The reader should be sensitive to the difference, and should, in conjunction with the posed legal solutions, realize that each individual jurisdiction has its own political, legal, and institutional contexts which may require different answers. Wise policy formulation requires that all such matters be taken into account. It is hoped after a weighing of considerations, rationality rather than expediency will prevail and the final decision will be based on reasoned judgment."

During the course of our research it became apparent that there are regional conflicts concerning multiple use of natural resources. State officials interviewed via telephone were asked to identify leasing issues that have emerged during their experience with regulatory programs for aquaculture. Of the nine key issues identified, three represent resource conflicts: Opposition from fishermen, Lack of suitable sites to locate aquaculture operations (due to competition with other users), and Aesthetics.

Resolving these issues does not depend upon implementing an innovative aquaculture leasing program, but in the value the public places upon aquaculture relative to residential development, water quality, habitat alterations, commercial fishing, and other competing uses of submerged lands. The outbreak of disease (especially MSX and Dermo, *Perkinsus marinus,* along the Atlantic coast) was identified as a fourth issue and is a biological problem that requires a biological solution.

The remaining five issues—the lack of clear leasing guidelines (regulatory constraints), the need to ensure public access to state lands and waters, the unnecessary degree of detail involved in leasing programs, the costs associated with leases (bids and royalties), and the limited political muscle of the aquaculture industry (due to a lack of organization and recognition)—would indeed benefit by the implementation of innovative aquaculture leasing programs throughout the United States.

In summary, many aquaculture leasing programs today have evolved primarily from traditional shellfish lease programs of the 1800s. Nevertheless, most states have not considered aquaculture in the broader sense; the focus remains on shellfish, primarily oysters and clams. As a result, current aquaculture technologies which require the use of submerged bottoms and superjacent waters cannot now be accommodated by many states.

While states support aquaculture development, they will have to make a conscious decision on how far that commitment shall go. The question must be asked, and answered: Will the promotion of aquaculture in the United States include a commitment of public resources to its development? Research and development efforts are leading to breakthroughs in aquaculture; e.g., improvements in

species survivability, growth rate, and disease resistance all contribute to the potential of aquaculture. However, if the commercial feasibility of aquaculture is limited by institutional and legal constraints, these advances will have limited value.

ACKNOWLEDGMENTS

The authors would like to thank the following individuals who provided information and assistance during this study: Tim Dillingham, Rhode Island Coastal Resources Management Council and Bill Lapin, Rhode Island Department of Environmental Management; Dewitt Davies, Long Island Regional Planning Board and Steve Hendrickson, New York State Department of Environmental Conservation; Joe Dobarro and Tom McClure, New Jersey Department of Environmental Protection; Charlie Lesser, Delaware Department of Natural Resources and Environmental Control; Ben Florence, Maryland Department of Natural Resources; Rob O'Reily, Virginia Marine Resources Commission; Mike Ednoff, Florida Department of Agriculture and Charles Futch, Florida Department of Natural Resources; Granvil Treece, Texas A&M Sea Grant Marine Advisory Service and C. E. Bryan, Texas Parks and Wildlife Commission; Rob Collins, California Department of Fish and Game; and Dr. Dave Jameson and Bob Hoyser, Washington (State) Department of Natural Resources.

Any errors and misrepresentations made herein are the sole responsibility of the authors.

We would also like to thank Margaret A. Davidson and John J. Manzi for reviewing the paper, and Virginia Beach for editing the manuscript. Support for this study was provided by the South Carolina Sea Grant Consortium.

REFERENCES CITED

Buck, E. H. & C. H. Dodge. 1984. Aquaculture: Status of technology and future prospects. Issue Brief Number IB83004. The Library of Congress, Congressional Research Service. 15 p.

Clay, G. S., S. Broder, R. Turner, D. S. Kitaoka, G. L. Rhodes & D. K. Yamase. 1981. Ocean Leasing for Hawaii. Hawaii Department of Planning and Economic Development. Honolulu.

Governor's Task Force on Agriculture and Rural Economic Development. 1988. Executive Summary of the Governor's Task Force. Columbia, S.C. 23 p.

Joint Legislative Committee on Aquaculture. In Press. Strategic Plan for Aquaculture Development in South Carolina. Columbia, S.C.

Joint Subcommittee on Aquaculture. 1983. National Aquaculture Development Plan: Volume I. Washington, D.C. 67 p.

Kundell, J. E., J. Kealey, R. Klant & L. Wilson. 1988. Management of Georgia's marshlands under the Coastal Marshlands Protection Act of 1970. Carl Vinson Institute of Government, University of Georgia, Athens. 34 p.

Owen, S. 1978. The response of the legal system to technological innovation in aquaculture: A comparative study of mariculture legislation in California, Florida and Maine. *Coastal Zone Mgnt. Jrnl.* 4(3):269–297.

Riggio, R. J. 1985. Building the perfect lease. Unpublished report. 40 p.

Wildsmith, B. H. 1982. Aquaculture: The Legal Framework. Emond-Montgomery Limited, Toronto, Canada. 313 p.

Journal of Shellfish Research, Vol. 8, No. 1, 241–248, 1989.

PASSIVE WATER REUSE IN A COMMERCIAL-SCALE HARD CLAM, *MERCENARIA MERCENARIA*, UPFLOW NURSERY SYSTEM

STEVE M. MALINOWSKI[1] AND SCOTT E. SIDDALL[2]

[1]*The Clam Farm, Inc.*
Box 402
Fishers Island, NY 06390 USA
[2]*Marine Sciences Research Center*
State University of New York
Stony Brook, NY 11794 USA

ABSTRACT The potential for recirculating ambient seawater through upflow silos was investigated in a hard clam (*Mercenaria mercenaria*) nursery system. Since greater flow is needed to accomplish uniform flow through seed bed masses than is needed to meet the food demands of the animals, a single pass of ambient seawater through upflow silos may not be the most efficient use of pumped water. Experiments conducted at The Clam Farm, Inc., Fishers Island, NY, USA, showed that at production-level stocking densities chlorophyll was removed and ammonia accumulated as seawater passed through consecutive tiers of upflow silos. As much as 84% of ambient chlorophyll was removed in four passes through the system; ammonia levels increased by 86% after four passes. The rate of chlorophyll removal and ammonia accumulation generally was a function of clam biomass. Growth in terms of whole dry weight and ash-free dry weight declined significantly after more than two passes of water through the system. Individual variability in whole dry weight and ash-free dry weight decreased as growth was limited with increasing water reuse.

Employing a passive water reuse design can at least double the production of upflow nursery systems per unit of ambient water pumped indicating that upflow systems are at least as efficient as traditional raceway systems with respect to water use. The demonstrated ability of effluent waters from upflow silos stocked at production-level biomasses to support maximum rates of growth of an additional and equal biomass of seed suggests that physical (such as minimum velocities of flow necessary to achieve uniform flow) rather than biological (such as food availability) factors determine maximum silo stocking densities.

KEY WORDS: hard clam, growth, passive recirculation, upflow system

INTRODUCTION

Upflow nursery systems have become a common method for the intermediate growout of bivalve seed. Seed clams, *Mercenaria mercenaria*, are normally introduced into these systems at a size of ~1 mm and grown to a size that can be introduced into a field culture system (6–10 mm). Since upflow systems are relatively easy to construct and maintain and yield excellent growth and survival, they have replaced traditional raceway systems as the primary system for production of hard clams.

While several studies have documented the production and operating protocol of upflow systems using artificially enriched seawater to culture oysters in Europe (e.g., Bayes 1979, Claus 1981, Lucas and Gerard 1981, and Rodhouse and O'Kelley 1981), only a single study (Manzi et al., 1987) has documented production, flow requirements and stocking densities for an upflow system using natural seawater to culture hard clam seed. The results of this study (Manzi et al., 1987) indicate that upflow systems use space much more efficiently than traditional raceway nursery systems since more than five times the biomass of animals can be supported per unit area. Flow rate requirements per unit biomass, however, were greater in the upflow system than were previously reported for raceways (Hadley and Manzi 1984). This is somewhat paradoxical given that the design of upflow systems causes 100% of the pumped water to pass directly through seed bed masses while some overlying water passing through a raceway is undoubtedly unavailable to the animals.

Manzi et al. (1987) found that even at optimal flow:biomass ratios, only 20% of the incoming chlorophyll-*a* in ambient seawater was removed. The authors' interpretation was that only 20% of the chlorophyll-*a* in the ambient seawater constituted a satisfactory source of food. It is possible, however, that to achieve maximum growth of clams in an upflow system with ambient seawater as the source, it is necessary to pass much more water by the animals than actually can be filtered and the low rate of chlorophyll-*a* removal may reflect the passage of a large quantity of unused water through the system rather than a relative measure of the useable chlorophyll-*a*. If this were the case then the surplus water (amounts exceeding that which the biomass of clams could filter) may be a physicial requirement of the system (minimum flow rates may be required to create uniform flow through seed bed masses) and it may be possible to increase the efficiency of the system by passing pumped seawater through silos more than once. Water reuse could significantly reduce production costs since costs for pumps and electricity can be substantial (Malinowski, personal observations).

In the present study, clam growth and selected characteristics of pumped, natural seawater were examined in an experimental, commercial-scale upflow system that employs water reuse. The purpose of the study was to determine the potential (if any) for water reuse as well as to gain insight into factors which limit production of upflow systems.

MATERIALS AND METHODS

All experiments were conducted in the facilities of The Clam Farm, Inc. a small-scale, hard clam producer oper-

ating on less than five acres (2.5 ha) of bay bottom in West Harbor, Fishers Island, New York, USA (see Fig. 1). The upwelling system used throughout this study is depicted schematically in Fig. 2.

Raw seawater pumped from a submerged intake line (40 m in length) in West Harbor was pumped into the topmost trough (tier 1) containing six 35.6 cm (14 in) diameter passive upwelling silos (design similar to the passive system described by Manzi and Hadley, 1984). Effluent from three of the silos in this first tier was collected and sequentially passed through three more troughs (tiers 2, 3 and 4) each contaning three 35.6 cm silos. The presence of the additional three silos in tier 1 was required to maintain sufficient commercial production of hard clam seed during the period of these experiments, however the experimental treatments at each of the four tiers was the same (three silos/tier). Flow rates varied from 17.1 l/min/silo at low tide (greatest pumping effort) to 21.7 l/min/silo at high tide (least pumping effort).

Three separate experiments were conducted during 1985 to determine the effects of water reuse on growth of hard clam (*M. mercenaria*) seed. The first experiment was begun on August 7 and continued for three weeks. Seed clams (purchased from Mook Sea Farms, Damariscotta, ME, USA) sieved through a 2.0 mm mesh sieve and retained on a 1.4 mm mesh sieve had a mean shell length of 2.5 mm (n = 50). A packed volume of 200 ml of these seed clams was distributed into each of the three silos in all four tiers. On August 28, clams in all upwelling silos in all tiers were pooled, sieved on a 2.8 mm sieve (mean shell length = 4.8 mm) and redistributed into the silos (600 ml packed volume of clams per silo) to establish the second experiment. On September 4, this week-long experiment was terminated by similarly collecting all clams from all silos, sieving on a 3.4 mm sieve (mean shell length = 5.8 mm), and redistributing 800 ml packed volume into each silo for the third and last experiment which was terminated on September 11. The experimental protocol is summarized in Table 1.

At the initiation of each experiment, 5 ml subsamples of the clams were frozen for later analysis. At the termination of each experiment (and at weekly intervals during Experiment 1), 5 ml of clams were subsampled from each tier (⅓ of the sample from each silo within a tier) and frozen for later analysis. Because of the routine accumulation of detritus, fecal material and fouling organisms on and around seed clams in these (or any) passive upwelling silos, all silos were cleaned daily. During this process, the trough of each of the four tiers was completely drained, and the clams within each silo were rinsed with seawater. Total clam biomass volumes were estimated at the termination of each experiment (and at weekly intervals during Experiment 1) by gently pouring all cleaned clams from a silo into a 1000 ml graduated cylinder through a funnel. This measurement provides an approximation of the packed volume of the clam biomass which is useful for relative comparisons among silos and sampling dates and for estimating silo stocking densities.

Frozen clam samples were analysed within two months as follows. The total number of seed clams in each 5 ml (packed volume) sample was counted. The displacement volume of the total sample was determined by adding the clams to a graduated cylinder filled to 50 ml with freshwater and recording the increase in freshwater volume. The clams were immediately removed from the freshwater, blotted dry and a random subsample of 50 selected for fur-

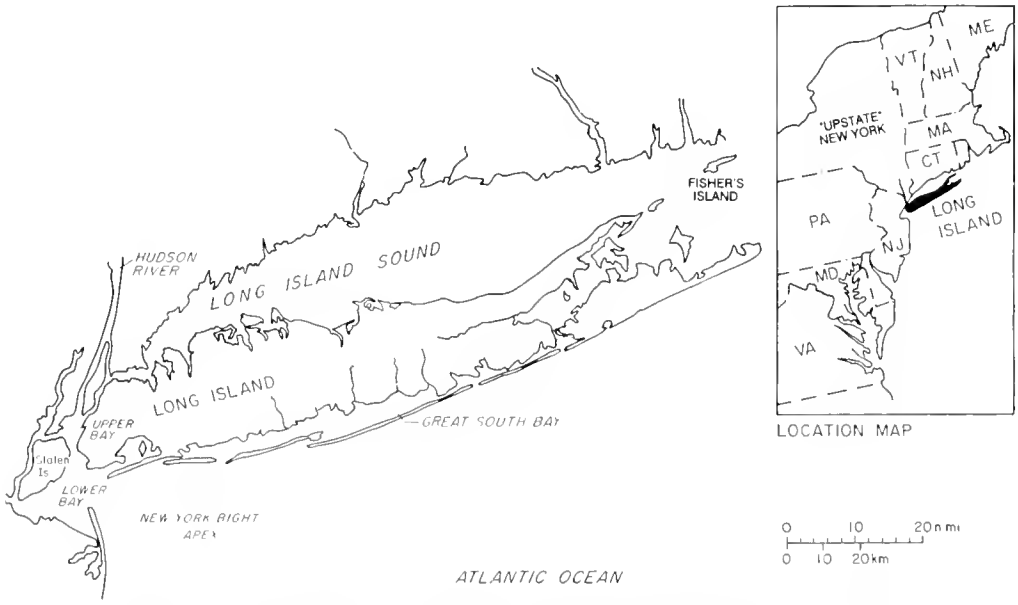


Figure 1. Location map for The Clam Farm, Inc. of Fishers Island, New York, USA.

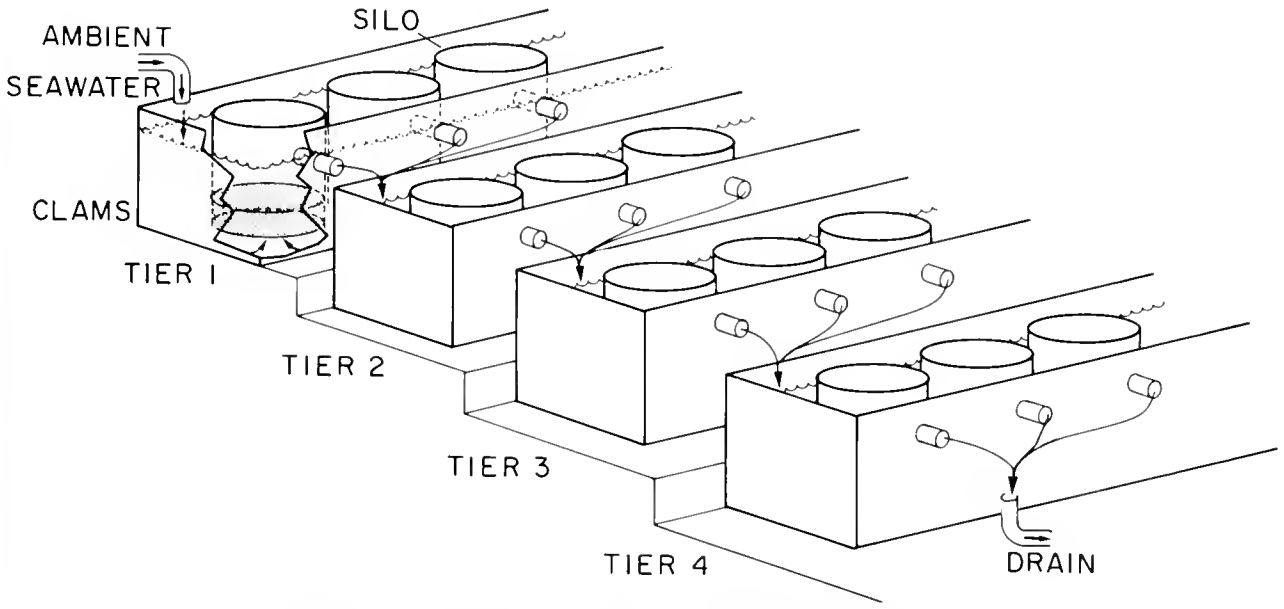


Figure 2. Schematic diagram of four tiers of troughs holding upwelling silos.

ther treatment. Hand-held venier calipers were used to measure the shell length (maximum dimension, parallel to the hinge line) of each clam. Each clam was placed in a numbered pan fabricated from aluminum foil. The weight of each foil pan had been measured on a Cahn Electrobalance Model 24 (±0.1 mg). Clam samples were then dried to a constant weight for 24–48 hours at 60°C then weighed on the same Cahn Electrobalance. Clam samples were then ashed at 450–480°C for 12–18 hrs in a muffle furnace and reweighed on the same balance. The aluminum pans were not ashed before use; two series of ten pans were weighed before and after the ashing process and found to change in weight by no more than 0.015%; therefore, pre-ashing the pans was deemed unnecessary.

Whole dry weights of individual clams were calculated as whole dry weight of clam and pan less pan weight. Ash-free dry weights of individual clams were calculated as whole dry weight less ashed clam and pan weight. Mean individual clam volumes were calculated as total sample displacement volume divided by total number of clams in the sample.

Several parameters of the seawater in the upwelling system were measured both on-site and in the laboratory from frozen seawater samples. A Turner Designs Portable Fluorometer (Model 10) was used to estimate chlorophyll-*a* (chl-*a*) content of seawater as it entered the upwelling system and cascaded through each tier of the silos. Additional discrete seawater samples were taken at each level in the system for independent, laboratory analyses of chl-*a* which could be used to calibrate the field measurements. For the discrete samples, 50 ml of seawater were taken from the appropriate source and immediately filtered

TABLE 1.

Experimental design of three upflow system experiments.

Experiment	Initiation Date	Sampling Date		Stock Volume[1]	Initial Clam Size[2]	Date Terminated
		Clams	Seawater			
I	8/07/85	8/07	8/12	200 ml	2.5 mm	8/28
		8/14	8/19			
		8/21	8/26			
		8/28				
II	8/28/85	8/28	9/03	600 ml	4.8 mm	9/04
		9/04				
III	9/04/85	9/04	9/11	800 ml	5.8 mm	9/11
		9/11				

[1] Packed volume/silo in 1000 ml graduated cylinder.

[2] Mean standard length (n = 50).

through a 1 cm glass-fiber filter which was held on ice and frozen immediately. Standard fluorometric procedures for chlorophyll-*a* analysis (acetone extraction, spectrophotometric analysis on a stationary and independently calibrated Turner Designs Fluorometer; see Strickland and Parsons, 1972) were followed.

Additional seawater samples were taken at each level in the system for nutrient analyses. Fifty ml seawater samples were filtered through glass fiber filters to remove phytoplankton and other particulates. Ten ml of the filtered sample were used to rinse acid cleaned sample bottles which were then frozen for ammonia, nitrite and nitrate analyses using standard methods of seawater analysis (Strickland and Parsons, 1972) on a Technicon Autoanalyzer.

Samples of raw seawater were taken at each level of the upwelling system and preserved in Lugol's iodine preservative for particle size analysis immediately upon return to the laboratory (6–12 hrs) using a Coulter Counter Model TA-II.

RESULTS

In experiment I, chlorophyll was removed (Fig. 3) and ammonia accumulated (Fig. 4) generally as seawater passed through consecutive tiers. Because clams were not removed or redistributed throughout Experiment I, clam biomass increased during the three week course of this experiment. The rate of chlorophyll removal and ammonia accumulation generally was a function of this increasing biomass. When clam biomasses were lowest (8/12 sampling), as little as 26% of ambient (incoming) chlorophyll was being removed. As clam biomasses within the four tiers increased by the 8/26 sampling, as much as 63% of ambient chlorophyll was being removed. Stocking densities in the silos of Experiments II and III were intentionally high (600 and 800 ml clams/silo, respectively) to permit detection of the effects of water reuse during normal commercial operating conditions. Chlorophyll was removed and ammonia accumulated in Experiments II (Table 2) and III (Fig. 3). Rates were also a function of clam biomass; as much as 84% of ambient chlorophyllI was removed by the clams in all four tiers.

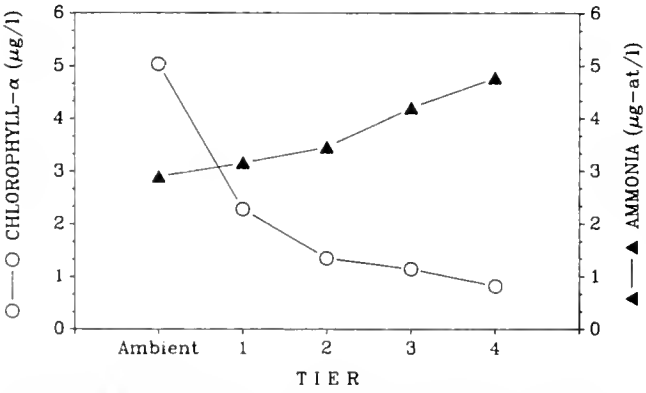


Figure 3. Mean chlorophyll-*a* (µg/l) and mean ammonia (µg-at/l) vs tier for Experiment III.

Throughout all three experiments, ambient chlorophyll levels varied from 0.68–6.08 µg/l (mean = 1.93 µg/l); there was no constant pattern of variation (flood vs ebb tide, morning vs afternoon measurements, August vs September samples). Neither nitrite nor nitrate was accumulated as water passed through the tiers.

It is not possible to calculate clam weight specific rates of chlorophyll removal or ammonia accumulation because clam sampling dates did not correspond to seawater sampling dates (see Table 1). Furthermore, because there are no long-term, continuous data records for chlorophyll, we cannot directly relate changes in growth to absolute values of chlorophyll.

Analyses of particle sizes in ambient seawater compared to effluent of tier four were conducted during Experiment I. In this analysis, it is not posible to differentiate between particles ingested by the seed clams and particles "trapped" or mechanically filtered out of the seawater by the mass of clams within the silos. Between 40 and 70% of incoming particles between 1.3 and 25 microns were removed, while there was a net accumulation of larger particles in the 32–40 micron size classes, possibly aggregated masses of phytoplankton, feces and other rejecta. The largest size class of a Coulter Counter analysis is "indeterminate" (in this case, it includes all particles 40 microns and larger) and must be interpreted with caution. Qualitative analyses of incoming phytoplankton and accumulated detritus were not possible.

The change in biomass of clams reared in each tier is given in Table 3. Mean increases in clam volume were the same for tiers 1 and 2. Tier 3 volume increases were 12% lower than those of tiers 1 and 2; tier 4 increases were 37% lower. More specific data on differences in growth among tiers were available for shell length, total dry weight and ash-free dry weight. Shell length, however, is not a reliable predictor of tissue growth, and the ash-free dry weight determinations for Experiment I (smallest clams) were based on weight measurements very near the lower limits for the balance (estimated precision of only ±63%). While precise ash-free dry weight measurements are perhaps the best indicator of tissue growth, it was felt that whole dry weight measurements (with an estimated precision of ±16% for all experiments) were a superior choice for analyses of growth in Experiment I. Similar problems with ash-free dry weights of the larger clams in Experiments II and III were not encountered; for purposes of comparison with Experiment I, both whole dry weight and ash-free dry weight analyses are presented for Experiment II in Table 2.

Effects of water reuse on total dry weight of individual clams became more pronounced with time. Effect of water reuse on whole dry weight was highly significant for the terminal samples of Experiment 1 ($p = 0.0023$) and Ex-

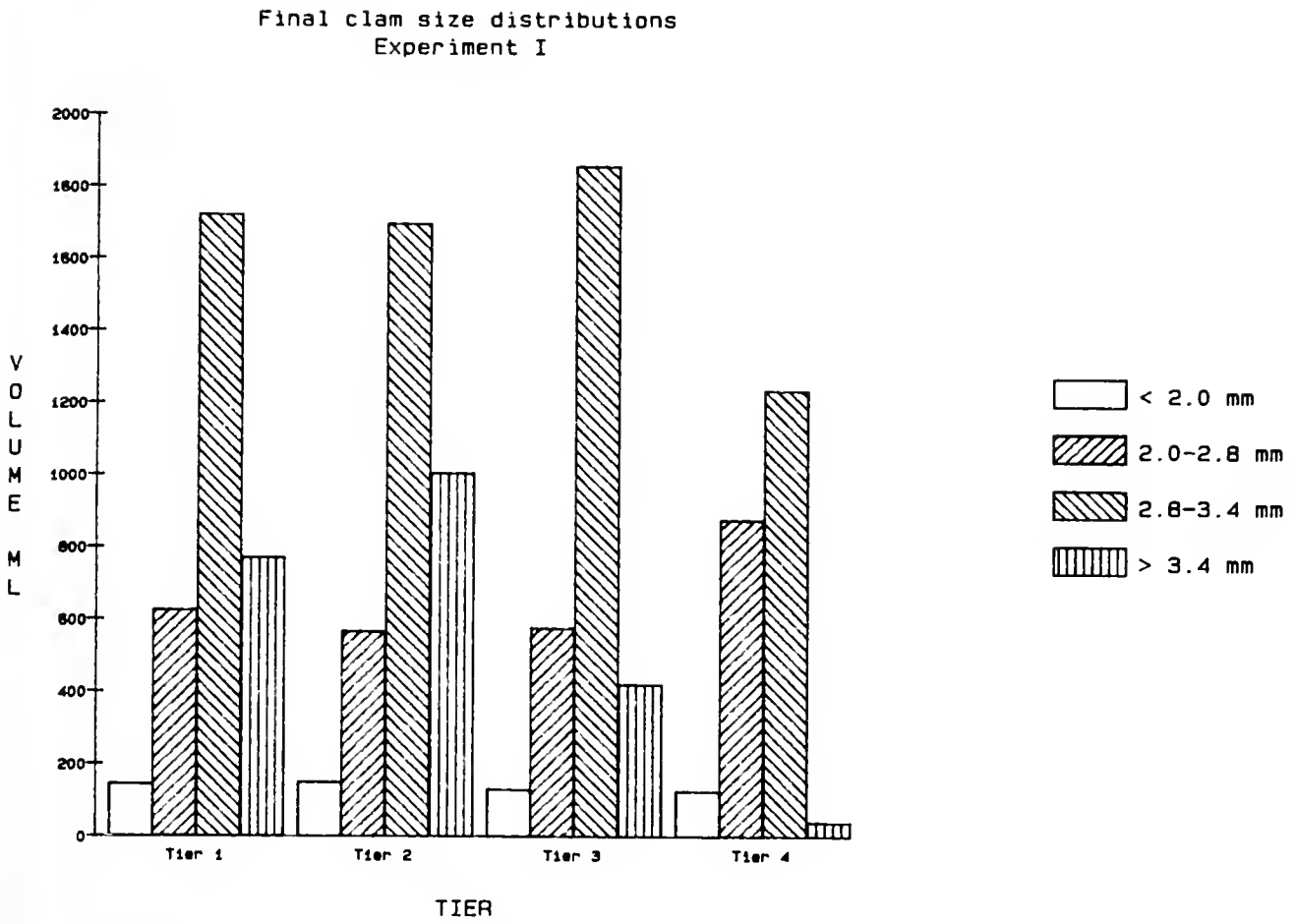


Figure 4. Clam size distributions (in sieve sizes, equal to minimum shell dimension) from terminal sample of Experiment I.

periment III ($p = 0.0001$) but not for Experiment II ($p = 0.4185$; however, overall growth of clams in Experiment II was poor). Student-Newmans-Keuls (SNK) multiple range tests showed that whole dry weights of individual clams in tiers 1 and 2 of both Experiments I and III were insignificantly different from each other, yet significantly higher than tiers 3 and 4. In other words, in Experiments I and II, water reuse through two consecutive tiers did not affect total dry weight of seed clams; water reuse through three or four tiers did decrease total dry weights. Sieving of all seed clams removed from the silos at the termination of Experiment I produced the size distributions presented in Fig. 4.

In Experiments I and III, ash-free dry weight of clams in the terminal sample decreased with increasing water reuse (Table 2; Fig. 5). This effect was significant in Experiment I ($p = 0.0413$) and highly significant in Experiment III ($p = 0.0001$). While the overall effect of water reuse on ash-free dry weight was significant in Experiment II, there were no significantly different subsets (SNK multiple range test), however, the ash-free dry weights of clams in tiers 1 and 2 of Experiment III were similar yet significantly different from ash-free weights of clams reared in tiers 3 and 4. In other words, water reuse through two consecutive tiers did not affect ash-free dry weights in either Experiments I and III, yet water reuse through three or four tiers decreases ash-free dry weight.

DISCUSSION

The production protocol routinely used at The Clam Farm, Inc. involves stocking silos at a density that yields maximum growth for one week. After one week, increases in biomass begin to limit growth and clams are removed from the system and redistributed at an appropriate stocking density. Size specific stocking densities and flow rates conforming to this protocol were determined experimentally during 1984 (Malinowski, unpublished data) and are in general agreement with those presented by Manzi et al. (1984). The stocking densities maintained throughout Experiments II and III and during weeks 2 and 3 of Experiment I were similar to those used in the commercial production system and therefore known to be near the maximum biomass of seed that can be grown at a maximum rate in a silo. The results of this study indicate that effluent waters from these optimally stocked silos can be used to

TABLE 2.

Results of water quality and growth analyses (means based on n = 50) from Experiments I and II.

Experiment	Tier	Chlorophyll-*a* (μg/l)	Ammonia (μg-at/l)	Mean Shell Length (mm)	Mean Whole Dry Weight (mg)	Mean Ash-free Dry Weight (mg)
I (wk 1)	Init	0.95	2.45	2.53	3.61	0.13
	1	0.92	2.45	3.11	6.59	0.34
	2	0.61	2.76	3.18	7.19	0.34
	3	0.67	3.38	3.21	7.25	0.57
	4	0.70	3.13	3.19	6.87	0.60
I (wk 2)	1	1.20	2.30	3.75	13.01	1.89
	2	0.81	2.86	4.02	14.49	1.29
	3	0.46	3.49	3.84	12.56	1.93
	4	0.43	3.93	3.72	11.20	1.47
I (wk 3)	1	2.01	3.35	4.70	23.91	1.67
	2	1.07	4.32	4.77	22.66	1.22
	3	0.93	5.08	4.51	20.03	0.94
	4	0.55	5.72	4.26	16.91	1.09
II	Init	0.83	5.83	4.77	21.93	1.25
	1	0.61	5.29	5.39	32.19	1.91
	2	0.52	6.94	5.65	34.24	1.70
	3	0.28	7.07	5.51	31.87	1.53
	4	0.19	6.80	5.55	31.48	1.56

support an additional, equal biomass of seed and therefore double seed production per unit of water pumped. Additional reuse (3 and 4 passes through seed clams) resulted in significantly reduced growth rates but survival was unaffected.

It was beyond the scope of this study to determine the relative roles of available food versus metabolite accumulation in determining the maximum potential for water reuse. Epifanio and Srna (1975) found that ammonia concentrations of 20–40 μg/l were necessary to decrease pumping rates of juvenile clams. Since ammonia accumulated to maximum concentrations of only 5–6 μg/l during the present study, it is suspected that food depletion, rather than metabolite accumulation was the primary cause of the significantly reduced rates of growth in tiers three and four.

In raceway systems, Kirby-Smith (1972) reported that scallop growth was depressed when more than 40% of the available chlorophyll-*a* was removed from ambient water while Rhodes et al. (1981) indicated that an absolute vlaue of 1 μg/l chlorophyll-*a* should be present in the effluent to achieve maximum scallop growth. In a nonrecirculating experimental-scale upflow system, Manzi et al. (1987) found that hard clam growth was reduced if more than 20% of the ambient chlorophyll-*a* was removed. Results from the present study indicate that after the first pass through a silo of seed clams approximately 17% of the ambient chloro-

TABLE 3.

Weekly volumetric increases in biomass for all three experiments. All values represent the mean (n = 3) packed volume (ml) of clams per silo in each of the four tiers.

		Packed Volume (% increase) Tiers			
Experiment	Date	1	2	3	4
I	8/07/85	200	200	200	200
	8/14/85	412 (106%)	422 (111%)	412 (106%)	402 (101%)
	8/21/85	705 (71%)	722 (71%)	683 (66%)	607 (51%)
	8/28/85	1073 (52%)	1115 (54%)	970 (42%)	760 (25%)
II	8/28/85	600	600	600	600
	9/04/85	862 (42%)	878 (46%)	872 (45%)	827 (38%)
III	9/04/85	800	800	800	800
	9/11/85	1270 (59%)	1237 (55%)	1158 (45%)	1033 (29%)
Mean volume increase		66.4%	67.4%	60.8%	48.8%

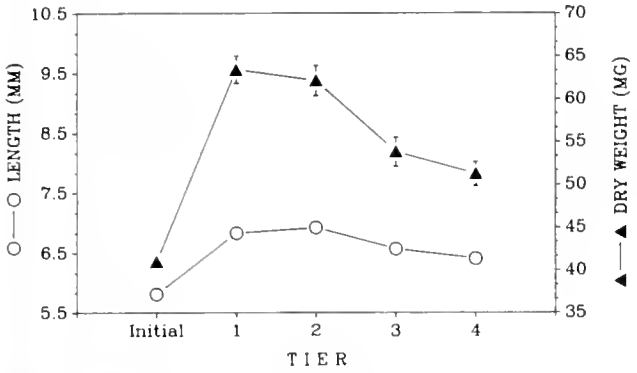


Figure 5. Mean whole dry weight (mg) and mean shell length (mm) vs tier for Experiment III.

phyll-*a* had been removed with an average of 1.3 μg/l remaining in the effluent. After the second pass through clams, however, the percentage chlorophyll-*a* which had been removed ranged from 36–73% (mean = 44%) which left from 0.52–2.81 μg/l (mean = 0.87 μg/l) remaining.

In the previous mentioned study (Manzi et al. 1986), growth rates were found to decrease with stocking density (flow rates were kept constant). The authors concluded that available food limited growth and suggested than an optimally operated upflow system would vary stock density as a function of the concentration of ambient chlorophyll-*a*. The results of the present study suggest that factors other than available food determine the maximum biomass of clams that can be grown at an optimal rate in an upflow silo. In agreement with the previous study, we found chlorophyll-*a* values reduced by ~20% (17% in this study) after water had passed through an initial group of silos stocked with an optimal biomass of clams. Our results indicate, however, that after water had passed through an initial group of clams, it could support maximum rates of growth of an additional, equivalent biomass of clams even though increasing the stocking densities of the initial silos would reduce rates of growth. In other words, production in the initial silos cannot be increased despite the fact that there is enough food in the incoming seawater to sustain at least twice the biomass. This clearly suggests that ambient food levels, measured as chlorophyll-*a*, do not limit seed clam production in these silos.

Furthermore, industry-wide standards for optimal stocking densities and flow rates for ambient water upflow culture systems appear to be emerging despite dramatic differences in the apparent quality and quantity of food in ambient source waters. For example, even though mean chlorophyll-*a* levels during this study were much lower than those reported by Manzi et al. (1987) (1.93 versus 12.7 μg/l) the optimal stocking densities and flow rates for the commercial production system of The Clam Farm, Inc. are comparable (on a per unit area basis) to those recommended by Manzi et al. (1984).

The results of the present study suggest that physical rather than biological factors may be most important in determining the upper limits of optimal stocking volumes in non-recirculating upflow nursery systems. Optimizing growth of clams throughout the system requires minimizing the small-scale environmental variability within silos. This can be accomplished by attaining as uniform a flow as possible throughout the entire volume of seed within a silo. Critical flow rates and stocking volumes are likely a reflection of the physical requirements necessary to achieve this uniform flow through the entire seed bed mass. Meeting the necessary food:biomass ratio then becomes a secondary concern. The commonly observed relationship between increasing seed size and increased maximum sustainable biomass in upflow systems may be the consequence of a physically limited system whereby properties of water flow (particularly the ability to achieve uniform flow) are influenced by the differential resistance of different sized particles (seed); many small seed clams probably impede water flow more than fewer large seed clams. Explaining production limits of upflow systems may be an engineering rather than biological problem.

Hadley and Manzi (1984) found that a flow rate of 8–9 l/min/l clams (approximately equivalent to 8–9 l/min/kg from Table 4, Manzi et al., 1985) was necessary to achieve maximum growth in a raceway system. Non-recirculating upflow systems make less efficient use of pumped water since flow rates of 15–20 l/min/kg are necessary to achieve maximum growth (Manzi et al. 1987; personal observations). The increased flow rates necessary for upflow systems are a physical requirement of the system rather than a biological requirement of the animals, however, and passive water reuse can double production yielding an effective production per unit of water pumped nearly identical to the raceway system but without the position effects frequently reported for raceways stocked with high densities of animals (e.g., Landers and Rhodes 1968; Hadley and Manzi 1984).

Substantial savings in both the equipment and operating costs required to operate an upflow nursery system may be realized by maximizing the use of water that must be actively pumped. Pumps may constitute as much as 25% of the total construction costs and the costs for electricity required to power pumps may be as much as 15% of all production costs. Utilizing a nursery design that recirculates 100% of the actively pumped water reduces these costs by 50%.

ACKNOWLEDGMENTS

The authors thank Nicholas Appelmans and Lee Crockett for assisting in the field and Brian Conklin, Gregg Rivara and Jennifer Epp for their help in processing samples. This research was funded by The New York State Department of Agriculture and Markets.

LITERATURE CITED

Bayes, J. C. 1979. How to rear oysters. In Proceedings of the 10th Annual Shellfish Conference. Shellfish Association of Great Britain, pp. 7–17.

Claus, C. 1981. Trends in nursery rearing of bivalve molluscs. pp 1–33. In C. Claus, N. Depauw, & E. Jaspers (eds.) Nursery Culturing of Bivalve Molluscs. European Mariculture Society Special Publication No. 7. EMS, Bredene, Belgium. 394 pp.

Epifanio, C. E. & R. F. Srna. 1975. Toxicity of Ammonia, Nitrite Ion, Nitrate Ion, and Orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. *Mar. Biol.* 33:241–246.

Hadley, N. H. & J. J. Manzi. 1984. Growth of seed clams (*Mercenaria mercenaria*) at various densities in a commercial scale nursery system. *Aquaculture* 36:369–378.

Kirby-Smith, W. W. 1972. Growth of the bay scallop: the influence of experimental water currents. *J. Exp. Mar. Biol. Ecol.* 8:7–18.

Landers, W. S. & E. W. Rhodes, Jr. 1968. Growth of young clams, *Mercenaria mercenaria*, in tanks of running seawater. *Proc. Nat. Shellfish Assoc.* 58:5. (Abstract)

Lucas, A. & Gerard. 1981. Space requirement and energy cost in some type of bivalve nurseries. In C. Claus, N. Depauw, and E. Jaspers (eds.) *Nursery Culturing of Bivalve Molluscs*. European Mariculture Society Special Publication No. 7. EMS, Bredene, Belgium. pp. 151–170.

Manzi, J. J. & Hadley, N. H. 1984. Hard clam, *Mercenaria mercenaria*, culture in a commercial-scale upflow nursery system. Abstract of the 76th Annual Nat. Shellfish Assoc. Annual Meeting. p. 17.

Manzi, J. J., N. H. Hadley, C. Battey, R. Haggerty, R. Hamilton, & M. Carter. 1984. Culture of the northern hard clam *Mercenaria mercenaria* (Linne) in a commercial-scale, upflow, nursery system. *J. Shellfish Res.* 4:119–124.

Manzi, J. J., N. H. Hadley, & M. B. Maddox. 1987. Seed clam, *Mercenaria mercenaria*, culture in an experimental-scale upflow nursery system. *Aquaculture* (In Press).

Rhodes, E. W., R. Goldberg, & J. C. Widman. 1981. The role of raceways in mariculture systems for the bay scallop, *Argopecten irradians irradians*, and the surf clam, *Spisula solidissima*. In C. Claus, N. Depauw, & E. Jaspers (eds.) Nursery Culturing of Bivalve Molluscs. European Mariculture Society Special Publication No. 7. EMS, Bredene, Belgium, pp. 227–251.

Rodhouse, P. G. & M. O'Kelly. 1981. Flow requirements of the oysters *Ostrea edulis* and *Crassostrea gigas* Thunb. in an upwelling column system of culture. *Aquaculture* 22:1–10.

Strickland, J. D. H. & T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. *Fish. Res. Bd. Can. Bull.*, 167. Second Edition, 310 pp.

Journal of Shellfish Research, Vol. 8, No. 1, 249–252, 1989.

AN AUTOMATED MASS CULTURE SYSTEM FOR PHYTOPLANKTON

PETER J. WANGERSKY, CHRISTOPHER C. PARRISH, AND CHARLES P. WANGERSKY
Department of Oceanography
Dalhousie University
Halifax, N.S. B3H 4J1 Canada

ABSTRACT An automated mass culture system based on the cage culture turbidostat was constructed for the mass culture of microalgae. The system consisted of a 200 l acrylic growth chamber through which growth medium was passed continuously. The population, confined within the chamber by filters, was monitored by the measurement of turbidity. Population density was held closely within limits by the removal of excess organisms (harvesting).

In the chamber configuration described in this paper, growth of the test organism, *Chaetoceros gracilis*, was limited by self-shading at concentrations greater than 10^6 cells/ml. At this concentration, with a 16:8 light:dark cycle, 4×10^{11} cells/day were harvested. The system maintained the phytoplankton culture in log phase growth until the end of the test period of two months.

KEY WORDS: culture, phytoplankton

INTRODUCTION

For those organisms whose diet consists largely of phytoplankton, the cost of growing the phytoplankton in mass culture is a large part of the expense of nursery culture (Persoone and Claus, 1980). As much as 80% of the cost of raising bivalves to marketable size can be attributed to the expense of culturing phytoplankton (Pruder and Bolton, 1981). Any decrease in the cost of phytoplankton production could result in higher profits or lower selling prices for the high cost items already being grown, and possibly in an extension of aquaculture techniques to species whose market prices are too low for profitable operations at this time.

One source of such savings is an increased automation of mass phytoplankton culture. For the past seven years our laboratory has been engaged in the study of growth patterns of phytoplankton, using a laboratory version of the cage culture turbidostat (Skipnes et al., 1980). With this apparatus, we have kept phytoplankton species such as *Phaeodactylum tricornutum, Dunaliella tertiolecta, Chaetoceros gracilis,* and *Protogonyaulax tamarensis* in log phase growth for several months at a time (Zhou and Wangersky, 1985, Parrish and Wangersky, 1987).

The cage culture turbidostat principle, while well suited to physiological experimentation because of the close control of experimental variables, was also an attractive choice for mass culture because of the ease with which it can be automated. Like the smaller laboratory version, the mass culture unit can run unattended for long periods. The replenishment of the concentrated nutrient supply was the only maintenance required.

THE TURBIDOSTAT

The cage culture turbidostat was first described in detail by Skipnes, et al. (1980). The unit used in our work (produced by Manna Marine Enterprises, Halifax), differed in several respects (Fig. 1) from the Skipnes model. Organisms were contained in a growth chamber, a 200 l container consisting of a 42.7 cm O.D. acrylic cylinder, 1.2 m in length, sealed at the top and bottom by acrylic plugs with silicone O-ring seals. This size was chosen because the diameter (18″) is a standard size for acrylic tubing, and the length (4′) matches that of the standard industrial fluorescent fixture.

The growth chamber was isolated from the system by 5 μm Acropore® membrane filters, 90 mm in diameter (Gelman Instrument Co., Ann Arbor, MI), held in a modified dual membrane stirred cell (Nuclepore, Pleasanton, CA). The cell modification consisted of the addition of perforated plastic support plates on either side of the stirrer, which permitted the pumping of seawater through the filters in either direction. The reversal of direction of flow was necessary in order to prevent clogging of the filters by the phytoplankton. Nylo-Seal fittings and black Poly-Flo (polyethylene) tubing (Imperial Eastman, Barrie, Ont.) were used for all connections in the growth chamber.

The growth medium used consisted of two solutions, the base and the nutrient concentrate. The base was seawater taken from the Northwest Arm of Halifax Harbour and filtered through the Dalhousie Aquatron sandbed filters. A further filtration step, through a 10 μm filter, was added just before the stirred cell. The seawater base was added to the growth chamber from a reservoir at a rate of 200–600 l/day. The minimum flow rate of medium was determined by the rate of harvesting; the medium removed in harvesting had to be replaced at least at the same rate, in order for the dilution of the culture to turn off the harvesting mechanism. A better flow rate would be at least twice the minimum rate, to ensure that bacteria and possibly harmful metabolites are swept from the growth chamber.

The seawater base was brought to an f/2 (Guillard and Ryther, 1962) concentration of nutrients, vitamins, and trace metals by the continuous pumping of a concentrated

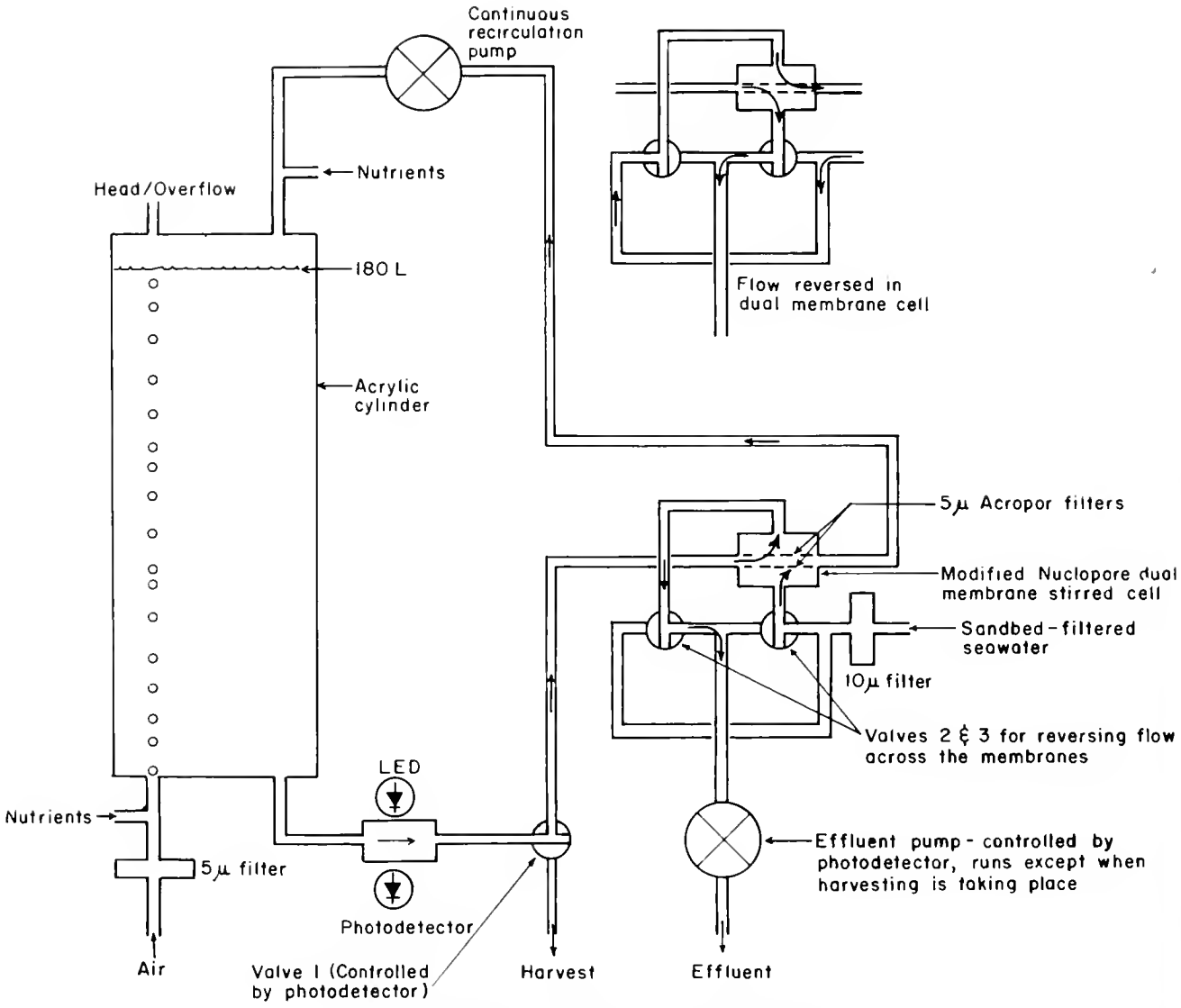


Figure 1. Diagram of the cage culture turbidostat system.

stock solution directly into the growth chamber. NH_4Cl was used as the nitrogen source instead of $NaNO_3$. This level of nutrients was higher that was actually needed for the growth of the phytoplankton at a concentration of 10^6 cells/ml; we have shown with *P. cornutum* that at such high concentrations of nutrients only a small proportion of the nutrients was used (Parrish and Wangersky, 1987). However, in the early experiments with the mass culture unit, we preferred to ensure that nutrients would not be limiting.

The culture was mixed by bubbling air from the bottom of the growth chamber. Since new seawater medium is added continuously CO_2 is not likely to be limiting. We did not investigate the use of air enriched with added CO_2; such addition would not be difficult, but would entail added expense for the grower.

The electronic control unit monitored and controlled the harvesting mechanism, determined the direction of flow of the medium through the growth chamber, and read and reported the turbidity. When the turbidity, which was proportional to the number of organisms present, reached a pre-set upper limit, organisms were removed from the growth chamber by opening the "harvest" valve until a pre-set lower limit was reached. The population was thus held within arbitrarily chosen narrow limits. The time of operation of the harvest valve was therefore a measure of the population growth rate; it was integrated hourly, and reported as the percentage of the hour spent harvesting. A close check could be kept on the population growth of the culture simply by inspection of the harvest times. Information was sent to a Hewlett-Packard 3390A computing integrator. The control units run continuously, typically requiring minor maintenance about once a year. We have

four control units which have been in operation almost continuously for four years.

The growth chamber was illuminated by six 4′ and three 2′daylight fluorescent tubes (standard industrial sizes), yielding 120 $\mu E/m^2/s$ at the center of the empty chamber. The nutrient, recirculation, and effluent pumps were all Masterflex (Cole-Parmer Instruments, Chicago, IL) peristaltic pumps. The temperature in the room and in the culture was held at 20°C by an industrial air conditioner.

DISCUSSION

We have normally optimized growth conditions for the species of phytoplankton chosen with the use of the laboratory version of the unit (Parrish and Wangersky, 1987). The smaller volume (250–350 ml) provided a faster response to changes in the nutrient supply or composition. However, fine tuning of the system must be done on the unit to be used for mass culture. The change in surface/volume ratio, as well as the increase in light attenuation in the larger growth chambers, can result in a shift in highest growth efficiency to lower population densities with lessened nutrient requirements.

With the species we have grown, the ultimate limitation on growth rate, and thus on yield, has been the amount of light available. Denser cultures or larger growth chambers could be employed if stronger sources of light are used. We have considered the use of light pipes in the growth chamber, or the use of doughnut-shaped growth chambers with fluorescent lights at the center, but these alternatives would add to the cost of construction and maintenance of the units. Such modificaations might be worthwhile for experimental use, but would not be indicated for commercial units, where initial cost and operational simplicity are major considerations.

Another alternative would be the use of continuous lighting, in place of the 16:8 light:dark cycle we employed. For the diatom species we have grown, growth rates of 2 divisions/day at 2×10^6 cells/ml, giving a yield of 8×10^{11} cells/day, can be maintained over periods of several months. With *Chaetoceros gracilis,* we have achieved growth rates as high as 3 div/day at 2×10^6 cells/ml and 24 hrs of light, or 1.2×10^{12} cells/day for periods of several weeks. Not all diatom species adapt well to growth under continuous light; under these conditions, we have found that our clones of *P. cornutum* grew well for periods of a month or more, after which the growth rate fell off drastically until the culture was lost. This drop in the growth rate was not a reversible condition; once the decrease was well started, the culture was invariably lost. If the larger units are held to a schedule of cleaning and restarting once a month, this behavior need not be a disadvantage, since the added yield resulting from the extra division/day might offset the cost of more frequent attention. If maximum yield were to be the sole criterion, we would certainly use continuous light and monthly cleaning and reinoculation.

No attempt was made to keep the mass culture axenic; the volume of culture medium used daily would make any sterilization scheme impractical. The microbial population in the incoming medium was reduced to some extent by the system of filtration, and the flow-through system ensured that neither the bacterial population nor the organic exudates and metabolic by-products were allowed to accumulate in the growth chamber. If bacterial growth became a problem, the use of finer filters for the inflow side of the "cage", or a shift to crossflow filtration of the incoming water could be instituted. While bacteria have not proved to be a problem, we have had long-standing cultures contaminated by various species of microalgae, possibly imported in the unsterilized medium. As a remedial measure, we have used tangential flow filtration during the summer months (Millipore, Ltd) for our mass cultures of *Protogonyaulax tamarensis* to eliminate all material with a molecular weight >1000 D.

Population size, as measured by turbidity, was monitored constantly by the cage culture turbidostat itself. In addition, our practice was to determine the cell number and mean cell size for all cultures daily, using a Coulter Counter Model 1015 ZB. Cultures were also examined under the microscope daily, since the effects of nutrient or growth factor deficiency can sometimes be seen as aberrant cell forms before they become evident as large decreases in growth rate. If the phytoplankton are grown as food, rather than as part of a study of phytoplankton physiology, the separate counts would not be necessary, since the relationship between cell numbers and turbidity was quite good ($r^2 = 0.99$, Fig. 2). Simple examination under the microscope as a check on gross irregularities would be sufficient.

The size of the mass culture unit is not limited to the 200 l unit used in this work; our choice of this volume was

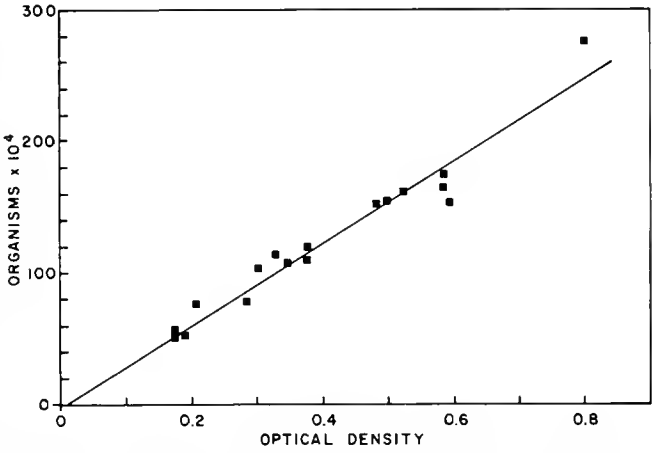


Figure 2. Cell counts, Coulter Counter, vs. turbidostat optical density.

dictated by practical considerations, as already noted. The standard sizes of acrylic tubing, 18″, 20″, and 24″, when made up into the 4′ lengths covered by a bank of fluorescent lights, will contain about 200, 250, and 500 l respectively. Again, these dimensions are given in inches because these are American standard sizes; use of the standard industrial sizes, rather than units constructed to order, considerably reduces the cost of manufacture. In our mass cultures of the dinoflagellate *P. tamarensis* we have used the 250 l size successfully.

We have taken the diatoms *Phaeodactylum tricornutum* and *Chaetoceros gracilis* and the dinoflagellate *Protogonyaulax tamarensis* from batch culture through laboratory scale turbidostat cage culture to mass culture. Several other species of microalgae, such as *Dunaliella tertiolecta* (Wangersky and Maass, 1988) and *Thalassiosira weissflogii*, have been brought into laboratory turbidostat cage culture, but not into mass culture. We feel that any species of microalgae which we can maintain in batch culture can eventually be moved into laboratory scale cage culture, and finally into mass culture in our unit. At least to this point we have had no failures, although we have had anxious moments.

The mass culture unit can run unattended between fillings of the nutrient concentrate vessel, as long as the harvested organisms are removed. We typically check the overnight records and inspect the cultures once a day, and look in on it during the day when we happen to be passing, to ensure that the plumbing is intact. In two years of operation, including construction and breaking-in time, we have had only one midnight summons. It would be possible to provide computer control over the unit, or several such units; the major advantage of such control would be the installation of signals warning of pump failures or leaky plumbing, the most common problems with these units. Otherwise, there is little to be gained from the substitution of computer control for manual control, unless banks of growth chambers are to be used. With several units in operation, computer control becomes less expensive and more reliable and flexible than control by inspection of a recorder output.

If a seawater line is already available, running costs for these units are minimal; power costs for the lights and pumps, and nutrient chemicals, average a few dollars a day at current rates. The major expense is still for labour. However, one technician can maintain several of these units, as well as the batch or turbidostat "mother" culture. Capital costs are harder to estimate; over a period of 18 months, the cost of building the growth chamber has increased by 25%. However, much of this increase was due to the demise of the only Canadian firm manufacturing acrylic tubing in the required size. The cost of building the prototype was about $3,000 for the culture chamber, $1000 for the control unit, and $1000 for the pumps. All costs are in Canadian dollars. Less expensive pumps could certainly be used, now that the flexibility of an experimental unit is no longer required. The prototype control unit was also more expensive than an equivalent production model would be.

CONCLUSIONS

The cage culture turbidostat offers economy of operation, as well as excellent control of variables, for the mass culture of microalgae. Once the operating variables for optimum production have been selected, the units can run almost unattended. Such units should be considered for the growth of mass cultures of algae for hatchery use.

ACKNOWLEDGMENTS

This project was funded by a contract with the Department of Supply and Services, with the Department of Fisheries and Oceans as scientific authority. We gratefully acknowledge the assistance of Regine Maass, Bryan Schofield, and Dr. Xianliang Zhou.

LITERATURE CITED

Guillard, R. R. L. & J. N. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) *Gran. Can. Microbiol.* 8:229–239.

Parrish, C. C. & P. J. Wangersky. 1987. Particulate and dissolved lipid classes in cultures of *Phaeodactylum tricornutum* grown in cage culture turbidostats with a range of nitrogen supply rates. *Mar. Ecol. Prog. Ser.* 35:119–128.

Persoone, G. & C. Claus. 1980. Mass culture of algae: a bottleneck in the nursery culturing of molluscs. In G. Shelef & C. J. Soeder (eds.) *Algal Biomass*, Elsevier/North Holland Biomedical Press, Amsterdam, pp. 265–285.

Pruder, G. & E. Bolton. 1981. Controlled-environment mariculture. In University of Delaware Marine Programme, College of Marine Studies, pp. 17–20.

Skipnes, O., I. Eide, & A. Jensen. 1980. Cage culture turbidostats: a device for rapid determination of algal growth rate. *App. Environ. Microbiol.* 40:318–325.

Wangersky, P. J. & R. L. Maass. 1988. Diurnal behavior of cultures of *Dunaliella tertiolecta*. *J. Plankt. Res.* 10:327–329.

Zhou, X. & P. J. Wangersky. 1985. Copper complexing capacity of *Phaeodactylum tricornutum*: diurnal changes. *Mar. Chem.* 17:301–312.

Journal of Shellfish Research, Vol. 8, No. 1, 253, 1989

AN ADDENDUM TO A CASE FOR SEQUESTERING OF PARALYTIC SHELLFISH TOXINS AS A CHEMICAL DEFENSE

RIKK G. KVITEK[1] AND MARK K. BEITLER[2]
[1]*Department of Zoology*
University of Washington
Seattle, WA 98195
[2]*Institute for Food Science & Technology*
University of Washington
Seattle, WA 98195

KEY WORDS: paralytic shellfish toxin standards, HPLC analysis, mouse bioassay, toxic shellfish extracts

In "A case for sequestering of paralytic shellfish toxins as a chemical defense" by Kvitek and Beitler, *J. Shellfish Res.* 7(4):629–636, 1988, HPLC values reported for saxitoxin (STX) concentrations were 40% too low. This was because the number used for the concentration of a STX secondary standard we prepared was found to be incorrect in a subsequent study.

In the previous investigation, a STX primary standard (STX1) containing HCl (100 μg STX/ml, pH 3.5, USFDA, Cincinnati, OH) was diluted 1:300 with 0.05 N HOAc to form a secondary standard (STX2). Using a mixed paralytic shellfish toxin standard coded MS-33;1:20 (USFDA, Seattle, WA), STX2 was found to contain 0.64 μM STX by HPLC analysis rather than an expected value of 0.90 μM STX. The former value was precise because it had an acceptably low coefficient of variation (% C.V.) of 12 (Boyer et al. 1986, Sullivan and Wekell 1987). In another study, STX1 was diluted 1:300 in an HCl solution (pH 3.5) rather than with 0.05 N HOAc to prepare another STX secondary standard (STX3). Using the MS-33 standard, the HPLC system correctly measured the concentration of STX3 as 0.89 μM STX (% C.V. = 8). This concentration for STX3 was considered to be accurate because a close correlation was obtained between mouse bioassay values and HPLC results for extracts of six species of shellfish analyzed on the same days as STX3 by HPLC. Therefore, the previously used value of 0.64 μM STX for STX2 was incorrect. The discrepancy between STX concentrations for STX2 and STX3 is believed to have occurred because STX2 contained HCl and HOAc and STX3 was prepared in only HCl.

To correct for the error in quantitating the concentration of STX in STX2, all HPLC results in the report expressed as μg STX equivalents/100 g tissue, μg STX/ kg body weight, and μg STX in Tables 1–2 and throughout the text should be multiplied by 1.4. These modifications do not change the lower limits of detection for STX or the conclusions drawn from the original findings.

When performing HPLC analyses, it is advisable to dilute the STX primary standard with only HCl (pH 3.5) and to precondition the analytical column with several injections of extracts from toxic bivalves.

LITERATURE CITED

Boyer G. L., J. J. Sullivan, R. J. Andersen, F. J. R. Taylor, P. J. Harrison & A. D. Cembella. 1986. Use of high-performance liquid chromatography to investigate the production of paralytic shellfish toxins by *Protogonyaulax* spp. in culture. *Marine Biology* 93:361–369.

Sullivan, J. J. & M. M. Wekell. 1987. The application of high performance liquid chromatography in a paralytic shellfish poisoning monitoring program, p. 357–371. In D. E. Kramer & J. Liston (eds.) *Seafood Quality Determination*. Elsevier Science Publishing Co., Inc., New York.

Journal of Shellfish Research, Vol. 8, No. 1, 255–257, 1989

PROCEEDINGS OF THE SPECIAL SYMPOSIUM: CRAWFISH INDUSTRY STATUS AND TRENDS

Presented at the 80th Annual Meeting

NATIONAL SHELLFISHERIES ASSOCIATION

New Orleans, Louisiana

June 26–30, 1988

Convened and edited by

ROBERT P. ROMAIRE
School of Forestry, Wildlife, and Fisheries
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

INTRODUCTION TO THE SYMPOSIUM, CRAWFISH INDUSTRY: STATUS AND TRENDS

Louisiana is a national leader in annual production of seafood commodities, and is first in the USA in annual production of shrimp, oyster, blue crab, and crawfish (crayfish). Not only is Louisiana the national leader in freshwater crawfish production, it is also the international leader. For centuries crawfish have been a highly esteemed seafood of native Louisiana Indians and French immigrants that settled Louisiana in the mid-1700's. World-wide there are over 500 species of crawfishes, and about 350 species are found in North America. However, there are only five genera of crawfishes that have major economic importance as food for human consumption: *Procambarus, Astacus, Pacifastacus, Cherax,* and *Orconectes*. Crawfishes are highly esteemed in certain areas of western Europe, notably Scandinavia (Sweden and Finland in particular), France, and Spain. Crawfishes are also consumed in moderate quantities in Australia. Crawfishes are not as widely recognized in national and international markets as are other popular seafoods such as marine shrimp, rainbow trout, salmon, oysters and clams; thus, consumption is largely restricted to the southern USA, principally Louisiana, and western Europe.

Louisiana produces over 80% of the world's annual harvest of crawfishes. Louisiana has an ideal wetland habitat, the Atchafalaya River Basin, for natural production of the red swamp crawfish, *Procambarus clarkii*, and the white river crawfish, *Procambarus acutus acutus*. These two species dominate world-wide crawfish production, and through intentional and non-intentional introductions these two species are the most cosmopolitan of all crawfish species. In addition to the large natural fishery for crawfish, about 2,000 producers cultivate over 55,000 ha of crawfish in man-made impoundments located principally throughout the French-Acadian areas of south Louisiana. Louisiana has an ideal environment for crawfish aquaculture because it has expanses of flat lands and fertile soils that are ideal for pond construction, plentiful surface and subsurface freshwater resources, a warm climate, an abundant labor force, and a historical tradition of seafood production and consumption.

Crawfish aquaculture in Louisiana developed very rapidly in the 1980's in response to increased demand for crawfish products, not only in Louisiana, but also in national and international markets as well. Crawfish aquaculture was easily integrated into existing agricultural operations such as rice, soybean, and sugarcane. Additionally, capital investment, and educational and management requirements necessary to establish a profitable "crawfish farm" were relatively low compared to other aquacultural commodities. The popularity of crawfish in many areas of the USA and the increased national and international demand for crawfish has spurred development of crawfish aquacultural industries throughout the southeastern USA. In 1985, a new crawfish aquacultural industry emerged, soft-shell crawfish production, and it is estimated that 300 producers will culture soft crawfish in Louisiana in 1989.

On June 30th 1988, a special symposium on the Louisiana crawfish industry was held in New Orleans in conjunction with the 80th Annual Meeting of the National Shellfisheries Association. Topics of interest to the crawfish industry were presented by representatives of academia who were instrumental in the development of technology currently used in the crawfish aquaculture industry. The purpose of this symposium was to provide a general overview of status and trends in the crawfish industry from a worldwide perspective, but with special emphasis on Louisiana. Topics included a review of national and international freshwater crawfish production; a review of crawfish cultivation management practices, including forages and feeding systems, and harvesting; soft crawfish production technology; and Louisiana crawfish processing, product markets, and marketing.

Journal of Shellfish Research, Vol. 8, No. 1, 259–265, 1989.

OVERVIEW OF INTERNATIONAL AND DOMESTIC FRESHWATER CRAWFISH PRODUCTION

JAY V. HUNER
Crawfish Center
College of Agriculture & Home Economics
University of Southwestern Louisiana
Lafayette, Louisiana 70504

ABSTRACT Freshwater crawfishes are the dominant macrobenthic invertebrates in many temperate aquatic environments. Over 500 species belong to three families: Astacidae (Europe and North America), Cambaridae (eastern Asia, North and Middle America (native) and Africa, Europe, and South America (introduced)) and Parastacidae (Australo-New Guinea region, southern South America, and Madagascar). Important commercial, subsistence and/or recreational genera are *Astacus*, *Austropotampobius*, *Cherax*, *Euastacus*, *Orconectes*, *Pacifastacus*, and *Procambarus*. Estimated annual production (1988) is at least 60,000, 6,800, 2,000, and 550 MT in the USA, Europe, Asia, and Australia, respectively. Several million hatchling (fry) and one-summer (juvenile) crawfishes are produced annually in Europe and Australia for restoration and aquacultural purposes. One species, *Procambarus clarkii*, accounts for about 85% of all crawfishes harvested. It is native to the southern USA and northeastern Mexico. It is now firmly established throughout the USA and western Mexico and perpetuating populations are present in the Caribbean, Central America, South America, Hawaii, Japan, Taiwan, mainland China, Africa, Cyprus, and Europe. It is cultured in 65,000 ha of earthern ponds, often in a multiple-crop rotation with rice, in the southern USA. North American species have been introduced into Europe to replace species decimated by the fungal disease, *Aphanomyces astaci*. N.A. species are highly resistant to the disease and are known vectors. They should, therefore, be excluded from contact with parastacid crawfishes which have no apparent resistance to the malady.

KEY WORDS: crawfish, Astacidae, Cambaridae, Parastacidae

INTRODUCTION

Freshwater crawfishes are the most signficant macroinvertebrates in many temperate freshwater environments (Momot 1984). They are important links between low and high trophic levels. Crawfishes are exploited for food and fish bait in areas with high crawfish population densities (Holdich and Lowery 1988b). Crawfishes range in size from dwarf species in North America and Australia that are as small as 2 cm total length (TL) and <0.1 g to the giant Australian species which are as large as 50 cm TL and >3 kg (Hobbs 1988). Most species exploited by man, however, attain maximum sizes of 10–12 cm TL and 30–80 g, but even dwarf crawfishes are used for food in Mexico.

Three families of freshwater crawfishes are currently recognized—Astacidae, Cambaridae, and Parastacidae (Hobbs 1988). The native ranges were: Astacidae, western North America, western Asia and Europe; Cambaridae, eastern Asia and North and Middle America; and Parastacidae, Australia-New Guinea region, southern South America, and Madagascar. Several North American and Australian species have been widely transplanted (see details below) and feral populations of at least one species, *Procambarus clarkii* are now present in eastern Africa (Huner 1977).

The Astacidae is the least diversified crawfish family with only three recognized genera: *Astacus*, *Austropotamobius*, and *Pacifastacus*, and 12 species. The astacid species all attain sizes in excess of 10 cm TL and several species are the subjects of major fisheries and aquacultural endeavors. The Cambaridae is the most diversified crawfish family with 12 genera and at least 360 species although representatives of only three genera, *Cambarus*, *Orconectes*, and *Procambarus*, are of major economic importance. The Parastacidae has the greatest number of crawfish genera, 14, but has only 145 species. The genera *Astacopsis*, *Cherax*, and *Euastacus* include species of major economic importance. Hobbs (1988) should be consulted for additional information about crawfish taxonomy. Table 1 includes a list of economically important crawfishes.

CRAWFISH PRODUCTS

Most crawfish are used for food and as bait for sport fishing. Lesser quantities are widely used for physiological research and for classroom dissections of representative arthropods (Holdich and Lowrey 1988b; Huner and Barr 1984). The primary form for crawfish as food and as fish bait is whole in both the hard, intermolt state and the soft, recently molted state. Soft shell crawfishes are usually 10–20 times more valuable than intermolt crawfishes in the USA but the volume of soft shell crawfish produced is small (Huner 1988c).

Where crawfish are abundant, as in Louisiana, they may be processed for abdominal muscle ("tail meat"). The yield of abdominal muscle from astacid and cambarid crawfishes ranges from 10–40% of total body weight depending on size, maturity, and whether or not chela muscle is recovered (Huner and Barr 1984, Huner 1988c, Huner et al. 1988). Australian parastacid species may have up to 50% recovery when the exoskeleton weight is included (Hutchings 1988a) but the yield is comparable to that of other crawfishes when the weight of the exoskeleton is considered (Morrissy 1988).

TABLE 1.

Crawfish production in the USA and Canada

Region	Wild	Aquaculture (tons)	Species
Southern USA	5,000–25,000	60,000 (65,000 ha)	*Procambarus clarkii* *P. a. acutus*
Northcentral USA	100	150 (500 ha?)	*P. clarkii* *P. a. acutus* *Orconectes immunis* *O. rusticus* *O. virilis*
Northeastern USA	?	25 (50 ha)	*O. immunis* *Orconectes* spp.
Western USA	300	n/a/	*Pacifastacus leniusculus* *P. clarkii*
Eastern Canada	10	?	*O. immunis* *O. rusticus* *O. virilis* *Cambarus robustus*

PRODUCTION

Total world production of freshwater crawfishes is probably 70,000–100,000 metric tons (MT) per annum. Accurate production data are difficult to obtain because sales are often based on cash transactions that are poorly recorded. For example, official Turkish data showed annual exports of about 4,000 MT in the mid-1980's while importation statistics in western Europe revealed imports closer to 8,000 MT (Laurent 1987a, b). Production of all crawfishes from natural fisheries and aquaculture changes dramatically depending on natural conditions such as disease and local weather as well as economic considerations. Natural fishery production in Turkey has declined to a few hundred MT because of mass mortality caused by the crawfish fungus plague, *Aphanomyces astaci*, (Laurent 1987a, b). Natural fishery production in Louisiana varies from 5,000–25,000 MT depending upon hydrological conditions in the principal fishing area (Huner and Barr 1984). Therefore, annual production is volatile and data presented in Tables 1, 2, and 3 may change dramatically.

Many species of crawfishes are exploited (Table 4); however, the single most important species of crawfish is the red swamp crawfish, *Procambarus clarkii*. This species accounts for about 85% by volume of all crawfishes harvested annually using 80,000 MT as the current figure for world production. Most of these crawfish come from the 54,000 ha of culture ponds and natural fisheries in Louisiana. Significant fisheries for this species occur elsewhere including Spain, 5,000 MT (Habsburgo-Lorena 1988) and the People's Republic of China, 2,000 MT (Shu 1988). Several hundred MT have been produced from fisheries in Kenya in recent years (Laurent 1987a, b) but production has been reduced significantly from drought that has reduced habitat (French 1988).

NORTH AMERICA

Natural Fisheries and Cultivation of Procambarus spp. in the USA

Most natural production of *Procambarus* spp. occurs in Louisiana, principally in the south-central part of the state in the Atchafalaya River Basin, the major distributary for the Mississippi River. Production, primarily *P. clarkii*, is estimated to range from 5,000–25,000 MT per year (Table 1) (Soileau et al. 1975; Huner and Barr 1984; LCES 1986), and is dependent upon the vagaries of nature. The Basin is dry during summer and early autumn with crawfish surviving and reproducing in burrows. They emerge with young when surface waters accumulate in late autumn. Pro-

TABLE 2.

Crawfish production in Europe[1].

Country	Production (tons)	Species
Spain	5,000	*Austropotamobius pallipes* *Procambarus clarkii*
Sweden	175	*Astacus astacus* *Pacifastacus leniusculus*
Turkey	500	*Astacus leptodactylus*
Finland	75	*A. astacus*
USSR	1,000	*A. astacus* *A. leptodactylus*
France	10	*Orconectes limosus* *P. leniusculus*
Norway	20	*A. astacus*
Great Britain	5	*P. leniusculus*
Greece	20	*A. astacus*
Other	75	Species listed above

[1] Aquaculture in the true sense of the word accounts for very little production of food size crawfish in Europe.

TABLE 3.

Food crawfish production in Australia.

Method	Tons	Species
Aquaculture	<50 (<50 ha)	*Cherax destructor* *C. quadricarinatus* *C. tenuimanus*
Wild Fishery	<500	*C. destructor* *C. tenuimanus* *Euastacus* spp. *Astacopsis gouldii*

duction in the Basin is then dependent on the length of time that low lying areas are covered by flood waters entering the Atchafalaya River from the Mississippi River. In a year when the region is flooded from early winter through late spring, a large crop of crawfishes is harvested. A reduction in the time that water covers crawfish habitat in the Basin reduces production with the total dependent on weather and climate in the middle and upper reaches of the Mississippi River Valley. In general, good crops of 25,000 MT or more are realized 2 out of every 5 years.

Area devoted to aquaculture of *Procambarus* spp., *P. clarkii* and *Procambarus acutus acutus,* is about 66,000 ha (Table 5) with 86% of the area being located in Louisiana. *Procambarus clarkii* is the principal species, accounting for at least 90% of the harvest, although *P. a. acutus* can be locally abundant. A conservative estimate of annual harvest would be 500–600 kg per ha (Pomeroy and Kahl 1987; Roberts and Harper 1988); however, well managed crawfish farms consistently produce over 1,000 kg per ha (Huner 1988b, d; Huner and Barr 1984).

Procambarid crawfish aquaculture has been largely limited to Louisiana and, to a lesser extent, Texas (Huner 1988c). Louisiana has a long history, several centuries, of exploiting crawfish populations and a crawfish "farm" is reported as early as the late 1700's near New Orleans (Huner and Barr 1984). Louisiana has ideal conditions for crawfish production including low, flat, poorly drained lands well-suited for aquaculture and extensive areas of rice cultivation that are easily converted to crawfish production either in a crop rotation with rice, soybeans, and or grain sorghum or in monoculture depending on agricultural economics at the time (Huner 1988b). Southeastern Texas is culturally and ecologically similar to southwestern Louisiana, a major crawfish and rice cultivation region. Therefore, it is not surprising that Texas is the second leading state in crawfish production. Procambarid crawfish aquaculture development elsewhere is slow but increasing with most farms established in the past 2–3 years. Whether or not massive development will occur is yet to be determined. The use of improved management practices could easily increase production significantly without need for increasing the area devoted to crawfish culture.

Some *Procambarus* spp. are produced in the midwestern and northeastern USA as bait for sport fishing (Huner 1988b, d). Both *P. clarkii* and *P. a. acutus* are cultured. An objection to the two species is that young-of-the-year grow so rapidly that they are too large for bait when the principal fishing seasons for game fish begin in July.

Procambarus clarkii are found in most states in the continental USA and Hawaii. [The species has become well established in predator-free irrigation systems far outside of its natural range (Huner and Barr 1984).] These crawfish are exploited to a limited degree when the irrigation

TABLE 4.

Species of freshwater crawfish of significant or developing importance.

Family	Species	Region
Astacidae	*Astacus astacus*	Europe
	Astacus leptodactylus	Europe
	Pacifastacus leniusculus	Europe, USA
Cambaridae	*Cambarus robustus*	Canada
	Orconectes immunis	Canada, USA
	Orconectes limosus	Europe
	Orconectes nais	USA
	Orconectes rusticus	Canada, USA
	Orconectes virilis	Canada, USA
	Procambarus acutus acutus	USA
	Procambarus clarkii	Africa, Asia, Europe, USA
Parastacidae	*Astacopsis gouldii*	Australia
	Cherax destructor	Australia
	Cherax quadricarinatus	Australia
	Cherax tenuimanus	Australia
	Euastacus armatus	Australia
	Euastacus spp.	Australia
	Cherax spp.	New Guinea
	Paranephrops spp.	New Zealand

TABLE 5.

Cultivation of *Procambarus* spp. in the USA[1].

State	Estimated Pond Area (hectares) 1988
Louisiana	56,580
Texas	7,300
Arkansas	200
Mississippi	100
Alabama	<50
Florida	800
Georgia	<100
South Carolina	445
North Carolina	<50
Maryland	<50

[1] These are "good faith" estimates based on conversations with commercial and governmental sources in all states involved. Those from Louisiana and Texas are most accurate. The other estimates may vary as much as 10–20% from the stated values. Furthermore, some production is present in some "cultivated" ponds in most states at mid- or lower latitudes but statistics, real or estimated, are not available.

systems are drained in the autumn for maintenance. *Procambarus clarkii* is abundant in California especially in the ricefields of the Sacramento River-San Joaquin River Delta (Somer and Goldman 1983; Somer 1984). Many thousands of kg of crawfish are concentrated in ricefield irrigation canals when the fields are drained in late summer prior to harvesting of rice. These populations are largely unexploited and farmers often poison crawfish in the fields because they damage, through burrowing, earthen levee systems in the fields.

Exploitation of Other North American Crawfish Species

While the bulk of the North American crawfish harvest comes from the southern USA, organized fisheries for *Orconectes* spp. have been, or are being, established in Wisconsin and Minnesota area (100 Mt) (Pagel 1988) and the Ontario, Canada area (10 MT) (Momot 1988b); and fisheries for *Pacifastacus leniusculus* are being established in California (100–300 MT) (Lowery and Holdich 1988) and Oregon (150–200 tons) (Anonymous 1987) (Table 1). These have developed largely as a consequence of European demand following the declines in Turkish production. These fisheries have potential to expand. However, if the fisheries are to persist, strong domestic markets must be developed because the European market is finite and domestic European production of crawfishes is increasing.

Crawfish aquaculture outside of the southern USA has focused on *Orconectes* spp., especially *Orconectes immunis*, the papershell crawfish, which is a hardy, though small, burrowing species common in the northcentral and northeastern USA (Momot 1988a). *Orconectes immunis* is cultured primarily for fish bait, and it is often a secondary species cultured in conjunction with conventional fish cultural operations where primary species are fingerling food fishes or bait minnows, neither of which are predators of *Q. immunis* (Huner 1976). The area used for *Orconectes* spp. culture is speculative because no surveys have been made (at least to this author's knowledge), but, there are probably at least 500 ha of finfish cultural ponds in these regions. If 50–100 kg per ha of *Orconectes* spp. were harvested incidental to other fish cultural operations, production would be 25–50 MT per annum.

EUROPE

Production of native European crawfishes is volatile as a consequence of the crawfish fungus plague. *Astacus astacus*, the noble crawfish, occurs at exploitable levels in Scandinavia largely because the high price ($1.00 US each retail, Huner 1988a) makes it feasible to harvest, and 100–200 MT is probably harvested annually in Norway, Sweden, and Finland. The crawfish plague occurs periodically in these countries but plague-infested waters are restocked, naturally and artificially, rapidly enough to sustain current low production levels (Huner and Lindqvist 1988). Present production levels of *A. astacus* are estimated to be only 5–10% of that realized around 1900 (Fjälling and Fürst 1988).

Astacus astacus is also harvested in Greece for export to western European markets. About 50 MT was exported to France in the mid-1980s (Laurent 1987a, b) but an episode of crawfish fungus plague in Greece has reduced harvest dramatically and continued production at commercial levels is questionable.

Austropotamobius pallipes is abundant in England and Ireland, but harvest for sports or commercial purposes is small. However, development of commercial fisheries for *A. pallipes* is questionable because of the recent appearance of the crawfish fungus plague in both countries (Laurent 1988). *Austropotamobius pallipes* once supported commercial production in excess of 800 MT annually in Spain and up to 75 MT anually in Italy (Laurent 1987a, b), but in the past decade, the crawfish fungus plague severely reduced harvest. The harvest in these countries is currently very small and efforts are being made to re-establish the species in waters where it was once abundant (Laurent 1988).

Commercial fisheries for *Astacus leptodactylus* in Turkey has also declined dramatically from 8,000 MT in the early 1980's to fewer than 500 MT currently in response to the appearance of the crawfish fungus plague (Laurent 1987a, b; Fürst 1988). The location of new, unexploited populations in plague free areas offers some hope of increased production if these populations can be protected from the plague. This is questionable, based on the past history of plague dissemination.

The Soviet Union appears to have good potential for harvest of *A. leptodactylus* and Brodsky (1985) reported annual production of 1,000 MT. No current production statistics are available and potential for expansion is unknown

although it could be significant. However, the potential of crawfish fungus plague exists.

Demand for native European crawfishes remains great. High prices, that reflect their scarcity, have generated much interest in the aquaculture and management of these native species as well as the introduced *P. leniusculus* in Europe. Several million hatchling (fry) and summerling (juvenile) species are produced annually for restoring natural populations. There is little aquacultural production of food-size European crawfishes. For reviews of these subjects see Holdich and Lowery (1988a), and Huner (1988).

Because of crawfish plague, the North American species *Orconectes limosus*, *P. leniusculus*, and *P. clarkii* have been introduced widely throughout western Europe. These species have a high degree of resistance to the disease.

Procambarus clarkii was introduced into Spain in the early 1970s and as much as 5,000 MT were harvested from feral ricefield and marsh populations in 1988 (Habsburgo-Lorena 1988). *Procambarus clarkii* is now established in areas of France and Portugal, and in time more successful introductions should result in increased production of this species. Application of aquacultural techniques used in the USA would increase production significantly.

Pacifastacus leniusculus, the signal crawfish, has been introduced into most western European countries but the most significant populations are found in Sweden. Over 1,000 Swedish lakes and streams have been stocked with the species (Fjälling and Fürst 1988) since the late 1960s. Annual production has reached 175 MT and may reach 1,000 MT before the year 2000. Swedish consumption of crawfish is about 2,000 MT annually (Huner *et al.* 1989). Thus, increases in production of *P. leniusculus* will have a positive impact on Sweden's economy. There is little doubt that *P. leniusculus* will expand further in Europe (see Lowrey and Holdich 1988).

Orconectes limosus was introduced into Europe in the late 1800s and it has expanded across the continent and can be found in most European countries except Scandinavia (Momot 1988a). *Orconectes limosus* is of minor economic importance. Although annual fishery production could probably generate several thousand MT, consumers will not buy *O. limosus* because it is small, rarely larger than 9 cm TL, and it frequents water bodies with poor water quality so it is considered to be unfit for human consumption. Although *O. limosus* has been widely harvested in parts of Europe (Kossakowski 1966), annual production is not more than 30 MT (Laurent 1987c).

Introduction of the various North American crawfishes into Europe has created a major controversy. All three N.A. species of commercial importance, *O. limosus*, *P. clarkii*, and *P. leniusculus*, are more fecund and aggressive than the native European species so they are at a competitive advantage. Additionally, N.A. species are vectors for the crawfish fungus plague (Alderman and Polglase 1988, Söderhäll et al. 1988, Vey et al. 1983). Even where the native European species are at a competitive ecological advantage, they are subject to elimination by the crawfish fungus plague.

AUSTRALIA

Australians have long exploited crawfishes (Olszewski 1980). Two groups of crawfishes are important, the slow growing, "spiny" species like *Astacopsis gouldii* and *Euastacus armatus*, and the rapid growing, "smooth" species of the genus *Cherax*. Natural fisheries for these species are currently estimated to be about 500 MT (Morrissy 1988). Both *A. gouldii* and *E. armatus* attain sizes in excess of 1 kg and *A. gouldii* can easily attain 4 kg (Morrissy 1983). Neither *A. gouldii* nor *E. armatus* or related species have much aquacultural potential because they grow slowly and have poor meat yields.

The potential for culture of several *Cherax* species is much greater because they grow rapidly and can be cultivated in earthen ponds (Morrissy 1983). About 500 ha of crawfish ponds is cultivated in the states of Western Australia, South Australia, New South Wales, and Queensland; however, most of the culture is concentrated on production of high valued hatchling (fry) and summerling (juvenile) crawfish for sale to other aquaculturists. Annual production of food-sized crawfish is probably not more than 50 MT per annum (Hutchings 1988b; Morrissy 1988). The important species are the yabbie, *Cherax destructor*, in eastern states, the marron, *Cherax tenuimanus*, in the southwest, and the Queensland red claw, *Cherax quadricarinatus*, in eastern states. While maximum sizes of these species is roughly 150 g, 2,000 g, and 400 g, respectively, these species should be cultivated to 40–100 g in size for commercial ventures to be viable (Morrissy et al. 1986, Staniford et al. 1987). Production data for Australian crawfishes are summarized in Table 5.

Cherax spp. are generally cultivated in Australia by stocking hatchery-produced young into earthen ponds at rates around 5 per m^2 (Morrissy 1983). Thus, hatchery production of young *Cherax* spp. is estimated at about 2.5 million to supply the 500 ha of ponds currently in production.

Australian crawfishes have been shown to be highly susceptible to the crawfish fungus plague in laboratory tests (Unestam 1975). Therefore, it could be a great ecological tragedy to introduce any North American crawfish, regardless of source, into Australia because they must be considered to be vectors for the disease until evidence to the contrary is produced. Exposure of Australian crawfishes to North American and European crawfishes through introductions outside of Australia would be unwise.

CONCLUSIONS

Crawfishes are commercially important in the USA, western Europe, parts of the People's Republic of China,

and Australia. The bulk of the world's supply comes from the State of Louisiana, which leads the world in both natural fishery and aquaculture production of crawfishes. The single most important and widely distributed species is *Procambrus clarkii*. The single most important factor limiting crawfish production in Europe is the crawfish fungus plague, *Aphanomyces astaci*, to which no European species is known to have resistance. The crawfish fungus plague also must be taken into account when considering the future of the culture and introduction of parastacid crawfishes outside their native ranges in the Southern Hemisphere because they are apparently highly susceptible to the plague. It is, therefore, of importance that North American species, which are the presumed vectors of the disease, be excluded from the native ranges of all parastacid crawfishes. Significant expansion of international crawfish production can be expected primarily through exploitation of transplanted North American species, especially in Europe. It is advisable for conservation reasons to maintain native species wherever possible. This can be achieved only through exclusion of non-native species and restoration of populations that can be protected from the crawfish fungus plague.

REFERENCES CITED

Alderman, D. J. & J. L. Polglase. 1988. Pathogens, parasites, and commensals. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management and Exploitation*. London: Croon Helm Ltd, pp. 167–212.

Anonymous. 1987. The crawfish connection. *Oregon Wild* 43(4):12.

Brodsky, S. J. 1985. Sur l'elevage en Union Sovietique des ecrevisses de riviere (Astacidae) par la methode industrielle et sur les perspectives de cette methode. *La Pisciculture Francaise* 78:5–9.

Fjälling, A. & M. Fürst. 1987. The development of a fishery for the crayfish *Pacifastacus leniusculus* in Sweden 1960–86. *Freshwater Crayfish* 7:223–230.

French, C. 1988. Personal Communication, Nairobi, Kenya.

Fürst, M. 1988. Personal Communication, Institute of Freshwater Research, S-170 11 Drottningholm, Sweden.

Habsburgo-Lorena, A. S. Personal Communication, Fuente Milano 2, 28035 Madrid 35, Spain.

Hobbs, H. H., Jr. 1988. Crayfish distribution, adaptive radiation and evolution. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management, and Exploitation*. London: Croom Helm Ltd., pp. 52–82.

Holdich, D. M. & R. S. Lowery. 1988a. *Freshwater Crayfish: Biology, Management and Exploitation*. Croom Helm Ltd., London, 498 p.

Holdich, D. M & R. S. Lowery. 1988b. Crayfish—an introduction. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management, and Exploitation*. London: Croom Helm Ltd., pp. 1–10.

Huner, J. V. 1976. Raising crawfish for fish bait and food: a new polyculture crop with fish. *Fisheries Bull.* 1(2):7–8.

Huner, J. V. 1977. Introductions of *Procambarus clarkii* (Girard)—an update. *Freshwater Crayfish* 3:193–202.

Huner, J. V. 1988a. Crayfish culture in Europe. *Aquaculture Magazine* 14(2):48–52.

Huner, J. V. 1988b. *Procambarus* in North America and elsewhere. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management and Exploitation*. London: Croom Helm Ltd., pp. 239–261.

Huner, J. V. 1988c. Comparison of the morphology and meat yields of red swamp crawfish and white river crawfish. *Crawfish Tales* 7(2):29–30.

Huner, J. V. 1989. Crayfish culture in North America. In J. Skurdal, K. Westman & P. I. Bergan (eds.) *Crayfish Culture in Europe. Report from the Workshop on Crayfish Culture, 16–19 Nov. 1987, Trondheim, Norway*. Lyngaas Form & Data, Lillehammar, Norway, pp. 126–135.

Huner, J. V., R. Gydemo, J. Haug, T. Järvenpää, and T. Taugbæl, 1989. Trade, marketing and economics. In J. Skurdal, K. Westman, & P. I. Bergan (eds.) *Crayfish Culture in Europe. Report from the Workshop on Crayfish Culture, 16–19 Nov. 1987, Trondheim, Norway*. Lyngaas Form & Data, Lillehammar, Norway, pp. 54–62.

Huner, J. V. & J. E. Barr. 1984. *Red Swamp Crawfish: Biology and Exploitation*. (Revised). Baton Rouge, La.: La. Sea Grant College Prog., La. State Univ., 136 p.

Huner, J. V. & O. V. Lindqvist. 1988. Response of a slow growing noble crayfish, *Astacus astacus* L., population from a pond in central Finland to several years of exploitation. *Freshwater Crayfish* 8:00–00.

Huner, J. V., O. V. Lindqvist & H. Könönen. 1988. Comparison of morphology and edible tissues of two important commercial crayfishes, the noble crayfish, *Astacus astacus* Linné, and the red swamp crayfish, *Procambarus clarkii* (Girard) (Decapoda, Astacidae and Cambaridae). *Aquaculture* 68:45–57.

Hutchings, R. 1988a. Many species, only a few with aquaculture potential. *Austasia Aquaculture Magazine* 2(9):3.

Hutchings, R. 1988b. Personal Communication. Freshwater Australian Crayfish Traders, "Leawarra", M.S. 461, Kalbar 4309, Australia.

Kossakowski, J. 1966. 1971. Crayfish. Panstowe Wydownectwo Polnicze: Lesne. Warsaw. Available from U.S. Dept. of Commerce Translation 77-55114 (Translated from Polish), Washington, DC, 163 p.

Laurent, P. J. 1987a. Les enseignements de quarante ans de statistiques douanieres interpretees par un astacologue. 1ere partie. *Aqua Revue* 14:15–19.

Laurent, P. J. 1987b. Les enseignements de quarante ans de statistiques douanieres interpretees par un astacologue. 2eme partie. *Aqua Revue* 15:35–39.

Laurent, P. J. 1987c. I.N.R.A., 75, Av. de Corzent, F-74203 Thonon les Bains Cedex, France.

Laurent, P. J. 1988. *Austropotamobius pallipes* and *A. torrentium*, with observations on their interactions with other species in Europe. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management and Exploitation*. London: Croom Helm Ltd., pp. 341–364.

LCES (Louisiana Cooperative Extension Service). 1986. Louisiana summary, agriculture and natural resources 1986. Louisiana Cooperative Extension Service, Louisiana State University, Baton Rouge, Louisiana, 200 p.

Lowery, R. S. & D. M. Holdich. 1988. *Pacifastacus leniusculus* in North America and Europe with details of the distribution of introduced and native crayfish species in Europe. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management and Exploitation*. London: Croom Helm Ltd. 283–308.

Momot, W. T. 1984. Crayfish production: a reflection of community energetics. *J. Crust. Biol.* 78:55–81.

Momot, W. T. 1988a. *Orconectes* in North America and elsewhere. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management and Exploitation*. London: Croom Helm Ltd., pp. 262–282.

Momot, W. T. 1988b. Personal Communication. Dept. of Biol., Lakehead Univ., Thunder Bay, Ontario P7B 5E1, Canada.

Morrissy, N. M. 1983. Crayfish research and industry activities in Australasia. *Freshwater Crayfish* 5:534–544.

Morrissy, N. M. 1988. Personal Communication. Western Aust. Mar. Res. Lab., Box 20, North Beach, Western Australia 6020, Australia

Morrissy, N. M., N. Hall & N. Caputi. 1986. Appendix 1. Bioeconomic model for semi-intensive pond grow-out of marron (*Cherax tenuimanus*). p. 94–139, Fish Mgmt. Discussion Paper No. 2. The Report of the Fish Farming Legislative Review Committee. Report by the Chairperson Mr. Rogers to the Minister of Fisheries, the Hon. J. F. Grill. Fish. Dept., Perth, Australia, 170 p.

Pomeroy, R. S. & K. H. Kahl. 1987. *Crawfish in South Carolina: development and current status of the industry*. South Carolina Agriculture Experiment Station, Clemson University, Clemson, South Carolina, 14 p.

Olszewski, P. 1980. A Salute to the Humble Yabby. Angus & Robertson Publishers, London, Sydney, Melbourne, Singapore, Manila, 150 p.

Pagel, R. 1988. Personal Communication. 217 W. Liberty, Deerfield, WI 53531.

Roberts, K. J. & C. D. Harper. 1988. Louisiana farm-raised crawfish production statistics, 1981–87. Louisiana Cooperative Extension Service, Louisiana State University, Baton Rouge, Louisiana

Shu, Xinya. 1988. Personal Communication. Hubei Fisheries Res. Inst., No. 18 Dong Hu Road Wuhan, Wuhan City, People's Republic of China

Soileau, L. D., K. C. Smith, R. Huner, C. E. Knight, D. M. Soileau, W. E. Shell, Jr. & D. W. Hayne. 1975. *Atchafalaya Basin Usage Study Final Report, July 1, 1971—June 30, 1974*. U.S. Army Corps of Engineers, New Orleans, Louisiana, 85 p.

Somer, T. R. 1984. The biological response of the crayfish, *Procambarus clarkii*, to transplantations into California rice fields. *Aquaculture* 41:373–384.

Somer, T. R. & C. R. Goldman. 1983. The crayfish *Procambarus clarkii* from California rice fields: ecology, problems, and potential for harvest. *Freshwater Crayfish* 5:418–428.

Söderhäll, K., M. W. Johansson & V. J. Smith. 1988. Internal defence mechanisms. In D. M. Holdich & R. S. Lowery (eds.) *Freshwater Crayfish: Biology, Management and Exploitation*. London: Croom Helm Ltd., pp. 213–238.

Stanford, A. J., J. Kuzhecovs & B. J. Mills. 1987. Economics of commercial aquaculture of the yabbie (*Cherax destructor*). South Aust. Dept. Fish., Adelaide, South Australia, Australia, 59 p.

Unestam, T. 1975. Defence reactions in and susceptibility of Australian and New Guinea freshwater crayfish to European crayfish plague fungus. *Aust. J. Exp. Biol. Med. Sci.* 53:349–357.

Vey, A., K. Söderhäll & R. Ajaxon. 1983. Susceptibility of *Orconectes limosus* Raff. to the crayfish plague *Aphanomyces astaci* Schikora. *Freshwater Crayfish* 5:281–291.

Journal of Shellfish Research, Vol. 8, No. 1, 267–275, 1989.

COMMERCIAL CRAWFISH CULTIVATION PRACTICES: A REVIEW

LAWRENCE W. DE LA BRETONNE, JR.[1] AND ROBERT P. ROMAIRE[2]

[1]*Louisiana Cooperative Extension Service*
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

[2]*School of Forestry, Wildlife, and Fisheries*
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

ABSTRACT Crawfish are cultivated in about 65,000 ha of ponds in the southeastern USA, and Louisiana produces 80% of USA crawfish crop. Crawfishes of the genus *Procambarus* are cultivated in earthen ponds using an extensive production technology that does not use hatcheries or formulated rations, but rather manipulates water levels to simulate conditions in natural production areas that maximize production. Ponds are stocked with broodstock in spring and drained over 2–4 weeks to stimulate burrowing and reproduction. Vegetation, such as rice, is planted in summer as crawfish forage. The ponds are filled with water in September–October and the crawfish are harvesed with baited wire traps from November through May/June. Crawfish are cultivated in wooded, semi-wooded, and permanent ponds, and in agricultural rotations with rice, soybeans, and sorghum. Crawfish yields range from 500–3,000 kg per ha and yield depends on pond size and design, water management, forage type, broodstock structure in spring, and harvesting intensity.

KEY WORDS: crawfish, *Procambarus*, aquaculture, management

INTRODUCTION

For years, most of the crawfishes of the genus *Procambarus* marketed in the USA were harvested from the Atchafalaya River Basin, a natural floodway for the Atchafalaya River-Mississippi River system (Huner and Barr 1984). About 33% of the water from the Mississippi River drainage system is diverted through the Basin which is 24 km wide by 121 km long. Good crawfish production in the Basin occurred in years when the summer was dry, and water levels in fall, winter and spring were high. The Atchafalaya Basin produced crawfish 3 years out of every 5, with one crop traditionally being a "bumper" crop. Crawfish were harvested in the Basin by trappers from March through June. The seasonality of the crawfish crop from the Basin and the year-round demand for crawfish in larger Louisiana cities stimulated development of crawfish aquaculture.

An intensive research program that began in the mid-1960's by the Louisiana Agricultural Experiment Station (Louisiana State University Agricultural Center), the Louisiana Department of Agriculture, and the University of Southwestern Louisiana, developed technology for commercial cultivation of crawfish. The research base and a strong technology transfer program by the Louisiana Cooperative Extension Service have led to the development of a crawfish aquaculture industry in Louisiana that is the largest in the USA, with about 55,000 ha in production (Roberts and Dellenbarger 1989). Other states that culture cambarid crawfish are Arkansas (200 ha), Alabama (<50 ha), Florida (800 ha), Georgia (<100 ha), Maryland (<50 ha), Mississippi (100 ha), North Carolina (<50 ha), South Carolina (500 ha), and Texas (7,300 ha).

Crawfish harvest in Louisiana averages about 50,000 metric tons (MT) per year from both the natural fishery and the aquaculture industry (Huner 1989). In 1987 crawfish captured from ponds comprised about 60% of total harvest, but the harvest fluctuates depending on production from the Basin. Pond cultivation of crawfish has extended the availability of crawfish from November through June and new research developments in "off-season" crawfish aquaculture should make crawfish available year-round (Romaire and de la Bretonne 1988). The species of crawfish of commercial importance in the southeast are the red swamp crawfish, *Procambarus clarkii*, and the white river crawfish, *Procambarus acutus acutus*, which comprise 90% and 10% of the catch, respectively (Avault and Huner 1985).

Cultivation technology and intensity used in cambarid crawfish cultivation is low compared to many other cultivated aquatic animals such as rainbow trout, channel catfish, and marine shrimp. No hatcheries are used to produce young crawfish for stocking ponds nor are formulated rations used to feed crawfish. Rather, young are produced by brood stock contained within the pond and vegetation is utilized as a forage for the crawfish. This article reviews cultivation practices used in the southeastern USA, principally Louisiana, for production of the red swamp crawfish and white river crawfish.

Approved by the Director of the Louisiana Agricultural Experiment Station as manuscript number 89-22-3274.

SITE LOCATION AND POND CONSTRUCTION

Site location and pond construction are important in successful crawfish aquaculture. Crawfish ponds should be located in flat, open areas and the soils should have sufficient clay to hold water. Clay loams, sandy clay, sandy clay loam, and silty clay loams are satisfactory (Coche 1985). Soils with high clay content are not good for cultivation of many agricultural crops because of poor drainage and susceptibility to flooding, but they are excellent for crawfish. Sandy soils are not conducive to crawfish production.

Perimeter levees (embankments) should have a core trench filled with clay to prevent water seepage, and the minimum perimeter levee base should be 3 m wide to prevent leakage from the burrowing activities of the crawfish. A levee system 1 m high is adequate to contain the 0.6–0.8 m deep water necessary to cultivate *Procambarus*. The land should have no more than a 15 cm slope between perimeter levees; otherwise, the area should be leveled or divided into two or more ponds. Interior baffle levees, 0.7–1 m high with a 1–3 m base, should be spaced at 50–100 m intervals to facilitate water circulation (Figure 1). A recirculation canal, external to the perimeter levee, and a re-lift pump is recommended to aid in water circulation and to minimize water usage (Baker 1987). Ponds designed to recirculate water are important in areas where water is scarce or where subsurface water must be pumped from great depths at high expense.

Drains should be matched with the pond size, pumping capacity, and projected rainfall. Two 25-cm water outlets are sufficient to drain a pond 10 ha in size, containing 50,000 m^3 of water. Crawfish ponds should be isolated from crops that require frequent use of insecticides (Romaire 1984, Jarboe 1988). Construction of crawfish ponds and factors to consider in site location are discussed by Craft (1980).

TYPES OF CRAWFISH PONDS

Crawfish ponds are generally categorized as follows: wooded, semi-wooded, and open ponds (Huner and Barr 1984). Open ponds are further categorized as being ricefield ponds, permanent ponds, and marsh ponds.

Wooded Ponds. The earliest type of pond used for crawfish cultivation in Louisiana was the wooded pond. These ponds are built in forested areas on heavy clay soils near drainage canals that are filled with precipitation from surface runoff. Wooded ponds produce 50–900 kg of crawfish per ha per year (Table 1). Production is limited by the inability to manage water effectively because these ponds

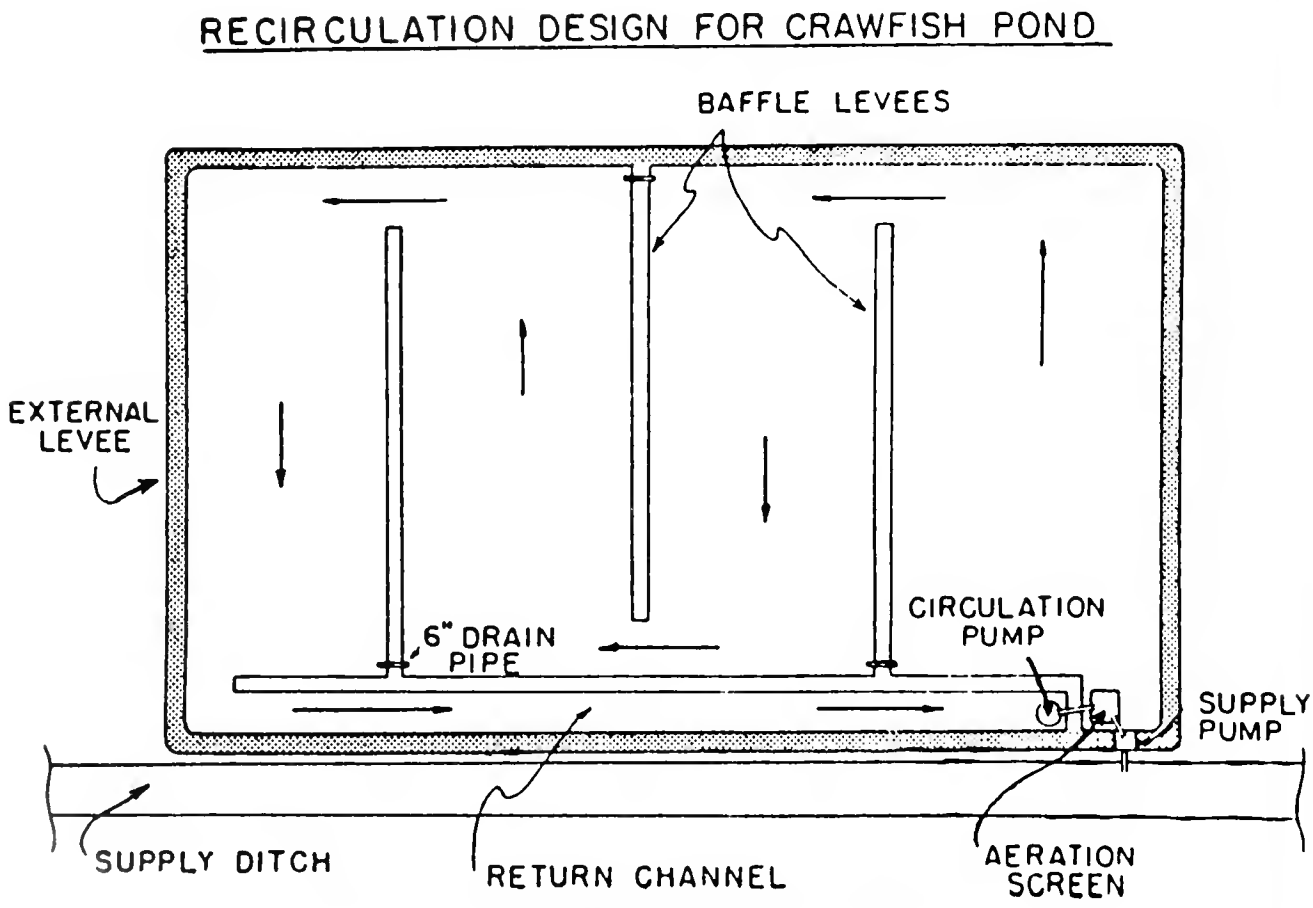


Figure 1. Recirculation design for crawfish pond (source: Baker 1987).

TABLE 1.

Types of crawfish ponds and production characteristics.

Type	Size (ha)	Yield (kg/ha)	Water Source
Wooded	20–120	560 (50–900)[1]	Surface
Semi-Wooded	20–120	670 (225–1,100)	Surface
Open			
Permanent	8–40	1,200 (340–3,240)	Surface/Subsurface
Ricefield	6–16	1,000 (450–2,800)	Subsurface
Marsh	20–80	450 (200–600)	Surface

[1] Mean Yield with Range in parentheses.

have internal borrow ditches that channel water directly to the drains thus impeding water circulation. Wooded ponds have poor stands of vegetative forage from shading, and leaf litter provides a significant amount of forage of the crawfish (Sanguanruang 1988). Crawfish harvest is difficult because the trees hinder movement of boats, so tree-free lanes are often cut to provide trapping areas.

Semi-wooded Ponds. Wooded ponds after several years of alternate flooding and drying have high mortality of hardwoods, thus reducing tree density and generally improving crawfish habitat. A vegetative forage base of terrestrial grasses and aquatic plants is developed which requires better water circulation and more intense water management. Properly managed semi-wooded ponds produce 15–30% more crawfish wooded ponds, but poorly managed semi-wooded ponds produce fewer crawfish than wooded ponds because water quality is often poorer. Semi-wooded and wooded ponds are large, generally 20–120 ha in surface area, and about one-third of the area in crawfish cultivation in Louisiana are wooded ponds, but few of these ponds are currently built.

Open Ponds. Open ponds, those that contain few or no trees, are recommended for crawfish aquaculture. Open ponds, which account for 65–70% of the production area in Louisiana and 100% in other states, range from 8–40 ha in size. Ricefield crawfish ponds are productive open ponds because they often have baffle levees, a good water supply, recirculation capability, and they are located on fertile soils. Crawfish yield from ricefield crawfish ponds is generally 1,000 kg per ha, with the better managed ponds routinely producing 1,400–2,200 kg of crawfish per ha (Table 1). Crawfish are commonly double-cropped with rice in Louisiana and Texas.

Permanent crawfish ponds are those constructed solely for the purpose of cultivating crawfish. Crawfish can be harvested in permanent ponds 1 or 2 months longer because there is no conflict with planting, draining and harvest schedules of other crops. Crawfish yield from permanent, open ponds is generally 10–30% higher than ricefield ponds in which rice and crawfish are double-cropped (Table 1). Marsh ponds, which are constructed in coastal areas, have low crawfish yields (200–600 kg per ha) because the soils are high in organic matter and water quality can not be maintained at levels conducive to good crawfish production (Table 1).

WATER SUPPLY

Water quantity and water quality are the most common limiting factor in crawfish aquaculture. A pumping capacity of 1.2 m^3 per min per ha (100 gallons per min per acre) is needed to exchange (turnover) water 0.6 m deep in 3.5 days. This exchange rate is essential to maintain satisfactory water quality in the fall and late spring when water temperature is highest. Crawfish producers can reduce pumping capacity to 0.72–0.84 m^3 per min per ha (60–70 gallons per min per acre) if the pond is filled to half the normal depth in late fall and the pond is flushed regularly with fresh, oxygenated water. A properly managed open crawfish pond will generally require nine water exchanges per production season to maintain acceptable water quality (Baker 1987). An aeration screen is used to oxygenate water as it is pumped into the pond.

Both subsurface and surface water are acceptable for crawfish cultivation. Subsurface water from wells provides predator- and disease-free water but wells have limited discharge capacity and greater expense in pumping. Subsurface water often contains soluble iron and hydrogen sulfide that must be removed before water enters the pond. Water recirculation systems are usually recommended for ponds that must rely on subsurface water. Surface water is desirable for large ponds because it is less expensive to pump, but it may not be reliable in quantity and quality, and it generally contains predaceous fishes that must be removed. Mechanical paddlewheel aerators, 0.6 to 1.2 HP per ha, can be used to aerate and circulate water in crawfish ponds more cost effectively than water replacement (Hymel 1988). Pond location and energy costs dictate the type of pump and power source used for crawfish ponds (Baker 1987).

WATER QUALITY

Over 99% of production problems in crawfish ponds can be directly attributed to improper water management (Avault et al. 1975). A good water quality management program requires that crawfish ponds be properly designed and constructed, and have a dependable supply of surface or subsurface freshwater. Inadequate concentration of dissolved oxygen (DO) is the most serious water management problem in crawfish aquaculture (Hymel 1987). Low concentration of DO results in high crawfish mortality; moreover, crawfish do not feed or grow well and can become predisposed to diseases in ponds with chronically low concentrations of DO.

Most serious water quality problems occur 2–6 weeks after flooding ponds, October–November, and from

April–June because the warm water (>20°C) in these months increases the biological oxygen demand (BOD) of inundated vegetation and decreases DO (Romaire and Hymel 1989). Dissolved oxygen should be maintained above 2 mg/liter for optimal crawfish production and significant mortality occurs when DO is less than 1 mg/liter (Melacon and Avault 1977). Dissolved oxygen should be measured daily when water temperature exceeds 20°C. Oxygen deficiency is corrected by replacing pond water with fresh, oxygenated water, or by recirculating the water with pumps or mechanical aerators.

Other water quality variables important in crawfish production are the pH, total hardness and total alkalinity, ammonia, nitrite, iron and hydrogen sulfide. The water pH should range from 6.5–7.5 at dawn, and both total hardness and total alkalinity should range between 50–250 mg/liter as $CaCO_3$ but 100 mg/liter is optimum (de la Bretonne et al. 1969). If the pH, hardness and alkalinity are low, agricultural limestone should be incorporated into the pond bottom (Boyd 1979, 1982). Un-ionized ammonia and nitrite are toxic to *Procambarus* at concentrations exceeding 2 and 4 mg/liter of N, respectively (Hymel 1985) but concentrations this high are not likely to occur in crawfish ponds because the crawfish production intensity is low and ammonia is rapidly assimilated by aquatic macrophytes (Romaire and Hymel 1989). Iron and hydrogen sulfide are toxic to crawfish at concentrations often found in subsurface well water; however, the two compounds are oxidized to non-harmful concentrations when well water is oxygenated prior to entering the pond.

CRAWFISH LIFE CYCLE

There are at least 32 described species of crawfishes in Louisiana but only *P. clarkii* and *P. acutus acutus* are cultivated. The red swamp crawfish is preferred over the white river crawfish because it produces more consistent yields and it is more valued in international and southern Louisiana markets. Unlike other cultured aquatic animals that require hatcheries to produce young for stocking, cambarid crawfish aquaculture as currently practiced relies on control of the hydrology of ponds to simulate optimal conditions that occur in the species natural habitat (de la Bretonne and Fowler 1976).

Mature *P. clarkii* and *P. acutus acutus* mate in open water in all months but mating peaks in May and June (Penn 1943). The female stores the spermatophore in a seminal receptacle for 2 to 8 months until spawning (Huner and Barr 1984). After mating, the female burrows into the levee, 10–15 cm above the water level. The burrow extends in depth to the water table, generally 1–1.2 m in Louisiana (Jaspers and Avault 1969). The burrow is capped with soil to maintain a humid environment. A male may occupy a burrow with the female. Crawfish ponds are slowly drained over 2–4 weeks in May and June to stimulate burrowing and reproductive activities of remaining crawfish population.

After an ovarian development period of 2–5 months (Penn 1943, Suko 1956, 1958) and while crawfish are in burrows, oocytes (eggs) are extruded through the oviducts, fertilized, and attached to the pleopods. On average about 300 eggs are extruded by females that are 85 mm total length (TL) with a range of 100–700 eggs depending on the size of the female (Penn 1943). The female repeatedly dips the eggs in water in the burrow chamber to keep them moist (Jaspers and Avault 1969). The eggs hatch in 2–3 weeks at 23–27C and can take as long as 3–4 months for eggs to hatch at lower temperatures (Suko 1956, 1958).

Crawfish ponds are filled in the fall to coincide with peak spawning of females in burrows (de la Bretonne and Avault 1977). When burrows are filled with water adults and young-of-the year (YOY) leave the burrow, and distribute themselves throughout the pond.

When crawfish ponds are initially stocked with brood crawfish in spring, ovaries of females should be checked to determine stage of maturity. Females with advanced oocyte development (tan to brown eggs) in May or June will spawn in August–September and females with yellow eggs will spawn in October–December (Romaire and Lutz 1989). Females with white eggs or undeveloped ovaries are immature and they do not spawn until March and April.

Young-of-the-year grow rapidly and can obtain harvestable size, 65 mm TL or larger (10 g or larger), in 2–3 months if water temperature is 26–30 C and other environmental conditions are optimal (Avault and Huner 1985). Crawfish hatched in late fall or mid-winter require 4–5 months to attain harvestable size. *P. clarkii* and *P. acutus acutus* have a natural life span of no more than 2 or 3 years in southern Louisiana (Huner and Barr 1984).

CRAWFISH PRODUCTION SYSTEMS

Crawfish are amenable to culture because they are hardy, the life cycle can be easily manipulated to fit a variety of cultural situations, and they can be easily integrated into agricultural crop rotations. The most common crawfish-agronomic crop rotations are rice-crawfish-rice, rice-crawfish-soybean, and crawfish-rice set-aside. The various crawfish culture cycles are as follows:

Rice-Crawfish-Rice

March–April—Plant rice
June—At permanent flood (rice 20- to 25-cm high) stock 50–60 kg of adult crawfish per ha
August—Drain pond and harvest rice
October—Reflood rice field
November–April—Harvest crawfish
April—Replant rice.

The rice-crawfish-rice rotation has been practiced for years (Chein and Avault 1978). Problems include pesticide use, poor water circulation, and a shorter crawfish harvest period. About 8,000–15,000 ha of crawfish are cultured in this rotation in Louisiana.

Rice-Crawfish-Soybeans

March–April—Plant rice
June—Stock 50–60 kg adult crawfish per ha at permanent flood
August—Drain field and harvest rice
October—Reflood rice field
November–May—Harvest crawfish
Late May–June—Plant soybeans
October–November—Harvest soybeans
November–March—Reflood pond and harvest crawfish or leave pond dry
March–April—Plant rice.

The rice-crawfish-soybean rotation allows for the production of three crops in two years, and has the additional advantage of a longer crawfish harvest season than the rice-crawfish-rice rotation. Pesticide use is also an important management consideration in this rotation.

Crawfish-Rice Set-Aside

April–May—Flood pond and stock 50–60 kg per ha of crawfish
May—Drain pond over 2–4 weeks
August—Plant rice according to Agricultural Stabilization and Conversion Service (ASCS) guidelines
October—Reflood pond
January–May—Harvest crawfish (according ASCS regulations)
May—Drain pond over a 2–3 week period.

In this rotation rice grain can not be legally harvested. The crawfish rice set-aside program allows rice farmers to use idle land registered in the federal rice set-aside program to cultivate crawfish. Restrictions on rice planting dates, crawfish harvest, and pond draining are regulated by the ASCS.

Permanent Crawfish Ponds

April–May—Stock 50–60 kg of adult crawfish per ha
May–June—Drain pond over 2–4 weeks
June–August—Plant rice, sorghum, or other vegetation as crawfish forage
October—Reflood pond
November–May/June—Harvest crawfish
May/June—Drain pond, and repeat cycle.

About 65–70% of the crawfish production area in Louisiana is permanent crawfish ponds. The permanent culture system allows producers to design the system for optimal crawfish production with no concerns regarding planting date requirements, and pesticide use for agricultural crops.

STOCKING BROOD CRAWFISH

Brood crawfish should be stocked in April–May. Mature crawfish, harvested from another crawfish pool, should be stocked within 2–3 hours after capture. Crawfish stored in a cooler should not be used. The majority of the crawfish should be *P. clarkii* and with a sex ratio of 1:1. At least 20% of the females should have tan to brown eggs in the ovary. About 50–60 kg per ha should be stocked in areas with established crawfish culture, and 70–80 kg per ha in new or recently established crawfish production areas.

Brood crawfish should be transported to the pond in a covered vehicle so as to not expose crawfish to wind and sun. Crawfish should be stocked throughout the pond in water 35–60 cm deep adjacent to baffle levees or perimeter levees. The water should be drained slowly over 3–4 weeks to stimulate burrowing by crawfish. Because of the inefficiency of the harvesting process there is usually a sufficient amount of mature crawfish after the first production season to supply YOY for the following production season; thus, restocking the pond is generally not necessary. However, crawfish producers should sample female crawfish prior to draining the ponds in May or June to insure enough females have advanced ovarian development to supply YOY in the next production season.

FORAGES

Crawfish are benthic omnivores and they rely on aquatic flora, fauna, and detritus for their energy needs (Sanguanrguang 1988). Crawfish are not fed formulated rations as are other cultured aquatic animals; rather, vegetation is either allowed to become established naturally in the summer months when the pond is dry or selected agronomic crops are planted as forage for the crawfish. Vegetation is the base of the detrital food web on which crawfish rely to satisfy their nutrient requirements.

Volunteer terrestrial grasses do not supply sufficient forage to support high levels of crawfish production (Brunson 1987a). Water quality is also poorer in ponds with large amounts of terrestrial vegetation (Romaire and Hymel 1989). Aquatic and semi-aquatic plants such as alligatorweed (*Alternathera philoxeroides*) and smartweed (*Polygonium* spp.) are superior to terrestrial grasses because they do not deteriorate water quality. However, like terrestrial grasses, alligatorweed and smartweed do not supply sufficient food to sustain good crawfish growth and high yields (Garces and Avault 1985). Additionally, aquatic plants can become so dense that they interfere with water circulation and crawfish harvest. Millets (*Echinochloa*

spp.) are sometimes used as a cultivated forage for crawfish but millets lodge soon after ponds are flooded which increases the severity of oxygen deficiency (Miltner and Avault 1981, Brunson 1987b). Millet, though easy and inexpensive to plant, is not recommended as a crawfish forage.

The preferred forage to plant for crawfish is rice, *Oryza sativa* (Brunson 1987b, Brunson et al. 1988). Rice is semiaquatic and it has less negative impact on water quality compared to terrestrial plants (Romaire and Hymel 1989). Rice can be planted for grain production with the post-harvest residue serving as crawfish forage or it can be planted solely as a crawfish forage. Rice as forage is normally planted from June–August at a seeding rate of 76–90 kg per ha. Procedures for planting rice as forage for crawfish including soil preparation, planting techniques, water management, recommended rice varieties, and fertilization are detailed in Linscombe (1985), Brunson et al. (1988) and Brunson (1989). Factors considered in rice variety selection include culture system (double-cropping), rice biomass, lodging characteristics, and rice regrowth (ratoon) potential. Some of the recommended rice varieties are Mars, Starbonnet, Newbonnet, Labelle, and Lemont.

Crawfish are highly susceptible to pesticides used to control insects in rice production (Romaire 1984, Jarboe 1988). Crawfish producers must either avoid the use of pesticides or apply them when crawfish are not exposed, for example, when crawfish are in burrows. Another problem with rice as a forage is that it is often depleted by late March or April in ponds with a large crawfish population. Forage depletion causes a cessation in crawfish growth and results in crawfish "stunting" at non-desirable market size.

Sorghum sudan grass hybrids may have good potential as forage for crawfish (Brunson and Taylor 1987). Sorghum hybrids produce large quantities of forage biomass and are less expensive to plant than rice. They also do not appear to significantly deteriorate water quality.

Crawfish are not fed formulated rations on a large-scale in the crawfish aquaculture industry (Romaire 1989a). Experimental studies are inconclusive as to whether or not it is economically feasible to feed crawfish, but it is unlikely to be feasible with current culture techniques without a concomitant decrease in other production costs. Formulated rations have the potential to increase crawfish growth and production, minimize or prevent crawfish stunting at submarketable sizes when vegetation is depleted, and extend the crawfish season into the summer for "off-season" production (Romaire and de la Bretonne 1988).

CRAWFISH POPULATION DYNAMICS

Although cambarid crawfish are relatively easy to culture, the dynamics of populations in ponds is complex. High crawfish yield is dependent on having multiple recruitment classes of crawfish during the September/October–May/June production season. A population of both mature females with various stages of ovarian development and immature females should be present in the pond prior to draining in May–June. This will insure that 5–8 recruitment classes will be hatched from October through March of the next production season, thereby maintaining a population of harvestable crawfish from late November through May. Although *P. clarkii* spawn in all months in which the pond is flooded, there is a primary peak of hatching in fall, and lesser, secondary peaks in mid-winter and spring (de la Bretonne and Avault 1977, Huner 1978, Romaire and Lutz 1989). *Procambarus acutus acutus* in culture spawn in fall and winter only (Romaire and Lutz 1989).

Mature females that have orange, tan, and brown eggs in May–June produce three to five recruitment classes of YOY over a two month period after the pond is flooded in September–October. If adequate environmental conditions are maintained in the fall, many of these YOY are marketable by late November–December ("early crop") and can be harvested with holdover adults from the preceding season. Poor water quality management in the fall often kills many YOY resulting in a low crawfish harvest in fall and winter when crawfish prices are highest.

Females with yellow eggs in May–June re-burrow 4–8 weeks after the pond is flooded, and one to two recruitment classes from these females are hatched in November–January. These mid-winter recruitment classes attain market size in late March through May (late crop). Large crawfish that were immature in May–June, mature after flooding, mate, and re-burrow in January–February. These adults produce one or two recruitment classes in March and April but the YOY do not attain market size before the ponds are drained in May or June. The pond can remain flooded through summer to harvest this YOY recruitment class but it is seldom done because by May forage is generally not adequate to sustain acceptable crawfish growth.

Crawfish should not be intensively harvested in October or November because a significant portion of the catch may consist of holdover adults that produce the mid-winter YOY recruitment classes. Harvest should be minimal until these holdover adults have burrowed. The population dynamics cycle of *Procambarus* in culture is depicted in Fig. 2.

Recruitment of YOY crawfish is monitored by pulling a dip net (6-mm mesh) along the pond bottom in various locations around the pond. As a general rule, the relationship between mean number of crawfish caught per dip 6–8 weeks post-flooding and the potential crawfish yield is as follows: 0–1 per dip, 500–600 kg per ha; 3–5 crawfish per dip, 1,000–1,500 kg per ha; and 8–20 crawfish per dip, 2,000 kg per ha or more.

Because the number of brood crawfish in ponds at draining is not controlled the number of YOY in the ponds

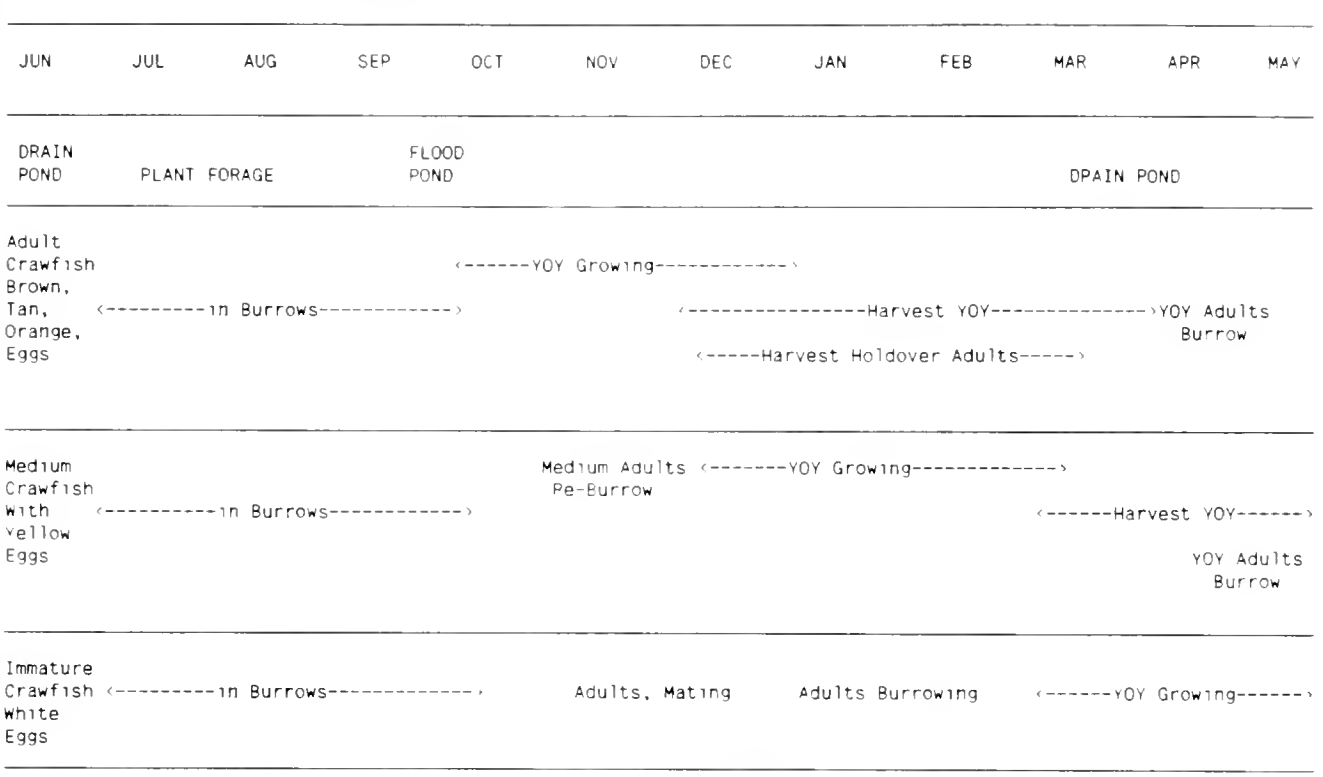


Figure 2. Crawfish population structure throughout the growing season [in a well-managed crawfish pond].

TABLE 2.

Estimated investment requirements and annual depreciation charges for 16-ha crawfish pond in southwestern Louisiana, 1987 (source: Dellenbarger et al. 1987).

Item	Investment/ha $USD	Depreciation/ha $USD
Pond Construction		
Dirt Moving	$ 387	
Water Control Structures	91	
Ground Cover	21	
Total Construction Cost	499	
Equipment		
Broodstock	51	5.12
Well	688	34.38
Oxygen Meter	38	9.38
Crawfish Combine	312	31.25
Truck	563	93.75
Traps	387	129.00
Cooler	75	14.94
Scale	6	1.19
Aerator	27	2.63
Mower	44	14.56
Waders	15	7.00
Pump	608	40.56
Engine-Gearhead	786	52.38
Total Equipment Cost	3,600	436.14
Total	4,099	436.14

after flooding in the fall is variable, with densities from 3–25 per m^2 (Romaire and Lutz 1989). Crawfish growth is 6–12 mm per week at 20–30°C, if food is not limited (Romaire and Lutz 1989). Growth declines to 1–3 mm per week during mid-winter when water temperature is less than 10°C. Crawfish mortality in the various recruitment

TABLE 3.

Estimated annual operating costs ($USD/ha) associated with a 16-ha crawfish pond in southwestern Louisiana, 1987 (source: Dellenbarger et al. 1987).

Variable Costs	
Forage	$ 102
Fuel—Well	115
Repairs and Maintenance	66
Labor ($5/h)	182
Herbicides	10
Sacks	10
Bait ($0.35/kg)	365
Total Variable Costs	850
Fixed Costs	
Depreciation	436
Interest (12%)	225
Total Fixed Cost	661
Total Annual Costs/ha	1,511

TABLE 4.

Estimated breakeven prices ($USD) associated with a 16-ha crawfish pond in southwestern Louisiana, 1987 (source: Dellenbarger et al. 1987).

Yield/ha (kg)	800	1,000	1,200	1,450	1,700
Total Yield (kg)	12,800	16,000	19,200	23,200	27,200
Break-even ($/kg)					
Variable	1.17	0.90	0.75	0.64	0.55
Fixed	0.90	0.70	0.57	0.48	0.42
Total	2.07	1.60	1.32	1.12	0.97

classes ranges from 2–6% per week (Romaire and Lutz 1989). Major sources of crawfish mortality are poor water quality (Romaire and Hymel 1989), predation by fishes (Huner and Barr 1984), and cannibalism following ecdysis (Romaire and Lutz 1989).

HARVESTING

Harvesting crawfish is labor intensive and accounts for 60–80% of production costs. Crawfish are generally harvested 120–180 days per production season (November–June) although if there is a poor fall and winter crawfish crop then harvest may be restricted to March–May (60–90 days). In well-managed ponds about one-third of the crawfish are harvested from November–February, one-third from March–April, and the remainder in May.

Crawfish are captured in traps constructed from 1.9-cm mesh wire, and baited with 100–150 g of either fish (cupleids, catastomids, cyprinids are most common), formulated bait, or a combination of both (Romaire and Osorio 1986). Traps are set at a density of 50–100 traps per ha, and they are baited and emptied 4–6 days per week depending on the catch, price structure for crawfish, and market demand (Romaire and Pfister 1983). More than 25 different traps designs are used but the most effective traps have two or three entrance funnels, are made from PVC-coated wire, have retainer bands or collars to minimize crawfish escape, and are set up-right in the water column (''stand-up'' traps) (Romaire and Pfister 1983, Romaire 1987, 1988). Daily crawfish catch is cyclic and it is influenced by many factors including water temperature, water quality, weather, forage type and forage quantity, crawfish growth and recruitment patterns, trap design, baits, and harvesting intensity (Romaire 1989b).

Crawfish can be effectively harvested in ponds less than 8 ha in area by one or more persons who ''walk'' the pond while pulling a small boat into which harvested crawfish are placed. About 400 traps per person per day can emptied using this technique. Crawfish harvesting boats that are powered by air-cooled engines increase harvesting efficiency by allowing harvest personnel to empty and re-bait 200–300 traps per hour. Harvest boats are indispensable for efficient crawfish harvest in ponds larger than 8 ha. Crawfish harvest techniques are reviewed by Romaire (1989b).

As crawfish are harvested they are placed into ventilated mesh bags or sacks that can hold 16–20 kg of crawfish. All debris such as vegetation and bait residue are removed before crawfish are placed in the sacks. The ''sacked'' crawfish are placed in a high humidity cooler within 2–3 hours of harvest, and if handled properly the crawfish can be stored alive for several days until they are resold or further processed (Moody 1989).

ECONOMICS OF CRAWFISH AQUACULTURE

The estimated investment requirements, depreciation charges, estimated annual operating cost, and breakeven price for a 16-ha crawfish pond supplied with well water in southwest Louisiana is presented in Tables 2–4. Pond construction cost are about $500 per ha and annual depreciation on equipment purchases is approximately $436 per ha. Bait ($365 per ha) purchases to harvest crawfish accounts for 43% of annual variable costs, and labor ($182 per ha), 22%. Most of the expense in labor is associated with harvest. Cost during the harvest season accounts for about 75% of annualized variable costs. The estimated breakeven prices when only variable costs are considered range from $1.17 to $0.55 per kg at crawfish harvest levels of 800 and 1,700 kg per ha, respectively. The breakeven prices are about doubled when annualized fixed costs are included in the estimates. Details on estimated investment requirements, production costs, and breakeven prices for crawfish aquaculture in Louisiana are detailed in Roberts (1984) and Dellenbarger et al. (1987).

LITERATURE CITED

Avault, J. W., Jr. & J. V. Huner. 1985. Crawfish culture in the United States. In J. V. Huner and E. E. Brown (eds.) *Crustacean and mollusk aquaculture in the United States*. AVI Publishing Co., Inc., Westport, CN Pp. 1–54

Avault, J. W., Jr., L. W. de la Bretonne, & J. V. Huner. 1975. Two major problems in culturing crayfish in ponds: oxygen depletion and overcrowding. In J. W. Avault (ed.) *Proc. 2nd Internat. Symp. Freshwater Crayfish*, Baton Rouge, LA. Pp. 139–148.

Baker, F. 1987. Pumps and pumping efficiency. In R. Reigh (ed.) *Proc. 1st Louisiana Aquacult. Conf.*, Louisiana Coop. Ext. Serv., Baton Rouge, LA. Pp. 70–73.

Boyd, C. E. 1979. Water quality in warmwater fish ponds. Alabama Agricult. Exp. Sta., Auburn Univ., Auburn, AL.

Boyd, C. E. 1982. Water quality management for pond fish culture. Developments in aquaculture and fisheries science, Volume 9, Elsevier Sci. Publ. Co., Amsterdam, The Netherlands.

Brunson, M. 1987a. Pre-flood evaluation of seven grasses (Graminae L.) as planted forage for crawfish. *J. World Aquacult. Soc.* 18:186–189

Brunson, M. 1987b. Crawfish forages: an overview. In R. Reigh (ed.) *Proc. 1st Louisiana Aquacult. Conf.*, Louisiana Coop. Ext. Serv., Baton Rouge, LA. Pp. 77–81.

Brunson, M. 1989. Forage and feeding systems for commercial crawfish culture. *J. Shellfish Res.*, 8:277–280.

Brunson, M. & R. Taylor. 1987. Evaluation of three sorghum as potential alternative forages for crawfish culture. *J. World Aquacult. Soc.* 18:247–252.

Brunson, M., R. Taylor, & B. J. Hoff. 1988. Characteristics of rice cultivars with implication for crawfish double cropping. *J. World Aquacult. Soc.* 19:8–13.

Chien, Y. H. & J. W. Avault, Jr. 1978. Double cropping rice *Oryza sativa* and red swamp crawfish *Procambarus clarkii*. In P. J. Laurent (ed.) *Proc. 4th Internat. Crayfish Symp.*, Thonon-les-Bains, France. Pp. 263–276.

Craft, B. R. 1980. Some basic considerations in crawfish pond construction. First National Crawfish Culture Workshop, Univ. Southwestern Louisiana, March 3–4, Lafayette, LA., 15 p.

Coche, A. G. 1985. Soil and freshwater fish culture. FAO Training Series Manual 6, Food and Agricultural Organization of the United Nations, Rome, Italy.

de la Bretonne, L., Jr. & J. W. Avault, Jr. 1977. Egg development and management of *Procambarus clarkii* (Girard) in a south Louisiana commercial crayfish pond. In O. V. Lindqvist (ed.) *Proc. 3rd Internat. Freshwater Crayfish Symp.*, Univ. of Kuopio, Kuopio, Finland. Pp. 133–140.

de la Bretonne, L., Jr., J. W. Avault, Jr., & R. O. Smitherman. 1969. Effects of soil and water hardness on survival and growth of the red swamp crawfish, *Procambarus clarkii*, in plastic pools. *Proc. Southeastern Assoc. Game and Fish Comm.* 23:263–266.

de la Bretonne, L., Jr. & J. F. Fowler, 1976. The Louisiana crawfish industry—its problems and solutions. *Proc. Southeastern Assoc. Game and Fish Comm.* 30:251–256.

Garces, C. & J. W. Avault, Jr. 1985. Evaluation of rice (*Oryza sativa*), volunteer vegetation, and alligatorweed (*Alternathera philoxeroides*) in various combinations as crawfish (*Procambarus clarkii*) forages. *Aquaculture* 44:177–176.

Huner, J. V. 1978. Crawfish population dynamics as they affect production in several small, open ponds in Louisiana. *Proc. World Maricult. Soc.* 9:610–640.

Huner, J. V. & J. E. Barr. 1984. Red swamp crawfish: biology and exploitation. Sea Grant No. LSU-T-80-001, LSU Center for Wetland Resources, Baton Rouge, LA.

Huner, J. V. 1989. Overview of international and domestic freshwater crawfish production. *J. Shellfish Res.*, 8:259–265.

Hymel, T. M. 1987. Water quality in crawfish ponds. In R. Reigh (ed.) *Proc. 1st Louisiana Aquaculture Conf.*, Louisiana Coop. Ext. Serv., Baton Rouge, LA. Pp. 74–76.

Hymel, T. M. 1988. Paddlewheel aerator update. *Crawfish Tales* 7(4):22–24.

Jarboe, H. H. 1988. The toxicity of pesticides to crawfish. *Crawfish Tales* 7(4):25–29.

Jaspers, M. & J. W. Avault, Jr. 1969. Environmental conditions in burrows and ponds of the red swamp crawfish, *Procambarus clarkii* (Girard), near Baton Rouge, Louisiana. *Proc. Southeastern Assoc. Game and Fish Comm.* 23:634–647.

Linscombe, S. 1985. Forage for crawfish-rice cultural practices. Louisiana Coop. Ext. Serv., Louisiana State Univ. Agricult Center, Baton Rouge, LA.

Melancon, E. & J. W. Avault, Jr. 1977. Oxygen tolerance of juvenile red swamp crawfish *Procambarus clarkii*. In O. V. Lindqvist (ed.) *Proc. 3rd Internat. Freshwater Crayfish Symp.*, Univ. of Kuopio, Kuopio, Finland. Pp. 371–380.

Miltner, R. M. & J. W. Avault, Jr. 1981. An evaluation of rice, *Oryza sativa*, and Japanese millet, *Echinochloa frumentacea*, as forage for red swamp crawfish, *Procambarus clarkii*. *Louisiana Agriculture* 24(3):8–10.

Moody, M. 1989. Crawfish processing: a review. *J. Shellfish Res.*, 8:293–301.

Penn, G. H. 1943. A study of the life history of the Louisiana red crawfish, *Cambarus clarkii* Girard. *Ecology* 24:1–17.

Roberts, K. & L. Dellenbarger. 1989. Louisiana crawfish product markets and marketing. *J. Shellfish Res.*, 8:303–307.

Romaire, R. P. 1984. Pesticides and crawfish production. *Crawfish Tales* 3(4):10–12.

Romaire, R. P. 1987. Crawfish harvesting. In R. Reigh (ed.) *Proc. 1st Louisiana Aquacult. Conf.*, Baton Rouge, LA. Pp. 82–87.

Romaire, R. P. 1988. Traps designs and their catchability. *Crawfish Tales* 7(1):35–37.

Romaire, R. P. 1989a. Use of formulated feeds in crawfish culture. *Crawfish Tales*, in press.

Romaire, R. P. 1989b. Overview of harvest technology used in commercial crawfish aquaculture. *J. Shellfish Res.* 8:281–286.

Romaire, R. P. & L. de la Bretonne, Jr. 1988. Off-season crawfish production. *Crawfish Tales* 7(4):36–40.

Romaire, R. P. & T. Hymel. 1989. Water quality dynamics in commercial crawfish ponds., unpublished manuscript.

Romaire, R. P. & C. H. Lutz. 1989. Population dynamics of red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus acutus acutus*), in commercial ponds. *Aquaculture*, in press.

Romaire, R. P. & V. Pfister. 1983. Effects of trap density and diel harvesting frequency on catch of crawfish. *North Am. J. Fish. Manag.* 3:419–424.

Romaire, R. P. & V. H. Osorio. 1986. Evaluation of manufactured crawfish baits. *Crawfish Tales* 5(4):24–29.

Sanguanruang, M. 1988. Bioenergetics of red swamp crawfish (*Procambarus clarkii*) and white river crawfish (*Procambarus acutus acutus*) in cultivated, noncultivated and wooded ponds in south Louisiana. Ph.D. Dissertation, Louisiana State Univ., Baton Rouge, LA.

Suko, T. 1956. Studies on the development of the crayfish. IV. The development of winter eggs. Science Repts. Saitama Univ., Vol II, No 2.

Suko, T. 1958. Studies on the development of the crayfish. VI. The reproductive cycle. Science Rept. Saitama Univ., Series B. Vol III, No. 1.

Journal of Shellfish Research, Vol. 8, No. 1, 277–280, 1989.

FORAGE AND FEEDING SYSTEMS FOR COMMERCIAL CRAWFISH CULTURE

MARTIN W. BRUNSON[1]
Rice Research Station
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Crowley, LA 70527-1429

ABSTRACT Commercially produced crawfish are not typically fed formulated rations, but organic substrates supporting rich microbial communities are provided for them to forage upon various components of a detrital food web. Crawfish readily accept a prepared feed, but the economic feasibility of intensive feeding has not compared favorably with the forage based system. Farmers traditionally relied upon native semiaquatic and aquatic vegetation such as alligatorweed (*Alternanthera philoxeroides*), smartweeds (*Polygonum* spp.), water primrose (*Ludwigia* spp.), and other terrestrial volunteer plants to provide a detrital substrate. These are undependable and often difficult to manage. Agronomic plants have recently gained popularity, and provide the opportunity to increase system intensity by double cropping with crawfish. Crawfish have been harvested as an incidental crop from Louisiana rice fields for years, and rice remains the mainstay crawfish forage. Grain sorghum has also recently been identified as a viable double cropping alternative to rice. The recurring problem of forage substrate depletion has renewed interest in feeding crawfish a formulated ration. Nutritional requirements of crawfish and the feasibility of supplemental and/or intensive feeding practices need to be addressed and defined before crawfish production can be further intensified.

KEY WORDS: crawfish, feeding, foraging

INTRODUCTION

Crawfishes are generally considered detritivores (Huner and Barr 1985), and their feeding activity is closely associated with the vegetative detrital system (Avault et al. 1983). Thus, the use of standing vegetation is an efficient food delivery strategy and, unlike most other cultured acquatic animals, crawfish are not typically fed a formulated ration. For many years crawfish producers relied upon volunteer natural vegetation as a food source for crawfish. However, with the emergence of crawfish aquaculture as a bona-fide commercial venture in the mid-1960's, producers realized the need for more dependable and productive food supplies. The use of a commercially prepared feed similar to those used in the catfish or salmonid industries appeared to be the next logical step in the development of crawfish feeding systems; however high costs of production, acquisition, storage and distribution of artificial feeds more than offset any advantage, in terms of crawfish production, over the use of standing vegetation.

Over the past 20 years three basic feed delivery strategies have been developed and tested through both applied research and the ingenuity and resourcefulness of the Louisiana crawfish farmer. These are:

1. use of volunteer natural vegetation
2. use of agricultural by-products and supplemental feeds, and
3. use of planted and cultivated forage crops.

NATURAL VEGETATION

The oldest, simplest and least expensive feed delivery strategy is the use of volunteer natural vegetation. Native plants are allowed to grow in the pond during the summer when the pond is dry and crawfish are in burrows. Ponds are then flooded in the fall. Many plant species, both aquatic and terrestrial, have been utilized in this manner, but aquatic genera are typically better suited to crawfish production. Aquatic and semiaquatic plants thrive in the moist conditions of a crawfish pond and persist longer under winter flooded conditions than do more xerophytic species. Among the most commonly used native plants are alligatorweed, water primrose, and smartweeds (Avault and Huner 1985). Others such as delta duckpotato (*Sagittaria platyphylla*) and wild rice (*Zizania aquatica*) have also been used. Generally, crawfish production in ponds containing volunteer vegetation is lower than that achieved when a cultivated forage crop is used (Clark et al. 1974, Chien and Avault 1980).

In the typical situation, one or more of these native plants is allowed to grow voluntarily in the pond during the summer and early fall. In some cases, limited cultivation and/or fertilizer is used to stimulate plant growth. At fall flooding, the farmer has a readily available forage base for crawfish production, and because these are aquatic or semiaquatic plants, they continue to thrive until killed by freeze.

Major disadvantages of utilizing volunteer aquatic vegetation are twofold. First, because these are volunteer plants, the certainty of obtaining an adequate stand and suitable biomass for crawfish forage is not guaranteed. Secondly, many of the native plants are considered weed species and as such are undesirable in agricultural fields where other crops, especially rice, will be grown in subsequent years. Thus, native vegetation is most often used in wooded or open ponds where cultivated forage crops are not feasible.

AGRICULTURAL WASTES AND SUPPLEMENTAL FEEDS

Many by-products and waste products from agricultural activity have been evaluated and used as feed for crawfish

[1]Present address: Department of Wildlife and Fisheries, Mississippi Cooperative Extension Service, P.O. Box 5446, Mississippi State, MS 39762.

(Romaire and Avault 1981). Among the materials tested are sugarcane (*Saccharum officinarum*), sugarcane bagasse, sugarcane filter cake, chicken manure, sweet potato (*Ipomoea batatas*) trimmings and vines, rye (*Secale cereale*) hay, soybean (*Glycine max*) stubble and hay, rice (*Oryza sativa*) hay, and bahiagrass (*Paspalum notatum*) hay and poultry manure (Smitherman et al. 1967, Goyert et al. 1975, Goyert and Avault 1977, Avault and Giamalva 1978, Rivas et al. 1978, Romaire et al. 1978, Johnson and Avault 1980, Johnson et al. 1983). Although crawfish growth was good on several of these substrates, only the hays have potential for use in existing large-scale commercial crawfish production. Hays such as rice and bahiagrass can be used to supplement food supplies in crawfish ponds late in the crawfish production season (spring) when the primary vegetation in the pond becomes limited or depleted (Romaire 1976). The addition of 300–500 kg/ha of hay provides vegetative matter which enters the detrital system, thus providing a suitable crawfish food source. This practice is commonly used, and although food conversion and economic efficiencies are not well documented, positive growth responses are usually observed.

Another method of feed supplementation is the use of formulated feeds (Meyers et al. 1970). Studies have been conducted using cattle range pellets (Cange et al. 1982), but crawfish have shown only moderate growth in ponds when exclusively fed such formulated feeds. However, when pellets were used to supplement food in rice-planted ponds crawfish production increased significantly. It appears that such supplementation can increase crawfish production (Clark et al. 1974, Huner et al. 1975), but there is little information as to the economic benefits of this practice. It has been generally assumed that the costs of production, acquisition, storage and daily labor for feed distribution to the pond would negate much, if not all, of the extra income generated through increased crawfish production.

Romaire (1989) lists several advantages to the use of formulated feeds including: rapid growth potential, rapid attainment of desirable large animals, increased production yields, possible higher dressout percentage, more productive and better conditioned broodstock, and intensification of cultural practices. He noted, however, that the lack of reliable experimental data concerning recently developed feeds is a major disadvantage and constraint. Research efforts are underway to re-examine the practicality and economy of using formulated feeds for crawfish production.

PLANTED FORAGE CROPS

Probably the most productive and dependable method of providing crawfish forage is through the use of an agronomic plant (Miltner and Avault 1983). Use of such a crop species has a number of advantages. The crawfish farmer can control the type of forage which will be available, instead of relying upon volunteer natural vegetation. Additionally, the concern over weed problems in subsequent years is eliminated. Finally, in many cases, the farmer can realize a cash crop from the forage plants in addition to supplying fodder for crawfish.

Several commercial plants have been used and evaluated as crawfish forage. Among these are rice, Japanese millet (*Echinochloa frumentacea*), sugarcane and soybean (Chien and Avault 1983, Day and Avault 1984, Miltner and Avault 1981). The most promising is rice (Brunson et al. 1985, 1988a, Brunson In Press). Crawfish have been harvested incidentally from rice fields for decades (Thomas 1963), and rice has been shown to be superior to other planted forages as well as natural vegetation for crawfish production (Chien and Avault 1983, Brunson 1987). With over one million hectares of rice in the United States, the potential for integrating rice and crawfish is great. Cultivation timing for rice fits nicely into the crawfish production cycle. Additionally, because rice is a semiaquatic plant, it tends to persist longer during the fall and winter months than would other species which are less well adapted to wet conditions.

Recent studies indicate that certain varieties of rice are better suited than others for crawfish forage (Brunson et al. 1988, Brunson In Press). Most of the rice/crawfish studies prior to 1984 utilized the rice varieties "Labelle" and "Saturn," which are popular varieties in Louisiana. Based on such forage parameters as straw biomass, lodging rate, rates of senescence, persistence under a winter flood, and nutritional quality, certain varieties appear to be superior to these for crawfish systems (Table 1). The more promising rice varieties can produce as much as 5,000 kg/ha of straw dry matter in addition to their high grain yields. Some of these varieties show slower senescence rates and their stubble persists longer under a winter flood than that of other varieties. The combination of these desirable varietal parameters results in one or more rice varieties which not only provide adequate crawfish forage during fall and winter, but will persist through the winter and into spring, thus providing crawfish forage at a time when other forage crops typically have been depleted.

There are basically two production strategies for rice/crawfish culture (Table 2). Double cropping is where both rice and crawfish are raised as cash crops. Rice is planted

TABLE 1.

Recommended rice varieties for use in crawfish ponds.

	Culture System	
Grain Type	**Double Crop**	**Monoculture**
Long	Labelle	Starbonnet
	Lemont	Newbonnet
		Bellevue
Medium	Mars	Mars
Short	Nortai	Nortai

TABLE 2.

Two strategies used in rice/crawfish production systems.

	Double-Cropping Rice and Crawfish
March–April	: Prepare land & plant rice
May–June	: Stock brood crawfish after permanent flood on rice
July–August	: Drain pond; harvest grain
October	: Reflood crawfish pond; rice stubble and regrowth will serve as crawfish forage
Winter–Spring	: Harvest crawfish
March–April	: Drain crawfish pond; prepare land & plant rice
	Mono-Cropping Crawfish
June–August	: Plant rice; periodic flushing will be necessary to maintain soil moisture if permanent flood is not used on rice
October	: Flood pond for crawfish; rice will be green and thriving
Winter–Spring	: Harvest crawfish
Spring–Summer	: Farmer has choice of extending crawfish harvest season and again planting late rice or draining pond in spring to plant rice for grain/crawfish double-crop. Market status and prices may dictate choice to make.

in early spring and harvested for grain in late summer. The rice stubble is left standing and ratoon growth (green rice regrowth) is encouraged. The field is reflooded in early fall and crawfish subsist on the decaying rice stubble and green regrowth. In the second strategy of monocropping, rice is planted solely for crawfish, with no concern for grain production. Under this strategy, rice is planted in mid to late summer and the grain, if produced, is not harvested. The entire rice plant them remains to provide crawfish forage. Monoculture is most often practiced by landowners participating in governmental "set-aside" programs which prohibit agronomic production but allow planting of a crawfish forage, or by producers who extend their crawfish harvest season into early summer, thereby necessitating late planting of rice.

Although crawfish have been harvested from rice fields for many years, only recently has intensive commercial crawfish culture in rice-field areas become common (Avault 1981). Thus, production techniques are not highly refined and many questions are yet unanswered (Brunson 1986). For example, some of the pesticides, especially insecticides such as Furadan, used on rice are highly toxic to crawfish. Consequently the farmer assumes he must choose which crop he wishes to maximize, rice or crawfish. To maximize crawfish production, use of rice pesticides is limited and many times grain production is reduced. Cultural and management practices for rice and crawfish must be developed to maximize production of both crops.

Other agronomic crops may have potential as crawfish forage and for double-cropping with crawfish. One group of plants recently identified as having potential is the sorghums (*Sorghum* spp.) (Brunson 1985, Brunson 1987, Brunson et al. 1988, Brunson and Griffin 1988, Brunson et al. 1985). Land area planted to grain sorghum, or milo, (*S. bicolor*) has increased dramatically in the Southeast (more than 20-fold in Louisiana since 1979), and concomitantly, so has interest in this plant as a crawfish forage. Preliminary studies indicate that milo is a viable and desirable alternative to rice for double cropping with crawfish (Brunson and Griffin 1988). Another sorghum that appears to have great potential is a sorghum sudangrass hybrid. This plant is most commonly planted for production of silage or hay and possesses great vegetative growth and ratoon potential. During the course of a summer, sorghum sudangrass can produce several tons of dry matter which may later be utilized as a crawfish forage (Table 3).

In rice producing areas, farmers typically rotate their rice fields into a terrestrial crop (traditionally soybeans) every third or fourth year for soil conservation and weed control purposes. Both sorghums are drought resistant and can provide the farmer with the necessary "dry land" crop in his normal cropping system rotation. Soybeans have little potential as crawfish forage, but the substitution of one of the sorghums provides the opportunity for production of a crawfish crop during the "off-year." Use of such a rotational system would result in an intensification of production such that four cash crops could be produced in two calendar years (Brunson and Griffin 1988).

FUTURE RESEARCH NEEDS

Research to date has attempted to establish baseline information, but the bulk of crawfish forage and feed research lies ahead. Perhaps the greatest need in the forage arena is the identification of plants which will provide food throughout the crawfish harvesting season. Forages currently in use are typically depleted in late winter and early spring, sometimes resulting in a need for feed supplementation and sometimes in stunted crawfish. Other forage crops and crop combinations also should be evaluated for potential in crawfish systems. New crawfish production techniques and new agronomic cultural practices are needed to fully integrate crawfish production with agronomic crops. Basic research such as food habit studies and

TABLE 3.

Biomass produced by various crawfish forages.

Forage	How Treated	kg/ha Dry Matter
Rice	Double Cropped	5000
Rice	Monoculture	2000
Milo	Double Cropped	4000
Milo	Monoculture	2000
Sudangrass	Early Summer	8–10,000
Sudangrass	Late Summer	2000
Japanese Millet	Mid-summer	3400
Native Plants	Volunteer	2900
Browntop Millet	Mid-summer	2500

food-web dynamics in crawfish ponds have been limited, but contribute to a better evaluation and utilization of crawfish feeds and forages. Recently completed, but as yet unpublished, studies at several southern universities have lent great insight into these topics.

The inevitable intensification of crawfish production practices, as well as diversifications such as soft crawfish production and possible year around production, will result in the need for more efficient and refined feeding strategies. The use of prepared feeds should be strongly considered, and development and acceptance of such feeds may well change the whole complexion of crawfish forage management. Such a revolution would mark the end of the era of "managing" semi-natural systems and the onset of truly intensive crawfish culture.

REFERENCES

Avault, J. W., Jr. 1981. Double cropping rice and crawfish. *Aquaculture* 7(2):40–41.

Avault, J. W., Jr., & J. V. Huner. 1985. Crawfish culture in the United States. In E. E. Brown (ed.) *Crustacean and Mollusk Aquaculture in the United States*. AVI Publishing, Westport, CN.

Avault, J. W., Jr., R. Hernandez, & M. Giamalva. 1978. Sugarcane waste products and chicken manure as supplemental feed for crawfish. *Sugar y Azucar* 73(6):42.

Avault, J. W., R. P. Romaire, & M. R. Miltner. 1983. Feeds and forages for red swamp crawfish, *Procambarus clarkii:* 15 years of research at Louisiana State University reviewed. *F.W. crayfish.* 5(1981):362–369.

Brunson, M. W. 1985. Alternative forage crops for crawfish production. *Crawfish Tales* 4(4):35–37.

Brunson, M. W. 1986. Rice/crawfish culture in south Louisiana. Agronomy Abstracts, American Society of Agronomy. 78:46–47.

Brunson, M. W. 1987. Pre-flood evaluation of seven grasses (Graminae L.) as potential alternative forages for crawfish culture. *Journal of the World Aquaculture Society* 18(4):247–252.

Brunson, M. W. In Press. Impacts of rice cultivar selection in double cropped crawfish/rice ponds. *Progressive Fish-Culturist.*

Brunson, M. W. & J. L. Griffin. 1988. Comparison of crayfish-rice and crayfish/grain sorghum double cropping systems. *Aquaculture* 72:265–272.

Brunson, M. W., J. L. Griffin, & D. K. Riecke. 1988a. A new forage crop for crawfish production. *Louisiana Agriculture* 31(3):3–4, 24.

Brunson, M. W., R. W. Taylor, & B. J. Hoff. 1988b. Characteristics of rice cultivars with implications for crawfish double cropping. *Journal of World Aquaculture Society* 19(1):8–13.

Brunson, M. W., C. R. Weirich, & S. M. Simon. 1985. Evaluation of three sorghums as potential alternative forages for crawfish culture. *Journal of the World Aquaculture Society* 18(4):247–252.

Cange, S., M. Miltner, & J. W. Avault, Jr. 1982. Range pellets as supplemental crayfish feed. *Prog. Fish-Cult.* 44(1):23–24.

Chien, Y-H., & J. W. Avault, Jr. 1980. Production of crayfish in rice fields. *Prog. Fish-Cult.* 42(2):67–71.

Chien, Y-H., & J. W. Avault, Jr. 1983. Effect of flooding dates and type of disposal of rice straw on the initial survival and growth of caged juvenile crayfish, *Procambarus clarkii,* in ponds. *F.W. Crayfish* 5(1981):351–361.

Clark, D. F., J. W. Avault, Jr., & S. P. Meyers. 1974. Effects of feeding, fertilization, and vegetation on production of red swamp crayfish, *Procambarus clarkii. F.W. Crayfish* 2:125–138.

Day, C. H. & J. W. Avault, Jr. 1982. Crayfish production in ponds receiving varying amounts of soybean stubble and rice straw as forage. *F.W. Crayfish* 6:247–265.

Goyert, J. C. & J. W. Avault, Jr. 1977. Agricultural by-products as supplemental feed for crayfish *Procambarus clarkii. Trans. Amer. Fish Soc.* 106(6):629–633.

Goyert, J. C., J. W. Avault, Jr., J. E. Rutledge, & T. P. Hernandez. 1975. Agricultural by-products as supplemental feed for crawfish. *LA Agric.* 19(2):10–11.

Huner, J. V., S. P. Meyers, & J. W. Avault, Jr. 1975. Response and growth of freshwater crawfish to an extruded water-stable diet. *F.W. Crayfish* 2:149–157.

Huner, J. V., & J. E. Barr. 1985. Red swamp crawfish: Biology and exploitation. Sea Grant Publ. No. LSU-T-80-001. Center for Westland Resources, LSU, Baton Rouge, LA.

Johnson, W. B. & J. W. Avault, Jr. 1980. Some effects of poultry manure supplementation to rice-crawfish (*Oryza sativa—Procambarus* spp.) experimental earthen impoundments. Abstract, Fish. Culture Section, Am. Fish Soc. 1980:15.

Johnson, W. B., L. L. Glasgow, & J. W. Avault, Jr. 1983. A comparison of delta duckpotato (*Sagittaria graminea platyphylla*) with rice (*Oryza sativa*) as cultured red swamp crayfish (*Procambarus clarkii*) forage. *F.W. Crayfish* 5(1981):351–361.

Meyers, S. P., J. W. Avault, Jr., J. S. Rhee, & D. Butler. 1970. Development of rations for economically important aquatic and marine invertebrates. Coastal studies Bull. No. 5. LSU.

Miltner, M. R. & J. W. Avault, Jr. 1981. Rice and millet as forages for crawfish. *LA Agri.* 24(3):8–10.

Miltner, M. R. & J. W. Avault, Jr. 1983. An appropriate food delivery system for low-levee pond culture of *Procambarus clarkii,* the red swamp crawfish. *F.W. Crayfish* 5(1981):370–378.

Rivas, Rao, R. P. Romaire, J. W. Avault, Jr. & M. Giamalva. 1978. Agricultural forage and by-products as feed for crawfish. *Procambarus clarkii. F.W. Crayfish* 4:337–342.

Romaire, R. P. 1989. Use of formulated feeds in crawfish culture. *Crawfish Tales* (In Press).

Romaire, R. P. 1976. Population dynamics of red swamp crawfish, *rocambarus clarkii* (Girard), in ponds receiving fertilization, and two agricultural forages as supplemental feed. M.S. Thesis, LSU, 103 pp.

Romaire, R. P. & J. W. Avault, Jr. 1981. Forages and agricultural by-products as feed for crawfish. *LA Agri.* 25(2):20–21.

Romaire, R. P., J.S. Forester, & J. W. Avault, Jr. 1978. Growth and survival of stunted red swamp crawfish (*Procambarus clarkii*) in a feeding-stocking density experiment in pools. *F.W. Crayfish* 4:331–336.

Smitherman, R. O., J. W. Avault, Jr., L. de la Bretonne, Jr., and H. A. Loyacano. 1967. Effects of supplemental feed and fertilizer on production of red swamp crawfish in pools and ponds. *Proc. Ann. Conf. S.E. Assoc. Game and Fish Comm.* 21:452–458.

Thomas, C. H. 1963. A preliminary report on the agricultural production of the red swamp crawfish in Louisiana rice fields. *Proc. Ann. Conf. S.E. Assoc. Game and Fish Comm.* 17:180–186.

Journal of Shellfish Research, Vol. 8, No. 1, 281–286, 1989.

OVERVIEW OF HARVEST TECHNOLOGY USED IN COMMERCIAL CRAWFISH AQUACULTURE

ROBERT P. ROMAIRE
School of Forestry, Wildlife, and Fisheries
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

ABSTRACT The harvest of crawfish is the major production cost associated with crawfish aquaculture generally accounting for 60–80% of total operating expenses. Crawfish are harvested with small, basket-shaped traps emptied 60 to 150 days per production season. Crawfish catch is affected by many factors including water temperature, water quality, forage, population density and structure, climate, trap design and trap density, and bait. Many traps are used by crawfish producers but the most effective designs are constructed from PVC-coated wire, have two or three entrances, and a retainer band or collar to minimize crawfish escape. The traps, typically set for 12–24 h at a density of 25–100 per ha, are emptied 3–5 days per week from November through May or June. Traps are baited with fishes, formulated bait, or a combination of the two. Fish baits are most effective in water less than 20°C, and a combination of fish and formulated bait is most effective at 20–30°C. Motorized flat-bottomed boats with one or two persons are the most efficient devices for accessing the traps. Trappers empty and re-bait 150–250 traps per h when motorized boats are used. Major improvements in crawfish harvesting efficiency will likely minimize the use of traps and baits.

KEY WORDS: crawfish, aquaculture, harvesting, bait, traps

INTRODUCTION

The extensive intensity level of production technology used to cultivate crawfish of the genus *Procambarus* necessitates a method of harvest that is atypical of methods used to harvest most cultured aquatic animals. Channel catfish, bait minnows, rainbow trout, shrimp, and other cultured aquatic animals are harvested one to several times per production cycle with nets and seines, by draining the culture system, or a combination of the two. However, the management system used to cultivate crawfish, in which vegetative forage is cultivated as a food source for the crawfish, presently precludes the use of seines and nets, and batch harvesting. Rather, pond-reared crawfish are harvested with small traps baited with fish or formulated baits, and the traps are commonly fished 120–180 days per production cycle (November–June) (Avault and Huner 1985). A trapper can empty 400–1,600 traps per day depending upon the harvest method and machinery used, and crawfish are harvested 4–6 days weekly. Crawfish are typically harvested from ponds beginning in late November, 6–8 weeks after ponds are flooded in September and October, and it continues through May or June when the ponds are drained. Some producers harvest crawfish from March through June only, particularly in states other than Louisiana.

Current methods of harvesting crawfish are labor intensive, and 60–80% of total production costs are associated with the harvest (Roberts 1984, Dellenbarger et al. 1987). Annual harvest expenses incurred by farmers normally range from $1,500–$1,250 per ha. Bait (40–55% of harvest cost) and labor to bait and empty traps (20–35%) are major expenses. Some crawfish producers lease harvest rights with the trappers receiving a percentage of the wholesale value of the crawfish (de la Bretonne and Fowler 1976). The percentage, normally 40–70%, is set based on items supplied by the crawfish producer and those supplied by the trapper. Trappers who supply their own boats, traps, and bait will normally receive 60–70% of the wholesale value of the crawfish caught that day. The producer will retain 50% if he supplies the trapper with bait and traps. The income received by both producers and trappers varies according to seasonal changes in the wholesale value of crawfish, and crawfish catch.

FACTORS THAT INFLUENCE CRAWFISH HARVEST

The daily crawfish trap catch in ponds is variable and it is influenced by many factors including water temperature (Taylor 1984, Hymel 1985, Somers and Stechey 1986), water quality (Avault et al. 1975, Hymel 1985, Araujo and Romaire 1989), type and quantity of forage (Romaire and Osorio 1989, Brunson 1989), weather (Araujo and Romaire 1989), crawfish reproduction and growth (de la Bretonne and Avault 1977, Momot and Romaire 1983, Romaire and Lutz 1989), trap design (Pfister and Romaire 1983), bait (Cange et al. 1986, Romaire and Osorio 1989), harvesting intensity (Romaire and Pfister 1983), and market value (Roberts 1984, Dellenbarger et al. 1987). The crawfish catch in well-managed ponds typically ranges from 0.1–1.5 kg per trap per day with a seasonal average of 0.4–0.6 kg per trap (Romaire and Osorio 1989). Crawfish catch varies as much as 200% from day to day, often unpredictably (Fig. 1).

Proper pond design, adequate water exchange capacity,

Approved by the Director of the Louisiana Agricultural Experiment Station as Manuscript Number 89-22-3273.

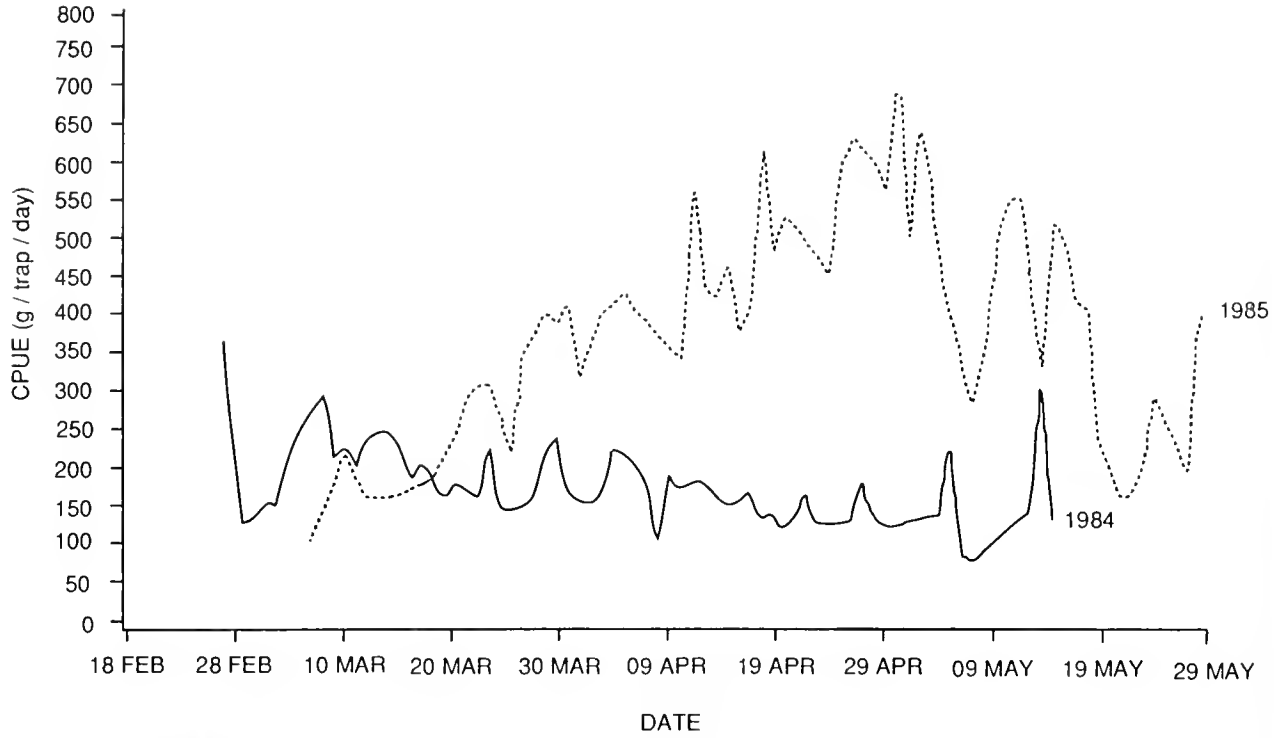


Figure 1. Mean daily variation in crawfish catch per unit trap effort in five ponds in 1984 and 1985, Baton Rouge, LA.

and good water and vegetation management are necessary to maximize crawfish harvest. Water temperature, crawfish population density and crawfish recruitment patterns are primary factors that influence daily crawfish catch. Crawfish are relatively inactive below 10°C and harvest is lowest in January and February. Crawfish catch is greatest at 20–30°C, November–December and March–May, and when the standing crop of harvestable crawfish is highest, March–May. The catch declines when water temperature exceeds 30°C, dissolved oxygen (DO) levels decline below 2 mg/liter, and when a large portion of the crawfish population matures and begins to burrow in late May and June.

Recruitment of young-of-the-year (YOY) crawfish and holdover crawfish, from the preceding year, into the harvestable population occurs continuously from September through June and this allows for an extended harvest season. However, the maximum standing crop of harvestable size crawfish, 65 mm total length (TL) or larger, occurs from mid-March through early May (Romaire and Lutz 1989), if the pond is flooded in early October. If the pond is flooded earlier or later than October, the maximum standing crop of harvestable crawfish will correspondingly be earlier or later (de la Bretonne and Romaire 1989). Crawfish cease feeding several days prior to, during and after ecdysis (Huner and Barr 1984), and during this period they do not enter traps. After the carapace has sufficiently hardened the crawfish resumes feeding and is susceptible to capture. Crawfish molting patterns and the continuous recruitment of YOY to harvestable sizes, and their subsequent depletion through continuous harvest account for much of the cyclic variation in daily crawfish catch, even when other factors are optimal.

Crawfish catch is affected by changes in water quality, weather, and lunar phase. Crawfish catch is reduced when crawfish are continually exposed to DO concentrations less than 2 mg/liter (Araujo and Romaire 1989). Low DO stresses crawfish and reduces feeding, growth, and activity, reducing their susceptibility to trapping. Rain showers of several minutes to several hours duration increase water circulation and enhances catch by increasing the dispersion of bait attractants (Baum 1987, Araujo and Romaire 1989). Crawfish catch declines with the approach of full moon and with passage of cold fronts (Morrissy and Caputi 1981, Araujo and Romaire 1989). Overcast weather associated with short duration cold fronts (1–3 days) reduces DO concentration and subsequent catch. Longer cold fronts (several days or more) reduce water temperature, and subsequent crawfish activity, and decreases crawfish catch. The relationship between environmental conditions, crawfish recruitment and molting patterns, and crawfish catch is complex and no single environmental factor can accurately forecast daily changes in crawfish catch.

TRAPS

Designs

Over 25 different types of crawfish traps are used to harvest cambarid crawfish, and the traps differ in design

and configuration, physical dimension, construction material and mesh size, number of entrances, presence or absence retainer bands or collars, and presence or absence of bait protection containers (Gary 1975, Bean and Huner 1978, Romaire 1983, Pfister and Romaire 1983, Romaire 1988). Most traps are constructed of 1.9-cm dia hexagonal mesh wire (19- or 20-gauge PVC-coated wire is the most common) or plastic netting. The mesh size is selective for crawfish 65–75 mm TL or larger. Traps made from 1.6-cm mesh wire are also used by some trappers but the smaller crawfish retained with this mesh size are not preferred by processors.

Crawfish traps are generally categorized as "stand-up" (upright position in the water with the top of the trap protruding above the water surface) or "pillow" (lays horizontally on the pond bottom and is completely submerged) (Pfister and Romaire 1983, Romaire 1983). Stand-up traps are made in several designs and are the most common. Pillow traps (named for their pillow-like shape) are used for trapping in waters too deep (>0.8 m) for most stand-up traps. A pillow trap set upright in the water column is referred to as a stand-up pillow traps.

Three basic trap designs are used by most crawfish producers: stand-up pillow, pyramid, and barrel (Fig. 2). The stand-up pillow trap is the most common followed by the pyramid and barrel designs. Stand-up pillow traps are oval in shape, pyramids are triangular, and barrel traps are cylindrical. The three designs are all effective and selection of a design is based on personal preference. Traps retail for $3–$12 each with $5 the most common price.

Several design factor enhance the effectiveness of a trap. Traps made from PVC-coated wire or plastic (e.g., Vexar™) catch 15–25% more crawfish than traps made from galvanized wire (Romaire 1983, Pfister and Romaire 1983). The color of the PVC coating or plastic is black, and colors other than black do not appear to enhance catch (Romaire 1988). Trap longevity is increased by using coated wire or plastic. Trappers often damage or destroy wire traps by careless operation of harvesting boats but damage to plastic traps is minimal. Traps with two entrances (stand-up pillow, barrel) and three entrances (pyramid, barrel) catch 2–3 times more crawfish than traps with one entrance (Pfister and Romaire 1983). Traps with three entrances catch 20% more crawfish in 12-h trap-sets than two-entrance traps of similar design; otherwise, traps with two or three entrances and set for 24-h catch equally.

Retainer bands (7.6-cm wide strips of thin aluminum on the inside circumference at the top of the open area of the trap) or collars (15-cm dia PVC pipe at the top of the trap) (Fig. 2), designed to minimize crawfish escape from traps, increase crawfish catch 15–20% compared to traps without retainers with 24-h trap-sets. Crawfish catch after 12-h trap-sets is not enhanced with retainer bands. Retainers prevent crawfish from escaping from the top of the open trap but they do not prevent crawfish from escaping through the entrances. On average, 15–20% of the crawfish that enter traps escape within 24 h by exiting the entrances and up to 40% escape after 48 h (Pfister and Romaire 1983). Traps with bait protection containers catch 40% fewer crawfish than traps with exposed bait. The inability of crawfish to masticate the bait placed in protection containers minimizes the release of bait attractants (Pfister and Romaire 1983).

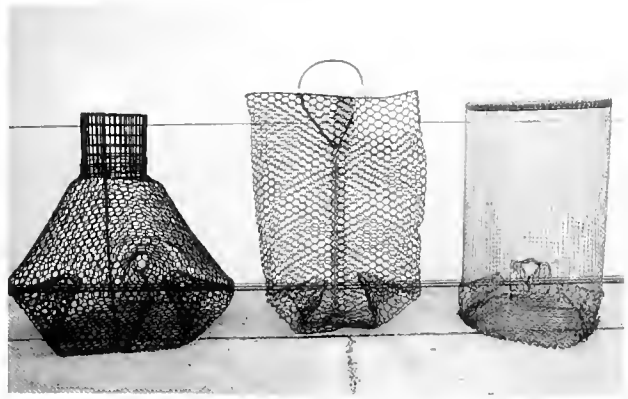


Figure 2. Three types of stand-up crawfish traps; pyramid (left), stand-up pillow (middle), and barrel (right).

Trap dimensions are varied by using wire of different heights and lengths. Larger traps (e.g., 0.76-m high × 0.4-m dia) retain more crawfish than smaller traps (e.g., 0.76-m high × 0.25-m dia) because smaller traps are more likely to saturate with crawfish, particularly in ponds with a large standing crop of harvestable crawfish (Baum 1987). Metal rods (1-cm dia) with handles are used to aid in maintaining stand-up pillow and barrel traps upright. Pyramid traps because of their wide, flat bottom normally do not require rods. Wind, birds and rodents often cause stand-up traps to topple if support rods are not used (Martin and Hamilton 1986).

Stand-up pillow and submerged pillow traps catch equally (Pfister and Romaire 1983) but stand-up traps can be more efficiently lifted, emptied and rebaited than pillow traps. Generally, a trapper can lift and empty 1.5–2 times more stand-up traps per unit time than pillow traps. The crawfish captured in an open-top stand-up trap can be emptied directly onto a sacking table in the harvest boat, the trap re-baited, and returned to the water within 15 seconds. In contrast, 25–30 seconds is required to lift a pillow trap to the surface via an attached line, after which it must be opened, emptied, re-baited and closed before it can be returned to the water. Submerged pillow traps also prevent crawfish from reaching the water surface during DO depletion often resulting in crawfish death (Avault et al. 1975).

Rodents often enter traps and eat the bait. In some ponds, turtles kill crawfish confined to traps by removing the abdomen from the cephalothorax if the crawfish abdomen protrudes from the trap.

Trap Density

A trap density of 50–75 per ha is recommended for well-managed crawfish ponds (Romaire and Pfister 1983) although the industry average is probably no greater than 30 traps per ha. Inadequate harvesting from using to few traps hastens forage depletion and causes crawfish to "stunt" at non-desirable market size (Romaire and Lutz 1989). In smaller ponds (<8 ha) 100 traps per ha can be used if the catch justifies the additional effort. A high trap density in areas of a pond with poor water quality is inefficient because the low catch seldom justifies the trapping effort. Traps are placed in rows to facilitate harvesting and the distance between traps depends on trap density. A spacing of 12–20 m between traps and rows of traps is common.

Trap-sets of 12 h will normally catch as many or more crawfish than the catch after 24-h sets, if water temperature exceeds 20°C and formulated baits are used (Romaire and Pfister 1983, Romaire and Osorio 1989). Traps are normally emptied once per 24 h in cool water (November through mid-March), but traps can be emptied two to three times per day (6- to 12-h trap-set) from March through May if the catch and crawfish price justifies the effort (Romaire and Pfister 1983). After several days of intense trapping the average size of crawfish harvested decreases as the density of harvestable crawfish is reduced temporarily (Romaire and Pfister 1983).

It is probably not possible to overfish a crawfish population with present harvest technology but it is common for "well-managed" ponds to be underfished. Insufficient harvesting can hasten forage depletion and cause crawfish to stunt at sub-marketable size (Romaire and Lutz 1989). Recent improvements in pond design and water management has increased survival of young-of-the-year (de la Bretonne and Romaire 1989) and this requires that intensive harvesting be practiced.

BAITS

Bait is the highest expense in crawfish harvest (Dellenbarger et al. 1987). Bait generally costs $0.3–$0.6 per kg, $225–$750 per ha per production season, depending on bait type, quantity used per trap, trap density, and frequency of harvest (days per season and trap sets per day) (Roberts 1984). Bait is replaced after each trap-set. About 15,000–30,000 tons of bait are used annually in the Louisiana crawfish industry (wild fishery and aquaculture) of which about 50% are natural baits and the remainder are formulated. Natural baits include mostly rough fishes. Gizzard shad (*Dorosoma cepidanum*) is the most widely used natural bait, but significant quantities of herrings (*Alsoa* spp.), menhaden (*Brevoortia patronus*), common carp (*Cyprinus carpio*), suckers (Catastomidae), and channel catfish (*Ictalurus punctatus*) and buffalofishes (*Ictiobus* spp.) offal, especially heads, are also used. Formulated baits were developed in 1981 largely from formulations developed by Louisiana Agricultural Experiment Station researchers (Avault et al. 1985, Burns and Avault 1985, Cange et al. 1986). Formulated crawfish baits are made from fish meal, fish solubles, cereal grains and grain by-products, attractants, and binders. At least 10 commercial feed companies manufacture and market formulated crawfish bait in the southeastern USA. Most formulated baits are cylindrical pellets, 0.4- to 1.25-cm dia and 1.25- to 1.5-cm long, that weight 50–75 g per pellet. Many trappers use both formulated bait and fish together in the same trap.

Natural baits have several disadvantages—supply and price are seasonal, freezers or coolers are required for storage, labor is required to cut the bait in 150 g pieces, they generally have an unpleasant odor, and bait residue must be disposed. The primary advantage of natural baits, particularly gizzard shad, menhaden and common carp, is that they are more effective in attracting crawfish in water less than 20°C than current formulated baits (Huner et al. 1989). In contrast, formulated baits are cost competitive with fish, they are easier to store and handle, and some brands leave no residue to dispose. The most effective formulated baits have a water stability of at least 12–18 h and a crude protein content of 17–20% (Romaire and Osorio 1989, Huner et al. 1989). Many formulated crawfish baits are as effective, or more effective than fish baits at a temperature above 20°C and when forage biomass is low (mid-March through May). Formulated baits compare favorably with each other in effectiveness when water temperature is greater than 30°C and when the pond has a high standing crop of harvestable crawfish (Huner et al. 1989). The habitat of the crawfish pond (open, rice or wooded ponds) has no interactive effect on the relative effectiveness of one type of bait compared to another (Romaire and Osorio 1989). If a bait performs good (or poorly) in one type of pond it should perform good (or poorly) in another.

A combination of formulated bait and fish used together will generally attract 15–30% more crawfish into a trap than either the same formulated bait or same fish used singly (Romaire and Osorio 1989, Huner et al. 1989) at temperatures of 20–26°C. Traps should be receive 100–150 g of bait, and no increase in catch is obtained by using more than 150 g even in ponds with large quantities of crawfish (Romaire and Osorio 1989). Because traps baited with formulated bait and set for 12 h catch up to 30% more crawfish than traps set for 24 h at 20°C or higher, many trappers rebait traps on 12-h cycles to increase catch.

HARVESTING MACHINERY

Traditionally, crawfish were harvested by trappers who "walked" the pond on foot while pulling a small boat into which the crawfish harvested were placed. Some producers with small ponds (<8 ha) still use this method because it

requires little investment even though it is laborious and inefficient. One person can normally empty no more than 400 traps per day using this method. In deeper ponds a trapper may use a "pirogue" or small boat that is propelled with a pole or paddle. This method is no more efficient than walking while pulling a boat. Boats with water-cooled outboard motors are used in some deeper ponds. Significant improvements in harvesting boats and machinery have occurred since 1980.

A more efficient harvesting system employs a large, flat-bottomed boat (4.7- to 5.3-m long × 1.2- to 1.5-m wide × 0.46- to 0.61-m high) made from aluminum and which is propelled with an air-cooled outboard motor (8–12 HP) with a long shaft and weedless propeller that is adapted for operation in shallow water (0.5–0.9 m). The propulsion unit is commonly referred to as a "Go-Devil" (Fig. 3). A gear box and cleated wheel to replace the propeller is added to some outboards. The wheel rolls along the pond bottom and pushes the boat forward. The boats generally require two persons to operate, one to empty and rebait the traps, and a second to guide the boat. The two-man harvesting system utilizing the Go-Devil can handle 200–300 traps per hour. The boat and motor cost $2,500–$3,500. Although these boats and outboards are efficient for use in harvesting crawfish they are difficult to operate in brisk wind.

Commercial crawfish farmers most commonly use a "crawfish combine" to access traps (Fig. 4). The combine is a large flat-bottomed boat (similar in size to "Go-Devil" boats) to which is attached a metal wheel(s), about 0.78 m in dia. The wheel has metal cleats welded to the bottom for traction. The wheel, powered with a hydraulic motor, is mounted to the front or rear of the boat. A cultivator blade, attached to the side or stern, is required to minimize boat drift in wind. Hydraulic pumps and motors are powered with a 8–12 HP air-cooled engine mounted inside the boat. The boat can be steered by a single person with hand valves or foot pedals. Foot pedals allow the driver's hands to be free to empty and rebait traps. A single operator can handle about 150–200 traps per hour and 250 traps per hour can be handled with two operators. Crawfish combines (boat, motor and wheels) retail for $4,500–$6,500. The crawfish combine can push or pull itself across small rice-field levees (0.5 m high).

The crawfish "mud-buggy" has been used by several crawfish farmers in Louisiana and Texas. The "buggy" is a four-wheel drive modified rice-field levee sprayer powered by a 16 HP air-cooled engine. The single operator, who sits on an elevated platform, lifts and empties traps as they pass between the wheels of the vehicle. A person using the crawfish mud buggy can empty about 200–250 traps per hour and the vehicle can easily cross large perimeter levees. The buggy has two disadvantages—it makes deep ruts in the pond bottom and requires frequent maintenance. The mud buggy retails for $7,000.

The most efficient crawfish harvesting method using powered boats employs a technique in which the trapper sets a baited trap about 1 m in front of the next trap in the row to be lifted. The trap is emptied and rebaited while the boat moves to the next trap in the row, and the process is continually repeated. The technique does not require the trapper to stop the boat at each trap thus significantly increasing the number of traps that can be handled per unit time.

FUTURE DEVELOPMENTS IN CRAWFISH HARVESTING

Current crawfish harvest practices are inefficient and labor intensive. Advances in crawfish harvesting technology can increase crawfish production efficiency and decrease the cost of crawfish production. Better trap designs, baits with enhanced effectiveness in cold and warm water, and improved harvesting machinery require development to improve crawfish harvest efficiency. Ideally, crawfish should be harvested with minimal use of traps, bait and labor. Active methods of harvesting crawfish with trawls,

Figure 3. Crawfish harvest boat, "Go-Devil" design.

Figure 4. Crawfish harvest boat, "crawfish combine" design.

seines, and boat-mounted push trawls have been tested (Cain and Avault 1983, Morgan et al. 1984) and though a few devices have shown promise, they have not yet proven to be economically feasible.

Cain and Avault (1983) developed a boat-mounted electro-trawl as a harvesting system for crawfish. The electro-trawl is presently used to harvest highly valued ($14 per kg) soft crawfish. Soft-shelled crawfish do not enter baited traps because of the cannibalistic traits of the animal. Morgan et al. (1984) developed an automated crawfish harvester using a modified linear-move irrigator. The traps on the irrigator are automatically set, raised and emptied at specific time intervals; after which the irrigator would moves to a different location in the pond. Morgan et al. (1984) reported that the crawfish catch from the automated system was equal to the catch from conventional traps in the same pond, but the high cost and special design requirements of the system has precluded its commercial development.

Baum (1987) demonstrated that water circulation can be used to induce crawfish migration into locations where the crawfish can be concentrated and harvested with large, unbaited hoop traps (1.8-m L × 0.48-m dia). The hoop traps were placed in channels formed by closely-spaced (4 m) parallel levees inside a 4 ha commercial crawfish pond (Fig. 4). Water currents, generated by flushing the pond with fresh water stimulated crawfish movement into the "channels". Average crawfish catch in each unbaited hoop trap was five times greater than baited conventional traps.

LITERATURE CITED

Araujo, M. & R. Romaire 1989. Effects of water quality, climate, and lunar phase on crawfish catch. *Prog. Fish-Cult.*, in press.

Avault, J. W., Jr., & J. V. Huner. 1985. Crawfish culture in the United States. In J. V. Huner, and E. E. Brown (eds) *Crustacean and mollusk aquaculture in the United States*. AVI Publ. Co., Inc., Westport, CN, pp. 1–54.

Avault, J. W., Jr., L. W. de la Bretonne, & J. V. Huner. 1975. Two major problems in culturing crayfish in ponds: oxygen depletion and overcrowding. In J. W. Avault (ed.) *Proc. 2nd Internat. Symp. Freshwater Crayfish*, Baton Rouge, LA, pp. 139–148.

Avault, J. W., Jr., B. A. Pollock, J. A. Collazo, R. P. Romaire, & S. W. Cange. 1985. Evaluating experimental crawfish baits. *Louisiana Agricult.* 28(2):4–5.

Baum, T. 1987. Evaluation of water circulation and a hoop trap to enhance crawfish harvesting. M.S. thesis, Louisiana State Univ., Baton Rouge, LA.

Bean, R. A. & J. V. Huner. 1978. An evaluation of selected crawfish traps and trapping methods. In P. Laurent (ed) Proc. 4th Internat. Symp. Freshwater Crayfish, Thonon-les-Baines, France, pp. 141–152.

Brunson, M. 1989. Forage and feeding systems for commercial crawfish culture. *J. Shellfish Res.*, 8:277–280.

Burns, C. & J. W. Avault, Jr. 1985. Artificial baits for trapping crawfish (*Procambarus* spp.): formulation and assessment. *J. World Aquacult. Soc.* 16:368–374.

Cain, C. D., Jr. & J. W. Avault, Jr. 1983. Evaluation of a boat-mounted electro-trawl as a commercial harvesting system for crawfish. *Aquacult. Eng.* 2(2):135–152.

Cange, S. W., D. Pavel, B. Carol, R. P. Romaire & J. W. Avault, Jr. 1986. Evaluation of eighteen artificial crayfish baits. In Per Brinck (ed) Proc. 6th Internat. Symp. Freshwater Crayfish, Lund, Sweden, pp. 270–273.

de la Bretonne, L. Jr., & J. F. Fowler. 1976. The Louisiana crawfish industry—its problems and solutions. *Proc. Southeastern Assoc. Game and Fish Comm.* 30:251–256.

de la Bretonne, L. W., Jr. & J. W. Avault, Jr. 1977. Egg development and management of *Procambarus clarkii* (Girard) in a south Louisiana commercial crawfish pond. In O. V. Lindqvist (ed) *Proc. 3rd Internat. Symp. Freshwater Crayfish*, University of Kuopio, Kuopio, Finland, pp. 133–140.

de la Bretonne, L. W., Jr. and R. P. Romaire. 1989. Commercial crawfish cultivation practices: a review. *J. Shellfish Res.* 8:267–275.

Dellenbarger, L. E., L. R. Vandeveer & T. M. Clarke. 1987. Estimated investment requirements, production costs, and breakeven prices for crawfish in Louisiana, 1987. D.A.E. Research Report No. 670, Louisiana Agricult. Exp. Sta., Louisiana State Univ. Agricult. Center, Baton Rouge, LA.

Gary, D. L. 1975. Commercial crayfish pond management in Louisiana. *Prog. Fish-Cult.* 37:130–133.

Huner, J. V. & J. E. Barr. 1984. Red swamp crawfish: biology and exploitation. Sea Grant No. LSU-T-80-001, LSU Center for Wetland Resources, Baton Rouge, LA

Huner, J. V., R. P. Romaire, V. Pfister & T. Baum. 1989. Effectiveness of commercially formulated and natural bait in attracting crawfish., unpublished manuscript.

Hymel, T. M. 1985. Water quality dynamics in commercial crawfish ponds and toxicity of selected water quality variables to *Procambarus clarkii*. M.S. thesis, Louisiana State Univ., Baton Rouge, LA.

Martin, R. P. & R. B. Hamilton. 1986. The extent and severity of crawfish predation by wading birds at commercial crawfish ponds. *Louisiana Agricult.* 28(4):3–5.

Momot, W. T., & R. P. Romaire. 1983. Use of a seine to detect stunted crawfish populations, a preliminary report. *J. World Maricult. Soc.* 12(2):284–390.

Morgan, K. L., R. J. Edling, & J. A. Musick. 1984. A mechanical crawfish harvester. *Louisiana Agricult.* 26(2):4–5.

Morrissy N. M. & N. Caputi. 1981. Use of catchability equations for population estimation of marron, *Cherax teniumanus* (Smith) (Decapoda: Parastacidae). *Australian J. Marine and Freshwater Res.* 32:213–225.

Pfister, V. & R. P. Romaire. 1983. Catch efficiency and retentive ability of commercial crawfish traps. *Aquacult. Eng.* 2:101–118.

Roberts, K. 1984 (revised). Crawfish production cost. *Crawfish Tales* 2(4):31–32.

Romaire, R. P. 1983. Catch efficiency of crawfish traps. *Crawfish Tales* 2(2):27–29.

Romaire, R. P. 1988. Traps designs and their catchability. *Crawfish Tales* 7(1):35–37.

Romaire, R. P. & V. Pfister. 1983. Effects of trap density and diel harvesting frequency on catch of crawfish. *North Am. J. Fish. Manag.* 3:419–424.

Romaire, R. P. & C. G. Lutz. 1989. Population dynamic of *Procambarus clarkii* (Girard) (Decapoda:Cambaridae) and *Procambarus acutus acutus* in commercial ponds. *Aquaculture*, in press.

Romaire, R. P. & V. Osorio. 1989. Effectiveness of crawfish baits as affected by habitat type, trap-set time, and bait quantity. *Prog. Fish-Cult.*, in press.

Somers, K. M. & D. Stechey. 1986. Variable trappability of crayfish associated with bait type, water temperature and lunar phase. *Am. Midland Naturalist* 116:36–44.

Taylor, R. C. 1984. Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemical Physiology* 77:513–517.

Journal of Shellfish Research, Vol. 8, No. 1, 287–291, 1989

SOFT-SHELL CRAWFISH PRODUCTION TECHNOLOGY

DUDLEY D. CULLEY, AND LEON DUOBINIS-GRAY[1]
School of Forestry, Wildlife, and Fisheries
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

ABSTRACT Commercial production of soft crawfish for human consumption was initiated in Louisiana in 1985. During the 1988–89 crawfish season, an estimated 300 producers were thought to exist in Louisiana, Texas, Mississippi, Florida, and the Carolinas. Immature red swamp crawfish (*Procambarus clarkii*) are collected from ponds and natural habitats, placed in culture trays at high density, and fed. Advanced premolt crawfish are transferred to a molting tray for molting and to control cannibalism. Freshly molted crawfish are packaged and frozen. Even though the soft crawfish is new to the seafood industry, repeat sales are a healthy sign that the product is becoming established.

KEY WORDS: crawfish, soft-shell, molting, *Procambarus*

INTRODUCTION

In 1985, technology for commercial production of soft-shell crawfish for human consumption, commonly referred to as soft crawfish, was released in Louisiana (Culley et al. 1985a, b). Soft crawfish are produced by holding marketable immature, intermolt crawfish at high density in culture trays and feeding the crawfish until they molt. Once molted, the crawfish are removed, processed, packaged and sold. In 1985–86, seven commercial producers marketed soft crawfish to restaurants in Louisiana. Thirty-five soft crawfish producers were identified in 1986–87, and about 150 producers in 1987–88. For the 1988–89 season approximately 300 producers were identified. Most of the soft crawfish operations are in Louisiana, but operations are also located in Mississippi, Texas, the Carolinas, and Florida.

In Louisiana there are two species of crawfishes of suitable size for soft crawfish systems. The red swamp crawfish (*Procambarus clarkii*) is the species of choice (Culley et al. 1985b). The white river crawfish (*P. acutus acutus*) has not responded well to intensive culture. World-wide there are many species of crawfishes that could be suitable for producing soft crawfish. However, other species may not molt adequately in culture systems designed for red swamp crawfish.

The following sections discuss general aspects of the crawfish life cycle and pond management, selection and collection of immature crawfish, types of facilities, procedures for producing and packaging soft crawfish, and economic consideration.

THE CRAWFISH LIFE CYCLE AND POND MANAGEMENT

In southern Louisiana young-of-the-year crawfish are produced in all months but major spawning in managed impoundments occurs in September and October with a lesser peak November–January and March–April (Avault and Huner 1985). Culture ponds are generally flooded in September and October and by late November harvesting begins, and it continues through May–June or until ponds are drained (Avault and Huner 1985). With some variation in time, production in natural areas follow a similar annual cycle, but rainfall affects the timing of the cycle.

The soft crawfish aquaculturist is dependent on this annual production of crawfish. If the season is long (November–June) soft crawfish can be produced 7 months. If the fall, winter, and spring are unusually cool, soft crawfish may be produced in 4 months, February–June.

Commercial production of soft crawfish focuses on the molt cycle. Molting is more frequent in young crawfish, and in warm water. Young crawfish may molt every 5–10 days. Older, immature crawfish usually molt within 30 days (Huner and Barr 1984). After the crawfish molts the carapace is soft for several hours, and at this stage the whole animal is edible, and thus harvested.

SELECTION, COLLECTION, AND TRANSPORTATION OF CRAWFISH

A reliable source of immature red swamp crawfish is essential for successful production of soft crawfish. The supply of hard-shelled (intermolt) crawfish comes from commercial ponds or natural habitats. The key to successful soft crawfish production is knowing which crawfish to select. Sexually mature crawfish molt infrequently and are not used. Immature crawfish molt readily in captivity when provided proper care. Crawfish, 70 mm total length or larger, are retained for use in soft crawfish shedding operations.

Ideally, soft crawfish producers should own, or have control of ponds where hard crawfish are collected (Culley and de la Bretonne 1988). Poor control over the source of intermolt crawfish increases risk of high mortality because poor handling of the crawfish reduces survival. Normally,

[1]Current address: Department of Biology, Murray State University, Murray, Kentucky 42071

Approved by the Director of the Louisiana Agricultural Experiment Station as manuscript number 89-22-3275.

the soft crawfish culturist is dependent on a pond owner or trapper to supply crawfish. The trapper must understand how crawfish are to be selected, collected, and delivered to soft crawfish producers.

Crawfish should be gently poured from traps in ponds onto a sacking table in the harvest boat. Attendants should carefully, but quickly, select immature crawfish to be sold to soft crawfish producers. Crawfish selected are placed in a container, usually a ventilated sack. About one-half of the sack should be filled (about 10 kg of crawfish). Sacks should be tied at the top, layed horizontally to evenly distribute the crawfish and placed in a protected area, to minimize exposure to extreme environmental conditions.

Transporation of crawfish is stressful. Factors that contribute to stress include high temperature, poor ventilation, vibration, time out of water, and condition of the crawfish. The longer the time between trapping and placement of crawfish in the soft culture system, the higher the mortality. Ideally crawfish should be transported in a van or covered truck.

ACCLIMATION TECHNIQUES

When hard-shelled crawfish are brought to the soft crawfish facility, they must be acclimated to the new environment (Culley and Duobinis-Gray 1988a). Acclimation time ranges from 24–72 hr. A good acclimation technique, developed by a commercial producer, is as follows:

1. Gently place crawfish into a dry, culture or acclimation tray;
2. Immediately cover the trays with an insulated top (most owners use 19-cm styrofoam insulation covered by aluminum or plastic film). Do not disturb the crawfish for at least 2 hr and up to 12 hr is acceptable;
3. Drip culture water into the tray from 8–16 hr but do not allow it to accumulate; and then
4. Increase water flow to a slow, steady stream. After several hours increase the water flow rate again and maintain the flow overnight. On day 2 increase the water flow to ½ the recommended rate for several hours and then increase the flow to the recommended rate of about 0.63 litres per minute per kg of crawfish. The removable overflow drain is installed to raise the water depth to 1.9–2.5 cm in the tray several hours after full flow is initiated.

A formulated crustacean ration is usually fed to crawfish daily 24 hours after initial acclimation. Crawfish will consume 3–5% of their body weight daily for several days after acclimation, but the feed consumption rate declines to 1–2% per day thereafter. Crawfish are fed 2–3 times daily.

CULTURE TRAY MANAGEMENT

During the crawfish season, immature crawfish collected from traps are placed in culture trays containing shallow water (Fig. 1). Feed is provided daily. Premolt crawfish, identified by a color change in carapace (Culley et al. 1985b), are removed once daily from the culture trays and placed in a molting tray. Molted crawfish are immediately collected, refrigerated, packaged and frozen the same day. Details of these steps are covered in the following sections.

Most commercial soft crawfish producers use trays that are 1-m wide × 2.7-m long. Soft crawfish culture trays should be maintained at a density of 4.9 kg per m^2,, or 270–320 crawfish per m^2 (Culley and Duobinis-Gray 1987a). Premolt and dead crawfish are removed once daily. Replacement crawfish and feed are then added to the trays. Most attendants replace premolt and dead crawfish once a week, but daily replacement is preferable.

Crawfish held at high density are not seriously cannibalistic if fed properly. However, if a crawfish molts in a tray with intermolt crawfish, it is usually eaten. Cannibalism is greatest when crawfish have been received in poor condition. If crawfish are not injured or stressed, mortality should not exceed 1% per day in culture trays and 0.3% per day in the molting trays (Duobinis-Gray and Culley 1989).

Premolt Development

As intermolt crawfish approach the advanced premolt stage (2 days from molting) the exoskeleton becomes distinctly darker in color, and brittle (Culley et al. 1985b). Each species of crawfish will have a different color pattern, and it may not be easy to use color change on some crawfish species. The following pattern and time sequence are evident in the red swamp crawfish at 23–25°C:

1. Dorsal area of carapace begins to darken, turning grayish brown to brown (7–10 days from molting);
2. Dark coloration migrates down the sides of the carapace, and the lower edge of carapace (immediately

Figure 1. A typical commercial soft-shell crawfish tray system in Louisiana (photograph courtesy of Lyle Soniat, LSU Sea Grant Program).

above the base of the periopod) is still firm when squeezed (5–7 days);
3. Sides of carapace continue to darken (3–5 days);
4. Carapace becomes uniformly dark, and lower edge of carapace begins to weaken, becoming flexible when pressed (2 days); and
5. Lower edge of carapace begins to slip upward, exposing the new underlying soft exoskeleton (<1 day). The crawfish molts within 24 hours.

When the crawfish is within 1–2 days of molt it is transferred from the culture tray to a molting tray to prevent cannibalism by intermolt crawfish. One molting tray is required for every 10 culture trays when the system is operated at full capacity. The density of the crawfish in the molting tray should not exceed the density in the culture trays (4.9 kg/m^2).

The temperature in the molting trays is maintained the same as in the culture trays, 25 ± 1°C. Crawfish must be removed within 3 hr of molting to be of prime market quality. If water temperature in the trays is 20–22°C crawfish remain soft for 8–10 hr, but for prime quality they should be removed within 6 hr. About 90% of the molting occurs from 0700–1800 hours (Culley and Duobinis-Gray 1987b), thus attendance of the system at night is seldom necessary.

The soft crawfish, when collected from the molting trays, are placed in a container without water. After collection, the soft crawfish are placed in cool water (<5°C). The cool water slows hardening of the exoskeleton, allowing the attendant to collect and hold soft crawfish over the entire day before packaging. If soft crawfish are left overnight without processing, the exoskeletons of some will harden to the point that the crawfish cannot be marketed, although they are still edible.

WATER QUALITY

Well water, surface water, or treated recycled water can be used in soft crawfish culture systems but the source water must be of acceptable quality. If a city water supply is used, chlorine must be removed either by aeration or with an activated charcoal filter. If chloramine is added to the water instead of chlorine, the charcoal filter will remove the chlorine, but not the ammonia. Thus, a biological filter must be added to the system.

Because a high volume of heated water is required, many commercial soft crawfish systems treat and recycle their water. A typical culture tray (1 m × 2.7 m) will hold 12 kg of crawfish and it requires a constant flow of water. The filtration system consists of a fluidized bed and upflow sand filter (Malone and Burden 1988). A total system volume of 42 to 84 liters of water are required for each kg of crawfish in the system.

Water temperature should be maintained around 25 ± 1°C, but temperature as high as 32°C is acceptable. Above 32°C the molting rate is rapid but mortality also increases. Below 21°C the molting rate significantly decreases, reducing production.

Water quality standards suitable for warm-water fish culture are also acceptable for crawfish: pH, 6.5–8.5; total hardness and total alkalinity (both as calcium carbonate) over 20 mg/l; and dissolved oxygen, above 3 mg/l (Culley et al. 1985b). High calcium in the water causes the soft crawfish to harden faster. Less than 20 mg/l of total hardness is not detrimental and may be beneficial by lengthening the time for calcification of the new exoskeleton.

Hydrogen sulfide (H_2S, toxic above 0.1 mg/l), saline water (8 ppt), pesticides, sewage, industrial waste, and nitrogenous compounds can also cause problems in some soft crawfish systems. An analysis of the quality of water to be used in the soft crawfish system should be made prior to facility development.

Crawfish excrete ammonia into the water. In addition, waste feed and fecal matter contributes to the total ammonia (NH_3 + NH_4) load. Total ammonia should be less than 0.5 mg/l (Culley and Duobinis-Gray 1988b, Malone and Burden 1988).

Nitrite (NO_2) is toxic to crawfish at low levels. In flow-through systems nitrite does not attain toxic concentrations. In recirculating systems bacteria convert ammonia to nitrite, and nitrite to nitrate (non-toxic). Soft crawfish producers operating recirculating systems must periodically monitor nitrite levels. Nitrite should not exceed 0.5 mg/l for more than 2–3 consecutive days, and it should be maintained below 0.2 mg/l (Culley and Duobinis-Gray 1988b).

Iron is extremely toxic to crawfish. Concentrations of iron as low as 0.1 mg/l have been observed to cause crawfish death, particularly in molting trays (Culley and Duobinis-Gray 1988b).

PROCESSING

Soft crawfish are rarely marketed as a fresh product because claws and legs may be damaged by repeated handling. Until 1989, most of the product was frozen in 1 liter plastic bags with 454–680 g of crawfish per bag. Water is placed in the bag to remove air, excess water is removed, and the bag is then flattened and immediately frozen. The ice prevents freezer burn and provides structural support to prevent loss of claws and legs.

Current new packaging techniques include vacuum, and vacuum shrink-wrap packing. Several factors must be considered in developing new packing techniques: air must be excluded; the product should be seen; the pack should be thin; the package should be tamper-proof; it must be capable of being frozen quickly; and packages must stack efficiently in storage.

Soft crawfish are processed by the buyer. Because a crawfish ceases to feed 24–48 hr before molting, the intestine is empty and need not be removed. The gastroliths (two hard hemispherical calcium carbonate secretions be-

hind the rostrum) must be removed. About 92% of the product is edible (Culley et al. 1985a). The internal organs, such as hepatopancreas, are rarely removed because they give distinctive flavors to crawfish dishes. If internal organs are removed, then 82% of the animal remains. Occasionally the carapace and the hepatopancreas (fat) is removed, leaving 72% edible product.

CRAWFISH SIZE AND MARKETING

Soft crawfish are successfully marketed in sizes ranging from 44–100 count per kg with the most common size being 60 count. Crawfish larger than a 44 count per kg size are not readily available in large quantities. Crawfish smaller than a 100 count per kg are frequently abundant but the soft crawfish producer must increase time and labor to molt smaller crawfish. Approximately 90% of all soft crawfish produced for human consumption are between 50–80 count.

STRUCTURAL CONSIDERATIONS

Soft crawfish must be cultured within an enclosed building, a greenhouse, or some other kind of structure that will provide protection from predators such as mammals and birds; offer personnel a comfortable work environment; protect equipment and electrical circuits; and provide reasonable environmental control. The building need not be elaborate.

Greenhouses are popular and one of the most economical units. They should be covered with black plastic, sheet metal, or if clear plastic is used, no less than a 92% shade cloth will be required to prevent solar radiation from injuring the crawfish. However, because of rapid heat loss and buildup in the culture tray water, covers will be required for each tray. Most producers use one-half inch styrofoam-type insulation panels used to insulate the walls of houses.

Metal, concrete and wooden buildings are widely used. Compared to green houses they are expensive, so their use is generally limited to existing structures. Temperature control in buildings is different from greenhouses. The buildings heat up much more slowly in the mornings, and on cloudy days remain quite cool in the winter. However, they tend to cool down more slowly at night.

ECONOMICS OF COMMERCIAL SYSTEMS

As the soft crawfish industry developed, cottage-type facilities were constructed. Until the 1988–89 season, most facilities contained less than 700 kg of crawfish (60 trays) with the average facility containing 300–400 kg of intermolt crawfish. In the 1988–89 season several facilities in operation, holding from 1,200–10,000 kg of intermolt crawfish (100–800 trays).

When a system is properly operated, the molting rate average over a 1–7 month season should be about 2%/tray/day/of the total population, or 0.26 kg (0.5 lbs)/tray/day. Most facilities are not this efficient, producing a 1–1.5% molting rate/day. When a system is first placed in operation, it takes up to 60 days to obtain a molting rate that will be above the seasonal average. There will be days when the molting rate will exceed 5%, but when averaged over the season the rate is less.

The breakeven cost for a well managed system is about $10/kg of soft crawfish ($4.50/lb). The true cost for commercial systems is probably about $13/kg ($5.75/lb) because of operational inefficiencies. Labor accounts for about 28% of the operating and fixed costs if the owner provides most of the labor. Purchase of crawfish will account for nearly 40% of the operating and fixed costs, assuming crawfish will cost $2.20/kg.

Wholesale prices range from $13–$22/kg ($6–10/lb) depending on where the producer markets the product. The lower prices are paid by seafood brokers. Very efficient systems will give net returns (profit) of about 40% if the producer can get $17/kg. The average commercial system probably has a maximum net return of 10%.

There are several ways of calculating the economics of soft crawfish production. Caffey (1988) used a 454 kg (1,000 lb) system as a standard unit for developing an economic budget. He assumed land would be available, but the owner would have to drill a well, treat and recycle water, purchase a greenhouse, and provide most of the construction time. He calculated that construction of the facility would cost $15,000. If properly managed, the income from sales ($17.60 kg) over a 6 month season would be $34,560. Operating costs were $18,153, and net returns were $16,407. Break-even prices were projected to be $9.24/kg ($4.20 lb) with a 2.5% molting rate per day. This level of efficiency is biologically obtainable, but has not been achieved outside of laboratory conditions.

The economic future of the industry is uncertain at this time. Prices fluctuate, and small producers are in the business primarily to provide supplemental income. It is difficult for them to find time to market their product, thus they depend on seafood brokers to market their product. The brokers currently pay the lowest prices.

The soft crawfish is a good product, and one positive aspect of this new product is that repeat sales are evident. Efficiency of operation and a consistent market demand will be the key to survival.

REFERENCES CITED

Avault, J. W. & J. V. Huner. 1985. Crawfish culture in the United States. In Huner and Brown (eds.) *Crustacean and Mollusk Aquaculture in the United States*, AVI, Westport, CN, pp. 1–61.

Caffey, R. H. 1988. An economic analysis of alternative soft-shell crawfish production facilities. *1988 Amer. Agri. Econ. Assoc. Annu. Meet.*, Knoxville, Tenn. (In press).

Culley, D. D. & L. de la Bretonne. 1988. Selection and collection of crawfish to support the soft crawfish industry. *Crawfish Tales* 7(4):30–33.

Culley, D. D. & L. Duobinis-Gray. 1987a. Molting, mortality, and effects of density in a soft-shell crawfish culture system. *J. World Aquacult. Soc.* 18:242–246.

Culley, D. D. & L. Duobinis-Gray. 1987b. 24-hour molting pattern of the red swamp crawfish (*Procambarus clarkii*). *Aquaculture* 64:343–346.

Culley, D. D. & L. Duobinis-Gray. 1988a. Selection and acclimation of crawfish used for soft crawfish production. In R. Reigh (ed.). *La. Aquacult. Conf.* La. State Univ. Agricult. Center, Baton Rouge, LA, pp. 30–32.

Culley, D. D. & L. Duobinis-Gray. 1988b. Ammonia, nitrite, and iron toxicity in soft crawfish culture systems. *Crawfish Tales* 7(3):24–26.

Culley, D. D., M. Z. Said, & P. T. Culley. 1985a. Procedures affecting the production and processing of soft-shell crawfish. *J. World Aquacult. Soc.* 16:183–192.

Culley, D. D., M. Z. Said, & E. Rejmankova. 1985b. Producing Soft Crawfish: A Status Report. La. Sea Grant College Program, La. State Univ.

Duobinis-Gray, L. & D. D. Culley. 1989. Molting response of red swamp crawfish to commercially available feeds. *Crawfish Tales* 8(1):15–18.

Huner, J. V. & J. E. Barr. 1984. Red swamp crawfish: biology and exploitation. La. Sea Grant College Program, La. State Univ., Baton Rouge, LA. 136 p.

Malone, R. F. & D. G. Burden. 1988. Design of recirculating soft crawfish shedding systems. La. Sea Grant College Program, La. State Univ., Baton Rouge, LA., 74 p.

Journal of Shellfish Research, Vol. 8, No. 1, 293–301, 1989.

PROCESSING OF FRESHWATER CRAWFISH: A REVIEW

MICHAEL W. MOODY
Louisiana Cooperative Extension Service
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

ABSTRACT The Louisiana crawfish processing industry is comprised of 102 processing facilities located in south central Louisiana. There are two commercially important crawfish species: the red swamp crawfish and the white river crawfish. The major crawfish product forms are: whole, live crawfish; whole, raw and frozen crawfish; whole, cooked and frozen crawfish; cooked, peeled and deveined crawfish meat. There are three processing phases: storing and cooking of the live crawfish; meat removal and waste disposal; and packaging and storing of the finished product. Specific processing steps include: receiving the live crawfish, grading; washing; cooking; meat removal; inspecting and packaging.

KEY WORDS: crawfish, aquaculture, processing, seafood

INTRODUCTION

The processing of freshwater crawfishes native to Louisiana (*Procambarus clarkii* and *P. acutus acutus*) and surrounding states is an important economic and cultural activity. Advances in crawfish aquaculture and adoption of recommended production management methods by commercial crawfish producers have made supplies of crawfish readily available to more than just regional markets. Crawfish produced in Louisiana have become important to international markets where native stocks have been depleted by disease or overfishing (Huner 1989, Roberts and Dellenbarger 1989).

Some processing methods and techniques currently practiced by the crawfish industry have evolved over decades. Others are relatively new but have been adopted by the industry to take advantage of changing marketing opportunities. Processing of crawfish probably developed from market demands for live crawfish. During the crawfish season (November–June), heaviest demand for live crawfish by consumers is traditionally, Friday through Sunday. Demand of crawfish Monday–Thursday was poor and consequently this resulted in problems in product flow.

Enterprising "processors" would blanch crawfish and peel out abdominal (tail) meat for sale to salvage crawfish that could not be sold live. Presently, crawfish processing is a modern industry that takes advantage of current technology and uses scientific principles to deliver a high quality finished product world-wide.

In 1989, there were 102 licensed crawfish processing facilities in Louisiana. The facilities are confined to the south-central portion of the state in areas of live crawfish production (Fig. 1). As crawfish aquaculture expands to other regions of the state, crawfish processing plants will follow.

Two major species of crawfishes of commercial importance are processed in Louisiana: the red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus acutus acutus*). Although the red swamp crawfish is the preferred species, because the public perceives that it is more desirable in taste and the attractive color of the hepatopancreas, both species are processed simultaneously. Marshall et al. 1988, reported that a sensory panel found no significant difference in taste between the two species, and that shear force measurements revealed no significant difference in meat texture. The only significant difference in processed meat from red swamp and white river crawfishes was color. Normally, the red swamp crawfish is the dominant species, generaly comprising 90% of the commercial harvest. The two species are harvested collectively.

The intensity of crawfish processing is determined by crawfish supply. Two sources of crawfish are available to processors:

1. crawfish cultured in managed impoundments, and
2. crawfish from the native wild stocks found in natural waterways throughout Louisiana, but especially in the Atchafalaya River Basin in south-central Louisiana.

Crawfish produced from aquaculture are available to processors as early as November. Wild stocks become available as early as late winter. Peak crawfish production from both sources occurs in March, April and May (Huner and Barr 1984), and crawfish harvested in these months are of highest quality.

Crawfish processing activities correspond to crawfish production activities. In late fall and early winter when crawfish availability is inconsistent and prices are volatile, there is minimal processing activity. Processing peaks when crawfish prices are lowest. Seasonal crawfish production is a significant problem to crawfish processors. Because production may be only 6 months, the processing facility may be idle for 6 months. Crawfish processors often process other species of seafood in the "off-season" to maximize utilization of the facility. For example, blue crabs are plentiful in the Gulf of Mexico in summer and fall when crawfish are not available. Because the same equipment used to process crawfish can be used to process crabs, some crawfish processors process crabs in the off season.

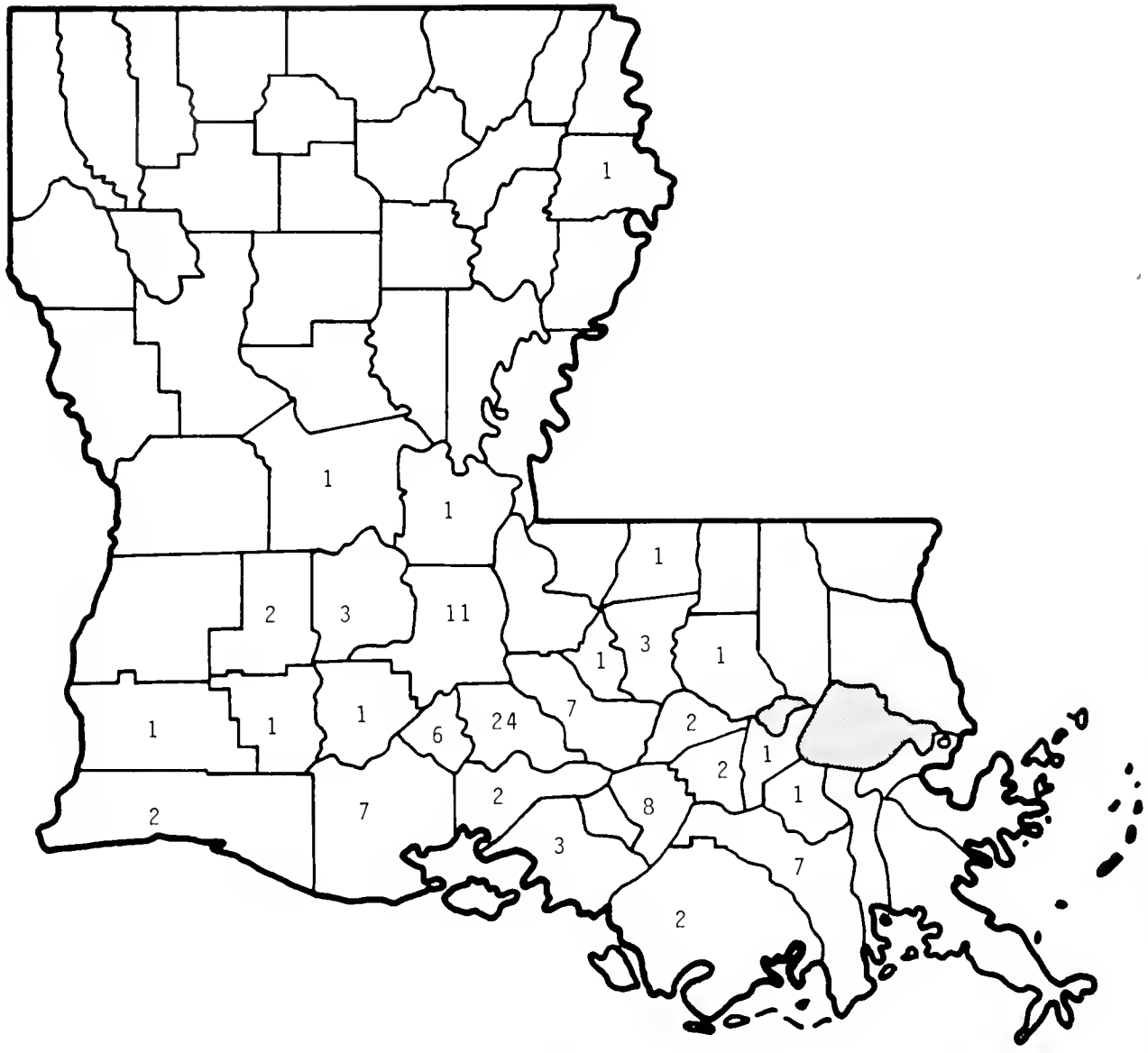


Figure 1. Number of Louisiana Crawfish Processing Plants by Parish.

Ownership of crawfish processing facilities is often diverse. Some are locally owned by individuals or families; others are owned cooperatives; and some are complex partnerships involving international interest.

Crawfish processing facilities vary in capacity of crawfish processed and procedures used. Small facilities process fewer than 450 kg of live crawfish on an average day during the peak of the season. A large facility may process more than 9,000 kg/day. The crawfish processing industry is labor intensive, with manual handling in nearly every phase of the processing scheme.

CRAWFISH PRODUCTS

Whole, Live Crawfish

Whole, live crawfish is one of the more important products. Consumption of whole, live crawfish is about 45% of annual production. Local markets, mostly households and restaurants, are the major purchasers of whole, live crawfish for boiling. Air shipments of whole, live crawfish to national and international markets are also important.

Generally, crawfish can be maintained alive and in good condition for several days when conditions are favorable. As crawfish are harvested, from either ponds or wild stocks, live crawfish are tightly packed into mesh bags or sacks. The bags are the same type used to package onions in U.S. markets. Packaging or bagging live crawfish in sacks has several advantages. First, tight packing restricts their movement, it minimizes their aggressive behavior, and it facilitates ease in handling and transportation. The mesh sacks also permit adequate air circulation and permeation needed to keep crawfish alive. Crawfish mortality in

sacks is increased when air circulation is restricted, and this can occur in sacks with wide labels or when sacks are excessive stacked.

Other factors contributing to the shelf-life of whole, live crawfish are storage conditions, especially relative humidity and temperature. To minimize dehydration of the crawfishes gills, 100% humidity should be maintained. Storage temperatures in coolers should be 1.6–4°C to slow metabolism and extend shelf-life. Some processors put a thin layer of crushed ice on top of the sacks to reduce crawfish metabolism.

A low velocity fan in the cooler will ensure uniform conditions throughout the storage facility (Wheaton 1985).

Whole, Raw and Frozen Crawfish

Although not as popular as other products, whole, raw and frozen crawfish are used by some customers. Live, whole crawfish are rapidly, and economically, quick frozen in sacks by immersion into a brine freezer (−20°C) (Bankston 1984). The end product is individually frozen crawfish will little or no ice glaze. Providing an ice glaze gives some product protection during handling. The main disadvantages of this product is dehydration during storage caused by large surface area exposure; and product damage that can result from rough handling of fragile frozen crawfish. This product, when thawed should be used quickly because raw crawfish decompose relatively fast.

Whole, Cooked and Frozen Crawfish

With the incorporation of more efficient chilling and freezing systems in modern crawfish processing facilities, many plants are producing whole, cooked and frozen crawfish. For local and national markets, this crawfish product often is spiced with Cajun seasoning. For European markets, especially in Scandinavia, dill seasoning is used. Crawfish are generally cooked, seasoned and packaged in 1 kg containers. A broth of the desired seasoning is usually packaged with the crawfish and the product is vacuum packed. The package is quick frozen using cryogenic freezers. Because of the state-of-the-art technology used to manufacture this product, excellent shelf-life quality is expected.

Cooked, Peeled and Deveined Crawfish Meat

The standard product from crawfish processing is cooked, peeled and deveined tail meat. It may be either fresh or frozen, and washed or unwashed. In its most desirable form, the meat is in a single unbroken piece and is mottled on the dorsal side with a red pigmented membrane. The vein (intestine) is removed and discarded during the process operation. The vein, which is black, is unappetizing in appearance and is considered a quality defect if packed with the finished meat. The two long, thin twin muscles (called "backstrap") covering the vein should be left attached to the tail meat. These muscles can become detached with rough handling during peeling or washing. Edible meat is also located in crawfish claws, but because of the difficulty and effort required to extract it in useable quantities, it is not considered economically feasible to remove it. Consequently, claws are discarded along with other crawfish waste.

Except for the red membrane, crawfish meat is opaque white to pink. Crawfish meat may be packed with or without the natural adhering hepatopancreatic tissue (called "fat"). Crawfish meat is generally used as an ingredient for the preparation of other dishes or products. Many times the product will be labeled "partially cooked" or "requires further cooking."

CRAWFISH PROCESSING

Crawfish processing plants are designed to accommodate three processing phases: storing and cooking of the live crawfish; meat removal and waste disposal; and packaging and storing of the finished product. Generally, plants are designed to facilitate efficient product flow from one phase to another and, at the same time, avoid cross contamination of the product.

Storage and Cooking of the Live Crawfish

Receiving the Live Crawfish

The quality of crawfish received at the processing plant depends on precautions and care taken at harvest and subsequent transportation to the processing plant. Crawfish should be protected from excessive heat on warm, sunny days and protected from dehydration caused by windy conditions. Sacks of crawfish transported to processing plants should not be exposed to the climatic elements and wind. Excess stacking of sacks of crawfish can crush and injure crawfish, especially early in the season when crawfish are immature and exoskeletons (shells) are delicate. Fishermen must avoid contamination of crawfish with fuels, oils and other foreign substances on the harvesting vehicle. At certain times and in certain areas, crawfish are stressed from poor water quality or other factors. These crawfish should be handled with care and processed rapidly. Dead and injured crawfish are rejected and discarded as waste.

Once received at the plant, crawfish are processed immediately or stored live in coolers until processing. Coolers are often detached from the main processing facility to avoid cross-contamination with finished products. Environmental conditions within the live coolers must be maintained as previously discussed to maximize the shelf-life of the live crawfish. Crawfish brought to the plant in good physical condition can be maintained alive and in prime condition for several days using recommended storage procedures.

Grading

With the recent development of whole, cooked crawfish as a major sales item, grading has become standard in

Figure 2. Sacked, live crawfish awaiting processing.

crawfish processing plants. Although some hand grading occurs at harvest and in processing facilities, most grading is done with equipment that minimizes injury to the live crawfish and is capable of separating large volumes of crawfish. Most graders use variable-spaced bars or slots through which different sizes of crawfish pass. For example, one type of grader is a tilted rotating drum with variable-spaced bars. The small to medium sized crawfish will fall through the bars as the drum is rotated, and the large crawfish discharged from the open-end. Other graders use small rotating paired cylinders that have graduated spacing. As the crawfish move down the cylinders from the narrowest spacing to the widest spacing, the crawfish are graded by falling between the cylinders at spacing that corresponds to crawfish size. Other grading devices use the same principle for separating crawfish but belts or grates may be used. Water sprayed on crawfish during grading improves the efficiency of the device.

Currently, crawfish are graded into two or three sizes. The largest are used in the whole, cooked crawfish market and for shipments of live crawfish. The small- to medium-sized crawfish are peeled.

Washing

Crawfish are washed prior to processing. Wash tanks are patterned after washers used in the shrimp and crab industries. A washer is a narrow rectangular tank, deep (about 1 M) at the feeder and shallow at the discharge. A revolving belt on the bottom of the washer lifts the crawfish from the tank at the discharge. Washing separates foreign material, including soil, grass and bait, from the live crawfish. It also permits inspection of crawfish on the conveyor belt before cooking, and it systematically fills cooking baskets.

Cooking

To prepare crawfish for meat removal or to prepare crawfish for the whole, cooked market, crawfish must first be heat treated or blanched. After washing, the crawfish are loaded into metal baskets for cooking. If the crawfish are processed for meat, the crawfish are boiled in clean, unseasoned water. If crawfish are cooked for the whole cooked market, the water may or may not be seasoned. The water may be heated by one of several ways. Many smaller to medium-sized processing plants use natural gas heaters to heat the water. Although this method is the easiest and most the economic it is also the slowest and least efficient. More progressive processing plants use steam to heat water for cooking the crawfish. Steam can injected directly into the water, or a steam jacketed kettle can be used to heat the water. Rather than using batch cooking procedures as described, some larger processing facilities use continuous cooking systems that cook with hot water or live steam.

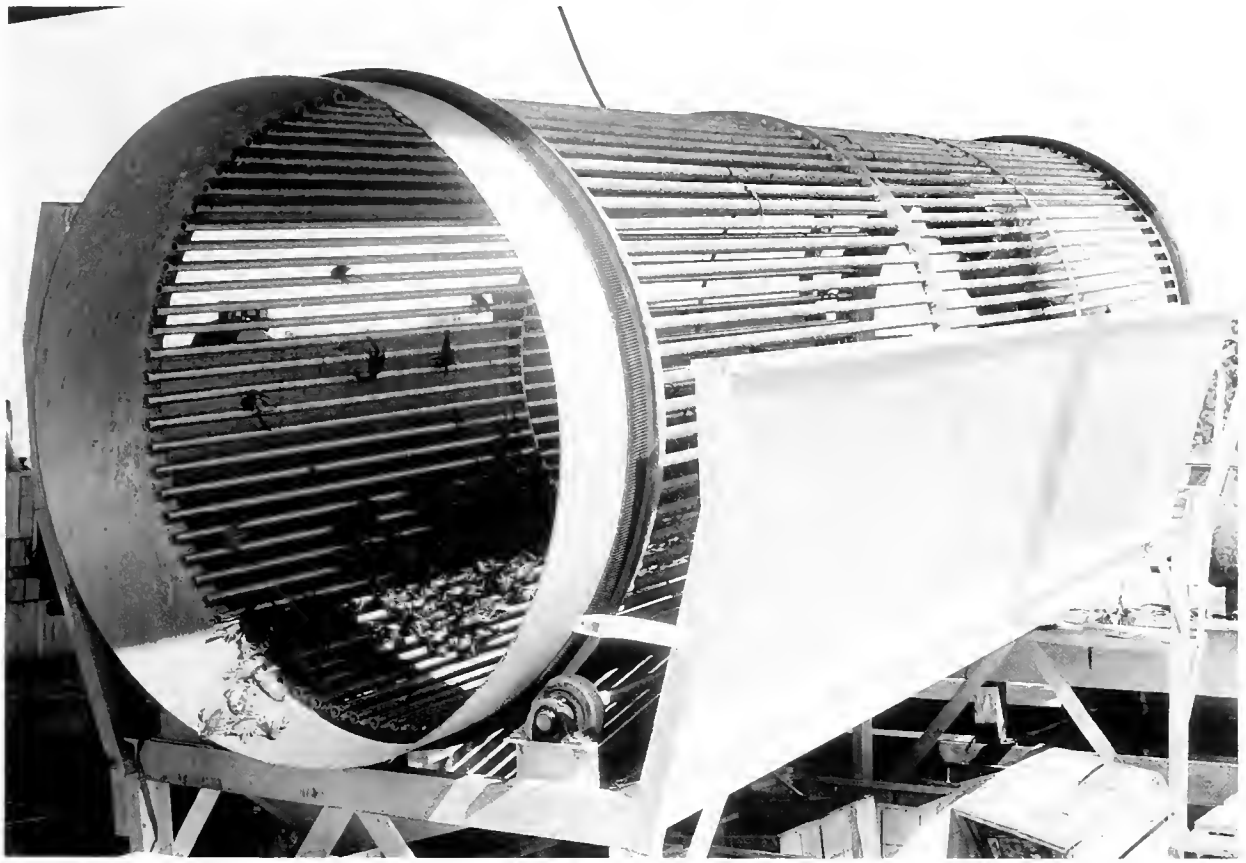


Figure 3. Crawfish Grader.

Cooking is the most critical step in processing crawfish to be peeled for meat. Excessive cooking makes meat removal difficult. A more serious problem occurs if the crawfish are undercooked. The hepatopancreas, an important flavor ingredient, is often packaged with ice-stored peeled tail meat. In some cases, it is packaged with frozen meat. The presence of natural adhering hepatopancreas has been implicated in textural problems in crawfish meat (Marshall et al. 1987). Crawfish that are undercooked have softer, mushier meat than crawfish that are adequately blanched. The soft meat texture is attributed to heat labile proteolytic enzymes in the hepatopancreas. A simple in-plant test using gelatin as a test substrate can be tested for the adequacy of blanching time for individual plants.

When crawfish have been adequately blanched they are removed from the water and air cooled. Some processors will use a spray of water to accelerate cooling. For whole, cooked crawfish products, crawfish may be cooled in a seasoned solution to intensify the desired flavor.

Meat Removal Processing

The extraction of meat from crawfish is very labor-intensive and requires a large work force. Although attempts have been made to mechanize crawfish meat removal, no devices are used on a commercial scale. In 1978, a crawfish peeling machine was patented (Rutledge 1978) and used for several years. The machine was effective and reduced labor cost. The Rutledge peeler used a patented brine freezing procedure to freeze the whole, live crawfish prior to peeling (Rutledge 1977). The freezing process facilitated the easy removal of the meat after the crawfish were thawed and run through the machine. The peeling machine used a roller device and a synchronized feeder belt to extract the whole, raw abdominal meat from the detached tail. Meat from this process was packaged raw and frozen. A newer, commercially untested machine, the Duzitall uses compressed gas to separate the cooked abdominal meat from the shell. A third device uses steam heating and manipulation of pressure to loosen the exoskeleton to ease hand peeling. This device is used by several processors to cook crawfish for the whole, cooked market.

Hand peeling takes place in a large room especially designed for the procedure. Peelers are stationed at long, stainless steel or aluminum tables. Peelers are normally paid according to the weight of meat processed. The number of peelers in any particular plant depend on the amount of table space available and the quantity of crawfish to be peeled, however, a large crawfish processing facility may use 75–100 peelers a day during the peak of the season. As in most seafood processing plants, peelers are

Figure 4. Crawfish washing prior to cooking.

provided all the necessities of a sanitary operation—running water, sanitizing hand dips, and hand washing facilities. No special tools or knives are used to extract the tail meat from the crawfish (Moody 1980).

Crawfish are spread in the middle of the peeling table while still warm for peeling. Warm crawfish are easier to peel than cold crawfish. The meat with natural adhering hepatopancreas is placed in a sanitized colander as it is removed from tail, and the shell including cephalothorax and claws is discarded as waste. Crawfish peelers remove extaneous material such as the vein and pieces of shell. Good hygiene procedures and practices must be closely adhered to in meat removal to ensure a high quality product with maximum shelf-life.

Yield

Meat yields (cooked abdominal meat to total live body weight) from cooked crawfish depends on several factors. Many processors assume an overall yield of 15%. Size and maturity of the crawfish have a significance influence on yield.

For example, Huner 1988, showed that smaller crawfish, regardless of sex, had a higher meat yield. Meat yield ranged from 10–26%. Also immature crawfish (both male and females) had meat yields that were consistently 4–5% greater than mature males. Marshall and Moody (1986) reported that cooking times and procedures also have an effect on meat yield.

Hepatapancreas

The hepatopancreas referred to as "fat" by processors and consumers is an important constituent in finished and packaged crawfish meat. Regulatory agencies consider naturally adhering hepatopancreas a part of the net weight of the finished peeled and deveined product. When crawfish are peeled by hand, hepatopancreas is usually extracted from the cephalothorax by adherence to the tail meat.

The amount of hepatopancreas tissue that adheres to the meat during peeling is influenced by several factors including blanching time (Marshall and Moody, 1985). Maximum hepatopancreas adherence is achieved using recommended blanching times. Peeling methods have an effect on the amount of adhering hepatopancreas. Marshall and Moody 1985 showed that the amount of adhering hepatopancreas varies with the season. In general, there is an increase in the percentage of hepatopancreas tissue with the progression of the wild crawfish season. The amount of hepatopancreas adhered to abdominal meat ranges from 2.2–13.1% meat weight. The seasonal average is 8.14%. Huner 1988 showed that crawfish meat with adhering he-

Figure 5. Crawfish packing line for whole, cooked crawfish.

patopancreatic tissue increased 1.5–2.0% yield compared to crawfish meat without adhering hepatopancreas.

Although the hepatopancreas is an important flavoring constituent in many crawfish dishes, this tissue is also responsible for undesirable flavors associated with frozen crawfish meat held under extended freezer storage.

Waste Disposal

The crawfish industry generates considerable waste from the peeling operation. About 85% of the total crawfish by weight received by crawfish meat processing plants is discarded as waste. Although some of this waste is dried for use in animal feeds, most is disposed in sanitary land fill.

PACKAGING AND STORAGE

Inspecting

Peeled, deveined crawfish meat is conveyed from the peeling tables to the packaging room on a predetermined schedule. To maintain high quality, most seafood processors allow the meat to remain in colanders for no more than 45 minutes before packaging occurs. After delivery to the packaging room, the meat is spread onto large trays and inspected for remaining pieces of digestive veins and bits of shell. The extraneous material is removed.

Packaging

Peeled and deveined crawfish meat is packaged according to shelf-life requirements, or according to specifications of customers. Crawfish meat for the fresh-market trade is generally packaged in polyethylene bags. The most common net weight for fresh crawfish meat is 0.454 kg although 0.341 kg packages can sometimes be obtained. The packaging process for fresh-meat packs involves "hand weighing" meat and adhering hepatopancreas, placing it in bags, and heat sealing the bag. Care must be taken to eliminate air from the bag before sealing. Most processors place sealed bags into an ice slush to chill the meat before storage. Shelf-life for fresh meat depends on the quality of the meat packaged, but typically, shelf-life is from 7–12 days if the packages are stored in crushed ice.

When extended shelf-life is required, crawfish meat is generally frozen. Marshall (1988) demonstrated that freezing crawfish meat caused a toughening of the crawfish tissue compared to non-frozen meat. The freezing methods, still freezing at −23°C, blast freezing at −40°C, or cryogenic freezing, did not have a differential effect on toughening or texture, but the time of storage did.

When crawfish meat is frozen, the hepatopancreas must be removed because the hepatopancreas is responsible for

Figure 6. Cyrogenic freezing system used to freeze whole, cooked crawfish.

off-flavor and rancidity. The hepatopancreas is water soluble and is easily removed by washing. Some processors freeze the meat with adhering hepatopancreas but they must take precautions to extend the shelf-life. For example, vacuum packaging, using laminated bags to prevent dehydration and to serve as an oxygen barrier, is commonly used in the industry to extend the shelf-life of frozen crawfish meat.

A problem that can occur in frozen crawfish meat is the development of a dark, blue discoloration. Although harmless and tasteless, the discoloration is unappetizing. Discoloration is most common after frozen meat has been thawed and reheated. Preliminary research indicates that chelating agents such as citric acid or EDTA are effective in minimizing discoloration (Moody and Moertle 1986). Some processors dip the washed crawfish meat in a citric acid solution (about 0.75–1.0% citric acid) to minimize discoloration. Because of FDA regulations, it is not likely that EDTA will be permitted to be used in crawfish meat. With proper processing, packaging and cold storage, crawfish processors can obtain a reasonable extended frozen shelf-life of crawfish meat.

Whole, Cooked Crawfish Processing

Whole, cooked crawfish have become an important product of commerce for crawfish processors. With the development of the European crawfish markets, especially in Sweden markets, Louisiana crawfish processors have responded with technologies and procedures capable of producing high quality, whole, cooked crawfish. Various techniques are used to process and package this specialty item. Cole and Kilgen (1987) compared various methods of freezing whole cooked crawfish to achieve maximum shelf-life. Live crawfish were processed in five different ways. Their research indicated that whole cooked, individually quick frozen (IQF) crawfish were superior. In another study, Cole and Kilgen (1988) compared quality of whole live, cooked crawfish with irradiated crawfish. Cooking reduced the number of microorganisms 2–3 log cycles whereas irradiated crawfish showed 2 log reduction in number of bacteria. Sensory analysis showed that irradiated crawfish produced the most stable product although irridated crawfish are not commercially available. An acceptable quality of cooked, whole crawfish was retained throughout a 48-week storage period.

LITERATURE CITED

Bankston, D. 1984. Brine Freezers. Louisiana Cooperative Extension Service, Louisiana State University Agricult. Center, Baton Rouge, LA.

Cole, M. T. & M. B. Kilgen. 1988. Characterization of the quality and shelf-life of whole frozen crawfish. Final Report for the Gulf and South Atlantic Fisheries Development Foundation, Inc., Tampa, FL.

Cole, M. T. & M. B. Kilgen. 1987. Characterization of the quality and

shelf-life of whole frozen crawfish. Final Report for the Gulf and South Atlantic Fisheries Development Foundation, Inc., Tampa, FL.

Huner, J. V. 1989. Overview of international and domestic freshwater crawfish production. *J. Shellfish Res.*, 8:259–265.

Huner, J. V. 1988. Comparison of the morphology and meat yield of red swamp crawfish and white river crawfish. *Crawfish Tales* 7:2.

Huner, J. V. & J. E. Barr. 1984. Red swamp crawfish: biology and exploitation. Sea Grant No. LSU-T-80-001, LSU Center for Wetland Resources, Baton Rouge, LA.

Marshall, G. A. 1988. Processing and freezing methods influencing the consistency and quality of fresh and frozen peeled crawfish (*Procambarus* sp.) meat. Ph.D. dissertation. Louisiana State University, Baton Rouge, LA.

Marshall, G. A., M. W. Moody, C. R Hackney & J. S. Godber. 1987. Effect of blanch time on the development of mushiness in ice-stored crawfish meat packed with adhering hepatopancreas. *J. Food Sci.* 52:6.

Marshall, G. A., M. W. Moody & C. R. Hackney. 1988. Difference in color, texture and flavor of processed meat from red swamp crawfish (*Procambarus clarkii*) and white river crawfish (*P. acutus acutus*). *J. Food Sci.* 53:1.

Marshall, G A. & M. W. Moody, 1986. Department of Food Science, Louisiana State University, Baton Rouge, LA, Unpublished data

Marshall, G. A. & M. W. Moody. 1985. Department of Food Science, Louisiana State University, Baton Rouge, LA Unpublished data.

Moody, M. W. 1980. Louisiana seafood delight—the Crawfish. Louisiana Cooperative Extension Service Publication #LSU-TL-80-002, Louisiana State University Agricult. Center, Baton Rouge, LA.

Moody, M W. & G M. Moertle. 1986. An evaluation of the effectiveness of specific chemical compounds on the prevention of discoloration development during the cooling of frozen crawfish meat. Annual Meeting of the Institute of Food Technologists, Dallas, TX.

Roberts, K. J. & Dellenbarger. 1989. Louisiana crawfish products markets and marketing. *J. Shellfish Res.*, 8:303–307.

Rutledge, J. W. 1978. Crustacean meat extraction means. U.S. Patent 4121322, Oct. 24, 1978.

Rutledge, J E. 1977. Hand-shelled crustacean meat recovery process. U.S Patent 4053964, Oct. 18, 1977.

Wheaton, F. W. & T. B. Lawson. 1985. "Processing aquatic food products " John Wiley and Sons, New York.

Journal of Shellfish Research, Vol. 8, No. 1, 303–307, 1989

LOUISIANA CRAWFISH PRODUCT MARKETS AND MARKETING

KENNETH J. ROBERTS[1], and LYNN DELLENBARGER[2]
[1]*Louisiana Cooperative Extension Service*
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803
[2]*Department of Agricultural Economics*
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

ABSTRACT The increase in crawfish production area in Louisiana slowed in 1987 and 1988. Post-1980 area increases in aquacultural production resulted in major supply increases. The combined influence of crawfish production from natural waterways and a significant economic slowdown in the major market, south Louisiana, resulted in major marketing problems. Market research and product development investments from public and private sources were part of the industry's response. New group efforts of crawfish product production and promotion were undertaken. Producer assessments are used to fund a crawfish marketing and promotion program. Promotion must be a permanent component of the crawfish industry. The ability of producers to respond to price increases will continually stress marketing companies. Additionally, new products such as soft crawfish require marketing programs in order to keep consumption in step with production increases.

KEY WORDS: crawfish, Louisiana, marketing

INTRODUCTION

Freshwater crawfishes are referred to as crayfishes, crawdads, mudbugs, and a host of local names. Over 350 species of crawfishes exist in the USA. Nomenclature and the many species of crawfishes can cause confusion when new customers for crawfish products are sought in non-traditional markets. The commercially exploited species dominant in the USA are the red swamp crawfish, *Procambarus clarkii*, and white river crawfish, *Procambarus acutus acutus*. These two species alone account for >95% of the USA commercial harvest.

The first reported commercial crawfish catch statistics for Louisiana date to 1880. The 1880 report documented only 10,600 kg harvested, whereas production from natural habitats in the 1980's ranged from 9–32 million kg. Additionally larger and more reliable supplies of crawfish have been made available for the past 15 years through aquaculture. Although culture of crawfish began around 1950, it took 20 years for the production area to reach 10,000 ha. The area devoted to crawfish cultivation in Louisiana alone in 1987 was 53,000 ha (Louisiana Cooperative Extension Service 1988). Concomitant with the large increase in area has been higher average crawfish yields from improved cultural practices. Total annual harvest of Louisiana crawfish from both aquaculture and the natural fishery exceeds 45 million kg (45,000 MT). The states of Texas, South Carolina, Arkansas, Florida, and Mississippi have crawfish aquaculture industries that are collectively about 6,000 ha. Thus, Louisiana supplies about 90% of the USA aquacultural production of crawfish.

Corresponding Author: Kenneth J. Roberts, Louisiana Cooperative Extension Service Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

PRODUCTION AND MARKETING HISTORY

An important element of crawfish aquaculture and crawfish marketing in Louisiana is the market information system. Essentially, in Louisiana it is non-existent. No data has been historically collected on the crawfish production area and associated harvest which impedes an understanding of crawfish supply and consequently establishment of an effectively structured marketing program. A series of estimates of area devoted to crawfish aquaculture is presented in Table 1. Several gaps exist in the 1960–1988 estimates because no program existed to provide estimates. Most estimates made prior to 1981 were made by different individuals or agencies using non-standardized procedures. The state of Louisiana and the Federal government do not include crawfish area and harvest estimates in official statistical series. Crawfish harvests are considered by some agencies as an agricultural function and by others as a fisheries matter. The result is an unsatisfactory depiction of crawfish supply and price. The seasonal supply of crawfish is not documented sufficiently to inform prospective buyers. A crawfish buyer's best market information system is that which evolves through purchases over time with various suppliers. Buyers new to the crawfish market, thus, proceed cautiously with small orders.

Crawfish are harvested 4–5 days weekly from November–June (Romaire 1989). The market cycle from harvest to consumption should occur within four days for crawfish marketed live. An historical perspective of market development must be secured from an evaluation of the industry growth depicted in Table 1. The 1971–1988 statistics are the most reliable. The area devoted to aquaculture from 71–88 increased at an annualized rate of 11.1%. The total supply of crawfish doubled every four years after adjustment for increased gains in per ha yield and this

TABLE 1.

Louisiana crawfish farm area, 1960–1988.

Year	Hectares	Year	Hectares
1960	809	1978	24,282
1966	2,428	1981	23,472
1968	4,047	1982	26,710
1969	4,856	1983	40,874
1970	7,285	1984	42,088
1971	9,713	1985	41,279
1973	17,807	1986	48,159
1975	18,211	1987	52,610
		1988	53,420

Source: Louisiana Cooperative Extension Service, Louisiana Summary: Agriculture and Natural Resources.

placed significant stress on the marketing system. The tendency of crawfish wholesalers to sell crawfish in live form to Louisiana markets only, compounded the difficulty in establishing a smooth functioning market.

As crawfish supply increased in the 1970's, local markets were easily saturated with live product. The marketing situation was described in an economic report (Hudson 1970). Hudson attributed the seasonality of crawfish supply as a contributor to marketing problems. Supply peaks in a brief season left too little time for owner-operated processing companies to truly develop markets. Sales concentrated in local markets caused producer prices to decrease to unacceptable levels.

Construction of crawfish processing plants increased and existing plants were expanded to increase value-added processing. Processing technology to cook, peel and package crawfish meat was necessary to establish new product forms to help alleviate saturation of the live crawfish resale market. The number of crawfish processing plants licensed in Louisiana increased from 35 in 1970 to 90 in 1987. Although processing of crawfish became more capital intensive through multi-product offerings, in-state marketing prevailed. Crawfish aquacultural production statistics estimated from a consistent reporting system established in 1981 are presented in Table 2.

An insight to crawfish marketing was provided in 1974 (Carroll et al. 1974). A survey of crawfish processors documented a pattern where only a few crawfish products (live, fresh meat) were marketed in a limited geographic area. Three states, other than Louisiana, received crawfish products. Combined local and out-of-state buyers bought 70% of the crawfish supply at the retail sales level, such as speciality seafood stores and supermarkets, and the remainder reached consumers via restaurant purchases. The dependence on retail sales indicated the limited processing capabilities of the industry (Carroll et al. 1974). Processors avoided discarding of excess crawfish that could not be sold alive by cooking, peeling, and packaging crawfish meat products.

The experience of "salvage" processing of crawfish discourage investment in processing and marketing that was necessary to match the increased crawfish production from aquaculture. All crawfish processors were single-unit operations with limited financial, management, and marketing capabilities. Mergers and acquisitions of processing plants were not used to pool resources, in part, because of the limited geographic area a plant could effectively service. Thus, the crawfish industry in the early 1970's marketed 65% of the crawfish in live, raw form, and 35% was processed into meat as "salvage". Salvaged crawfish products were not afforded management and financial resources to be effectively marketed.

RECENT PROCESSING AND MARKETING DEVELOPMENTS

In the mid-1970's efforts were made to develop processing procedures that capitalized on emerging market opportunities. Research revealed a potential for increased sales of cooked, peeled and deveined crawfish meat (Carroll et al. 1974) for use in restaurants. The effort was necessary but also premature.

The economic structure of the crawfish industry, in part, limited success in restaurant sales. Entry into large-scale, out-of-state restaurant markets did not occur until the early 80's. Crawfish harvesting and processing was influenced by supply instability and product inconsistency. The new market approach that targeted restaurant sales was dependent on a crawfish supply harvested from natural habitats. The expansion of cultured crawfish from ponds decreased annual supply variations. However, the 50–70% supply coming from natural habitats in the 70's made early purchases and sales in the market risky. With crawfish supplies from natural habitats available primarily from March to May, processors could not effectively market the November to May supply of crawfish from ponds. Buyers would withhold major purchases of meat until inevitable lower supply prices in April–May. No collective, cooperative action among processors developed which would smooth marketing problems, and pooling of product to meet buyer needs for large crawfish product volumes of consistent quality, year-round, was not possible. The crawfish market remained current, that is, numerous small-sales with minimal product inventorying by processors to reduce downward pressure on prices.

Crawfish processing technology remained a constraint to better crawfish marketing. Large numbers of processing companies, each with small shares of overall supply, restricted standardization and technological improvements in processing. Mechanization of peeling procedure to increase product quality and reduce labor costs was investigated. Hand-labor had been used to peel cooked crawfish. With 6 kg of live crawfish producing about 1 kg of meat and cost of peeling labor in the early 80's at $1.32/kg, the market price for a kg of processed meat was above competing shrimp products. Several machines were developed without

TABLE 2.

Louisiana pond raised crawfish production statistics, 1981–87.

Year	Hectares	kg (millions)	kg/ha	Producer Value-USD (millions)	Price/kg
1981	23,472	14.4	613	$26.7	$1.85
1982	26,710	19.7	738	35.0	1.78
1983	40,874	31.6	773	33.1	1.05
1984	42,088	29.8	708	27.6	0.93
1985	41,279	29.6	717	29.4	0.99
1986	48,159	30.1	625	33.1	1.10
1987	52,610	32.6	620	32.3	0.99
1988	53,420	30.4	569	27.7	0.91

Source: Louisiana Cooperative Extension Service, Louisiana Summary: Agriculure and Natural Resources.

major success. Presently, no commercially utilized mechanical procedure of meat removal from crawfish exists. Currently, workers peel crawfish by hand for the piece rate of $2.20/kg. The addition of employment taxes results in an actual cost of $2.75/kg.

Processing techniques had been oriented to provide fresh cooked crawfish meat including hepatopancreas (fat) to local buyers (Moody 1989). The instability of the fat in terms of affecting product shelf-life became a marketing problem until off-season and new markets were developed. Discolored product sometimes reaching the consumer and incidences of rancid fat from frozen product added to other marketing problems; seasonality, lack of consumer knowledge about crawfish, and price instability.

Processors responded to improved crawfish products with better plant design, proper cook time determination, product stabilization, freezing techniques, quality control programs, and packaging improvements. A comprehensive study of the Louisiana crawfish processing industry in 1983 provided information on processing/marketing companies (Dellenbarger et al. 1986). The processing plants averaged 580 square meters (5,200 square feet) with five year-round, full-time employees, and four seasonal employees paid on a wage basis. The plants averaged 27 crawfish meat peelers paid on a piece-rate basis. Established markets in 35 states and four countries were identified. Eight crawfish product forms reached needs of varied buyers (Table 3). The usage of live crawfish purchased by processors was about 60% resold in shell-on products and 40% was peeled for meat products. Markets for live crawfish, meat products and prepared entrees by region are shown in Table 3. Crawfish product categories most desired in out-of-state markets were frozen meat, purged live, frozen whole raw, frozen whole cooked, and prepared entrees. Among these products only frozen meat had more in-state sales than out-of-state sales.

A 1985 survey of 236 USA seafood distributors revealed that 23% sold crawfish products (NMFS 1985), a significant increase from the early 1970's. Large markets for crawfish remain to be developed but impediments exist. Processors cited lack of quality control and undesired sizes of crawfish supplied to plants as critical factors. Presently there are no standardized size categories for whole crawfish or meat and few guidelines for acceptable amounts that can be packaged with meat. Seafood wholesalers experience problems associated with profitable crawfish marketing including a lack of consumer awareness and unfamiliarity with sources of crawfish products. Prospective out-of-state buyers recommended menu clip-ons, in-store tasting, advertising art, and information for employees as the high priority needs to help sell crawfish.

INDUSTRY ORGANIZES TO EXPAND MARKETS

Efforts to structure the crawfish processing/marketing industry to capitalize on increasing seafood consumption occurred from 1983 to 1987. Restructuring of programs was the approach of an industry trade group. Other changes involved the creation of new trade structures. The Louisiana Crawfish Farmers Association (LCFA) initiated a program to promote increased crawfish consumption by developing the International Crawfish Tasting and Trade Show. The Trade Show increased the general public's awareness of crawfish food products and the shows increased interaction between processors, other vendors of crawfish products, and prospective crawfish buyers worldwide. Thus, crawfish farmers have become activists in the market development of their products. This development indicates a maturing of the crawfish aquacultural industry, and a commonality with marketing activities of other USA food commodity growers. Crawfish producers do not yet differentiate pond-raised crawfish from wild supplies because there is no foundation on which to differentiate separate markets for the two sources of crawfish.

New trade structures have also been developed. The Associated Crawfish Processors of Louisiana was formed in 1983. Its function is to unify Louisiana crawfish processors into an effective organization that will advance the industry and act in the interest of the individual processor. Specific

TABLE 3.

Estimated regional markets for crawfish by product type for Louisiana crawfish industry, survey of 38 crawfish processing plants, Louisiana, 1984.

Product	Louisiana	South Central	South East	Mid-Atlantic	North Central	Far West	Export	Total
Unpurged (kg)	13,471,005	1,690,416	301,669	109,969	129,978	141,790	(0)	15,844,827
Live (%)	(85)	(10)	(2)	(1)	(1)	(1)	(0)	(100)
Fresh meat (kg)	6,775,429	1,019,365	47,449	188,520	95,172	61,105	0	8,187,040
(%)	(83)	(12)	(1)	(2)	(1)	(1)	(0)	(100)
Frozen meat (kg)	1,223,042	350,597	249,435	152,375	951	20,252	0	1,996,652
(%)	(62)	(18)	(12)	(7)	(0)	(1)	(0)	(100)
Purged Live (kg)	152,228	94,245	6,340	44,680	19,047	19,047	0	335,587
(%)	(45)	(28)	(2)	(13)	(6)	(6)	(0)	(100)
Frozen whole raw (kg)	29,045	18,154	18,154	76,104	0	3,631	11,618	156,706
(%)	(19)	(12)	(12)	(48)	(0)	(2)	(7)	(100)
Frozen whole cooked (kg)	3,098	32,942	17,935	18,404	15,612	484	60,511	148,986
(%)	(2)	(22)	(12)	(12)	(11)	(0)	(41)	(100)
Prepared product (kg)	30,507	100,058	19,902	1,264	3,122	1,749	0	156,602
(%)	(19)	(64)	(13)	(1)	(2)	(1)	(1)	(100)
Other (kg)	25,623	87	0	0	0	0	0	25,710
(%)	(100)	(0)	(0)	(0)	(0)	(0)	(0)	(100)

Note: The weight estimates are product weights, not live weight equivalents.
South Central: Texas, Mississippi, Alabama, Florida
South East: Virginia, North Carolina, South Carolina, Georgia
Mid-Atlantic: Maryland, Delaware, New Jersey, New York, Pennsylvania, Massachusetts, Connecticut, Rhode Island, New Hampshire, Maine
North Central: Dakotas east to Ohio; Oklahoma east to Tennessee
Far West: Rocky Mountain states west to the coast

activities deal with out-of-state marketing, establishment of standards to provide customers quality products, and achieve technological advances.

Secondly, a Louisiana Crawfish Promotion and Research Board (LCPRB) was approved in 1983 by vote of crawfish producers during a referendum. The LCPRB provides a voluntary method of raising revenues to promote and develop markets for Louisiana crawfish, and fund research to increase crawfish production. All segments of the industry from culturist to marketer are presented on the 10 member governing board. The board is funded through assessments on crawfish baits and sacks. The board has addressed the need for printed material to aid in crawfish product introductions and consumer education.

The 1985 Louisiana legislature established the Louisiana Crawfish Market Development Authority (LCMDA). In 1985, a major food service establishment sought purchase of 320,000 kg of crawfish tail meat. Several independent processors pooled efforts to meet the order but could provide only half the contracted amount. To meet standards of the buyer, the processors had to contract with a food packaging company in another state to do the final packaging. This first access to large national markets proved to be less than ideal for both contract participants.

The LCMDA has progressed toward developing a centralized processing, packaging, and inventory facility. In 1987, about 20 processor members of the corporation, Louisiana Crawfish Wholesalers, Inc., emerged from the Authority's actions in 1986, built a large centralized processing facility. In 1989, a multi-species processing and marketing company purchased the facility but will continue crawfish product sales.

NEW CRAWFISH MARKETS AND PRODUCTS

Louisiana consumers prior to 1985 consumed 80% of the state's total crawfish supply. By 1988 in-state crawfish consumption had decreased to 70%. The profitable entry into new markets and successful new products included a major export initiative and introduction of soft crawfish (Culley 1989).

A decline in the supply of crawfish from Turkey opened markets for Louisiana Crawfish products in Europe (Huner 1989). In 1987, six Louisiana companies sold 1.4 million kg in Sweden. The exacting specifications and seasonality of the market in Europe resulted in only minor marketing efforts there prior to 1987. The success in delivering carefully sized, and cooked crawfish packaged for retail sale in Sweden during August to October, 1987 resulted from coordination between Louisiana companies and Swedish importers. Louisiana companies expanded their commitment to markets in Sweden and France in 1988. Both countries used graded whole frozen tray packed crawfish but with different preparation procedures. Louisiana companies were unable to supply contracted agreements for crawfish in 1988 because of inadequate supplies of 25–35 count/kg

crawfish. The European market, accepted Louisiana crawfish when prepared to buyer specifications. Exports of crawfish to Europe will likely be 3–5% of Louisiana's production in the foreseeable future.

The export market was also a significant buyer of the newly developed soft crawfish. Production of soft crawfish reached marketable levels in 1987. Production of soft crawfish was 7,000–9,000 kg in 1987. By 1988 expansion took place in both number of producers and capacity per operation. The result was that production increased to 27,000–36,000 kg. About 90% of soft crawfish are used in the food service sector. A typical entree includes 4–5 soft crawfish. A kg can provide 11–15 entrees. About 15–20% of soft crawfish were sold in Louisiana, 25–30% as foreign exports, and the remaining 50–60% in states other than Louisiana. The producer price increased throughout 1988 to end the season at $19.80/kg. This is more likely a short-run result of promotion efforts exceeding the ability of production systems to produce the product. In 1989 prices received by producers decreased to about $13/kg. Educational material for consumers and training programs for food service employees were developed by the Louisiana Seafood Marketing and Promotion Board to enhance marketing efforts by wholesalers.

LITERATURE CITED

Carroll, J. C. & H. C. Blades, Jr. 1974. A Quantitative Analysis of the Amounts of South Louisiana Crawfish That Move to Market Through Selected Channels of Distribution. Office of Institutional Research, University of Southwestern Louisiana, Lafayette, Louisiana.

Culley, D. D. 1989. Soft-shell crawfish production technology. *J. Shellfish Res.*, 8:287–291.

Dellenbarger, L. E., K. J. Roberts, S. S. Kelly & P. W. Pawlyk. 1986. An Analysis of the Louisiana Crawfish Processing Industry and Potential Market Outlets. D.A.E. Research Report No. 654, Louisiana Agricul. Exp. Sta. Louisiana State University Agricul. Center, Baton Rouge, Louisiana.

Hudson, J. F. & W. J. Fontenot. 1970. Profitability of Crawfish Peeling Plants in Louisiana. D.A.E. Research Report No. 408, Louisiana Agricul. Exp. Sta. Louisiana State University Agricul. Center, Baton Rouge, Louisiana.

Huner, J. V. 1989. Overview of international and national freshwater crawfish production. *J. Shellfish Res.*, 8:259–265.

Louisiana Cooperative Extension Service. 1988. Louisiana Summary: Agriculture and Natural Resources. Louisiana State University Agricultural Center, Baton Rouge, Louisiana.

Moody, M. W. 1989. Processing of freshwater crawfish: a review. *J. Shellfish Res.*, 8:293–301

NMFS (National Marine Fisheries Service). 1985. Survey of Seafood Distributors. U.S. Department of Commerce, Little Rock, AR.

Romaire, R. P. 1989. Overview of crawfish harvest technology. *J. Shellfish Res.*, 8:281–286.

Journal of Shellfish Research, Vol. 8, No. 1, 309–313, 1989.

CRAWFISH CULTURE IN SOUTH CAROLINA: AN EMERGING AQUACULTURE INDUSTRY[1]

ARNOLD G. EVERSOLE[2] AND ROBERT S. POMEROY[3]
[2]*Department of Aquaculture, Fisheries and Wildlife*
Clemson University
Clemson, SC 29634
[3]*Department of Agricultural Economics and Rural Sociology*
Clemson University
Clemson, SC 29634

ABSTRACT Crawfish aquaculture started in South Carolina in 1978 and has grown to where operations (n = 50) are now in over half of the counties of the state. Most of the farms are <8 ha and family run. Crawfish producers manage their operations either for a single crop of crawfish or double-crop crawfish and waterfowl. The most commonly-used vegetative forages are rice and Japanese millet. State production levels average 500–800 kg ha^{-1}. Break-even cost of production for 8 ha pond is \$1.45 kg^{-1} for operating costs and \$1.98 kg^{-1} for operating fixed costs. Average price is currently \$2.81 kg^{-1}. Potential for the increase in crawfish aquaculture in the state is reviewed.

KEY WORDS: crawfish, aquaculture, marketing, economics, production

INTRODUCTION

Aquaculture is not a new industry in South Carolina, but a re-emerging one. The oyster was the first animal cultured in South Carolina. Known locally as "Mill Pond Oysters," these were grown primarily for private use from 1830–1869 in tidal sawmill ponds originally built for power generation (Keith and Gracy 1972). By 1890 several commercial companies extensively cultured oysters in tidal creeks. As early as 1800 diamondback terrapins and shad were also cultured (Anon. 1983). Presently in South Carolina, the species cultivated include shrimp, crabs, crawfish, clams, oysters, catfish, trout, carp, tilapia, hybrid striped bass and several species of game fish. Culture systems range from the extensive management of oyster leases in tidal creeks to the intensive monitoring and care of trout in raceways. Much of the current interest and growth in aquaculture in South Carolina can be traced to the most recent financial difficulties encountered by the American farmer. Other reasons include the successes of catfish and crawfish aquaculture in Mississippi and Louisiana, the continued national trend of increased fish consumption, and the abundant available natural resources within the State.

One of the fastest developing aquaculture enterprises in South Carolina is the crawfish (*Procambarus* spp.) industry. Crawfish is a relatively recent cultured species in the State. The industry began in 1978 when a U.S. Soil Conservation Service employee made arrangements for the transport of broodstock from Louisiana and successfully stocked ponds in 2 South Carolina counties. From this beginning, the crawfish industry has grown (and is still growing) to where some 50 operations are found in 28 of the 46 counties of the State. Not unlike Louisiana, most of the production is centered in one area, the coastal plain, with 2 counties, Georgetown and Berkeley, accounting for approximately 75% of South Carolina's production. While the crawfish industry is relatively new, area devoted to crawfish culture has increased during the decade. From the initial stocking of 9 ha of ponds in 1978, there are presently about 445 ha in production (Table 1). The largest increases in area occurred in the early 1980's, when area doubled for several consecutive years making crawfish farming the largest aquaculture industry, in terms of area, in the State. The expansion in area has primarily involved the utilization of existing farm ponds and old ricefields (impoundments), in addition to the construction of new ponds. The crawfish industry in South Carolina has expanded because of increased market demand for the product, increased interest in crawfish culture as an alternative agricultural enterprise, increased technical support from state agencies, and cooperative support from fellow crawfish producers.

A study of the South Carolina crawfish industry in 1986–1987 by Pomeroy and Kahl (1987) provided baseline information on its status. Several producers in South Carolina have cultured crawfish for >7 years, but most of the crawfish producers in South Carolina have been producing crawfish <4 years. The majority of crawfish are produced in South Carolina as a part-time business activity. Although some of the producers are involved in other agricultural enterprises, the primary occupation of most producers is not agriculture. Crawfish operations are small, compared to Louisiana. In South Carolina, the area per farm devoted to crawfish culture ranges from 0.2–71 ha. The majority of operations are <8 ha with an average of 2 ponds per enterprise. In comparison, the average crawfish producer in Louisiana in 1980 had 62 ha devoted to production (Avault and Huner 1985). Individual crawfish ponds in South Carolina are smaller (2–5 ha), on average, than ponds in Loui-

[1]Technical Contribution No. 2955 of the South Carolina Agricultural Experiment Station.

TABLE 1.

Crawfish production in South Carolina, based on information from Pomeroy and Kahl (1987), Pomeroy (1988) and Whetstone (1988).

Year	Area (ha)	Yield (kg ha^{-1})	Value ($ US)
1978	9	280	6,900
1979	9	280	6,900
1980	9	280	6,900
1981	10	280	7,800
1982	50	336	46,900
1983	101	336	93,800
1984	212	534	312,500
1985	324	420	375,000
1986	334	544	460,000
1987	405	448	460,000
1988	445	511	600,000

siana (8–16 ha). Due to the small scale of crawfish operations in South Carolina, most producers rely primarily on family labor.

Concurrent increases in both production and economic value of the crawfish crop have occurred over the last 10 years (Table 1). Production increases are thought to be the result of improved culture efficiency and management skills of the producers. These production estimates, however, are more than likely underestimates of true production figures (Pomeroy and Kahl 1987). Production levels in the State probably average closer to 840 kg ha^{-1} (Whetstone 1988). In contrast, production averages reported for Louisiana crawfish farmers are 500–1500 kg ha^{-1} (Avault and Huner 1985). Most of the South Carolina farmers are new at crawfish aquaculture and are in the beginning stages of the learning curve. We anticipate these crawfish farmers will approach the higher levels of production experienced in Louisiana with experience, exchange of information, and with technical assistance from state agencies.

Historical markets for South Carolina crawfish have been in state with most crawfish being sold directly to customers at the farm. Other markets include local restaurants, seafood markets, and use at crawfish festivals. In the last 2–3 years more of the production has been marketed out of state, and ~50% of state crawfish production in 1988 was shipped to markets such as the Baltimore and Washington, D.C. areas and Chicago. South Carolina has a transportation advantage over Louisiana in reaching Middle Atlantic and Northeastern markets with a live product. Several producers have differentiated their product with the brand "South Carolina" or "Carolina" produced crawfish. Currently all the trade is in live crawfish, but a few producers are considering limited processing and the selling of cooked tail meat.

The price received by producers has remained stable at about $2.75–$3.30 kg^{-1} for the last several years and is higher than prices received for crawfish in Louisiana. The geographic distance between Louisiana and South Carolina and the difficulty in transporting live crawfish prevent crawfish producers in Louisiana from effectively competing in the South Carolina market.

The crawfish industry in South Carolina is well organized and is the only aquaculture commodity group within the State. The S.C. Crawfish Growers Association has been influential in obtaining institutional assistance and having input in legislative issues related to aquaculture.

Crawfish producers in South Carolina follow much the same management scheme as that used by Louisiana farmers because the Louisiana crawfish production technology was used to start the industry. A brief review of the management scheme follows, stressing the major differences between the 2 states and variations of systems used in South Carolina. Details of management practices used in Louisiana can be found in other papers in this proceedings (e.g., R. P. Romaire and L. W. de la Bretonne, J. V. Huner, R. P. Romaire, and M. W. Brunson).

CURRENT PRACTICES

South Carolina crawfish producers manage operations in one of 2 ways, either as a single crop for crawfish (monoculture) or double cropped for crawfish and waterfowl. The production and economic goals of each system are different.

Crawfish/Waterfowl Systems

Crawfish/waterfowl double-crop operations are managed with the primary purpose of attracting waterfowl, with crawfish being a secondary product of the management system. Currently, these crawfish/waterfowl systems constitute 10% of the operations and 30% of the total crawfish area in the State (Whetstone 1988).

Crawfish/waterfowl double-crop operations are almost exclusively located along major coastal river systems in former ricefields. These ricefields, built in the late 1700's and early 1800's (Doar 1936), utilize a system of trunk and gates and harness tidal action to flood and drain the impoundments (Morgan et al. 1975). Managers usually plant rice (*Oryza sativa*), Japanese millet (*Echinochlea crusgalli*) or a combination of these 2 as a forage with the latter being the most common. Japanese millet is inferior to most rice varieties because millet has high senescence and rapid decomposition which adversely impacts water quality (Brunson 1987). Occasionally, crawfish/waterfowl systems are managed for natural forage and smartweed (*Polygonum* spp.) is the preferred species because it is an excellent forage for both crawfish (Alon and Dean 1980, Avault and Huner 1985) and waterfowl (Landers et al. 1976). Crawfish/waterfowl systems are normally drained one month earlier (May) and flooded one month later (November), than the management scheme practiced by most of the managers who single-crop crawfish. The longer dry period fa-

cilitates seed production and increases waterfowl use (Epstein and Joyner 1986).

Crawfish are not harvested during the fall in crawfish/waterfowl systems because of the perceived conflicts with duck hunting. For this reason, and because few ricefields have adequate pumps for good water quality management, crawfish production is lower than the state average (Table 1). Because of increased taxes and operating costs, higher economic returns are needed from these systems (Gresham and Hook 1982), and so it is anticipated that ricefield owners will make the necessary changes to improve water management to increase crawfish production.

Crawfish/waterfowl systems provide income from both the lease of hunting rights and the harvest of crawfish. An economic study of these systems has not been conducted to date. It is anticipated that the management practices of crawfish/waterfowl systems will intensify as crawfish production and profits increase and as crawfish/waterfowl management techniques are refined.

Single Cropping of Crawfish Systems

South Carolina producers who single-crop crawfish use rice as a forage, primarily Melrose and Mars rice varieties. These producers do not harvest rice grain, so many of the management practices used by farmers who double-crop rice and crawfish in Louisiana (e.g., flooding schedules and pesticide use) are not applicable in the South Carolina culture system.

Adult crawfish are stocked as early as April in old tidal ricefields and as late as May and June in upland ponds. Generally local crawfish producers provide broodstock for farmers. Occasionally, growers will buy broodstock from Louisiana. Farmers usually stock at 56–84 kg ha^{-1}, a 50:50 sex ratio and with a size range of 27–35 g, a higher rate than generally used in Louisiana. South Carolina producers typically manage upland crawfish ponds (as opposed to "old ricefields") for 2 harvest periods, fall and spring. The fall harvest season is usually from mid-October–November and the spring season from mid-March–May. Some producers extend the spring harvest into summer (June and July) to take advantage of higher prices and less competition. Ponds managed on this schedule follow the same sequence of events as in the standard management regime except activities (e.g., draining, planting rice and reflooding) are delayed 4–6 weeks.

An experimental evaluation of the extended spring harvest management strategy was undertaken to determine if the departure from the standard crawfish managment schedule significantly impacted harvest. Average yield for 2 years in the extended managed ponds (966 kg ha^{-1}) was not different ($P > 0.05$) from ponds managed in the standard manner (1,006 kg ha^{-1}) (Eversole unpubl. data). The yield figures are comparable with yields in Louisiana (Avault and Huner 1985) and are much higher than yields for commercial crawfish operations in South Carolina (Table 1). Some South Carolina producers are considering extending the grow-out and harvest season through summer by supplementing vegetative forages with formulated feeds. Regardless of the management strategy used, South Carolina producers manage ponds to produce a uniformly large animals (≈100 mm total length) that are more readily marketable.

Enterprise budgets have been prepared for commercial crawfish aquaculture in South Carolina (Pomeroy et al. 1988). The budgets represented 5 operation sizes of 2, 4, 8, 16 (2 8-ha ponds) and 32 (4 8-ha ponds). The ponds are upland, embankment-type ponds that are rectangular and use well water. No budgets have been prepared for existing ricefield (impoundment) ponds.

The establishment year budget reflects costs and returns during the first year of operation when yields are normally low at 450 kg ha^{-1}. The full production year budget reflects costs and returns during subsequent years when yields are 1,000 kg ha^{-1}. A producer price of \$2.81 kg^{-1} was used.

The estimated total capital investment for a 8-ha pond is \$24,385, which includes cost of pond construction, water pumping system (excluding well cost), a boat for harvest, and other equipment. It does not include the cost of land purchase.

An enterprise budget for the second year of operation of the 8-ha crawfish pond is presented in Table 2. Pumping and repair and maintenance are the major preharvest operating costs while bait and labor are the major harvest costs. Broodstock is the major cost in the establishment year. The break-even price-cash of production (which includes only operating costs) for the 8-ha operation is \$1.45 kg^{-1}, and the breakeven price-all costs (including operating and fixed costs) is \$1.98 kg^{-1}.

The 5 crawfish production systems (2–32 ha) were determined to be profitable, with break-even prices—all costs ranging from \$1.80–\$2.00 kg^{-1}. Negative returns were obtained with each system during the first or estab-

TABLE 2.

Estimated costs and returns for 8-ha upland crawfish pond system with 1000 kg ha^{-1} yield in South Carolina, 1988 (Source: Pomeroy et al. 1988).

Gross Receipts (price \$2.81 kg^{-1} and 8,000 kg total yield)	\$22,500
Variable Costs:	
Preharvest	\$ 4,616
Harvest	\$ 7,192
Total Variable Costs	\$11,808
Fixed Costs	\$ 3,068
Other Costs (land charge, overhead)	\$ 1,345
Total Costs	\$16,221
Returns to Management, Risk and Family Labor	\$ 6,279
Breakeven Price—Cash \$1.45 kg^{-1}	
Breakeven Price—All Costs \$1.98 kg^{-1}	

lishment year because of the low yield. Crawfish producers can obtain net returns of \$0.75–\$1.00 kg^{-1}. Further improvements in production technology and management should reduce production costs and increase cash.

In comparison, the capital investment requirement for a 8-ha crawfish operation in southwest Louisiana is \$30,110 (Dellenbarger et al. 1987). The major operating costs in Louisiana are bait and labor. The breakeven cost-cash for a yield of 1,000 kg ha^{-1} is \$0.95 kg^{-1} with operating costs and \$1.70 kg^{-1} with all costs in southwest Louisiana. The production costs in Louisiana are lower because input costs (e.g., bait, labor and pumping) are lower than in South Carolina.

POTENTIAL

South Carolina has the necessary natural resources, climate and soil characteristics for aquacultural development. Approximately 12,000 ha of privately-owned farm ponds and 28,500 ha of impounded coastal wetlands (i.e., old ricefields) hold potential for crawfish aquaculture (Gresham and Hook 1982, Nussman 1983). In addition there are 4 major river systems which average 125×10^9 l of in-stream discharge per day (Nussman 1983). Nussman (1983) reported that 84% of the length of thse rivers meet the federal classification for "fishable/swimmable" waters. Other surface freshwater resources in the State include 241,000 ha of inland reservoirs (Nussman 1983) and more than 26,000 ha of freshwater marshes (Tiner 1979). Ground water sources, which are excellent, are provided by 6 major aquifers within the State (Nussman, 1983).

South Carolina has a mild climate. The State has ample rainfall, averages 100 cm or more rainfall annually, and appears to have sufficient runoff to supplement aquacultural water requirements (Foltz and Smith 1983). Foltz and Smith (1983) categorized the regions of South Carolina as for aquaculture potential based on 3 thermal provinces: areas with average air temperature <16°C and suitable for coolwater aquaculture; average temperatures 16–18°C suitable for warmwater culture; and >18°C optimal for warmwater culture. Based on thermal regimes about 90% of the State is suitable for the culture of warmwater species such as crawfish (Huner and Barr 1980).

Geographic distribution of soil types indicate that about 70% of the soils in South Carolina are satisfactory for pond construction and water management (Foltz and Smith 1983). Over 60% of the area in South Carolina is suitable for warmwater aquaculture when soil types and average air temperatures are considered (Foltz and Smith 1984). Areas rated the highest for warmwater aquaculture in the State are in the lower coastal plain counties where the crawfish industry is centered. With its old ricefields, this area has the greatest potential for expansion of the crawfish industry in South Carolina.

Both in-state and out-of-state markets for South Carolina crawfish should continue to expand. Increased consumer awareness and demand should stimulate in-state market expansion for crawfish. Out-of-state markets will primarily be in the Middle Atlantic and Northeastern states. Crawfish farming is expanding in North Carolina, Virginia and Maryland, and this has helped expand markets for South Carolina crawfish. The S.C. Department of Agriculture has a aquaculture marketing specialist to assist producers in promotion and market development.

The profitability of crawfish aquaculture should continue to attract new entrants into the industry. Crawfish producers are also investigating ways to increase economic returns, and the most recent development is soft-shell crawfish aquaculture. There are currently 2 soft-shell crawfish producer in the State.

To help stimulate the aquaculture industry in South Carolina, the state legislature requested that a strategic plan for aquaculture development in the state be drafted (DeVoe 1987). This work was completed in 1987. The recommendations of the plan cover regulatory, financial, marketing, and research, education and technical assistance issues. A positive result of this plan is the establishment of a permit coordination office to assist new aquaculturists in getting started.

ACKNOWLEDGMENTS

Authors thank South Carolina crawfish growers and Jack M. Whetstone for sharing information about the industry. We also express our thanks for Dr. Robert P. Romaire for including us in the program and providing such a thorough review on an earlier manuscript draft. This manuscript and its preparation was funded in part by S.C. Sea Grant Consortium, S.C. Agricultural Experiment Station and Clemson University Extension Service.

LITERATURE CITED

Alon, N. C. & J. M. Dean. 1980. Production guidelines for crawfish farming in South Carolina. *Univ. S.C. Spec. Publ.* USC-BI-80-1. 71 pp.

Anonymous. 1983. South Carolina's aquaculture history. *Coastal Heritage Bull.* No. 5. 6 pp.

Avault, J. W., Jr. & J. V. Huner, 1985. Crawfish culture in the United States. In J. V. Huner and E. E. Brown (eds.) *Crustacean and Mollusks Aquaculture in the United States.* AVI Publ. Co., Inc. Westport, CT, pp. 1–61.

Brunson, M. W. 1987. Pre-flood evaluation of seven grasses (Graminae L.) as planted forage for crawfish. *J. World Aquacult. Soc.* 18(3):186–189.

Dellenbarger, L. E., L. R. Vandeveer, & T. M. Clarke. 1987. Estimated investment requirements, production costs, and breakeven prices for crawfish in Louisiana, 1987. *La. Agric. Expt. Stn., D.A.E. Res. Rep.* No. 670. 37 pp.

DeVoe, R. (ed.). 1987. Strategic plan for aquaculture development in South Carolina. Prepared for S.C. Joint Comm. on Aquaculture, Sept. 1, 1987. Columbia, 130 pp.

Doar, D. 1936. Rice and rice planting in South Carolina low country. *Charleston Mus. Contrib.* No. 8. 69 pp.

Epstein, M. B. & R. L. Joyner. 1986. Use of managed and open tidal marsh by waterbirds and alligators. In M. R. DeVoe & D. S. Baughman (eds.) *South Carolina Coastal Wetland Impoundments. Ecological Characterization, Management, Status and Use.* Vol. II. *Technical Synthesis. S.C. Sea Grant Consortium Tech. Rep.* SC-SG-TR-86-2. Charleston, SC, pp 529–579.

Foltz, J. W. & B. R. Smith. 1983. Freshwater aquaculture resources in South Carolina. *S.C. Agric. Expt. Stn. Circ.* 192. 7 pp.

Gresham, C. A. & D. D. Hook. 1982. Rice fields of South Carolina: A resource inventory and management policy evaluation. *Coastal Zone Manage. J.* 9(2):183–203.

Huner, J. V. & J. E. Barr. 1980. Red swamp crawfish: Biology and exploitation. *L.S.U. Ctr. Wetland Resour., Sea Grant Publ.* No. LSU-T-80-001. 148 pp.

Keith, W. J. & R. C. Gracy. 1972. History of the South Carolina oyster. S.C. Wildl. Mar. Resour. Dep., *Mar. Resour. Ctr. Educational Rep.* No. 1. 19 pp.

Landers, J. L., A. S. Johnson, P. H. Morgan, & W. P. Baldwin. 1976. Duck foods in managed tidal impoundments in South Carolina. *J. Wildl. Manage.* 40(4):721–728.

Morgan, P. H., A. S. Johnson, W. P. Baldwin, & J. L. Landers. 1975. Characteristics and management of tidal impoundments for wildlife in a South Carolina estuary. *Proc. Annu. Conf. Southeast. Assoc. Game Fish Comm.* 29:526–539.

Nussman, M. 1983. Aquaculture in South Carolina. *S.C. Sea Grant Consortium Rep.* 42 pp.

Pomeroy, R. S. 1988. South Carolina aquaculture situation and outlook. *Carolina Aquacult. News* 6(3):14–15.

Pomeroy, R. S. & Kahl, K. H. 1987. Crawfish in South Carolina: Development and current status of the industry. *S.C. Agric. Expert. Stn. Bull.* No. 661. 14 pp.

Pomeroy, R. S., D. B. Luke, & J. M. Whetstone. 1988. Budgets and cashflow statements for South Carolina, crawfish production. EER 83, *Dept. Agric. Econ.* EER 83, 37 pp.

Tiner, R. W., Jr. 1977. An inventory of South Carolina's coastal marshed. *S.C. Wildl. Mar. Resour. Dept. Tech. Rep.* No. 23. 33 pp.

Whetstone, J. M. 1988. Personal communication—aquaculture extension specialist. Clemson Univ. Extension Service, Georgetown, S.C.

Journal of Shellfish Research, Vol. 8, No. 1, 315–325, 1989

ABSTRACTS OF TECHNICAL PAPERS

Presented at the 42nd Annual Meeting

PACIFIC COAST OYSTER GROWERS ASSOCIATION

&

NATIONAL SHELLFISHERIES ASSOCIATION
(Pacific Coast Section)

Olympia, Washington

September 22–24, 1988

CONTENTS

A COMPARISON OF TANK AND BAG CULTURE OF MICROALGAE. J. Harold Beattie, Washington State Department of Fisheries Shellfish Laboratory, Brinnon, WA 98320.

The Point Whitney Laboratory geoduck hatchery began using bag culture for microalgae production two years ago. Last year we reported in a general way on the configuration and production levels using this type of system. Based on our experience so far, we can expect algal densities to be 3–5 times higher from bag culture than from tank culture. In the past year, we have had numerous inquiries about our system, and two commercial hatcheries and one experimental hatchery have begun using bag culture systems similar to ours.

This paper compares and contrasts our bag culture system with a commercial hatchery using a tank culture system, and one using a combination of the two. We have included for analysis the input of labor in man-hours, other costs and algal production levels in terms of cells per day.

UNIDENTIFIED PROTOZOAN PARASITES ASSOCIATED WITH DISEASE IN BIVALVES FROM BRITISH COLUMBIA. Susan M. Bower, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6.

To date, three unidentified protozoan parasites have been found to cause disease in bivalves in British Columbia. One protozoan is gregarine-like and occurs between the cells of the digestive tract epithelium and associated connective tissue of Manila clams (*Tapes philippinarum*) and in the connective tissues of Pacific oysters (*Crassostrea gigas*). Although this parasite appears nonpathogenic to adult bivalves, it may cause mortalities on oyster spat. The second protozoan is a microcell which is found intracellularly in vesicular connective tissue cells around green pustules of infected Pacific oysters. This parasite causes Denman Island disease and has been found at 8 localities in B.C. The parasite only occurs in the spring, but over 50% of the 2+ year-old oysters low on the beach can succumb to infection. The third protozoan is *Perkinsus*-like and occurs in the connective tissue of all organs of experimentally cultured Japanese scallops (*Patinopectin yessoensis*). Within two months at one grow-out site, this parasite caused mortalities in about 40% of the scallops. The current lack of knowledge concerning the etiology of these and other shellfish pathogens emphasizes the necessity of implementing precautions to prevent the spread of disease whenever live bivalves are transported.

SURVIVAL OF HATCHERY-GROWN GEODUCK (*PANOPE ABRUPTA*) SEED IN PUGET SOUND, WASHINGTON. Alex Bradbury, Washington State Department of Fisheries, 1000 Pt. Whitney Rd., Brinnon, WA 98320.

Washington Department of Fisheries has been experimentally seeding subtidal tracts in Puget Sound with juvenile geoducks since 1976. Our objective has been to augment the naturally low recruitment rate of geoducks so that such tracts may be commercially exploited on a more frequent basis. This year, due to improvements in both hatchery culture and planting methods, we have been able to begin seeding of commercial-sized tracts.

Since 1985, 13.7 million hatchery-grown juveniles between 2 and 22 mm shell length have been seeded subtidally either by divers or by an apparatus which scatters the seed on the surface. Follow-up survival estimates are made via dredge samples or diver counts and have ranged from 0%–10.7% after two years. A survival rate of 5% would make the program cost-efficient. Survival appears highest in tracts relatively free of predators such as flatfish, shrimp, snails, and starfish. Predation by the ubiquitous basket snail (*Nassarius* sp.) can be avoided with healthy, uncracked seed. Long-term predation appears lowest in mud/sand substrates rather than pure sand. Other variables apparently affecting survival (and their optimal values) include seed size (10 mm), seed density (30 seed/m^2), season (late spring/early summer), and proximity to adult geoducks.

IMPOSEX IN PACIFIC COAST NEOGASTROPODS RELATED TO TRIBUTYLTIN CONTAMINATION. Doug A. Bright, and **Derek V. Ellis,** Department of Biology, University of Victoria, P.O. Box 1700, Victoria, B.C., Canada, V8W 2Y2.

IMPOSEX, the development of male sex characters in the female, has been described for several Atlantic species of neogastropods. The relationship between imposex and TBT contamination in U.K. dogwhelks, *Nucella lapillus*, has been confirmed by Bryan and Gibbs (1986), and imposex has been proposed as an index of TBT contamination. TBT has known detrimental effects on oyster culture and a variety of other bivalves.

Surveys of neogastropod populations in southern British Columbia indicate that imposex is a widespread phenomenon occurring in all neogastropods examined (*Nucella lamellosa, N. canaliculata, N. emarginata, Searlesia dira, Neptunea phoenecia, Colus halli, Ocenebra lurida*) with the exception of *Ampissa columbiana*. Blockage of the genital pore by growth of the vas deferens leads to sterilization of females in only *N. lamellosa* due to differences in morphological patterns of sex character development. In a comparison of imposex between 3 local species of *Nucella*, it was found that in all populations examined except one, 100% of females examined had a penis. The severity of morphological response, as well as total body burden of TBT, was independent of body size. Typical whole body burdens for populations examined were from 0.1–0.3 μg/g. dw tin as TBT. Site to site variation in the population relative penis size (mean bulk of female penes/mean bulk of male penes) showed parallel trends for all three species.

Although the etiology of imposex has not been confirmed in Pacific Coast neogastropods, all three species of *Nucella* examined show promise as bioindicators of TBT contamination. Dif-

fering life histories may alter the interpretation of imposex observations. Examination of different sites suggests that chronic TBT contamination, sufficiently high to induce imposex, is widespread in southern B.C.s coastal waters.

THE JAPANESE OYSTER DRILL, *CERATOSTOMA INORATUM*, IN BRITISH COLUMBIA. Robert K. Cox, Aquaculture Operations Section, Agriculture and Fisheries, 808 Douglas St., Victoria, B.C., Canada V8W 2Z7.

The Japanese oyster drill, *Ceratostoma inoratum* was introduced into British Columbia along with early imports of the Japanese oyster, *Crassostrea gigas*. By the 1930's it had become well established at a number of locations within the Strait of Georgia. Currently, six areas of the B.C. coast are under restrictions that prohibit movement of shellstock and equipment to prevent the spread of the drill.

Little data is available to support the rationale for existing regulations with most work to-date consisting of ad hoc qualitative surveys.

In order to provide more detailed information a comprehensive survey of one restricted area, Comox Harbour, was undertaken in May, 1988. Results from this survey showed that adult drills and egg clusters were present in high numbers (1.3 and .4/m^2 respectively) but distribution was strongly clumped. The observed aggregated pattern was likely associated with egg laying.

Survey data indicated the continuing necessity of regulatory control and suggest operational constraints for shellstock movement.

GROWTH RATE OF SIBLING DIPLOID AND TRIPLOID OYSTERS, *CRASSOSTREA GIGAS*. Jonathan P. Davis, School of Fisheries, University of Washington, Seattle, WA.

The rate of growth was compared between diploid and triploid Pacific oysters over two years. Sibling diploid and triploid oysters were produced in the hatchery and grown in suspension culture in Quilcene Bay and Westcott Bay, Washington. Internal cavity volume, dry weight and wet weight was measured in individual oysters at approximate three month intervals. At both sites, triploids outperformed diploids after twenty-four months. In both bays, the growth rate of triploids exceeded that of diploids during the spring and summer months. At Quilcene Bay, a productive site with peak water temperatures exceeding 20° Celsius in July and August, the growth rate of triploids relative to diploids was greater than in Westcott Bay, a productive but cooler site where peak water temperatures only reach 16° Celsius.

This pattern of growth is discussed with respect to energetic considerations relating to the observation that oysters generally spawn every year at Quilcene Bay. In Westcott Bay, oysters develop an extensive gonad, generally do not spawn and resorb gametes during the fall and winter months.

The pattern of growth is discussed with reference to differential glycogen content and utilization, gametogenesis and spawning activity in diploid and triploid Pacific oysters.

RECRUITMENT OF DUNGENESS CRAB IN PUGET SOUND FROM OCEANIC STOCKS. Paul Dinnel, David Armstrong, Karen Larsen, Janet Armstrong, and **Bob Pacunski.** School of Fisheries WH-10, University of Washington, Seattle, WA 98195

Settlement of Dungeness crab (*Cancer magister*) post-larvae in Puget Sound was detected in early June 1988, although the year class probably started settling in early May. The magnitude of this settlement was monitored at about ten locations around the Sound throughout the Summer of 1988.

Very heavy recruitment of first and second instar crab was found at Dungeness Spit, Port Townsend and Useless Bay (Whidbey Island) with average densities of 80–200 juvenile crab/m in intertidal areas with 50–100% cover of eelgrass and/or macroalgae. Settlement densities were much less (0–20 crab/m^2) in the central and northern portions of Puget Sound.

This May settlement of Dungeness crab differed from the typical Puget Sound pattern in both timing and sizes of the 1st instars. Typical settlement in Puget Sound from 1984 through 1987 occurred in late July and August and the size range of 1st instar juveniles is about 4–5 to 6.0 mm Carapace Width (CW). The first instars of the May cohort ranged in size from about 6.0–7.5 mm CW. Both the timing and size match the settlement characteristics of coastal populations measured in the Grays Harbor/Willapa Bay region.

A pattern is emerging which suggests that recruitment of oceanic stocks of Dungeness crab in Puget Sound may occur sporadically depending on the vagaries of winter/spring meterological and oceanographic conditions. The effect of this early settlement of oceanic Dungeness crab on th population dynamics of this species in Puget Sound is presently unknown.

THE BENEFITS OF IMPROVED REFUGE ASSOCIATED WITH COMMERCIAL OYSTER CULTURE FOR THE SURVIVAL OF JUVENILE DUNGENESS CRAB. Daniel C. Doty, David A. Armstrong, and **Brett R. Dumbauld,** School of Fisheries WH-10, University of Washington, Seattle, WA 98195.

Ground culture of the Pacific oyster in Washington State estuaries such as Willapa Bay and Grays Harbor appears to benefit the Dungeness crab resource, and possibly the fishery, by providing critical habitat for 0+ crab newly settled to the intertidal zone of such estuaries. However, culture practices over the last 25 years have included the use of an insecticide Sevin sprayed to control burrowing shrimp whose activities inhibit oyster survival and growth. Incidental mortality of juvenile crab which occurs as a consequence of spraying Sevin is viewed by commercial crab fishermen as a threat to their industry. Studies of areas treated with Sevin suggest that the impacts are confined primarily to the treated areas, with the majority of crab killed being 0+ crab di-

rectly exposed to the spray and older crab which forage on the sites in the 24–48 hour period after treatment. There is growing evidence that crab loss during treatment with Sevin are substantially replaced during subsequent years of oyster culture by virtue of an increase in optimal shell habitat for 0+ crab. Measurements of intertidal 0+ crab density suggest that shell habitat supports higher densities (2–16 crab/m^2) of crab than does eelgrass (0–3 crab/m^2) or open ground. In addition, growout beds with 2–3 year old oyster support higher densities of crab than do newly planted seed beds.

ESTIMATING POLYPLOID PERCENTAGES USING OYSTER LARVAE: A VALUABLE HATCHERY MANAGEMENT AND RESEARCH TOOL. Sandra L. Downing, School of Fisheries WH-10, University of Washington, Seattle WA 98195.

Space limitations often force hatcheries to throw out larvae. By flowing trochophores (1-day-old larvae) or older larvae to determine how many polyploids are in a treated group, a hatchery could concentrate its efforts on the higher percentage groups. An example from our hatchery will be described. In addition, research can be expedited because experiments can be stopped or scaled down after only one week compared to the normal 4–6 week duration.

Details will be presented, but generally the technique involves putting hundreds to thousands of *Crassostrea gigas* larvae (1–15 days old) into a test tube. DAPI, a fluorescent dye, is then added and the larvae are run collectively on the flow cytometer. The polyploid percentages for each treated group are indicated by the relative areas of the generated histograms. To confirm the accuracy of this method, the ploidy of 25–30 spat from each group were determined individually.

Trochophores generally give wider, less accurate histograms than older larvae. After one week, there is little difference among samples; the standard deviation is around 10%. For example, in one treated group, flowing trochophores suggested a 50% triploid group. Seven day larvae gave an estimate of 66%, 12 day—80%, 15 day—70% and 57%, for an average of 68 ± 9.5%. Spat yielded 67% triploidy.

Besides the practical uses, this technique by sampling throughout the larval period will permit testing for differential survival between diploids and triploids. Furthermore, by comparing larvae and spat percentages, the setting success of treated groups can be assessed.

BURROWING SHRIMP: NEW BAIT FISHERY RESOURCE AND HISTORICAL PEST TO THE OYSTER INDUSTRY: A PRELIMINARY LOOK AT THEIR BIOLOGY IN WASHINGTON COASTAL ESTUARIES. Brett R. Dumbauld, David A. Armstrong, and **Dan C. Doty,** School of Fisheries, University of Washington, Seattle, Washington, 98195.

An investigation into the biology of the mud shrimp *Upogebia pugettensis* and ghost shrimp *Callianassa californiensis* in Washington state was initiated in April of this year as part of related studies on Dungeness crab in Willapa Bay. Previous and ongoing studies have focused on crab mortality caused by application of the insecticide SEVIN to oyster culture grounds to kill these burrowing shrimp. A growing fishery for the shrimp, which are harvested as bait for sport fisheries, prompted the Washington State Department of Fisheries to classify them as a commercial entity and bring them under management jurisdiction.

Analysis of qualitative data taken in 1987 and early 1988 indicates recruitment cycles for the 2 species of shrimp differ. Ovigerous female *Upogebia* were rarely encountered after May of each year whereas female *Callianassa* carried their egg clutches well into the summer. Newly recruited *Upogebia* (5 mm carapace length) began to appear in large numbers in samples taken in late June and early July of 1988. *Callianassa* appears to recruit in small numbers year round but major settlement probably occurs in late summer. Ramifications of these results with regards to both the spray schedule and the commercial bait fishery are discussed. A quantitative sampling technique is now being used to study and monitor populations of both species in Willapa Bay. Additional work related to the pesticide program includes a study of the efficacy of several Sevin concentrations to kill shrimp and experiments to further examine toxicity of contaminated shrimp to crab in the field.

RED ABALONE CULTURE IN THE PACIFIC NORTHWEST. Thomas B. Ebert, Ocean Resource Consulting Associates, POB 3334, Salinas, California 93912; **John McMullen,** Ab Lab, % NCEL, Port Hueneme, California 93043.

Ab Lab, a commercial abalone mariculture facility in southern California, developed a successful technique for growing abalone contained in modified plastic barrels (~55 gallons). The barrels are suspended from piers, rafts or longlines. This barrel culture technique provides: (1) natural growing conditions for the abalone, (2) minimal start-up and operational costs, (3) containment for the abalone while protecting them from predators, (4) easy access for feeding, managing, and harvesting, (5) a compatible, productive use of the coastal waters which can generate substantial primary or additional income for mariculturists.

Due to limited availability and access to growing areas in California, Ab Lab began searching for other potential growing sites. The Pacific Northwest (PNW) represents an area of tremendous potential for exploiting this technology due to the numerous quiet bays and on-going oyster, mussel, and salmon facilities. Although this area is outside the natural range of the red abalone, *Haliotis rufescens*, previous data suggests that they will grow, but not reproduce. Presently, Ab Lab is evaluating the feasibility of growing red abalone, utilizing the barrel culture technique, from a seed size (~¼″) to a marketable sized animal (2–3″) in the PNW.

AQUACULTURE HEALTH MANAGEMENT AND DISEASE CONTROL IN THE PACIFIC NORTHWEST. Ralph Elston, Battelle Marine Research Laboratory, 439 West Sequim Bay Road, Sequim, WA 98382.

Infectious diseases of farmed and natural populations of marine invertebrates can have a significant impact of the production and harvest of these species. The risk of exotic diseases which can cause the most serious impact can be reduced by controlling and the importation of exotic species. This control consists of pathological and historical evaluation of the species and population proposed for introduction. The impact of enzootic diseases (those already present in the region) can be reduced by developing husbandry management methods through applied research. In order to effectively control the importation of exotic diseases and allocate research funds for the management of enzootic diseases it is first necessary to develop an inventory of regional invertebrate diseases, estimate the economic impact of each disease and prioritize their importance. The status of this inventory will be discussed. Case histories, including bonamiasis of flat oysters, hemic neoplasia of mussels, nocardiosis of the Pacific oyster, oyster velar virus disease, ligament disease of juvenile oysters, vibriosis of larval oysters, Denman Island disease and the Pacific razor clam NIX will be used to illustrate known distribution and impact of these diseases and methods known for their management.

NOCARDIOSIS OF ADULT PACIFIC OYSTERS, *CRASSOSTREA GIGAS*. Carolyn S. Friedman, Blaine L. Beaman, Ronald P. Hedrick,[1] J. H. Beattie[2] and **Ralph A. Elston,[3]** [1]Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616. [2]School of Fisheries, University of Washington, Seattle, WA 98195. [3]Battelle Marine Research Laboratory, 439 W. Sequim Bay Rd., Sequim, WA 98382.

Focal necrosis of adult Pacific oysters (*Crassostrea gigas*) has been reported to coincide with recurrent mid to late summer mortalities in Matsushima Bay, Japan. The disease, now designated Pacific oyster nocardiosis (PON), has also been observed among Pacific oysters in the state of Washington, U.S.A., where it is believed to cause significant mortalities during mid summer to early fall. The nature and significance of the disease to oyster populations is still poorly understood. We have examined oysters from 10 sites in Washington and three sites in British Columbia, Canada in an effort to better characterize the etiology, pathogenesis, and distribution of PON.

The principal lesion is composed of host inflammatory cells (amoebocytes) surrounding colonies of gram-positive, acid-fast, beaded and branched actinomycete-like bacteria. Lesions are primarily found within oyster vesicular connective tissue cells surrounding the gut and digestive diverticulae. A bacterium with the same tinctorial and morphological properties noted above has been isolated from diseased oysters collected in several of the sample sites. Thin-layer and gas liquid chromatographic analyses of extracted bacterial cell wall mycolic acids (lipids) indicate that the bacteria belong to the genus *Nocardia*.

Injection of the isolated bacterial cultures into the branchial vein of Pacific oysters reproduces the same gross and histological pathology observed in naturally infected animals. The same bacterium has been reisolated from challenged oysters indicating that the nocardial bacterium is the etiological agent of PON. Cohabitation experiments in which diseased oysters are incubated with uninfected animals indicate that the disease is not easily transmitted via the water. Further experiments regarding the taxonomic placement of the pathogen and transmission of the disease are currently in progress.

CELL FUSION IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*. I. FORMATION OF POLYPLOID CELLS VIA OOCYTE FUSION. Ximing Guo, William K. Hershberger, Kenneth K. Chew and **Paul Waterstrat,** School of Fisheries WH-10, University of Washington, Seattle, Washington 98195.

The use of cell fusion to construct "new" combinations of genetic material in Pacific oyster has some very exciting and useful applications in oyster culture. For example, the fusion of two fertilized eggs could lead to the production of tetraploid oysters; these tetraploids could then be crossed with diploids to produce 100% triploid groups for grow-out. Many other useful combinations could be produced also. Consequently, this study was undertaken to determine the feasibility of successfully fusing Pacific oyster cells and to define the parameters for optimizing the yield of fused cells.

Mature oocytes were obtained from conditioned oysters and kept in calcium- and magnesium-free seawater. The oocytes were treated with trypsin to remove the vitelline membrane and then exposed to a urea solution. Polyethylene glycol was applied as a fusogen to induce fusions. Most fusions occurred 10 min after the treatment with fusogen, and fusion levels ranged from 1–24% of the treated oocytes. A number of the fused oocytes were collected by micropipet and fertilized. About 30% of these exhibited embryonic development, and chromosomal analysis revealed all were polyploids (mostly mosaic). Additional research is needed to define the conditions required to increase the yield of fused eggs and assure more consistent development of tetraploids.

DOMESTICATION AND BREEDING OF PACIFIC OYSTERS. Dennis Hedgecock, Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923.

Domestication usually conveys the image of docile, productive farm animals much changed from their wild ancestors. To a large extent, however, domestication involves changes in human behavior that make possible breed development and improvement. Most fundamental are the assignment of economic value to the species, to traits, and to individual broodstock and the keeping of

pedigrees, without which breeding value cannot be judged and loss of genetic diversity through accidental inbreeding is likely.

Though the life cycle of Pacific oysters is now controlled by the west coast industry, many behaviors conducive to domestication and improvement are still absent: pedigreeing, stock development, identification of traits and their economic value, and assignment of breeding values to individual broodstock. These needs are being addressed by a collaborative research project funded by the USDA's Western Regional Aquaculture Consortium, involving the University of California, the University of Washington and Coast Oyster Co. Participation of other PCOGA collaborators is being solicited.

The WRAC project is producing families of known pedigree whose growth to market sizes and sexual maturation in different growout areas will be measured. The objectives are to learn how to pedigree oysters, to produce pedigreed stocks, and to partition variability in growth and reproduction into genetic and environmental components. Five experimental crosses, a total of 120 families, have been completed; most of these have been deployed to growout areas in Puget Sound, WA, and Humboldt Bay, CA. Pedigreeing is difficult and not easily put to commercial practice. Industry must cease mass spawnings, admixing of spawns at all phases of growout, and haphazard selecting of broodstock in order to make progress towards domestication.

INFLUENCE OF WATER QUALITY ON LARVAE OF THE RED ABALONE *HALIOTIS RUFESCENS*. W. B. Jaeckle, and **D. T. Manahan,** Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371.

Larval abalone (*Haliotis* spp.) are structurally incapable of ingesting particulate food and the energy necessary for development is thought to come solely from endogenous reserves. These larvae are considered to be energetically independent of the environment and abalone hatcheries raise their larvae in water that has been treated to eliminate bacterial contaminants ("biological filters" and UV-oxidation). However, these treatments are known to decrease the amount of certain dissolved organic compounds present in seawater. Abalone trochophore and veliger larvae can take up dissolved free amino acids from seawater and metabolically use these transported compounds. As dissolved organic material in seawater represents the sole source of exogenous energy available to developing abalone, any manipulation that changes the organic chemistry of seawater can have an impact on the larvae.

Abalone larvae, raised in static batch culture, increase in biomass during the first two days of development when raised in seawater that has only been mechanically filtered (to 0.2 μm). Following this initial growth, there was little change in biomass during the remainder of the larval life. Larvae reared in 0.2 μm-filtered seawater, that had previously been treated with a biological filtration system, decreased in biomass during the first two days of development. Biomass decreases in larvae raised in biologically-filtered water were reversed by changing the source and treatment (only mechanical filtration) of the seawater. Thus, the organic chemistry of seawater provides energy to developing larvae. These data suggest that water treatments (biological filtration and UV-oxidation) employed in hatcheries are reducing the only source of exogenous food for developing abalone. For abalone larvae, such decreases in dissolved organic materials were correlated with a decrease in larval biomass during development.

UTILIZATION OF CELLULOSIC DETRITUS AND BACTERIA AS A FOOD SOURCE BY MARINE SUSPENSION-FEEDERS. Christopher J. Langdon, and **Dan A. Kreeger,** Department of Fisheries and Wildlife, Hatfield Marine Science Center, Newport, Oregon 97365; **Roger I. E. Newell,** Horn Point Laboratories, University of Maryland, Cambridge, Maryland 21613.

We have examined the ability of two species of bivalve molluscs to utilize cellulosic detritus and bacteria as sources of dietary carbon and nitrogen. The ribbed mussel *Geukensia demissa* commonly inhabits marshes of the east coast of the United States and is adapted to digest and absorb refractory cellulosic carbon and filter bacteria from suspension more efficiently than the American oyster *Crassostrea virginica*. We have estimated that less than 5% of the total carbon requirements of the oyster could be met by utilization of cellulosic detritus and bacteria in the marsh habitat. In contrast, the mussel could obtain 15% of its total carbon requirements from cellulose plus 38% from utilization of bacteria. We estimated that bacteria could also provide the mussel with 87% of its metabolic nitrogen requirements.

REQUIREMENTS FOR SPECIFIC AMINO ACIDS AND PROTEINS BY OYSTER LARVAE (*CRASSOSTREA GIGAS*). Donal T. Manahan, and **S. Nourizadeh,** Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371.

Techniques to culture oyster larvae are well established. However, the biochemical basis for the observed growth is not understood. Analysis of the amino acid composition of the proteins of *C. gigas* larvae showed that glycine and alanine were the predominant neutral amino acids at 12% and 8%, respectively. Electrophoretic analysis of the proteins of embryos and larvae, which were exposed to 14C-alanine, showed that the pattern of incorporation of 14C-alanine was very different for each developmental stage. In embryos, most of the 14C-alanine was found in proteins with molecular weights ranging from 13.6–15.0 kD. Larvae, in contrast, had no low-molecular weight proteins labeled by 14C-alanine; the smallest proteins labeled had a molecular weight around 39.5 kD.

Taurine, a sulfur-containing amino acid, was found to be the major (70%) organic osmolyte in *C. gigas* larvae. The absolute amount of taurine per larva increased during growth. A larva with 150 μm shell length had 220 pmoles of taurine/larva; a 300 μm larva had 375 pmoles. Analysis of the amino acids in a commonly-used larval food (*Isochrysis galbana*) revealed that there was no taurine present in this alga. The sulfur-containing amino acids, Cys and Met, are usually precursors for taurine biosynthesis. However, in studies with both axenic and nonaxenic *C. gigas* larvae of different sizes, no taurine biosynthesis was observed from either 35S-labeled Cys or Met. These findings suggest that taurine is an essential amino acid for the growth of oyster larvae, but that this requirement is not supplied by the algal diet.

CERTIFICATION POLICY FOR IMPORTATION OF *CRASSOSTREA GIGAS* SPAT INTO THE STATE OF ALASKA. Theodore R. Meyers, Alaska Department of Fish and Game, Fisheries Rehabilitation and Enhancement Division, P.O. Box 3-2000, Juneau, Alaska 99802.

In Alaska the Japanese oyster is the only shellfish species permitted by state regulations for import and does not include stocks from Korea, the Gulf of Mexico and the Atlantic coast of North America. Any grower within Alaska having intent to import Japanese oysters of a particular stock is required to submit a Fish Transport Permit application for approval by the ADF&G. Part of this approval is based upon successful disease certification of the proposed stock by the FRED fish pathology lab. The current pathology policy allows only spat or seed (animals <1 yr-old) to be imported due to the increased risk of transporting exotic diseases which may infect the older and larger animals. Certification is contingent upon the vendor having a stable brood source which does not change from year to year and no detection of disease organisms having transport significance within the considered oyster stock. Certification does not mean disease-free but that no significant agents were detected within the limits of the diagnostic examination. Certification procedures follow American Fisheries Society guidelines regarding sample sizes. Sixty adults of the parent stock, 200 spat and about 1–2 ml of larvae (if available) are the required samples. Renewal of certification is on a yearly basis, requiring examination of 60 spat from the year class to be imported, and an updated disease history and hatchery performance review of the hatchery stocks from the vendor for the previous growing season. A certification will become invalid if a disease outbreak occurs within stocks at the facility, or if an uncertified stock is brought into the rearing facility or grow-out area. Three facilities are currently certified for oyster importation into Alaska: one in British Columbia, Canada; one from California and one from Washington State.

INTERIM RESULTS OF THE KODIAK, ALASKA SCALLOP MARICULTURE FEASIBILITY STUDY. William P. Osborne, Kodiak Area Native Association, 402 Center Avenue, Kodiak, Alaska; **Tomizo Sakamoto, Kiyoshi Iwagishi,** and **W. Michael Kaill,** Alaska Department of Fish and Game, 211 Mission, Kodiak, Alaska.

The Kodiak Area Native Association, the Overseas Fishery Cooperation Foundation of Japan, and the Alaska Departments of Fish and Game and Commerce and Economic Development have been cooperatively conducting a feasibility study of scallop mariculture around Kodiak Island, Alaska, since May 1987. The goal of the project is to encourage economic development in Alaskan coastal communities. Weekly plankton samples were taken from May to September and oceanographic measurements were made throughout the year at several sites around the island. Japanese style spat collectors were set out and recovered at intervals to determine the timing of spat settlement. Of the scallop spat collected, 90% were *Chlamys rubida* and 10% were *Chlamys hastata*. Growth rates and the potential markets for these scallops are being studied.

STORAGE AND TRANSPORTATION OF STRAIGHT-HINGE OYSTER LARVAE. L. Panggabean, P. R. Waterstrat, S. L. Downing and **J. L. Beattie,** School of Fisheries WH-10, University of Washington, Seattle, WA 98195.

Northwest oyster hatcheries routinely transport eyed-larvae for remote setting at farming or grow-out sites. The advent of this technique has contributed to the economic success of commercial oyster hatcheries by allowing the efficient use of hatchery capacity and manpower. Storage and transportation of straight-hinge larvae can provide further gains in hatchery efficiency and versatility. Limited tank capacities in hatcheries often preclude rearing all the larvae available from a spawn to setting size. Storage of excess larvae until tank space is available or the transport of these larvae to hatcheries with available space would allow more efficient use of the effort expended in obtaining viable larvae. Transportation of straight-hinge larvae would also allow the expanded use of larvae obtained through specialized conditioning regimens, spawning techniques or genetic manipulation. For example, triploid straight-hinge larvae could be produced in commercial quantities in the small experimental hatchery at the University of Washington and shipped to larger commercial hatcheries for rearing to eyed larvae.

To investigate both the feasibility of straight-hinge larval storage and the conditions suitable for storage, straight-hinge larvae were stored for 48 hours under a variety of test conditions and evaluated for subsequent survival and growth under standard hatchery rearing protocols. Three different containers, plastic beakers, polyethylene bags and nytex screening, as well as storage temperature and larval density have been examined.

Preliminary evidence indicates that 48 hr storage of straight-hinge larvae is feasible. Evaluation of storage conditions suggests that larvae stored at 5°C exhibit greater survival than larvae held at room temperature.

SOLVING PROBLEMS WITH REMOTE SETTING PACIFIC OYSTER LARVAE IN BRITISH COLUMBIA. W. G. Roland, T. A. Broadley, and **I. R. Sutherland,** Ministry of Agriculture and Fisheries, Parliament Buildings, Victoria, British Columbia.

Remote Setting of oyster larvae is widely recognized as the key to solving the chronic need for a reliable and economical source of seed oysters in British Columbia. Lack of sound guidelines has resulted in low or variable average setting and spat survival rates for the more than 50 farms using the process.

The strategy to solve problems associated with remote setting has included:

1. publications and courses on the use of the process;
2. annual workshops for problem identification and exchange of new technology;
3. a standardized data collection method for industry to use in assessing their success; and
4. experimental investigations of variables that affect the percentage of larvae setting, their distribution on cultch, and post-set survival of spat. These will give a good basis from which sound guidelines can be constructed within the coming year.

These initiatives should provide a sound basis for future growth of oyster culture in British Columbia.

GENETIC DRIFT AND EFFECTIVE POPULATION SIZES IN COMMERCIAL STOCKS OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*, ON THE U.S. WEST COAST. Fred Sly, and **Dennis Hedgecock,** UC Davis Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA 94923.

Culture of the Pacific Oyster, *Crassostrea gigas*, along the west coast of the United States relies almost exclusively on seed produced by a few major commercial hatcheries. Because this introduced species reproduces naturally in only a few localities, commercial stocks have in recent years been isolated from natural stocks. While isolation makes possible domestication and genetic improvement, it necessitates the careful management of these captive gene pools. Improper broodstock management and ill-conceived breeding programs result in inbreeding, reductions in genetic diversity, and declines in performance.

Using allozyme analysis we have scored individual differences at 14 polymorphic enzymes in samples of natural set from Dabob Bay, WA, and in samples of third generation hatchery stocks derived from such natural set and reared on commercial growout beds in Willapa Bay, WA and Humboldt Bay, CA. Hatchery stocks differ markedly from the naturally occurring population at many loci. Assuming that the Dabob Bay sample represents the population from which the commercial stocks were derived, we calculate that the genetically effective population sizes of the Willapa and Humboldt stocks are only 40.6 ± 13.9 and 8.7 ± 2.1, respectively. Continued use of restricted effective population sizes can lead rapidly to extensive inbreeding of commercial stocks and declines in growth and reproductive performance.

COMPARING DIPLOID AND TRIPLOID GAMETES AND ZYGOTES FROM PACIFIC OYSTERS (*C. GIGAS*) USING A SCANNING ELECTRON MICROSCOPE. L. B. Stephens, and **S. L. Downing,** School of Fisheries WH-10, University of Washington, Seattle, WA 98195.

Eggs and sperm from mature adult diploid and triploid Pacific oysters will be fixed and examined under a scanning electron microscope. Differential sizes of sperm and unfertilized eggs will be determined. Size of micropyles will be compared between the diploid and triploid eggs. Observations will also be made on fertilized eggs treated with cytochalasin B to induce triploidy.

CONTROLLED PURIFICATION—A POLICY OPTION FOR THE MANAGEMENT OF WASHINGTON STATE COMMERCIAL SHELLFISH RESOURCES. Marilou M. Taylor, Student Institute for Marine Studies, University of Washington and Registered Sanitarian, King County Dept. of Health, Seattle, Washington 98195.

Controlled purification is a technical process which provides a clean seawater environment in which bivalve molluscan shellfish may actively cleanse themselves. The process is employed globally. Currently, commercial purification plants are located in the United Kingdom, France, Spain, Japan, and Australia; the process is mandatory for commercially harvested species in Spain and Australia. In the United States, 22 controlled purification facilities are in operation in eight states. The majority of United States plants are located on the eastern seaboard. To date, controlled purification has never been employed in Washington State.

This paper addresses the question of whether the process of controlled purification should be permitted in Washington State. The discussion of the issue is presented through an analysis of the present Washington State controlled purification policy, which is a prohibitive one, and two alternative policy options for the management of commercial shellfish resources.

FLATWORM PREDATION ON YOUNG CROPS OF REMOTE SET MUSSELS, *MYTILUS EDULIS*, L. George A. Trevelyan, University of California, Bodega Marine Laboratory, Bodega Bay, CA 94923.

In an attempt to gain more experience in the setting and growout of hatchery-reared mussel spat, 2 crops, totalling 5 × 10 spat (0.9–1.9 mm) were set and planted at Tomales Bay, CA in early

and late March, 1988. The bottom of the set tank was lined with scour pad material in net tubing. Spat were allowed to attach for 1–3 days before planting onto longlines. The percent of spat that failed to attach, and thus were left behind in the set tank, ranged from 1–4%.

A polyclad flatworm settled in early March onto the early crop (at a mean density of 148/m^2), but not onto the late crop. The early crop suffered 96% mortality to day 43 while the late crop had much lower mortality and developed into a heavy seed crop. In the field, the flatworms were observed with the whole meats of the small (1 cm) mussels in their guts. Thousands of empty mussel shells were also present on the early crop. In the laboratory at 9–14°C, the flatworms ate 4–12 mm mussels at a mean rate of 0.6 mussels/worm/day. They preferentially ate mussels at the base of mussel clumps, thereby destabilizing whole clumps. These flatworms were intolerant of freshwater; a 30 second dip being sufficient to kill them.

The spat used in this study were derived from larvae reared at Whiskey Creek Oyster Farm and metamorphosed at Kuiper Mariculture Inc.

INFORMATION FOR CONTRIBUTORS TO THE *JOURNAL OF SHELLFISH RESEARCH*

Original papers dealing with all aspects of shellfish research will be considered for publication. Manuscripts will be judged by the editors or other competent reviewers, or both, on the basis of originality, content, merit, clarity of presentation, and interpretations. Each paper should be carefully prepared in the style followed in Volume 3, Number 1, of the *Journal of Shellfish Research* (1983) before submission to the Editor. Papers published or to be published in other journals are not acceptable.

Title, Short Title, Key Words, and Abstract: The title of the paper should be kept as short as possible. Please include a "short running title" of not more than 48 characters including space between words, and approximately seven (7) key words or less. Each manuscript must be accompanied by a concise, informative abstract, giving the main results of the research reported. The abstract will be published at the beginning of the paper. No separate summary should be included.

Text: Manuscripts must be typed double-spaced throughout one side of the paper, leaving ample margins, with the pages numbered consecutively. Scientific names of species should be underlined and, when first mentioned in the text, should be followed by the authority.

Abbreviations, Style, Numbers: Authors should follow the style recommended by the fourth edition (1978) of the *Council of Biology Editors [CBE] Style Manual*, distributed by the American Institute of Biological Sciences. All linear measurements, weights, and volumes should be given in metric units.

Tables: Tables, numbered in Arabic, should be on separate pages with a concise title at the top.

Illustrations: Line drawing should be in black ink and planned so that important details will be clear after reduction to page size or less. No drawing should be so large that it must be reduced to less than one third of its original size. Photographs and line drawings preferably should be prepared so they can be reduced to a size no greater than 17.3 cm × 22.7 cm, and should be planned either to occupy the full width of 17.3 cm or the width of one column, 8.4 cm. Photographs should be glossy with good contrast and should be prepared so they can be reproduced without reduction. Originals of graphic materials (i.e., line drawings) are preferred and will be returned to the author. Each illustration should have the author's name, short paper title, and figure number on the back. Figure legends should be typed on separate sheets and numbered in Arabic.

No color illustrations will be accepted unless the author is prepared to cover the cost of associated reproduction and printing.

References Cited: References should be listed alphabetically at the end of the paper. Abbreviations in this section should be those recommended in the *American Standard for Periodical Title Abbreviations*, available through the American National Standard Institute, 1430 Broadway, New York, NY 10018. For appropriate citation format, see examples at the end of papers in Volume 3, Number 1, of the *Journal of Shellfish Research* or refer to Chapter 3, pages 51–60 of the *CBE Style Manual*.

Page Charges: Authors or their institutions will be charged $50.00 per printed page. If illustrations and/or tables make up more than one third of the total number of pages, there will be a charge of $30.00 for each page of this material (calculated on the actual amount of page space taken up), regardless of the total length of the article. All page charges are subject to change without notice.

Proofs: Page proofs are sent to the corresponding author and must be corrected and returned within seven days. Alterations other than corrections of printer's errors may be charged to the author(s).

Reprints: Reprints of published papers are available at cost to the authors. Information regarding ordering reprints will be available from The Sheridan Press at the time of printing.

Cover Photographs: Particularly appropriate photographs may be submitted for consideration for use on the cover of the *Journal of Shellfish Research*. Black and white photographs, if utilized, are printed at no cost. Color illustrations may be submitted but all costs associated with reproduction and printing of such illustrations must be covered by the submitter.

Corresponding: An original and two copies of each manuscript submitted for publication consideration should be sent to the Editor, Dr. Sandra E. Shumway, Department of Marine Resources, and Bigelow Laboratory for Ocean Science, West Boothbay Harbor, Maine 04575.

THE NATIONAL SHELLFISHERIES ASSOCIATION

The National Shellfisheries Association (NSA) is an international organization of scientists, management officials and members of industry that is deeply concerned and dedicated to the formulation of ideas and promotion of knowledge pertinent to the biology, ecology, production, economics and management of shellfish resourses. The Association has a membership of more than 900 from all parts of the USA, Canada and 18 other nations; the Association strongly encourages graduate students' membership and participation.

WHAT DOES IT DO?

—Sponsors an annual scientific conference.
—Publishes the peer-reviewed *Journal of Shellfish Research*.
—Produces a Quarterly Newsletter.
—Interacts with other associations and industry.

WHAT CAN IT DO FOR YOU?

—You will meet kindred scientists, managers and industry officials at annual meetings.
—You will get peer review through presentation of papers at the annual meeting.
—If you are young, you will benefit from the experience of your elders.
—If you are an elder, you will be rejuvenated by the fresh ideas of youth.
—If you are a student, you will make most useful contacts for your job search.
—If you are a potential employer, you will meet promising young people.
—You will receive a scientific journal containing important research articles.
—You will receive a Quarterly Newsletter providing information on the Association and its activities, a book review section, information on other societies and their meetings, a job placement section, etc.

HOW TO JOIN

—Fill out and mail a copy of the application blank below. The dues are 30 US $ per year ($20 for students) and that includes the *Journal* and the Newsletter!

NATIONAL SHELLFISHERIES ASSOCIATION—APPLICATION FOR MEMBERSHIP
(NEW MEMBERS ONLY)

Name: _____ For the calendar year: _____ Date: _____
Mailing address: _____
_____
_____
Institutional affiliation, if any: _____
Shellfishery interests: _____

Regular or student membership: _____
Student members only—advisor's signature REQUIRED: _____

Make cheques (*MUST* be drawn on a US bank) or international postal money orders for $30 ($20 for students with advisor's signature) payable to the National Shellfisheries Association and send to Dr. Tom Soniat, Dept of Biology, University of New Orleans, New Orleans, Louisiana 70148 USA.

CONTENTS (Continued)

COVER PHOTO: American lobster, *Homarus americanus* suffering from shell disease. Photo courtesy of Maine Department of Marine Resources.

JOURNAL OF SHELLFISH RESEARCH

Vol. 8, No. 1 June 1989

CONTENTS

CONTENTS CONTINUED ON INSIDE BACK COVER

JOURNAL OF SHELLFISH RESEARCH

VOLUME 8, NUMBER 2 DECEMBER 1989

The *Journal of Shellfish Research* (formerly *Proceedings of the National Shellfisheries Association*) is the official publication of the National Shellfisheries Association

Editor

Dr. Sandra E. Shumway
Department of Marine Resources
and
Bigelow Laboratory for Ocean Science
West Boothbay Harbor
Maine 04575

EDITORIAL BOARD

Dr. Monica Bricelj
Marine Sciences Research Center
State University of New York
Stony Brook, New York 11794-5000

Dr. Anthony Calabrese
National Marine Fisheries Service
Milford, Connecticut 06460

Dr. Kenneth K. Chew
College of Fisheries
University of Washington
Seattle, Washington 98195

Dr. Charles Epifanio
College of Marine Studies
University of Delaware
Lewes, Delaware 19958

Dr. Paul A. Haefner, Jr.
Rochester Institute of Technology
Rochester, New York 14623

Dr. Robert E. Hillman
Battelle Ocean Sciences
New England Marine Research Laboratory
Duxbury, Massachusetts 02332

Dr. Herbert Hidu
Ira C. Darling Center
University of Maine
Walpole, Maine 04573

Dr. Lew Incze
Bigelow Laboratory for Ocean Sciences
McKown Point
West Boothbay Harbor, Maine 04575

Dr. Louis Leibovitz
Marine Biological Laboratory
Woods Hole, Mass. 02543

Dr. Roger Mann
Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

Dr. Gilbert Pauley
College of Fisheries
University of Washington
Seattle, Washington 98195

Dr. Les Watling
Ira C. Darling Center
University of Maine
Walpole, Maine 04573

Journal of Shellfish Research
Volume 8, Number 2
ISSN: 00775711
December 1989

IN MEMORIAM

Robert Winston Menzel, Sr.
1920–1989

The aquatic science community lost a colleague and dear friend in June with the death of Dr. Robert Winston Menzel, Sr. I learned of Winston Menzel's passing from Sandy Shumway some three weeks after the event. Sandy asked me to share some thoughts about Winston and the significant impact he had on our profession. What follows are some of my remembrances that have endeared him to me. That is followed by lists of the graduate students who took degrees under him and his list of publications. I am proud to be included in the former.

In the academic world, where egos reign supreme and it seems as though it is always possible to find someone with an ego that is slightly larger than the most gigantic one you've ever seen, Winston Menzel was something of an enigma. He was, at least in the years that I knew him (slightly over 20), among the most humble human beings in my acquaintance. His manner (relaxed, almost plodding) and speech (a slow, downhome Virginia drawl) belied his enthusiasm, keen wit, and outstanding intellectual ability. Winston always spoke fondly of his former students and was intensely interested in their careers. He seemed much less interested in having any glory heaped upon himself, though his research productivity led to some such heaping, which he accepted with humility.

While he was not at all dynamic in the classroom, students who had the good fortune to have him available to them outside the lecture hall found him to be an absolute fountain of information. Winston was knowledgeable in virtually every aspect of marine biology, and in a wide variety of subject areas outside of that broad discipline. In his many conversations with the students that surrounded him, he always had some tidbit of information that none of us had previously known, and he frequently could drag out a reprint to underwrite his information. A trait that meant perhaps as much to me as any, was Winston's availability to his students.

Winston joined the faculty at Florida State University in 1954 after taking his Ph.D. from Texas A&M University under Sewell Hopkins. By 1968, when I got to Florida State, Winston had moved up to a spacious closet office in what had been Bachelor Officer Quarters during World War II. The buildings—I seem to recall that there were five of them—

housed the Oceanography Department during its first few years of existence. Other students, such as Ed Cake, Ed Bault, and Pat McCaffrey who were all contemporaries of mine, were housed in one end of a BOQ with Winston. His door was always open to us and many hours were spent learning about the wonders of marine science in his presence.

Winston introduced me to my first raw oyster; actually to my first oyster of any kind. We were at the old Florida State marine laboratory on Alligator Harbor when he found a cluster of oysters on a piling. He whipped out his pocket knife, deftly shucked a large *Crassostrea virginica,* and passed it my way. That was the start of a love affair that continues to this day. It has been difficult to make up for nearly 30 years of deprivation. Winston gulped down several of the delicacies on that trip, and there were many other opportunities at various seafood restaurants that were obligatory pit stops on our field trips to Alligator Harbor and the then new Turkey Point laboratory to observe Winston's gustatory pleasures being accommodated.

Winston, in fact, had a reputation for eating nearly anything. He admitted to having consumed the world's largest stone crab before anyone had a chance to measure it for the record books. Somewhat less probable is the story about the recovery of the only known living trilobite. After bringing it aboard the ship, the scientific party, with one exception, began congratulating each other about their amazing find. In the meantime, the subject of their delight disappeared. Winston Menzel was observed leaving the deck of the ship with a leg of some type of sea creature protruding from the corner of his mouth. Or, so the story goes.

I attended Florida State University for a variety of reasons, though none of them involved any attempt with linking myself with a particular professor. Becoming associated with Winston Menzel was largely chance. While I was a student, I knew him as a mollusc biologist who had done some interesting work on oyster genetics, had demonstrated the economic and biological feasibility of rearing quahog clams in Florida long before any commercial aquaculturist made an attempt to do so, and as a person who kept algae cultures going and spawned the occasional shellfish.

The greater breadth and depth of the man came to be appreciated in large part after I left his tutelage. There was one episode, however, that was humiliating for me while at the same time providing me with a much greater appreciation for Winston's scope of expertise. I had written a term paper for one of Winston's classes in which I reviewed the literature on catfishes and their culture. When he returned my graded paper, he indicated that it was acceptable but that I had missed two critically important references. That didn't seem likely in that I felt my literature search had been painstakingly thorough. The two papers in question involved the catfish fishery of Virginia and an incidence of albinism in catfish in the same state. Both papers had been written by R. Winston Menzel. My face was red, and I've subsequently cited both papers (they were Winston's first two publications) on many occasions.

My first job after completing my education was at Skidaway Institute of Oceanography in Savannah, Georgia where I worked under the direction of David Menzel (no relation). In a conversation with David and my wife one day, the fact that I had been associated with two Menzels arose. David made some comment to the effect, "I didn't know you had studied under the famous Menzel." Certainly, both Menzels can lay claim to fame, but between my wife and I, Winston has always been the "famous" Menzel.

Some of our fondest memories of Florida State University, Tallahassee, and the Menzel's were the get-togethers that Margaret (now deceased) and Winston hosted at their home. We always felt very welcome, and they tolerated and even seemed to enjoy the small children that came along with students like myself. The Menzel's always made the students feel comfortable and were truly interested in us and in our families.

The homespun demeanor, slow drawl, and easygoing nature demonstrated by Winston did nothing to detract from the respect that we as graduate students held for him. None of us would have ever thought of calling him by his first name, at least while we were students. Some referred to him as 'doc,' but that was as familiar as it became. In fact, it was several years before I could address him as other than 'Dr. Menzel,' and my wife continues to refer to him by that name. Yet, Winston did not demand respect in any outward way. Instead, he genuinely earned it. The longer I knew him, the more respect I developed.

Shortly after I joined the faculty of Texas A&M University in 1975, I had the opportunity of having dinner with Winston Menzel and Sewell Hopkins. It occurred to me as I sat in the presence of two eminent scientists that while publications provide a legacy, an important part of what academicians leave behind is the knowledge and the philosophy that are passed down through their students to later generations. Those of us who were Winston's students can only hope that some of the knowledge, dedication, high ethical standards, and love of life that was exhibited by him have been kept alive in us and passed along to our students.

I'll miss you, Winston, and I'll always wonder if you really did eat that trilobite!

Robert R. Stickney
Seattle
July, 1989

M.S. Students who completed degrees under R. Winston Menzel

1955 T. M. Smith. "The distribution and breeding of chaetognaths of the northwest coast of Florida" (Now M.D.)
1956 F. E. Nichy. "The effects of predators on the mortality of oysters in a high salinity area in Florida"
1956 B. C. Townsend, Jr. "A study of the spot, *Leiostomus xanthurus* Lacepede in Alligator Harbor, Florida"
1957 R. R. Hathaway. "Studies on the crown conch, *Melongena corona* Gmelin" (Ph.D. later)
1958 F. K. Little. "The sponge fauna of St. George's Sound, Apalachee Bay and Panama City regions of Florida Gulf Coast" (Ph.D. later)
1958 J. M. Branham. "An ecological survey of the ascidians of Alligator Harbor, Florida and the adjacent Gulf of Mexico" (Ph.D. later)
1961 R. O. Waller. "Ostrocods of the St. Andrew Bay System"
1962 F. O. Perkins. "The maintenance of oyster cells in vitro" (Ph.D. later, vide below)
1965 M. H. Zilberberg. "An assessment of the biological, chemical and physical factors of a tidewater marsh area before impoundment"
1966 H. Matthews. "Primary production studies on an artificial reef"
1966 L. W. Yeater. "Studies on the ecology of the commensal crab *Pinnotheres maculatus* Say" (Ph.D. later)
1966 S. M. Z. Naqvi. "Effects of predation in infaunal invertebrates of Alligator Harbor, Florida region" (Ph.D. later)
1967 T. P. Ritchie. "Chemical and physical factors that influence the setting of the larvae of the American oyster, *Crassostrea virginica*".
1968 R. O. Adams. "The color variation of *Neosima* (Mollusca: Gastropoda) with notes on the natural history" (Ph.D. later).
1968 William J. Tiftany III. "The life cycle and ecology of the beach clam *Donax variables* Say (Mollusca Pelecypoda: Donacidae)" (Ph.D. later)
1969 Edward I. Bault. "A study of the distribution and the zoogeography of the Polychaetous Annelids of the continental shelf in the northeastern Gulf of Mexico"
1969 Patrick M. McCaffrey. "Cytotaxonomy of the Florida species and evolution of the genus *Fundulus* (Pisces: Cyprinodontidae)" (Ph.D. later, vide below).
1970 Edwin W. Cake, Jr. "Some predator-prey relationships involving the Sunray Venus clam, *Macrocallista nimbosa* (Lightfoot) (Pelecypoda: Veneridae) along the Gulf coast of Florida" (Ph.D. later vide below)
1971 Martha Moulton. "An inquiry into the use of plastic 'grass' as a substitute for *Thalassia*"
1972 Luis A. Soto. "Decapod shelf fauna of the northeastern Gulf of Mexico: Distribution and zoogeography" (Ph.D. later)
1973 David LeBlanc. "The ecology, diversity, and biomass of nearshore polychaetes in Ochlockonee Bay, Florida"
1973 L. A. Olsen. "Food and feeding in relation to the ecology of two estuarine clams, *Rangia cuneata* (Gray) and *Polymesoda caroliniana* (Bosc)" (Ph.D. later, vide below)
1977 R. C. Dalton. "The reproductive cycles of the northern and southern Quahogs, *Mercenaria mercenaria* (L.) and *M. campechiensis* (Gmelin), and their hybrids, with a note on their growth"
1978 Gregg Gitschlag. "Salinity effects on survival and growth of larvae of the Quahog clam *Mercenaria mercenaria, M. campechiensis* and their hybrids"
1978 J. Michael Lyons. "Distribution and abundance of the larvae of *Decapterus punctatus* (Pisces, Carangidae) and *Bothus* spp. (Pisces, Bothidae) in the Florida and Yucatan Straits"
1978 J. Dugan Whiteside. "Evaluation of the nutritional aspects of certain cultivable marine organisms"
1979 Robert H. Blanchet. "The distribution and abundance of ichthyoplankton in the Apalachicola Bay, Florida area"
1979 Paul F. Hayes. "The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico and its implications for population recruitment"
1980 Joseph Hendricks. "The salinity tolerance of the squid *Lolliguncula brevis*"
1980 Jan Mandrup-Poulsen. "Changes in selected blood serum constituents, as function of salinity variations in the marine elasmobranch, *Sphyrna tiburo*"
1981 Steven Glomb. "Speciation in the oyster genus *Crassostrea:* It's not just a shell game"
1981 Boris Fabre. "Abundance, distribution and species composition of demersal finfish off northeast South America."
1983 Craig F. Feeny. "Effects of salinity on the vertical distribution of the larvae of *Crassostrea virginica* and *Ostrea equestris*."
1984 David W. Arnold. "The effect of salinity on size increase of the blue crab, *Callinectes sapidus* Rathbun"
1984 Darly E. Joyner. "Mariculture of bay scallops, *Argopectin irradians* (Lamarck)
1985 Lawrence E. Eaton. "The Interrelationships Between the Bay Scallop *Argopectin irradians* and Its Associated Fauna"

1986 Samuel A. Johnson, Jr. "Electrophoretic investigations of black mangrove (*Avicennia gerinans* L.) Populations and Their Chemotaxonomic Implications"
1987 James Loftin. "The distribution of crown conch egg capsules"
1987 Irma Olguin-Espinoza. "The reproductive cycle of the oyster *Crassostrea virginica* (Gmelin) in the Apalachicola Bay, Florida"
1987 Steven H. Wolfe. "The mouthparts and fore gut morphology of larvae and juveniles of the spiny lobster, *Panilirus argus*, with ecological implications"

Ph.D. Students who completed degrees under R. Winston Menzel

1957 G. C. Grice. "The copepods of the Florida West coast"
1958 N. C. Nulings. "An ecological study of the recent ostracods of the Gulf coast of Florida"
1962 A. M. Sastry. "Studies on the bay scallop, *Aequipectin irradians concentrictus* in Alligator Harbor, Florida"
1962 M. L. Forbes. "Studies of *Ostrea permollis* and aspects of its relationship to the host sponge *Stellata grubii*"
1964 T. L. Hopkins. "The plankton of the St. Andrews Bay System"
1966 F. O. Perkins. "Life history studies of *Dermocystidium marinum*, an oyster pathogen"
1970 R. R. Stickney; "The effects of various dietary lipids on the growth and lipid metabolism of channel catfish"
1974 Edwin W. Cake, Jr. "Larval and postlarval cestode parasites of shallow water, benthic mollusks of the Gulf of Mexico from the Florida Keys to the Mississippi Sound"
1975 M. Lynn Haines. "The reproductive cycle, larval development, culture, and tolerances of the sunray venus clam *Macrocallista nimbosa* (Lightfoot 1786)"
1976 L. A. Olsen. "Reproductive cycles of *Polymesoda caroliniana* (Bosc) and *Rangia cuneata* Gray, with aspects of dessication in the adults, and fertilization and early larval stages in *P. caroliniana*"
1977 P. M. McCaffery. "Studies on demersal ichthyofauna of the northeastern Gulf of Mexico continental shelf"

Publications of R. Winston Menzel

1943 Menzel, R. W. The catfish fishery of Virginia. Trans. Amer. Fish. Soc., 73:364–372.
1944 Menzel, R. W. Albino catfish in Virginia. Copia, 2:124.
1945 Mackin, J. G. and R. W. Menzel. Research seeks to expand new fishery (mussels). The Commonwealth, 12:3–10.
1945 Newcombe, CL. L and R. W. Menzel. Future of the Virginia oyster industry. The Commonwealth, 12:3–11.
1951 Menzel, R. W. Early sexual development and growth of the American oyster in Louisiana waters. Science, 113:719–721.
1952 Hopkins, S. H. and R. W. Menzel. Methods for the study of oyster planting. Proc. Nat'l. Shellfish Assoc., 43:109–112.
1953 Hopkins, S. H., J. G. Mackin and R. W. Menzel. The annual cycle of reproduction, growth and fattening in Louisiana oysters. Proc. Nat'l. Shellfish Assoc., 44:19–24.
1953 Menzel, R. W. The prodissoconchs and setting behaviour of three species of oysters. Proc. Nat'l. Shellfish Assoc., 44:104–112.
1954 Menzel, R. W. and S. H. Hopkins. Effect of two parasites on the growth of oysters. Proc. Nat'l. Shellfish Assoc., 45:184–186.
1955 Menzel, R. W. and S. H. Hopkins. Crabs as predators of oysters in Louisiana. Proc. Nat'l. Shellfish Assoc., 46:177–184.
1955 Menzel, R. W. Marking of shrimp. Science, 121:446.
1955 Menzel, R. W. Some phases of the biology of *Ostrea equestris* Say, and a comparison with *Crassostrea virginica* (Gmelin). Publ. Inst. Mar. Sci. (Univ. Texas). 4:70–153.
1955 Menzel, R. W. and S. H. Hopkins. The growth of oysters parasitized by the genus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. Journ. Parasit., 41:333–342.
1956 Menzel, R. W. The effect of temperature on the ciliary action and other activities of oysters. Florida State University Studies, 22:25–36.
1956 Menzel, R. W. Some additional differences between *Crassostrea virginica* and *Ostrea equestris* in the Gulf of Mexico area. Proc. Nat'l Shellfish Assoc., 47:76–81.
1957 Menzel, R. W. Marine Biology of Alligator Harbor, Florida. ASB Bull., 4:51–54.
1957 Gunter, G. and Menzel, R. W. The crown conch, *Melongena corona*, as predator upon the Virginia oyster. Nautilus 70:84–87.

1958 Menzel, R. W., Hulings, N. C. and Hathaway, R. R. Causes of depletion of oysters on St. Vincent's Bar, Apalachicola Bay, Florida, Proc. Nat'l Shellfish Assoc., 49:66–71.
1958 Menzel, R. W. Further notes on the albino catfish. Journ. Heredity, 49:1p.
1958 Menzel, R. W. and Nichy, F. E. Studies on the distribution and feeding habits of some oyster predators in Alligator Harbor, Florida, Bull. Mar. Sci., Gulf and Caribb., 8:125–145.
1960 Nichy, F. E. and Menzel, R. W. Mortality of intertidal and subtidal oysters in Alligator Harbor, Florida. Proc. Nat'l Shellfish Assoc., 51:39–41.
1961 Menzel, R. W. Shellfish mariculture. Proc. Gulf Caribb. Fish Inst., 14:195–199.
1961 Menzel, R. W. Seasonal growth of the northern quahog *Mercenaria mercenaria* and the southern quahog *M. campechiensis* in Alligator Harbor, Florida. Proc. Nat'l Shellfish Assoc., 52:37–46.
1962 Sastry, A. N. and Menzel, R. W. Influence of hosts on the behavior of the commensal crab, *Pinnotheres maculatus* Say. Biol. Bull., 123:388–395.
1962 Menzel, R. W. Seasonal growth of the northern and southern quahogs *Mercenaria mercenaria* and *M. campechiensis*, and their hybrids in Florida. Proc. Nat'l Shellfish Assoc., 53:111–119.
1962 Menzel, R. W. and H. W. Sims. Experimental farming of hard clams. Mercenaria
1964 Perkins, F. O. and R. W. Menzel. Maintenance of oyster cells *in vitro*. Nature, 204:1106–1107.
1965 Perkins, F. O. and R. W. Menzel. Morphological and cultural studies of a motile stage in the life cycle of *Dermocystidium marinum*. Proc. Nat'l Shellfish Assoc., 56:23–30.
1965 Menzel, R. W. and M. Y. Menzel. Chromosomes of two species of quahogs and their hybrids. Biol. Bull., 129:181–188.
1966 Menzel, R. W., N. C. Hulings and R. R. Hathaway. Oyster abundance in Apalachicola Bay, Florida, in relation to biotic associations influenced by salinity and other factors. Gulf Research Rpts., 2:73–96.
1967 Perkins, F. O. and R. W. Menzel. Ultrastructure of sporolation in the oyster pathogen *Dermocystidium marinum*. Journ. Invert. Path., 9:204–229.
1968 Menzel, R. W. Cytotaxonomy of species of clams (*Mercenaria*) and oysters (*Crassostrea*). Symposium of Mollusca, Mar. Biol. Assoc. of India. Part 1:75–84.
1968 Menzel, R. W. Chromosome number in nine families of marine pelecypod mollusks. Nautilus, 82(2):45–50; 53–58.
1969 Ritchie, T. P. and R. W. Menzel. The influence of light on the setting of the larvae of the American oyster *Crassostrea virginica*. Proc. Nat'l Shellfish Assoc., 59:116–120.
1971 Menzel, R. W. Selective breeding in oysters. Conference on Artificial Propagation of Commercially Valuable Shellfish. Eds. K. S. Price, Jr., and D. L. Maurer, University of Delaware. pp. 81–92.
1971 Menzel, R. W. The role of genetics in molluscan mariculture. Amer. Malacol. Union Bull. 1971 (Feb 1972):13–15.
1971 Menzel, R. W. Possibilities of molluscan cultivation in the Caribbean. "Symposium on Investigations and Resources on the Caribbean Sea and Adjacent Regions." FAO Fish. Res. Div. Fish. Rep. 712:183–200.
1971 Menzel, R. W. Quahog clams and their possible mariculture. Proc. World Mariculture Soc., II:23–36.
1972 Menzel, R. W. Effects of man's activities on estuarine fisheries. Underwater Naturalist, Am. Litt. Soc., 7(2):19–31.
1973 Menzel, R. W. Selection and hybridization in the mariculture of oysters and clams. Proc. World Mariculture Soc., III:309–317.
1974 Menzel, R. W. Portuguese and Japanese oysters are the same species. Journ. Fish. Res. Board Canada, 31:453–456.
1974 Menzel, R. W. Clams and oysters for mariculture in the United States. Marine Technology Society, 10th:611–617.
1975 Menzel, R. W., E. W. Cake, M. L. Haines, R. W. Martin and L. A. Olsen. Clam mariculture in northwest Florida: A field study of mortality. Proc. Nat'l Shellfish Assoc., 65:59–62.
1975 Sunderlin, J. B., M. Brenner, M. Castagna, J. Hirota, R. W. Menzel and O. E. Roels. Comparative growth of hardshell clams (*Mercenaria mercenaria* and *Mercenaria campechiensis*) and their F_1 cross in temperate, subtropical and tropical natural waters and in a tropical artificial upwelling mariculture system. Proc. World Mariculture Soc. VI:171–183.
1977 Menzel, R. W. Selection and hybridization in quahog clams. Proc. World Mariculture Soc. VIII:507–521.
1979 Clams and Snails (Mollusca: Pelecypoda [except Oysters] and Gastropoda). In: *Pollution Ecology of Estuarine Invertebrates*, Academic Press. pp. 371–396, Chapter 17.
1980 Cake, E. W. Jr. and R. W. Menzel. Infections of *Tylocephalum metacestodes* in commercial oysters and three predacious gastropods of the Eastern Gulf of Mexico. Proc. Natl. Shellfish. Assoc., 70:94–104.

1981 Hayes, P. F. and R. W. Menzel. The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico, and its implication for population recruitment. Biol. Bull., 160:80–88.
1983 Rodney Dalton and Winston Menzel. Seasonal development of young laboratory spawned southern (*Mercenaria compechiensis*) and northern (*M. mercenaria*) quahog clams and their reciprocal hybrids in Northwest Florida. J. Shellfish Resch., 3(1):11–17.
1987 Winston Menzel. Hybridization of oysters and clams. Proc. World Symp. on Selection, Hybridization and Genetic Engineering in Aquaculture. Bordeau, France. 27–30 May 1966, Vol II:47–59.
1989 Winston Menzel. The Biology, Fishery and Culture of Quahog Clams, *Mercenaria*. In: *Clam Mariculture in North America*, Elsevier Science Publishers. J. J. Manzi and M. Castagna, Editors. (In press).
1989 Menzel, W. (Editor) The biology and culture of bivalve mollusks, worldwide CRC Press (in Press).
1989 Menzel, W. Quahog clams in the United States. In The Biol. and Cult. of Bivalve Mollusks, CRC Press. (In press).
1989 Menzel, W. The oyster, *Crassostrea virginica* in the Southeast United States. In the Biol. and Cult. of Bivalves Mollusks. CRC Press (in press).

Journal of Shellfish Research, Vol. 8, No. 2, 327–334, 1989.

GAMETOGENIC CYCLES OF THREE BIVALVES IN WASSAW SOUND, GEORGIA. III. *GEUKENSIA DEMISSA* (DILLWYN, 1817)

PETER B. HEFFERNAN AND RANDAL L. WALKER
University of Georgia
Marine Extension Service Shellfish Laboratory
Skidaway Island
P. O. Box 13687
Savannah, Georgia 31416-0687

ABSTRACT The gametogenic cycle of the ribbed mussel, *Geukensia demissa*, was studied from December 1983 to March 1985 in Wassaw Sound, Georgia. Staging criteria were used to describe gametogenic development from histological preparations. Several features were quantitatively analyzed for males (% gonad area and % spermatozoa area) and females (% gonad area, % oocyte area, and egg number) using photo-planimetry (image analysis). A unimodal gametogenic cycle was evident among mussels in 1984 and 1985. Major gonadal development commenced during February–April each year. Peak maturity levels were attained by July (1985)–August (1984). Spawning extended from July to September (1985) and August to October (1984). In both years male spawning was more protracted than that of females. Gonadal redevelopment was relatively rapid following spawning, but remained at relatively low levels during the winter months before the spring burst. Sex ratios were 1:1. Gonadal area levels were consistent from year to year. Males and females produced similar amounts of gametes during 1985, while females had significantly higher levels during 1984. Mean oocyte diameter values calculated from photomicrographs were demonstrated to be useful for evaluating mean sample values, but were unsatisfactory for mean female values.

KEY WORDS reproductive cycle, gametogenesis, ribbed mussel, *Geukensia demissa*, image analysis

INTRODUCTION

The ribbed mussel, *Geukensia demissa*, ranges from the Gulf of St. Lawrence to northeastern Florida with the subspecies, *G. demissa granosissima* (Sowerly 1914), ranging from both coasts of Florida to the Yucatan (Abbott 1974). *G. demissa* (Dillwyn 1817), is a dominant macro-invertebrate inhabiting the salt marshes of the eastern and Gulf coasts of the United States (Kuenzler 1961, Jordan and Valiela 1982, Bertness 1984). As the Georgia coast is dominated by marshlands, which comprise approximately one third of the total saltmarsh area along the Atlantic coast, *G. demissa* is an important member of the coastal ecosystem.

The basic ecology of the ribbed mussel in coastal Georgia was determined by Kuenzler (1961). In his study of a mussel population at Sapelo Island, Georgia, Kuenzler (1961) reported that spawning occurred from July to August–September and that it took two years for juvenile mussels to reach sexual maturity. He determined the spawning period by observing the loss of body weight during this time period and attributed that weight loss to spawning. No histological study of the reproductive cycle has been determined for *G. demissa* in the coastal waters of Georgia. Histological descriptions of gametogenesis in *G. demissa* have been performed in two eastern United States locations, Connecticut (Brousseau 1982) and South Carolina (Borrero 1987). It was the purpose of this study to histologically determine the gametogenic cycle of *G. demissa* in Wassaw Sound, Georgia.

MATERIALS AND METHODS

Sampling and Tissue Processing

An average of 19.5 (14–21) ribbed mussels, *Geukensia demissa*, were collected monthly from a shallow sheltered creek on the northern end of Wassaw Sound, Georgia from December 1983–March 1986. This is the same site from which northern quahogs, *Mercenaria mercenaria*, and American oysters, *Crassostrea virginica*, were collected for similar studies (see Heffernan et al. 1989a, b). Mussels were located amongst oysters in an intertidal oyster bed, which was exposed for ca. 12 hours per day. Shell length measurements and tissue processing (histology) were performed as described previously (Heffernan et al. 1989a, b).

Qualitative Reproductive Analysis

The staging criteria described by Brousseau (1982) were employed for comparative purposes. Individual specimens were thus ascribed, on the basis of morphological observations, to one of the following stages: Inactive; Male or Female Developing; Male or Female Ripe; Male or Female Partially Spawned; and Male or Female Spent.

Quantitative Reproductive Analysis

Two fields per specimen with a minimum separation of 80–100 μm (more often ca. 300 μm) within the tissue block were printed on a Javelin[1] video printer via a TV

[1]Mention of a trade name does not signify endorsement by The University of Georgia.

camera system mounted on a standard compound microscope (10× objective). These prints contained elements of epithelia, gonadal, connective, and digestive tissues and had a standard field size of 7,566 mm^2. Given a mean magnification factor of 244.9× (±2.1, SE) this represents an actual field area of 0.125 mm^2.[2]

Using total field area as the standard, several male and female area measurements were calculated from prints using a Sigma Scan[1] digitizing tablet, and expressed as percentage values. We have termed this method of image analysis "photo-planimetry." Thus, mean values calculated from data measured on both specimen prints were evaluated for each individual, depending on sex, for the following: Males—% Gonad and % Spermatozoa; and Females—% Gonad, % Eggs, and Egg Number (manually counted). The percentage value indicates how much of the field area was occupied by the gametogenic feature being measured, e.g., egg area. Oocyte diameter values (nucleolated eggs only) were measured by 2 methods, ocular micrometer analysis (N = 30 per specimen) and direct measurement from the two prints (N ranged from 4 to 15 per specimen), for the periods of peak maturity (August and September) during 1984 and 1985. Mean monthly oocyte diameter values derived by both techniques using all oocytes measured, were statistically compared using Student t-tests (Sokal and Rohlf 1981) in order to evaluate the

[2]Enforced camera (TV) replacement during the course of the study required replacement of prints taken with the original system. Consequently, the presently reported field dimensions differ unavoidably, due to the differing magnification factors of the two TV cameras, from those reported in an earlier Research Note (Heffernan and Walker 1988). Those dimensions were based solely on data generated by the original camera system.

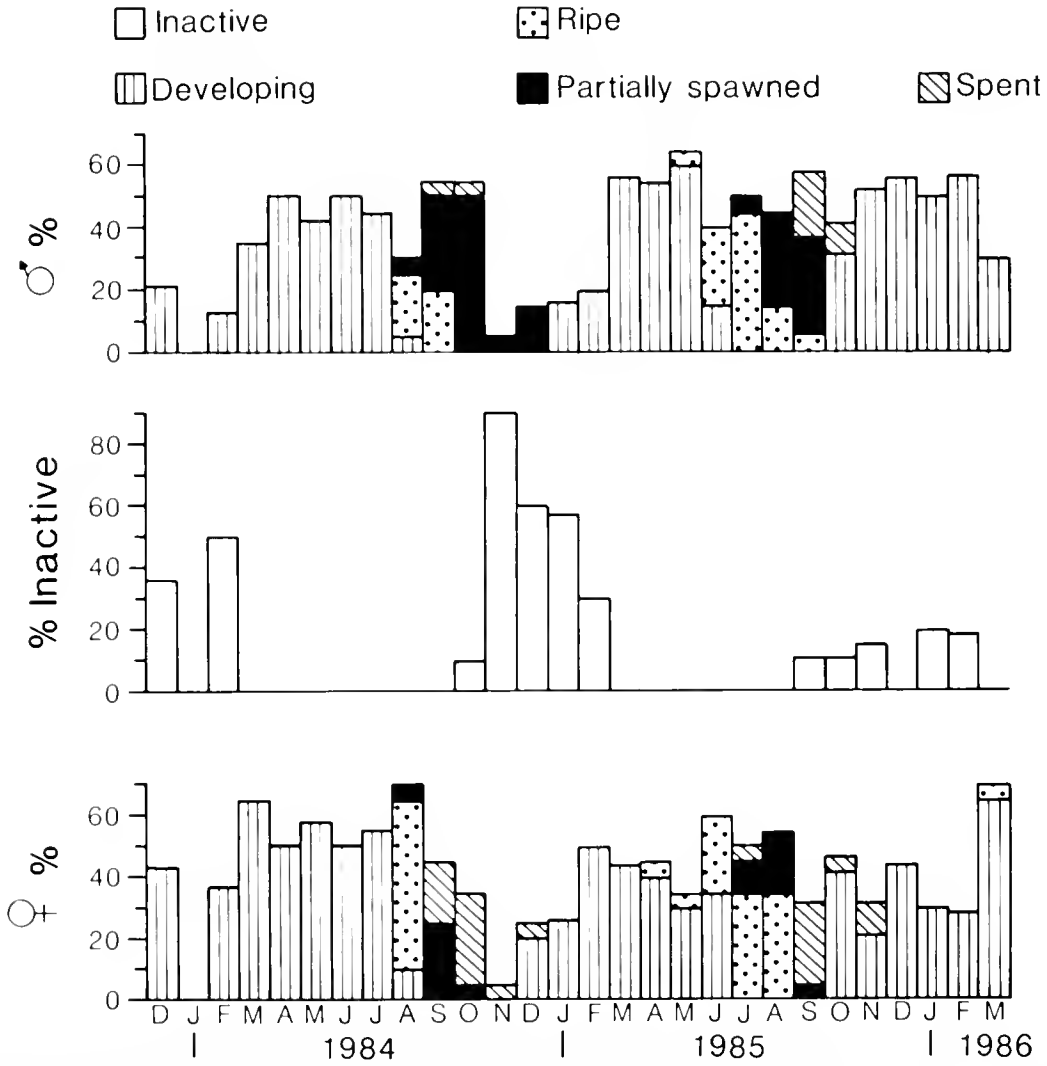


Figure 1. Qualitative data illustrating the sex and developmental stages of ribbed mussels from Wassaw Sound, Georgia. The length of each area represents the percentage frequency of mussels in each developmental stage.

merits of direct egg measurement from prints as a rapid and less labor intense calculation method. Individual female mean oocyte diameter values calculated by both methods were also compared and a regression analysis was performed with print values as the independent variable (after Sokal and Rohlf 1981). Sex ratios were tested against a 1:1 ratio with chi-square tests (Steel and Torrie 1960).

RESULTS

Unimodal annual gametogenic cycles were elucidated by both qualitative (Figs. 1–2) and quantitative (Figs. 3–4) analyses for *Geukensia demissa* during 1984 and 1985. Over one half of the mussels sampled in February 1984 (50.1%) were still inactive (Fig. 1). Male quantitative values were as low as 7.8% and 6.1% for gonad and spermatozoan area, respectively. Similarly, low values were also recorded during February for female gonad area (9.4%), oocyte area (5.9%), and egg number (18.7) (Fig. 2). Qualitative data (Fig. 1) showed gametogenic development rose sharply from February to March 1984 and sexually developing individuals constituted 100% of the mussels sampled. However, gametogenesis appeared to plateau from March through July, when all of the mussels sampled remained in the developing stage. Quantitative data (Figs. 2–3) during this period gave us a more detailed picture, indicating that the major burst in gonadal development took place a little later, during March to April. This is indicated by significant increases ($p < 0.05$) in both male [gonad area (16.9%) and spermatozoan (22.5%) levels] and female data [gonad area (28.7%), oocyte area (14.5%), and

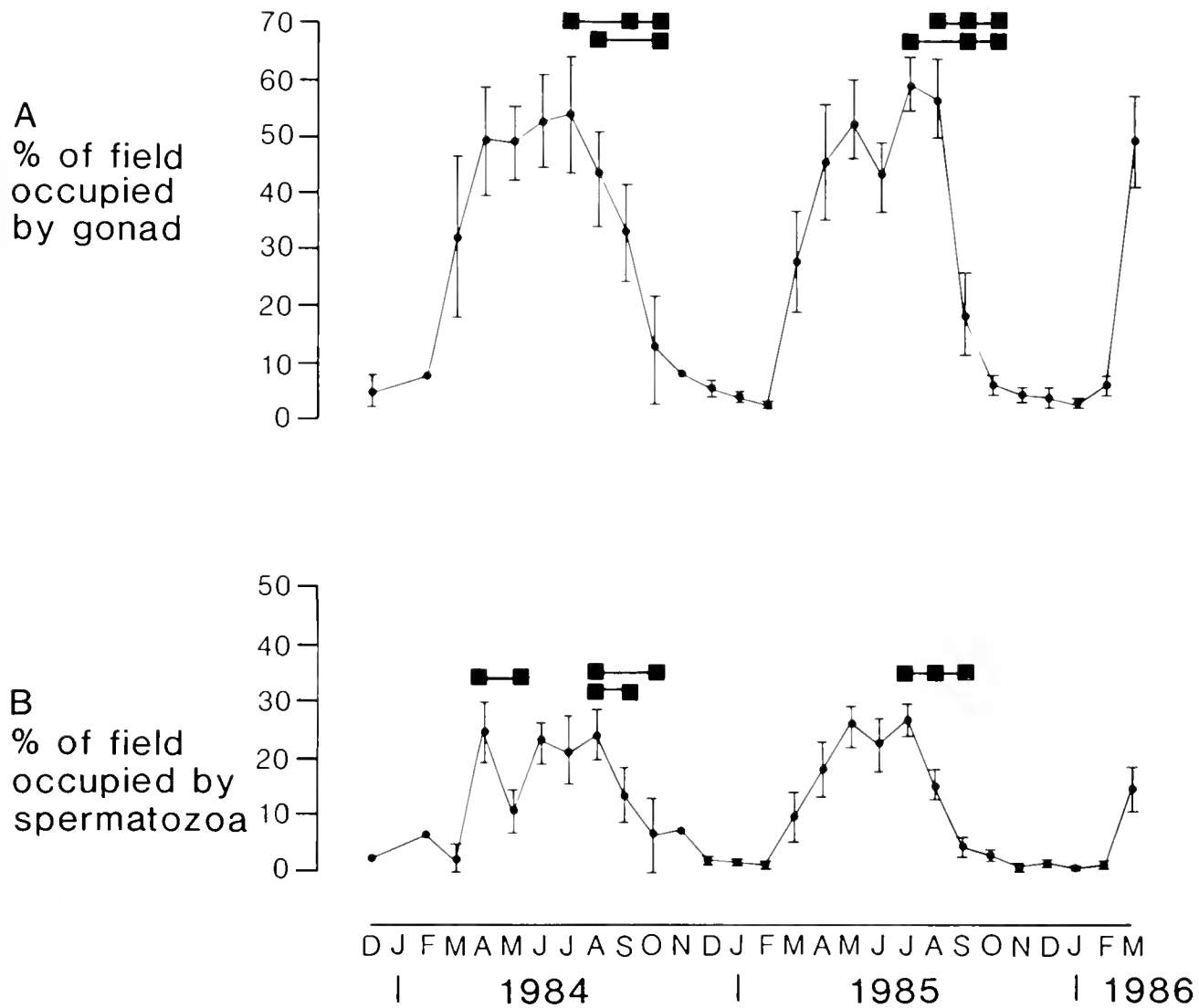


Figure 2. Composite of quantitative data (obtained using image analysis of prints of microscopic fields) representing the state of gonad condition for *Geukensia demissa* males in Wassaw Sound, Georgia. A. Mean percentage of print field area occupied by gonad. B. Mean percentage of print field area occupied by spermatozoa. Vertical bars represent 2 standard errors about the mean. Horizontal bars indicate periods of statistically significant (t-test) declines in the feature being measured. These have been included to highlight likely spawning events.

egg number (8.7)] during March–April (Figs. 2–3). Most gametogenic data sets (Figs. 2–3) indicated a developmental plateau from April to July. (Egg number, Fig. 3C, was the only data set which showed a significant rise during this time period). There was an unexplained drop (significant) of 14.1% in spermatozoan area (Fig. 2B) levels from April to May 1984. While an early male spawning was considered highly unlikely, it could not be ruled out entirely.

It appeared that some spawning activity commenced as early as August (1984) when 10% of mussels sampled were partially spent while 75% of the mussels sampled were ripe (Fig. 1). Male gonad area values peaked in July (54.1%), but were statistically similar from June through August (Fig. 2A). Spermatozoan area was also stable during this period, while its peak mean value was in August (24.1%, Fig. 2B). All 3 female features measured (Fig. 3A–C) indicated peak mean values during August (Gonad Area = 57.8%; Oocyte Area = 33.3%; and Oocyte Number = 45). Gonad Area (by 17.6%) and Egg Numbers (by 14.2) increased significantly from July to August (Fig. 3A, C), while oocyte area remained relatively stable (Fig. 3B).

Major spawning occurred between August and October 1984 (Fig. 2). Of mussels sampled during September, 55% were Partially Spawned with another 25% Spent (Fig. 1).

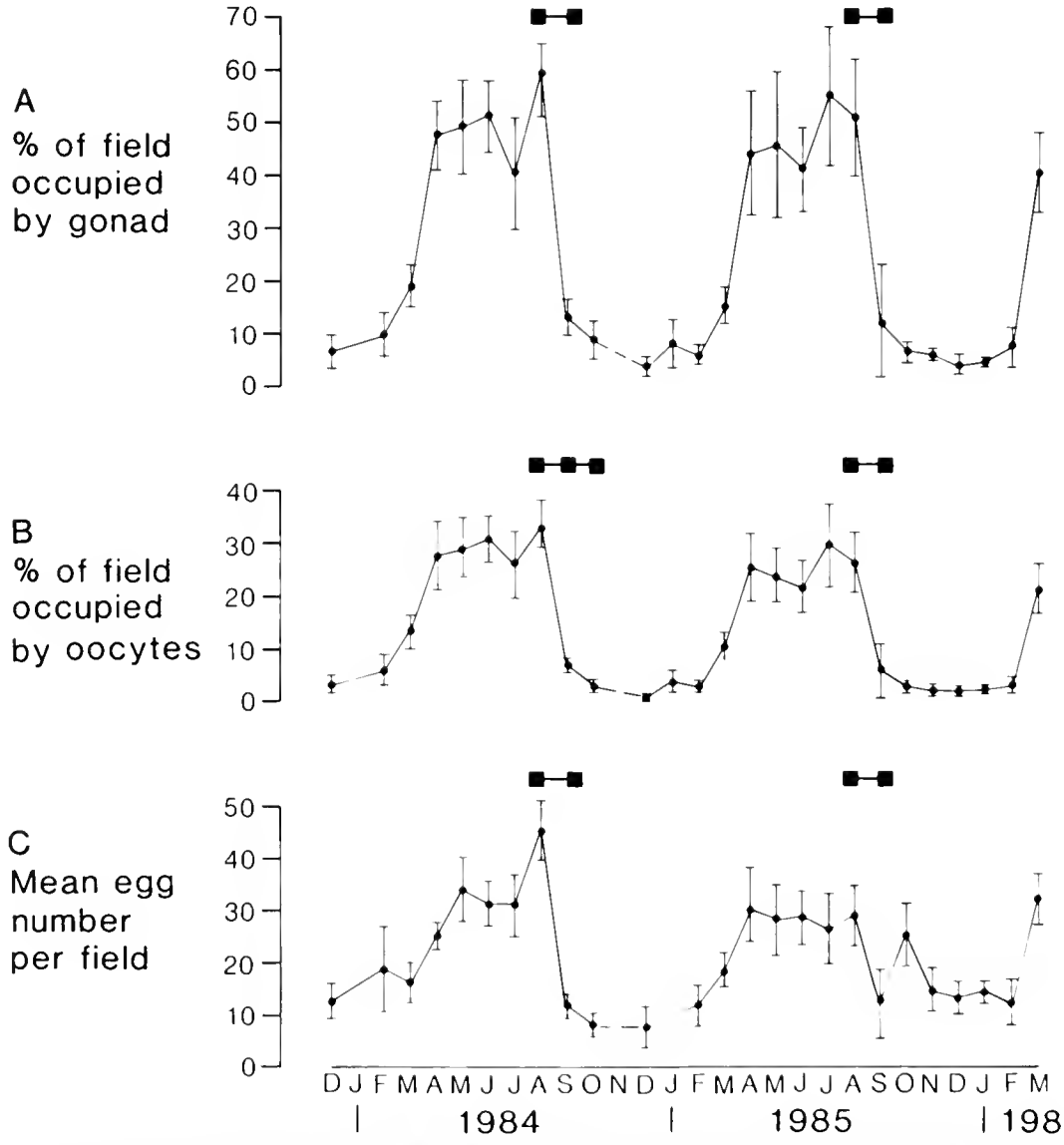


Figure 3. Composite of quantitative data (obtained using image analysis of prints of microscopic fields) representing the state of gonad condition for *Geukensia demissa* females in Wassaw Sound, Georgia. A. Mean percentage of print field area occupied by gonad. B. Mean percentage of print field area occupied by oocytes. C. Mean number of eggs per print field area. Vertical bars represent 2 standard errors about the mean. Horizontal bars indicate periods of statistically significant (t-test) declines in the feature being measured. These have been included to highlight likely spawning events.

By October, 55% and 35% were Partially Spawned and Spent, respectively. Quantitative male data indicated a protracted spawning with significant declines recorded in gonad area from August to October (decline = 30.4%) and September to October (decline = 20.8%) (see Fig. 2A). Spermatozoan values also declined significantly during this period, with a drop of 10.5% from August to September and an overall drop of 17.9% from August to October (Fig. 2B). Female spawning (1984) appeared less protracted than males, with most activity occurring during August to September (Fig. 3). Gonad area (44.9%), oocyte area (26.6%), and egg number (33.5) all declined significantly during August–September, while oocyte area was the only feature which also showed a significant fall from September to October (3.9%) (Fig. 3). By November, 90% of the mussels sampled were Inactive (Fig. 1). While the Inactive stage remained dominant through January 1985 (57.8%) (Fig. 1), redevelopment was limited to an increase in the number of specimens with detectable gonadal material, as the quantitative data on gametogenic material indicated consistently low values in both sexes (Figs. 2–3) through February 1985. Male gonad and spermatozoan area values were from 8.5–3.1% and 7.2–1.4% from November to February, respectively (Fig. 2). Female gonad area (8.3–5.6%), oocyte area (2.8–2.6%), and egg numbers (7.9–11.6) displayed a similar trend during October 1984–February 1985 (Fig. 3).

Major gonadal development commenced during February–March 1985 (Figs. 1–3). During this period the Inactive portion dropped to zero while 100% of mussels sampled were at the Developing stage (Fig. 1). There were significant increases in male gonad area (25.2%) and spermatozoan (8.4%) levels during February–March 1985, while both female gonad area (9.4%) and oocyte area (5.5%) displayed significant increases (Fig. 3A–B). There also was a NS ($p > 0.05$) rising trend evident in egg number, (Fig. 3C). While the staging criteria (Fig. 1) with the developing stage dominant, indicated a period of little gametogenic development during March–May 1985, the quantitative data generated by photo-planimetry (Figs. 2–3) showed significant increases in gametogenic material in both sexes. Male gonad area and spermatozoan levels rose by 25.3% and 16.4%, respectively, during this period (Fig. 2). Females exhibited significant increases in gonad area (28.9%), oocyte area (15.0%), and egg number (12.9) during March–April 1985 (Fig. 3).

Peak mussel maturity levels were indicated for July 1985 by staging criteria (Fig. 1), with 80% of the mussels sampled Ripe. Final gonadal maturation appeared to be a gradual process as indicated by quantitative data patterns for males and females (Figs. 2–3). While male gonad and spermatozoan areas values (Fig. 2) and female gonad and oocyte area levels (Fig. 3A–B) all displayed unexplained declines (NS) during June, this is not interpreted as a spawning event (see discussion). Male gonad (59.7%) and spermatozoan (27.0%) areas were at peak values in July, as were female gonad (54.9%) and oocyte (29.5%) areas (Figs. 2–3). While egg numbers had peak values earlier in April (30.9), they did not vary significantly through August (28.8) (Fig. 3C).

Spawning commenced during July–August 1985 and extended into September, with major spawning in August. The levels of Partially Spawned and Spent mussels rose from 20% in July to 50% in August, and 84.6% in September (Fig. 1). Male spawning (July–September) was more protracted than that of females (August–September (Figs. 1–3) during 1985. There were significant declines in spermatozoan areas during July–August (11.5%), and August–September (11.4%) (Fig. 2B). Male gonad area, on the other hand, had a non-significant decline during July–August (2.5%) while significant declines were evident during August–September (38.5%) and September–October (12.2%). Staging criteria (Fig. 1) indicated male spawning activity from July to September. Female mussels spawned intensely during August–September 1985, as evidenced by significant declines in gonad area (38.4%) oocyte area (20.7%), and egg number (16) (Fig. 3). Staging criteria showed spawning females from July to September (Fig. 1). However, the lack of significant declines on quantitative data sets (Fig. 3A–B) lead us to consider the July–August spawning activity as a relatively minor event. Furthermore, as the September value (5.3%) for spawning females was much lower than that for spawning males (31.6%) (Fig. 1), we concluded that the major burst of female spawning was confined to August–September.

Gametogenic redevelopment in the fall and winter of 1985–86 progressed from a September low to a March 1986 high (Fig. 1). Inactive stages (Fig. 1) were less abundant than in the previous year, with peak values of only 20% (January 1986), as opposed to 90% in 1984 (November). Both male and female quantitative data sets exhibited consistently low values from October 1985 to February 1986 (Figs. 2–3). Thus, it appeared that early gametogenic stages rapidly reappeared in both sexes following spawning in 1985, but that further gonadal development was delayed until February to March 1986. During this latter period there were significant increases in male gonad area (43.4%) and spermatozoan area (13.6%), as well as in female gonad area (33.2%), oocyte area (18.3%), and egg number values (19.6) (Figs. 2–3).

Computation of a monthly (sample) mean oocyte diameter value by microscope and print measurements were shown to be statistically similar (Table 1). However, calculation of mean individual (specimen) oocyte diameter based on print data, (from only 2 prints), was shown to be unreliable (Table 2).

Sex ratios did not deviate significantly from 1:1 with mean values for the study period at 41.1% ± 3.3 (SE) males and 43.9% ± 2.9 (SE) females. No hermaphroditic mussels (out of 498 processed) were detected during the study period. Monthly mean shell length values for mussels

TABLE 1.

Comparisons between *Geukensia demissa* mean oocyte diameter (μm) values derived from measurements of (a) N = 30 eggs (nucleolated) per specimen using an occular micrometer (at 100×); (b) direct measurement of all eggs (nucleolated) present in both prints per specimen (N ranged from 4–15, $\bar{N}$ = 9.64 ± 1.04 SE).

	N	Mean Diameter (μm)	SE
(a) Microscope measurements	420	44.99	0.71
(b) Print measurements	135	46.04	1.22

t-test (a) vs. (b): t-stat = −0.741; df. = 233; $0.5 < p > 0.2$

used in this study ranged from 60.9 ± 0.04 (SE) mm to 85.3 ± 0.06 (SE) mm.

DISCUSSION

The unimodal gametogenic cycle of *Geukensia demissa* described herein for Wassaw Sound, Georgia is very similar to that reported for mussels from the North Inlet Estuary in South Carolina (Borrero 1987, Borrero and Hilbish 1988). The development sequence and spawning pattern elucidated by stereologic techniques (after Lowe et al. 1982) for the lower intertidal mussel population of Borrero (Fig. 2, Site 1; 1987) bear striking similarities to our results for the same time periods during 1984. The spawning period (July–September) reported by Kuenzler (1961) for mussels on Sapelo Island, Georgia is also in agreement with our data. Brousseau (1982) reported an earlier onset of spawning, in June, at her site in Westport, Connecticut, with spawning finished by August. However, as Borrero (1987) clearly demonstrated, these differences are just as likely to be due to habitat (microgeographic) differences as to latitudinal trends in gametogenic patterns. Spawning periods were of approximately three months duration in both Georgia and Connecticut (Kuenzler 1961; this study; Brousseau 1982).

Looking at the gametogenic cycle of *G. demissa* in our study area during 1984 and 1985, one can see that male (Fig. 2) and female (Fig. 3) quantitative data showed similar gametogenic production levels each year (Table 3). There were no significant differences in male gonad area or spermatozoan levels at maturity during 1984 and 1985 (Table 3). Similarly, female gonad and oocyte areas were the same each year. While mean egg numbers (Fig. 3C) were significantly ($p < 0.001$) higher during August 1984 than August 1985, mean egg size was significantly lower ($p < 0.001$), however no significant differences were observed in percent area occupied by eggs (Fig. 3B, Table 3) indicating no difference in net production between years. Kraeuter et al. (1982) have shown a strong correlation between egg size and larval survival for *Mercenaria mercenaria* and *Argopecten irradians* and it is interesting to speculate on the subsequent effects of the smaller eggs produced by *G. demissa* during 1984 as compared to 1985, especially in regard to recruitment success. Unfortunately, no data is available on ribbed mussel recruitment patterns at this site. Similar differences in egg size were also detected among *M. mercenaria* and *C. virginica* from the same site, with 1985 levels consistently larger than those of 1984 (Heffernan et al. 1989a, b). Male spawning was more protracted than females in both years, while the general developmental cycle was similar in both sexes (Figs. 2–3).

TABLE 2.

A comparison between individual female mean oocyte diameter values derived from occular micrometer and print measurements illustrate that print values are unreliable estimates (no doubt due to the great reduction in egg numbers available for computation of a mean from just 2 standard print fields). N = number of nucleolated eggs measured.

Microscopic Mean (N = 30) (μm)	Significance	Print Mean (μm)	(N)
50.83 ± 2.54 (SE)	NS	52.87 ± 2.09 (SE)	(9)
43.83 ± 1.98	NS	46.16 ± 3.35	(13)
35.33 ± 2.31	NS	44.24 ± 4.82	(9)
49.00 ± 2.31	NS	48.13 ± 6.69	(7)
52.17 ± 2.71	NS	54.12 ± 6.43	(6)
43.67 ± 2.38	S	66.91 ± 6.25	(9)
39.67 ± 3.11	NS	38.93 ± 3.09	(13)
41.66 ± 2.71	NS	46.43 ± 8.88	(4)
40.17 ± 2.24	NS	39.89 ± 2.97	(13)
45.50 ± 2.30	NS	49.02 ± 8.14	(5)
51.13 ± 2.96	S	34.20 ± 4.90	(12)
44.67 ± 2.19	NS	43.88 ± 2.94	(6)
47.33 ± 2.24	NS	47.10 ± 2.23	(14)
44.83 ± 3.05	NS	51.93 ± 5.10	(15)

A regression analysis between print values (independent variable) and microscopic values (dependent variable) gave a regression equation y = 39.5 + 0.12 x; and an R-squared = 3.55%, while the correlation coefficient was 0.183. ± values represent SE.

TABLE 3.

Gametogenic peak production values for *Geukensia demissa* during 1984 and 1985. *Denotes significant differences, ± values represent SE.

	1984		1985		t-stat	df	p
Female							
% Gonad	57.8% ± 11.7	(Aug.)	54.9% ± 6.6	(July)	0.391	13	$p > 0.05$
% Eggs	33.3% ± 2.2	(Aug.)	29.5% ± 3.9	(July)	0.853	14	$p > 0.05$
# Eggs	45.0 ± 2.8	(Aug.)	28.8 ± 2.9	(Aug.)	4.026	25	$p < 0.001$*
Egg Diam.	44.9 μm ± 0.7	(Aug.)	58.1 μm ± 2.3	(July)	4.539	81	$p < 0.001$*
Male							
% Gonad	54.1% ± 5.2	(July)	59.7% ± 2.4	(July)	−0.967	11	$p > 0.05$
% Sperm.	24.1% ± 3.1	(Aug.)	27.0% ± 1.4	(July)	−1.106	10	$p > 0.05$

Spawning commenced earlier (July) in 1985 than 1984 (August), with subsequent shifts in the timing of maximum spawning (September 1984, August 1985). There appears to be a subtle separation in the timing of major spawning events between the three sympatric bivalves we have studied in Wassaw Sound (Heffernan et al. 1989a, b, this study). While *M. mercenaria* has its major spawning period from March–May or June, *C. virginica* spawns most intensely from June–September and *G. demissa* spawns from August or September to October or November. There is some overlap between *G. demissa* and *M. mercenaria* during the latter's second annual spawning. However, this second clam spawn is much smaller than the spring event (Heffernan, et al. 1989a). It would appear that the three dominant bivalve species in this habitat have evolved with discreetly separated reproduction periods, probably contributing to their collective success as cohabitants.

When one compares mussel gametogenic production (gonad area) levels for each sex during peak maturity periods, one sees similar levels for males and females in 1985) (Table 4a). However, during 1984, females produced significantly higher levels of gametes (ova) than did males (spermatozoa) (Table 4a), as evidenced by the area occupied by each gamete type (Figs. 2B and 3B). This pattern of higher female gamete levels was also observed in the Iceland scallop, *Chlamys islandica* (Sundet and Lee 1984 and Vahl and Sundet 1985). However, the equal gonad area levels of male and female mussels during 1985 and for oysters from the same Georgia location during both years shows that one cannot assume higher gonadal-output levels for female bivalve molluscs (Tables 4a and c). Furthermore, we saw a reversal of this trend in the sympatric northern quahogs (Table 4b). While, in the majority of cases, area levels were equal for sperm and eggs of northern quahogs, spermatozoa area levels were significantly higher than those of ova on two occasions, Fall 1984 and Spring 1985 (Table 4b). While it was long assumed that the energetic cost of oogenesis was higher than that

TABLE 4.

Comparison of gonadal production levels among ripe males and females of (a) *Geukensia demissa*, (b) *Mercenaria mercenaria* and (c) *Crassostrea virginica* at the same site in Wassaw Sound, Georgia during 1984 and 1985.

	GONAD AREA (%)					GAMETE AREA (%)				
	Male		Female		Sign.	Sperm		Ova		Sign.
(a) *G. demissa*										
1984	54.1	(5.2)-Jul.	40.2	(5.3)-Jul.	NS	24.0	(2.3)-Aug.	33.3	(2.2)-Aug.	S ($p < 0.02$)
1985	59.7	(2.4)-Jul.	54.9	(6.6)-Jul.	NS	27.0	(1.4)-Jul.	29.5	(3.9)-Jul.	NS
(b) *M. mercenaria* (Data from Heffernan *et al.* 1989a)										
1984										
Spring	94.5	(1.4)-Feb.	94.3	(1.4)-Mar.	NS	38.9	(4.5)-Mar.	33.6	(2.2)-Mar.	NS
Fall	91.4	(2.1)-Sept.	92.5	(1.8)-Sept.	NS	32.1	(1.6)-Aug.	21.5	(1.0)-Sept.	S ($p < 0.001$)
1985										
Spring	90.0	(2.2)-Jan.	96.5	(1.1)-Jan.	NS	67.1	(4.4)-Apr.	26.5	(0.7)-Mar.	S ($p < 0.001$)
Fall	92.7	(1.0)-Sept.	95.3	(1.6)-Sept.	NS	16.0	(1.5)-Sept.	19.0	(1.9)-Sept.	NS
Winter	93.4	(1.1)-Dec.	92.0	(1.9)-Nov.	NS	28.4	(3.0)-Dec.	23.9	(0.6)-Dec.	NS
(c) *C. virginica* (Data from Heffernan *et al.* 1989b)										
1984	85.1	(7.4)-Apr.	64.4	(9.8)-Jul.	NS	28.3	(2.3)-Apr.	22.3	(4.2)-Jul.	NS
	96.9	(0.7)-Jun.	99.6	(0.8)-Jun.	NS	52.7	(4.2)-Jun.	44.3	(4.1)-Jun.	NS

spermatogenesis, this has recently been questioned (Vahl and Sundet 1985). As our data show both equal and unequal gonad area levels among ripe male and female bivalves which vary from one cycle to the next, the physiological dynamics associated with such events strongly suggest themselves for future study.

Sex ratios did not deviate significantly from 1:1 during the study and no hermaphroditic mussel was detected. This latter observation is similar to that of Borrero (1987) and leads us to agree, on the basis of our data, with the view of Fretter and Graham (1964) that *G. demissa* is a strict gonochoristic hermaphrodite. However, it must be noted that Brousseau (1982) detected hermaphrodites in her study and concluded *G. demissa* was a stable gonochoric species.

There were several instances in the course of this study where the employment of image analysis methodology proved beneficial, in addition to that outlined above (gonad production levels). One example of this was in elucidating details of the rapid bursts in gametogenic development during February–March–April each year (Figs. 2, 3). Staging criteria failed to demonstrate these events (Fig. 1). Furthermore, while one could have been mistakenly led to believe, on the basis of staging criteria, that gonadal development was progressing more rapidly in Active stage mussels during the winter of 1985 than during 1984 (Fig. 1), quantitative data (Figs. 2, 3) clearly refuted this. We suggest that the only difference between 1984 and 1985 post-spawning periods was the earlier progress to the Developing stage by mussels in 1985. However, as clearly demonstrated in Figures 2 and 3, the amounts of gametogenic material present during November–February of each gametogenic cycle were relatively constant. Similarly, the lack of statistical significance associated with the declines in quantitative features (Figs. 2–3) during June 1985 suggested this was not a major spawning event. Borrero (1987) similarly demoted declines in gonad volume fraction values during June in South Carolina mussels to a "lesser intensity event." Finally, the employment of quantitative image analysis methodology in future studies of ecosystem production dynamics, such as that of Jordan and Valiela (1982), could be very useful in detailing energy allocations to, for example, gonadal development.

ACKNOWLEDGMENTS

This work was supported by Georgia Sea Grant Project number NA84AA-D-00072. Mr. J. Carr and Dr. H. Langston helped greatly at the histological and analytical stages of this work, respectively. The loan of an autotechnicon and microtome from Georgia Institute of Technology greatly facilitated this research and the two earlier papers in this trilogy. Dr. R. Hanson allowed us to use his digitising system and Mr. C. Robertson provided much appreciated assistance during this phase of the study. Mr. G. Paulk, Mr. D. Head and Ms. P. Adams provided much appreciated technical assistance. Ths statistical advice of Dr. J. W. Crenshaw, Jr. and Dr. D. M. Gillespie is duly acknowledged. Dr. A. G. Eversole is thanked for his critical review of an earlier draft. Mrs. J. Haley typed the manuscript. Ms. A. Boyette and Ms. S. McIntosh provided all the graphic materials.

LITERATURE CITED

Abbott, R. T. 1974. *American Seashells*. Second Edition. Van Nostrand Reinhold, New York. 663 pp.

Bertness, N. D. 1984. Ribbed mussels and *Spartina alterniflora* production in a New England salt marsh. *Ecology* 65:1794–1807.

Borrero, F. J. 1987. Tidal height and gametogenesis: reproductive variation among populations of *Geukensia demissa*. *Biol. Bull.* 173:160–168.

Borrero, F. J. & T. J. Hilbish. 1988. Temporal variation in shell and soft tissue growth of the mussel, *Geukensia demissa*. *Mar. Ecol. Prog. Ser.* 42:9–15.

Brousseau, D. J. 1982. Gametogenesis and spawning in a population of *Geukensia demissa* (Pelecypoda: Mytilidae) from Westport, Connecticut. *The Veliger* 24:247–251.

Fretter, V. & A. Graham. 1964. Reproduction. K. M. Wilbur and C. M. Younge (eds.). Physiology of the mollusca. Academic Press, New York. 127–156 p.

Heffernan, P. B. & R. L. Walker. 1989. Quantitative image analysis methods for use in histological studies of bivalve reproduction. *J. Moll. Stud.* 55:135–137.

Heffernan, P. B., R. L. Walker & J. L. Carr. 1989a Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: I. *Mercenaria mercenaria* (L.). *J. Shellfish Res.* 8: (In press).

Heffernan, P. B., R. L., Walker & J. L. Carr. 1989b. Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: II. *Crassostrea virginica* (Gmelin). *J. Shellfish Res.* 8: (In press).

Jordan, T. E. & I. Valiela. 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a New England salt marsh. *Limnol. Oceanogr.* 27:75–90.

Kuenzler, E. J. 1961. Structure and energy flow of a mussel population in a Georgia salt marsh. *Limnol. Oceanogr.* 6(2):191–204.

Kraeuter, J. N., M. Castagna & R. Van Dessel. 1982. Egg size and larval survival of *Mercenaria mercenaria* (L.) and *Argopecten irradians* (Lamarck). *J. Exp. Mar. Biol. Ecol.* 56:3–8.

Lowe, D. M., M. N. Moore & B. L. Bayne. 1982. Aspects of gametogenesis in the marine mussel *Mytilus edulis* L. *J. Mar. Biol. Ass. U.K.* 62:133–145.

Sokal, R. R. & F. J. Rohlf. 1981. *Biometry*. Freeman, New York. 859 p.

Steel, R. & J. Torrie. 1960. *Principles and Procedures of Statistics*. McGraw Hill Book Co., Inc. New York. 481 p.

Sundet, J. H. & J. B. Lee. 1984. Seasonal variations in gamete development in the Iceland scallop, *Chlamys islandica*. *J. Mar. Biol. Assoc. U.K.* 64:411–416.

Vahl, O. & J. H. Sundet. 1985. Is sperm really so cheap? J. S. Gray and M. E. Christiansen (eds.). Marine Biology of Polar Regions and Effects of stress on Marine Organisms. John Wiley & Sons Ltd. Chichester. 281–285 p.

Journal of Shellfish Research, Vol. 8, No. 2, 335–344, 1990.

ASPECTS OF GONADOMORPHOGENESIS AND REPRODUCTIVE CYCLE OF *SCAPHARCA INAEQUIVALVIS* (BRUG.) (BIVALVIA; ARCIDAE).

M. GRAZIA CORNI[1] AND OTELLO CATTANI[2]
[1]*Dip. Biologia E.S.*
[2]*Dip. Biochimica*
Università di Bologna—Italia

ABSTRACT Some aspects of gonadomorphogenesis and the reproductive cycle of *Scapharca inaequivalvis* (Brug.) from Cesenatico (Adriatic Sea) were examined during the period April 1984–March 1985. Histomorphological aspects of gametogenesis, minimal size at sex differentiation and gonadal development are described. Gametogenesis clearly starts in May and extends until October. Spawning occurs from June to October with differentiating activity. A reproductive pause, almost total, was observed from November to April.

KEY WORDS: gametogenesis, spawning, *Scapharca inaequivalvis*.

INTRODUCTION

The reproductive cycle of *Scapharca inaequivalvis* (Brug.), an Indopacific species recently found in the Adriatic Sea (Ghisotti and Rinaldi 1976), has been examined in another work to correlate the stored energy metabolism (glycogen, total lipids and proteins) with the gonadal activity differentiated by a concise modified scale (Lubet 1959) over a whole year (Cattani et al. 1986). In this work histomorphological and cytological aspects of gonadomorphogenesis and gametogenesis of the species concerning the same period are examined in more detail to point out the first phases of sexual differentiation and gonadal growth patterns in relation to water temperature.

MATERIALS AND METHODS

Three samples of juvenile specimens were carried out to study histologically the primary gonad up to sex differentiation (Figs. 1–6). They were collected offshore at Cesenatico during February, August, and November 1981 by means of a pump. For each sample three series of juvenile specimens ranging in shell length (SL) from 1.5 to 20.0 mm were examined. The size class was about 1.5–2 mm, so a tenth of individuals progressing in size were observed of each series. About thirty specimens for each sample were examined comprehensively.

The reproductive cycle of *Scapharca* was studied during the period April 1984–March 1985 in a fixed station (qualified as N° 14 for other periodical investigations) in waters 1.5–2 m deep, 100–150 m offshore at Cesenatico (Adriatic Sea). Hydrological data (pH, temperature and salinity) and chlorophyll "a" ($\mu g \cdot l^{-1}$) as an indicator of primary productivity, related to this station were supplied by the "Study Center" of Cesenatico. Salinity and temperature graphics are reported in Fig. 13. The samples were collected by means of a grating rake to select specimens longer than 30 mm, to insure that they were adults (Figs. 7–12). The shell length (SL) was used as an indicator of age. A subsample of 50 adult individuals, with SL from 30 to 55 mm, was examined every month. These specimens (as were the juvenile specimens) were fixed in Bouin's fluid and buffered formalin (10%), embedded in paraffin, cut into 8 μm sections and stained with Mayer's acid and haemalum-eosin.

Gonad development was categorized using Tranter's classification as reported by Lucas (1965) which includes five stages of female (Fd1 → Fd5) and male (Md1 → Md5) gonadal development and three regressive stages (Fr1 → 3; Mr1 → 3). Composite stages often occurred either in different acini of the same gonad (e.g., Md4 + Md5) or differential phases overlapped in the same acinus (e.g., Md5/Mr1). Resting and/or sexually undeterminable specimens were qualified as "undifferentiated".

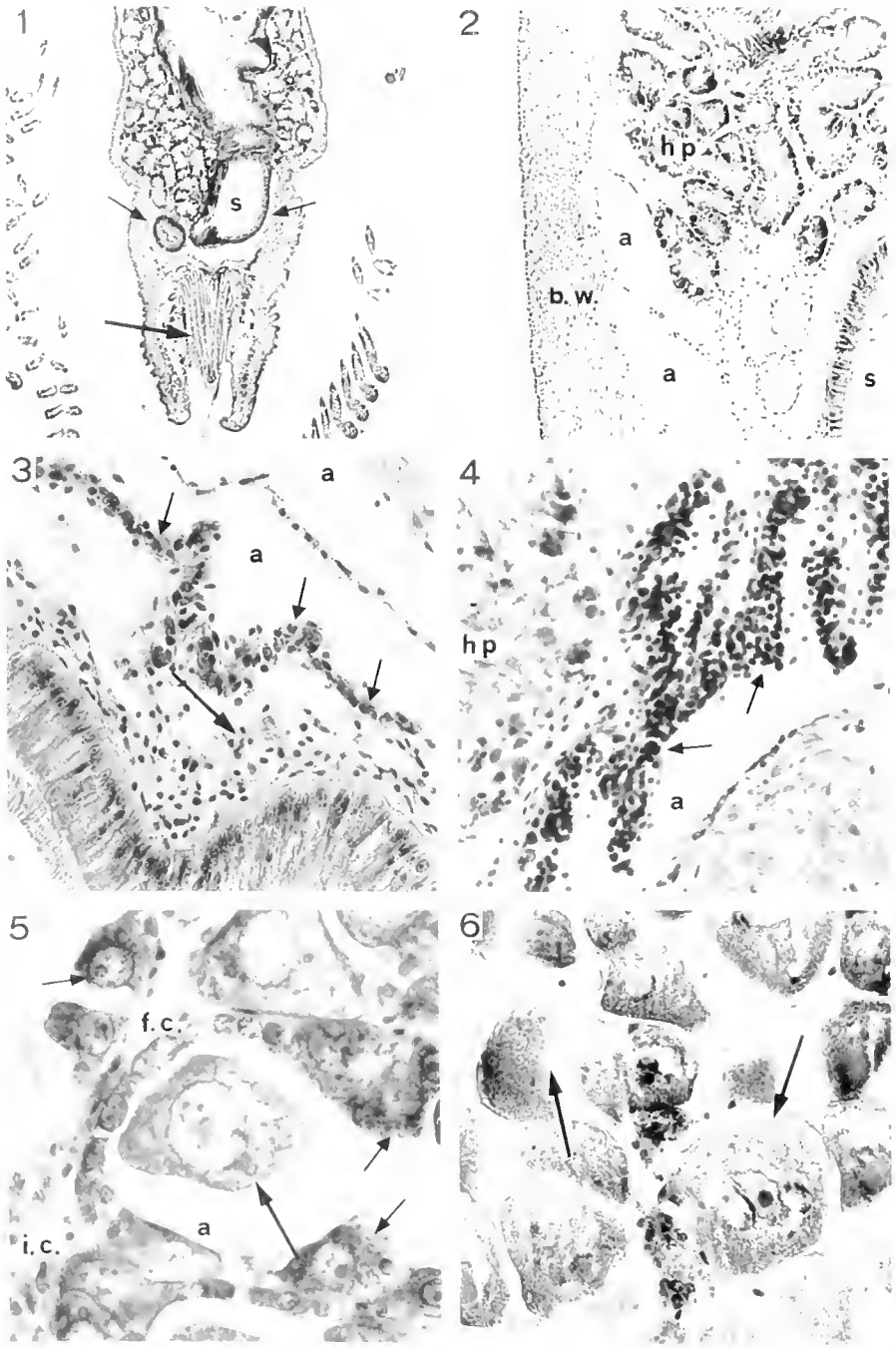
Frequencies (in %) of the three adult principal categories ("undifferentiated", females, males) were calculated both for the whole study period and for the monthly samples. (Table 1; Fig. 14 A, B). Monthly values from April to October were compared with water temperature (Fig. 15).

The percentage frequency of gonadal stage of each sex, based on the total number of females and males examined for every month, was calculated in the most active gametogenetic period, from May to October 1984, even considering the resting stages sexually recognizable by residual gamete presence in acini (Table 2, Figs. 16, 17).

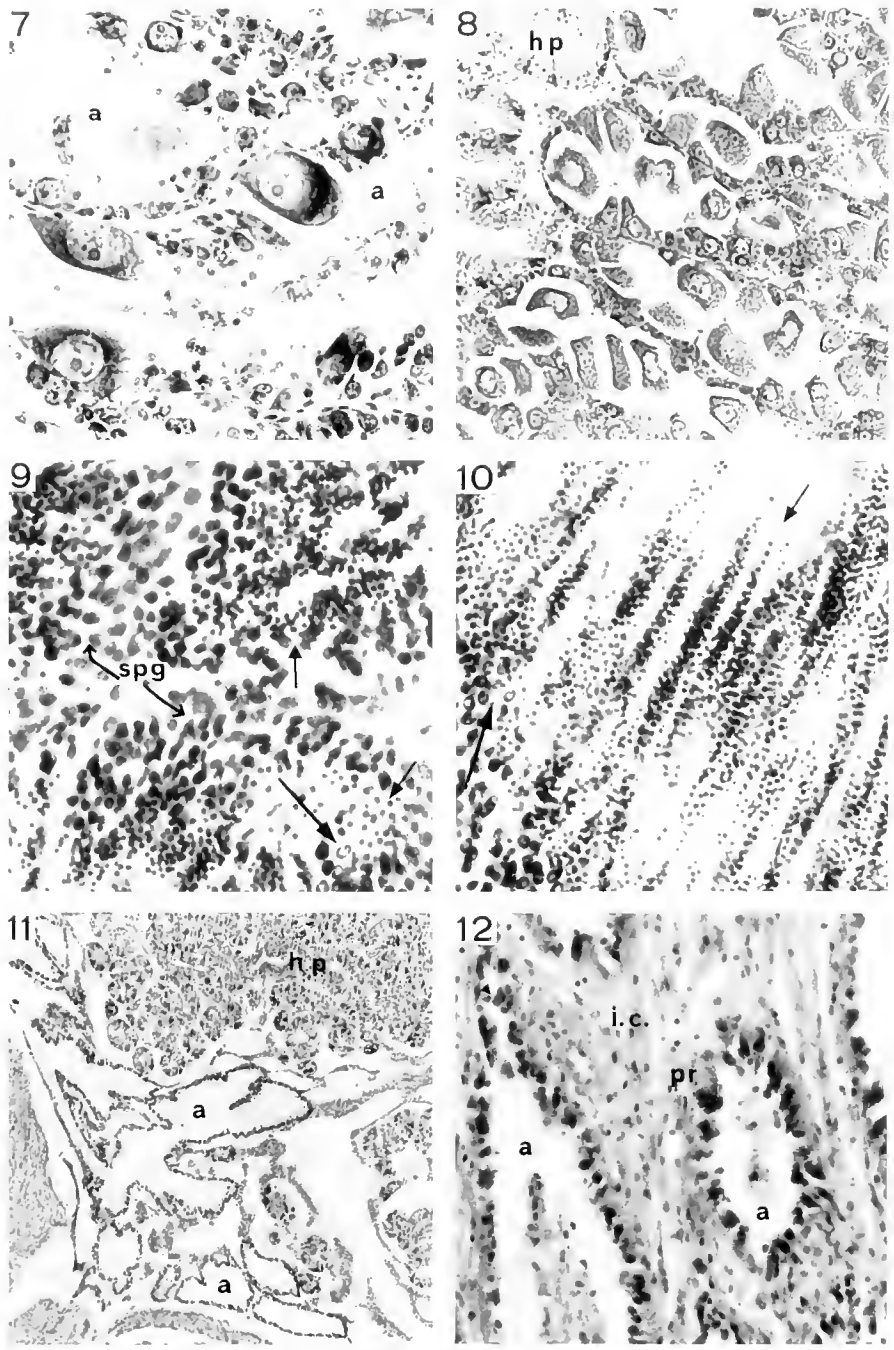
The gonadal histological patterns were correlated to the growth of the energy reserve tissue (as perigonadic and perigastric connective tissues) which in Arcidae and in Mitilidae consists of two kinds of cells: the vesicular cells, Leidig's or Langer's cells, rich in glycogen, and adipogranulous cells (Lubet 1959, Bayne et al. 1982).

The reproductive cycle of the exotic species *Scapharca* was compared with that of *Chamelea gallina* (L.) (Veneridae) (Corni et al. 1985), an autochthonous bivalve economically important, to evidentiate the eventual affinities or distinctions in the reproductive strategy. *Chamelea*

Research carried out with the financial help of M.P.I. (60%).



Figures 1–6. GONADAL AND GAMETOGENETIC STAGES IN JUVENILE SPECIMENS (SL < 20 mm): (1) Cross section of a young: SL = 3.5 mm—Between the visceral mass and the body lateral walls there are few empty acini (small arrows) of the primary acini where no primordial element is histologically evident. The byssus gland (large arrow) is active in the foot. s—stomach. (53×). (2) Cross section of a young: SL = 8.7 mm. It results sexually undifferentiated. a—acinus; b.w.—body wall; hp.—hepatopancreas. (130×). (3) Cross section of a young: SL = 10.0 mm. Protogonia (8 × 10 μm, diam.) (small arrows) project from the acinus wall next to the perigastric connective tissue (large arrow). (320×). (4) Cross section of a young male: SL = 10.8 mm. Germinal elements (arrows) invade the acinus lumen. (Md1 + Md2 stage). (320×). (5) Cross section of a young female: SL = 17.3 mm. Oogonial "nests" and precocious oocytes with very basophilic cytoplasm (small arrows) with some oocytes probably at the start of vitellogenesis (45 × 37 μm, diam.) (large arrow) project from the acinus wall (Fd1 + Fd2 stage). f.c.—follicular cells; i.c.—intragonadic connective. (530×). (6) Parasagittal section of a young female: SL = 14.0 mm. Several vitellogenetic oocytes (arrows) fill the acinus lumen. (Fd3 + Fd4 stage). (320×).



Figures 7–12. GONADAL AND GAMETOGENETIC STAGES IN SPECIMENS WITH SL > 30 mm: (7) Cross section of an ovary: SL = 33.1 mm. (Fd1 stage). (320×). (8) Cross section of an ovary: SL = 38.4 mm. (Fd2 + Fd3 stage). (130×). (9) Cross section of a male gonad: SL = 38.4 mm. (Md2 + Md3 stage). Acinus in active spermatogenesis: at the periphery there are spermatogonia (spg) succeeded by spermatocytes (large arrows) and spermatids (small arrows). (320×). (10) Cross section of a male gonad: SL = 39.9 mm. (Md5/Mr1 stage). Male in spermiohistogenesis; spermatocytes (large arrow) and spermatids-sperms disposed in radial braids (small arrow) are visible. (320×). (11) Cross section of a specimen that has completely spawned SL = 35.8 mm. (53×). (12) Cross section of a resting specimen: SL = 39.0 mm i.c.—intragonadic connective; pr—protogonia. (320×).

seems now to be partially threatened by the copresence of *Scapharca* which presents, in the area studied, high density because it is not fished and shows a greater resistance to environmental ipoxia conditions as also attested by biochemical and physiological works (Cortesi et al. 1985, Carpené 1985).

RESULTS AND DISCUSSION

The series of juvenile specimens with SL from 1.5 to 20 mm collected during February, August and November 1981 demonstrated the minimal size at which the sexual differentiation takes place. All the young observed were sexually undifferentiated until to SL = 10 mm. Differentiated spec-

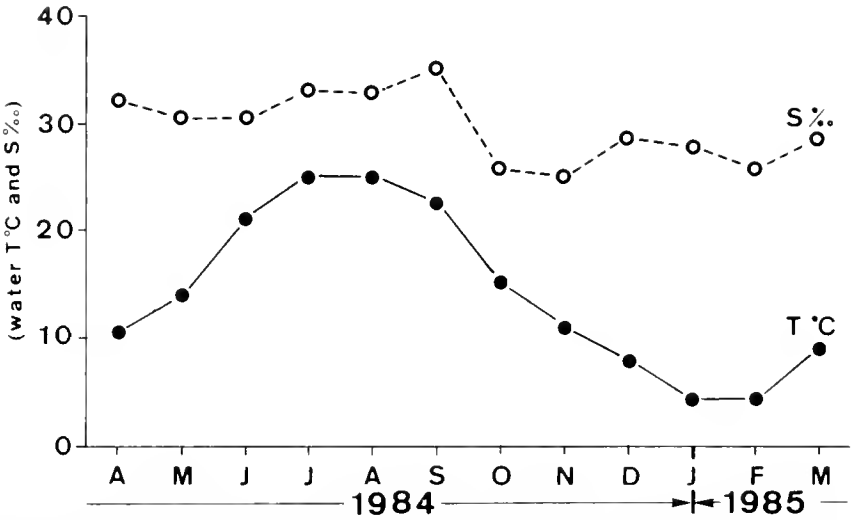


Figure 13. Seasonal temperature and salinity trends in the fixed station N° 14 at each sampling (April 1984–March 1985; Cesenatico, Adriatic Sea).

imens were observed in August and November series. Changes in the gametogenetic condition of the gonad in histological preparations from the series of individuals of different shell length are described below. The prospection in size is assumed to represent an equal progression in age of very young individuals but this criterion is not rigorous because after the size SL = 10 mm not all the specimens of the same size show similar gonadomorphogenic stages. In fact the smallest sexually differentiated specimens of November do not have equivalents in the series parallel of August.

SL = 1.5 mm—In cross medial section, both on the sides of the viscera, between hepatopancreas (liver) and body walls, small and empty acini separated by mesodermal thin areolae are visible. No primordial germinal cells are histologically evident. Byssus gland is already present.

SL = 3.5 mm—The number of acini was greater. Gonadal tubules spread above the visceral mass but are not sexually discriminable. Byssus gland is clearly active in some specimens (Fig. 1).

SL = 8.7 mm—Gonadal acini become more numerous

TABLE 1.

Hydrological data and percent frequency of "undifferentiated"*, females (ff) and males (mm) of *Scapharca inaequivalvis* (Brug.) (SL > 30 mm) relative to station N° 14 in the period April 1984–March 1985 (Cesenatico, Adriatic Sea).

Sampling dates	pH	T °C	S ‰	Chl.a (μg · l⁻¹)	"undiff." %	ff %	mm %
11-04-1984	7.75	10.5	32.3	14.80	100		
16-05-1984	7.80	14.0	30.5	6.16	60	20	20
19-06-1984	8.20	21.0	30.5	3.21	14	38	48
13-07-1984	8.07	25.0	32.9	8.34	20	24	56
02-08-1984	7.90	25.0	32.7	3.98	4	36	60
07-09-1984	7.80	22.5	34.9	12.71	16	38	46
26-10-1984	7.46	15.0	25.7	1208.71	62	28	10
22-11-1984	8.70	11.0	24.9	1975.56	92	6	2
13-12-1984	8.20	8.0	28.5	4.13	90	6	4
24-01-1985	7.92	4.5	27.7	1.76	88	8	4
22-02-1985	8.58	4.5	25.7	16.45	100		
25-03-1985	8.06	9.0	28.6	0.53	100		
					Global annual composition:		
					"undiff." %	**ff %**	**mm %**
					63.10	15.14	21.76

* "undifferentiated" = sexually undeterminable.

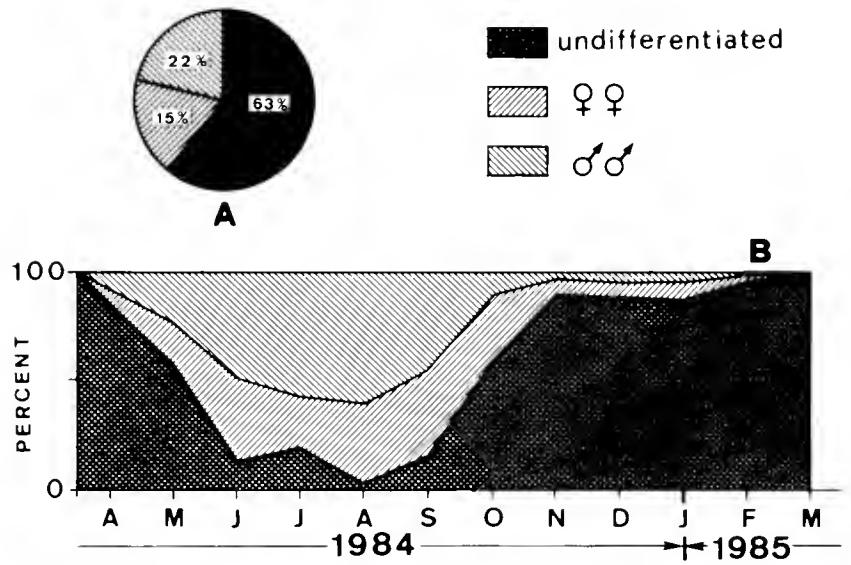


Figure 14. (A) Global annual percentages of the three principal categories ("undifferentiated", females and males with SL > 30 mm) of *Scapharca inaequivalvis* (Brug.) (April 1984–March 1985; Cesenatico, Adriatic Sea). (B) Monthly percentage composition of the three principal categories.

and surround the visceral mass but are still undifferentiated (Fig. 2).

SL = 10.0 mm—Germinal cells, clearly basophilic, project from the acinous wall close to the perigastric connective tissue (Fig. 3). This stage at this size is present only in one of the series of November.

SL = 10.8 mm—Basophilic elements, explanable as spermatogonia (size: 4.5 × 4.5 μm), project from the acinus internal wall and invade the lumen (Fig. 4). They are associated with spermatocytes-spermatids elements (stage Md1 + Md2). This specimen is the shortest male observed. This stage at this size is present only in one of the series of November.

SL = 14.0 mm—From our data this value corresponds to the shortest female observed, but it is already in advanced gametogenesis. Cytologically the ovary presents previtellogenetic and vitellogenetic oocytes (major cell diameters about 55 × 50 μm) (Fd3 + Fd4 stage) (Fig. 6). Also this stage at this size is present only in one of the series of November.

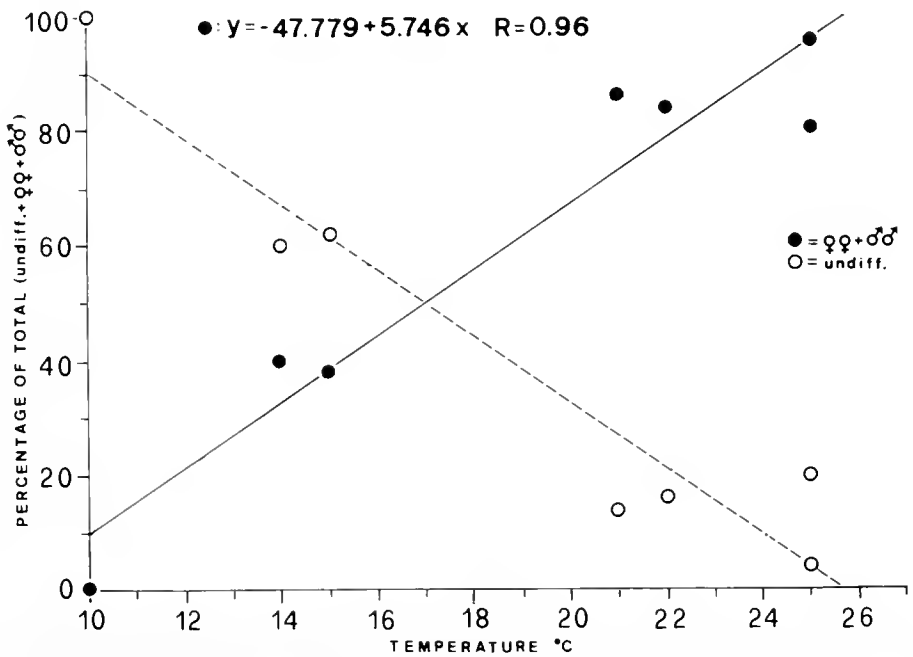


Figure 15. A positive correlation between the specimens of *Scapharca inaequivalvis* (Brug.) (SL > 30 mm) active in gametogenesis and water temperature is evident ($p < 0.01$ for d.f. = 6). undiff. = undeterminable.

TABLE 2.

Percent frequency of female (ff) and male (mm) gonadal stages of *Scapharca inaequivalvis* (Brug.) (SL > 30 mm) in the period May–October 1984. (Cesenatico, Adriatic Sea).

Stages (Tranter) ff	1984 Sampling Dates					
	16 May %	19 June %	13 July %	2 August %	7 Sept. %	26 Oct. %
Fd1	100.00	52.63	33.40			
Fd1 + Fd2		21.05				7.14
Fd2 + Fd3		10.53		16.70		
Fd3 + Fd4		5.26	16.60			7.14
Fd4 + Fd5		10.53	41.60	38.90	15.80	
Fd5/Fr1				33.40	47.40	7.14
Fd1 + Fr3						21.43
Fr2–3			8.40	11.00	36.80	57.15
mm	%	%	%	%	%	%
Md1	90.00	33.30	3.57			
Md1 + Md2	10.00	16.70	3.57			
Md2 + Md3		8.40				
Md3 + Md4		4.10				
Md4 + Md5		8.40		6.70	30.43	20.00
Md5/Mr1		25.00	78.58	93.30	69.57	80.00
Md2 + Mr3		4.10	7.14			
Mr2–3			7.14			

Fd1 → Fd5; Md1 → Md5: gametogenesis
Fd4 → Fd5; Md4 → Md5: maturation
Fr1 → Fr3; Mr1 → Mr3: emission

SL = 16.65 mm—The gonad of this individual is in Md1 + Md2 stage. This specimen is the shortest male observed in August series.

SL = 17.3 mm—In the gonad there are visible oogonial "nests" and precocious oocytes with very basophilic cytoplasm associated with some oocytes probably at the start of vitellogenesis as shown by an initial cytoplasmatic vacuolisation (45 × 37 μm) (Fd1 + Fd2 stage). The intragonadic connective is well developed (Fig. 5). This individual is the shortest female observed in August series.

The smallest sexually differentiated young were found in the November 1981 series, a month not favourable to gametogenesis as shown by the gonadal patterns of specimens with SL > 30 mm examined, in an equivalent period, during 1984, which are predominantly in a resting phase. Further investigations on the rate of shell growth and gametogenetic patterns in the different months are needed. Hypothetically they might be the young born at the start of the reproductive cycle. In the August 1981 series, the two sexes are clearly distinguishable only in specimens with SL greater than 16 mm, and not in all specimens. It appears that it takes the young one whole season to mature and that they do not reproduce until the second summer.

The different gonadal maturation observed in the various specimens is also probably referable to a differed gonadal growth, a reproductive strategy common in Bivalves.

Other gonadal and gametogenetic patterns were deduced from the histological study of the reproductive cycle (from May 1984 to March 1985) including specimens with SL > 30 mm, either differentiated (Figs. 7–10) or sexually indiscriminable. In fact a secondary sexual *differentiation* occurs in specimens that have completely spawned (Fig. 11) and in those in the resting stage (Fig. 12). Adult composition of the population for the whole sampling period consisted of three principal categories: specimens with empty acini or in the resting stage, identified together as "undifferentiated" (63%), females (15%), and males (22%) (Table 1; Fig. 14 A). The highest percentages of resting and reproductively active specimens were distributed in two distinct periods. The resting stages were, indeed, dominant in autumn and winter months; the active stages, were dominant in spring and summer months. Fluctuations in percent values of the three categories were evident during the whole period studied. In April 1984 all the specimens examined were in resting stages and sexually indiscriminable. In May 1984 several individuals were either in the resting phase or at the start of gonadic revival (60%) but some others were already sexually recognizable. From June to September 1984 the differentiated specimens were dominant and the "undifferentiated" stages represented by individuals that had already spawned. From October 1984 to January 1985 the "undifferentiated" stages were clearly prevalent. The females and males were recognizable either from rare still active specimens or from residual gametes in empty acini. In February and in March 1984 all the specimens examined were in resting stages. A positive correla-

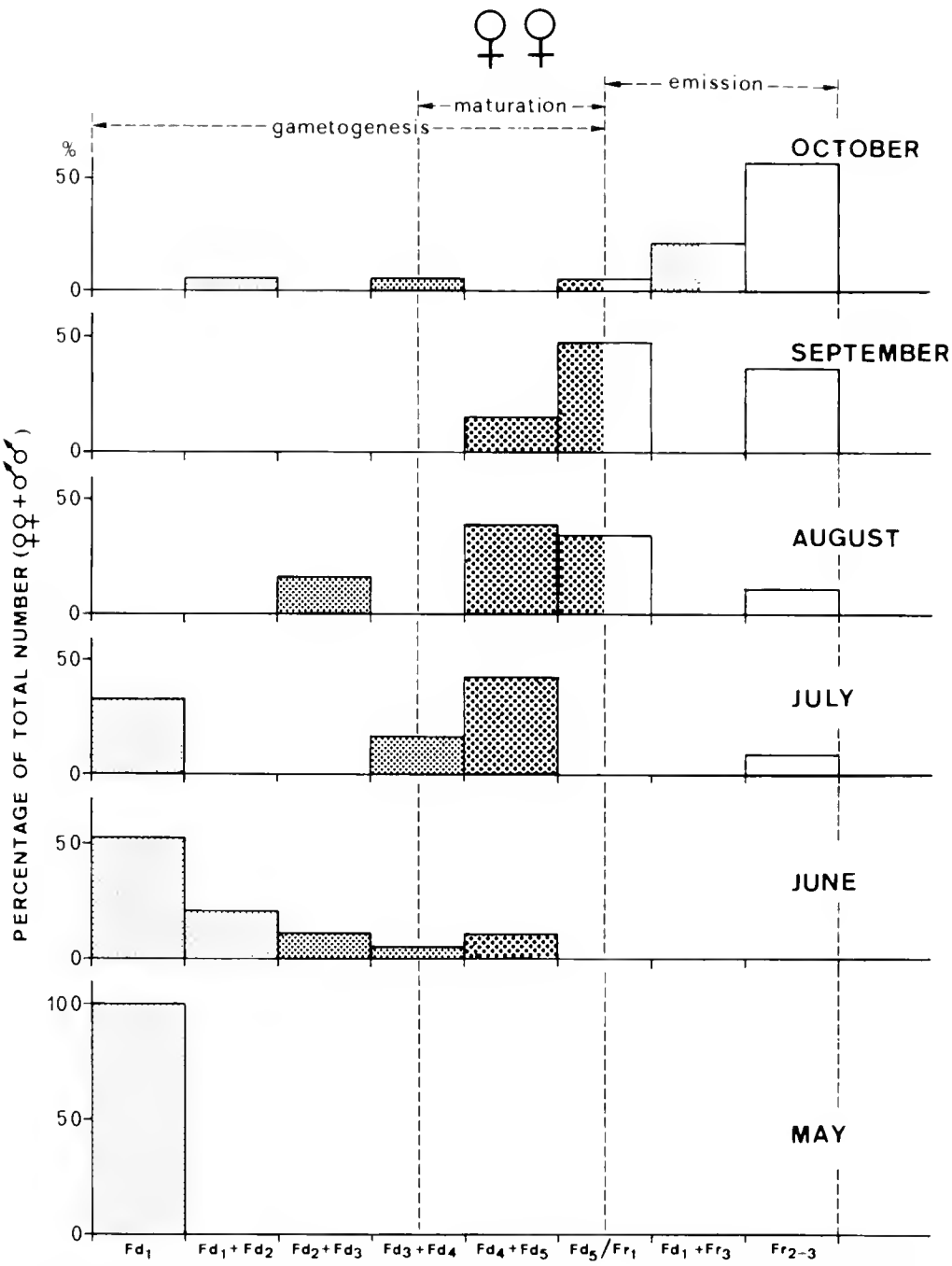


Figure 16. Gonadal growth stages of females of *Scapharca inaequivalvis* (Brug.) (SL > 30 mm) (in % on the total of females and males sampled monthly) in the period May–October 1984 (Cesenatico, Adriatic Sea).

tion existed between active gonadal stages (ff + mm) and water temperature; on the contrary, an inverse relationship existed between this parameter and specimens in the resting stage (Fig. 15).

The reproductive period, from May to October 1984, was further examined by identifying the different gametogenetic stages in females and males. The dominant developmental stages in both sexes and their relative frequency (in %), based on the total number of females and males examined monthly, showed distinct fluctuations (Table 2; Figs. 16, 17). In May gonocyte multiplication and differentiation were present in many specimens (Fd 1 = 100.00%; Md1 = 90.00%, Md1 + Md2 = 10.00%). In June the early gametogenetic stages were already dominant (Fd 1 = 52.63%, Fd 1 + Fd2 = 21.05%; Md1 = 33.30%, Md1 + Md2 = 16.70%). Intermediary and final stages of gametogenesis were also present (Fd3 → Fd5; Md3 → Md5). Some males showed acini partially empty (Md5/Mr1, Md2 + Mr3) or empty (Mr2–3) because of spawning. In the mature acini spermatogonia, spermato-

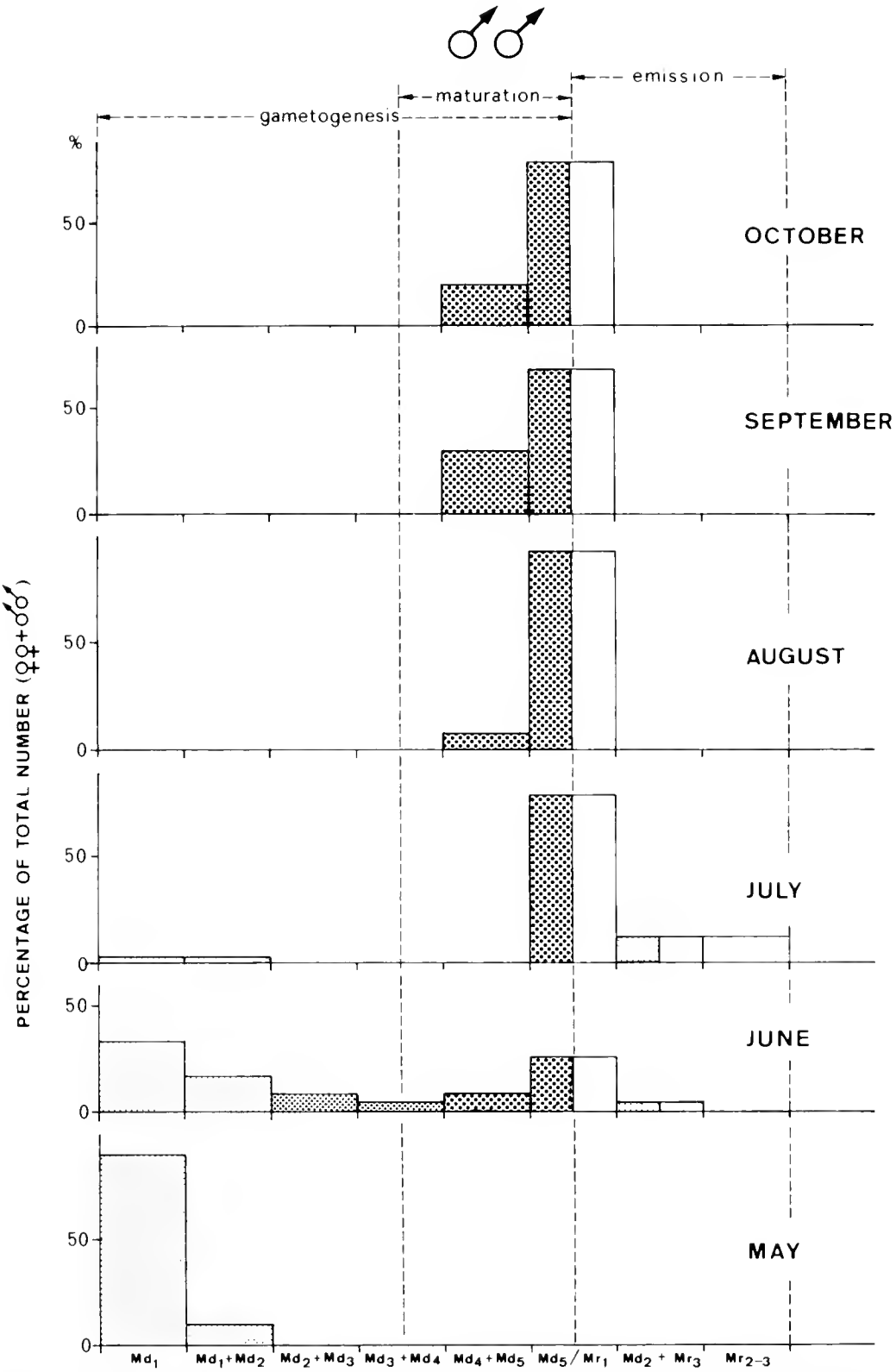


Figure 17. Gonadal growth stages of males of *Scapharca inaequivalvis* (Brug.) (SL > 30 mm) (in % on the total of females and males sampled monthly) in the period May–October 1984 (Cesenatico, Adriatic Sea).

cytes and sperms were regularly distributed from the periphery to lumen. In July early ovogenesis stages were already frequent (Fd1 = 33.40%) but the ripe stages (Fd 4 + Fd5 = 41.60%) were dominant. Intermediary (Fd 3 + Fd4 = 16.60%) and spawning (Fr2–3 = 8.40%) stages were also evident. In this month the early spermatogenetic stages were not frequent; gamete emission was, on the contrary, very evident (Md5/Mr1 = 78.58%). In August ovogenetic intermediary stages (Fd2 + Fd3 = 16.70%), and ripe stages (Fd4 + Fd5 = 38.90%) were well represented. Many females were spawning (Fd5/Fr1 = 33.40%). Another fraction has just spawned (Fr2–3 = 11.0%). Ripe males were clearly in emitting stages (Md5/Mr1 = 93.30%). In September several females showed mature stages (Fd4 + Fd5 = 15.80%, Fd5/Fr1 = 47.40%) and many others have just spawned (Fr2–3 = 36.80%). Several males were ripe and spawning (Md4 + Md5 = 30.43%, Md5/Mr1 = 93.30%). In October a few females were in early and intermediary gametogenetic stages (Fd1 + Fd2 = 7.14%, Fd3 + Fd4 = 7.14%), and some others had partially spawned (Fd5/Fr1 = 7.14%, Fd1 + Fr3 = 21.43%); the majority of them, however, has almost completely spawned (Fr2–3 = 57.15%). The males were in ripe (Md4 + Md5 = 20.00%) and spawning (Md5/Mr1 = 80.00%) stages. The percent values of this month were, indeed, not very significant since the little number of specimens still reproductively active.

Gametogenesis occurs at a water temperature higher than 14°C. Gonocyte multiplication, supported by active mitoses, is particularly evident at the start of gametogenesis in May, and in residual immature acini, as ascertained in a separate karyological study (Corni and Trentini, 1988). Oogonium (as is spermatogonium) sizes are similar (about 4.5 × 4.5 μm in diameter). During the reproductive period continuous successive spermatogenesis maturation and spawning keep place as showed by the numerous males already in emitting stages by June. Ovogenesis proceeds more slowly as shown by the numerous intermediary stages, and vitellogenesis appears more influenced by water temperature, with a peak of the Fd4–5 stages in July and August at the highest water temperature (25.0°C). The mean size of the most mature eggs, probably at the end of vitellogenesis, is about 60 × 65 μm. The sex ratio, from our data, is rarely balanced, most often favouring the males.

Since October the gonadal inactivity gradually takes place. In November the majority of specimens were in a resting stage. Only rarely did any specimen show gonads at the first phases of gametogenesis and a few specimens had just spawned. In December the reproductive inactivity was quite general. The same situation was evident in January–March 1985 with rare exceptions; in January a male, apparently active at an early stage of gametogenesis was found at a water temperature of 4.5°C. It probably represents a case of incomplete resorption of gametes during the winter period.

Summing up, the seasonal pattern of gonads consisted of about six months of continuous gametogenetic activity, from May to September–October, followed by about six months of total gonadal inactivity.

The intragonadic reserve tissue is histologically well developed in the first phase of gametogenesis up to Fd1–2 and Md1–2 stages, in reconstituting acini, and during the resting stages (Fig. 12). It considerably decreases in the final stages of gametogenesis and is atrophic around the acini that have been completely emptied (Fig. 11). The perigastric connective tissue is always well developed. These histological observations are supported by a separate biochemical study (Cattani et al. 1986).

Compared with the reproductive cycle of *Chamelea, Scapharca* shows a different strategy. The start and progression of gametogenesis, in *S. inaequivalvis* sustained continuously from May to September, seemed to constitute a single seasonal cycle more so than in *Chamelea gallina*. In that species, after a gonad developmental period from April to July and the massive spawning in July–August, a short pause of one–two months occurs (in September–October), followed by a fast revival of gametogenetic activity in November. Spermatogenesis, in particular, occurs in some specimens also in Winter; vitellogenesis, however, seems to be generally inhibited at low temperature in that species too.

ACKNOWLEDGMENTS

We wish to thank Mr. F. Monte and Mr. G. Vitali for their technical assistance.

REFERENCES CITED

Bayne, B. L., A. Bubel, P. A. Gabbott, D. R. Livingstone, D. M. Lowe & M. N. Moore. 1982. Glycogen utilization and gametogenesis in *Mytilus edulis* L. *Mar. Biol. Lett.* 3(2):89–106.

Carpené, E., W. Zurburg, P. Cortesi, G. Isani & O. Cattani. 1985. Effetti biochimici dell'anaerobiosi in *Venus gallina* L. e *Scapharca inaequivalvis* (Bruguière). *Boll. Soc. It. Biol.* 61(5):707–714.

Cattani, O., M. G. Corni, E. Carpené, G. Isani, G. Vitali & P. Cortesi. 1986. Seasonal variations of glycogen, proteins and lipids in tissues of *Scapharca inaequivalvis* (Bruguière) for the reproductive cycle. *Nova Thalassia* 8(suppl. 3):187–195.

Corni, M. G., M. Farneti & E. Scarselli. 1985. Histomorphological aspects of the gonads of *Chamelea gallina* (Linné) (Bivalvia; Veneridae) in autumn. *J. Shellfish Res.* 5(2):73–80.

Corni, M. G. & M. Trentini. 1988. *Scapharca inaequivalvis* (Brug.) chromosomes. *Oebalia* (in press).

Cortesi, P., D. Fabbri, G Isani, O. Cattani & E. Carpené. 1985. Characterization of pyruvate kinase isolated from the adductor muscle of the mollusc *Scapharca inaequivalvis*. *Comp. Biochem. Physiol.* 81B(1): 123–129.

Ghisotti, F. & E. Rinaldi. 1976. Osservazioni sulla popolazione di *Scapharca* insediatasi in questi ultimi anni su un tratto del litorale romagnolo. *Conchiglie* (Milano), 12(9–10):183–195.

Lubet, P. 1959. Recherches sur le cycle sexuel et l'émission des gamètes chez les Mytilides et les Pectinides (Moll. Bivalves). *Rev. Trav. Inst. Pêches maritimes* 23(4):389–548.

Lucas, A. 1965. Recherche sur la sexualité des Mollusques Bivalves. *Bull. Biol. Fr. Belg.* 99(2):117–247.

Journal of Shellfish Research, Vol. 8, No. 2, 345–354, 1989.

EFFECTS OF INTENSIVE FISHING EFFORT ON THE POPULATION STRUCTURE OF QUAHOGS, *MERCENARIA MERCENARIA* (LINNAEUS 1758), IN NARRAGANSETT BAY*

MICHAEL A. RICE,[1] CHARLES HICKOX,[2] AND ITRAT ZEHRA[3]

[1]*Department of Fisheries and Aquaculture*
The University of Rhode Island
Kingston, Rhode Island 02881 U.S.A.
[2]*College of Business Administration*
The University of Rhode Island
Kingston, R.I. 02881 U.S.A.
[3]*Centre of Excellence in Marine Biology*
University of Karachi
Karachi, Pakistan

ABSTRACT Quahogs, *Mercenaria mercenaria*, and sediment samples were collected from three locations in Narragansett Bay: Greenwich Cove, Greenwich Bay, and the West Passage of Narragansett Bay. Greenwich Cove has been closed to shellfishing for several decades. The average density of quahogs in the cove was 190/m^2, ranging from 32 m^2–500/m^2 in 30 quadrats. The average valve length of quahogs in Greenwich Cove was 62 mm. Adjacent to Greenwich Cove in Greenwich Bay which has been heavily fished since the 1930s. The average density of quahogs in Greenwich Bay was 78/m^2, ranging from 8/m^2–184/m^2. The average valve length was 31 mm. There were no significant differences in salinity, Secci disk turbidity or total organic content of sediments between these two sites. There was a slightly higher content of very fine-grained sands (<125 μm), silts, and clays in the Greenwich Cove sediments. The average *Mercenaria* density at another closed site on the West Passage of Narragansett Bay was 46/m^2 with an average valve length of 61 mm. The lower density may be due to higher silt and clay content of the sediments. There were significantly more juvenile (<40 mm) quahogs in the heavily fished area ($p < 0.01$, ANOVA). Determination of age by shell growth rings showed that quahogs in the bay were 12 years of age or less. Ages were greater in the closed areas and exceeded 25 years in the largest individuals. Growth data from quahogs in the closed areas was fit to the von Bertalanffy growth equation. This yielded asymptotic valve length maxima (L_{max}) of 110 mm ± 9.6 (SE) in the West Passage and 86 mm ± 4.7 (SE) in the cove, suggesting density-dependent stunting in the latter site. Active fishing tends to remove adults from the population and enhance either the set or survival of juvenile quahogs. The mechanism for increasing the juvenile density is not understood; possible explanations include removal of competing adults and sediment disturbance/turnover as a result of the fishing methods. Reburrowing of quahogs placed on the sediment surface was studied. Results indicate that the largest adults (>86 mm valve length) have the least ability to reburrow.

KEY WORDS: *Mercenaria*, population structure, Narragansett Bay, shellfishery, fishing effort

INTRODUCTION

Several studies in Narragansett Bay have focused on populations of the northern quahog, *Mercenaria mercenaria* (Linnaeus 1758). Many of these studies have correlated bottom type with clam abundance (Pratt 1953, Pratt and Campbell 1956, Saila et al. 1967). Another study has elucidated the infaunal community structure by correlating clam populations with the distribution of other infaunal species (Stickney and Stringer 1957). Other studies have been undertaken to assess the standing stock of adult clams with the aim of developing a database to aid the management of the *Mercenaria* fishery (Kovach 1968, Kovach et al. 1968, Sisson 1976).

In all of the aforecited studies, *Mercenaria* and/or sediments were sampled by using either clam rakes or tongs, or a grab sampler from a boat. Clam tongs or rakes will not effectively sample *Mercenaria* less than about 30 mm in valve length which escape through the teeth of the sampling device (Kovach 1968). A "clam shell" grab sampler has been used for other studies (Stickney and Stringer 1957, Saila et al. 1967). Quantitative grab sampling may possibly retain juvenile *Mercenaria*, however data on the size of individual clams were not reported.

In more recent study, Walker and Tenore (1984) sampled intertidal areas in Wassaw Sound, Georgia, by placing 1.0 m^2 quadrats intertidally during low tide and sieving the contents of the quadrats through a 1.0 mm mesh screen. They reported the size and numbers of *Mercenaria* to assess modestly exploited stocks with the aim of expanding the quahog fishery.

Unlike areas in the South, the quahog fishery in Narragansett Bay had begun early in this century and was well established by 1928. At that time, Greenwich Bay was one of the major areas of quahog production in Rhode Island due to its shallow waters. Only 8% of Greenwich Bay is deeper than 13 feet, making the quahog stocks well within reach of tongs (Pratt 1988). Greenwich Bay remains one of the most heavily exploited quahog grounds in Narragansett Bay (Campbell 1961; Ganz 1987). Greenwich Cove, adja-

*This study was sponsored by the Rhode Island Sea Grant Marine Advisory Service and the Rhode Island Cooperative Extension Service.
This is publication no. 2469 of the Rhode Island Agricultural Experiment Station.

cent to Greenwich Bay, has been closed to shellfishing since the 1930s (J. Migliore, RI Department of Environmental Management; J. Martin, EPA Narragansett Bay Project, pers. comm.). With an area long-closed to shellfishing in such close proximity to heavily exploited beds, changes in the populations of *Mercenaria* due to fishing pressure may be readily studied by comparing the two areas.

Concomitant with a well developed fishery, efforts have been taken by management agencies to enhance the quahog fishery, especially in areas with intense fishing pressure. A state sponsored program to transplant quahogs from beds closed to shellfishing to actively fished areas has been carried out intermittently in Narragansett Bay since the 1950s (Ganz 1987, Pratt 1988). Typically, large adult quahogs are removed from dense assemblages in closed areas and moved to areas with certified clean waters. The transplanted quahogs remain undisturbed for at least 3 months before the transplant area is opened for fishing. Although this program has been carried out for several years and has the popular support of fishermen, little has been done to investigate the survival of quahogs during the transplant process.

This study aims to compare the population structure of quahogs in two areas closed to shellfishing with an area which is heavily fished. The ability of quahogs to reburrow is studied, because it is considered a key factor to the survival of transplanted quahogs.

MATERIALS AND METHODS

The main study area was in the Greenwich Cove area in the northwest quadrant of Narragansett Bay (Fig. 1). Greenwich Cove has been closed to shellfishing because of the presence of a sewage treatment plant and several marinas. The pollution closure line runs between Chepiwanoxet Point and Long Point which form the mouth of the Cove, providing easy landmarks for enforcement purposes. The actual study site is 2 km from the sewage treatment plant outfall and 300 meters away from boat moorings. East of the pollution closure line is Greenwich Bay which is considered one of the most productive commercial and recreational shellfishing areas in Rhode Island (Ganz 1987). Tidal currents at the mouth of Greenwich Cove are semidiurnal with a maximum velocity of approximately 0.5 m/s (Spaulding and Swanson 1984). The Greenwich Bay site is approximately 100 m east of the pollution closure line and 200 m east of the Greenwich Cove site. The Greenwich Cove and Greenwich Bay sites have been the location of a recent in-depth study of tidal exchange and nutrient loading (Dettmann et al. 1989). A secondary study area was chosen on the West Passage of Narragansett Bay adjacent to the University of Rhode Island Narragansett Bay Campus (Fig. 1). This area has been closed to shellfishing for two decades because of a small sewage treatment plant one km to the south and a nearby experimental nuclear reactor. Thermal effluents by the reactor are neglegable because of its small power output. Due to its location in the open bay, this site is subject to greater tidal current velocities than the relatively enclosed Greenwich Cove area. Maximum current velocities at the West Passage site are approximately 2.5 m/s (Spaulding and Swanson 1984).

Salinity and turbidity measurements were taken on a weekly basis in each of the sites by a hand-held refractometer and a 25 cm diameter Secci disk. Sediment samples from each of the study sites were taken by SCUBA divers using short core tubes. The sediment cores were approximately 10 cm deep. Sediment was analyzed according to Folk's methods (1968). To determine grain size, sediment samples were dried overnight at 100°C and passed through a standard series of six sieves, beginning with a 2 mm mesh (-1.0 phi) with sequential halving to a final sieve of 62.5 μm mesh ($+4.0$ phi). The retained sediments on each of the sieves were weighed and expressed as a percentage of the total sediment dry weight. The sediment fraction passing through the final 62.5 μm mesh sieve was treated similarly. The total organic content (TOC) of the sediments was determined by weight loss after ignition at 550°C (Gross, 1972).

Samples of *Mercenaria* were taken from 0.25 m^2 quadrats placed by SCUBA diver at each of the three sites. In Greenwich Bay and Greenwich Cove, the quadrats were placed in a haphazard fashion approximately 100 m east or west of the pollution closure line in 3–5 m of water. The 100 m distance was chosen well within or well outside the pollution closure area to minimize the possibility of sampling an area which might have occasional errant commercial harvesting. At the West Passage site, quadrats were placed haphazardly along the 7-meter depth contour as determined by diver depth gauges. The upper 10 cm sediment content of the quadrat was removed by a small garden trowel and placed in a nylon mesh bag constructed with 5 mm octagonal meshes. While underwater, the mesh bag was shaken to allow sediments to filter out. It was assumed that quahogs greater or equal to 5 mm in valve length stayed in the mesh bag. The retained contents of the mesh bag were then transferred to polyethylene bags for transport and live storage pending sorting in the laboratory. The anterio-posterior valve lengths of all collected quahogs were measured by vernier calipers. Some quahogs were set aside for other morphometric analyses. The relationships between valve length, hinge width, and shell-free wet weight of fresh blotted tissue were determined. Age of selected quahogs was estimated by counting growth rings deposited on the exterior of the shell along the edge of the hinge plate, adjacent to the ligament. It was assumed that each major ring corresponded to the annual cessation of growth at the onset of winter. The counting of external rings was verified by cutting and polishing a small subsample of shells from umbo to ventral edge and counting major dark bands in the prismatic layer corresponding to the winter

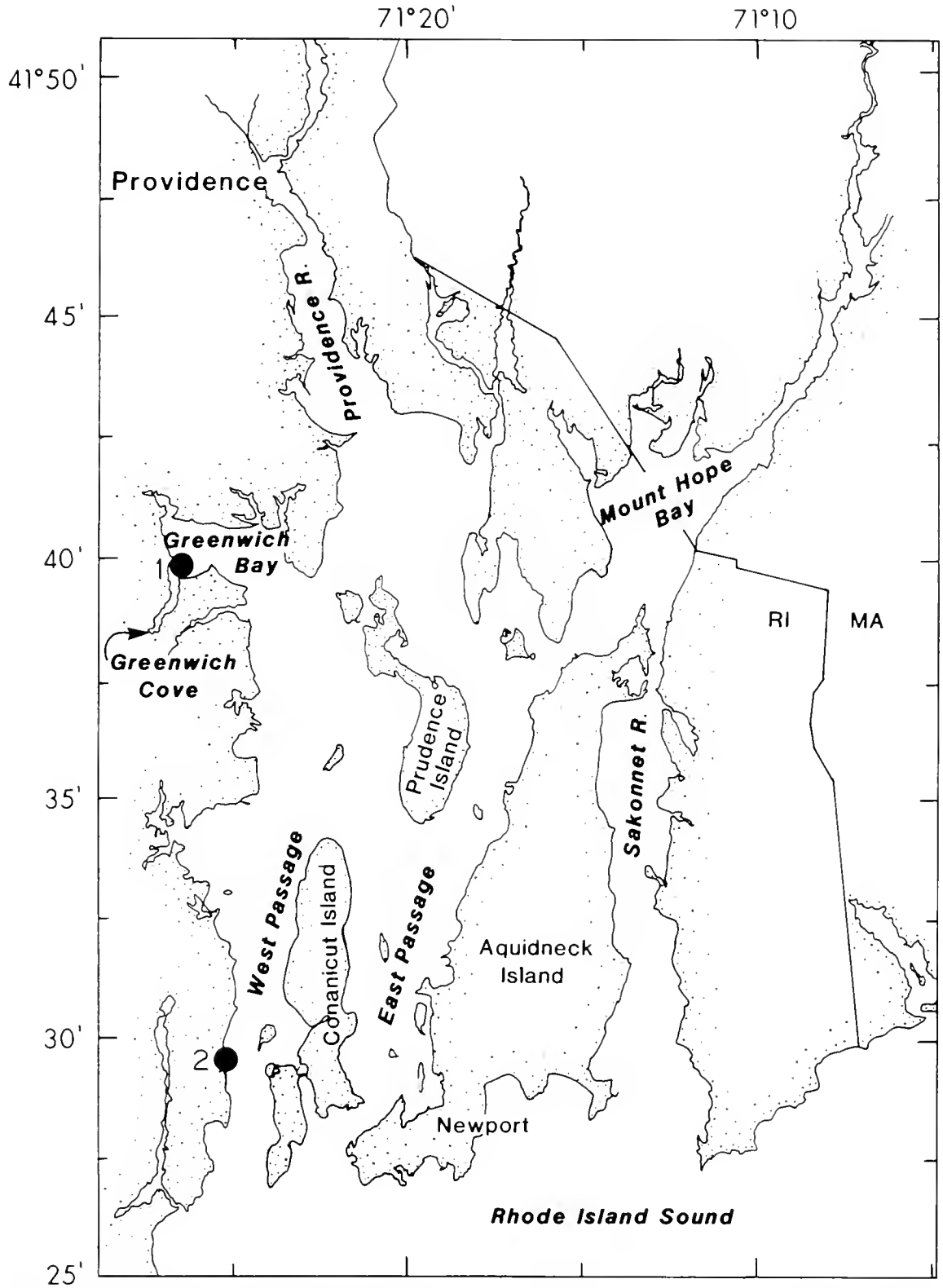


Figure 1. Narragansett Bay, Rhode Island. The study locations are on the western shore of the Bay. Location number 1 is at the mouth of Greenwich Cove, a narrow inlet off Greenwich Bay. Location number 2 is at South Ferry, on the West Passage of Narragansett Bay.

break in growth (Grizzle and Lutz 1988). Reliable age estimates of quahogs older than 15–20 years were possible only by sectioning the shell because of the close proximity of successive growth lines at the ventral edge.

Individual quadrat data were tabulated and analyzed by a microcomputer spreadsheet program (Lotus 123). Statistics and calculations using pooled data from individual sites were analyzed using the Fishery Science Application System (FSAS) (Saila et al., 1988). Subroutines of FSAS used for this study include univariate statistics, length-frequency analysis and non-linear least squares regression for curve fitting of the von Bertalanffy growth equation.

To determine the reburrowing of harvested quahogs, three subsets of quahogs ranging from 25–100 mm in valve length were selected. The shells of the quahogs were washed in fresh water, allowed to air dry, and painted with yellow enamel spray paint for easy underwater identification. Divers then returned the quahogs to their collection site, placing them on the surface of the sediments. An iron bar was inserted to project from the sediments at the center of the pile of painted quahogs, serving as a fixed reference point. After one week, the painted quahogs were retrieved from the surface or excavated from the sediments.

RESULTS

Measurements of salinity of surface waters and Secci disk turbidity were taken on a weekly basis from May to July, 1989. The mean salinity in Greenwich Cove and Greenwich Bay was 27 ppt (23–30 ppt range). Secci depths at the two sites averaged 1.8 m (1.5–2.5 m range). There were no differences in the individual daily salinity and turbidity measurements between the cove and the bay. Mean salinity and Secci depths at the West Passage site were 29.5 ppt (28–32 ppt range) and 2.5 m (1.5–4 m range).

The sediments collected at the Greenwich Cove and Greenwich Bay sites have a similar composition (Table 1). At both locations, the sediments are sandy in character with minor silt/clay and gravel components. There are, however, significant differences in some of the grain size fractions. The actively fished area (Greenwich Bay) has comparatively more medium to fine sands and significantly less very fine sand, silts, and clays ($p < 0.05$; ANOVA). The sediments collected at the West Passage site have a significantly higher percentage of shell fragments indicated by higher percentages in the 2.0 mm and 1.0 mm gravel and coarse sand fractions. The West Passage samples also contain considerably more silts and clays as indicated by a higher percentage in the <63 μm fraction. The mean percentage of TOC in Greenwich Cove and Greenwich Bay sediments was 2.53 ± 1.09 (SD; n = 9) and 2.93 ± 1.90, respectively. These values, likewise, are not significantly different ($p > 0.1$; ANOVA). The TOC of sediments collected at the West Passage site was 3.91 ± 0.52 (SD; n = 9) percent which is significantly higher ($p < 0.05$; ANOVA) than either of the Greenwich sites.

In Greenwich Cove (Fig. 2A), a total of 1426 quahogs was collected from 30 quadrats. This corresponds to an average quahog density of 190 m^2. The mean and median size of quahogs was 62 mm and 63 mm, respectively. The distribution is unimodal with the mode in the 61–63 mm size class. The number of quahogs per quadrat in Greenwich Cove was highly variable, indicating patchiness of distribution. The mean number of quahogs per quadrat was 47.6 ± 39.4 (SD), ranging from 8–125 per quadrat. In adjacent Greenwich Bay (Fig. 2B), 578 quahogs were collected in 30 quadrats. This corresponds to an average density of 78/m^2. The mean and median size of quahogs was 31 mm and 30 mm, respectively. The size distribution is polymodal with a majority of the quahogs less than 50 mm in valve length. The mean number of quahogs per quadrat was 19.4 ± 10.1 (SD), ranging from 2–46. In comparing the size distribution of quahogs in Greenwich Cove and Greenwich Bay, there are significantly higher numbers ($p < 0.01$) of juvenile quahogs (<40 mm valve length) in Greenwich Bay. There is no significant difference in the

TABLE 1.

Sediment samples from the pollution closure area and the fished area were dried and sieved. Units are percent of total sample weight. Significant differences between sediment fractions in the open and closed area were determined by one-way analysis of variance and Fischer's least significant difference test. All values except for those indicated by an asterisk (*) are significantly different ($p < 0.05$; ANOVA) from sediments in closed area of Greenwich Cove.

Mesh Size	(Phi)	Greenwich Cove Closed Area (mean ± SD) (n = 9)	Greenwich Bay Open Area (mean ± SD) (n = 9)	West Passage Closed Area (mean ± SD) (n = 9)
2.0 mm	(−1.0)	3.60 ± 1.23	0.76 ± 0.47	13.18 ± 4.35
1.0 mm	(0.0)	1.68 ± 0.39	1.26 ± 0.56*	7.23 ± 2.27
500 μm	(+1.0)	3.89 ± 0.71	3.94 ± 1.95*	8.40 ± 3.24
250 μm	(+2.0)	19.55 ± 2.76	24.63 ± 3.52	19.39 ± 4.71*
125 μm	(+3.0)	40.50 ± 3.16	49.26 ± 4.13	32.32 ± 8.50
63 μm	(+4.0)	28.29 ± 2.72	19.07 ± 2.71	9.76 ± 1.05
<63 μm	(<4.0)	2.47 ± 0.61	1.06 ± 0.28	9.68 ± 2.32

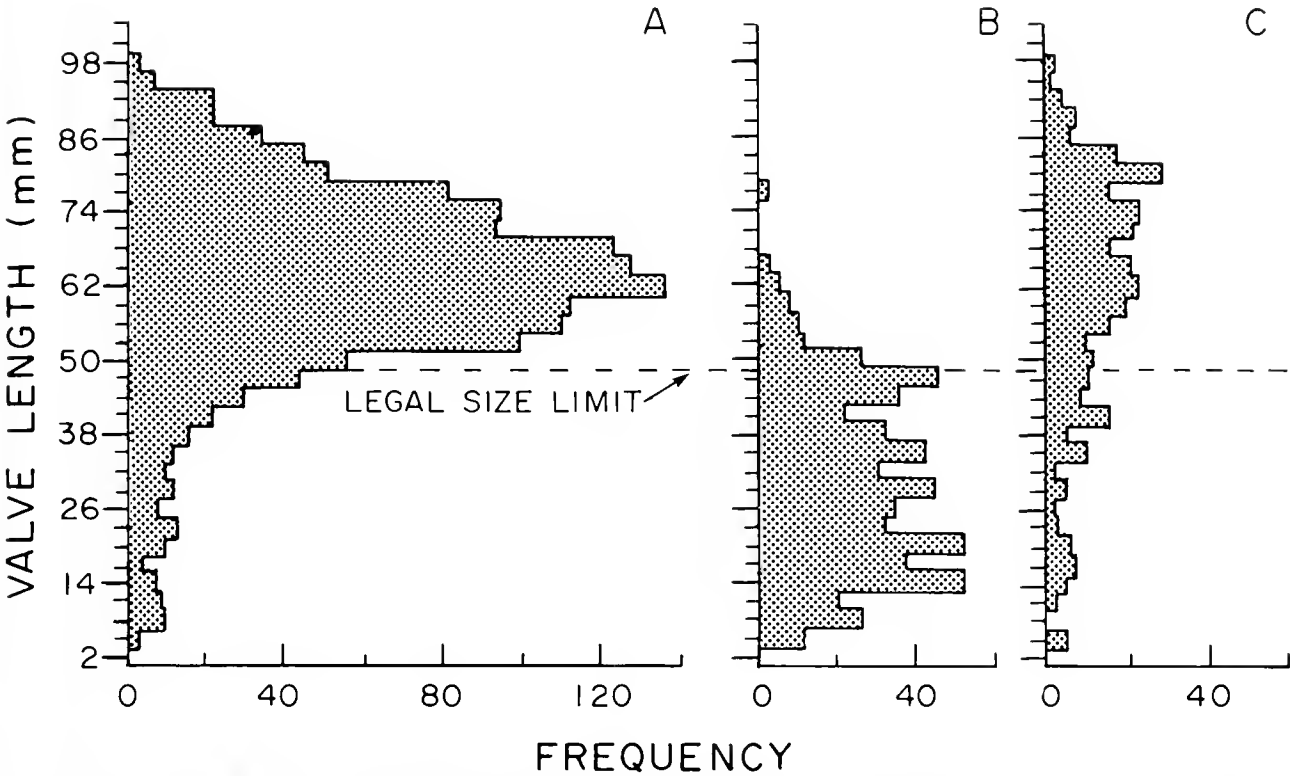


Figure 2A–C. Quahogs, *Mercenaria mercenaria*, were collected by SCUBA divers from 30 quadrats (0.25 m²) in each of three sites in Narragansett Bay. The sites are: (A) Greenwich Cove, (B) Greenwich Bay, and (C) South Ferry, West Passage. Histograms represent total numbers of quahogs in size classes of 3 mm increments. The indicated valve lengths are the size class midpoints. The dashed line represents the Rhode Island legal size limit for quahogs which is a one-inch hinge width, which corresponds approximately to a valve length of 48 mm.

numbers of quahogs in the 40–48 mm size classes between the two sites. This suggests that there may be general compliance among fishermen to the laws prohibiting the harvest of undersized shellfish. The lowest densities of quahogs were found at the West Passage site (Fig. 2C). A total of 344 quahogs was collected in 30 quadrats, representing an average density of 46/m². The mean and median size of quahogs at the West Passage site was 61 mm and 64 mm, respectively, with the major mode in the 79–81 mm size class. The distribution of quahogs in the West Passage is also patchy with an average 11.5 ± 6.4 (SD) per quadrat, ranging from 0–25 per quadrat.

Quahogs of varying size from each of the three sites were selected for age estimation. The maximum age and corresponding size of the quahogs were 33 years and 111 mm from West Passage; 34 years and 87 mm from Greenwich Cove; and 12 years and 74 mm in Greenwich Bay. The relation between size and estimated age of quahogs at each of the sites was plotted (Fig. 3). The data from Greenwich Cove and the West Passage Site were fit to the von Bertalanffy growth equation:

$$L_{(t)} = L_{max}(1 - e^{-K(t-t0)}) \qquad (1)$$

where $L_{(t)}$ is length at time in years (t); L_{max} is the maximum theoretical valve length; t_0 is time zero; and K is an empirically determined growth constant. Estimates for L_{max} and K with West Passage quahogs were 110 mm ± 9.6 (SE) and $8.7 \times 10^{-2} \pm 2.9 \times 10^{-2}$ (SE) respectively. The L_{max} and K estimates for Greenwich Cove quahogs were 86 mm ± 4.7 (SE) and $1.0 \times 10^{-1} \pm 2.8 \times 10^{-2}$ (SE). One criterion of the goodness of fit of the von Bertalanffy growth curve to the data is the estimated value of t_0. In both cases of data from West Passage and Greenwich Cove, the abscissa origin of the data plot falls within one standard error of the estimated value of t_0. The estimated von Bertalanffy growth parameters for quahogs collected in Greenwich Bay are not reported because insufficient numbers of large quahogs were able to be collected to give a confident estimate of L_{max} (Knight, 1968). The largest quahog from Greenwich Bay to be aged was 77 mm in valve length and 10 years old.

In Rhode Island, as well as other states, one-inch hinge width has been established as the legally harvestable size for *Mercenaria*. To compare our data readily with the legal harvestable size limits of quahogs, we determined the relationship between valve length and hinge width (Fig. 4). These linear measurements correlate directly with a correlation coefficient (r) of 0.988. From this regression, valve length is 1.78 times the hinge width.

The relationship between the valve length and the shell-free wet weight of *Mercenaria* tissues can be described by the allometric equation:

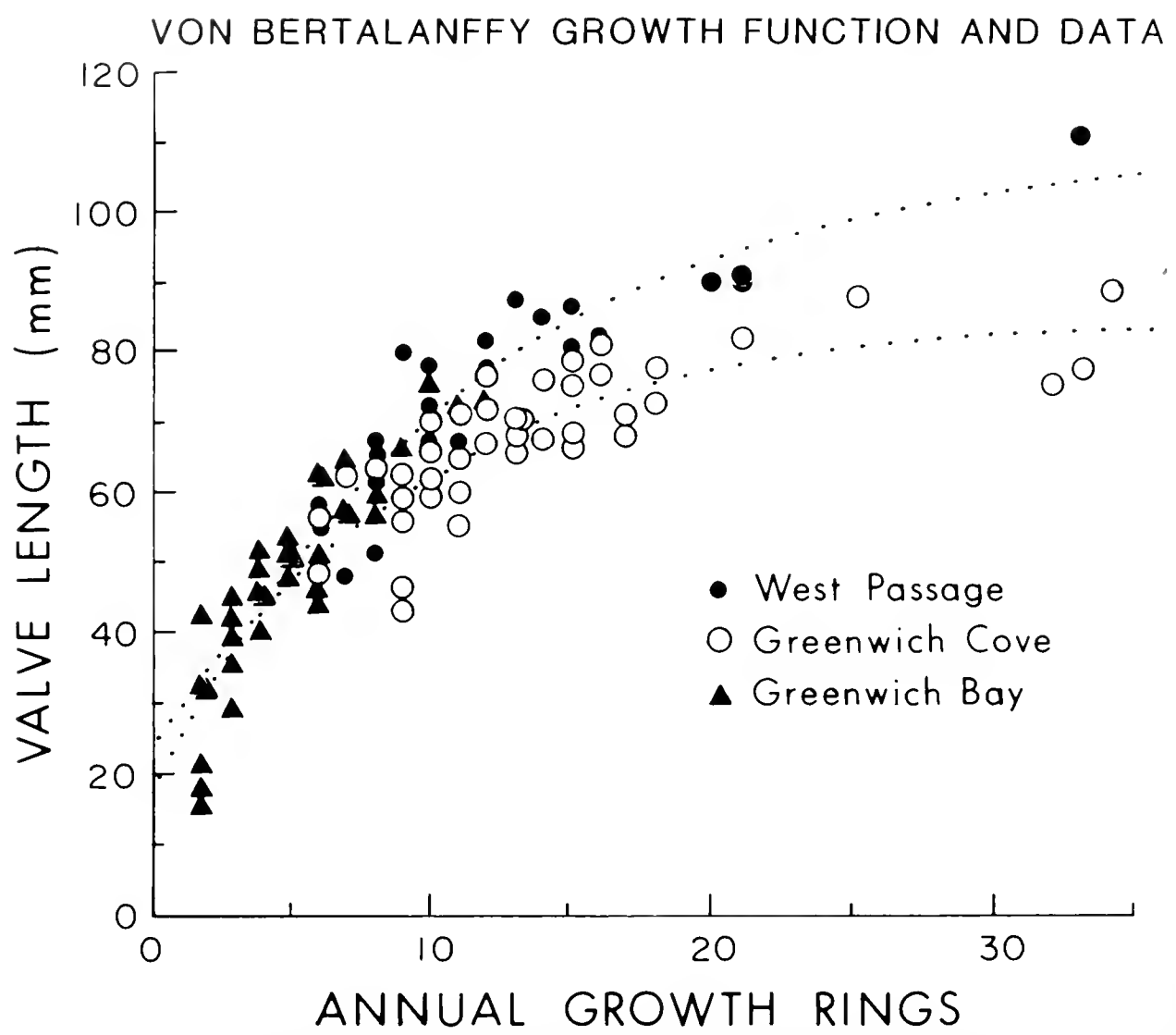


Figure 3. The valve length of quahogs from the three study sites are plotted as a function of estimated age. Von Bertalanffy growth curves were fit to Greenwich Cove and West Passage data. The L_{max} asymptotic values are 110 mm for West Passage quahogs and 86 mm for Greenwich Cove quahogs. Refer to the text for discussion of other von Bertalanffy parameters.

$$W = aL^b \quad (2)$$

where W = weight in g; L = valve length in mm; and a and b are allometric coefficients. The allometric equation can be transformed to the linear form:

$$\log W = b \log L + \log a \quad (3)$$

This transformation was used to estimate the allometric coefficients by linear regression (Fig. 5). The allometric coefficients are: $a = 9.51 \times 10^{-5}$ and $b = 2.81$.

A total of 21 quahogs was recovered from the sediment surface, representing an average recovery of 6.82% ± 1.11 (SD, n = 3). The average size of the quahogs on the surface was 90.2 mm ± 4.5 (SD, n = 21). The minimum valve length of the quahogs on the sediment surface was 83 mm. The recovery of quahogs from the sediment surface can be expressed as a percentage of the released quahogs which were ≥86 mm in valve length. This percentage is 18/33 or 54.5%. All but three of the 310 released quahogs were recovered from the surface or by removal from the sediment.

DISCUSSION

Horizontal seston fluxes, the product of phytoplankton concentration and current speed, are known to affect the growth of *Mercenaria* (Grizzle and Lutz 1989). Our Secci depth measurements show that there is no significant difference in turbidity between the Greenwich Cove and Greenwich Bay sites. This indirectly suggests that there is no difference in phytoplankton concentration between the two sites. Recently summer and winter fluorescent dye

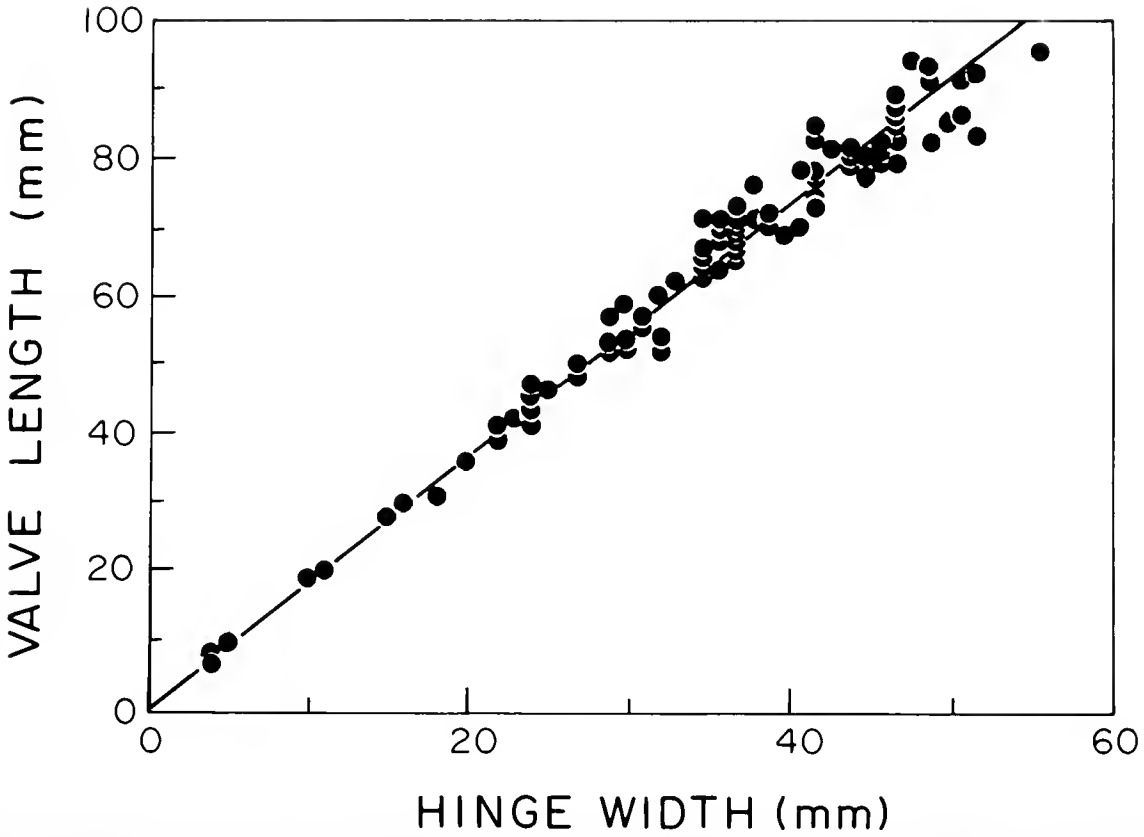


Figure 4. The value length and hinge width of *Mercenaria mercenaria* are linearly correlated (r = 0.988; n = 104). The calculated regression line has a positive slope of 1.78 and an intercept of 0. Quahogs were collected at the West Passage site and ranged from 7–95 mm in valve length.

surveys were conducted in Greenwich Cove with the aim of studying flushing rates of the cove and nutrient loading by the sewage treatment plant (Dettmann et al. 1989). In both surveys, dye was added to the cove waters at a continuous rate through three tidal cycles, which was sufficient for the dye to reach steady-state concentrations throughout the cove and the adjacent waters of Greenwich Bay. Sampling transects crossed the areas which we collected quahogs for this study. The data indicated no significant difference in steady-state dye concentrations between our Greenwich Cove and Greenwich Bay study sites. From this we can conclude that differences in seston fluxes or nutrient loading is not a likely explanation for the observed differences in quahog populations between the two sites. Likewise, salinity does not appear to be a factor which could explain differences in quahog populations between the two sites. Elevated total organic carbon (TOC) in sediments has been considered an indication of sewage pollution (Gross 1972). The data show that TOC in Greenwich Cove and Greenwich Bay are the same, which supports our assertion that pollutant levels from the nearby sewage treatment plant are not a factor in the distribution of quahogs. It is unlikely that the slight difference in the sediment grain size between the open and closed area can account for the gross differences in quahog densities (Table 1). Previous studies suggest that quahog densities increase as the percentage of silts and clays in the sediments decreases (Pratt 1953, Pratt and Campbell 1956, Saila et al. 1967). In this study, there is much less biomass of mature *Mercenaria* in the sandier sediments. Thus the difference between quahog densities in the open and closed sites in the Greenwich Cove area probably results from differences in fishing effort.

Comparisons can be made between populations of quahogs in the two closed sites. The unimodality of the size-frequency distribution of quahogs from Greenwich Cove prevents the direct identification of any distinct year classes (Fig. 2). Although the size-frequency distribution of quahogs from West Passage exhibits some polymodality, it is not likely that this represents individual year classes. Our age-length data (Fig. 3) show considerable variability in size at any given age. This variability would be expected to obfuscate identification of individual year classes of quahogs.

Physically, quahogs collected from Greenwich Cove tended to be more blunted. The modal size of quahogs in the West Passage is 83 mm; in Greenwich Cove, 62 mm (Fig. 2). The data (Fig. 3) show that the estimated ages of quahogs in the two sites are similar. The observation of higher asymptotic growth maxima of West Passage quahogs in comparison to Greenwich Cove quahogs may

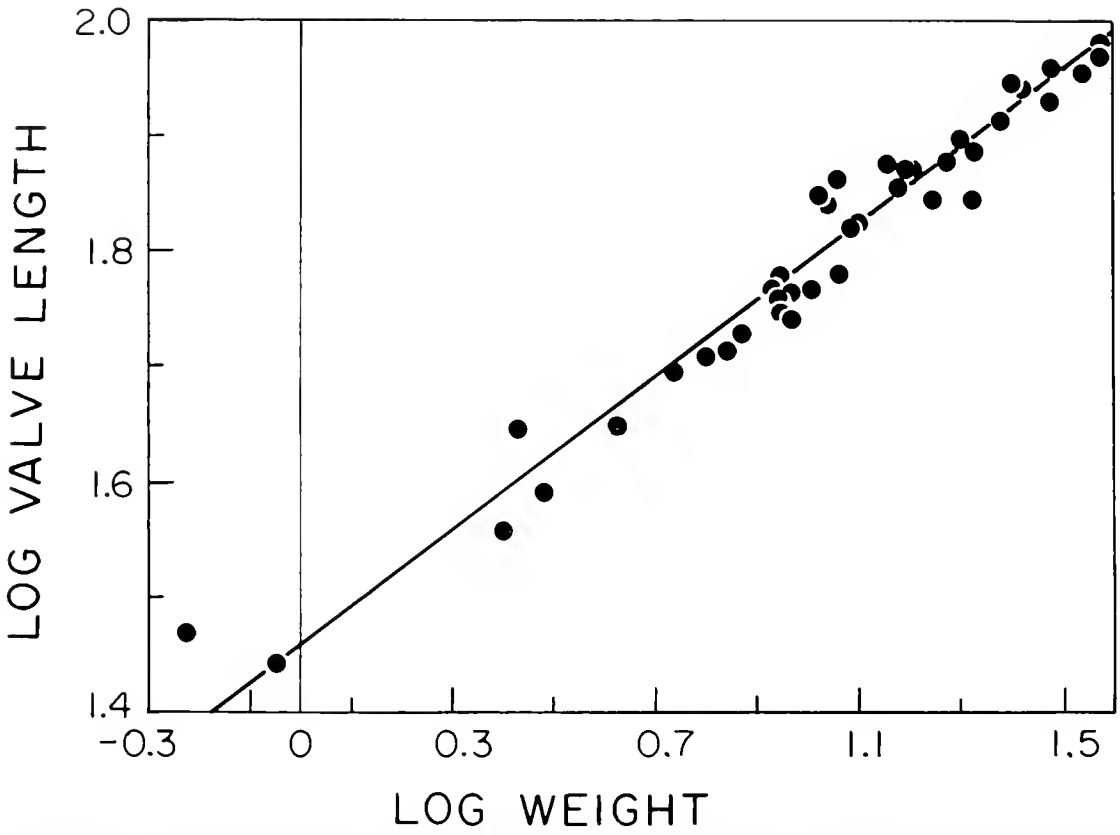


Figure 5. The valve length and shell-free tissue wet weight of *Mercenaria* can be correlated by using a linear transformation of the allometric equation. The correlation coefficient (r) is 0.973 with a sample size (n) of 40. Quahogs were collected at the West Passage site and ranged from 26–95 mm in valve length. Refer to text for discussion of regression coefficients.

suggest density-dependent stunting in the latter. The average density of 190/m^2 and the maximum density of 500/m^2 in Greenwich Cove are quite high. These densities of *Mercenaria* appear to be quite rare in nature and are more characteristic of densities of quahogs in intensive mariculture. Castagna (1984) suggests that field grow-out of hatchery-reared juvenile quahogs should be at densities of 250–1000/m^2 depending on the site. *Mercenaria* at higher densities exhibited stunting characterized by blunt valve margins and slower growth. The biomass of 1000 seed quahogs with valve lengths of 20 mm would have a total shell-free wet weight of 440 g (Fig. 5). The comparable biomass of 190 quahogs in Greenwich Cove with an average valve length of 62 mm would be 2000 g. Thus the natural standing crop biomass of quahogs in Greenwich Cove greatly exceeds the recommended stocking densities for mariculture. Natural populations of mussels *Mytilus edulis* are known to exhibit density-dependent stunting (Ricketts et al. 1968). The infaunal bivalves *Protothaca staminea* and *Chione undatella* have been shown to have depressed growth rates at high densities (Peterson 1982), as did *Anadara granosa* (Broom 1982), *Anomalocardia squamosus* and *Circe lenticularis* (Peterson & Black 1987). Peterson et al. (1985) postulated that *Mercenaria mercenaria* maintained at 80/m^2 (artificially high for their study area) exhibited stunting as evidenced by a lowered rate of deposition of new shell at the ventral edge.

A number of studies have been conducted to investigate the effects of large suspension-feeding bivalves on the settlement of larvae (Hunt et al. 1987, Ertman and Jumars 1988, Black and Peterson 1988). These studies have focussed upon the manipulation of densities of large adults in small (0.1 m^2–1.0 m^2) plots. The conclusion of all of these studies was that there was no localized inhibition of larval settlement by the presence of adult suspension-feeders in the plots. These studies however do not exclude the possibility of the inhibition of larval settlement by dense beds of adult suspension-feeding bivalves on a large scale. No large scale manipulation experiments similar to the aforecited small scale studies have been conduted. By using a correlative analysis approach, inferences as to inhibition of larval settlement and survival by adult populations can be made. Using this approach, Hancock (1973) showed that dense infaunal assemblages of the cockle *Cardium edule* inhibited settlement of juveniles.

The data show that there are significantly higher numbers of juvenile *Mercenaria* ($p < 0.01$, ANOVA) in the area open to fishing than in either of the two closed areas. There are a number of possible explanations for this observation. The first explanation relates to the large scale

removal of the adult quahogs. Woodin (1976) and Williams (1980) found that the survival of larval and post-set juveniles of various bivalve mollusks or other infaunal invertebrates is enhanced when there are lower densities of adults. At high densities of filter-feeding adults, there is the potential for larval loss through larviphagy and less available space for settlement and growth. More recent work (Butman 1986) suggests that hydrodynamic factors such as excurrent siphonal currents of dense assemblages of bivalves may act to prevent larval settlement. Meadows and Campbell (1972) concluded that the presence of some adult infaunal invertebrates may provide various chemical cues which promote settlement of the larval forms. More recent work has shown that receptors for Γ-aminobutyric acid (GABA) or GABA-mimetics are responsible for the settlement of the red abalone *Haliotis rufescens* (Morse and Morse 1984, Baxter and Morse 1987, Trapido-Rosenthal and Morse 1986). There is evidence that ammonia produced by beds of oysters induces settlement (Khalil et al. 1988, Coon et al. 1988). The data in this study, particularly concerning Greenwich Cove/Greenwich Bay, show that large numbers of juveniles are not coincident with large numbers of adults, nor are large numbers of adults coincident with large numbers of juveniles. Thus for *Mercenaria*, chemical cues (or pheromones) from the adults may not be a major factor in the initiation of settlement. The possibility of chemical cues inhibiting settlement in *Mercenaria* is not excluded.

The presence of large numbers of adult quahogs in Greenwich Cove may be a factor in the recruitment of juvenile quahogs in Greenwich Bay. The average density of *Mercenaria* in the portion of Greenwich Cove sampled for this study was about 190/m^2. Greenwich Cove is 106.8 ha, assuming that the average density of quahogs in all of Greenwich Cove is 25% of the sample area, there would be a standing crop in excess of 50 million individuals. The shell-free wet weight biomass of this many quahogs would be approximately 0.5 million metric tons (Fig. 5).

Quahogs harvested from very dense beds well inside Greenwich Cove have been harvested as part of a state sponsored transplant program (Ganz 1987). As stated earlier, little has been done previously to evaluate the reburrowing of quahogs harvested and translocated under these relay programs. Our data suggest that all quahogs less than 83 mm in valve length were able to reburrow within a week. Quahogs exceeding 83 mm show the lowest capability of reburrowing. These quahogs have the lowest direct commercial value but are potentially quite fecund. It might be argued on this basis that transplants from some dense, predominantly adult assemblages may reduce the production of larvae destined for settlement in actively exploited shellfish grounds. Further research is necessary to evaluate the maintenance of "spawner sanctuaries" and transplant programs as tools for shellfish management.

As previously noted, there have been several studies which have shown that population numbers and growth of *Mercenaria* is greater in sediments which have a lower percentage of silts and clay muds. We speculate that the process of fishing in Greenwich Bay resuspends sediments and results in a higher percentage of sands. This may be an explanation for the slightly lower silt/clay fraction in Greenwich Bay sediments (Table 1). This slightly higher sand content of the Greenwich Bay sediments may be another factor in the increased settlement and survival of juvenile *Mercenaria*. As early as the 1900s, bottom cultivation or stirring the sediments to allow fine silts and clays to drift away with the currents has been promoted and practiced as a means of enhancing the settlement of various species of bivalves (Belding, 1931; Rask, 1986). Controlled experiments are necessary to confirm the anecdotal observations of the efficacy of bottom cultivation.

In summary, we have shown that intensive commercial and recreational shellfishing results in significant changes in the population structure of *Mercenaria*. The shellfishing results in lowered numbers of mature adults and enhances the settlement and/or survival of juveniles. The exact mechanism for enhancement of settlement and/or survival of juvenile *Mercenaria* is not understood. Further studies are necessary to elucidate the relative effects of adult removal and sediment disturbance.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Joseph DeAlteris, Dr. Robert Bullock and Ms. Kathleen Castro for critical reviews of this manuscript. A special thanks to Dr. Conrad Recksiek who offered suggestions concerning von Bertalanffy analysis. This is contribution No. 2469 of the URI, AES. Support of URI AES and URI, SG/MAS is appreciated.

REFERENCES

Baxter, G. & D. E. Morse. 1987. G protein and diacylglycerol regulate metamorphosis of planktonic molluscan larvae. *Proc. Natl. Acad. Sci. USA* 84:1867–1870.

Belding, D. 1931. The quahog fishery of Massachusetts. Massachusetts Department of Conservation, Marine Fisheries Series Leaflet No. 2.

Black, R. & C. H. Peterson. 1988. Absence of preemption and interference competition for space between large suspension-feeding bivalves and smaller infaunal macroinvertebrates. *J. Exp. Mar. Biol. Ecol.* 120:183–198.

Broom, M. J. 1982. Analysis of the growth of *Anadara granosa* (Bivalvia: Arcidae) in natural, artificially seeded and experimental populations. *Mar. Ecol. Prog. Ser.* 9:69–79.

Butman, C. A. 1986. Larval settlement of soft-sediment invertebrates: Some predictions based on an analysis of near-bottom profiles. *In:*

J. C. J. Nihoul (ed.). Marine Interface Ecohydrodynamics, Elsevier Science Publishing Co., New York, 670 pp.

Campbell, R. 1961. A summary report of the fleet plotting program in Narragansett Bay. Rhode Island Division of Fish and Game Leaflet No. 4.

Castagna, M. 1984. Methods of growing *Mercenaria mercenaria* from post-larval to preferred-size speed for field planting. *Aquaculture* 39:355–359.

Coon, S. L., M. Walsh, W. K. Fitt, D. B. Bonar & R. M. Weiner. 1988. Induction of settlement behavior in oyster larvae by ammonia. *Am. Zool.* 28:70A (abstract).

Dettmann, E. H., J. F. Paul, J. S. Rosen & C. J. Strobel. 1989. Transport, fate and toxic effects of a sewage treatment plant effluent in a Rhode Island estuary. Report no. 1003 Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Narragansett, RI. 134 pp.

Ertman, S. C. & P. A. Jumars. 1988. Effects of bivalve siphonal currents on the settlement of inert particles and larvae. *J. Mar. Res.* 46:797–813.

Folk, R. L. 1968. Petrology of Sedimentary Rocks. University of Texas, Austin. 170 pp.

Ganz, A.R. 1987. Shellfish stock relocations: The Greenwich Bay experience. Narragansett Bay Symposium, April 22–25, 1987. p. 113.

Grizzle, R. E. & R. A. Lutz. 1988. Descriptions of macroscopic banding patterns in sectioned polished shells of *Mercenaria mercenaria* from southern New Jersey. *J. Shellfish Res.* 7:367–370.

Grizzle, R. E. & R. A. Lutz. 1989. A statistical model relating horizontal seston fluxes and bottom sediment characteristics to growth of *Mercenaria mercenaria*. *Mar. Biol.* 101:95–105.

Gross, M. G. 1972. Marine waste deposits near New York. *Mar. Poll. Bull.* 3:61–63.

Hancock, D. A. 1973. The relationship between stock and recruitment in exploited invertebrates. Rapports et Proces-Verbaux des Reunions Conseil International pour l'Exploration de la Mer 164:113–131.

Hunt, J. H., W. G. Ambrose, Jr. & C. H. Peterson. 1987. Effects of the gastropod, *Ilyanassa absoleta* (say), and the bivalve, *Mercenaria mercenaria* (L.), on the larval settlement and juvenile recruitment of infauna. *J. Exp. Mar. Biol. Ecol.* 108:229–240.

Khalil, A., W. K. Fitt, S. L. Coon & D. B. Bonar. 1988. Production of ammonia by fed and starved oysters *Crassostrea gigas* in relation to metabolism and metamorphosis. *Am. Zool.* 28:122A (abstract).

Knight, W. 1968. Asymptotic growth: An example of nonsense disguised as mathematics. *J. Fish. Res. Board Can.* 25:1303–1307.

Kovach, K. A. M. 1968. Shellfish Survey of Wickford Harbor. Rhode Island Division of Fish and Game Leaflet No. 24.

Kovach, K. A. M., M. T. Canario & G. Gray. 1968. Shellfish Survey of the West Passage of Narragansett Bay. Rhode Island Division of Fish and Wildlife Leaflet No. 26.

Meadows, P. S. & J. I. Campbell. 1972. Habitat selection by aquatic invertebrates. *Adv. Mar. Biol.* 10:271–382.

Morse, A. N. C. & D. E. Morse. 1984. Recruitment and metamorphosis of *Haliotis* larvae are induced by molecules uniquely available at the surfaces of crustose red algae. *J. Exp. Mar. Biol. Ecol.* 75:191–215.

Peterson, C. H. 1982. The importance of predation and intra- and interspecific competition in the population biology of two infaunal suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella*. *Ecol. Monogr.* 52:437–475.

Peterson, C. H., P. B. Duncan, H. C. Summerson & B. F. Beal 1985. Annual band deposition within shells of the hard clam, *Mercenaria mercenaria:* Consistency across habitat near Cape Lookout, North Carolina. *Fish. Bull.* 83:671–677.

Peterson, C. H. & R. Black. 1987. Resource depletion by active suspension feeders on tidal flats: Influence of local density and tidal elevation. *Limnol. Oceanogr.* 32:143–166.

Pratt, D. M. 1953. Abundance and growth of *Venus mercenaria* and *Callocardia morrhuana* in relation to the character of bottom sediments. *J. Mar. Res.* 12:60–74.

Pratt, D. M. & D. A. Campbell. 1956. Environmental factors affecting growth in *Venus mercenaria*. *Limnol. Oceanogr.* 1:2–17.

Pratt, S. D. 1988. Status of the hard clam fishery in Narragansett Bay. Draft Report—Narragansett Bay Project, U.S. Environmental Protection Agency. 89 pp. + 26 figures.

Rask, C. 1986. The effect of hydraulic harvesting on sediment characteristics related to shellfish abundance. Massachusetts Cooperative Extension Leaflet, Barnstable, Massachusetts.

Ricketts, E. F., J. Calvin & J. W. Hedgpeth. 1968. Between Pacific Tides. (4th ed.). Stanford University Press, Stanford, California, 614 pp.

Saila, S. B., J. M. Flowers & M. T. Canario. 1967. Factors affecting the relative abundance of *Mercenaria mercenaria* in the Providence River, Rhode Island. *Proc. Natl. Shellfish. Assoc.* 57:83–89.

Saila, S. B., C. W. Recksiek & M. H. Prager. 1988. Fishery Science Application System (FSAS). BASIC Fishery Science Programs: A Compendium of Microcomputer Programs and Manual of Operation. Elsevier Scientific Press, Amsterdam. 230 pp.

Sisson, R. T. 1976. Hard clam resource assessment study in the upper Narragansett Bay and the Providence River, Rhode Island. Rhode Island Division of Fish and Wildlife Leaflet No. 49.

Spaulding, M. & C. Swanson. 1984. Tides and Tidal Currents of Narragansett Bay. Sea Grant Marine Technical Report No. 35, The University of Rhode Island, Narragansett Bay Campus.

Stickney, A. P. & L. D. Stringer. 1957. A study of the invertebrate bottom fauna of Greenwich Bay, Rhode Island. *Ecology* 38:111–122.

Trapido-Rosenthal, H. G. & D. E. Morse. 1986. Availability of chemoreceptors in down-regulated by habituation of larvae to a morphogenetic signal. *Proc. Natl. Acad. Sci. USA* 83:7658–7662.

Walker, R. L. & K. R. Tenore. 1984. The distribution and production of the hard clam, *Mercenaria mercenaria* in Wassaw Sound, Georgia. *Estuaries* 7:19–27.

Williams, J. G. 1980. The influence of the adults on the spat of the clam, *Tapes japonica*. *J. Mar. Res.* 39:735–743.

Woodin, S. A. 1976. Adult-larval interactions in dense infaunal assemblages: Patterns of abundance. *J. Mar. Res.* 24:25–41.

Journal of Shellfish Research, Vol. 8, No. 2, 355–357, 1989.

GROWTH AND SURVIVAL OF CULTCHLESS SPAT OF *OSTREA EDULIS* LINNAEUS, 1750 PRODUCED USING EPINEPHRINE AND SHELL CHIPS

MUKI SHPIGEL,[1] STEVEN L. COON,[2] AND PHILIP KLEINOT[1]
[1]*National Center for Mariculture*
Israel Oceanographic and Limnological Research
Eilat, Israel
[2]*Department of Zoology*
University of Maryland
College Park, Maryland 20742, USA

ABSTRACT Growth and survival of cultchless spat of *Ostrea edulis* L. produced using three treatments were compared. Exposure of oyster larvae to either epinephrine (EPI) or shell chips alone resulted in 20% and 26% metamorphosis, respectively. In contrast, exposure to an integrated treatment of EPI and shell chips simultaneously resulted in 78% metamorphosis. No differences in subsequent growth and survival were observed between spat from the three treatments when followed for 112 days post-metamorphosis. Thus, exposing competent oyster larvae to EPI, either alone or in combination with shell chips, is a practical method for producing cultchless spat.

INTRODUCTION

Cultchless oysters have several advantages over attached oysters for both commercial and laboratory application. For oyster hatcheries advantages include superior shape, uniformity, ease of shipping and shucking, and elimination of costs of handling cultch material (Dupuy and Rivkin 1972). In laboratories cultchless oysters can be easily manipulated and measurements are not affected by attached cultch. Obtaining cultchless oysters by dislodging young spat soon after they have attached to a substrate damages the spat and requires laborious sorting. Alternatively, a widely employed method is to set larvae on small shell chips; after a 2–3 weeks growth they are essentially cultchless (Hidu et al. 1981). Recently Coon et al. (1986) demonstrated that the neurotransmitter, epinephrine (EPI), induces larvae of the Pacific oyster *Crassostrea gigas* Thunberg and the Atlantic oyster *C. virginica* (Gmelin) to metamorphose without attaching, thus directly producing cultchless oysters. These authors, however, did not closely examine the long term effects of EPI treatment on subsequent growth and survival of cultchless spat. The aim of the present study was to compare the use of EPI and/or shell chips to produce cultchless spat of the European oyster *Ostrea edulis* L. both in terms of the percentage which metamorphosed and the rates of subsequent growth and survival.

MATERIALS AND METHODS

Eyed larvae of *O. edulis* were supplied for this study by the National Center for Mariculture, Israel Oceanographic and Limnological Research, Eilat, Israel (IOLR). The larvae were cultured at 25 ppt and 25°C; seawater was changed on alternate days. A mixed diet of *Isochrysis galbana* and *Chaetocerus calcitrans* was added daily to a final concentration of 1×10^5 cells/ml. Larvae used for experiments were 8 days post-hatching. All experiments were conducted at the IOLR from February to June, 1988. All statistical comparisons were conducted using one-way analysis of variance at a 95% level of significance.

For the production of cultchless spat, three treatments were used. First, eyed larvae were exposed to 100 μM EPI for 1 h according to the methods of Coon et al. (1986). Preliminary experiments had shown that exposure of *O. edulis* larvae to 100 μM EPI for times ranging from 45 min to 24 h did not affect the percentage which metamorphosed. In the second treatment, larvae were exposed to shell chips for 48 h. For this study shell chips were made from pulverized oyster shell which had aged over 1 year. Shell chips were sieved to a size range from 250–750 μM and were incubated in seawater for 1 day prior to use in experiments. Larvae in the third treatment were exposed to an integrated regime of 100 μM EPI plus shell chips for 1 h. The shell chips were retained with the larvae when the EPI was removed. In all three treatments unmetamorphosed larvae were removed after 48 hr. Two experiments using these treatments were conducted as detailed below.

The first experiment was conducted in plastic tissue culture plates (24 well; Falcon #3047) using 1 μM filtered seawater, 25 ppt salinity at 25°C. In each of the 6 replicate treatments, 25 eyed larvae were placed in a total volume of 1.5 ml. For treatments involving shell chips, a single layer of shell chips was placed on the bottom of each well. After 48 h the wells were examined to determine the number of larvae which had metamorphosed as indicated by new shell growth. Unmetamorphosed larvae were removed from the wells and the spat grown in the culture plates for 21 days. Fifty percent of the water was changed daily and an algal diet of *I. galbana* was supplied at 1×10^5 cells/ml/day. Spat mortality and shell lengths were measured using a dissecting microscope at the end of the experiment.

The second experiment was conducted at a larger scale in plastic trays (50 × 30 × 10 cm) closed at the bottom

with 150 μM nylon screening. In each tray, approximately 5×10^4 larvae were exposed to one of the three treatments as described above. Neither percentage metamorphosis nor survival were determined in the early phase of this experiment. The trays were placed in a water table (220 × 100 × 20 cm) in which seawater was circulated from an external tank (80 × 80 × 70 cm), sprayed down into the plastic trays, and then returned to the external tank. Water temperature was kept at 25°C, 40 ppt salinity and 100% oxygen saturation (measured with a YSI model 57 dissolved oxygen meter). The flow rate was 4 ± 0.5 liter/h/tray and the water was changed on alternate days. A mixed diet of *I. galbana* and *C. calcitrans* was added daily to a final concentration of 1×10^5 cells/ml. After 30 days, the shell length of random samples of 20 spat were measured weekly for 82 days (total 112 days post-metamorphosis).

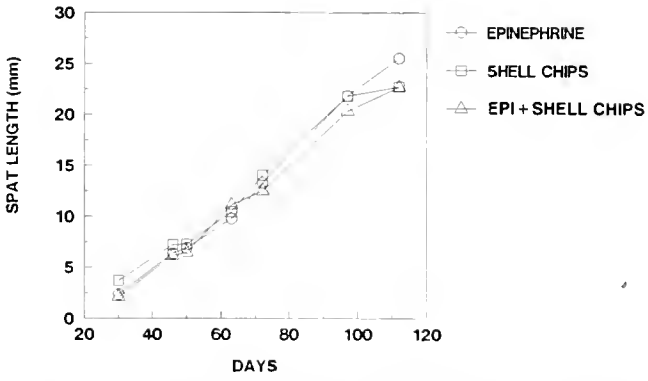


Figure 1. Growth of cultchless spat of *Ostrea edulis* beginning 30 days after metamorphosis following exposure to epinephrine alone, shell chips alone, or epinephrine plus shell chips. Each point represents the mean of 20 spat. Data are from March 24, 1988 to June 16, 1988.

RESULTS

The percentage of *O. edulis* larvae induced to metamorphose by the EPI alone and the shell chips alone were 20% and 26%, respectively (Table 1). Exposure of larvae to EPI and shell chips simultaneously resulted in a significantly higher percentage, 78%, metamorphosing with 16% unattached and 62% attached to shell chips. Larvae did not attach to the plastic culture plate in any of the treatments. The survival rates in the three treatments during the first 48 h were not significantly different. After 21 days, survival among the three treatments was still not significantly different. Additionally, after 21 days there were no significant differences in mean spat length between any of the treatments. All treatments had similar size frequency distributions (data not shown).

In the larger-scale, longer-term experiment, no significant differences in growth rates were observed (Fig. 1). The daily growth rates for EPI alone, shell chips alone and EPI plus shell chips were 2.0%, 1.5% and 2.0%, respectively (Table 2). Survival rates for the three treatments ranged from about 90–95%, but was not measured rigorously.

DISCUSSION

The results of this study demonstrate that treatment of *O. edulis* larvae with EPI to produce cultchless spat does not have a negative effect on either subsequent growth or survival. The use of EPI may therefore provide a useful tool for some applications in both mariculture and research where cultchless spat are advantageous.

The percentage of *O. edulis* larvae metamorphosing in response to EPI alone or shell chips alone was low compared to the percentage metamorphosing in the integrated EPI plus shell chips treatment, indicating perhaps a synergistic effect. Typically, >90% of *C. gigas* larvae metamorphose in EPI alone and although *C. virginica* larvae have a lower and variable response to EPI, metamorphosis rates often reach 75% (Coon et al. 1986, unpub. obs.). Low induction rates are often caused by having a large percentage of larvae in the population which are not yet competent to

TABLE 1.

Percentage metamorphosis, survival and growth of larvae and spat in the experiment conducted in tissue culture plates. Each treatment started with 6 replicates of 25 larvae; unmetamorphosed larvae were removed after the 48 h observation. Data for percentages are means ± (95% confidence interval). Shell lengths were measured for all spat and are shown as means ± s.d.; numbers in brackets are the total number of spat remaining.

	48 hour observation		21 day observation	
Treatment	% metamorphosis	% survival	% survival	spat length (mm)
Epinephrine alone	20.0 (14.0–27.2)	100 (97.6–100.0)	80 (61.4–92.3)	2.4 ± 1.9 [24]
Shell chips alone	26.0 (19.3–33.7)	96 (91.5–98.5)	85 (70.3–94.1)	2.5 ± 1.6 [33]
Epinephrine + Shell chips	[a]78.0 (70.6–84.2)	100 (97.6–100.0)	84 (76.0–90.1)	2.1 ± 0.4 [96]

[a] 16% unattached + 62% attached to shell chips.

TABLE 2.

Shell length and growth rates of spat from the long term experiment conducted in large trays. Data are taken from Figure 1 and are means ± s.d. (n = 20).

Treatment	Initial length (mm)	Final length (mm)	Average growth rate (μm/day)	Relative growth per day (%)
Epinephrine alone	2.4 ± 0.4	24.5 ± 3.1	270	2.0
Shell chips alone	3.7 ± 0.7	22.7 ± 4.8	230	1.5
Epinephrine + Shell chips	2.1 ± 0.4	22.7 ± 5.1	250	2.0

respond to EPI; in these cases aging the larvae will usually increase the percentage metamorphosis. Thus, under optimized conditions *O. edulis* may exhibit higher percentages of metamorphosis in EPI, as has been observed (Coon, Shpigel, unpub. obs.). The 26% metamorphosis observed in the shell chips alone treatment is not unexpectedly low for a 48 h exposure period, based on general hatchery experience with shell of various sizes (Shpigel, unpub. obs.). What was unexpected, however, was the large increase in the percentage metamorphosis when EPI and shell chips were used together.

In the integrated EPI plus shell chips treatment, about 80% of the metamorphosed spat were attached. Some of the spat became attached by shell growth after metamorphosis but apparently most were stimulated to attach during settlement and metamorphosis by treatment with EPI. In the EPI alone treatment no larvae were attached to the surface of the plastic culture plate, possibly indicating a larval preference for shell over polystyrene.

If *O. edulis* spat set on shell chips are acceptable or useful we currently recommend an integrated treatment of EPI plus shell chips for producing cultchless oysters although many other potential treatments have not been thoroughly examined. If shell chips are undesirable, then EPI alone is recommended. Current efforts are underway to increase the efficiency of EPI treatment to yield over 80% cultchless spat in *O. edulis*. Further research is needed to understand both the exogenous and endogenous mechanisms that control settlement and metamorphosis of oysters.

ACKNOWLEDGMENTS

The authors thank Dr. D. Popper and Mr. R. Chasan for reviewing this manuscript and making helpful suggestions, and they also thank Mr. R. Fridman for his assistance. This research was supported by the IOLR and the Maryland Industrial Partnership Program grant #119.23 to SLC.

REFERENCES

Coon, S. L., D. B. Bonar & R. M. Weiner. 1986. Chemical production of cultchless spat using epinephrine and norepinephrine. *Aquaculture* 58:255–262.

Dupuy, J. L. & S. Rivkin. 1972. The development of laboratory techniques for the production of cultch-free spat of the oyster, *Crassostrea virginica*. *Chesapeake Sci.* 13:45–52.

Hidu, H., S. R. Chapman & D. Dean. 1981. Oyster mariculture in subboreal (Maine, United States of America) waters: cultchless setting and nursery culture of European and American oysters. *J. Shellfish Res.* 1:57–67.

Journal of Shellfish Research, Vol. 8, No. 2, 359–365, 1989.

EFFECTS OF INTRASPECIFIC COMPETITION ON GROWTH OF THE EUROPEAN OYSTER, *OSTREA EDULIS* LINNAEUS, 1750

PADERMSAK JARAYABHAND[1] AND GARY F. NEWKIRK[2]

[1]*Sichang Marine Science Research and Training Station*
Chulalongkorn University
Bangkok 10500, Thailand
[1,2]*Department of Biology*
Dalhousie University
Halifax, NS B3H 4J1, Canada

ABSTRACT The effect of intraspecific competition on growth was studied by growing European oysters under various degrees of competition, including no-competition. Individual tagging was used to provide more information than the usual technique based on group data. The results demonstrate a clear non-random effect of communal rearing (intraspecific competition) on growth performance of this species. Growth rate is suppressed more for small oysters than for large oysters. It is expected that this competition will interfere considerably with the genetic expression of oysters grown in a communal environment as is used in selection programs.

KEY WORDS: competition, size effects, *Ostrea edulis*, European oyster

INTRODUCTION

Competition is one of the most important sources of growth variation in aquatic species, especially in such confined areas as cages, nets, ponds etc. (Purdom 1974, Malecha 1977, 1983, Kinghorn 1983, Doyle and Talbot 1986). When animals of the same species interact with each other during the process of seeking or occupying any common resources which may or may not be in short supply (i.e. are resource dependent or resource independent, respectively) they are engaged in intraspecific competition. The common resources may be food, space, social rank, mates, etc.

One effect of intraspecific competition, either resource dependent or resource independent, is to increase the degree of variability measured as a coefficient of variation and the third moment functions of size at age distribution (Wilbur and Collins 1973, Purdom 1974, Brett 1979, Malecha 1977, 1983, Doyle and Talbot 1986). Increased variability and skewness of size at age distributions imply a non-random effect of intraspecific competition. Examples of this non-random phenomenon were reported in brown trout fry (Brown 1946a, b, c, 1957); medaka (Magnuson 1962); the common carp (Nakamura and Kasahara 1955, 1956, 1957, 1961, Wohlfarth 1977 cited in Tave 1986, Moav and Wohlfarth 1974); goby (Yamagishi et al. 1974); plaice/flounder hyrids (Purdom 1974); freshwater prawns (Fujimura and Okamoto 1972, Malecha 1977, 1983, Ra'anan and Cohen 1984a, b); tadpoles (Wilbur and Collins 1973); the American lobster (Aiken 1977, Nelson et al. 1980). An extreme type of intraspecific competition can be seen in the Siamese fighting fish, which results in death of one of the contestants (Brown 1957).

A quantitative geneticist views growth as a polygenic, metrical or quantitative character. Such characters are likely to be controlled by a large number of so-called "additive" genes, each one having a small contribution to the trait. To improve this important economic trait by means of selection, the additive genetic variance is exploited. Basically, this is done by mating the best with the best to produce better offspring. But these traits are influenced not only by the genotype but by environmental factors and their interaction. The problem is how to judge from the phenotype, the best genotype. The decision is not easy and perhaps more difficult in aquatic species than in terrestrial ones. This is partly due to a non-random size effect of competition in aquatic environments which violates the assumption of quantitative genetic models that there is a random effect of environmental variation on phenotypic variation.

While there are many studies of intraspecific competition in fish and crustaceans, this information is limited for bivalves, especially in the European oyster, *Ostrea edulis* L. An application of the available knowledge from other species to this oyster species does not seem appropriate. Fish and crustaceans are motile species whereas the oyster is sessile after metamorphosis. In addition, we have little knowledge of the mechanisms of intraspecific competition in bivalve cultivation systems. In Nova Scotia, oysters are grown together in pearl nets or lantern nets suspended from longlines. It is expected that if the effect from intraspecific competition in this species is non-random, it will differentially affect the expression of the genotypes in the population. This will result in a weak correlation between phenotypic and genotypic values.

This experiment was designed to investigate the effects of intraspecific competition on growth of the European oyster grown in various degrees of competition including no competition.

MATERIALS AND METHODS

Experimental Design

In June of 1985, three year classes of hatchery-produced oysters (1983, 1984 and 1985) were obtained from Ostred

Sea Farm Ltd. Three discrete size groups based on their shell length (anterior-posterior direction) were separated for the 2 oldest year classes: 1983 (small ≤42 mm, medium 46 to 54 mm, and large ≥60 mm), and 1984 (small ≤20 mm, medium 24 to 28 mm, and large ≥32 mm). For the 1985 year class only two discrete size groups (small ≤5 mm, and large ≥7 mm) were used. For each year class, a mixed group was made up with equal numbers of each size group. It should be pointed out that the mixed group does not represent the population from which the oysters were sampled. This was done to ensure an adequate number of individuals of each size group. However, this should not seriously affect the result as our objective is to look at interactions of size groups and stocking density.

Twelve lantern nets of mesh size 12 mm, each with four levels (approximate area of 1930 cm^2/level) were used for the 1984 (level 1 and 2) and the 1983 (level 3 and 4) year classes. Since no comparisons will be made between year classes, the blocking of year classes in different depths will reduce any depth difference. Three small pearl nets of mesh size 3 mm were used for the 1985 year class but each net was divided into 4 compartments (approximate area of 290 cm^2/compartment). Periwinkles, *Littorina littorea* Linne, were used as a biological control for fouling organisms at a density around 0.04 periwinkle/cm^2 (Enright et al. 1983 and Muise et al. 1986). These nets were suspended from longlines about one meter below the surface at Ostred Sea Farm (Blind Bay, Nova Scotia).

Three stocking densities were maintained in the culture nets for 1983 (low = 25 individuals/level, medium = 75 individuals/level, high = 150 individuals/level), and 1984 (low = 50 individuals/level, medium = 150 individuals/level, high = 300 individuals/level). Two stocking densities for 1985 year class (low = 125 individuals/compartment, high = 750 individuals/compartment) were used. The stocking densities used in the analysis will be weight per unit area (g/cm^2) (Table 1).

The controls (same size interval as in the mixed group) in all three year classes were maintained by hanging the animals individually and widely spaced (5–10 cm apart) from plastic frames (Fig. 1). This was expected to eliminate the effect from intraspecific competition.

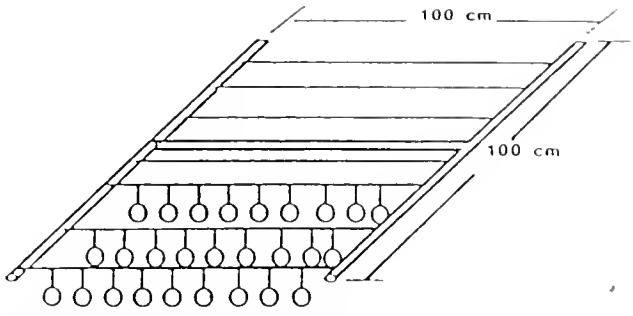


Figure 1. Diagrams showing the structures designed for the control culture condition. Note: oyster = O

Some of the animals used in this experiment were tagged individually by using Dymo tape and epoxy cement. For the controls and low stocking density of 1983 and 1984 year classes and the control of 1985 year class, all animals were tagged. In the medium and high stocking densities groups of 1983 and 1984 year class only 50% of the animals were tagged. Only 50 and 100 individuals per compartment of 1985 year class were tagged for low and high stocking densities. The numbers of labelled animals were 1224 out of 2045 for 1983; 2320 out of 4120 for 1984, and 1200 out of 5550 for 1985 year class, respectively.

Whole live weight (g) was measured at the start in July 1985 (WT0), then in December 1985 (WT1) for 1983 and 1984 year classes. The 1985 year class the same parameter was measured in August and December 1985.

Data Analysis

Multiple regression has been used to find the best fit growth model of the oyster in the control and mixed conditions. This has been done separately for each year class. Individual instantaneous growth rate (GI = ln(WT1/WT0)) was used as the dependent variable, whereas, whole live weight at the beginning (WT0), initial stocking density (g/cm^2; STKO), and replication (REP) or depth (DEPTH) were used as independent variables. All variables in the model can be quantified and the experimental design allows us to obtain individual growth data (high degrees of freedom). The final best fit models can have, to a certain

TABLE I.

Initial stocking densities in term of biomass (g/cm^2) used in the mixed condition of the 1985, 1984, and 1983 year classes.

Year Class	Stocking density (g/cm^2)					
	Low		Medium		High	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1985	0.019	0.016	—	—	0.101	0.100
1984	0.060	0.060	0.186	0.200	0.371	0.399
1983	0.155	0.152	0.524	0.504	1.065	1.114

extent, predictive roles. An analysis was started from the most complicated model with every possible interaction term. The back elimination technique (Neter et al. 1983) was used to find the best models. The significance level of every independent variable was ≤ 0.05.

Since there are non-linear relationships between specific growth rate and both initial weight and stocking density (from scatter plots), these two variables have been transformed to the natural logarithmic form.

RESULTS

The best fit growth models are shown in Table 2. The equations are for control and mixed conditions. In each case in the 1985 year class, log-transformed initial weights (LNWT0) in the control and mixed conditions show significant positive relationship with individual growth rate (G1). This implies that the large animals grow at a higher (specific) rate than the small ones during the time of the experiment. However, contributions of the initial weights to the final models are very small (about 2.1, and 6.6% for control and mixed conditions respectively).

For the control in the 1984 and 1983 year classes, initial size (LNWT0) shows a significant negative relationship with individual instantaneous growth rate (G1). This means that specific growth rate decreases as animals get bigger. The effect of depth in the control of 1984 year class is not significant. The two levels are only 30 cm apart (130 and 160 cm from surface for level 1 and 2). In the control of the 1983 year class depth is significant as is the interaction between depth and initial size. The control r^2 for the 1984 and 1983 year class G1 is quite high (0.708 and 0.903). The equation obtained can be satisfactorily used as a forecasting model for growth rate to predict size of the animals in the following year. Instantaneous growth rate shows a significant negative relationship with log-initial stocking density (LNSTK0) for the mixed conditions.

In the mixed condition for the 1984 and 1983 year classes, the best fit models are more complicated than the model in the control condition. The growth rate (G1) has a negative relationship with depth and initial stocking density but has a positive relationship with an interaction term between initial weight and initial stocking density. In the 1983 year class there is a negative relationship with initial weight.

The coefficient of determination for the 1985 year class (r^2 of the final models) increases from 0.044 in the control to 0.739 in the mixed condition. This is due to more significant independent variables in the model and a non-significant effect from the constant in the final model. However, the contribution from initial weight is still low. The r^2 of the control in the 1984 and 1983 year classes are higher than those obtained from the mixed group.

Plots of the best fit models for the control and mixed culture conditions are shown in Figure 2. The lines demonstrate that, individual growth rates in the control are higher than in the mixed condition (except for the large oysters in the 1984 year class). This is true over the range of initial sizes. The fitted lines allow one to compare instantaneous growth rate between animals of the same initial size grown under different conditions.

It is shown in Figure 3 (by calculating expected growth curve of oysters grown under different stocking densities) that growth rate of oysters decreases as stocking density increases. As the stocking density increases in the 1984 and 1983 year classes growth rate of the small oysters decreases faster than the growth rate of the large oysters (Fig. 3b). It should be mentioned that expected growth curves in the 1983 year class of the control and the low stocking density are very close to each other.

Since in the mixed conditions of the 1984 and 1983 year classes r^2's of the best fit model are lower than the controls, a further attempt was made to see how the relationship between growth rate and initial size changes with the degree of competition. This is done by finding correlation coeffi-

TABLE 2.

Best fit models obtained from the multiple regression technique of 1985, 1984, and 1983 year classes grown in control, and mixed conditions. The coefficients of multiple determination (r^2), contribution from initial size (in bracket) and sample size (number) are also shown.

Best fit model	r^2	Number
1985 year class		
Control		
G1 = 1.958 + 0.172 (REP) + 0.091 (LNWTO)	0.044 (0.021)	196
Mixed		
G1 = 0.092 (LNWTO) − 0.329 (LNSTKO)	0.739 (0.066)	176
1984 year class		
Control		
G1 = 2.029 − 0.531 (LNWTO)	0.708 (0.708)	60
Mixed		
G1 = 1.207 − 0.074 (DEPTH) − 0.248 (LNSTKO) + 0.140 (LNWTO*LNSTKO)	0.397 (0.238)	281
1983 year class		
Control		
G1 = 1.952 − 0.176 (DEPTH) − 0.475 (LNWTO) + 0.075 (DEPTH*LNWTO)	0.909 (0.903)	83
Mixed		
G1 = 1.062 − 0.085 (DEPTH) − 0.419 (LNSTKO) − 0.164 (LNWTO) + 0.114 (LNSTKO*LNWTO)	0.501 (0.329)	166

G1 = individual instantaneous growth rate (ln WT1/WTO); REP = replication (for 1985 year class); DEPTH = depth (for 1984 and 1983 year classes); LNWTO = natural log transformed of initial whole live weight (g); LNSTKO = natural log transformed of initial stocking density (g/cm^2).

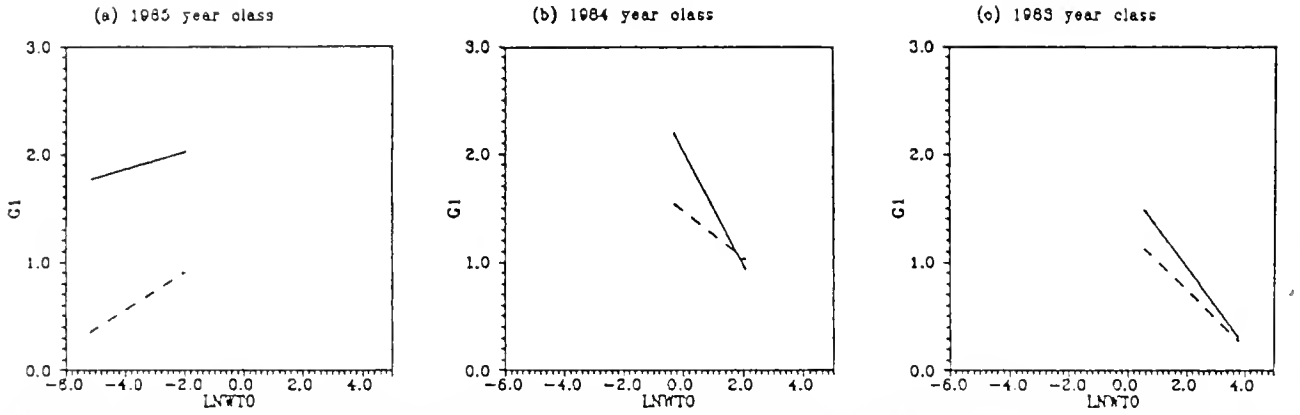


Figure 2. The best-fitted lines derived from a multiple regression technique for the 1985 (a), 1984 (b) and 1983 (c) year classes grown in the control (———) and mixed (– – – –) conditions (see Table 1 for the equations used).

cients between growth rate and initial size for each stocking density. The results show that the correlation coefficients decrease as stocking density increases. This is true for both the 1984 and 1983 year classes for the mixed conditions (Table 3).

Another way of looking at the effect of competition in this species is to see how the arcsine-transformed correlation coefficient between instantaneous growth rate and initial size of the animals changes with the degree of competition. There are two opposite forces controlling the correlation coefficient. A physiological force causes a negative correlation between growth and initial size, whereas, a competition force tends to work in the opposite direction (large animals have an advantage over relatively small animals).

As animals in this experiment are individually tagged it is possible to follow how animals of each size group react in relation to the others at different levels of competition. The relationships are shown in Figure 4 a and b for the 1984 and 1983 year classes. It is seen that small size groups of both the 1984 and 1983 years classes show a greater change in the correlation coefficient with a change in stocking density (as the competition increases) than the other two size groups.

DISCUSSION

The effects of stocking density in decreasing growth rate and increasing growth variation in this oyster are not new. However, a non-random effect of stocking density on growth performance was shown.

In most cases, initial weight (LNWT0) is a single or a major contributor to the growth model. High r^2's of the models mean high predictabilities of the models to explain growth. This is true for high r^2's of the initial weight.

Very low r^2's in the 1985 year class of both the models and the initial size imply a very poor predictability of initial size to explain subsequent growth rate. Although the r^2 of the model under the mixed condition is high (no constant), the relative contribution of the initial size to the model is still low (6.6%). For this year class, the animals are young and differences in age may be between 1 to 6 weeks. Such age differences are small if we are dealing with 1 or 2 year old animals, however, they could be important for at an early stage like the 1985 year class. More study is needed with young animals of known age.

The r^2's of both the growth models and the initial size increase from the 1984 to the 1983 year classes (i.e., with animals' age) when the comparisons are made within the

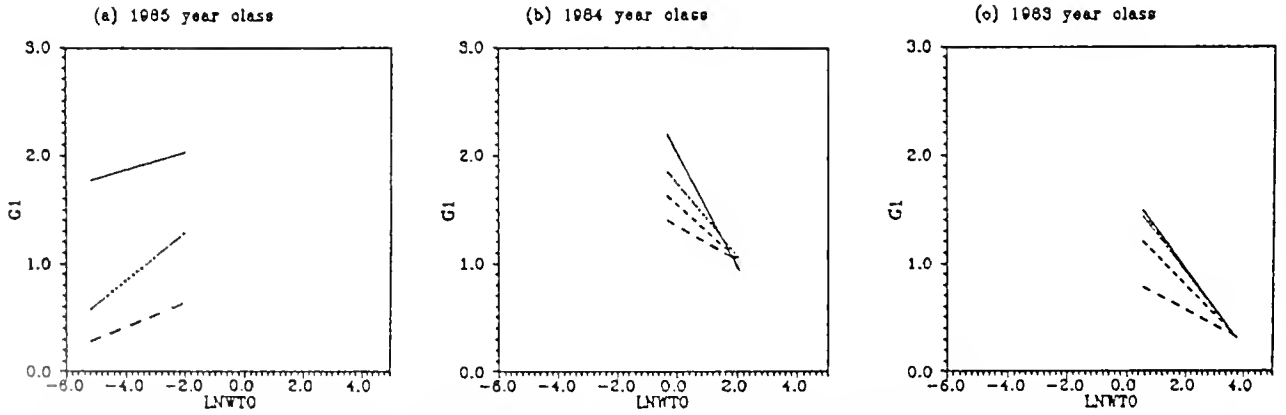


Figure 3. The expected lines derived from multiple regression equations for the 1985 (a), 1984 (b) and 1983 (c). The lines are for control (———), low (·····), medium (-----); only for the 1984 and 1983 year classes), and high (– – – –) stocking densities.

TABLE 3.

Correlation coefficient (r) and coefficient of determination (r^2) between instantaneous growth rate and initial weight after the first (July–December 1985) growing season of the 1984 and 1983 year class (Sample size is n). Note that the levels were combined.

Condition	Stocking density	r	r^2	n
1984 year class				
Control		−0.841	0.707	60
Mixed	Low	−0.825	0.681	50
	Medium	−0.580	0.336	79
	High	−0.390	0.152	152
1983 year class				
Control		−0.950	0.903	83
Mixed	Low	−0.829	0.687	30
	Medium	−0.674	0.454	48
	High	−0.443	0.196	88

same culture condition. The r^2's are high for the control condition and low for the mixed condition. This is mainly due to smaller contributions of initial size in the mixed condition (even though more variables are added).

Increased contribution of initial size to the growth model with age, especially under the control condition, means that growth is more constant in the older animals than in the younger ones. Higher r^2's (both model and initial size for 1984 and 1983 year classes) in the control condition mean growth is more constant under non-competitive conditions than in the competitive environment. This suggests that the control animals grow without interference from each other.

Evidence is presented to support the non-random size effect of intraspecific competition on oysters. The plots of G1 against initial weight for all year classes (Fig. 2) show the same patterns. First, the control lines are the topmost. This means that throughout the initial size ranges, except for large animals of the 1984 and 1983 year classes, spe-

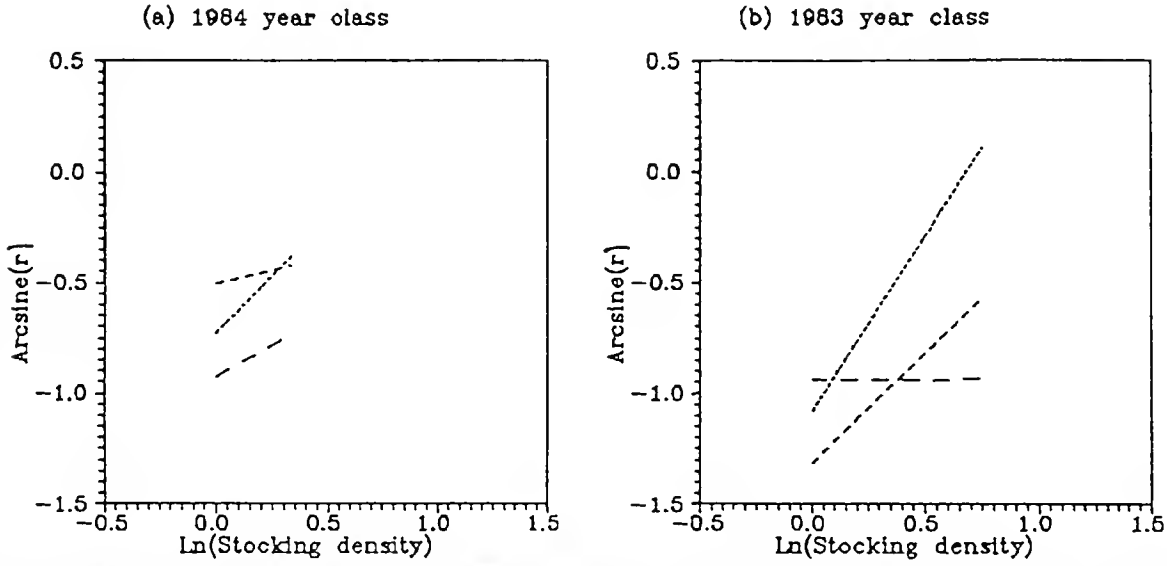


Figure 4. Plots between arcsine-transformed correlation coefficients (between instantaneous growth rate and initial weight: r) and natural log-transformed stocking density of the 1984 (a) and 1983 year classes grown in the mixed condition. The plots are separated for small (·····), medium (-----), and large (−−−−−) size groups. The weighted regression equations are:

(a) the 1984 year class

Small $Y = -0.73 + 1.03X$; $N = 103$, $r^2 = 0.09$, $P = .00$
Medium $Y = -0.51 + 0.24X$; $N = 116$, $r^2 = 0.00$, $P = .50$
Large $Y = -0.93 + 0.56X$; $N = 122$, $r^2 = 0.14$, $P = .00$

(b) the 1983 year class

Small $Y = -1.08 + 1.60X$; $N = 61$, $r^2 = 0.45$, $P = .00$
Medium $Y = -1.32 + 0.99X$; $N = 79$, $r^2 = 0.77$, $P = .00$
Large $Y = -0.94 + 0.01X$; $N = 107$, $r^2 = 0.00$, $P = .87$

where X = log-transformed stocking density
Y = arcsine-transformed correlation coefficients (r).

cific growth rates in the control oysters tends to be higher than the ones in the mixed conditions. Second, the distances between the control and mixed fitted lines decrease as they approach the large initial sizes.

The effects of stocking density on growth rate of oysters can be seen by comparing the fitted lines of the control and the mixed culture conditions. It is clear from those fitted lines that small oysters suffer from the effect of increasing stocking density more than the large oysters; small oysters decrease their specific growth rates more than that of the large ones (Fig. 3b and c). The results suggest a non-random effect of intraspecific competition. This relationship is not obvious for the 1985 year class.

The slopes of the relationships between the arcsine-transformed correlation coefficients (between growth rate and initial size) and stocking densities increase going from the large to the small size group (Fig. 4). This means that as stocking density increases the relationship between growth rate and initial size of the small oyster is affected more than that of the large oysters.

If some animals develop reproductive tissue, this may slow down their growth. This will result in decreasing r^2, as animals of the same initial size may have considerably different growth rates. However, this is not always the case since in the control r^2's are still high. There may be some chemical mechanisms either slowing down or speeding up growth rate of animals. There are some reports on hormonal inhibiting growth (HIG) in crustaceans (Malecha, 1977; 1983; Ra'anan and Cohen, 1984a, b). This has not been reported in oysters, but there is no reason to overlook this possibility. Unfortunately, we are not be able to test this possibility in our experiment.

Another possible explanation of this declining in r^2 is the position of animals in the nets. Physically, animals are stacked up in the net. The packing of individuals may in some circumstances support growth of small animals while inhibiting growth of large animals or the other way around.

Under the conditions used in this experiment one could argue that there are many other factors affecting the growth of the oysters under both the net and the control conditions, e.g. flow rate of water, food supply, fouling organisms, effects from periwinkles etc. One would expect that variation in these variables could affect all size classes similarly in each unit. However, all the results tend to show the same non-random effects of stocking density in nets on the growth rates of various sized oysters (control vs mixed as a whole and with various stocking densities). Intra-specific competition is most likely to be the cause of this non-random effect while other variables may change the level of competition. If increase in stocking density were unrelated to competition, it would result in an equal suppression of growth of all oysters. Thus it would not effect the slope of the relationship between initial size and growth rate. However, the results obtained show a change in slope when the stocking density (intraspecific competition) increases (Fig. 3). This experiment was not designed to determine the mechanism of intraspecific competition.

In most selection programs, the oysters were outplanted in nets, a communal environment. It is very likely that competition will interfere with the expression of the genotype of oysters grown in competitive conditions. Thus, the grow-out methods presently used for selective breeding of this species may not be suitable. This has been studied by selecting oysters for growth rate and testing their growth performance in environments with different levels of competition (Jarayabhand and Newkirk, in preparation).

ACKNOWLEDGMENTS

The senior author was supported by a postgraduate scholarship from Chulalongkorn University. The research was supported by the Natural Sciences and Engineering Research Council of Canada to the second author and through the Dalhousie University Aquatron.

REFERENCES CITED

Aiken, D. E. 1977. Molting and growth in decapod crustaceans, with particular reference to the lobster Homarus americanus. In: B. F. Phillips and J. S. Cobb (Editors), *Workshop on lobster and rock lobster ecology and physiology*. CSIRO, Division of Fisheries and Oceanography, Melbourne, Australia, pp. 41–73.

Brett, J. R. 1979. Environmental factors and growth. In: W. S. Hoar, D. J. Randall and J. R. Brett (Editors), *Fish Physiology Vol. VII. Bioenergetics and Growth*. Academic Press, New York, pp. 559–675.

Brown, M. E. 1946a. The growth of brown trout (*Salmo trutta* Linn.) I. Factors influencing the growth of trout fry. *J. Exp. Biol.* 22:118–129.

Brown, M. E. 1946b. The growth of brown trout (*Salmo trutta* Linn.) II. The growth of two-year-old trout at a constant temperature of 11.5° C. *J. Exp. Biol.* 22:130–144.

Brown, M. E. 1946c. The growth of brown trout (*Salmo trutta* Linn.) III. The effect of temperature on the growth of two-year-old trout. *J. Exp. Biol.* 22:145–155.

Brown, M. E. 1957. Experimental Studies on Growth. In: M. E. Brown (Editor), *Physiology of Fishes, Vol. 1*, Academic Press, New York, pp. 361–400.

Doyle, R. W. & A. J. Talbot. 1986. Artificial selection on growth and correlated selection on competitive behaviour in fish. *Can. J. Fish. Aquat. Sci.* 43:1059–1064.

Enright, C., D. Krailo, L. Staples, M. Smith, C. Vaughan, P. Ward, P. Gaul & E. Borgese. 1983. Biological control of fouling algae in oyster aquaculture. *J. Shellfish Res.* 3:41–44.

Fujimura, T. & H. Okamoto. 1972. Notes on progress made on developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. In: T. V. R. Pilley (Editor), *Coastal Aquaculture in the Indo-Pacific Region*. Fishing News (Books) Ltd., Surrey, England, pp. 313–327.

Kinghorn, B. P. 1983. A review of quantitative genetics in fish breeding. *Aquaculture* 31:283–304.

Magnuson, J. J. 1962. An analysis of aggressive behavior, growth and competition for food and space in medaka (*Oryzias latipes*, Pisces, Cyprinodontidae). *Can. J. Zool.* 40:313–363.

Malecha, S. R. 1977. Genetics and selective breeding of *Macrobrachium rosenbergii*. In: J. A. Hanson and H. L. Goodwin (Editors), *Shrimp and prawn farming in the western hemisphere*, Dowden, Hutchinson and Ross, Inc., Stroudsburg, PA, pp. 328–355.

Malecha, S. R. 1983. Crustacean genetics and breeding: An overview. *Aquaculture* 33:395–413.

Moav, R. & G. W. Wohlfarth. 1974. Magnification through competition of genetic differences in yield capacity in carp. *Heredity* 33:181–202.

Muise, B., L. Macleod, K. Henderson & R. Truen. 1986. Cultivation of the European Flat oyster (*Ostrea edulis*) in Nova Scotia: General Growth and Economic Model. Manuscript and Technical Report Series, Project Report No. 86-01, Nova Scotia Department of Fisheries, 183 pp.

Nakamura, N. & S. Kasahara. 1955. A study on the phenomenon of the tobi-koi or shoot carp-I. On the earliest stage at which the shoot carp appears. *Bull. Jpn. Soc. Sci. Fish.* 21:73–76. (English translation in Wohlfarth (1977).)

Nakamura, N. & S. Kasahara. 1956. A study on the phenomenon of the tobi-koi or shoot carp-II. On the effect of particle size and quantity of the food. *Bull. Jpn. Soc. Sci. Fish.* 21:1022–1024. (English translation in Wohlfarth (1977).)

Nakamura, N. & S. Kasahara. 1957. A study on the phenomenon of the tobi-koi or shoot carp-III. On the result of culturing the modal group and the growth of carp fry reared individually. *Bull. Jpn. Soc. Sci. Fish.* 22:674–678. (English translation in Wohlfarth (1977).)

Nakamura, N. & S. Kasahara. 1961. A study on the phenomenon of the tobi-koi or shoot carp-IV. Effects of adding a small number of larger individuals to the experimental batches of carp fry and of culture density upon the occurrence of shoot carp. *Bull. Jpn. Soc. Sci. Fish* 27:958–962. (English trans. in Wohlfarth (1977).)

Nelson, K., D. Hedgecock, W. Borgeson, E. Johnson, R. Daggett & D. Aronstein. 1980. Density-dependent growth inhibition in lobsters, *Homarus* (Decapod, Nephropidae). *Biol. Bull.* 159:162–176.

Neter, J., W. Wasserman & M. H. Kutner. 1983. Applied Linear Regression Models. Richard D. Irwin, Inc., Homewood, Illinois. 547 pp.

Purdom, C. E. 1974. Variation in Fish. In: F. R. Harden Jones (Editor), *Sea Fisheries Research*. Elek Science, London, pp. 347–355.

Ra'anan, Z. & D. Cohen. 1984a. Characterization of size distribution development in the freshwater prawn *Macrobrachium rosenbergii* (De Man) juvenile populations. *Crustaceana* 46(3):271–282.

Ra'nan, Z. & D. Cohen. 1984b. The effect of group interactions on the development of size destribution in *Macrobrachium rosenbergii* (de Man) juvenile populations. *Biol. Bull.* 166:22–31.

Tave, D. 1986. *Genetics for fish hatchery managers*. AVI Publishing Company, Inc. Westport, Connecticut. 299 pp.

Wilbur, H. M. & J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. *Science* (Wash., DC), 182:1305–1314.

Wohlfarth, G. W. 1977. Shoot carp. *Bamidgeh* 29:35–56.

Yamagishi, H., T. Maruyama & K. Mashiko. 1974. Social relation in a small experimental population of *Odontobutis obscurus* (Temminck et Schlegel) as related to individual growth and food intake. *Oecalogia* (Berlin) 17:187–202.

Journal of Shellfish Research, Vol. 8, No. 2, 367–373, 1989.

COMPARATIVE STUDY OF THE HEMOCYTES OF TWO OYSTER SPECIES: THE EUROPEAN FLAT OYSTER, *OSTREA EDULIS*, LINNAEUS, 1750 AND THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* (THUNBERG, 1793)

MICHEL AUFFRET
Laboratoire de Biologie Marine
Faculté des Sciences
Université de Bretagne
Occidentale, 29287 Brest Cedex, France

ABSTRACT Comparable hemocyte types have been observed in hemolymph from two oysters, *Ostrea edulis* and *Crassostrea gigas*. In both species, a type of granular hemocyte (granulocyte) was characterized by specific cytoplasmic granules exhibiting conspicuous morphological and cytochemical similarities. These granules are believed to be storage organelles rich in lysosomal enzymes. *Crassostrea gigas* had a supplementary granular type, the acidophilic granulocytes. The hyalinocytes formed a hemocyte population with several of the morphological characteristics of poorly differentiated cells. A new cell type, named vesicular hemocyte, is believed to be an immature cell associated with granulocytes. An homogeneous nomenclature and classification of hemocyte types for crassostreid and ostreid oysters is supported by morphological and ultrastructural similarities described here and in previous research.

KEY WORDS: *Ostrea edulis*, *Crassostrea gigas*, hemocytes, ultrastructure, enzyme cytochemistry

INTRODUCTION

There has been increasing interest in studying molluscan blood cells, or hemocytes, after their prominent role in internal defense was emphasized (Feng 1967, Tripp 1974). Because several economically important bivalve molluscs suffer severe mortalities due to parasitic and non-parasitic diseases (Farley and Durfort 1986), a working knowledge of hemocytes in healthy oysters is a preliminary step toward understanding the roles of these cells in internal defense. Morphological and cytochemical description of bivalve hemocytes can help to elucidate cellular defense mechanisms, such as phagocytosis and intracellular digestion.

Furthermore, the need for basic information on hemocytes of the common bivalve species is evident when one considers that certain physiological (Fisher and Newell 1986, Fisher et al. 1987) and pathological (Balouet et al. 1986, Poder and Auffret 1986, Cooper et al. 1982) hemocyte characteristics have been useful biological responses to variations in the environment.

As reviewed by Cheng (1981), abundant information is available about the blood cells of several marine bivalve molluscs but, unfortunately, most of the data are only partial descriptions and use heterogeneous nomenclature. Following the pioneer study by Takatsuki (1934), several types of hemocytes have been described by light microscopy in tissues from European flat oysters, *Ostrea edulis* (Franc 1975, Brereton and Alderman 1979), but always under various pathological conditions. From these studies, no clear, reusable nomenclature arose and there still lacks an ultrastructural description of the cell types. This paper makes the comparison with the Pacific oyster, *Crassostrea gigas*, which is now the most important commercial oyster species in France. Ruddell (1971a, b and c) and Feng et al. (1977) have described, respectively, the infiltrating and the circulating hemocytes of this oyster, but did not characterize the lysosomal system of these cells. In the present study, hemolymph cells of *Ostrea edulis* and *Crassostrea gigas* were identically processed and examined qualitatively by light and electron microscopy to provide a consistent comparison with respect to cell types, cytology, and ultrastructural morphology. The *in situ* activity of a typical hydrolase (acid phosphatase) was used to cytochemically reveal the lysosomal system of these cells.

MATERIAL AND METHODS

Two-year-old specimens of European flat oyster, *Ostrea edulis*, and Pacific oyster, *Crassostrea gigas*, were collected from reared populations, respectively, in Saint-Brieuc Bay (deep water areas) and in Aber Benoit river (Brittany, France), and held until processing in recirculating sea water in the laboratory. Hemolymph was collected from the heart in a 2 ml syringe fitted with a 0.8 × 38 mm hypodermic needle, after the shell had been notched near the posterior margin. The same fixative was used for all cytological preparations of hemolymph (1.25% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 supplemented with NaCl, osmolarity = 1010 $mOsm.kg^{-1}$). For light microscopy, drops of fresh hemolymph were placed on glass slides which were kept in a moist chamber for 10 to 20 min to allow the cells to settle. Some slides were observed without fixation under a phase contrast microscope. On other slides, the cell monolayer was stained using a modified Pappenheim's technique (Gabe, 1968) as follows. After 5 min fixation, slides were rinsed in tap water, air-dried and incubated for 3 min in May-Grünwald strain (R.A.L. reagents). Phosphate buffer (0.1 M, pH 7.2) was added (vol:vol) and gently mixed. After 3 min, this mixture

was replaced withoug rinsing by freshly diluted (1:20) Giemsa stain (R type, R.A.L. reagents) in phosphate buffer. After 10 min, the slides were gently rinsed in tap water, air dried and covered with immersion oil prior to examination.

For examination by transmission electron microscopy (TEM), the hemolymph sample was collected in cold fixative (4°C) and immediately centrifuged at 1500 rpm. The pellets were then immersed for 30 min in fresh fixative at 4°C, and post-fixed for 30 min in 1% osmium tetroxide in 0.15 M veronal-acetate buffer (pH 7.4).

For enzyme cytochemistry, the hemocytes were fixed in 1.25% glutaraldehyde in 0.1 cacodylate-HCl buffer (pH 7.4) supplemented with sucrose (osmolarity = 1000 $mOsm.kg^{-1}$) for 30 min at 4°C and then rinsed overnight in buffer. After centrifugation, the pellets were immersed for 60 min in preincubated medium (37°C, 60 min) containing 10 ml of 1.25% Na-β-glycerophosphate in water, 10 ml of Tris-maleate buffer 0.2 M (pH 5.0), 10 ml water, 20 ml of 0.2% lead nitate (aqueous) and 3.25 g of sucrose. An incubation medium without substrate (Na-β-glycerophosphate) was used for control preparations. Two rinses in water-diluted (vol:vol) Tris-maleate buffer and one rinse in 0.15 M veronal-acetate buffer (pH 7.4) preceded post-fixation as described above. Both localization and intensity of intracellular hydrolytic activity were assessed by the amount of lead phosphate deposits in ultrathin tissue sections observed by TEM.

All materials prepared for TEM were dehydrated in ethanol series, transferred in propylene oxide and embedded in epoxy resin. Except for cytochemistry, ultrathin sections were stained in uranyl acetate and lead citrate solutions, and observed in a JEOL JEM 100S electron microscope operated at 60 kV.

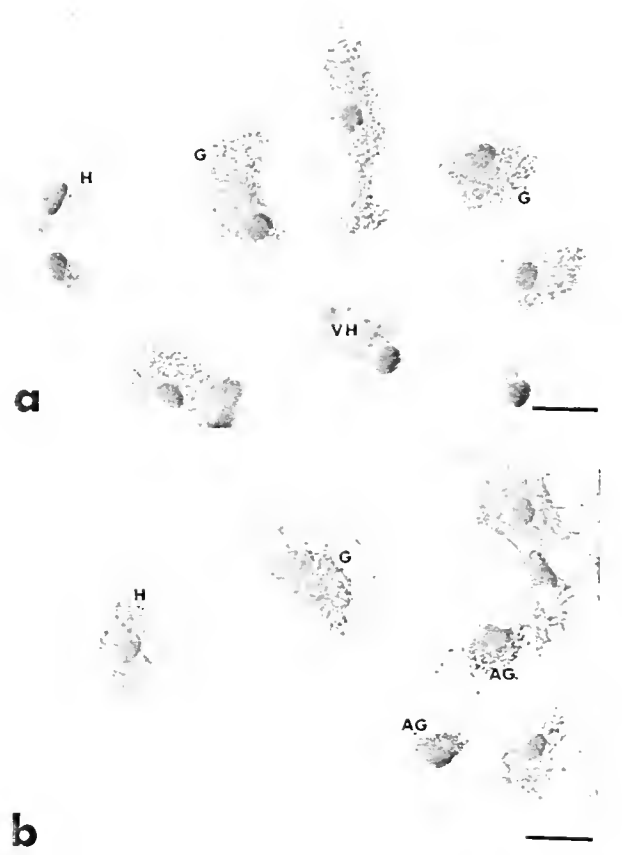


Figure 1. Hemolymph cell monolayers stained with May-Grünwald and Giemsa stains after 20 min settling. Scale bars = 10 μm. (a) *O. edulis*. Several granulocytes (G) and a vesicular hemocyte (VH) have migrated on the glass slide by amoeboid movement. A large hyalinocyte (H) shows spindle-shaped projections. (b) *C. gigas*. G: Granulocyte, AG: acidophilic granulocytes, H: hyalinocyte.

RESULTS

In stained monolayers from both species, the granulocytes were characterized by (1) eccentric nucleus with abundant, condensed chromatin, (2) well developed cytoplasm with a prominent endoplasm including the cytoplasmic organelles. In unfixed monolayers, the granulocytes were highly mobile. After spreading, the lucent ectoplasm formed on one side of the cell, a veil bounded with numerous thin filopodia (Fig. 1a, b). In stained cell monolayers from *O. edulis*, the cytoplasm of the granulocytes was filled with neutrophilic granules measuring about 1 μm (Fig. 1a). However, some granulocytes had smaller azurophilic granules measuring about 0.5 μm. The larger granulocytes from *C. gigas* had basophilic granules (Fig. 1b). In this species, large hemocytes with the same morphology as these cells had no granules but lucent vacuoles and were considered degranulated cells. Acidophilic granulocytes were observed exclusively in hemolymph from *C. gigas* and differed from the other granulocytes by their smaller size and, in stained monolayers, by numerous small acidophilic granules filling the cytoplasm and often covering the nucleus (Fig. 1b). Observed by TEM, the granulocytes from both species had regular profiles with a few thin cytoplasmic projections and a low nuclear-cytoplasmic ratio (Fig. 2a, b, c). *O. edulis* granulocytes and *C. gigas* basophilic granulocytes exhibited a rounded, eccentric nucleus with large clumps of chromatin. Their cytoplasm contained well developed vesicular smooth endoplasmic reticulum, and cytoplasmic granules, rounded or ovoid in shape, measuring 0.5 to 1.0 μm in *O. edulis* (Fig. 2a) and 0.4 to 1.2 μm in *C. gigas* (Fig. 2b). These granules had a conspicuous morphology, that is an electron-lucent core and a cortex made of electron-dense material condensed along the limiting membrane. Observed by TEM, acidophilic granulocytes had spherical granules measuring 0.6 μm and containing a homogenous electron-dense matrix. Their nucleus was smaller than in other granulocytes and often elongated in shape. Furthermore, it contained larger clumps of chromatin (Fig. 2c).

In both species, cells without cytoplasmic granules but with a nucleus similar to the nucleus of the granulocytes

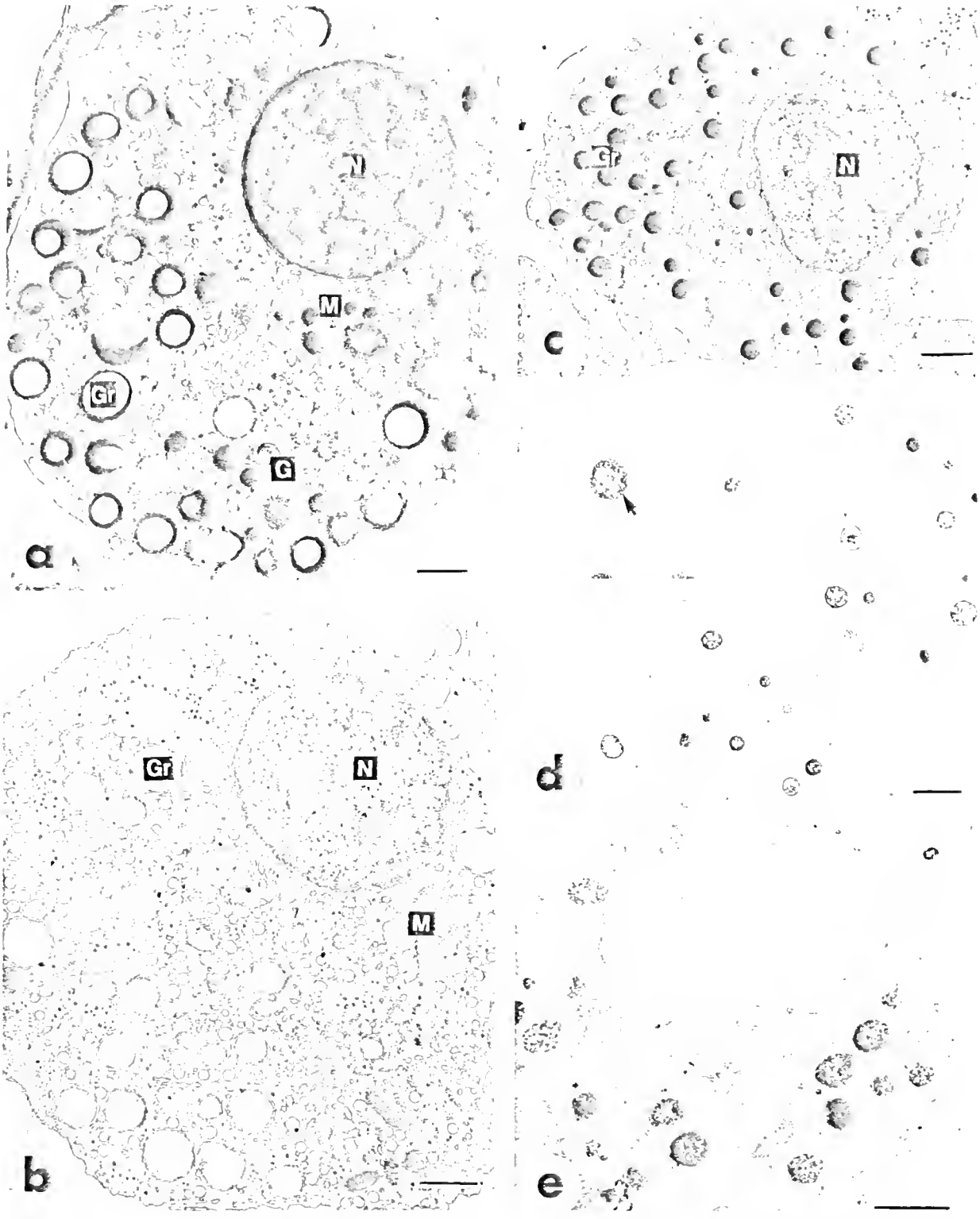


Figure 2. a-h-c: Electron micrographs of granulocytes. Scale bars = 1 μm. (a) *O. edulis*. Numerous specific granules (Gr) with an electron-lucent core in the cytoplasm. The nucleus (N) contains condensed chromatin. G: Golgi apparatus, M: mitochondrion. (b) Basophilic granulocyte from *C. gigas*. The specific granules (Gr) have a similar morphology as in *O. edulis* granulocytes. The nucleus (N) is round, eccentric with condensed chromatin. M: mitochondrion. (c) Acidophilic granulocyte from *C. gigas* exhibiting specific granules (Gr) with a homogenous electron-dense contents. The elongated, eccentric nucleus (N) has large aggregates of chromatin. d-e: Electron micrographs of granulocytes incubated for *in vitro* cytochemical demonstration of acid phosphatase activity revealed by electron-dense deposits in the granules. Scale bars = 1 μm. (d) *O. edulis*. Inset: Detail of a granule showing that the deposits are in the lumina. (e) *C. gigas*.

(neutrophilic and basophilic) were named vesicular hemocyte. In stained cell monolayers, they had a homogenous, pinkish, frequently vacuolated cytoplasm (Fig. 1a). Small, lymphocyte-like cells had only a rim of basophilic cytoplasm. Observed by TEM, the cytoplasm of the vesicular hemocytes was typically filled with vesicular, smooth endoplasmic reticulum (Fig. 3a, b). In *C. gigas,* several vesicular hemocytes had a few profiles of rough endoplasmic reticulum (Fig. 3b).

In unfixed monolayers observed by light microscopy, the hyalinocytes of *O. edulis* and *C. gigas* showed a weak mobility after spreading. In stained preparations, they were characterized by a large and frequently ovoid nucleus, with stippled chromatin and one or two large, conspicuous nucleoli. The lightly colored cytoplasm had frequently a heterogeneous aspect due to numerous vacuoles. Smaller cells exhibited a scanty, basophilic cytoplasm but a comparable nucleus as above. The largest hyalinocytes produced long filopodia including a rod-like axis and came to fibrocytic cells (Fig. 1a, b). In *O. edulis,* they contained a few azurophilic bodies measuring about 1 μm. Observed by TEM, the hyalinocytes had irregular profiles and a high nuclear-cytoplasmic ratio. The roundish, centrally situated nucleus had stippled chromatin. The most conspicuous organelles in their cytoplasm were numerous mitochondria, most of them being distributed in "juxtanuclear bodies" (Hawkins and Howse 1982) which are aggregates of mitochondria among a slightly granular material (Fig. 3c, d). In most hyalinocytes, long profiles of rough endoplasmic reticulum surrounded the nucleus. Few small, electron-dense vesicles occurred in *O. edulis* hyalinocytes, but they were morphologically different from the specific granules of the granulocytes (Fig. 3c).

In both species, strong acid phosphatase activity was demonstrated in the granules of the granulocytes, except in the acidophilic granulocytes of *C. gigas.* Lead phosphate deposits were localized in the core of the granules (Fig. 2d, e), which appeared electron-lucent in standard preparations. Small cytoplasmic vesicles and Golgi cisternae were also positive. A weak positive reaction was noted in the vesicular arrays of vesicular hemocytes. No or weak deposits were seen in control preparations.

DISCUSSION

In marine bivalve molluscs, the reference nomenclature based on hemocyte morphology includes granular hemocytes (granulocytes) and hyalinocytes (Cheng 1981). More recently, a complementary ontogenetic concept arose with the introduction of non-granular hemocytes (vs. granular hemocytes), since there are cells without cytoplasmic granules that are not hyalinocytes (Auffret 1988). In both species examined here, two types of non-granular hemocytes were identified: vesicular hemocytes and hyalinocytes. In view of several morphological similarities, especially the nucleus, vesicular hemocytes and granulocytes may be related. The cytoplasm of vesicular hemocytes frequently contained endocytotic vacuoles but no residual bodies or other signs of phagocytic activity. Thus, vesicular hemocytes are not belived to be aging cells of the granular population, such as the fibrocytes described in *C. virginica* by Cheng (1975), but rather granulocytes at an earlier stage of maturation. In previous descriptions of oyster hemocytes, small, roundish cells with scanty, basophilic cytoplasm have been called hyalinocytes (Foley and Cheng 1972). In the present study, such cells were identified as young cells related, as discussed below, to prohemocytes. Hyalinocytes are also described here, but these may be large cells with abundant cytoplasm. In fact, our nomenclature is consistent with cytological characteristics and not only cell size. Our ultrastructural and cytochemical observations did not reveal any conspicuous cellular differentiation in the hyalinocytes. According to Mix (1976), these cells should be intermediate between stem cells and functional cells including the granulocytes. This model implies that some hyalinocytes give rise to granulocytes. In oysters, this would mean a maturation of hyalinocytes toward vesicular hemocytes. This hypothesis is supported by the morphological relationship of several large hyalinocytes with vesicular hemocytes. However, this hypothesis does not exclude proper functions for the hyalinocytes and only complementary functional studies could elucidate their role.

Two types of cells with the characteristics of young, immature cells (scanty, basophilic cytoplasm and nucleus with abundant euchromatin) were found in the cell monolayers. Neither the origins of hemocytes nor the hemopoietic sites have been established in any bivalve mollusc, even if hemocytic hyperplasia and neoplastic proliferations of hemocyte-related cells were described in pathological conditions (see Sparks 1985 and Feng 1988 for reviews). This may support the idea that maturation of the hemocytic types takes place in hemolymph and/or in other tissues, before being released into the interstitial hemolymphatic spaces. Indeed, immature cells were described here, which are probably prohemocytes that could be classified on the basis of nucleus morphology as (1) hyalinocytes (large, oval nucleus with conspicuous nucleoli) and (2) granulocytes or related cells (small, round nucleus). The occurrence of two types of prohemocytes strongly enhanced the possibility (Cheng 1981) that there are two distinctive cell lines in oyster hemolymph arising from the first stages of cell differentiation. Moreover, our observation of hyalinocytes at various stages of cell maturation supports the idea that a complete cell line, from young to mature cells, may be present in the circulating compartment of these molluscs.

The cytoplasmic granules found in oyster granulocytes were of two types. The first type occurred in *O. edulis* granulocytes and *C. gigas* basophilic granulocytes. These granules ultrastructurally resembled the granules of *C.*

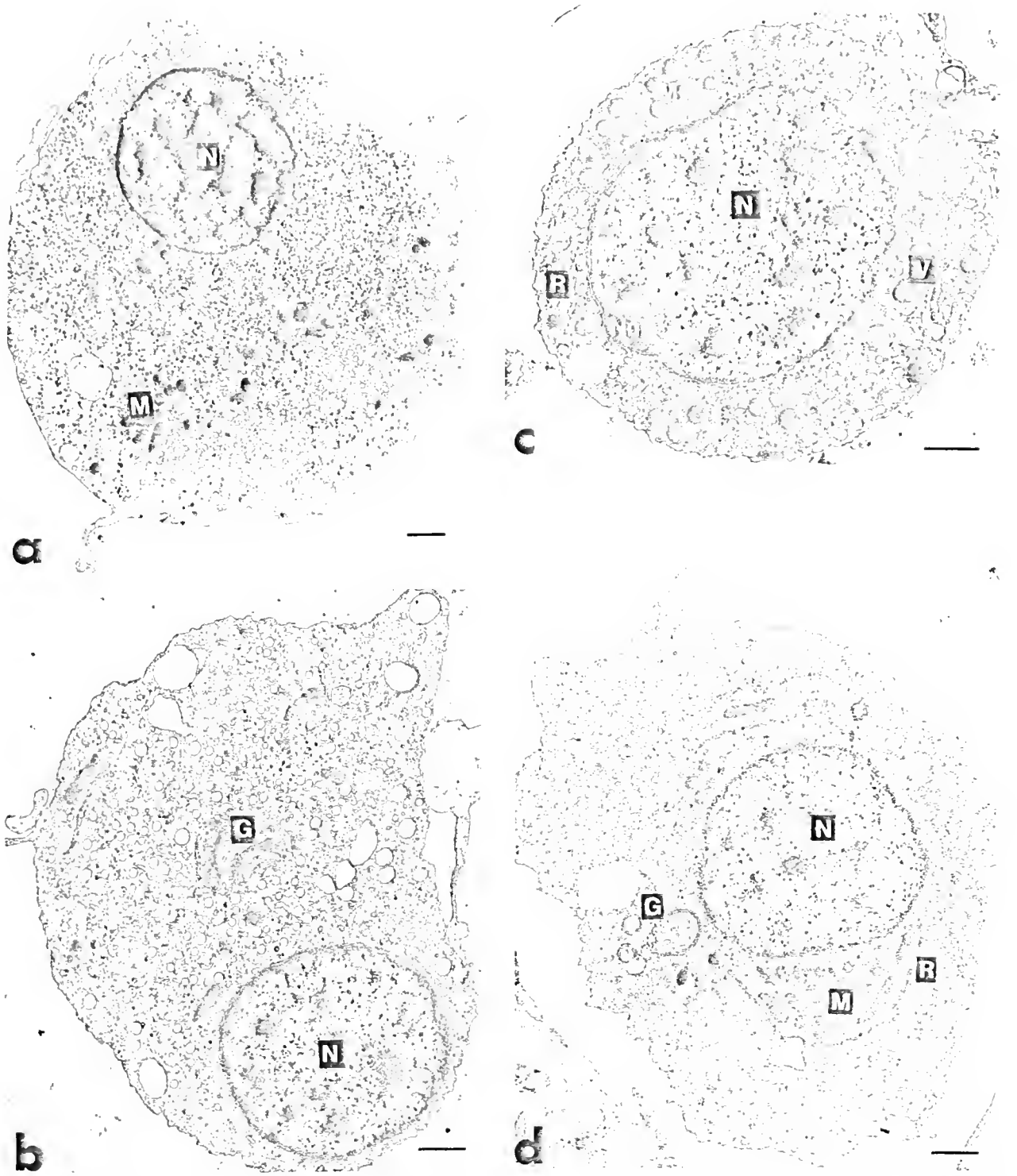


Figure 3. a-b: Electron micrograph of vesicular hemocytes. Scale bars = 1 μm. (a) *O. edulis*. The round, eccentric nucleus (N) has a similar morphology as in granulocytes (see figure 2a, b). Large cytoplasmic areas are filled with vesicles of smooth endoplasmic reticulum. M: group of mitochondria. (b) *C. gigas*. Same comments as in a). N: Nucleus, G: Golgi apparatus. c-d: Electron micrographs of hyalinocytes. Scale bars = 1 μm. (c) *O. edulis*. The nucleus (N) contains a large nucleolus (Nu) and stippled chromatin. M: aggregate of mitochondria, G: Golgi apparatus, R: profiles of rough endoplasmic reticulum, V: electron-dense vesicles. (d) *C. gigas*. N: Nucleus, G: Golgi apparatus, R: profiles of rough endoplasmic reticulum, M: aggregate of mitochondria.

virginica granulocytes (Feng et al. 1971, Cheng 1975, Hawkins and Howse 1982). Consequently, this type of granule and granulocyte are believed to be common structures in oyster genera. The morphological similarities strongly implicate a functional identity which is not entirely elucidated. Cytochemical investigations revealed a strong hydrolytic activity in the granules of this type of granulocyte. Comparable results have been obtained in *C. virginica* by Feng et al. (1971) and *Mercenaria mercenaria* by Yoshino and Cheng (1976). Feng et al. (1977) observed the fusion of hydrolase-containing granules and phagosomes in the mussel *Mytilus coruscus*. In *O. edulis*, phagocytized bacteria have been observed within cytoplasmic granules (Auffret 1986). Consequently, these organelles belong to the lysosomal system of the granulocytes and are involved in intracellular digestion of particulate material.

Another function of granulocyte granules is the sequestration of metals (George et al. 1978). These authors report the occurrence of two types of granules in *O. edulis* granulocytes with variable metal accumulation. After fixation of the tissues in presence of H_2S, one type of granule, with high copper concentration, contained crystalline inclusions. These were not found in the present study. However, the fact that the cells with such granules were predominantly found by George et al. (1978) in epithelial tissues suggests that such cells are not circulating hemocytes under usual conditions. The mechanisms of compartmentalisation in lysosomes or other membrane-limited vesicles has not been completely elucidated but their involvement in the metabolism and detoxication of trace-metals is a working hypothesis (Moore 1981).

In the present study, the cytological staining revealed in this type of granule either a basophilic or a neutrophilic affinity, respectively in *C. gigas* and *O. edulis*. In the latter however, variability in size and staining affinity of the cytoplasmic granules of the granulocytes probably reflects a polymorphism among a single population of granules. Preliminary technical assays had shown that extended aldehyde fixation reduced the granular basophilic staining in *C. gigas* granulocytes. Takatsuki (1934) observed in *O. edulis* that starvation did not affect the number of hemocyte granules and concluded that they did not contain metabolites. Hawkins and Howse (1982) observed glycogen particles in the granules of *C. virginica* granulocytes but, in *O. edulis* and *C. gigas* granulocytes, glycogen particles and lipid droplets were localized in the hyaloplasm. So, it appears that these specific granules are probably not involved in metabolic processes of the granulocytes. Their major characteristic is so far their richness in acid phosphatase. This suggests that they are storage organelles for lysosomal enzymes.

The other type of cytoplasmic granules occurred only in acidophilic granulocytes first described by Ruddell (1971c) in traumatized tissues from *C. gigas*. These granules were probably not lysosomes, since they exhibited no acid phosphatase activity. Rather, in the present study, they had ultrastructural characteristics of secretory granules. Ruddell (1971c) suggested that they could release humoral compounds during the inflammatory response. In this oyster at least, the occurrence of distinct types of granules suggests the possibility of distinct functions, as has been found for the different granulocytes in vertebrates.

According to Feng et al. (1971), the granules in *C. virginica* granulocytes could be formed from endoplasmic reticulum cisternae. The Golgi apparatus, well developed in all granulocyte types and giving rise to numerous secretory vesicles, could be involved in granule formation. This is known to occur in the granulocytes of vertebrates (Bernard et al. 1972), crustaceans (Bauchau 1981), and insects (Brehelin et al. 1978). A few electron-dense vesicles occurred in the hyalinocytes from *O. edulis*, and probably corresponded to the small azurophilic bodies observed in the cell monolayers. These results do not clearly elucidate their nature except that they are probably not lysosomes.

This study revealed that, in both oyster species, the fine morphology of the hyalinocytes and one type of granulocytes are highly similar. *Crassostrea gigas* has an additional granular type, the acidophilic granulocytes, which has not been observed in any other oyster. These results, in addition with previous descriptions in other species (Auffret 1988), support the concept that there are characteristic morphological similarities among the species in bivalve families.

ACKNOWLEDGMENTS

This work was part of a 3e cycle thesis from the University of Bretagne Occidentale, Brest, France. The author would like to thank W. S. Fisher for reviewing a part of the manuscript.

LITERATURE CITED

Auffret, M. 1986. Internal defence in bivalve molluscs: Ultrastructural observations on the fate of experimentally injected bacteria in *Ostrea edulis* granular hemocytes. In: Pathology in Marine Aquaculture. C. P. Vivarès, J.-R. Bonami, E. Jaspers eds., European Aquaculture Society, Special Publication, Bredene, Belgium. N° 9:351–356.

Auffret, M. 1988. Bivalve hemocyte morphology. In: Disease processes in marine bivalve molluscs. American Fisheries Society, Special Publication, 18:169–177.

Balouet, G., M. Poder, A. Cahour & M. Auffret. 1986. Proliferative hemocytic condition in European flat oyster (*Ostrea edulis*) from Breton coasts: A six-year survey. *J. Invertebr. Pathol.* 48:208–215.

Bauchau, A. G. 1981. Crustaceans. In: Invertebrate Blood Cells, N. A. Ratcliffe, A. F. Rowley eds., Academic Press, London/New York. Vol. 2:385–420.

Bernard, J., J. P. Levy & B. Varet. 1972. Etude morphologique de la lignée granulocytaire neutrophile. In: Hématologie, Flammarion Médecine Sciences, Paris, pp. 1243–1258.

Brehelin, M., D. Zachary & J. A. Hoffman. 1978. A comparative ultra-

structural study of blood cell from nine insect orders. *Cell Tiss. Res.* 195:45–57.

Brereton, J. D. & D. J. Alderman. 1979. Wound healing in the European oyster, *Ostrea edulis* L. *Aquaculture* 16:147–151.

Cheng, T. C. 1975. Functional morphology and biochemistry of molluscan phagocytes. *Ann. N.Y. Acad. Sci.* 266:343–379

Cheng, T. C. 1981. Bivalves. In: Invertebrate Blood Cells, N. A. Ratcliffe, A. F. Rowley eds., Academic Press, London/New York. Vol. 1:231–300.

Cooper, K. R., R. S. Brown & P. W. Chang. 1982. Accuracy of blood cytological screening techniques for the diagnosis of a possible hematopoietic neoplasm in the bivalve mollusc, *Mya arenaria*. *J. Invertebr. Pathol.* 39:281–289.

Farley, C. A. & M. Durfort. 1986. Parasites and diseases of marine bivalves: Summary. In: Marine Bivalve Pathology, W. S. Fisher, A. J. Figueras eds., Maryland Sea Grant Publication, University of Maryland, pp. 9–13.

Feng, S. Y. 1967. Responses of molluscs to foreign bodies with special references to the oyster. *Fed. Proc.* 26:1685–1692.

Feng, S. Y. 1988. Cellular defense mechanisms in oysters and mussels. American Fisheries Society Special Publication, 18:153–168.

Feng, S. Y., J. S. Feng, C. N. Burke & L. H. Khairallah. 1971. Light and electron microscopy of the leucocytes of *Crassostrea virginica* (Mollusca, Pelecypoda). *Z. Zellforsch. Mikrosk. Anat.* 120:222–245.

Feng, S. Y., J. S. Feng & T. Yamasu. 1977. Roles of *Mytilus coruscus* and *Crassostrea gigas* blood cells in defence and nutrition. In: Comparative Pathobiology: Invertebrate Immune Response, L. A. Bulla, T. C. Cheng eds., Plenum, London. Vol. 3:31–67.

Fisher, W. S. 1986. Structure and functions of oyster hemocytes. In: Immunity in Invertebrates, M. Brehelin ed., Springer Verlag, Heidelberg, pp. 25–35.

Fisher, W. S. & R. I. E. Newell. 1986. Salinity effects on the activity of granular hemocytes of American oysters, *Crassostrea virginica*. *Biol. Bull.* 170:122–134.

Fisher, W. S., M. Auffret & G. Balouet. 1987. Response of European oyster (*Ostrea edulis*) hemocytes to acute salinity and temperature changes. *Aquaculture* 67:179–190.

Franc, A. 1975. Hyperplasie hemocytaire et lésions chez l'huitre plate (*Ostrea edulis* L.). *C.R. Acad. Sci. Paris, Série D* 280:495–497.

Foley, D. A. & T. C. Cheng. 1972. Interaction of molluscs and foreign substances: the morphology and behaviour of hemolymph cells of the American oyster, *Crassostrea virginica, in vitro*. *J. Invertebr. Pathol.* 19:383–394.

Gabe, M. 1968. Techniques histologiques. Masson, Paris, 1113 pp.

George, S. G., B. J. S. Pirie, A. R. Cheyne, T. L. Combs & P. T. Grant. 1978. Detoxication of metals by marine bivalves: An ultrastructural study of the compartimentation of copper and zinc in the oyster *Ostrea edulis*. *Mar. Biol.* 45:147–156.

Hawkins, W. E. & H. D. Howse. 1982. Ultrastructure of cardiac hemocytes and related cells in the oyster *Crassostrea virginica*. *Trans. Am. Microsc. Soc.* 101:241–252.

Moore, M. N. 1981. Elemental accumulation in organisms and food chains. In: Analysis of Marine Ecosystems, A. R. Longhouse ed., Academic Press, London, pp. 535–569.

Poder, M. & M. Auffret. 1986. Sarcomatous lesion in the cockle *Cerastoderma edule*: I. Morphology and population survey in Brittany (France). *Aquaculture* 58:1–8.

Poder, M., M. Auffret & G. Balouet. 1983. Etude pathologique et épidémiologique des lésions parasitaires chez *Ostrea edulis* L. Premiers résultats d'une recherche prospective comparative chez les principales espèces de mollusques des zones ostréicoles de Bretagne-Nord. IFREMER, Actes de Colloques 1:125–138.

Rasmussen, L. P. D., E. Hage & O. Karlog. 1985. An electron microscope study of the circulating leucocytes of the marine mussel, *Mytilus edulis*. *J. Invertebr. Pathol.* 45:158–167.

Ruddell, C. L. 1971a. The fine structure of oyster agranular amoebocytes from regenerating mantle wounds in the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* 18:260–268.

Ruddell, C. L. 1971b. The fine structure of the granular amoebocytes of the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* 18:269–275.

Ruddell, C. L. 1971c. Elucidation of the nature and function of the granular oyster amoebocytes through histochemical studies of normal and traumatized oyster tissues. *Histochemie* 26:98–112.

Sparks, A. K. 1985. Synopsis of invertebrate pathology. Elsevier, Amsterdam, 423 pp.

Takatsuki, S. 1934. On the nature and functions of the amoebocytes of *Ostrea edulis*. *Quart. J. Micr. Sci.* 76:379–431.

Tripp, M. R. 1974. Molluscan immunity. *Ann. N.Y. Acad. Sci.* 234:23–27.

Yoshino, T. C. & T. C. Cheng. 1976. Fine structural localization of acid phosphatase in granulocytes of the pelecypod *Mercenaria mercenaria*. *Trans. Am. Microsc. Soc.* 95:215–220.

Journal of Shellfish Research, Vol. 8, No. 2, 375–385, 1989.

CHANGES IN THE ABUNDANCE AND DISTRIBUTION OF THE PRINCIPAL AMERICAN OYSTER PUBLIC FISHING GROUNDS IN THE SOUTHERN GULF OF ST. LAWRENCE, CANADA

T. W. SEPHTON AND C. F. BRYAN
Canada Department of Fisheries and Oceans
Science Branch, Gulf Region
P.O. Box 5030
Moncton, New Brunswick
Canada, E1C 9B6

ABSTRACT The changes in the distribution, abundance and population structure of the American oyster (*Crassostrea virginica* Gmelin) in the Public Fishing Grounds of Caraquet Bay, NB, and the Dunk River, Bedeque Bay, PEI, Canada, were observed by comparing the quantitative study for 1987 with previous studies of the areas. There were changes over time in the size, shape and location of high (>100 individuals m^{-2}), medium (10–100 m^{-2}) and low (<10 m^{-2}) density beds of both populations. In 1987, the average density of total (length > 5 mm) and market size (length > 75 mm) oysters for the entire bed was 31.8 and 3.3 m^{-2}, respectively, for Caraquet Ground. This was similar to 1979 data (31.5 and 4.7 m^{-2}) and much lower than in 1974 (63.5 and 11.3 m^{-2}). In 1987, the average density of total and market size oysters in Dunk Ground was 21.6 and 5.3 m^{-2}, respectively. This was significantly ($p < 0.05$) lower than that observed in 1977 (89.3 and 8.8 m^{-2}), 1972 (69.2 and 11.7 m^{-2}) and 1969 (47.4 and 2.8 m^{-2}). Recruitment to each population was intermittent and the high levels of recruitment observed in the past have not occurred recently (1985–1987). The similarity of recent recruitment patterns in both Grounds suggested that they may have similar functional processes of larval retention even though their physical embayments are different. Both Grounds now have an abundance lower than that previously recorded but still high enough to support commercial fisheries.

KEY WORDS: American oyster, *Crassostrea virginica*, population structure, recruitment, distribution, abundance, annual variability

INTRODUCTION

The northernmost commercially exploited populations of American oyster, *Crassostrea virginica* (Gmelin), are located in Caraquet Bay, New Brunswick (latitude 47°50′N) and Bedeque Bay, Prince Edward Island (latitude 46°22′N) in the southern Gulf of St. Lawrence (Medcof 1961, Lavoie 1977). Following their decimation by Malpeque disease this century (Prince Edward Island from 1915–1939, New Brunswick from 1950–1960) (Medcof 1961), substantially smaller populations reestablished themselves and remain the most productive in their respective provinces (Rowell 1975, Lavoie 1977). Of the total landings recorded for the entire southern Gulf of St. Lawrence in 1985 (2060 t, NAFO 1987), approximately half were directly from these Public Fishing Grounds (T. Sephton, unpublished data).

These Grounds are the most important sources of legal size (minimum length from hinge to bill end >75 mm) oysters for the leasehold relay and market fisheries for their respective provinces (Lavoie and Bryan 1980, 1981a, Hawkins and Rowell 1985a). While both Grounds have autumn fisheries (September 15 to November 30), only Bedeque Bay has a spring relay fishery (May 1 to July 15). Legal size oysters, which are only marginally contaminated by bacteria, are harvested and transferred to leases for cleansing prior to the autumn fisheries. The spring fishery may be of benefit to cohort recruitment as fishing exposes cultch for spat to settle on (Lavoie and Bryan 1981b).

The Dunk River Public Fishing Ground of Bedeque Bay, the object of extensive stock assessment surveys from 1969 to 1977, has not been studied quantitatively since 1977. The preliminary results were used to design enhancement projects to increase the total area of the oyster-producing grounds (Hawkins and Rowell 1985a). One aspect of these projects was to transfer oysters from overcrowded beds of the Ground to suitable unpopulated areas nearby (Lavoie and Bryan 1981b). Lavoie and Bryan (1981b) reported that the new areas were suitable "grow-out" sites, but were not self sustaining due to lack of natural recruitment. These results are similar to earlier studies conducted in Chesapeake Bay by Sieling (1950) and more recently by Abbe (1988).

The Public Fishing Ground of Caraquet Bay was sampled quantitatively in 1972 and 1974 (Lavoie 1977) and again in 1979 (Lavoie and Robert 1981) to provide the preliminary information required for development and management purposes. No further studies have been conducted since that time even though the private and public sectors of the Caraquet Bay oyster fishery are highly dependent upon this important Public Fishing Ground (Lavoie 1978).

In light of the initiation of development projects within the last 5 years designed to revitalize the industry, the lack of more recent oyster surveys in both Grounds is of concern. The basic objective of the present study was to document any major changes in the spatial distribution, abundance and structure of the oyster population of the Public Fishing Ground of Caraquet Bay and the Dunk River Public

Fishing Ground of Bedeque Bay. This paper summarizes and compares research conducted in 1987 with the work conducted previously in each of the respective Grounds, and spans a period of 15 to 18 years.

MATERIAL AND METHODS

The location of the Public Fishing Ground of Caraquet Bay (hereafter referred to as Caraquet Ground) and the Dunk River Public Fishing Ground of Bedeque Bay (Dunk Ground) are shown in Figure 1. They were sampled in June and July of 1987, respectively, using the transect method described by Lavoie (1977) and Hawkins and Rowell (1985a), briefly outlined here.

The 1987 survey of the Caraquet Ground sampled the same area as in previous studies (Lavoie 1977, Lavoie and Robert 1981). The 1987 survey of the Dunk Ground sampled the same area as the 1969 (Hawkins and Rowell 1985a) and 1972 surveys (Hawkins and Rowell 1985b) and covered an area between Oyster Point and Hurd point in the west, to Murray Island in the east (see Fig. 3 for reference). The 1977 survey covered a slightly smaller area than that of 1972 and 1987, extending from midway between Oyster and Hurd Points to Lower Bedeque in the west, to just south of Murray Island in the east (Hawkins and Rowell 1985c). Table 1 summarizes the number of transects and samples obtained in each survey for the two study sites.

Shore to shore transects were drawn on a hydrographic chart at approximately 200 m intervals and located in the field using a surveying quadrat. Samples were collected at 150 m intervals along the transect and averaged 15 samples per transect. Sample position was recorded using a surveying quadrat. A single 1 m^2 sample was collected at each station by divers hand picking all material within the sample area. Diver observations of the benthic habitat along the transect line were recorded to assist the mapping of the oyster distribution boundaries. Individual samples were sorted from debris in the laboratory. All live oysters (>5 mm) were enumerated and maximum length from hinge to bill end was measured to the nearest 1 mm. These data were used to map the distribution of the oyster population and delineate the low (<10 oysters m^{-2}), medium (10–100 m^{-2}) and high (>100 m^{-2}) density beds to facilitate comparisons with previous studies (Lavoie 1977). The hand drawn maps were digitized and a computer aided drafting package was used to produce the final maps and calculate the areas of the different beds.

The published reports for the Caraquet Ground for 1974 (Lavoie 1977) and 1979 (Lavoie and Robert 1981) contained density distribution maps and summary information for each year. The maps were digitized and redrawn to scale for comparison with 1987. Unfortunately, the quantitative sample data to allow statistical comparisons among years were not available.

The published reports for the Dunk Ground containing quantitative data for 1969 (Hawkins and Rowell 1985a), 1972 (Hawkins and Rowell 1985b) and 1977 (Hawkins and Rowell 1985c) was re-analyzed using the original field and diver observations (Rowell, Lavoie and Bryan, unpublished data). The distribution maps, which had not been created previously, were drawn for 1969, 1972 and 1977 and the average oyster density calculated for each of the beds.

The quantitative data for the Dunk Ground allowed statistical comparisons between past and present studies. The density estimates for each of the beds and the total bed (excluding the channel) for 1969, 1972, 1977 and 1987 were used in the calculation of analysis of variance and orthogonal contrasts (SAS GLM) (SAS Institute 1985) to identify significant ($p < 0.05$) changes in the average numerical abundance of oysters between years. The square root ($x + 0.05$) transformation was used to stabilize the variances.

Von Bertalanffy growth curves were used to show the year classes in the size frequency histograms. The growth equation used for the Caraquet Ground was:

$$L(cm) = 21.7\ (1\text{-}e^{-0.071[t-0.24]})$$

(Lavoie and Robert 1981)

and for the Dunk Ground was:

$$L(cm) = 15.3\ (1\text{-}e^{-0.186[t-0.16]})$$

(Lavoie and Bryan 1981b).

RESULTS AND DISCUSSION

Spatial Distribution

A) Caraquet Ground

Changes in the overall size of the Caraquet Ground from 1974 to 1987 are shown in Table 2. The total area (excluding the channel beds) increased from 240 ha in 1974 to 290 ha in 1979 and decreased slightly to 270 ha in 1987. The decrease from 1979 to 1987 may be due to an overall decrease in the extent of the low density peripheral bed. These decreases occurred in the vicinity of the river channels and along the north and south shores where previously suitable oyster habitats have apparently deteriorated. Diver observations in 1987 indicated that the substrate is soft and muddy.

The distribution of the low, medium and high density beds and channel beds for 1974, 1979 and 1987 are shown in Figure 2. The high density beds of 1974 were not seen in 1979 but were reestablished by 1987. The size frequency histogram for the high density beds of 1987 (Sephton and Bryan 1988) also reflected this density change and showed few animals of the 1979+ year classes which was the time when the high density beds were absent. These beds decreased in total area by 55% and became less contiguous from 1974 to 1987. The size and shape of the medium density bed remained relatively constant from 1979 to 1987, while both channel beds increased in size from 1979 to 1987. Oysters were found in clumps in the channel beds.

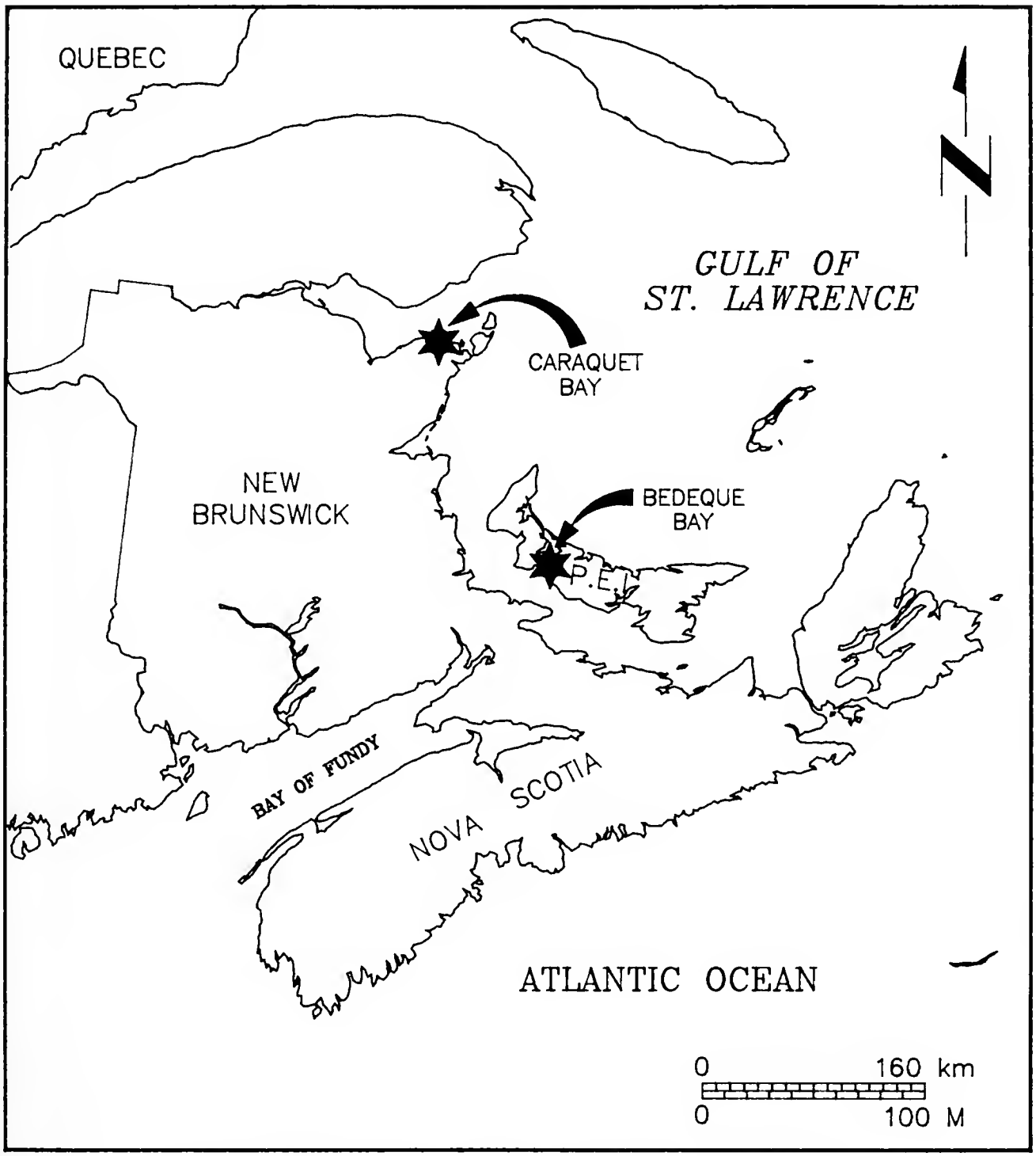


Figure 1. Location of the two study sites in the southern Gulf of St. Lawrence, Canada.

The increase in area may be due to the reduced fishing pressure on these beds because of the poor shell quality of the oysters (Lavoie 1977).

B) Dunk Ground

The distribution of the low, medium and high density beds and channel beds of the Dunk Ground for 1969, 1972, 1977 and 1987 are shown in Figure 3. The total area of the Ground was the same in 1969 and 1987 but lower in 1972 and 1977 (Table 2). The centrally located high density bed increased in size from 1969 to 1972, became moderately diffuse by 1977 and decreased to a medium density bed by 1987. Concurrent with this was the development of, and changes in the size of, high density beds near Oyster Point

TABLE 1.

Comparison of the total number of transects and samples obtained for each survey of the Public Fishing Ground of Caraquet Bay in 1974, 1979 and 1987, and for the Dunk River Public Fishing Ground of Bedeque Bay in 1969, 1972, 1977 and 1987.

	Caraquet Ground			Dunk Ground			
	1974 (n)	1979 (n)	1987 (n)	1969 (n)	1972 (n)	1977 (n)	1987 (n)
Transects	10	15	17	14	13	16	18
Samples	122	234	213	110	148	186	200

from 1969 to 1987 and along the north side of the channel. The high density bed near Murray Island was observed in 1969 and 1977 but not in 1972. The size of the channel bed increased from 1969 to 1972 and diminished gradually to 1987. The beds observed previously in the vicinity of Murray Island had almost completely disappeared by 1987 (Fig. 3). In light of this, the increase in the total area of the Ground back to the 1969 level was due to the expansion of the western side of the low density beds towards Hurd Point.

The apparent change in location and expansion of the Dunk Ground from east to west was not a sampling artifact since the same general area was surveyed in all studies. Although sampling intensity varied among years, diver observation notes from previous surveys corroborate the findings (Bryan, unpublished data). The data suggested that it resulted from the combined effect of the deterioration of once suitable oyster beds and fortuitous spatfall. Diver observations in 1987 indicated that the substrate of the former high and medium density beds near Murray Island was heterogeneous (areas of soft and firm mud and hard sand) and barren of oyster cultch (clean empty shell). This differed from the 1969 survey observations when moderate quantities of cultch were found (Hawkins and Rowell 1985a). In 1977, fortuitous spatfall and survival probably resulted in the appearance of the high density bed near Murray Island, as indicated by the data of Hawkins and Rowell (1985c), which showed a high proportion of juvenile oysters (length < 35mm) in this area.

Observations from both Grounds showed that river channels and some high density beds supported not only high concentrations of adult oysters but also juveniles (Sephton and Bryan 1988, 1989). The clumps of adult oysters and empty shells found in these beds provided a readily available cultch for spat to settle on (Crisp 1967, Haven et al. 1987). The large percentage of juvenile oysters found there over time compared with other beds suggested that they may be stable zones of natural recruitment: an area of larval retention and spatfall (Haven et al. 1987, Abbe 1988). The evidence presented above also suggested that some areas of recruitment may be transient and shift with time. For example, the westward expansion of the low density beds of the Dunk Ground may have resulted from fortuitous spatfall and survival, and it remains to be seen if they remain intact and are self sustaining, or gradually disappear. A gradual disappearance would support this contention of transient fortuitous spatfall, with the end result similar to that observed for other beds in the vicinity of Murray Island, other restocking projects in close proximity of the Dunk Ground (Lavoie and Bryan 1981b) and elsewhere (Sieling 1950, Abbe 1988).

There was a deterioration of productive oyster beds in

TABLE 2.

Comparison of the physical area (ha) of the density beds of the Public Fishing Ground of Caraquet Bay for 1974, 1979 and 1987, and of the Dunk River Public Fishing Ground of Bedeque Bay for 1969, 1972, 1977 and 1987.

	Caraquet Ground			Dunk Ground			
Bed	1974[1] (ha)	1979[2] (ha)	1987 (ha)	1969 (ha)	1972 (ha)	1977 (ha)	1987 (ha)
Low	61	86	60	47	27	34	78
Medium	139	204	188	77	52	42	58
High	40	N/A	22	18	25	28	7
Channel	N/A	3	11	8	11	11	9
Total (excluding channel)	240	290	270	142	104	104	143

[1] Lavoie 1977.

[2] Lavoie and Robert 1981.

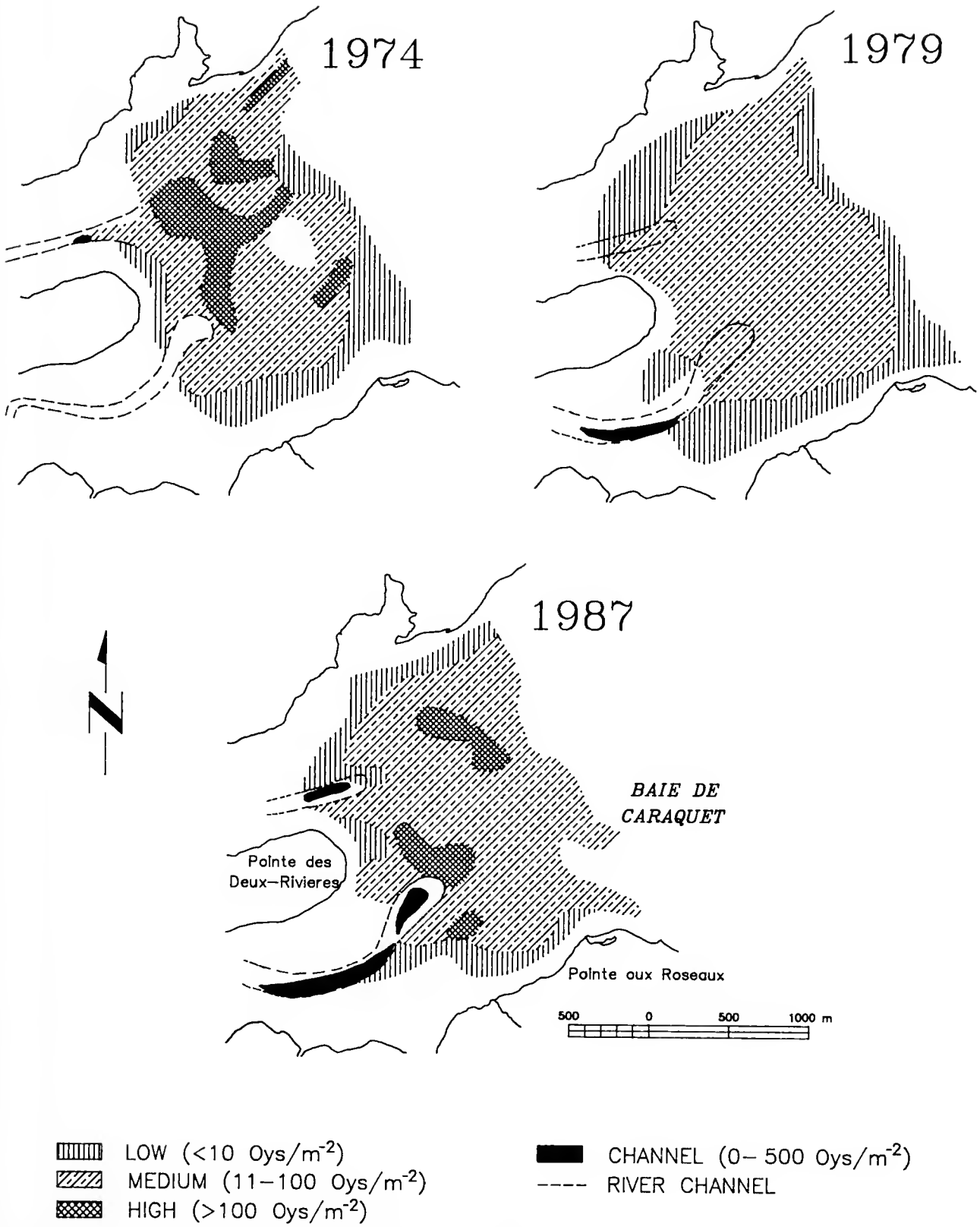


Figure 2. Distribution of the American oyster population of the Public Fishing Ground of Caraquet Bay, NB, showing the density beds in 1974, 1979 and 1987.

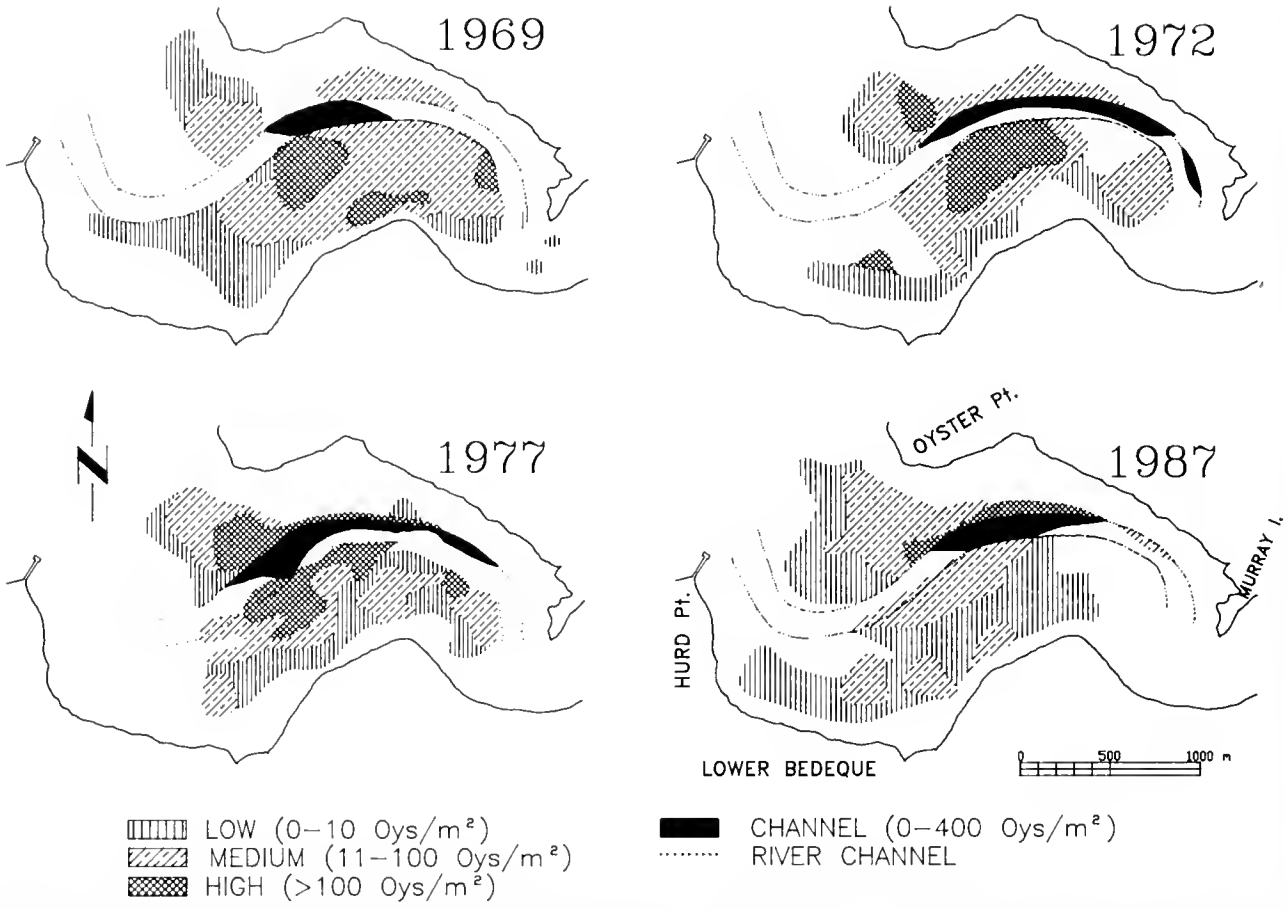


Figure 3. Distribution of the American oyster population of the Dunk River Public Fishing Ground of Bedeque Bay, PEI, showing the density beds in 1969, 1972, 1977 and 1987.

and adjacent to shallow (<1 m) headwater inflow areas in both Grounds. This deterioration probably resulted from the combined effects of silt deposition from river inflows (Mackenzie 1970) and winter ice scour which eventually eliminates the cultch and modifies substrate conditions (Medcof 1961, Galtsoff 1964). Complaints from Caraquet Bay leaseholders that peat moss effluent from a local commercial operation may have adversely affected the growth and survival of their oysters resulted in the construction of a containment system to solve the effluent problem in 1987. The effects of the peat moss appeared localized because the 1987 survey did not reveal any deterioration of the Caraquet Ground closest to the impacted area. Siltation is a constant management problem for most watersheds in PEI because of extensive agricultural land use. The present study showed that the Dunk Ground has been affected by silt, as indicated by the disappearance of productive oyster beds, and further preventive measures are required. Similar changes in the overall distribution of oyster beds and loss of oyster grounds were observed on much larger scales in areas of Chesapeake Bay by Haven and Whitcomb (1983) and Whitcomb and Haven (1987).

Average Density

A) Caraquet Ground

Table 3 summarizes the densities of total and legal size (market) oysters for each of the different beds and the total bed for 1974, 1979 and 1987. The total density decreased 50% from 1974 (63.5 m^{-2}) to 1979 (31.5 m^{-2}) and remained constant in 1987 (31.8 m^{-2}) (Table 3). The density of the low density bed almost doubled from 1974 to 1987 (Table 3) while that of the high density bed more than halved. The density of the medium density bed has fluctuated and is now at levels lower than recorded previously. The channel bed density increased almost 4 fold from 1979 to 1987.

The density of market oysters decreased continually from 1974 to 1987 (Table 3). Although the surveys were conducted prior to the autumn fishing season and would presumably provide an estimate of the fishable biomass, there is no way to gauge the accuracy of the predictions because landing statistics are not available for the Ground.

TABLE 3.

Comparison of the total and legal size (length > 75mm) average density (no. m^{-2}) for each of the density beds and the total bed of the Public Fishing Ground of Caraquet Bay for 1974, 1979 and 1987. An estimate of the available legal size biomass as total metric tons and boxes is also presented.

Bed	1974[1] Total no. m^{-2}	1974[1] Legal no. m^{-2}	1979[2] Total no. m^{-2}	1979[2] Legal no. m^{-2}	1987 Total no. m^{-2} ± SE	1987 Legal no. m^{-2} ± SE
High	250.5	46.1	N/A	N/A	101.6 ± 15.7	6.8 ± 1.5
Medium	36.2	5.9	43.7	6.7	30.9 ± 2.1	3.7 ± 0.4
Low	3.6	0.8	2.7	0.4	6.8 ± 2.8	0.7 ± 0.2
Channel	N/A	N/A	142.5	28.3	420.3 ± 70.4	119.6 ± 37.1
Total excluding channel	63.5	11.3	31.5	4.7	31.8 ± 2.8	3.3 ± 0.3
Estimated Boxes Legal Oysters*	56628 boxes		31410 boxes		19800 boxes	
Estimated Tons Legal Oysters*	2574 t		1282 t		808 t	

[1] Lavoie 1977.

[2] Lavoie and Robert 1981.

* One metric ton contains 24.5 standard oyster boxes which contain 450–500 legal size (length > 75mm) oysters per box (Lavoie 1977).

B) Dunk Ground

Table 4 shows the densities of total and legal size oysters for each of the different beds and the total bed for 1969, 1972, 1977 and 1987. The results of analysis of variance and orthogonal contrasts are summarized in Table 5.

In general, the total densities for most beds and the entire bed increased from 1969 to 1977 and decreased in 1987 to levels at or below those originally observed (Table 4). The density for the entire bed was not significantly different from 1969 (47.4 m^{-2}) to 1977 (89.3 m^{-2}) but decreased significantly from 1977 to 1987 (21.6 m^{-2}). Statistical differences were detected for most beds (Table 5); the medium bed decreased significantly from 1969 to 1972 and remained at those levels through to 1987 while the high density beds remained at higher levels until decreasing significantly in 1987. Variability in the data was great, which is typical of the aggregated distributions exhibited by oysters (Galtsoff 1964) and benthos in general (Green 1979).

The average density of market oysters changed between

TABLE 4.

Comparison of the total and legal size (length > 75mm) average density (no. m^{-2}) for each of the density beds and the total bed of the Dunk River Public Fishing Ground of Bedeque Bay for 1969, 1972, 1977 and 1987. An estimate of the legal size biomass as total metric tons and boxes is also presented.

Bed	1969 Total no. m^{-2} ± SE	1969 Legal no. m^{-2} ± SE	1972 Total no. m^{-2} ± SE	1972 Legal no. m^{-2} ± SE	1977 Total no. m^{-2} ± SE	1977 Legal no. m^{-2} ± SE	1987 Total no. m^{-2} ± SE	1987 Legal no. m^{-2} ± SE
High	159.8 ± 10.1	10.0 ± 0.9	205.2 ± 24.6	33.7 ± 4.9	233.9 ± 27.8	21.1 ± 4.1	118.8 ± 23.8	29.6 ± 12.2
Medium	47.0 ± 3.8	2.4 ± 0.3	30.9 ± 3.4	5.6 ± 0.8	42.8 ± 4.6	5.1 ± 0.7	34.2 ± 3.6	8.1 ± 1.1
Low	3.1 ± 0.4	0.5 ± 0.1	3.2 ± 0.8	0.7 ± 0.2	3.5 ± 0.9	1.1 ± 0.2	2.3 ± 0.4	0.9 ± 0.1
Channel	61.3 ± 19.0	2.0 ± 1.1	187.2 ± 69.9	23.6 ± 7.5	155.9 ± 80.9	14.9 ± 7.7	60.2 ± 36.9	27.5 ± 16.9
Total excluding channel	47.4 ± 5.2	2.8 ± 0.3	69.2 ± 12.2	11.7 ± 2.1	89.3 ± 12.6	8.8 ± 1.5	21.6 ± 3.3	5.3 ± 0.9
Estimated Boxes Legal Oysters*	8371 boxes		25613 boxes		18720 boxes		15947 boxes	
Estimated Tons Legal Oysters*	342 t		1045 t		764 t		651 t	

* One metric ton contains 24.5 standard oyster boxes which contain 450–500 legal size (length > 75mm) oysters per box (Lavoie 1977).

TABLE 5.

Summary of the results of analysis of variance and orthogonal contrasts to determine significant differences ($p < 0.05$) in the density of the total and legal size oysters between years over the density beds and the entire bed of the Dunk River Public Fishing Ground of Bedeque Bay.

Comparison	df	F	p	Orthogonal Contrasts
Total Oysters				
High Density Bed	3,62	3.10	*	1969 = 1972 = 1977 ≠ 1987
Medium Density Bed	3,177	2.81	*	1969 ≠ 1972 = 1977 = 1987
Low Density Bed	3,128	1.50	NS	[1969 = 1972 = 1977 = 1987]
Entire Bed (excluding channel)	5,373	3.06	*	1969 = 1972 = 1977 ≠ 1987
Legal Size Oysters				
High Density Bed	3,62	4.91	**	1969 ≠ 1972 ≠ 1977 = 1987
Medium Density Bed	3,177	12.65	***	1969 ≠ 1972 = 1977 ≠ 1987
Low Density Bed	3,128	1.33	NS	[1969 = 1972 = 1977 = 1987]
Entire Bed (excluding channel)	5,373	13.87	***	1969 ≠ 1972 ≠ 1977 = 1987

NS, not significant and years enclosed in [ ] brackets.
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

years and, again, most changes were statistically different (Table 4,5). The density over the entire bed increased significantly from 1969 (2.8 m^{-2}) to 1972 (11.7 m^{-2}) while decreasing and remaining at lower levels from 1977 (8.8 m^{-2}) to 1987 (5.3 m^{-2}). The density of market oysters of the medium beds increased significantly from 1969 to 1972 and remained at this level through 1977 until increasing significantly once more in 1987. Since all surveys were conducted after the spring relay fishery, the residual fishable biomass of legal oysters increased from 1969 to 1972 and then decreased between survey years to 651 t in 1987 which remains higher than those observed in 1969.

The densities presently observed in both Grounds should continue to support commercial fisheries at levels lower than previously observed. The overall density of oysters of both Grounds has decreased over time but remains higher than those reported recently for oyster reefs of Chesapeake Bay: Pocomoke Sound (3.9–6.0 m^{-2}) (Whitcomb and Haven 1987) and Flag Pond Bar (~23 m^{-2}) (Abbe 1988).

Population Structure

A) Caraquet Ground

The histograms for 1974, 1979 and 1987 were all bimodal but to differing degrees (Fig. 4). The high levels of juvenile recruitment belonging to the 1972 and 1973 year classes observed in 1974 have not been witnessed recently. The low percentage of juveniles in the 1986 year class is supported by comments from local oystermen that 1986 and 1987 were extremely poor years for spat collection. The Caraquet Ground has been sustained by intermittent recruitment, as shown by the year class partitioning, and the present fishery is based on the 1981 and 1982 cohorts.

B) Dunk Ground

The 1969, 1972 and 1977 histograms are unimodal, but skewed towards the larger size classes (Fig. 5). The 1987 histogram is bimodal with a peak occurring at size intervals 10 to 20 mm and a second, broader peak between 45 to 80 mm size intervals. The large percentage of oysters in the 1979 year class also include those larger animals (frequently found in the channel bed) which are not marketable due to their poor shell quality. Once again, high levels of juvenile recruitment are intermittent in nature and were observed for year classes 1967, 1971, 1976 and perhaps 1983 and 1984. The low levels of recruitment observed recently (1985 to 1987) are confirmed by reports from local oystermen that spatfall was a dismal failure in 1986 and 1987. The present fishery of the Dunk Ground is being sustained by the 1983 and 1984 year classes.

The population structure and intermittent recruitment observed in both Grounds are typical of most bivalve mollusc populations (Loosanoff 1966, Powell and Cummins 1985). The recruitment process is rarely controlled by a single factor but is the end result of an interaction of environmental and biological variables (Ricker 1975, Cushing 1981). Biological factors include the reproductive potential of adults and larval growth and development, and environmental variables such as temperature, salinity, water currents, tidal mixing and meteorological events (Galtsoff 1964, Drinnan and Stallworthy 1979, Mann 1988). In light of this, Abbe (1988) reported that recent studies of recruitment patterns of oysters in Chesapeake Bay have successfully correlated spatfall with salinity. Years of good recruitment coincided with high salinities (>16 ppt) while years of poor spatfall followed lower salinities (Abbe 1988). Unfortunately, long term salinity records are not

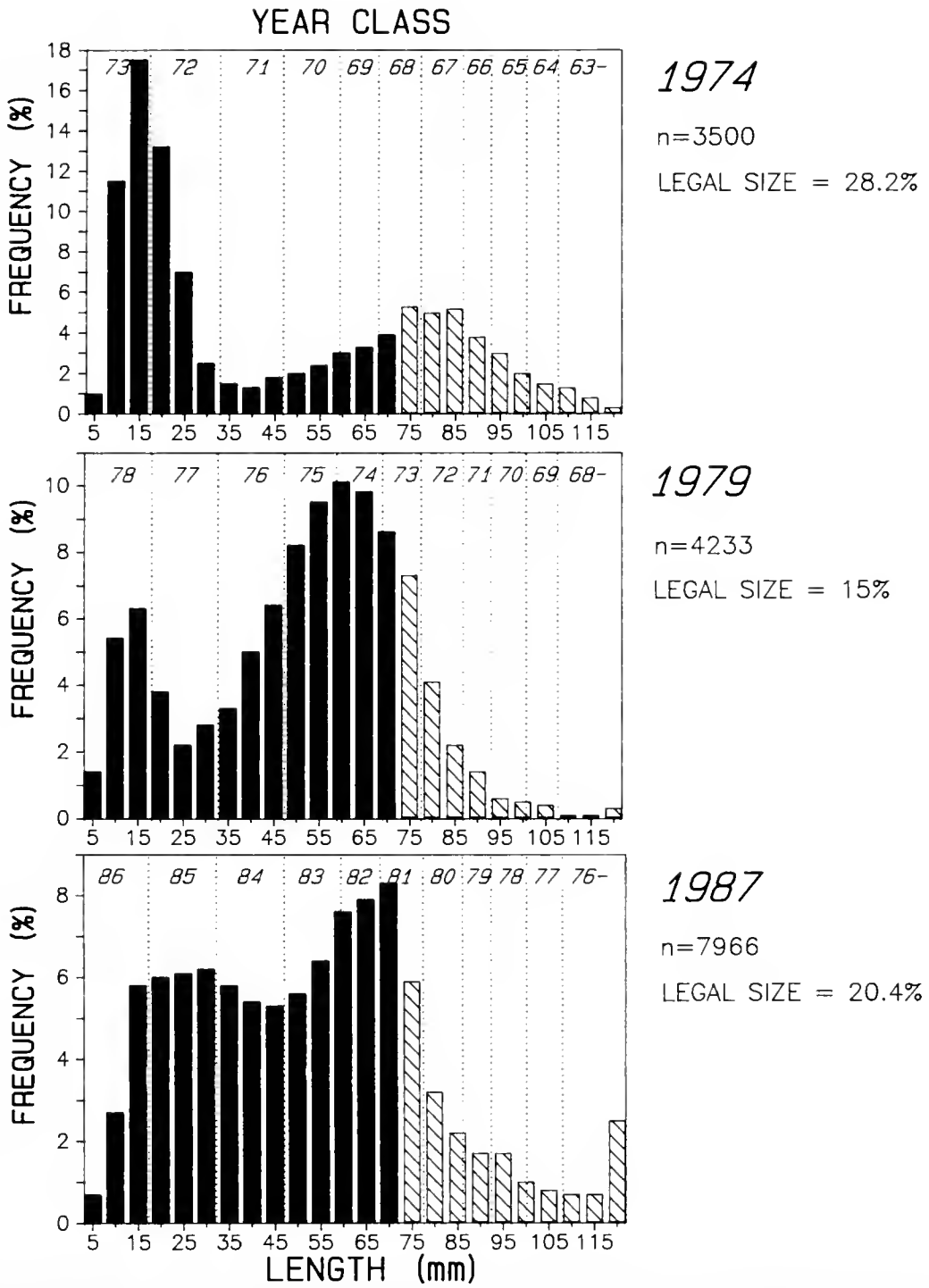


Figure 4. Size frequency distributions of the American oyster population of the Public Fishing Ground of Caraquet Bay, NB in 1974, 1979 and 1987. The histograms are partitioned to show year (age) classes, with the years shown at the top of the histogram. The diagonally striped bars indicate legal size (length > 75mm) oysters.

available for either of the Grounds of the present study. The similar patterns of recruitment observed for both Grounds in the last few years suggest that large scale meteorological events may be an important factor affecting the process. This was suggested by Drinnan and Stallworthy (1979) when they observed the adverse effects of a midsummer storm event and temperature change on the oyster larval abundance and distribution in a PEI estuary.

The Grounds are separated geographically and have different physical embayments. Caraquet Bay is enclosed with

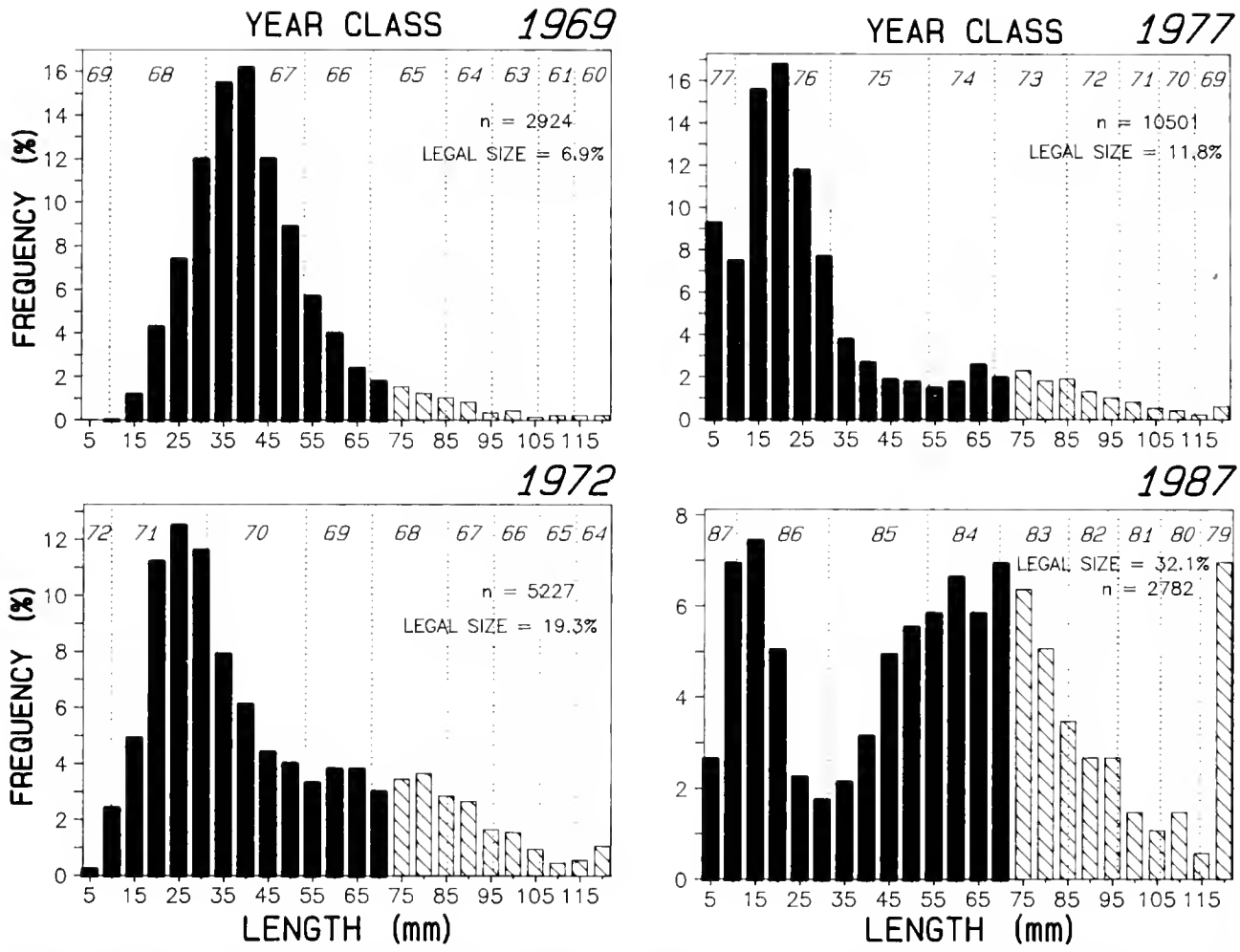


Figure 5. Size frequency distributions of the American oyster population of the Dunk River Public Fishing Ground of Bedeque Bay, PEI in 1969, 1972, 1977 and 1987. The histograms are partitioned to show year (age) classes, with the years shown at the top of the histogram. The diagonally striped bars indicate legal size (length > 75mm) oysters.

a narrow opening to the open water while the Dunk River-Bedeque Bay estuary is sheltered but open to water from Northumberland Strait. Research is required to identify the main oceanographic retention and dispersal mechanisms of larvae in these Bays to help explain the constancy of these populations in their respective Grounds and the lack of larval recruitment in other areas close to the Grounds. Physical oceanographic information was essential to understanding the distribution and dispersal patterns of oyster larvae in the James River of Chesapeake Bay (Mann 1988). Also, data from a regional spatfall monitoring program conducted in the 1970's may provide additional information on regional recruitment patterns of oysters in the southern Gulf of St. Lawrence.

The prognosis for both fisheries, based on the size of the 1985 to 1987 year classes, is that they will probably continue to decline. The excellent spatfall reported for both Grounds in 1988, however, should be formally assessed to estimate the success of the recruitment and the eventual impact on the respective fisheries. Although no growth rate estimates were attempted with the 1987 data, the fishable biomass may continue to decline in both areas over the next few years until the 1988 cohort is recruited to the fisheries. A confounding factor in the Caraquet Ground may also be effects of heavy metals on growth and survival of all stages. High concentrations of zinc were found in oysters examined during the "domoic acid toxic mollusc crisis" of 1987 in Atlantic Canada (Bates et al.). These results, combined with comments from Caraquet Bay aquaculturists of stunted growth of oysters in certain areas, have prompted new research on the effects of heavy metals on the growth and physiology of juvenile oysters.

In conclusion, a comparison of information, spanning a period of 15–18 years, indicates that the oyster populations of both Grounds are presently at levels lower than that recorded previously, with a similar population structure. Both populations remain self sustaining with only intermittent recruitment.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical and field assistance provided by the following individuals: Trevor Henry, Keith McCanna, Shawn Gilchrist, Jacques Arsenault, Randy Angus, Don Jones and Claude Leger. Local arrangements and laboratory facilities were facilitated and graciously provided in Caraquet by Francois Blanchard, Paul Cormier, and Jean-Andre Blanchard, and in PEI by Allan Morrison and the DFO PEI Area Office. We thank R. Lavoie, T. W. Rowell, R. B. Angus, J. Smith, J. Worms and D. H. Sephton for reviewing earlier drafts of the manuscript.

REFERENCES

Abbe, G. R. 1988. Population structure of the American oyster, *Crassostrea virginica*, on an oyster bar in central Chesapeake Bay: changes associated with shell planting and increased recruitment. *J Shellfish Res.* 7:33–40.

Bates, S. S., C. J. Bird, A. S. W. deFreitas, R. Foxall, M. Gilgan, L. A. Hanic, G. R. Johnson, A. W. McCulloch, P. Odense, R. Pocklinton, M. A. Quilliam, P. G. Sim, J. C. Smith, D. V. Subba Rao, E. C. D. Todd, J. A. Walter & J. L. C. Wright. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. *Can. J. Fish. Aquat. Sci.* 46:1203–1215.

Cushing, D. H. 1981. *Fisheries biology. A study in population dynamics. Second edition.* Univ. Wisconsin Press, Madison, Wisconsin. 295 pp.

Crisp, D. J. 1967. Chemical factors inducing settlement in *Crassostrea virginica* (Gmelin). *J. Anim. Ecol.* 36:329–335.

Drinnan, R. E. & W. B. Stallworthy. 1979. Oyster larval populations and assessment of spatfall, Bideford River, P. E. I., 1958. Fish. Mar. Serv. Tech. Rep. 792:32 pp.

Galtsoff, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. Fish. Bull. U.S. Fish. Wildl. Serv. 64:480 pp.

Green, R. H. 1979. *Sampling design and statistical methods for environmental biologists.* John Wiley & Sons, Inc., New York. 257 pp.

Haven, D. S. & J. P. Whitcomb. 1983. The origin and extent of oyster reefs in the James River, Virginia. *J. Shellfish Res.* 3:141–151.

Haven, D. S., J. M. Zeigler, J. T. Dealteris & J. P. Whitcomb. 1987. Comparative attachment, growth and mortalities of oyster (*Crassostrea virginica*) spat on slate and oyster shell in the James River, Virginia. *J. Shellfish Res.* 6:45–48.

Hawkins, C. M. & T. W. Rowell. 1985a. Population survey of the oyster *Crassostrea virginica* in the Dunk River Estuary, Prince Edward Island, 1969 and 1970. Can. MS Rep. Fish. Aquat. Sci. 1857:107 pp.

Hawkins, C. M. & T. W. Rowell. 1985b. Population survey of the oyster *Crassostrea virginica* in the Dunk River Estuary, Prince Edward Island, 1972. Can. MS Rep. Fish. Aquat. Sci. 1858:45 pp.

Hawkins, C. M. & T. W. Rowell. 1985c. Population survey of the oyster *Crassostrea virginica* in the Dunk River Estuary, Prince Edward Island, 1977. Can. MS Rep. Fish. Aquat. Sci. 1863:108 pp.

Lavoie, R. E. 1977. The oyster population of the Public Fishing Area, Caraquet Bay, New Brunswick. Fish. Mar. Serv. Tech. Rep. 735:39 pp.

Lavoie, R. E. 1978. The oyster leasehold industry in Caraquet Bay, New Brunswick. Fish. Mar. Serv. Tech. Rep. 805:48 pp.

Lavoie, R. E. & C. F. Bryan. 1980. Impact of stock thinning and relocation on oyster quality and value—Dunk River, P. E. I. (Part 1 of 2). Can. MS Rep. Fish. Aquat. Sci. 1587:15 pp.

Lavoie, R. E. & C. F. Bryan. 1981a. Medium-term value of stock thinning for oyster resource enhancement—Dunk River, P. E. I. (Part 2 of 2). Can. Tech. Rep. Fish. Aquat. Sci. 1010:13 pp.

Lavoie, R. E. & C. F. Bryan. 1981b. Oyster bed development in the Dunk River, P. E. I., 1975–1980. Can. Tech. Rep. Fish. Aquat. Sci. 1015:14 pp.

Lavoie, R. E. & G. Robert. 1981. Distribution and biological parameters of the oyster population of the public fishing area of Caraquet Bay, New Brunswick. Can. Tech. Rep. Fish. Aquat. Sci. 1039:20 pp.

Loosanoff, V. L. 1966. Time and intensity of setting of the oyster, *Crassostrea virginica*, in Long Island Sound. *Biol. Bull.* 130:211–227.

Northwest Atlantic Fisheries Organization. 1987. Tabular summaries of nominal catches, 1985. NAFO Stat. Bull. 35:59–61.

Mackenzie, C. L., Jr. 1970. Causes of oyster spat mortality, conditions of oyster setting beds, and recommendations for oyster bed management. *Proc. Natl. Shellfish. Assoc.* 60:59–67.

Mann, R. 1988. Distribution of bivalve larvae at a frontal system in the James River, Virginia. *Mar. Ecol. Prog. Ser.* 50:29–44.

Medcof, J. C. 1961. Oyster farming in the Maritimes. Fish. Res. Bd. Canada Bull. 131:158 pp.

Powell, E. N. & H. Cummins. 1985. Are molluscan maximum life spans determined by long term cycles in benthic communities? *Oecologia* 67:177–183.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fish. Res. Bd. Canada Bull. 191:382 pp.

Rowell, T. W. 1975. Maritime oyster culture, development potentials and limitations. Proc. Bras d'Or Aquaculture Conf., College of Cape Breton Press, Sydney, N.S., pp 130–139.

SAS Institute Inc. 1985. *SAS/STAT guide for personal computers.* Cary, N.C. 1028 pp.

Sieling, F. W. 1950. Intensity and distribution of oyster set in Chesapeake Bay and tributaries. *Proc. Natl. Shellfish. Assoc.* (1949):28–32.

Sephton, T. W. & C. F. Bryan. 1988. Abundance, distribution and dynamics of the American oyster population of the Public Fishing Area of Caraquet Bay, New Brunswick. Can. Tech. Rep. Fish. Aquat. Sci. 1654:18 pp.

Sephton, T. W. & C. F. Bryan. 1989. Changes in the abundance and distribution of the American oyster population of the Dunk River Public Fishing Area of Bedeque Bay, Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci. 1677:21 pp.

Whitcomb, J. P. & D. S. Haven. 1987. The physiography and extent of public oyster grounds in Pocomoke Sound, Virginia. *J. Shellfish Res.* 6:55–65.

Journal of Shellfish Research, Vol. 8, No. 2, 387–389, 1989.

EVALUATION OF SOME SHELLS FOR USE AS NUCLEI FOR ROUND PEARL CULTURE

R. B. ROBERTS[1] AND R. A. ROSE[2]

[1] *CSIRO Division of Applied Physics*
Lindfield, Australia, 2070

[2] *WA Marine Research Laboratories*
Perth, Australia, 6020

ABSTRACT Thermal expansion, thermal conductivity and hardness of shells of species of giant clam (*Tridacna squamosa*), pearl oyster (*Pinctada maxima*) and freshwater mussel (Family: Unionidae) were measured to assess differences in physical properties relevant to the performance of nuclei or beads made from these shells for the production of round or spherical pearls. All shells measured were strongly anisotropic. The giant clam shell is sufficiently close to the already successful (but expensive) freshwater mussel shell to warrant testing as an alternative source of pearl nuclei.

KEY WORDS: giant clam (*Tridacna squamosa*), pearl oyster (*Pinctada maxima*), freshwater mussel (Unionidae), thermal expansion, thermal conductivity, shell hardness

INTRODUCTION

The cultured pearl industry in Western Australia produces primarily round or spherical pearls and secondarily half or blister pearls (as well as mother-of-pearl-shell) which are exported as raw material for the jewellery industry. The estimated value of the pearl industry is about $AUS 65 million per annum and at least 90% or more of its income is derived from round pearls.

However, between $AUS 500,000 and 600,000 of this income is offset by imports from Japan of nuclei or beads made from the shells of freshwater mussels (Unionidae) used to initiate the process of round pearl cultivation. Besides their cost these beads also have the disadvantage of being limited to a maximum diameter of 13.5 mm. The technology for manufacturing beads is simple and inexpensive. The object of this study was to determine whether the physical characteristics of local bivalve shells are suitable for initiating pearl formation in place of freshwater mussel shells.

Within the same tropical region of Australia as the cultured pearl industry, there are several recently established clam farms capable of culturing several species of giant clam, such as *Tridacna squamosa*. As with all the large clams, the present salable product is the meat while little or no market exists for the shells. However, the shells are particularly thick and it is conceivable that beads up to 15 mm diameter could be produced from them.

The shells of clams, mussels, and oysters are generally similar chemically, all being composed primarily of calcium carbonate. However, they are distinct physically, each being composed of different fractions of the two principal polymorphs: calcite and aragonite. It seems intuitively obvious that a prime requirement for the substrate of a cultured pearl is that it have physical properties not unlike those of a natural pearl, which is primarily aragonite (Wada 1968). It was therefore of interest to measure the thermal expansivity, thermal conductivity, and hardness of the shell of *Tridacna squamosa* in order to compare these characteristics with those of the pearl oyster and freshwater mussel.

MATERIALS AND METHODS

Thermal expansion of samples of shell were measured by means of a silica pushrod dilatometer operating in a stirred bath of kerosene which was controlled over a temperature range of −20 to 50°C. The temperature was measured by means of a miniature platinum thermometer whose resistance was monitored by a digital multimeter which automatically compensated for thermal voltages in the leads. Resolution was equivalent to 0.001 deg. C and the maximum absolute error of the thermometry was less than 0.03 deg. C. The system for measuring length resolved expansions to 0.03 μm, so that in a sample 10 mm long, the resolution of change in length was about 3 ppm. Systematic errors inherent in the instrument over the temperature range limited the useful absolute resolution to about 10 ppm even after calibration against silicon. However, since changes in length were typically some thousands of ppm, the resolution was considered adequate.

The layers within all shells studied were not isotropic. Coloured bands appeared closely parallel to the "plane" of the shell. Samples were cut to allow measurement of thermal expansion in two directions: parallel to the plane of the shell and perpendicular to the plane of the shell.

Beads made from the nacreous layers of mussel shell were lapped to form cubes about 4 mm thick. One pair of faces of each cube was oriented parallel to the coloured bands. To constitute a sample long enough for the dilatometer, three cubes were stacked together in the same orientation with respect to the bands and the cubes could be stacked to allow expansion measurements in both directions.

The sample of clam shell was cut from what Kobayashi (1969) called the inner and middle calcareous layer by means of a diamond saw and ground on emery to produce a rectangular block 12 × 12 × 8 mm with the pair of 12 × 8 mm faces almost parallel to the coloured bands. Measurements of thermal expansion were made in the plane of the coloured bands and at right angles to them.

The pearl oyster shell was cut with a diamond saw to

yield a thin rectangular block of nacre. The ends of the block were lapped parallel with emery and this sample was used for the measurements of expansion in the plane of the shell. To provide a sample sufficiently thick for measurements perpendicular to the plane of the shell, several "buttons" each about 2 mm thick were cut by means of a hollow diamond drill. The buttons were lapped flat and parallel and stacked to form a sample 12 mm thick.

Samples were also measured on a Microhardness tester to determine the distance a diamond anvil could penetrate the shell under specified load conditions. The results were a measure of the strengh of the shell material [as they gave the area required to sustain a known load]. Area measured was about 0.03 mm square, so many readings were taken to give reproducible results. The microstructure of these shells was not uniform. Scatter in measurements on a particular surface was less than 15%.

Thermal conductivity measurements were done on lapped sections of shell, by means of a Tye thermal comparator (Lafayette Instrument Co. Model TC-1000). This device was calibrated against fused quartz, a special Corning glass (7740), and a titanium alloy of known thermal conductivity. The error expected from this type of measurement is typically less than 5%.

RESULTS AND DISCUSSION

Thermal expansion of all shell samples both in the plane of the shell and perpendicular to it was almost linear over the range −20 to 50°C (Fig. 1). There was a small positive curvature (i.e. expansion coefficient increasing with temperature) when measured perpendicular to the plane of the shell. The linear expansion coefficients were about 14 ppm/°C for all samples in the plane of the shells, and much larger in the direction perpendicular to the shells, being 29, 35, and 47 ppm/°C for clam, mussel, and oyster shell, respectively.

For comparison the expansion of a multi-twinned (pseudo hexagonal) crystal of natural (inorganic) aragonite was also measured over the same temperature range. In the direction of the c-axis, the expansion coefficient was 34 ppm/°C. The expansion coefficients along the a- and b-axes derived from measurements across the flats of the hexagonal base, averaged 15 ppm/°C. These values are in general agreement with measurements of Kozu and Kani (1934) who found $a_a \sim 6$ ppm/°C, $a_b \sim 14$ ppm/°C, $a_c \sim 25$ ppm/°C over the range from 20 to 100°C. The expansivities of calcite are 23 ppm/°C parallel to the c-axis, and -5 ppm/°C parallel to the a-axis (Touloukian 1977).

Thermal conductivity of all shell samples in both orientations were almost indistinguishable, being 1.6 ± 0.2 watt/m.K. The mussel shell was at the top of the range and the clam at the bottom, but these discrepancies probably reflect the difficulties in mounting the small specimens rather than differences in the material itself. (The conductivity of vitreous silica is typically 1.4 watt/m.K.).

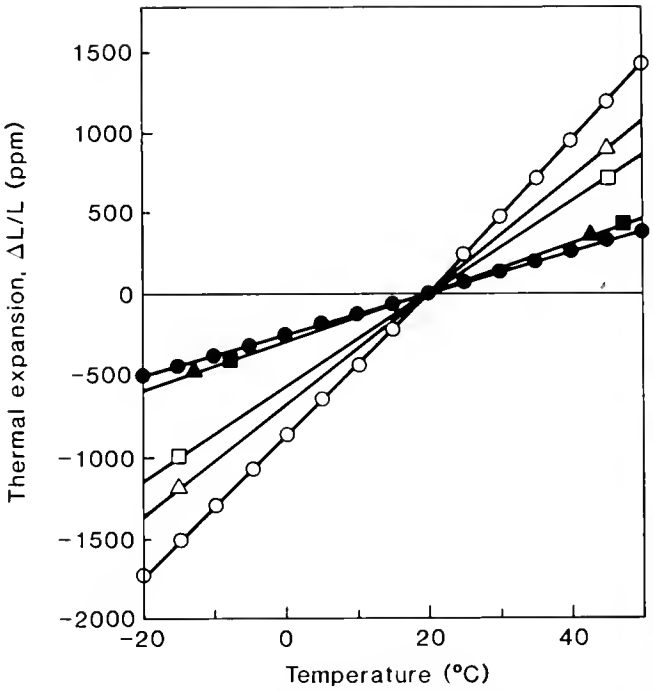


Figure 1. Thermal expansion of shells. Most of the data points have been omitted for clarity. ○ = Pearl oyster, perpendicular to banding; ● = pearl oyster, parallel to banding; △ = freshwater mussel, perpendicular to banding; ▲ = freshwater mussel, parallel to banding; □ = giant clam, perpendicular to banding; ■ = giant clam, parallel to banding.

The hardness of shells was measured by means of a Matsuzawa microhardness tester type MHT-1 with a 100 g load sustained for 20 sec (Table 1). The shell of the giant clam is the strongest and most homogeneous of the three. This is in keeping with its observed thermal expansion being the smallest and least anisotropic.

The results indicate that beads manufactured from clam shells of *T. squamosa* may be a suitable alternative to those from Unionidae freshwater mussels. The technology required for producing beads from clam shells should also be similar except that the equipment required may have to be more robust as clam shell is harder than that of mussel (Table 1). However, since the costs of beads from freshwater mussels are continuously rising and are now $AUS 7.30 and 20.20 each for beads 11–12 mm and 13.5 mm in

TABLE 1.

Shell strength as given by Microhardness test (units: kg/mm²).

	A	B
Freshwater Mussel	240	160
Pearl Oyster	210	180
Giant Clam	250	220

Column A: perpendicular, B: parallel to banding.

diameter, respectively, our results suggest that beads from locally aquacultured clam shells may be an economically attractive, alternative source. Further, because clam shells are so thick, they have the added advantage of being able to produce larger (15 mm) diameter beads which in turn would allow oysters to produce larger, more valuable pearls.

ACKNOWLEDGMENTS

Funds from the Australian Commonwealth Fishing Industry Research and Development Council partially supported this study.

REFERENCES

Kobayashi, I. 1969. Internal Microstructure of the Shell of Bivalve Molluscs. *Am. Zoologist*, 9:663–672.

Kozu & Kani. 1934. Geological Society of America Memoir 97 Handbook of Physical Constants. In S. P. Clark, Jr. (ed.) *Proc. Imp. Acad. Japan*, 10:222.

Touloukian, Y. S. 1977. Thermophysical Properties Research Centre, Vol. 13, Thermal Expansion of Non-Metals, Plenum, New York.

Wada, K. 1968. Cultured pearl and its structure, Symposium on Molluscs. *Mar. Biol. Assoc. India*, Series 3:972–974.

Journal of Shellfish Research, Vol. 8, No. 2, 391–397, 1989.

IDENTIFICATION OF SOFT-SHELL CLAM (*MYA ARENARIA* LINNAEUS, 1758) STOCKS IN EASTERN CANADA BASED ON MULTIVARIATE MORPHOMETRIC ANALYSIS

T. AMARATUNGA* AND R. K. MISRA
Department of Fisheries and Oceans
Halifax Fisheries Research Laboratory
P.O. Box 550
Halifax, Nova Scotia, Canada B3Y 2S7

ABSTRACT Recent escalation of clam price has contributed to an increase in fishing effort for the soft-shell clam, *Mya arenaria*, in eastern Canada. Fluctuations in reported landings demonstrate production variability and thus the desirability to manage stocks in an attempt to reduce the interannual variability. The first step is to identify stock differences and multivariate analysis is used for the first time on morphometrics of clams taken from 14 important fishing areas. Multivariate analysis, has the advantage of encompassing all data in one analysis. It yields information concerning relationships, interdependence and relative importance of biological characters. The computer program, multivariate analysis of covariance (MANCOVA), was employed to "adjust" morphometric characters (the variates width, height and weight) for variations in size (standard length), which was used as the covariate. The union-intersection procedure was used in the comparisons of adjusted mean vectors of samples and multiple comparisons of sample character combinations. The analysis suggests that three identifiable stocks exist in the study area: one on the Atlantic side of Nova Scotia, and two in the Bay of Fundy. The analysis also showed two further areas within the Bay of Fundy (Annapolis Basin and Passamaquoddy Bay) which were significantly different, each showing independent characteristics. The study presents new data for clam fishery management, and demonstrates the strength and value of multivariate analysis for stock delineation of sedentary species.

KEY WORDS: multivariate analysis, morphometrics, stock identification, soft-shell clam *Mya arenaria*

INTRODUCTION

The soft-shell clam *Mya arenaria* Linne is a valuable indigenous bivalve resource in eastern Canada. The fishery of this easily accessed and cheaply harvested intertidal clam dates back to aboriginal exploitation. Modern commercial exploitation began before the turn of the last century. The industry, which has historically been low valued compared to other molluscan fisheries such as scallop and oyster, has changed significantly over the last decade. Escalating prices based on both strong domestic and export markets, have contributed to steady increases in fishing effort. Reported annual landings in the study area, which exclude unreported part-time and recreational landings (which will elevate these values by probably more than an order of magnitude), increased by 15% from 1985 to 4,500 t in 1986 with a corresponding landed value of upward of $5.7 million. Fluctuations are often recorded in clam landings. The substantial decrease to less than 3,000 t in 1988, typically demonstrates high variability in production and the uncertainties to the fishermen who depend on the resource. Resource managers recognize the desirability to manage stocks or maintain production at a high level.

Successful resource management depends on the accuracy of knowledge of distribution and biological characteristics of individual stocks. This is because stocks are characterized by their own parameters such as mortality rates, ages-at-maturity and growth rates, and current models of population dynamics are generally inadequate when applied to mixed stock fisheries (Gulland 1969). Delineation of stocks is therefore vital for an effective fisheries management program.

Several techniques in use to identify stock differences, especially in fish stocks, include distributional (e.g. tagging studies), biological (e.g. growth rates), biochemical, meristic, morphometric and electrophoretic analyses. Sharp et al. (1978) observed that morphometrics offered greater potential than meristics in separating stocks, and suggested the use of morphometrics as a tool in separating capelin stocks.

Often univariate analyses on individual morphometric characters are employed to investigate differences among population (stock) means. However, researchers have long since recognized that they cannot rely solely on the univariate design (Harris 1975). It has been observed that several univariate analyses carried out separately for each morphometric variable are not adequate and can be misleading because such analyses ignore the correlations among the variables (Bliss 1970, Kshirsagar 1972). Morphometric characters such as length, width, height and weight are controlled by numerous genes (Falconer 1972) and the genetic processes may also contribute to mutual correlations of these characters. Bock (1975) noted that (i) multivariate statistical methods are emphasized because they make it possible to encompass all data from an investigation in one analysis, and (ii) multivariate approach results in a clearer, better organized account of the investigations than do piece meal analyses of portions of the data. Multivariate analysis yields additional information concerning relationships, interdependence and relative importance of characters. It is

*Present address: Northwest Atlantic Fisheries Organization, P.O. Box 638, Dartmouth, Nova Scotia, Canada B2Y 3Y9.

therefore desirable to employ multivariate analysis to compare populations.

Multivariate morphometric analyses have been employed widely in studies pertaining to geographic variation, racial affinities and phenetic relationships between related populations, in general (Gould and Johnston 1972, Thorpe 1976, Reyment et al. 1981, Libosvarsky and Kux 1982, Johnson et al. 1983) and in stock delineation in particular (Sharpe et al. 1978, Casselman et al. 1981, Ihssen et al. 1981, Almeida MS 1982). Bliss (1970) states that even where two similar species can be identified with a single measurement, a combined criterion of two or more may increase the separation between them.

When comparing closely related populations where only slight morphometric differences occur, sampling bias caused by varying sizes of specimens will restrict the scope of analysing morphometric data (Misra and Ni 1983). Use of ratios where the denominator is a body dimension (such as length) used as a proxy for size, will not overcome this difficulty (Atchley et al. 1976, Misra and Ni 1983, Pimentel 1979). In their redfish study Misra and Ni (1983) overcame this difficulty by employing multivariate analysis of covariance or MANCOVA (Morrison 1976, Srivastarva and Carter 1983) to "adjust" morphometric characters (variates) for variations in size (standard length) which was used as the covariate. At the univariate level the use of the covariance procedure is recognized (e.g. Marr 1955, Royce 1964). An added advantage of the analysis of covariance procedure is that by this statistical adjustment of data higher precision of comparisons of group means may be achieved (Snedecor and Cochran 1967).

Studies addressing clam stocks or populations have generally focussed on growth characteristics (Newcombe 1935, Newcombe 1936, Turner 1948, Brousseau 1979, Brosseau and Baglivo 1987). Effects of particularly environmental factors (Appeldoorn 1983) such as sediment type (Swan 1952, Newell and Hidu 1982), temperature (MacDonald and Thomas 1980) on individual growth characters are often reported. However, to date multivariate morphometrics have not been employed in stock delineation of clams. In this presentation the multivariate method based on MANCOVA of morphometric data is employed to identify stock differences in soft-shell clam (*Mya arenaria*) from fourteen geographical locations (stations) chosen from major clam producing areas in eastern Canada.

MATERIALS AND METHODS

Sampling Procedures

Fourteen stations (Fig. 1) from important clam fishing areas were selected from along the Nova Scotian and southern New Brunswick shorelines. Stations were geographically distributed such that a minimum of three stations represented each of the three major geographic areas (Areas A, B and C) as shown in Figure 1 and referred to in Table 1. Area A consists of stations from the Atlantic Ocean side of Nova Scotia while Areas B and C consist of stations from western Nova Scotia in Bay of Fundy. For analytical purposes in this study, Area B was subdivided into Minas Basin (Area B_1) and Annapolis Basin (Area B_2). Sampling was carried out during January and February 1987 when clams under winter conditions were considered to be inactive reproductively and in shell growth. *M. arenaria* were dug from the mid-tidal level, from areas where the sediments ranged from sand to mud. An attempt was made to collect a reasonably large sample (approximately one hundred clams) at each station using a rule of thumb to collect 35% from <20 mm shell length, 30% from between 20 mm and 40 mm and 35% from >40 mm. Selection of a wide range of values of the covariate (length) increases efficiency of regression based analyses such as the analysis of covariance (Li 1964). Field samples were rinsed and transported in fresh sea water, for laboratory study within 36 hrs after collection.

Morphometric characters Yj (j = 1 for width, = 2 for height, = 3 for weight and = 4 for length) were recorded for each clam. Length measurement represented the greatest antero-posterior dimension; width the dorso-ventral dimension taken from the umbo to the shell margin; and height the greatest lateral dimension of tightly closed animals. Linear measurements were taken to the nearest 0.1 mm using vernier calipers. The total wet weight of clams were taken to the nearest 0.1 g after extraneous materials were washed off and excess surface moisture was dampdried using paper towels.

Analysis

Only specimens for which all four measurements were available were employed in the multivariate analysis, because "missing observations virtually destroy morphometrics" (Pimentel 1979). Measurements Yj (j = 1, . . ., 4) were transformed to their common logarithms Xj (Hemmingsen in Bliss 1967, Pimentel 1979) recognizing that: (a) the distribution of body size is often log normal, (b) both linearity and multivariate normality are more closely approximated by logarithms than by original variables, and (c) the convention is to use common logarithms.

Table 1 lists sample (station) sizes, means and ranges of Xj (j = 1, . . ., 4). The computer program for the MANCOVA was written by one of the authors (RKM) following the methodology explained in Morrison (1976) and Srivastava and Carter (1983). In this presentation the vectors derived for each variable are denoted by an underscored letter. In the MANCOVA: (i) X_4 was employed as the covariate to adjust the station mean vectors of Xj, j = 1, 2, 3 for individual values of X_4. In univariate regression analysis, length has been used as the covariate in the past (Newcombe and Kessler 1936). Large overlaps between samples in the range of X_4 (Table 1) provides additional support for its use as the covariate (Snedecor and Cochran

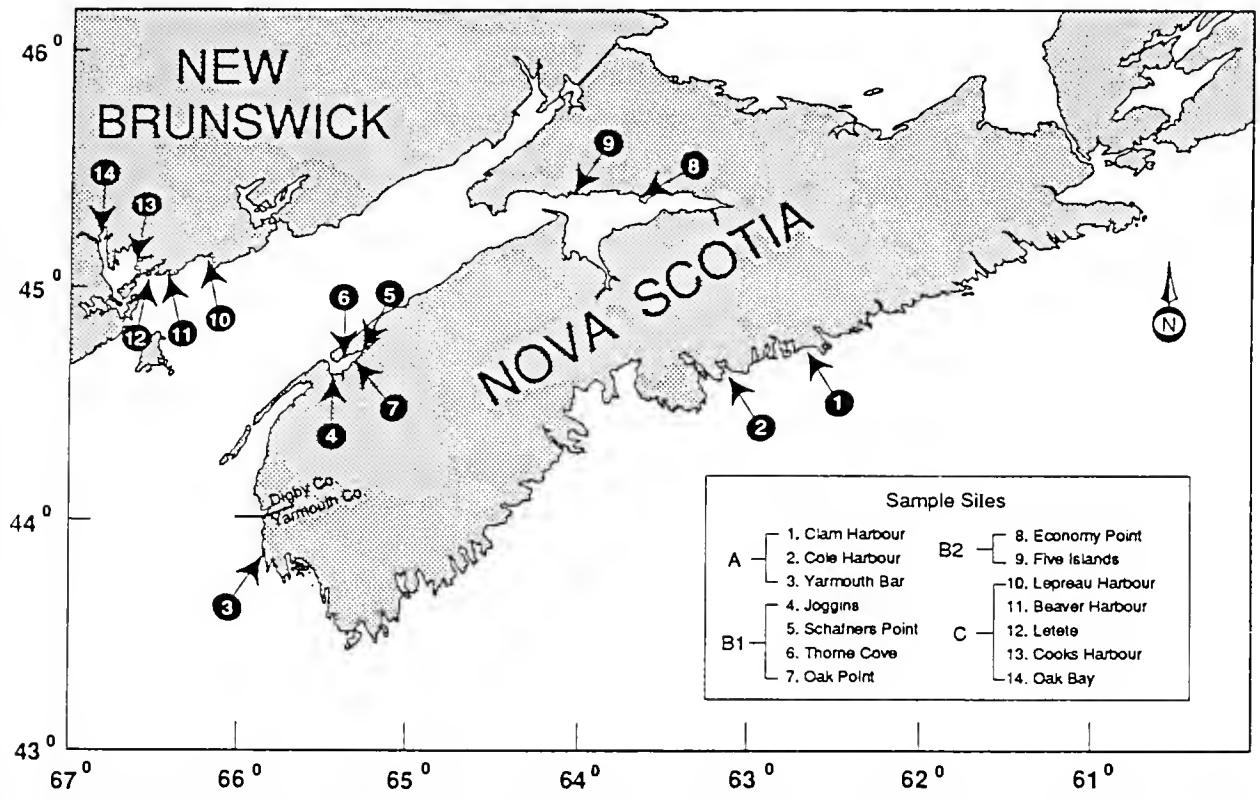


Figure 1. Station numbers, names and locations in the study area showing boundaries between Areas A, B (i.e. B_1 and B_2) and C at Digby/Yarmouth County line and Nova Scotia/New Brunswick Provincial line.

1967), (ii) comparisons of adjusted mean vectors of samples and multiple comparisons of sample-character combinations were done by the union-intersection procedure (Morrison 1976). This procedure may appeal to the practitioner "because of its greater heuristic and didactic values" (Harris 1975). It is the most general method of performing multiple comparisons of groups and/or variables and is particularly useful when the first characteristic root in the test of the multivariate general linear hypothesis is considerably larger than the other roots (Harris 1975, Morrison 1976), (iii) all tests of hypotheses were done at 5% probability level (p) of significance unless stated otherwise.

RESULTS

Vector *b* of coefficients bj (j = 1, 2, 3) of regression of the variates Xj (j = 1, 2, 3) on the covariate X_4 was computed from "within sample" sums of squares and products (SS and SP). Estimates of bj were $b_1 = 1.0541$, $b_2 = 0.9881$ and $b_3 = 3.0527$. The test statistic θ for the null hypothesis $b = 0$ of no linear regression was estimated as 0.9921 with values 1, 0.5 and 708.5 for parameters s, m and n respectively and equivalent value of the variance ratio F was 59553.4 with degrees of freedom (df) = 3 and 1419. This yielded p = 0+ (i.e. close to zero) indicating that the regression coefficient vector was highly significant and MANCOVA would provide an effective analysis for comparing samples. For test of the null hypothesis of equal vectors *X* of adjusted means of variates for fourteen samples, θ was estimated as 0.2529 with s = 3, m = 4.5 and n = 708.5 yielding p = 0+. This indicated that sample means were not all equal on at least one Xj (j = 1, 2, 3) variable and/or significant linear combination(s) of samples and variables existed. As a follow-up of this finding, paired comparison among sample adjusted mean vectors were done (Fig. 2), and an itemized summary of observations is as follows:

Area A - Eastern Nova Scotia

1. Stations 1 and 2 were similar. Station 3 was similar to Station 2 but significantly different from Station 1 at a low level of probability ($0.01 < p \leq 0.05$).

Area B_1 - Western Nova Scotia, Annapolis Basin

2. Station 4 was significantly different from all other stations except Station 14.
3. Station 7 was significantly different from all other stations except Stations 11 and 13.
4. All stations in this area (i.e. Stations 4–7) were significantly different from each other.

Area B_2 - Western Nova Scotia, Minas Basin

5. Stations 8 and 9 were similar to each other. The pair was also similar to Station 10 (Area C), but showed significant difference from all other stations in Areas B and C.

Area C - Southern New Brunswick

TABLE 1.

Sample size and means and ranges of morphometric characters Xj (i = 1, · · 4) at each station. X_1 = width (mm), X_2 = height (mm), X_3 = weight (g) and X_4 = length (mm). All measurements were transformed to common logarithms.

Area	Station Number–Name	Sample Size	Mean Width (Range)	Mean Height (Range)	Mean Weight (Range)	Mean Length (Range)
Eastern Nova Scotia (A)	1–Clam Harbour	100	0.0653 (−0.3979 to 0.4314)	0.3005 (−0.2218 to 0.6232)	0.6052 (−0.9586 to 1.5924)	0.5114 (0.0000 to 0.8129)
	2–Cole Harbour	100	0.0278 (−0.6990 to 0.4914)	0.2728 (−0.5229 to 0.7076)	0.5114 (−2.0000 to 1.8420)	0.4891 (−0.3010 to 0.9243)
	3–Yarmouth Bar	95	0.3527 (0.0792 to 0.5315)	0.5791 (0.3424 to 0.7324)	1.4554 (0.7067 to 1.9441)	0.7924 (0.5798 to 0.9494)
Western Nova Scotia (B_1)	4–Joggins	134	−0.0072 (−1.0000 to 0.4771)	0.2236 (−0.5229 to 0.7076)	0.3800 (−1.5229 to 1.8118)	0.4080 (−0.3979 to 0.9138)
	5–Schafner Point	63	0.2083 (−0.1549 to 0.5185)	0.4468 (0.0792 to 0.7324)	1.0436 (0.0000 to 1.9494)	0.6718 (0.3010 to 0.9445)
	6–Thorne Cove	149	0.1271 (−0.6990 to 0.4914)	0.3510 (−0.5229 to 0.7076)	0.7670 (−2.0000 to 1.8775)	0.5634 (−0.3010 to 0.9191)
	7–Oak Point	98	0.0412 (−0.3979 to 0.3802)	0.2654 (−0.0969 to 0.6021)	0.4654 (−0.7959 to 1.4615)	0.4644 (0.0792 to 0.8062)
Western Nova Scotia (B_2)	8–Economy Point	100	0.1591 (−0.2218 to 0.4150)	0.3890 (0.0414 to 0.6128)	0.9301 (−0.1308 to 1.6043)	0.6169 (0.2788 to 0.8326)
	9–Five Island	100	0.1285 (−0.3979 to 0.4771)	0.3595 (−0.1549 to 0.6721)	0.8266 (−0.6990 to 1.7483)	0.5806 (0.0792 to 0.8751)
Southern New Brunswick (C)	10–Lepreau Harbour	100	0.0611 (−1.0000 to 0.5315)	0.3066 (−0.6990 to 0.7160)	0.6311 (−2.000 to 1.9597)	0.5287 (−0.3979 to 0.9542)
	11–Beaver Harbour	100	0.0870 (−0.5229 to 0.4624)	0.3254 (−0.3010 to 0.6532)	0.6622 (−1.3010 to 1.7428)	0.5219 (−0.0969 to 0.8573)
	12–Letete	97	−0.0033 (−0 6990 to 0.4150)	0.2382 (−0.3010 to 0.6128)	0.4113 (−2.0000 to 1.5863)	0.4361 (−0.1549 to 0.8195)
	13–Cooks Harbour	100	0.0648 (−0.5229 to 0.4771)	0.2935 (−0.3010 to 0.6232)	0.5907 (−1.2218 to 1.6425)	0.4986 (−0.0969 to 0.8261)
	14–Oak Bay	100	0.0807 (−0.3979 to 0.4472)	0.3055 (−0.1549 to 0.6128)	0.6339 (−0.8861 to 1.5856)	0.5031 (0.0000 to 0.7993)

6. Stations 11, 12, 13 and 14 were similar to each other except in one instance where Stations 11 and 12 of a pair differed from each other at a low level of significance ($0.01 < p \leq 0.05$).
7. Station 10 was significantly different from all other stations (11 to 14) in Area C. However, Station 10 was found similar in the paired relationship patterns to stations in Area B_2 (8 and 9).

Cases where significant difference occurred between samples of a pair indicated that one or more linear combination of characters contributed to this difference. Number of possible linear combinations can be staggeringly large and identifying these is therefore a difficult task. However, as individual characters are of obvious interest, an attempt was made to identify those that separately contributed significantly to the difference between mean vectors of a pair. This was done based on 95% simultaneous confidence interval (CI) of individual characters Xj (j = 1, 2, 3) in each paired comparison of samples. Contribution of a Xj to the difference between two mean vectors would not be significant if the CI for it included zero. It is noted that two samples may differ significantly in their adjusted mean vectors and yet this difference may not show in individual characters (Johnson and Wichern, 1982; Misra and Carscadden, 1987).

Individual characters in relation to the similarities and differences in paired comparisons (Fig. 2) described above may be summarized as follows:

1. In all instances where paired stations were similar (e.g. summary items 1, 4, 5 and 6 above) individual characters Xj also did not show any significant differences. There were many instances, however, where station pairs were significantly different (e.g. Stations 1, and 3, 6 and 7, 11 and 12 etc.; 18 pairs in all) but no individual character showed significant difference. This was especially true for stations that differed at 5% level (but not at 1% level). Only one pair, Stations 6 and 8, that differed at $p \leq 0.001$ had no individual characters showing significant differences.
2. In 69% of the cases where paired stations showed significant differences, characters X_1 (width) and/or X_2 (height) contributed significantly to the differences. In all other cases, character X_1 along with

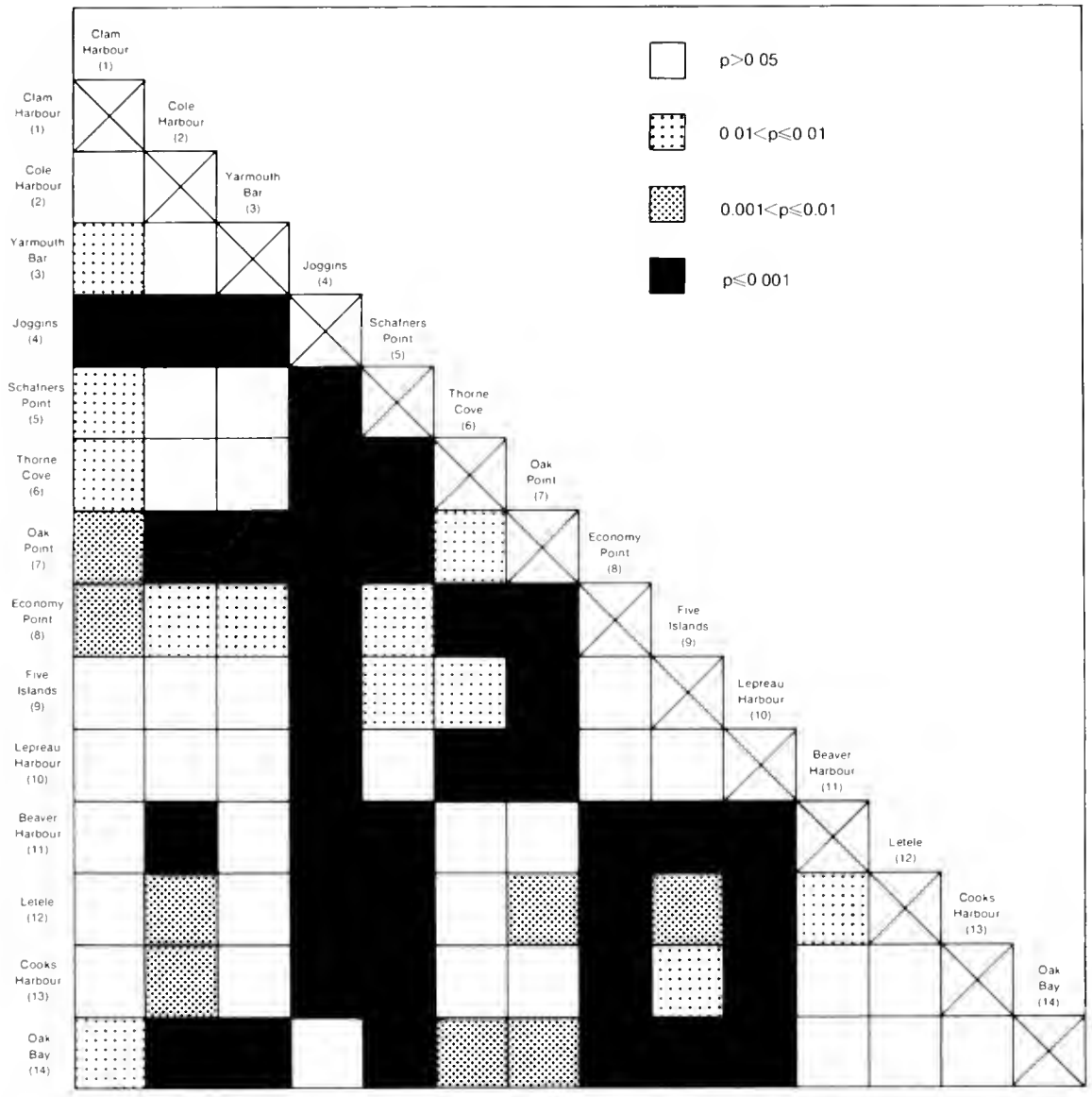


Figure 2. Paired comparisons of the fourteen stations showing probability level of significance (p).

characters X_2 and X_3 (weight) or with X_3, contributed to the differences. The major contributing (individual) character in this study was therefore X_1 - the width.

3. Station 4 was considered to be anomalous because (as stated above) it was significantly different from all other stations except Station 14. All three characters X_1, X_2 and X_3, jointly contributed to these differences when comparing with Stations 1 to 10 while X_3 was the major contributing character for differences with Stations 11, 12 and 13. Therefore, in this station alone X_3 (weight) was considered to be the major individual character contributing to between station differences.

DISCUSSION

This analysis of morphometric characters suggest that identifiable *M. arenaria* stocks exist in the study area. Cohesive station groupings in Area A, Area B_2 which included Station 10 from Area C, and Area C provide a basis for stock delineation. The arbitrary geographic boundary line between Areas B_2 and C established during sample collection (Fig. 1) did not prove to be correctly placed for separation of stocks. The analyses showed that a more appropriate boundary lies between Stations 10 and 11, with Station 10 included in area B_2. It is therefore proposed that there are at least three stocks in the study area, which are correspondingly labeled Stock A, Stock B_2 (including Station 10 in Fig. 1) and Stock C. Stock A is on the Atlantic Ocean side of Nova Scotia and the other two Stocks B_2 and C are in the Bay of Fundy.

It is not surprising to find such identifiable station groupings. In known Atlantic scallop aggregations studied in relation to physical oceanographic characteristics for example, it was interpreted in many instances that self-sustaining aggregations existed, some in close proximity to

each other and others considerable distances apart (Sinclair et al. 1985).

The covariate used in this study has been commonly used in clam population studies focussing on growth characteristics. Although they were not stock delineation studies, Newcombe (1935) reported that *M. arenaria* growth rates varied little among widely separated parts of the Bay of Fundy, whereas differential growth rates were observed between the Bay of Fundy and the Gulf of St. Lawrence (Newcombe 1936). Researchers have also found geographic variations of annual water temperatures to be directly related to growth rates (Newcombe and Kessler 1936, Turner 1948, Brousseau and Baglivo 1987). Appeldoorn (1983) reported the same trend studied from a different perspective, where growth rate was negatively correlated to northness in latitude. The geographic extent of the present study was relatively small. However, there may be a similar trend implied in the observations of Stock A on the Atlantic Ocean side. Here the most geographically distant Stations 1 and 3 differed from each other only at a low significance level ($0.01 < p \leq 0.05$), while they did not show significant difference on individual characters. This difference may relate to the geographic distance between stations. Coastal oceanographic currents (Drinkwater et al. 1979), on the other hand, are likely to be supportive of larval transport from Stations 1 and 2 to Station 3, to promote mixing and influencing a high level of similarity among the stations.

A similar argument may be extended to the Bay of Fundy Stock B_2. The counter clockwise circulation pattern in the Bay of Fundy and Minas Basin (Godin 1968, Greenberg 1983) is likely to support larval transport from Stations 8 and 9 to promote similarity with the distant Station 10, and result in the identifiable Stock B_2. This mixing, however, does not appear to influence stock in Area B_1 (Annapolis Basin) and in Area C (Passamaquoddy Bay) significantly. The analysis showed that they are significantly different and they each show strong independent characteristics. The anomalous situation between Stations 11 and 12 in Area C may be attributed to their location on the periphery of Passamaquoddy Bay where some external mixing can occur. It is noted, however, that the difference between the stations was only at a low significant level ($0.01 < p \leq 0.05$) and they showed no significant differences on individual characters.

The apparent lack of consistency and pattern among stations in Annapolis Basin (B_1) is not inconceivable. It is known that morphometric characters are influenced by both genetic and environmental variations (Barlow 1961, Falconer 1972, Todd et al. 1981). From the environmental side, it is possible that local conditions play an important role on the morphometrics of these clams, as often highlighted by researchers (Belding 1930, Newcombe and Kessler 1936, Swan 1952, Newell and Hidu 1982). Station 4 for example, which was the significantly different one in the entire study, was the only station placed adjacent to a relatively large urban centre - Digby town. Station 7, the next significantly different station, was one that changed dramatically between 1983 (Angus et al. 1985) and 1986 (Amaratunga, unpublished data). During these three years the sampling station recorded a loss of approximately 40% of the clam flat due to accumulation of silt and mud in the lowtide area. The clam population at the station was also apparently affected as observed in the change in size composition and significant depletion of both harvestable size classes (<51 mm) and prerecruits (>42 mm). The overall Annapolis Basin clam fishery has also experienced recent changes. Landings in 1980 which accounted for 68% of the total Nova Scotia landings diminished to only 28% of the total by 1986. Many environmental studies were recently initiated by the Department of Fisheries and Oceans, Canada, to assess relationships with this stock decline.

The multivariate analyses of morphometric characters suggest the possible occurrence of three discrete stocks of *M. arenaria*, along with a fourth (Annapolis Basin) which showed diverse characteristics. From the genetic standpoint, the occurrence of discrete stocks may be rationalized. It is known that organisms over a large region are usually distributed in patches rather than continuously, and these discontinuities in distribution subdivide a large species into number of isolates (Li 1958). On the other hand Dobzhansky (1951) described extreme diversity of local forms and an excellent example of random fixation of genes resulting in differentiation of local forms of a Hawaiian snail is cited by Li (1958). Todd et al. (1981) suggest the cisco fish groups they studied were represented by locally adapted and partly isolated populations that are genetically distinct enough to be differentially subject to exploitation and extinction but are not completely independent genetically. The diversity found in Annapolis Basin may be rationalized in this respect.

The lack of studies addressing stock delineation of clams have generally hampered the development of management strategies for the clam fishery. Many researchers in the past have assessed the importance of various biological factors, and edaphic factors of the environment on *M. arenaria* populations. Appeldoorn (1983) observed that these investigators were obliged to study these interrelated factors individually, without an adequate tool to separate stocks.

In the present study interrelated biological characteristics were utilized to delineate clam stocks in eastern Canada. While presenting new data to the fisheries managers, the study demonstrates the strength and value of the multivariate analysis for stock delineation, not only of clams but other sedentary species as well.

ACKNOWLEDGMENTS

We gratefully acknowledge field and laboratory assistance from Patrick Woo, Rod MacFarland and Tom McLane; Bob Semple for the computer graphic work and

Carolyn Harrie for assistance in computations. Appreciation is extended to Bob Miller and Glyn Sharp for the critical internal review of the manuscript and Victoria Clayton and Dorothy Auby for kindly typing the manuscript.

REFERENCES

Almedia, F. P. MS 1982. An analysis of the stock structure of silver hake, *Merluccius bilinearis*, in NAFO Subareas 5 and 6. NAFO SCR Doc., No. 82/81, Ser. No. N587: 36 p.

Angus, R. B., C. M. Hawkins, P. Woo & B. Mullen. 1985. Soft-shell clam survey of the Annapolis Basin, Nova Scotia—1983. *Can. Man. Rep. Fish. Aquat. Sci.* 1807:viii + 133 p.

Appeldoorn, R. S. 1983. Variation in the growth rate of *Mya arenaria* and its relationship to the environment as analyzed through principal components analysis and the ω parameter of the von Bertalanffy equation. *Fish. Bull.* 81(1):75–84.

Atchley, W. R., G. T. Gaskins & D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. *Syst. Zool.* 25:137–148.

Barlow, G. W. 1961. Causes and significance of morphological variation in fishes. *Syst. Zool.* 25:105–117.

Belding, D. L. 1930. The soft-shell clam fishery of Massachusetts. Mass. Dept. Conserv., Div. Fish. Game, Mar. Fish. Sect., Mar. Fish., Ser. 1, 65 p.

Bliss, C. I. 1967. Statistics in biology. Vol. 1. McGraw-Hill Book Company, New York, N.Y. 558 p.

Bliss, C. I. 1970. Statistics in biology. Vol. 2. McGraw-Hill Book Company, New York, N.Y. 639 p.

Bock, R. D. 1975. Multivariate statistical methods in behavioral research. McGraw-Hill Book Company, New York, N.Y. 623 p.

Brousseau, D. J. 1979. Analysis of growth rates in *Mya arenaria* using the von Bertalanffy equation. *Mar. Biol.* 51:21–227.

Brousseau, D. J. & J. A. Baglivo. 1987. A comparative study of age and growth in *Mya arenaria* (soft-shell clam) from three populations on Long Island Sound. *J. Shellfish Res.* 6(1):17–24.

Casselman, J. M., J. J. Collins, E. J. Crossman, P. C. Ihssen & G. R. Spangler. 1981. Lake whitefish (*Coregonus clupeaformis*) stocks of Ontario waters of Lake Huron. *Cn. J. Fish. Aquat. Sci.* 38:1771–1789.

Dobzhansky, Th. 1951. Genetics and the origin of species, 3rd Ed. Columbia University Press, New York, N.Y. p. xi + 364 p.

Drinkwater, K., B. Petrie & W. H. Sutcliffe, Jr. 1979. Seasonal geostrophic volume transports along the Scotian Shelf. *Estuarine and Coastal Mar. Sci.* 9:17–27.

Falconer, D. S. 1972. Introduction to quantitative genetics. Oliver and Boyd, Edinburg, U.K. 365 p.

Godin, G. 1968. The 1965 current survey of the Bay of Fundy - A new analysis of the data and an interpretation of results. Man. Rep. Ser., No. 8, Mar. Sci. Br., Energy, Mines and Resources, Ottawa. 29 p.

Gould, S. J. & R. F. Johnston. 1972. Geographic variation. *Ann. Rev. Ecol. Syst.* 3:457–498.

Greenberg, D. A. 1983. Modeling the mean barotropic circulation in the Bay of Fundy and Gulf of Maine. *J. Phys. Oceanogr.* 13(5):886–904.

Gulland, J. A. 1969. Manual of methods for fish stock assessment. Part. I. Fish population analyses. FAO Man. Fish. Sci., 4, FRS/M4, FAO, Rome. 154 p.

Harris, R. A. 1975. A primer of multivariate statistics. Academic Press, New York, N.Y. 332 p.

Ihssen, P. E., H. E. Bodre, J. M. Casselman, J. M. McGlade, N. R. Payne & F. M. Utter. 1981. Stock identification: materials and methods. *Can. J. Fish. Aquat. Sci.* 38:1838–1855.

Johnson, C. R., D. A. Ratkowsky & R. W. G. White. 1983. Multivariate analysis of phenotipic relationships of the species of *Paraglaxias* and *Galaxies* (Pisces: Galaxiide) in Tasmania. *J. Fish. Biol.* 23:49–63.

Johnson, R. A. & D. W. Wichern. 1982. Applied multivariate statistical analyses. Prentice-Hall, Inc., Englewood Cliffs, N.J. 594 p.

Kshirsagar, A. M. 1972. Multivariate analysis. Marcel Dekker, Inc., New York, N.Y. 534 p.

Libosvarsky, J. & Z. Kux. 1982. Multivariate analysis of five morphometric traits in the genus *Gobio*. *Folia Zool.* 31:83–92.

Li, C. C. 1958. Population genetics. The University of Chicago Press, Chicago, Illinois. 366 p.

Li, J. C. R. 1964. Statistical Inference, Vol. II. Edwards Brokers, Inc., Ann Arbor, Michigan. 575 p.

MacDonald, B. A. & M. L. H. Thomas. 1980. Age determination of the soft-shell clam *Mya arenaria* using shell internal growth lines. *Mar. Biol. (Berl.)* 58:105–109.

Marr, J. C. 1955. The use of morphometric data in systematic racial and relative growth studies. *Copeia* 1955(1):23–31.

Misra, R. K. & J. E. Carscadden. 1987. A multivariate analysis of morphometrics to detect differences in populations of capelin (*Mallotus villosus*). *J. Cons. Int. Explor. Mer.* 43:99–106.

Misra, R. K. & I-H. Ni. 1983. Distinguishing beaked redfish, *Sebastes mentella* and *S. fasciatus*, by discriminant analysis (with covariance) and multivariate analysis of covariance. *Can. J. Fish. Aquat. Sci.* 40:1507–1511.

Morrison, D. F. 1976. Multivariate statistical methods. McGraw-Hill, New York, N.Y. 415 p.

Newcombe, C. L. 1935. Growth of *Mya arenaria* L. in the Bay of Fundy Region. *Can. J. Res.* 13(6):97–137.

Newcombe, C. L. 1936. A comparative study of the abundance and the rate of growth of *Mya arenaria* in the Gulf of St. Lawrence and Bay of Fundy. *Ecology* 17(3):418–428.

Newcombe, C. L. & H. Kessler. 1936. Variations in growth indices of *Mya arenaria* L. on the Atlantic coast of North America. *Ecology* 17(3):429–443.

Newell, C. R. & H. Hidu. 1982. The effects of sediment type on growth rate and shell allometry in the soft-shell clam *Mya arenaria* L. *J. Exp. Mar. Biol. Ecol.* 65:285–295.

Pimentel, R. A. 1979. Morphometrics. Kendall/Hunt Publishing Company, Dubuque, IA. 276 p.

Reyment, R. A., R. E. Blackith & N. A. Campbell. 1981. Multivariate morphometrics. Academic Press, New York, N.Y. 233 p.

Royce, W. F. 1964. A morphometric study of yellowfin tuna (*Thunnus albacares* (Bonnaterre)). *Fish. Bull.* 63(2):395–443.

Sharp, J. C., K. W. Able, W. C. Leggett & J. E. Carscadden. 1978. Utility of meristics and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters. *Can. J. Fish. Aquat. Sci.* 35:124–130.

Sinclair, M., R. K. Mohn, G. Robert & D. L. Roddick. 1985. Considerations for the effective management of Atlantic scallops. *Can. Tech. Rep. Fish. Aquat. Sci.* 1382:vii + 113 p.

Snedecor, G. W. & W. G. Cochran. 1967. Statistical methods. Iowa State University Press, Ames, Iowa. 593 p.

Srivastarva, M. S. & E. M. Carter. 1983. An introduction to applied multivariate statistics. North-Holland, N.Y. 349 p.

Swan, E. F. 1952. The growth of the clam *Mya arenaria* as affected by the substratum. *Ecology* 33:530–534.

Thorpe, R. S. 1976. Biometric analysis of geographic variation and racial affinities. *Biol. Rev.* 51:407–452.

Todd, T. N., G. R. Smith & L. E. Cable. 1981. Environmental and genetic contributions to morphological differentiation in ciscoes (Coregoninae) of the Great Lakes. *Can. J. Fish. Aquat. Sci.* 38:59–67.

Turner, H. J., Jr. 1948. Report on investigations of the propagation of the soft-shell clam, *Mya arenaria*. Div. Mar. Fish. Dept. Conservation, Commonwealth of Mass., Boston, No. 462:11–42.

Journal of Shellfish Research, Vol. 8, No. 2, 399–405, 1989.

FOOD VALUE OF EURYTOPIC MICROALGAE TO BIVALVE LARVAE OF *CYRTOPLEURA COSTATA* (LINNAEUS, 1758), *CRASSOSTREA VIRGINICA* (GMELIN, 1791) AND *MERCENARIA MERCENARIA* (LINNAEUS, 1758)

ANTONIETO TAN TIU,[1] DAVID VAUGHAN, THOMAS CHILES,[2] AND KIMON BIRD

Harbor Branch Oceanographic Institution
Division of Applied Biology
5600 Old Dixie Highway
Fort Pierce, Florida 34946 USA

ABSTRACT Food values of eurytopic microalgae obtained from Solar Energy Research institute were examined by measuring larval development and growth of the bivalve species *Crassostrea virginica* (American oyster), *Cyrtopleura costata* (angel wing clam) and *Mercenaria mercenaria* (hard clam). Unialgal batch cultures of microalgae *Ellipsoidon* sp. (strain Ellip1), *Nannochloris* sp. (strain Nanno2), *Chaetoceros muelleri* Lemmermann (strain Chaet14) and *Isochrysis* aff. *galbana* Green (strain T-Iso) were grown at 30°C using 30 ppt salinity ocean water enriched with f/2 medium, silicate and urea. Larval feeding experiments were conducted in 30°C and 25 ppt salinity water. Shell length (growth) varied depending on the type of diet offered to the larvae. Whereas the number of metamorphosed larvae seems positively correlated with shell length, percent survival was not. Among the unialgal diets, *I.* aff. *galbana* and *Ellipsoidon* sp. were comparable to each other in supporting the greatest growth in *M. mercenaria* and *C. virginica*. However, *I.* aff. *galbana* supported better growth of *C. costata* larvae than *Ellipsoidon* sp. *Isochrysis*. aff. *galbana* and *Ellipsoidon* sp. were the only unialgal diets that supported metamorphosis in these bivalves within the experimental period. *Chaetoceros muelleri* and *Nannochloris* sp. could be used as feeds for larvae of these three bivalve species only when combined with other microalgae. The mixed diet consisting of equal numbers of the species *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* was found to be of greatest value, based on its overall capacity to support growth and metamorphosis of larvae of all three bivalve species. Moreover, its capacity to promote larval development may be attributed to balanced nutrients as suggested by the combined chemical composition.

KEYWORDS: Food value, food chemistry, microalgae, *Isochrysis* aff. *galbana, Nannochloris, Ellipsoidon, Chaetoceros muelleri, Cyrtopleura costata, Crassostrea virginica, Mercenaria Mercenaria*, bivalve larvae

INTRODUCTION

Since unicellular microalgae were recognized as a food source for bivalve larvae about half a century ago (Cole 1937, Bruce et al., 1939), few algal species have been shown to support complete larval development in pelecypods. One of the best diets discovered was *Isochrysis galbana* Parke. Unfortunately, *I. galbana* among other temperate microalgae is intolerant of high culture temperatures (Ukeles 1961). Loss of viability at 27°C has been reported in *I. galbana* by Ukeles (1961). Therefore, its usefulness is limited in tropical or subtropical hatcheries.

As interest in mariculture in tropical and subtropical regions grew, scientists obtained *Isochrysis galbana* and other temperate strains for trials with tropical molluscs. Laboratory studies showed their suitability as feeds for tropical molluscan larvae and juveniles. It was not until commercial hatchery operations were commenced that the problem of temperature tolerance with these strains emerged. Tropical hatcheries operate in warmer climates of the world. Typically, temperatures in such hatcheries vary seasonally between 14–40°C. Temperature-related problems are pronounced in summer months, where temperatures can range from 26–44°C.

To date, *Isochrysis* aff. *galbana* clone T-Iso is the only warm-water adapted phytoplankton strain available to many tropical hatcheries. This strain has been found to be a good food source for some molluscs such as *Mercenaria mercenaria* L. and *Tapes semidecussata* Reeve, but was less than satisfactory for oysters, *Crassostrea gigas* Thunberg and *C. rhizophorae* Guilding (Helm and Laing 1987). It appears obvious that the availability of phytoplankton strains for use in tropical hatcheries needs to be increased.

The Solar Energy Research Institute is engaged in fuel production from microalgae and has identified and compiled a list of microalgal strains that have a high energy yield, and a wide range of pH, temperature, salinity and light intensity tolerances (Ewart and Pruder 1981, Barclay et al. 1986, 1987, Carlson et al. 1986). Although such eurytopic microalgae were primarily selected for their potential use in biomass fuel production, they are also potentially valuable food sources either as alternatives or supplements to temperate microalgae in tropical or subtropical hatcheries. This manuscript reports the food values of four microalgal species (Solar Energy Research Institute collection) fed individually or in mixtures to larvae of three species of bivalves under tropical experimental conditions (i.e. both microalgae and larvae were cultivated at 30°C). The microalgal species tested were *Chaetoceros muelleri* Lemmermann (strain Chaet14), *Ellipsoidon* sp. (strain Ellip1), *Isochrysis* aff. *galbana* Green (strain T-Iso) and *Nannochloris* sp. (strain Nanno2). The bivalve species used

[1]Current address: Department of Biology, Indiana University of Pennsylvania, 114 Weyandt Hall, Indiana, PA, 15705-1090, USA.
[2]Current address: The University Hospital, Boston University Medical Center, 75 East Newton St., E-501, Boston, MA 02118, USA.

in the feeding experiments were the American oyster *Crassostrea virginica* Gmelin, the angel wing clam *Cyrtopleura costata* L. and the hard clam *Mercenaria mercenaria* L.

MATERIALS AND METHOD

Algae

Ten microalgal strains, ranging in cell size from 4 to 10 μm (maximum linear dimension), were obtained from the Solar Energy Research Institute microalgae collection. Unialgal batch cultures of these microalgae were grown in Fernbach flasks filled with 1.5 L of sterile (autoclaved for 25 minutes at 121°C and 1.05 kg/cm^2 pressure) ocean water of 30 ± 1 ppt salinity. The ocean water was collected from the Atlantic Ocean fronting the Florida Institute of Technology Vero Laboratory, Florida. The ocean water was enriched with f/2 medium, silicate (Guillard and Ryther 1962, Guillard 1975) and urea (final concentration = 0.05 mg urea in 1 L culture water). Culture temperature was maintained at 30 ± 1°C using an incubator. Cultures were illuminated (photosynthetic photon flux fluence rate = 2.0 × 10^{15} − 4.4 × 10^{15} quanta/s-cm^2) using six Sylvania F/15T12/CW fluorescent bulbs on a 14th light: 10h dark cycle. Pairs of fluorescent bulbs were placed about 30 cm apart vertically in the incubator.

Microalgae grown in Fernbach flasks were used in preliminary feeding experiments with larval *Mercenaria* (Tan Tiu and Vaughan 1988) if they attained a density of one million cells per ml in eight days and were either motile or easily resuspended in the water column after mechanical agitation. Microalgal strains that allowed metamorphosis to occur in *Mercenaria* (Tan Tiu and Vaughan 1988) were used in feeding experiments reported here. The strains and dimensions of the microalgae, whose food values were investigated for three species of bivalve larvae are: *Chaetoceros muelleri* Lemmermann (Chaet14, 6 × 4 μm), *Ellipsoidon* sp. (Ellipp1, 4–8 × 2 μm), *Isochrysis* aff. *galbana* Green (T-Iso, 7–5 × 4 μm) and *Nannochloris* sp. (Nanno2, 4 μm).

For analyses of gross biochemical constituents, microalgae were cultured in duplicate Fernbach flasks as described above, centrifuged at 6,000 g for 10 minutes (4.0°C), rinsed and resuspended in 3.2% (w/v) aqueous ammonium formate. The resuspended cells were rapidly frozen in a dry-ice acetone bath and either lyophilized immediately or stored frozen at −40°C. Total cellular carbohydrate was determined using the modified phenol-sulfuric acid method with glycogen as a standard (Dubois et al. 1956). Cellular protein was determined by the method of Lowry et al. (1951) using a bovine serum albumin standard. Ash content was determined by combusting pre-dried microalgae (dried to constant weight at 60°C) at 550°C for 2 h. Such combustion temperature and duration was empirically determined to be within optimal range. Triplicate samples of *Chaetoceros muelleri* combusted at 30 min intervals for 4 h indicated no appreciable change in ash free dry weight from 2 to 4 h.

The nutritional component estimated by subtracting the sum of protein and carbohydrate contents from the ash free dry weight was presumed to be lipid. Protein, lipid and carbohydrate contents were expressed as proportions (%) of dry weight. The equivalent calories of the three nutritional components were calculated using the factors 5.65, 4.10 and 9.45 cal/mg for protein, carbohydrate and lipid respectively (Crisp 1971) and expressed per million cells. Values of chemical composition of mixed diets were calculated from those of unialgal diets.

Bivalves

Adult bivalves were collected from various Indian River Lagoon habitats in Florida. The angel wing clams, *Cyrtopleura costata*, were collected from Cook Point, St. Lucie County, whereas, the American oysters, *Crassostrea virginica*, and the hard clams *Mercenaria mercenaria* and *M. m. notata* Say, were harvested from Harbor Branch Oceanographic Institution cultivated clams in Sebastian, Indian River County. Some *C. virginica* were also collected from Link Port, St. Lucie County. The bivalves were spawned on the day of collection or the following day. The bivalves were induced to spawn by fluctuating temperature (22° to 29°C) or addition of sperm suspension according to the method described by Loosanoff and Davis (1963). Spawning male and female bivalves were transferred into separate containers. Eggs of several females were thoroughly mixed and fertilized by sperm from several males. In hard clams, eggs of *M. mercenaria* were fertilized by sperm from *M. m. notata* (Chanley 1961). About 100,000 to 200,000 fertilized eggs were placed in each of three Nalgene cylindrical vessels containing 15 L aerated and filtered Indian River Lagoon water of 25 ± 1 ppt salinity and 30 ± 1°C (density = 6–13 eggs/ml). Larvae were allowed to develop for 22 ± 1 h into straight-hinge stage before starting the feeding experiments. All larval culture water was maintained at 30 ± 1°C using a water bath, and illuminated with Sylvania F/15T24/CW fluorescent bulbs (photosynthetic photon flux fluence rate = 3.0 × 10^{14} − 4.0 × 10^{14} quanta/s · cm^2) on a 12 h light: 12 h dark cycle. All seawater used was ultimately filtered using a Millipore filter of 0.45 μm pore diameter after prefiltration that included a charcoal filter and exposure to uv light.

Feeding experiments were conducted in 1 L Nalgene beakers. Each treatment was run in quadruplicate. Larval density in each beaker was adjusted to 800 larvae per 800 ml (density = 1 larva/ml) of filtered but unaerated water of 25 ± 1 ppt salinity and 30 ± 1°C. The microalgae used in the feeding experiments were harvested at stationary growth phase and cell density was determined using a haemacytometer. The amount fed was adjusted initially to 25,000 cells per ml of larval culture water (25,000 cells/larva). Algal species were offered to the larvae either as a

unialgal diet or as a mixed diet once every two days from day 1 to day 9 for *M. mercenaria*, and day 1 to day 17 in *C. virginica* and *C. costata*, after the complete renewal of larval culture water. The mixed diet consisted of equal proportions, based on cell number, of its algal components at a total concentration of 25,000 cells/ml. Non-fed larvae as well as larvae fed with *Isochrysis* aff. *galbana* were run with other treatments in all feeding experiments.

One hundred to 200 larvae were sampled from each well-mixed replicate, prior to water renewal, once every two days for *Mercenaria mercenaria* or once every four days for *Crassostrea virginica* and *Cyrtopleura costata*. The number of surviving larvae was counted, and the maximum anterior posterior lengths of 30 larvae were measured for each of the four replicates. Only the data of the final sampling dates are presented here. The final average shell lengths (= growth) of each treatment for each experiment were compared by Model I ANOVA. If the ANOVA were significant, Student-Newman-Keuls (SNK) Tests were carried out on the data. F and Q statistics were evaluated at the 0.05 significance level. The percentage survival of each treatment for each experiment was likewise compared statistically (as in shell length data) after arcsine transformation. For experiment CC2, one of two experiments using *C. costata*, data on the 9th day were used instead of the final sampling date because of ciliate contamination on the latter date.

The metamorphosed larvae of *C. virginica* and *C. costata* were detected by their dissoconch shell. The numbers of metamorphosed larvae in *C. virginica* and *C. costata* were counted. The number of eyed-larvae in *C. virginica* was also counted. These larval stages were all expressed as percentages. The dissoconch shell of *M. mercenaria* is difficult to distinguish from its prodissoconch shell. Moreover, the larval foot is difficult to discern in a preserved hard clam specimen. Therefore, only qualitative data were obtained for the final stages of larval development in *M. mercenaria*.

RESULTS

Larval Feeding Experiments

Shell Length

Larvae of *Crassostrea virginica*, *Cyrtopleura costata* and *Mercenaria mercenaria* fed different diets showed significantly different mean shell lengths as indicated by one-way ANOVA. The mean shell lengths on the final sampling dates and their groupings as determined by SNK tests are summarized in Table 1.

Among diets consisting of individual species, *Isochrysis* aff. *galbana*, *Ellipsoidon* sp. and *Chaetoceros muelleri* supported larval growth, while *Nannochloris* sp. did not support growth in any of the three bivalve species. *Ellipsoidon* sp. was equally as good as *I.* aff. *galbana* in supporting larval growth in *Mercenaria mercenaria*, *Crassostrea virginica* but not *Cyrtopleura costata* (Table 1).

Diets composed of more than one species can enhance growth (Table 1). For example, *Mercenaria mercenaria* fed on diets consisting of the three species *Chaetoceros muelleri*, *Ellipsoidon* sp. and *Isochrysis* aff. *galbana* or *Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp. or the diet consisting of all four species *C. muelleri*, *Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp., exhibited more rapid growth than on any of the component species of unialgal diets. Of the mixed diets, the three-species diet consisting of *C. muelleri*, *Ellipsoidon* sp. and *I.* aff. *galbana* was the most advantageous. It was as good as or better than the best unialgal diets in supporting growth in all three bivalve species (Table 1).

Survival

Data on survival of non-fed larvae were variable. Whereas all fed larvae showed some survival until the final sampling dates, this was not the case for all non-fed larvae. The non-fed larvae of *Mercenaria mercenaria* in both experiments MM1 and MM2, *Crassostrea virginica* in experiment CV1 and *Cyrtopleura costata* in experiment CC1 survived until the final sampling date. In contrast, non-fed larvae of *C. virginica* in experiment CV2 and *C. costata* in experiment CC2 were all dead on the 13th or 9th day, respectively, after fertilization.

Whereas the analysis of variance of the survival percentages of bivalve larvae indicated significant differences among treatments in all experiments, the Student-Newman-Keuls test failed to differentiate among treatments in experiment CV1 (Table 1). Moreover, survival of larvae did not seem to correlate with either unialgal or mixed diets offered.

Metamorphosis

Crassostrea virginica larvae fed unialgal diets of *Chaetoceros muelleri*, *Isochrysis* aff. *galbana* or *Ellipsoidon* sp. developed into eyed-larvae, but not those fed with *Nannochloris* sp. nor the non-fed larvae. *Ellipsoidon* sp. and *I.* aff. *galbana* supported metamorphosis in *C. virginica* of 1.3% and 5.0%, respectively. Doubling the amount of *I.* aff. *galbana* offered to *C. virginica* quintupled the number of metamorphosed larvae. *Ellipsoidon* sp. was the only unialgal diet that allowed metamorphosis to occur in *Cyrtopleura costata* larvae.

Of the mixed diets composed of three or four species, the three-species diet composed of *C. muelleri*, *Ellipsoidon* sp. and *I.* aff. *galbana* yielded 23.6%, while the four species diet composed of *C. muelleri*, *Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp. yielded 4.9% metamorphosed larvae in *C. virginica*. The high percentage of metamorphosed larvae in *C. virginica* fed on the three-species diet *C. muelleri*, *Ellipsoidon* sp. and *I.* aff. *galbana* may be

TABLE 1.

Average shell lengths (μm) and arcsine transformed survival percentages (p′) of bivalve larvae fed on different algal diets on subsequent times (days) after fertilization. p′ = arcsin $\sqrt{p}$, where p = proportion of surviving larvae. Averages with superscripts of similar letters are not significantly different according to Student-Newman-Keuls test (α = 0.05). Feeding ration was based initially on 25,000 cells/larva, unless otherwise indicated. md = missing data, nd = no data, n = number of replicates (beakers).

Diet	*Mercenaria mercenaria* Length	n	Survival	n	*Crassostrea virginica* Length	n	Survival	n	*Cyrtopleura costata* Length	n	Survival	n
	Experiment MM1 (Day 9)				*Experiment CV1 (Day 17)*				*Experiment CC1 (Day 17)*			
C. muelleri	155^{D}	4	60^{AB}	4	192^{B}	4	46^{A}	4	120^{C}	4	58^{C}	4
Ellipsoidon sp.	160^{CD}	4	79^{A}	4	243^{A}	4	68^{A}	4	205^{AB}	4	56^{CD}	4
I. aff. *galbana*	160^{CD}	4	65^{AB}	4	236^{A}	4	57^{A}	4	232^{A}	4	76^{AB}	4
Nannochloris sp.	99^{E}	4	66^{AB}	4	96^{C}	4	43^{A}	4	80^{D}	4	42^{D}	4
Not Feed	95^{E}	3	70^{AB}	3	120^{C}	4	42^{A}	4	77^{D}	4	54^{CD}	4
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana*	198^{A}	3	67^{AB}	3	247^{A}	3	68^{A}	4	240^{A}	4	82^{A}	4
C. muelleri *Ellipsoidon* sp. *Nannochloris* sp.	170^{BC}	4	76^{A}	4	229^{A}	4	66^{A}	3	182^{B}	4	67^{BC}	4
C. muelleri *I.* aff. *galbana* *Nannochloris* sp.	149^{D}	3	13^{B}	4	180^{B}	4	50^{A}	4	224^{A}	4	41^{D}	4
Ellipsoidon sp. *I.* aff. *galbana* *Nannochloris* sp.	177^{B}	4	45^{B}	4	189^{B}	4	60^{A}	4	175^{B}	4	69^{BC}	4
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana* *Nannochloris* sp.	179^{B}	4	47^{AB}	4	238^{A}	3	63^{A}	3	242^{A}	4	60^{C}	4
Error Mean Square	38.9		620.8		413.4		107.2		351.7		54.6	
I. aff. *galbana*	186^{A}	4	61^{AB}	4	198^{AB}	4	70^{A}	4	161^{A}	4	29^{B}	4
Not Feed	117^{D}	4	69^{A}	4	md	md	0^{B}	4	md	md	0^{C}	4
C. muelleri *Ellipsoidon* sp.	147^{C}	4	48^{ABC}	4	163^{BC}	4	70^{A}	4	151^{A}	3	64^{A}	3
C. muelleri *I.* aff. *galbana*	175^{AB}	4	52^{ABC}	4	201^{AB}	4	78^{A}	4	191^{A}	4	58^{AB}	4
C. muelleri *Nannochloris* sp.	133^{C}	4	36^{C}	4	135^{C}	4	60^{A}	4	88^{B}	4	44^{A}	4
Ellipsoidon sp. *I.* aff. *galbana*	185^{A}	4	64^{AB}	4	203^{AB}	4	66^{A}	4	55^{B}	4	34^{A}	4
Ellipsoidon sp. *Nannochloris* sp.	145^{C}	4	53^{ABC}	4	164^{BC}	4	70^{A}	4	73^{B}	4	51^{AB}	4
I. aff. *galbana* *Nannochloris* sp.	166^{B}	4	37^{C}	4	133^{C}	4	74^{A}	4	164^{A}	4	35^{A}	4
I. aff. *galbana* 50,000 cells/ml	195^{A}	4	63^{AB}	4	214^{A}	4	75^{A}	4	157^{A}	4	71^{A}	4
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana*	183^{A}	3	39^{BC}	4	211^{A}	4	78^{A}	4	nd	nd	nd	nd
Ellipsoidon sp. 50,000 cells/mL	nd	nd	nd	nd	nd	nd	nd	nd	71^{B}	3	61^{A}	3
Error Mean Square	79.9		112.9		397.6		85.8		491.1		210.8	

misleading because metamorphosis occurred in only one of four replicates. In *C. costata*, the four-species diet composed of *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana Nannochloris* and the three-species diet composed of *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana* and *C. muelleri, I.* aff. *galbana, Nannochloris* sp. produced 12.8, 0.5 and 0.4% metamorphosed larvae, respectively.

Metamorphosed larvae of *Mercenaria mercenaria* were difficult to discern in preserved specimens and therefore were not quantified. Nevertheless, qualitative observations

suggested that the three-species diet composed of *C. muelleri, Ellipsoidon* sp., and *I.* aff. *galbana* yield the highest proportion of pediveligers in hard clams.

Microalgal Composition

The gross chemical composition of microalgae and their equivalent calories are shown in Table 2. Among the unialgal diets, *Isochrysis* aff. *galbana* had the highest lipid content, while *Chaetoceros muelleri* had the highest protein and ash contents. *Ellipsoidon* sp. had the highest carbohydrate content, while *Nannochloris* sp. had the lowest protein content (Table 2). Total caloric equivalent per million microalgal cells was greatest in *I.* aff. *galbana* and lowest in *Ellipsoidon* sp. The major food component that contributed to the total caloric content in all four microalgae *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp. was presumed to be lipid (Table 2).

DISCUSSION

Planktotrophic larvae of *Crassostrea virginica, Cyrtopleura costata* and *Mercenaria mercenaria* rely on external food sources to nourish most of their development because their eggs have limited amounts of yolk. In our experiments, the energy and nutrients associated with *Ellipsoidon* sp. seem sufficient to support larval growth in *M. mercenaria, C. virginica* but not *C. costata*. The total caloric equivalent of *Ellipsoidon* sp. is 9 times lower than that of *I.* aff. *galbana*, yet it was as good as *I.* aff. *galbana* in supporting growth in *M. mercenaria* and *C. virginica*. More-

TABLE 2.

Gross chemical composition of different diets. All values are from 2 replicate cultures except for the carbohydrate contents of *C. muelleri* and *Ellipsaidon* sp., the protein content of *Ellipsaidan* sp. and "other" content of *C. muelleri* (1 replicate). "Other" was presumably lipid and was calculated from the difference between ash free dry weight and the sum of protein and carbohydrate in each diet. Equivalent calories were calculated using the factors 5.65, 4.10, 9.45 cal/mg for protein, carbohydrate and lipid respectively (Crisp, 1971). All values for mixed diets were calculated from unialgal diets.

Diet	Dry Weight ($\mu g/10^6$ cells)	Ash (percentage of dry weight)	Protein (percentage of dry weight)	Carbohydrate (percentage of dry weight)	"Other" (percentage of dry weight)	Protein (calories/10^6 cells)	Carbohydrate (calories/10^6 cells)	"Other" (calories/10^6 cells)	Total (calories/10^6 cells)
C. muelleri	118	19.76	32.20	20.33	27.71	0.21	0.10	0.31	0.62
Ellipsoidon sp.	17	12.50	29.40	35.30	22.90	0.03	0.02	0.04	0.09
I. aff. *galbana*	118	16.53	11.40	9.80	62.20	0.08	0.05	0.69	0.82
Nannochloris sp.	59	10.14	7.60	22.50	59.70	0.03	0.05	0.33	0.41
C. muelleri *Ellipsoidon* sp.	68	16.13	30.80	27.82	25.31	0.12	0.06	0.18	0.36
C. muelleri *I.* aff. *galbana*	118	18.15	21.80	15.07	44.96	0.15	0.08	0.50	0.72
C. muelleri *Nannochloris* sp.	89	14.95	19.90	21.42	43.71	0.12	0.08	0.32	0.52
Ellipsoidon sp. *I.* aff. *galbana*	68	14.52	20.40	22.55	42.55	0.06	0.04	0.37	0.46
Ellipsoidon sp. *Nannochloris* sp.	38	11.32	18.50	28.90	41.30	0.03	0.04	0.19	0.25
I. aff. *galbana* *Nannochloris* sp.	89	13.34	9.50	16.15	60.95	0.06	0.05	0.51	0.62
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana*	84	16.26	24.33	21.81	37.60	0.11	0.06	0.35	0.51
C. muelleri *Ellipsoidon* sp. *Nannochloris* sp.	65	14.13	23.07	26.04	36.77	0.09	0.06	0.23	0.37
C. muelleri *I.* aff. *galbana* *Nannochloris* sp.	98	15.48	17.07	17.54	49.87	0.11	0.07	0.44	0.62
Ellipsoidon sp. *I.* aff. *galbana* *Nannochloris* sp.	65	13.06	16.13	22.53	48.27	0.05	0.04	0.35	0.44
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana* *Nannochloris* sp.	78	14.73	20.15	21.98	43.13	0.09	0.06	0.34	0.49

over, some diet mixtures such as the three-species diet composed of *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* whose total caloric content (Table 2) were also lower than *I.* aff. *galbana,* supported comparable growth (Table 1) suggesting that the total energy demand of the larvae was satisfied at an energy level lower than that contained in *I.* aff. *galbana,* and that some factors in addition to energy content of food were responsible for promoting growth. Davis and Guillard (1958) observed that there was little difference in growth rate of *M. mercenaria* larvae (density = 10–15 larvae/ml) over the range of 50,000 to 400,000 *Isochrysis galbana* Parke cells per ml larval culture water. Riisgard (1988) predicted that the minimum algal concentration for maximum growth in *M. mercenaria* veligers is 40,000 to 60,000 *I. galbana* cells per ml larval culture water (larval density = 11–18 larvae/ml). Increasing the number of *Ellipsoidon* sp. cells offered to *M. mercenaria* from 25,000 to 525,000 per larva (Tan Tiu and Vaughan, unpubl. data) did not increase larval growth suggesting that sufficient energy for growth was obtained from the 25,000 *Ellipsoidon* sp. cells. Doubling the number of *I.* aff. *galbana* cells offered to the larvae of *C. virginica* (experiment CV2) and *C. costata* (experiment CC2) from 25,000 to 50,000 also did not improve their growth. These suggest once again that in our experiments, the initial ration of 25,000 cells per larva was sufficient for larval growth. Note however that the number of *C. virginica* larvae that metamorphosed increased five folds when the number of *I.* aff. *galbana* offered was doubled. This suggests a limiting factor that influences metamorphosis more than the growth of shell length. Doubling the number of *Ellipsoidon* sp. offered to *C. costata* (experiment CC2) did not improve larval growth relative to those fed on *I.* aff. *galbana,* suggesting the relative inferiority of *Ellipsoidon* sp. as feed for *C. costata.*

According to Webb and Chu (1983), the total concentration of cellular protein within algal cells may be important in determining the quality of food. The low amount of protein in *Nannochloris* sp. (Table 2) may be responsible for its poor food quality. The negative effect of *Nannochloris* sp. was diminished when its concentration was decreased to one fourth as in the four-species diet composed of *Chaetoceros muelleri, Ellipsoidon* sp., *Isochrysis* aff. *galbana* and *Nannochloris* sp. Thus, the positive effect possibly due to the balance micronutrients in this four-species diet overcame the negative effect of *Nannochloris* sp., allowing *C. virginica* and *C. costata* larvae fed on such diet to grow and metamorphose. Dupuy (1975) also observed that *Nannochloris oculata* when fed in combination with *Pyramimonas virginica* Pennick, *Pseudoisochrysis paradoxa* (F. Ott, nom. nud.), and *Chrysophaeropsis planktonicus* (Dupuy, nom. prov.) yielded eyed-larvae in *C. virginica* in 11 to 14 days.

The poor food value of some green algae including several species of *Nannochloris* sp. has been attributed to their indigestibility. *Nannochloris* sp., being a chlorophyte, may possess a cell wall; however, since we performed our experiments at 30°C, the cell wall may not have presented a digestibility problem. According to Davis and Calabrese (1964), *Chlorella autotrophica* Shihira and Krauss [=Chlorella 580, (Walne 1970)] was utilized at higher temperatures (up to 30°C) by both *C. virginica* and *M. mercenaria,* although at lower temperature (about 25°C) its cell wall rendered it indigestible, especially to *C. virginica* larvae (Davis and Guillard 1958, Babinchak and Ukeles 1979). Several other factors that influence the nutritional value of food were reviewed by Ukeles (1975) and Webb and Chu (1983). The food value of *Isochrysis* aff. *galbana* has been tested previously with larvae of *C. virginica* (Ewart and Epifanio 1981), *M. mercenaria* (Helm and Laing 1987, Tan Tiu and Vaughan 1988) and *C. costata* (Cresswell and Schilling 1985, Gustafson et al. 1988). Other than Tan Tiu and Vaughan (1988) however, experimental conditions employed by other researchers differed from ours making comparison difficult.

The three-species diet, composed of equal numbers of *Chaetoceros muelleri, Ellipsoidon* sp. and *Isochrysis* aff. *galbana* was the most advantageous mixed diet for larvae of the three bivalve species. This diet supported rapid larval growth (Table 1) in all three bivalve species. The ability of food mixtures to foster growth has been attributed by others to balanced essential nutrients (Bayne 1983, Webb and Chu 1983). Such good food quality in the three-species diet composed of *Chaetoceros muelleri, Ellipsoidon* sp. and *Isochrysis* aff. *galbana* may likewise be due to its more balanced essential nutrients. In this three-species diet, the lipid content is contributed largely by the *I.* aff. *galbana,* while the protein content is furnished mostly by *C. muelleri* (Table 2). *Ellipsoidon* sp. provided the main source of carbohydrate. Additional factors that could influence the food value of these microalgae for these three bivalve species are currently uncertain.

We have yet to find a unialgal diet that can surpass the performance of *Isochrysis* aff. *galbana* in supporting larval growth in *Crassostrea virginica, Cyrtopleura costata* and *Mercenaria mercenaria* under tropical conditions. Nevertheless, we have shown that among the unialgal diets, *Ellipsoidon* sp. can be as good as *I.* aff. *galbana* in supporting larval growth and metamorphosis in *M. mercenaria* and *C. virginica.* The other two microalgae, *Chaetoceros muelleri* and *Nannochloris* sp. can be used as food for these bivalve larvae in some diet mixtures described above. Some of these mixed diets were as good as and/or better than *I.* aff. *galbana* as feeds for bivalve larvae. The performance of the three-species diet composed of *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana,* and the unialgal diet of *Ellipsoidon* sp. as feeds for bivalve larvae should be explored further. As these strains all grow at temperature up to 35°C, they may find great utility in hatcheries located in subtropical or tropical countries.

ACKNOWLEDGMENTS

We are grateful to Dr. William Lellis for reviewing the manuscript and helping with statistical analyses. Suggestions of the two anonymous reviewers and Dr. Sandra E. Shumway substantially improved the presentation of this manuscript. The help of DAB staffs in the collection of broodstocks, maintenance of experimental facilities, and discussions is greatly appreciated. We also extend thanks to the Solar Energy Research Institute, Golden Co., for providing reports and microalgal strains. This study was supported by a Harbor Branch Postdoctoral Fellowship to A.T.T. Harbor Branch Oceanographic Institution, Inc. publication contribution number 720.

REFERENCES

Barclay, W., J. Johansen, P. Chelf, N. Nagle, P. Roessler & P. Lemke. 1986. Microalgae Culture Collection 1986–1987. Prepared by Solar Energy Research Institute Research Group for the U.S. Department of Energy. Contract No. DE-AC02-83CH10093.

Barclay, W., K. Terry, N. Nagle, J. Weissman & R. Goebel. 1987. Potential of new strains of marine and inland saline-adapted microalgae for aquaculture. *J. World Aquacul. Soc.* 18(4):218–228.

Babinchak, J. & R. Ukeles. 1979. Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. *Mar. Biol. (Berlin).* 51:69–76.

Bayne, B. L. 1983. Physiological ecology of marine molluscan larvae. In: K. Wilbur (Ed), The Mollusca, Vol. 3. Academic Press, New York. pp. 299–343.

Bruce, J. R., M. Knight & M. W. Parke. 1939. The rearing of oyster larvae on an algal diet. *J. Mar. Biol. Assoc. U.K.* 24:337–374.

Carlson, R., J. Ryther, P. Pendoley, P. Jensen & M. Blakeslee. 1986. Collection and characterization of saline microalgae from South Florida. Solar Energy Research Institute Final Report. Contract No. XK-4-0436-03.

Chanley, P. E. 1961. Inheritance of shell markings and growth in the hard clam, *Venus mercenaria*. *Proc. Natl. Shellfish. Assoc.* 50:163–169.

Cole, H. A. 1937. Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, with special reference to the food of the larva and spat. *Fish. Invest., London.* Series 2. 15:1–28.

Cresswell, R. L. & M. Schilling. 1985. Hatchery production and nursery culture of the angel wing clam, *Cyrtopleura costata*, a potential bivalve species for culture in subtropical waters. I.C.E.S. Shellfish Committee. CM 1985/F:46.

Crisp, D. J. 1971. Energy flow measurements. In: N. A. Holmes and A. D. McIntyre (Eds). Methods for the Study of Marine Benthos. I.B.P. Handbook No. 16. Blackwell Scientific Publications, Oxford. pp. 284–372.

Davis, H. C. & A. Calabrese. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *Fish. Bull.* 63(3):643–655.

Davis, H. C. & R. R. Guillard. 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *Fish. Bull.* 58:239–304.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rabers & F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350–356.

Dupuy, J. L. 1975. Some physical and nutritional factors which affect the growth and setting of the larvae of the oyster, *Crassostrea virginica*, in the laboratory. In: F. J. Vernberg (Ed), Physiological Ecology of Estuarine Organisms. University of South Carolina Press, Columbia. pp. 319–331.

Ewart, J. W. & C. E. Epifanio. 1981. A tropical flagellate food for larval and juvenile oysters, *Crassostrea virginica* Gmelin. *Aquaculture* 22:297–300.

Ewart, J. W. & G. D. Pruder. 1981. Comparative growth of *Isochrysis galbana* Parke and *Isochrysis* aff. *galbana*, clone T-Iso at four temperatures and three light intensities. *J. World Maricult. Soc.* 12(1):333–339.

Guillard, R. R. 1975. Culture of phytoplankton for feeding marine invertebrates. In: W. L. Smith and M. H. Chanley (Eds), Culture of Marine Animals. Plenum Publishing Corporation, New York. pp. 29–60.

Guillard, R. R. & J. H. Ryther. 1962. Studies on marine plankton diatoms I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8:229–239.

Gustafson, R. G., D. Vaughan & J. H. Ryther. 1988. Experiments in the artificial rearing of the angel wing clam, *Cyrtopleura costata*, a potential aquaculture species. *J. World Aquacult. Soc.* 19(1):34A.

Helm, M. M. & I. Laing. 1987. Preliminary observations on the nutritional value of 'Tahiti *Isochrysis*' to bivalve larvae. *Aquaculture* 62:281–288.

Loosanoff, V. L. & H. C. Davis. 1963. Rearing of bivalve mollusks. In: F. S. Russell (Ed), Advances in Marine Biology. Academic Press, New York. pp. 1–136.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall. 1951. Protein measurement with folin-phenol reagent. *J. Biol. Chem.* 193:265–275.

Riisgard, H. U. 1988. Feeding rates in hard clam (*Mercenaria mercenaria*) veliger larvae as a function of algal (*Isochrysis galbana*) concentration. *J. Shellfish Res.* 7(3):377–380.

Tan Tiu, A. & D. Vaughan. 1988. Food value of tropical microalgae to *Mercenaria mercenaria* larvae; a preliminary study. *J. Shellfish Res.* 7(1):206–207.

Ukeles, R. 1961. The effect of temperature on the growth and survival of several marine algal species. *Biol. Bull.* 120:255–264.

Ukeles, R. 1975. Views on bivalve nutrition. In: K. S. Price, Jr., W. N. Shaw, and K. S. Dunburg (Eds), Proceedings of the First International Conference on Aquaculture Nutrition. University of Delaware, Newark, Delaware. pp. 127–162.

Walne, P. R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus*. *Fish. Invest.*, Series 2. 26:1–62.

Webb, K. L. & F. L. Chu. 1983. Phytoplankton as food source for bivalve larvae. In: G. D. Pruder, C. Langdon and D. Conklin (Eds), Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge, Louisiana. pp. 272–289.

Journal of Shellfish Research, Vol. 8, No. 2, 407–417, 1989.

ABSTRACTS

PACIFIC COAST OYSTER GROWERS ASSOCIATION

NATIONAL SHELLFISHERIES ASSOCIATION
(Pacific Coast Section)

Forty-third Annual Meeting

September 21–23, 1989

Seattle Radisson Hotel

Seattle, Washington

CONTENTS

AQUACULTURE POTENTIAL OF THE SEA MUSSEL, *MYTILUS CALIFORNIANUS*. **Sylvia Behrens Yamada*** and **J. B. Dunham,** Zoology Department, Oregon State University, Corvallis, Oregon 97331-2914.

The aquaculture potential of the sea mussel, *Mytilus californianus*, was investigated by growing it next to the more traditional food mussel, *M. edulis*, on subtidal longlines in Winchester Bay, OR. For mussels of similar initial length (24–39 mm) *M. californianus* grew 24 mm versus 14 mm for *M. edulis* from April 27 to September 25, 1988. AT the end of the growth trial *M. californianus* contained 2.5 times as much dry meat as *M. edulis*. The condition index (dry meat weight, g × 100/shell volume, ml) was 19 for *M. californianus* versus 11 for *M. edulis*. The results from this and other studies suggest that the aquaculture potential of *M. californianus* merits further investigation.

OYSTER CULTURE IN CUBA. **Neil Bourne,** Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada. V9R 5K6.

Two species of oysters occur in Cuba, the mangrove oyster, *Crassostrea rhizophorae* and the eastern oyster, *C. virginica*. The only species used in commercial fisheries is the mangrove oyster and landings in recent years have averaged about 2,500 tonnes (whole weight). The industry has relied on obtaining natural sets for their seed source and most growout has been by suspended culture in the intertidal area. Growth to commercial size occurs in eight to nine months. Most of the product is shucked and sold fresh or frozen. In 1986 an experimental hatchery was built in the Varadero area of northern Cuba and methods have been developed to produce mangrove oyster seed throughout the year for the industry. In 1989 they intend to produce about 100 million mangrove oyster seed. Plans are now being developed to construct commercial-size oyster hatcheries in Cuba and to increase production over the next few years. In the spring of 1989 I had an opportunity to visit Cuba, observe the oyster industry and discuss past, present and future work with research people there. Some observations of this trip are presented.

EPIZOOTIOLOGY OF HEMIC NEOPLASIA IN *MYTILUS TROSSULUS* WITHIN WASHINGTON STATE. **Kenneth M. Brooks,*** and **Ralph A. Elston,** Battelle Marine Sciences Laboratory, 439 West Sequim Bay Road, Sequim, Washington 98382.

An epizootiological study of hemic neoplasia in feral and cultured populations of *Mytilus trossulus* has been undertaken in Washington State. Feral populations at 67 locations within Washington State have been examined hemocytologically for this disease. A neoplastic index has been developed to characterize the incidence and severity of the disease within a population. This index has been correlated with six indices describing substrate type, circulation, productivity, population density, year classes present, osmotic stress and thermal stress. The disease is ubiquitous within Puget Sound at sites with low circulation and high mussel population densities. On the Pacific coast, the disease was significant only at the Westport Coast Guard Station.

A growth and mortality study at a mussel farm in Puget Sound indicates a cumulative mortality rate of 22% from mid December until mid May 1989 in each of two year classes of mussels. Current challenge experiments suggest that various races of mussels found on the west coast differ in their susceptibility to the disease. The implications of these findings for intensive mussel culture operations in Puget Sound are discussed.

This research has been supported in part by the National Cancer Institute, the U.S. Department of Agriculture and the U.S. Department of Energy under contract DE-AC06-76RL0-1830 to the Battelle Memorial Institute.

GROWTH RATES OF THE MANILA CLAM, (*VENERUPIS JAPONICA*), IN VARIOUS SUBSTRATE: PEBBLE-GRAVEL, SAND, AND MUD-SILT. **Payton W. Carling,*** Olympia Clams Incorporated, Olympia, Washington 98502.

Manila clams were measured for shell length, weight, and volume. The clams went into cages and were placed at the +2 tidal height in three different beaches in Eld inlet, Southern Puget Sound. The substrate composition of the three beaches differed in particle size, (pebble-gravel, sand, mud-silt). Substrate was also transplanted, placed into buckets with 100 clams in each, and set into the same beach to eliminate locational variables.

After a 58 day growing period from 03/19/89 to 05/23/89 the total weight of the clams increased 18.4% in sand, 16.6% in mud-silt, and 13.6% in gravel. The clams in transplanted substrate showed a 13.6% weight increase in gravel, 13.3% in sand, and 13% in mud-silt.

OPTIMAL SUBSTRATE STRATEGY FOR SURVIVAL AND GROWTH OF EARLY JUVENILE GEODUCKS, *PANOPE ABRUPTA* IN A SAND NURSERY. **Lauran R. Cole,*** School of Fisheries, WH-10, University of Washington, Seattle, WA 98195; **J. H. Beattie,** Pt. Whitney Shellfish Laboratory, 1000 Pt. Whitney Rd., Brinnon, WA 98320; **K. K. Chew,** School of Fisheries, WH-10, University of Washington, Seattle, WA 98195.

Little is known about optimal conditions for survival and growth of juvenile geoducks either in the hatchery or in the wild. Present techniques at the State Shellfish hatchery at Pt. Whitney place newly metamorphosed *Panope abrupta* in 20′ × 12′ sand raceways, which have increased survival and growth dramatically over the original upwell system. But survival from metamorphosis to 8 mm seed is still only about 7%, and growth and health are extremely variable.

Hatchery observation and recent work at the University of Victoria indicate that early juveniles are mobile, with a well-developed foot, and remain in the upper centimeter of substrate for at

least one month post metamorphosis. Juveniles are capable of using byssal threads to attach to the substrate or each other until well after they become sedentary at 5–6 mm.

A study during the summer of 1989 will test three substrate variables and their interactions to determine optimal substrate strategy for juveniles ≤2 mm shell length at the Pt. Whitney nursery. The variables include: 1) grain size (from <250 microns to 2 mm); 2) substrate depth (<1 cm or >3 cm) and 3) bottom surface (smooth tank bottom or construction fabric). We expect to increase survival and growth in the nursery, to reduce costs through conservative use of sand, and to find clues to preferred recruitment substrate in the wild.

POLYPLOID PACIFIC OYSTERS PRODUCED BY INHIBITING POLAR BODY I AND II WITH CYTOCHALASIN B. Ken Cooper,* Coast Oyster Company, Quilcene, WA 98376; **Ximing Guo,** School of Fisheries, University of Washington, Seattle, WA 98195.

The Pacific oyster *Crassostrea gigas* can become polyploid by treating fertilized eggs with cytochalasin B (CB) during meiosis. Polyploid oysters are produced as a result of the drug acting to disrupt the normal segregation of chromosomes during meiotic development. CB acts primarily by inhibiting polymerization of actin filaments, thereby disrupting actin networks of the cytoskeleton, and indirectly the microtubule networks within the egg. The actin and microtubule networks are involved in the establishment of division planes, the organization of cytoplasm and in the segregation of chromosomes in the egg during meiosis. In this study we examined the interaction of the timing of CB treatment on the segregation of chromosomes during meiosis as evidenced by analysis of ploidy (flow cytometry and chromosome counts) and direct staining of chromosomes.

Results show that fertilized eggs treated with CB at 25°C may be: 1) diploid, triploid or tetraploid if CB treatment effects the formation of polar body I, 2) triploid if CB treatment effects the formation of polar body II, and 3) pentaploid if CB treatment effects the formation of both polar bodies I and II. Triploids produced by retaining the chromosome set from polar body II develop normally without apparent effects on survival after reaching D-stage larvae. Pentaploids develop into abnormal trochophores, do not develop into D-stage larvae and suffer 100% mortality by 72 hours post-fertilization. Similarly, tetraploids suffer 100% mortality within 72 hours post-fertilization. Indirect evidence suggest that triploids produced following CB treatment during the formation of polar body I exist as two subsets, with one group suffering mortality after becoming D-stage larvae and the second group not suffering mortality as larvae compared to diploids. Subsequent studies are evaluating the occurrence of aneuploidy in Pacific oysters following treatment with cytochalasin B.

EFFECT OF BACTERIA ON THE CULTURE OF LARVAE OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THUNBERG). Philippe Douillet, Oregon State University, Hatfield Marine Science Center, Newport, Oregon 97365.

The West Coast shellfish industry for Pacific oysters depends on the production of larvae from hatcheries in order to obtain seed for planting in bays and estuaries. Algae has been considered to be the principal food of oyster larvae, but concentrations of algae do not appear to be high enough to meet the energy requirements of larvae in their natural habitat. Therefore, larvae may also utilize non-algal food sources (dissolved organic matter, detritus, bacteria) for their nutrition.

Many investigators have studied the effects of bacteria on the growth of cultured bivalve larvae with conflicting results. These previous studies suffered from the inability of researchers to control the nature of bacteria present in larval cultures. A novel approach has been used to overcome this problem whereby bacteria-free larvae are obtained and cultured under aseptic conditions with innoculations of isolated strains of bacteria.

The first objective of the research has been to determine the effect of additions of isolated bacteria strains on survival and growth of bivalve larvae fed algae under axenic conditions. Twenty-one bacteria strains have been tested and most of them reduced larval growth and survival. However, additions of three bacteria strains consistently improved larval survival from 22 to 60% and one of these three strains (strain CA2) repeatedly enhanced mean larval growth by 19 to 26%, compared with that of larvae which were fed algae in the absence of bacteria.

Experiments in progress are concerned with a better understanding of the mechanisms by which bacteria improve larval growth and survival, as well as the development of techniques for use of selected beneficial bacteria strains in commercial hatcheries. Understanding of the nutritional requirements of oyster larvae, as well as the contributions from each of the various potential food components present in the culture environment, will ultimately lead to more reliable and successful oyster industry in Oregon and the Pacific Northwest.

BURROWING SHRIMP RECRUITMENT TO WASHINGTON COASTAL ESTUARIES: A NEW APPROACH TO AN OLD PROBLEM. Brett R. Dumbauld,* David A. Armstrong, Dan C. Doty and **Greg C. Jensen,** School of Fisheries, University of Washington, Seattle, Washington, 98195.

An investigation into the ecology of the mud shrimp *Upogebia pugettensis* and ghost shrimp *Callianassa californiensis* in Washington state was initiated in 1988 as part of related studies on Dungeness crab in Willapa Bay. Previous and ongoing studies have focused on crab mortality caused by application of the insecticide carbaryl to oyster culture grounds to kill these burrowing shrimp. Studies in Oregon have shown that a pool of shrimp

larvae, made up of individuals from more than one parent estuary, exists seasonally in the nearshore coastal zone. Without some form of intervention in, or control of the recruitment process itself, the practice of spraying carbaryl to kill adult and juvenile shrimp on affected oyster beds appeared to be a temporary solution to the problem. We collected temporal life history information on both species of shrimp in 1988 and initiated settlement and recruitment experiments in 1989.

Recruitment cycles for the 2 species of shrimp differ. Ovigerous female *Upogebia* were found from October through April and rarely encountered after May, while female *Callianassa* carried their egg clutches well into the summer. Newly recruited *Upogebia* (5 mm carapace length) appeared in large numbers in samples taken in August of 1988 only. *Callianassa* appears to recruit over a much broader period in late summer (August–October). Ramifications of these results with regards to the current spray program are, that application of the pesticide in July may entirely miss all newly recruited shrimp, unless the pesticide remains toxic in the sediments for at least a month. Results of field experiments on shrimp settlement behavior that may lead to management solutions are discussed.

MUSSEL CULTURE IN BRITISH COLUMBIA: IMPORTANCE OF SEED SOURCE. Glen S. Jamieson,* and **Dwight G. Heritage,** Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

Previous investigations have established that both the source of cultured blue mussels (*Mytilus edulis*) and the specific environmental conditions (site) under which they are cultured influence growth and survival rates. In Atlantic mussels, site appears to primarily influence mortality whereas population (genotype) is a major determinant of growth effects. However, in the northeast Pacific, mortality of 1 year olds in suspended culture has been unacceptably high at most sites investigated. In a recent series of studies in British Columbia, we have further investigated the potential for minimizing Pacific mussel mortality after their first spawning by selection of seed source. Preliminary results using both local and exotic (Nova Scotia) populations are presented. These are discussed in the context of solving the problem in the northeast Pacific of high natural mortality just at the time mussels are reaching a size of about 50 mm shell length, which is preferred in the high price, live product market.

CONTINUOUS PLANKTON SAMPLING: THE SPATIAL PATTERN OF DUNGENESS CRAB MEGALOPAE IN THE STRAIT OF GEORGIA, BRITISH COLUMBIA. Glen S. Jamieson,* and **Antan Phillips,** Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

For the past three years, the nocturnal neustonic abundance of Dungeness crab (*Cancer magister*) megalopae has been monitored along transects crossing the Strait of Georgia. We present here both the general pattern of distribution of megalopae observed and a comparison of two survey methodologies: discrete time gear hauls and continuous venturi pumping of the neuston net codend. With discrete time hauls, the net is typically hauled in every 10 min and the contents removed, which at a normal haul speed of 4 kn, gives a distance sampled of 1.25 km. With continuous sampling, the contents can be sampled over time periods as short as 1 min, allowing greater resolution of spatial patterns in relation to oceanographic conditions such as tidal fronts and river plumes. The most serious problem with continuous sampling is the possibility of floating debris or seaweed entering the net and partially blocking it, thereby temporarily biasing results. Crab megalopae were collected throughout the summer and were particularly abundant around in the southern areas of Georgia Strait.

INDUCTION OF METAMORPHOSIS OF THE JAPANESE SCALLOP (*PATINOPECTEN YESSOENSIS*). Brian C. Kingzett* and **N. Bourne,** Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada. V9R 5K6; **K. Leask,** Department of Biology, University of Victoria, Victoria, B.C., Canada.

Hatchery reared larvae of Japanese scallop, (*Patinopecten yessoensis*), will settle and metamorphosis on a suitable substrate when the larvae possess a developed eyespot, foot and gill rudiment, and have attained a shell length greater than 260 um. Larvae were treated with various neurotransmitters including epinephrine, norepinephrine, L-Dopa, Seratonin and glutamic acid, to test the ability of these compounds to increase percent settlement and metamorphosis in the absence of a suitable substrate. Thermal shock and the addition of potassium chloride (KCL) and ammonia (NH_3) were also assayed for their effect on mature larvae. Exposure to epinephrine and norepinephrine and chilling were found to increase percent metamorphosis. Effects were dependant on concentration of the neurotransmitter, temperature of the chilled seawater and duration of exposure.

THE INFLUENCES OF A COMMERCIAL SALMON FARM UPON SUSPENDED CULTURE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*. Trevor O. Jones* and **G. K. Iwama, 1989,** Department of Animal Science, University of British Columbia, Vancouver, B.C.

The objective of this study is to determine the suitability of culturing Pacific Oysters and Salmon in one site. This project investigates the possible effects of a Salmon culture facility on Oyster growth by monitoring any variations in temperature, salinity and available food. Absolute Growth, Condition Indices, Dry Meat Weight:Dry Shell Weight Ratio and Survival Rate were

determined for a common broodstock of oysters grown in Jervis Inlet, British Columbia, Canada over a 3 month period. Six stations were utilized at the salmon grow out facility and 2 controls were implemented at traditional oyster culture sites.

The potential for Bioaccumulation of antibiotics will be analysed during the fall by establishing measurement techniques for antibiotic residues from oysters collected during the study. Completed results of this ongoing study will be presented.

COMPARISON OF TWO CAPSULE TYPES FOR THE DELIVERY OF DIETARY PROTEIN TO THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*. Christopher J. Langdon, Hatfield Marine Science Center, Dept. of Fisheries & Wildlife, Oregon State University, Newport, Oregon 97365.

In order to efficiently deliver microencapsulated dietary protein to marine suspension-feeders under non-axenic conditions, it is necessary to ensure that capsules are not susceptible to bacterial degradation. In this study, the preparation and use of glyceride-coated, nylon-protein-walled (GNP) capsules are described as an alternative to protein-walled (P) capsules for the delivery of dietary protein to oysters. GNP capsules lost only 14% ^{14}C-protein compared with losses of up to 38% ^{14}C-protein from P capsules, when incubated in GF/C-filtered seawater at 25°C for 24 h. Both P and GNP capsules were equally digested *in vitro* by extracellular style enzymes of *C. gigas;* however, *in vivo* feeding experiments with *C. gigas* indicated that ^{14}C-protein from GNP capsules was assimilated with an efficiency of only 29%, while oysters assimilated ^{14}C from P capsules with a significantly higher efficiency of 39%. Selection of capsule type to maximize utilization of encapsulated protein by marine suspension-feeders should depend on both the degree of capsule breakdown by bacteria in the culture system and the relative ability of the organism to assimilate material encapsulated within different capsule types.

THE PAST, PRESENT AND FUTURE OF GEODUCK (*PANOPE ABRUPTA*) SEEDING EFFORTS IN PUGET SOUND. Amy R. Leitman, Washington Department of Fisheries, Point Whitney Shellfish Laboratory, Brinnon, Washington 98320.

Hatchery seed of *Panope abrupta* from the Point Whitney Shellfish Laboratory have been planted in Puget Sound since 1976. The slow recruitment and fast growth rate of these bivalves make them extremely amenable candidates for re-seeding efforts in previously harvested areas.

The first experiments (1976–1979) used relatively small seed (0.05–7 mm) and were planted directly from the hatchery. Only a small percentage of the seed were recovered. In later years the seed was transferred from the hatchery to a nursery facility which allowed the geoduck seed to be planted at a larger size (13–15 mm); these seed afforded greater success.

Areas that have been sampled 2 years post-seeding will be discussed in terms of their successes and failures of both the planting and sampling methods. The present seeding methods and future recommendations for achieving higher seed recovery rates will also be investigated.

ESTIMATING THE ABUNDANCE OF AGGREGATED POPULATIONS. Robert A. McConnaughey,* School of Fisheries, University of Washington, Seattle, WA 98195; **Loveday L. Conquest,** Center for Quantitative Science, University of Washington, Seattle, WA 98195; **David A. Armstrong,** School of Fisheries, University of Washington, Seattle, WA 98195.

The spatial distributions of marine biota are frequently patchy. This pattern is well-represented among marine fish and invertebrate taxa during all life history stages. Samples taken from these populations are characterized by values which are mostly small, relative to the expected value, and a few that are very large. Because of this, it is often difficult to obtain reliable estimates of abundance using conventional methods. The inconsistent and confusing statistical treatment of such data in the fisheries literature has prompted this analysis.

Monte Carlo simulations, based on trawl data for Dungeness crab collected off the coast of Washington and in Willapa Bay indicate that, even tough the sample average is theoretically unbiased, single estimates of the arithmetic mean (and thus population estimates obtained using area-swept) may be too low and are overly sensitive to an extreme value; confidence intervals capture the true value at a level well-below that prescribed. Evidence is presented that trends in stock abundance could actually be the *reverse* of those indicated by conventional analysis. We have investigated alternative analytical procedures having more desirable statistical properties. These may increase accuracy associated with conventional fisheries stock assessment practices and thus provide for more effective management of overdispersed stocks.

COMMERCIAL CULTURE OF THE GIANT RED SEA URCHIN *STRONGYLOCENTROTUS FRANCISCANUS* IN HAWAII. Robert M. Miller,* Ocean Farms of Hawaii, Keahole Pt, Kailua-Kona, Hawaii 96740.

Demand for sea urchin roe, primarily by the Japanese, has created flourishing fisheries throughout the littoral Pacific basin. However, increased demand for "uni" has resulted in a drastic depletion of many local wild stocks and concurrent increases in market value (up to $6.72/oz during December, 1988). For the first time the culture of sea urchins has assumed importance and has resulted in the development of a unique land-based commercial mariculture effort.

Current culture endeavors involve the giant red sea urchin *Strongylocentrotus franciscanus*. Larvae are reared in a static system through a 40 ± 10 day cycle during which time temperature is maintained at 16°C and food is provided in the form of the green flagellate *Dunaliella tertiolecta*. Settlement stimulus is pro-

vided by a cultured bacterial (*Pseudomonad*) substrate after which juveniles are reared on a succession of naviculite and chained diatoms. Nursery growth is continued until a mean test diameter of 15 mm is achieved after which a macroalgal diet of *Macrocystis pyrifera* is introduced. At mean growout temperatures of 14°C, growth rates of 40.4 ± 2.4 mm/year (27.3 ± 4.2 g/year) have been achieved. AT this rate harvestable animals (approximately 100 mm test diameter) are expected within 2.5 years.

Among the critical obstacles to culture success, ongoing research efforts have identified larval stocking density, mechanical and chemical management of pathogens, feeding rates, and settlement induction as key areas of concern. Further research has resulted in the innovative use of urchins as biofouling reduction agents in abalone culture tanks. This polyculture technique has important implications for minimum-cost production of roe.

Advantages of urchin mariculture include acceleration of growth rates, year-round roe production and growth cycles, consistency of roe quality, and the potential use of the species as a solution to a variety of cleanup applications. With no reduction in world demand for urchin roe anticipated in the future, the concept of urchin culture has emerged as a practical and reliable production method.

DIAGNOSIS AND ALTERNATE PATHOGENESIS OF HEMIC NEOPLASIA IN PUGET SOUND *MYTILUS* POPULATIONS. James D. Moore* and **Ralph A. Elston,** Center for Marine Disease Control, Battelle Marine Sciences Laboratory, 439 West Sequim Bay Road, Sequim, WA 98382.

Disorders in which atypical cells proliferate in the vascular spaces of bivalve molluscs have been recorded for over fifteen species worldwide. In Puget Sound *Mytilus*, use of flow cytometry has demonstrated that the neoplastic cells correspond with a cell population having 5 × haploid (5n) DNA content, which cycles to 10n. A rare form of the disease has been seen in which neoplastic cells are tetraploid (4n) and cycle to 8n.

In the present study, analyses utilizing flow cytometry, hemocytology, and histology were correlated and used to: a) examine the utility of each method for diagnosis and population assessment of the disease; and b) describe the early stages and the two alternate forms of the disease.

Preliminary estimates of the relative proportions of the two alternate forms of the disease (5n/10n and 4n/8n) were 84% and 16% of affected mussels respectively. Flow cytometry was found to be the only reliable method for distinguishing the two forms, in addition to providing a quantitative representation of the degree of severity in mid to later stages of either form. However, flow cytometry was found to be less sensitive in detecting early stages of the disease than hemocytology and histology. Histology was found to be the most sensitive detection technique, as foci of neoplastic cells in variable tissue locations were observed at stages of early progression and remission of the disease. Hemocytology was considered to be the most efficient method for routine population assessment.

Work supported by National Cancer Institute.

SCALLOP CULTURE IN WASHINGTON STATE. Yun-Wook Rhee,* School of Fisheries, University of Washington WH-10, Seattle, Washington 98195.

At least four different species of scallops are available for potential fishery in the Washington waters. These are the Weathervane Scallop (*Pecten caurinus*), Rock Scallop (*Crassodoma gigantea*), Pink Scallop (*Chlamys rubida*), and Spiny Scallop (*Chlamys hastata*).

Interest by the Washington department of Fisheries and the fishermen have been on and off for the past twenty years to harvest these underutilized scallops. Current research, harvest, and the aquaculture potential of these scallop species will be discussed.

GAMETOGENIC CYCLE OF THE KUMAMOTO OYSTER (*CRASSOSTREA GIGAS*) IN YAQUINA BAY AND CONDITIONING OF OYSTERS FOR SPAWNING UNDER LABORATORY CONDITIONS. Anja Robinson, Department of Fisheries and Wildlife, Oregon State University, Hatfield Marine Science Center, Newport, Oregon 97365.

Kumamoto oysters from commercial oyster grounds of the Yaquina Bay were sampled once a month over a three year period for determination of their reproductive cycle. Gonads contained some ripe gametes throughout the year, reaching maximum frequency in September–October and declining rapidly in November to a minimum in March. Gametogenesis started again in May and the first new ova appeared in June–July.

Conditioning trials were performed at 20°C and 24°C several times a year. The higher conditioning temperature resulted in accelerated production of gametes early in the year but the number of ova released during spawning was lower. The duration of the conditioning period was also dependent on the stage of gonadal development of oysters at the beginning of the conditioning period.

INVESTIGATIONS INTO REMOTE SETTING PACIFIC OYSTER LARVAE. W. G. Roland* and **T. A. Broadley,** Ministry of Agriculture and Fisheries, Parliament Buildings, Victoria, B.C. V8W 2Z7.

The oyster industry in B.C. requires an economical and reliable source of seed oysters for future expansion. In the early 1980's, industry began using remote setting to fulfill seed requirements but by 1986 problems with low and inconsistent yields of purchased larvae became evident. In 1987, investigations were initiated to increase the percentage of larvae that metamorphosed on cultch and to create an even distribution of these larvae on cultch in the setting tanks. The 1988 studies focused on defining

criteria for siting natural nursery areas that would facilitate good growth and survival of spat oysters. The proportion of larvae setting was affected by temperature, salinity, feeding levels, water circulation rate, and cultch type. Distribution on cultch was related to water circulation rate and pattern and method of adding larvae to tanks. Growth at nursery sites was related to water temperature or chlorophyll a. Nursery survival was related primarily to cover of fouling animals. A procedure manual for remote setting has been written based on these results.

BIOLOGICAL FEASIBILITY OF INTERTIDAL AQUACULTURE OF THE GEODUCK CLAM, (*PANOPE GENEROSA*). F. Randolph Shuman and **T. L. Roberts,*** Applied Marine Research, Inc., P.O. Box 51212, Seattle, WA 98115.

The project goal was to test the feasibility of intertidal culture of geoduck clams, *Panope generosa*. Seed clams, obtained from a shellfish hatchery at the Point Whitney Shellfish Laboratory, were planted in the bottom under netting and in nursery tubs filled with screened substrate and covered with netting.

Growth trials were run in four intertidal sites in Washington State. In-bottom plantings were unsuccessful in three of four sites, mostly due to very high initial mortality. This mortality was the result of washout, predation, and other factors. Growth and survival at the fourth site were encouraging.

The nursery tub plantings were successful at all sites, showed close to 100% survival and promise an efficient method for nursery rearing and final growout of geoduck clams. Recommendations are given for planting strategies and commercial growout techniques.

MUSSEL CULTURE IN BRITISH COLUMBIA: THE INFLUENCE OF SALMON FARMS ON GROWTH IN MUSSELS. Barbara E. Taylor,* Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, V6T 2A9/Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

In order to realise the potential for mussel culture in British Columbia, mariculture research must focus on identifying specific environmental conditions, and therefore locations, which promote maximum growth in mussels. The present study investigates the possible advantages, through nutritional enrichment, of salmon farms as sites for mussel culture.

Mussels are being cultured at different distances around two salmon farms on the east coast of Vancouver Island (Departure Bay and Genoa Bay). Three parameters of mussel growth: condition index, polysaccharide content and nitrogen content, have been monitored in these mussels at 3 to 6 wk intervals since September 1988. While distinct seasonal differences in the three growth parameters have been observed, up to spring 1989 there has been no significant difference in any parameter between mussels with respect to their proximity to a farm. It is expected, however, that such a difference may become evident during the summer months—typically a time of increased growth and reproduction.

Contrary to prediction, the farms have not appeared to influence the availability of foodstuffs for mussels. Measures of seston and chlorophyll content, made concurrently with the mussel collections, indicate that neither a direct contribution of nutrients in the form of feed particles, nor an indirect contribution of waste ammonia augmenting phytoplankton production, has so far been made by the salmon farms. This is despite the fact that measurements of currents indicate that they have flowed, for at least part of each tidal cycle, in such a direction as to pick up potential nutrients from the farms and carry them past the mussels.

IMPROVED METHODS OF HANDLING JUVENILE SCALLOPS IN AN INTENSIVE CULTURE OPERATION. L. Townsend,* and **N. Bourne,** Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada. V9R 5K6.

An efficient nursery system is an essential part of a culture operation. Juveniles are held in spat bags until they are about 1 cm. shell height at which time they must be thinned and transferred to larger mesh nets for continued growth. This transfer is labour intensive and extensive mortalities can occur to the juveniles if they are not handled correctly. Experimental studies were carried out to improve handling techniques during this transfer. Spat bags were collected and held in a trough with running water whose temperature did not exceed 12°C. Juveniles were washed off the cultch (kinran) with a gentle flow of water. The cultch was also agitated by hand to insure all the juveniles were removed. Juveniles were collected in a shallow tray filled with water. Sorting by size was accompanied by gently washing the juveniles through a series of screens that ranged in size from 9–5 mm. Juveniles were counted and placed in pearl nets to be suspended in the water for continued growth and development in the nursery system.

AN OVERVIEW OF THE WASHINGTON DEPARTMENT OF FISHERIES PUGET SOUND ENHANCEMENT PLAN FOR *CRASSOSTREA GIGAS* AND *TAPES PHILIPPINARUM*. Doug S. Thompson,* Washington Department of Fisheries, Point Whitney Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, Washington 98320.

Pacific oyster and Manila clam enhancement is proceeding throughout Puget Sound in response to the increasing harvest of these species by tribal and nontribal user groups. Pacific oyster enhancement is by traditional seeding methods. This year 800 cases (4000 bags) of seed have been planted on 18 acres of state owned tideland. Test plots are established on exposed beaches to

determine the feasibility of planting single seed, which can lodge between rocks; and spreading larvae which can attach to stable rock surfaces.

Manila clam enhancement is by two methods. The first, beach graveling, is being used to create new clam habitat on mud and mud/sand beaches. One to five acre test plots will be established at six sites in 1989–90. Base composition is 0.5 cm to 1.9 cm washed or unwashed gravel. The gravel will be mixed with crushed oyster shell in 50:50 and 70:30 ratios of gravel to crushed shell. Application depths from 2.5 cm to 10.0 cm will be tested.

The second method is to enhance natural gravel beaches by planting seed or spreading larvae. Replicate plots are established at seven sites to compare the efficacy of planting seed and spreading larvae. Beaches chosen for the study are beaches with good clam habitat that have a low natural recruitment of clams; and good clam producing beaches that are heavily harvested.

NEW ALGAL FEEDS FOR BIVALVE MOLLUSKS. Andrew D. Taylor, Cell Systems Limited, Orwell House, Cowley Road, Cambridge, United Kingdom, CB4 4WY.

Bivalve mollusc larvae and juveniles are particulate filter feeders. Traditionally, rearing them in hatcheries has depended on live algal feeds, the reliable and large-scale production of which is a laborious, technically difficult and expensive procedure.

Some types of marine unicellular algae can be grown heterotrophically, i.e. in the dark, using sugars rather than light as the energy source. Very high cell concentrations are obtained and the product can be preserved by spray drying.

Growth trials with a variety of bivalve species have shown the nutritional value of spray dried algal cells to be very similar to that of live cells. Spray dried algae requires less time and fewer facilities to prepare and is more convenient to use than live algae. Spray dried algae is now being used in hatcheries in Europe, the United States, South America and South East Asia.

The information presented describes the result of experimental and commercial growth trials with spray dried algae, and its applications in existing bivalve hatcheries.

Heterotrophically grown, spray dried algae is produced by Cell Systems Limited of Cambridge, England. Feeds for bivalve molluscs are marketed under the trading name of CELSYS ALGAL MICROFEEDS.

OYSTER FARMING DEMONSTRATION PROJECT USING THE FLEXIBLE BELT SYSTEM IN APALACHICOLA BAY, FLORIDA. David E. Vaughan,* Leslie Sturmer, John Holt and **LeRoy Creswell,** Mollusc Culture Department, Division of Applied Biology, Harbor Branch Oceanographic Institute, Inc., Fort Pierce, Florida 34936.

The Oyster Farming Demonstration Project was initiated in 1988 when the Governor of Florida requested emergency federal funding for dislocated oyster workers. The intent of the program is to train participants in the techniques of oyster farming. Harbor Branch Oceanographic Institute, a private research facility, has developed a unique molluscan culture methodology for cultivating oysters subtidally. This system uses a flexible belt apparatus and cultivates oysters intensively with minimal labor and with the potential for simple mechanization.

Over 100 participants have received over 20,000 oyster seed each (micro-cultch, 3–9 mm) and training in the use of the flexible belt system in the first phase of the program. The second phase, initiated in July (1989), has the capacity to train an additional 200 participants. The training site is described, and the growth and survival results form the training plots are summarized. Preliminary production results from a commercial scale (1 acre, 1 million seed) demonstration plant using the flexible belt system are presented.

Journal of Shellfish Research, Vol. 8, No. 2, 419–488, 1989.

ABSTRACTS OF TECHNICAL PAPERS

Presented at the 1990 Annual Meeting

NATIONAL SHELLFISHERIES ASSOCIATION

Williamsburg, Virginia

April 1–5, 1990

CONTENTS

RED TIDE

CLAM BIOLOGY AND CULTURE

GENETICS/POLYPLOIDY

VIBRIO

STATUS AND TRENDS

SETTLEMENT AND RECRUITMENT

REPRODUCTIVE BIOLOGY OF MOLLUSCS

GENETIC VARIATION IN GAMETOGENIC CYCLES OF AMERICAN OYSTER STOCKS. Bruce J. Barber,* College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA 23062; **Susan E. Ford** and **Robert N. Wargo,** Rutgers University, Shellfish Research Laboratory, Port Norris, NJ 08349.

The gametogenic cycles of four stocks of American oysters, *Crassostrea virginica* (Gmelin) (Long Island native, Long Island 6th generation inbred, Delaware Bay native, and Delaware Bay 5th generation inbred) were compared in Delaware Bay between March and October 1987 using three methods (gonad cross sectional area, oocyte diameter, and gamete volume fraction). All three methods indicated that both the Long Island stocks initiated gametogenesis earlier in the year, began spawning earlier in the year, and spawned over a shorter duration than both the Delaware Bay stocks. Both inbred stocks had gametogenic cycles that resembled their respective native stocks. Thus after 5–6 generations of inbreeding in Delaware Bay, gametogenic cycles characteristic of the site of origin were maintained, indicating that gametogenesis in oysters is largely under genetic control.

This is NJAES publication no. K-32405-3-89, supported by state funds.

ARTIFICIAL BREEDING OF SCALLOPS IN HATCHERIES. Neil Bourne, Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. Canada V9R 5K6.

Scallop resources in British Columbia are too small to sustain a continuous large fishery so establishment of a significant industry will have to rely on culture. Initially at least, scallop culture operations will have to rely on hatcheries to produce an adequate supply of juveniles. Artificial breeding work with two species of scallops is described; the Japanese scallop, *Patinopecten yessoensis*, and the rock scallop, *Crassadoma gigantea*. Conditioning regimes include raising ambient water temperatures and holding broodstock at conditioning temperatures for extended periods of time as well as feeding them large quantities of cultured algae. Gonadal development and gonadal index are followed closely. Combined effects of temperature and salinity are important and differ with species and results of rearing embryos under different temperature and salinity regimes are described. Broodstock of both species have been maintained in spawning condition for as long as six months, however, there is a deterioration in egg quality after they are held for approximately four months and results of this work are discussed.

QUANTITATIVE EVALUATION OF GONADAL PROTEINS IN MALE AND FEMALE OYSTERS (*CRASSOSTREA VIRGINICA*) USING AN IMMUNOLOGICAL TECHNIQUE. Kwang-Sik Choi,* Department of Oceanography; **Donald H. Lewis,** Department of Veterinary Microbiology and Parasitology; **Eric N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

A polyclonal antibody has been produced against egg protein and sperm protein of the American oyster for obtaining a weight-based gonadal index. Goat anti-rabbit alkaline phosphatase-labeled conjugates were used in an indirect enzyme-linked immunosorbent assay (ELISA) to quantify oyster egg protein. The gonadal index was then expressed in the form: mg gonadal protein/g wet wt oyster tissue.

The polyclonal antibody against oyster sperm protein was produced by stripping ripe sperm into phosphate-buffered saline (0.15 M NaCl, pH 7.3) (PBS II). The sperm was sieved over a 0.1 mm mesh to separate sperm from other tissue debris. The subsequent preparation was layered onto 100% Percol and centrifuged at 12000 g for 45 min. Sperm was collected, homogenized using an ultrasonicator, and injected into an Albino New Zealand rabbit. Rabbit anti-oyster sperm IgG (10 μg/ml) detected 0.2 to 6.0 μg of sperm protein.

To assess the usefulness of this method, 50 female oysters were collected from Deer Island, Galveston Bay, Texas from June through October, 1988. During that period, monthly gonadal index (for females) varied between 107 mg/g (June) and 22 mg/g (October). The maximum gonadal index recorded was recorded in July, 365 mg/g, and the minimum in October, 0.012 mg/g. Maximal indices in July indicate that the gonad may account for as much as 36% of the body weight during the spawning season.

COSTS OF REPRODUCTION IN PACIFIC OYSTERS. Jonathan P. Davis, School of Fisheries, University of Washington, Seattle, WA 98110.

Oysters and other estuarine bivalves generally exhibit high phenotypic plasticity with respect to somatic and germinal production as a function of environmental variables such as food availability and to a lesser extent temperature. As a consequence of the large annual diversion of energy to gamete production, Pacific oysters may incure significant costs in terms of changes in physiological rates relating to feeding and respiration and seasonal patterns of glycogen storage during gametogenesis (up to 70% of total body mass is devoted to reproductive tissue in *Crassostrea gigas*). Physiological costs may be integrated and observed as changes in somatic growth rate relative to germinal production during gametogenesis.

The goal of this research was to examine somatic growth over three years in hatchery populations of Pacific oysters transplanted into two grow-out environments in Washington state differing significantly in total organic seston load and temperature during the

period of gametogenesis (spring and early summer). Hatchery produced Pacific oysters were placed in Westcott Bay (San Juan Island) and lower Quilcene Bay (Hood Canal) as juveniles. Age and size specific changes in reproductive output and glycogen storage patterns relative to somatic growth were measured. In Quilcene Bay, temperatures ranged between 8 and 24°C with seston levels peaking early in the spring (April), and then dropping rapidly to less than 1 mg/l total POM by June. In Westcott Bay, temperatures ranged between 7 and 16°C. Organic seston levels were consistently high during this period (April–July). Both somatic and germinal production were reduced in Quilcene Bay transplants relative to Westcott Bay oysters. In Westcott Bay, somatic growth in tagged oysters continued during the period of gametogenesis (based on measurements on total volume on individual oysters), whereas in Quilcene Bay oysters, shell growth slowed or ceased during the late spring coincident with peak gametogenic activity. Glycogen content in Quilcene Bay oysters at peak gametogenesis was significantly lower than glycogen content in Westcott Bay oysters at peak gametogenesis.

Physiological rate changes in oysters were measured in the laboratory over the course of an artificial conditioning period where temperature and food levels were controlled. Oysters were measured weekly over a four week conditioning period and exhibited changes in respiration and feeding rates coincident to physiological changes occurring during gametogenesis. These results are discussed with reference to the integrated growth response (i.e. somatic and germinal production) during gametogenesis in Pacific oysters.

EFFECTS OF GAMETE STORAGE ON FERTILIZATION IN *MERCENARIA MERCENARIA*. **Joy G. Goodsell*** and **A. G. Eversole,** Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson, SC 29634-0362.

Investigations into the genetics of *Mercenaria mercenaria* require that the researcher be able to produce paired matings between specific individuals. Eggs or sperm must often be stored for several hours before the selected mating can be accomplished. Trials were run to determine the effects of delayed fertilization on eggs and sperm. In the first set of trials, freshly-spawned eggs were challenged with four different concentrations of sperm (10^4, 10^5, 10^6, and $2 * 10^6$ sperm/ml) that had been held at 23 and 4°C for 0, 1, 3, 5 and 24 hours. In the second set of trials, the procedure was reversed; 3 concentrations of freshly-spawned sperm (10^4, 10^5, 10^6) were used to challenge eggs which had been held for 0, 3 and 24 hours. After a period of 6 hours, embryos were counted to determine % fertilization. Initial challenges (0 hrs) yielded fertilization rates of 90–100%. Sperm which had been held at 4°C remained more active than sperm held at 23°C for all storage times. Higher concentrations of sperm yielded higher % fertilization in all delayed fertilizations. Fertilization of eggs stored 3 hours was severely reduced (20–30%) and no live embryos resulted from eggs held 24 hours. These results will be discussed with ways to improve storage procedures and fertilization success.

NEGATIVE LARVAL RESPONSE TO SELECTION FOR INCREASED GROWTH RATE IN THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*. **Peter B. Heffernan,* R. L. Walker,** and **J. W. Crenshaw, Jr.,** Marine Extension Service, Shellfish Research Laboratory, University of Georgia, P.O. Box 13687, Savannah, Georgia 31416-0687.

Larval (F_2) progeny of Georgia *Mercenaria mercenaria* selected for rapid growth rate were significantly smaller (shell length) than larval progeny of control parents at both 10 and 18 days of age in two experimental trials. Survival rates were similar for both progeny lines from 2–18 days of age. Earlier studies by our group have demonstrated significantly higher embryonic mortality rates (2 days) in the progeny of parents selected for rapid growth. Control line progeny in both experiments set earlier (10–14 days) than those of the select line parents (14–18 days). Parental lines were subjected to truncation selection (16% intensity level) for increased rate of growth. This negative larval response for increased growth rate (in adults) brings into question the merits of hatchery culling practices for smaller larvae. A long term approach to the study of the reproductive potential of bivalve broodstock lines selected for increased rate of growth is called for on the basis of these results.

DISTRIBUTION AND PREVALENCE OF GONADAL NEOPLASMS WITHIN THE INDIAN RIVER CLAM (*MERCENARIA* SPP.) POPULATION. **Donald M. Hesselman,*** FDNR, Shellfish Environmental Assessment Section, Punta Gorda, FL 33950; **William S. Arnold,** FDNR, Marine Research Institute, St. Petersburg, FL 33701.

The incidence and distribution of gonadal neoplasms in hard shell clams, *Mercenaria* spp., from the Indian River, Florida has been monitored since May, 1985. The neoplasms appear as proliferations of atypical germ cells arising from the germinal epithelium of both male and female clams. In extensive cases, the neoplasm completely fills the lumen of the follicles thereby causing the arrest of normal gametogenesis and reduction in the reproductive potential of the population. Examination of 3,643 clams revealed 340 (9.3%) clams were infected with the neoplasm with peak prevalences appearing during the summer months. Although the neoplasms are found in clams from all areas of the river, a higher prevalence occurred within the shellfish harvesting Body C. Recent results of enzyme electrophoresis of infected clams may be presented which will reveal if either *M. mercenaria*, *M. campechiensis* or their hybrids are more prone to neoplastic changes.

USE OF SHELTER BY THE SMALL PATAGONIAN OCTOPUS, *OCTOPUS TEHUELCHUS* d'ORBIGNY: AVAILABILITY, SELECTION AND EFFECTS ON FECUNDITY. **Oscar Osvaldo Iribarne,** CQS-HR20 University of Washington, Seattle, WA 98195

This study examines shelter use by *O. tehuelchus* in the sandy subtidal zone of San Matias Gulf (41°S, Argentina). Samples were taken monthly during 1986 and 1987 to address the following questions: 1) Is shelter use affected by size, sex or sexual maturity? 2) Is fecundity affected by shelter type or shelter quality? 3) Can shelter be considered a limiting resource in this area? and if so, 4) Are shelters equally limiting along the octopus life span?

The results show that most shelters were of biological origin and were used in a size specific way. Small individuals use shells of gastropods and of two clams (*Pittar rostratum* and *Amiantis purpuratus*). Intermediate size octopuses use mostly empty shells of *Ostrea puelchana*. Due to the shortage of large shelters, the biggest octopuses used only shelters composed by mixed parts, which appear to be of lower quality. Eggs are placed in the most concave areas of any shelters. In the unequivalve shell of *O. puelchana* most eggs (±90%) are attached to the concave valve, but they were equally distributed among the two shells of equivalve bivalves. Large size octopuses may be limited by shelter availability. The quality of shelters appears to affect reproductive output, mostly of larger females. The ecological significance of these results, and their possible implications for habitat enhancing will be discussed.

SEROTONIN ACTION ON *SPISULA* GAMETES: INDUCTION OF OOCYTE MATURATION AND STIMULATION OF SPERM MOTILITY. **S. S. Koide,* A. L. Kadam, P. A. Kadam, T. Haneji, A. H. Bandivdekar** and **S. J. Segal,** Population Council, New York, NY 10021, and Marine Biological Laboratory, Woods Hole, MA 02543.

Serotonin (5-hydroxytryptamine, 5-HT) induces spawning of gametes when injected into the gonads of the surf clam (*Spisula solidissima*), triggers *in vitro* maturation of *Spisula* oocytes and stimulates motility of cold-immobilized *Spisula* sperm. The 5-HT agonists, 8-OH-DPAT (5-HT_{1A}) and α-methyl-5-HT (5-HT_2), induce oocyte maturation. 5-HT maturation-inducing activity was blocked by mianserin (5-HT_1, 5-HT_2) and ketanserin (5-HT_2). Binding of [^{3}H]5-HT to isolated plasma membranes of *Spisula* oocytes was performed. The Kd of [^{3}H]5-HT binding was 17.5 nM and the maximum binding capacity was 7.9 pmoles/mg protein. The order of decreasing potency by 5-HT agonists was 5 HT > 5-CT > 8-OH-DPAT > 2-methyl-5-HT > α-methyl-5-HT and that of the antagonists was ICS-250-930 > mianserin > methysergide > BMY-73787 > ketanserin. Motility of cold-immobilized *Spisula* sperm is stimulated by 5-HT and its analogs, 8-OH-DPAT (5-HT_{1A}), α-methyl-5-HT (5-HT_2) and 2-methyl 5-HT (5-HT_3). Binding of [^{3}H]5-HT to isolated sperm plasma membranes was performed. The Kd of [^{3}H]5-HT binding was 27 nM and the maximum binding capacity was 11.25 pmoles/mg protein. The order of decreasing potency in the displacement of [^{3}H]5-HT binding by 5-HT agonists was 2-methyl-5-HT > 8-OH-DPAT > 5-HT > 5-CT > α-methyl-5-HT and by antagonists was ICS-205-930 > BMY-7378 > mianserin > methysergide. The present results demonstrate that *Spisula* gametes are sensitive to various site selective 5-HT analogs, suggesting that the receptor is mixed or complex type. During 5-HT induction of *Spisula* oocyte maturation, GTP-mediated protein phosphorylation and $^{45}Ca^{2+}$uptake are stimulated. 5-HT stimulation of $^{45}Ca^{2+}$ uptake is blocked by mianserin and by verapamil. The present results suggest that 5-HT may promote gating of receptor-operated Ca^{2+} channels and may activate a GTP-mediated kinase activity.

Supported by a grant from The Rockefeller Foundation.

LIPIDS, PEPTIDES AND LIPOPROTEINS IN BIVALVE EGGS. **Richard F. Lee,*** Skidaway Institute of Oceanography, Savannah, GA 31416; **Peter B. Heffernan,** Marine Extension Services Shellfish Research Laboratory, University of Georgia, Savannah, GA 31416.

The buildup of lipids by adult bivalves, followed by transfer of these lipids to the eggs, is essential for good reproductive success since egg-derived lipids play a major role in growth and survival of bivalve embryos. Within the egg, the lipids are found in membranes, oil droplets, and water soluble lipoproteins. Membrane lipids have a structural function and are predominantly phospholipids and sterols. Oil droplets have a storage function and are primarily triglycerides. Water soluble lipoproteins, which are supramolecular complexes of lipids and polypeptides, have a role in transport of lipids between their sites of synthesis, storage and utilization. The uptake and metabolism of lipids can be controlled by apoproteins, i.e., the peptides in lipoproteins. Invertebrate lipoproteins have phospholipids, sterols and small amounts of neutral lipids.

In our studies we analyzed the eggs of hard clams (*Mercenaria mercenaria*), oysters (*Crassostrea virginica*) and ribbed mussels (*Geukensia demissa*). The major egg constituent was protein followed by lipid and finally carbohydrate. The composition of clam eggs included the following: protein—62 ng/egg; carbohydrate—16 ng/egg; lipid—24 ng/egg. A similar composition was found in oyster and ribbed mussel eggs. Approximately half of the egg protein is in the cytosol and the major cytosolic protein is a very high density lipoprotein (density 1.3 g/ml) containing a peptide with a molecular mass of 20,000 daltons. This contrasts with the major peptides in marine arthropod eggs which are high molecular weight (77,000–190,000 daltons). Also, the arthropod lipoproteins are generally high density lipoproteins (density—1.12–1.21 g/ml). Our current studies involve the purification and characterization of the major peptide associated with clam egg lipoprotein.

This work was funded by Georgia Sea Grant/Project Number NA 88AA-D-56098.

LARVAL ECOLOGY OF THE SCALLOP, *PLACOPECTEN MAGELLANICUS*, IN THE MIDDLE ATLANTIC BIGHT: THE FUNCTIONAL SPAWNING SEASON. **Roger Mann,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The scallop, *Placopecten magellanicus*, is a member of the Arctic-Boreal molluscan fauna of the eastern continental shelf of North America. Despite its commercial value comparatively few studies have investigated its reproductive biology. The larvae have been described from laboratory culture. Larval distribution with depth and time have been described for locations north of the Georges Bank where summer thermoclines are moderate or absent. South of Cape Cod the adult distribution exhibits submergence with the inshore limit at increasing depth to the southerly limit at approximately Cape Hatteras. Two major spawning peaks have recently been reported for populations in the southerly portion of the Mid Atlantic Bight. This presentation uses a modification of larval growth models previously developed for *Arctica islandica* to address the question as to which of these peaks (if any) is the major functional spawning with respect to larval survival.

SEMIANNUAL REPRODUCTION IN THE CALICO SCALLOP, *ARGOPECTEN GIBBUS*. **Michael A. Moyer,*** and **Norman J. Blake,** Department of Marine Science, University of South Florida, 140 Seventh Avenue South, St. Petersburg, FL 33701-5016.

Extensive commercial fishing for the calico scallop (*Argopecten gibbus*) occurs off the east coast of Florida centered around Cape Canaveral. Periodic fluctuations in the population level of the calico scallop leads to financial difficulties for the scallop industry. Since October, 1983 we have been studying the reproductive biology and growth rate of the calico scallop along with various environmental factors. The main goal of this research has been to determine what factors cause the fluctuations in the population level in order to develop methods of predicting periods of decreased productivity.

Unlike most members of the Pectinidae family which spawn annually, our research has shown that the calico scallop normally spawns semiannually. The primary spawn occurs in the late spring while a secondary spawning event occurs in the fall or early winter. Occasionally the secondary spawn has not taken place apparently due to unusual environmental conditions. The effects of environmental factors upon spawning and population level is discussed.

FACTORS REGULATING REPRODUCTION AND RECRUITMENT IN POPULATIONS OF THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA*. **Roger I. E. Newell,* Thomas J. Jones, Victor S. Kennedy** and **S. Alspach,** Horn Point Environmental Laboratories, University of Maryland, PO Box 775, Cambridge, MD 21613.

Interannual variability in recruitment success is common in many bivalve molluscs species. The objective of the work presented here was to identify factors that are controlling reproduction, larval settlement, and recruitment to populations of the American oyster *Crassostrea virginica* in the mesohaline portion of Chesapeake Bay.

Our histological analyses have shown that the timing of the adult gametogenic cycle and the amount of germinal tissue produced were similar over a five year period, despite orders of magnitude difference in larval oyster settlement. This suggests that factors affecting larval survival are of paramount importance. However, we found there to be little influence of food availability, as measured by the chemical composition of different size classes (<3 μm, >3 to 10 μm., and >10 μm) of phytoplankton, on larval settlement. Instead, in these particular habitats of central Chesapeake Bay, low salinity (<9 ppt) during the reproductive period was highly correlated with low spatfall, indicating that physiological stress of low salinity may be important in adversely affecting larval development. In years with salinities >9 ppt, larval settlement on cement plates suspended 10 cm off-bottom was high but recruitment to adjacent natural oyster beds was 99.9% lower. Using predator exclusion cages we demonstrated that high juvenile mortality on natural oyster bars was principally due to benthic micropredators, such as flatworms (*Stylochus ellipticus*). Juvenile growth appeared little affected by temporal and spatial variability in food availability. The precipitous decline in oyster recruitment that has occurred in Maryland over the last three years appears to be due to the loss of broodstock from the recent disease epizootics rather than the adverse influence of environmental factors.

PARAMETERS ASSOCIATED WITH THE REPRODUCTIVE SUCCESS OF THE OYSTER *CRASSOSTREA VIRGINICA*. **Julia S. Rainer,*** and **Reinaldo Morales-Alamo,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Although fecundity has generally been used as an indicator of reproductive condition and implicated in determining reproductive success, the qualities of an egg to produce a larvae are largely unknown. Fecundity was determined by egg counts and gonad volume fraction, and egg quality measured as egg lipid content, size and ratio of fertilized eggs to subsequent D stage larvae. Measurements were taken weekly from June through October on oysters collected from the James River, VA. Egg counts varied between 50,000 and 500,000 per 2.5 inch female. Similar periodicity was found between both types of fecundity measurements. D stage larvae developed only during the period July 21 through August 18. An inverse relationship exists between the number of eggs produced and the number of larvae developed from those eggs. Resource partitioning to the gonad to determine reproductive success is correlated to the ability to produce an egg competent to develop into a larva.

REPRODUCTIVE CYCLE OF THE KUMAMOTO OYSTER *CRASSOSTREA GIGAS KUMAMOTO* (THUNBERG), AND ITS IMPLICATIONS FOR ARTIFICIAL CONDITIONING, AND REARING. **Anja M. Robinson,*** Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365.

Kumamoto oysters from commercial oyster grounds of the Yaquina Bay were sampled once a month over a three year period for determination of their reproductive cycle. Gonads contained some ripe gametes throughout the year. Maximum frequency occurred in August–September and declined rapidly in October–November to a minimum in March. Gametogenesis started again in May and the first new ova appeared in June–July.

Conditioning trials were performed at 20°C and 24°C four times a year between January and September. The conditioning temperature of 24°C resulted in accelerated production of gametes somewhat faster than 20°C. The duration of the conditioning period needed was the shortest in May and June. Also the larval survival and number of spat collected was better than earlier in the year.

The optimum temperature for larval rearing was 26°C and optimum salinity was 25 ppt.

THE GAMETOGENIC CYCLE OF *PLACOPECTEN MAGELLANICUS* IN THE MID-ATLANTIC BIGHT. **Anne C. Schmitzer,* William D. Dupaul,** and **James E. Kirkley,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Due to an apparent lack of information on the reproductive cycle of sea scallops (*Placopecten magellanicus*) located in the mid-Atlantic bight and the importance of this information to the management of the fishery, a detailed study on the gametogenic cycle was undertaken. From January to December 1988, histologically prepared gonad tissue was quantified into volume fraction components using stereological techniques to determine gametogenic events. Histological results concluded that sea scallops in the study area underwent a semiannual gametogenic cycle, with major spawning occurring in May and November. Scallops from this area were characterized as having an opportunistic reproductive strategy. Differences in the gametogenic cycle between sex, latitude, depth, and season are discussed, as well as environmental parameters which may influence the gametogenic cycle. A strong relationship between seasonal changes in adductor muscle weight and gonad weight was detected. The adductor muscle generally decreased in weight as the gonad developed, increased immediately upon spawning, and remained in an improved condition until recovery of the gonad was initiated. Implications of semiannual spawning to the fishery are discussed.

SEASONALITY IN SEA SCALLOP SOMATIC GROWTH AND REPRODUCTIVE CYCLES. **Fredric M. Serchuk,*** NMFS, Northeast Fisheries Center, Woods Hole, MA 02543; **Ronald J. Smolowitz,** NMFS, Northeast Region, Gloucester, MA 01930.

Monthly changes in growth and reproductive condition of sea scallops (*Placopecten magellanicus*) in the New York Bight area (south of Long Island) and on Georges Bank were evaluated from 46 commercial samples collected between November 1986 and October 1988. Estimates of average meat weight, average meat count [# meats/lb], average ovary weight, and reproductive condition factor [ovary weight/(ovary weight + meat weight)] were summarized by 5 mm shell height intervals from 9,400 individual scallops.

Monthly patterns in average meat weight at shell height indicate that scallops ≤92 mm shell height remain above 30 meat count irrespective of seasonal fluctuations, while scallops >102 mm shell height remain below 30 count throughout the year. Meat weights for all shell heights decreased from April to October but increased by the following April. Ovary weights for all shell heights increased to peak levels in September and October during the two spawning cycles covered in the study. The period of peak spawning [as indicated by a sharp decline in gonad weights and reproductive condition] coincides with the period of minimum meat size [maximum meat count] suggesting an inverse relationship between meat weight and ovary weight.

The implications of seasonal variations in sea scallop growth and reproductive condition are discussed with respect to management policies promulgated for the USA sea scallop fishery under the Fishery management Plan for the Atlantic Sea Scallop Fishery.

THE GAMETOGENIC CYCLE OF *ARGOPECTEN CIRCULARIS*. **Janzel R. Villalaz,** College of Marine Studies, University of Delaware, Lewes, DE 19958.

Studies of tropical ecosystems have failed to assess the relation between reproduction in scallops and seasonal changes of environmental parameters. A laboratory study was carried out in Delaware to assess the relationship of gametogenesis in *Argopecten circularis* to seasonal changes in temperature and phytoplankton densities by using relative dry weight changes in gonads, digestive gland, gills, mantle and adductor muscle.

Salinity and temperature of the water were measured with a salinity-temperature probe meter (YSI). Phytoplankton densities were recorded by direct count with a hematocytometer. Reproductive condition was determined from gonads processed histologically.

This study is a contribution to the reproductive biology of *A. circularis* and fisheries management of the tropical scallop.

EFFECTS OF ANTHROPOGENIC INPUTS ON BIVALVES

EFFECTS OF POLLUTANT-EXPOSURE ON HEMOCYTE-MEDIATED IMMUNE FUNCTION. **Robert S. Anderson,***

University of Maryland System, Chesapeake Biological Laboratory, P.O. Box 38, Solomon, MD 20688.

Environmental xenobiotics have been shown to exert immunosuppressive effects in mammals, usually manifested by decreased resistance to infectious agents including microorganisms and metazoan parasites. Evidence for comparable phenomena in marine invertebrates is sparse. This research examines modulation of internal defense mechanisms of the hard clam *Mercenaria mercenaria* by sublethal exposure to the model marine pollutants hexachlorobenzene (HCB) and pentachlorophenol (PCB). Bivalve molluscs have components of the nonspecific immune system: phagocytic cells and agglutinating and/or cytotoxic humoral molecules. Adaptive immune responses are minimal because of the absence of lymphocytes and immunoglobulins.

The ability of *M. mercenaria* to clear an injected dose of *Flavobacterium* sp. strain 807098, was compromised by exposure to HCB or PCP. In vivo inhibition of bacterial clearance was modeled in vitro: bactericidal capacity of whole hemolymph from unexposed clams was reduced by in vitro exposure to PCP, and whole hemolymph from exposed clams showed decreased bactericidal activity in vitro. To define the basis of this response, the relative contributions of hemocytes and serum factors were studied. Cell-free hemolymph alone had only slight bactericidal and opsonic properties; the role of serum lectins in immune recognition has been postulated. Hemocytes were primarily involved in bacterial killing and probably represent an important target cell for immunotoxicants in *M. mercenaria*.

BIOACCUMULATION OF ORGANIC CONTAMINANTS IN MARINE BIVALVE MOLLUSCS: EFFECTS ON BIOENERGETICS AND REPRODUCTIVE EFFORT. Judith Capuzzo,* Bruce A. Lancaster and **Dale F. Leavitt,** Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543; **John W. Farrington,** Environmental Sciences Program, University of Massachusetts-Boston, Boston, MA 02125 and Chemistry Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Uptake and bioconcentration of organic contaminate by marine bivalves are dependent on the bioavailability of specific compounds, the duration of exposure, and the physiological condition of populations. Species differ in their rate of uptake due to differences in filtration rates, lipid content, and habitat. Bioconcentration patterns may be influenced by physico-chemical properties such as molecular configuration or stearic properties of specific compounds that influence biotransformation and membrane transfer kinetics and biological factors such as the partitioning between storage lipids and structural lipids and differential distribution of contaminants among different tissues. Biological effects associated with bioconcentration of lipophilic contaminants have been attributed to the uptake of specific compounds and/or their metabolites, rather than the total body burden of hydrocarbons or chlorinated hydrocarbons. Empirical data suggest that linkages clearly exist between (1) developmental and reproductive abnormalities; (2) the physiological and molecular processes involved in uptake, retention and loss of contaminants; and (3) the toxicity and/or transformation of lipophilic contaminants. Critical factors that may contribute to the impairment of reproductive and developmental processes in response to exposure to lipophilic contaminants include: (1) deposition of contaminants in gametes and developing embryos; (2) lysosomal dysfunction associated with oocyte resorption; (3) interference with feeding mechanisms, such that exposure mimics starvation responses; (4) failure to incorporate sufficient yolk in oocytes; (5) morphological abnormalities during embryogenesis resulting from failure of morphological systems to develop properly; (6) limited capacity of developmental stages to metabolize or depurate contaminants; and (7) limited capacity of early developmental stages and reproducing adults to draw on excess energy reserves. Thus, responses can be categorized as interfering with energetic processes (3, 4, and 7), biosynthetic processes (4), and structural development (2, 5) in addition to direct toxic effects (1, 6). An understanding of the relationship between bioavailability, bioconcentration, and mechanisms of biological damage warrants further consideration.

A DISCUSSION OF VARIOUS APPROACHES FOR ASSESSING THE EFFECTS OF ANTHROPOGENIC INPUTS ON BIVALVES: CURRENT TRENDS AND SUGGESTIONS FOR THE FUTURE. Michael P. Crosby, Belle W. Baruch Institute for Coastal Research and Marine Biology, University of South Carolina, P.O. Box 1630, Georgetown, SC 29442.

The myriad of methodologies available for investigators to utilize in studying the effects of anthropogenic inputs on bivalves defy attempts at classifying them into a limited number of separate categories. The sheer number of different methods combined with the lack of sharp, distinct boundaries between the different method types preclude any coherent categorization of them. However, the focal point of a given study using a particular method(s) is generally one of several specific levels of biological organization within a bivalve species. The levels of organization may be broadly defined as population, organism, organ/tissue, cell/organelle and biochemical/molecular. Over the past 2–3 decades, the approaches employed for assessing the effects of pollution inputs on bivalve species has shifted focus from generally holistic levels of biological organization to more reductionistic orientation. While studies at the organismal and population levels continue to occur, investigations at the cellular and biochemical levels of organization seem to currently dominate this area of bivalve research. Reductionistic types of studies have and will continue to provide a great deal of extremely valuable information. As the scientific community progresses in its ability to investigate and understand how toxic substances effect bivalve cellular and biochemical levels of functioning, we must not lose sight of the fact

that one of the primary reasons for conducting these studies should remain to more fully understand the underlying mechanisms of how and why various pollutants stress bivalve populations. The question should always be asked, "Will a specific alteration or modification of a certain biochemical process or immune response effect the bivalve at the organismal or population level?" and, if so, "Will the effect be positive or negative?"

Specific suggestions for future studies include the development of reliable, quick, "cookbook" type assays for conditions of stress in general, as well as for specific stressing agents; the establishment of normal responses to all stress assays for a given species; determination of whether given physiological, biochemical and histopathological responses to a particular stress are the same from species to species; and investigations into the positive vs. negative synergistic effects on bivalves due to combinations of pollutants. Future studies would, ideally, coordinate holistic and reductionistic approaches into joint efforts in order to determine how effects of various stress-inducers effect the interactions between the several levels of biological organization within a bivalve species and how these internal "spatial" interactions progress temporally.

IMMUNOSUPPRESSION OF OYSTERS BY TRIBUTYLTIN. William S. Fisher,* University of Texas Medical Branch, Marine Biomedical Institute, Galveston, TX 77550; **Fu-Lin E. Chu,** Virginia Institute of Marine Science, Gloucester, VA 23062; **Arieh Wishkovsky,** University of California, School of Veterinary Medicine, Davis, CA 95616.

Cellular defense activities of eastern oysters *Crassostrea virginica* and Pacific oysters *C. gigas* were examined during acute *in vitro* exposure to tributyltin (TBT), the active ingredient in certain marine antifouling paints. Eastern oysters were collected from two sites in Chesapeake Bay and Pacific oysters were imported from Puget Sound, Washington and quarantined in closed-system aquaria. Chemiluminescent activity of hemocytes incubated with zymosan particles was measured from each of these stocks as a presumptive indicator of phagocytosis. In all cases, chemiluminescence was increased slightly upon exposure to low (0.4 ppb) levels of TBT but was reduced at 40 ppb TBT exposure and nearly eliminated at 400 ppb. Hemocytes were not affected by acute TBT exposure in their ability to spread to an ameboid shape or regulate changes in external salinity, but the rate of hemocyte locomotion was decreased upon acute exposure to 40 and 400 ppb TBT. A positive correlation between rate of locomotion and phagocytic activity, previously established by other studies, was supported by these results. Endocytosis underlies both of these activities and "capping" of hemocyte membranes may be the cause of decreased activity. Acute exposure to TBT retarded locomotion and phagocytosis at concentrations 1–2 orders of magnitude higher than ambient levels. However, when rates of bioaccumulation and chronic exposure are considered, it can be deduced that ambient levels of TBT cause immunosuppression in oysters.

THE USE OR USELESSNESS OF FREE AMINO ACIDS AS A BIOCHEMICAL INDICATOR OF POLLUTION. Herman Hummel,* Roelof Bogaards, Lein de Wolf, and **Jan Sinke,** Delta Institute for Hydrobiological Research, Vierstraat 28, 4401 EA Yerseke, The Netherlands.

In a previous study on the mussel *Mytilus edulis* in the Dutch Delta area it was shown that spatial and temporal fluctuations in total or individual free amino acids were primarily related to differences in salinity. The applicability of 2 stress-indices, i.e. taurine/glycine ratio and the sum of the threonine and serine concentrations, was not unambiguous, because annual and spatial fluctuations in the indices were too large. Therefore, it was not possible to discriminate between mussels from the polluted Westerschelde estuary or from relatively un-polluted areas (Oosterschelde sea-arm and brackish lake Grevelingen).

To test whether the suggested biochemical stress-indices are applicable or not under even extreme levels of stress, mussels were sampled in the Fal estuary in England. In this estuary, because of nearby mining activities, the mussels have to cope with ten-fold higher concentrations of heavy metals (especially zinc and copper) in the water, than in the Dutch Delta area.

Ecophysiological parameters (beside free amino acids also other parameters such as condition, glycogen and enzyme activities) showed again a relation with salinity. The values of these parameters were not very different from those in the Dutch Delta area.

It might be concluded that in case mussels are able to live in a certain (polluted) area, environmental parameters other than pollution, such as salinity, food or wave exposure, determine the measured ecophysiological parameters.

THE METABOLIC TRANSFORMATION OF AROMATIC AMINES IN MARINE BIVALVES AND IMPLICATIONS FOR GENOTOXIC EFFECTS. John P. Knezovich, Environmental Sciences Division L-453, Lawrence Livermore National Laboratory, Livermore, CA 94550.

The impact of many organic contaminants on shellfish populations may depend on the organisms' ability to metabolically activate or detoxify such compounds. This is particularly true for aromatic amines, which are often responsible for the mutagenic activity of fossil fuels and municipal effluents. When these contaminants enter the marine environment they may adversely impact the survival and reproduction of shellfish as a result of alterations to genetic material. These studies were undertaken to define the metabolism of aromatic amines in two commercially important bivalves (*Mytilus edulis* and *Crassostrea gigas*) and to examine potential genotoxic effects.

The mussels and oysters investigated were found to be capable

of activating and detoxifying several aromatic amines *in vivo* and utilized metabolic pathways that are responsible for normal biochemical roles. Transformations occurred exclusively at the nitrogen atom and consisted of *N*-oxidation and conjugation (methylation, acetylation, and formylation) reactions. Aromatic-ring oxidations and conjugations that are typically found in vertebrate species were not detected. Based on the low levels of activated products that were formed, limited impacts on genetic material were predicted from the exposure of these species to aromatic amines. Accordingly, evidence of DNA adducts, single-strand breaks, and sister chromatid exchanges were not detected in mussels exposed to aromatic amines but were demonstrated in mussels exposed to mutagens that do not require metabolic activation. (This work was performed under the auspices of the Ecological Research Division of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.)

HEMATOPOIETIC NEOPLASIA IN THE SOFT SHELL CLAM: POSSIBLE INTERACTIONS WITH ANTHROPOGENIC INPUTS INTO THE ENVIRONMENT. **Dale F. Leavitt*** and **Judith McDowell Capuzzo,** Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Hematopoietic neoplasia (Hn) has been observed in *Mya arenaria* (L.), the soft shell clam, from the northeastern U.S. (north of Chesapeake Bay) since 1952. Hn is characterized by increased numbers of morphologically altered cells circulating in the hemolymph. When compared to non-diseased hemocytes the neoplastic cells differ in that they are round with a higher nuclear to cytoplasmic ratio and usually have a distinct nucleolus. Hn cells have lost the ability to phagocytize, a primary function of bivalve hemocytes. Although remission has been reported to occur in earlier stages, Hn is considered fatal when it advances to later stages of the disease.

Before investigating the interrelationship of anthropogenic inputs to Hn, basic information on the etiology and impact of the disease are necessary. Previous work suggests that Hn follows a seasonal pattern of prevalence with high levels in the winter months and low levels in the spring/summer months. It has been suggested that the seasonal pattern is controlled by rates of mortality and remission of diseased clams as well as recruitment into the size classes which have been shown to have higher prevalences of Hn.

When comparing *M. arenaria* from contaminated sites (eg. New Bedford, MA) to clams from relatively clean sites (eg. Buzzards Bay, MA) significant differences are noted both in the prevalence of Hn and the physiological condition of the animals. New Bedford Harbor clams have significantly higher prevalence of Hn and significantly higher condition index as well. In contrast Buzzards Bay clams appear to be in better condition from an energetic standpoint. These differences will be discussed in terms of previous research on the correlation of hematopoietic neoplasia to anthropogenically perturbed environments.

LETHAL AND SUBLETHAL EFFECTS OF AGRICULTURAL NONPOINT-SOURCE INSECTICIDE RUNOFF ON THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN): IMPLICATIONS OF UPLAND MANAGEMENT PRACTICES. **James M. Marcus*** and **Fluor Daniel,** P.O. Box 19019, Greenville, SC 29602; **Geoffrey I. Scott,** Department of Environmental Health Sciences, University of S.C., Columbia, SC 29208.

Stormwater runoff from two agricultural areas (treatments 1 and 2) and natural forested lands (control) in coastal South Carolina have been monitored since the 1987 crop growing season to assess the levels of insecticides lost from tomato fields and associated physiological effects on the American oyster, *Crossostrea virginica*. Pesticides of interest are endosulfan, fenvalerate, methyl parathion and azinphosmethyl, since all four are surface-applied for pest control in these fields.

Oysters were exposed to endosulfan and fenvalerate during the 1987 and 1988 growing seasons at treatment 1 (Leadenwah Creek) although the exposure level was lower in 1988. In 1987, significant differences were observed in oyster condition and gonadal indices between treatment 1 and control after a major runoff event. In 1988, there were no significant differences observed between treatment 1 and the control. Oysters at treatment 2 (Kiawah Island) were exposed to elevated levels of endosulfan and azinphosmethyl during both seasons. Subsequently, significant differences in the physiological indices were observed between the treatment 2 and the control sites in both 1987 and 1988. A retention pond was built at treatment 1 between the 1987–88 seasons which reduced the volume of uncontrolled nonpoint-source runoff by approximately 50%. The levels of fenvalerate and endosulfan in the receiving stream in 1988 after rain events were effectively reduced by this control measure while insecticide levels in the receiving stream at uncontrolled treatment 2 were comparable to levels measured in 1987.

These chemical and physiological data demonstrate that management practices for uplands are important links to receiving stream water and biological quality. These data suggest that water quality protection should begin with proper land-use planning and management practices. A simple, yet effective, control system at treatment 1 demonstrated that the management of upland activities can offer protection to aquatic fauna and lessen impacts. This approach would seem to be preferential to attempts at impact mitigation after the fact for the restoration of water and biological quality.

RESPONSE OF OYSTER METALLOTHIONEINS TO CADMIUM EXPOSURE. **G. Roesuadi,** Chesapeake Biological Laboratory, University of Maryland, Solomons, MD 20688.

Recent work in this laboratory resulted in the identification of two cadmium-induced, metal-binding proteins of *Crassostrea virginica* as class I metallothioneins. They appear to be identical in structure, with the exception of a NH_2-terminal block in one of the two proteins. Although currently unidentified, the blocking moiety has been shown to confer increased hydrophobicity to this protein. The physiological behavior of these two proteins was examined in more detail in experiments that focused on the response of gills. Under experimental conditions utilized in this laboratory, these metallothioneins are induced within one to four days of cadmium exposure. The increased cadmium-binding rates associated with the induced metallothioneins are also associated with decreased rates of binding to other intracellular structures, thus appearing to spare these other structures from binding cadmium. Examination of the two individual metallothionein forms showed that the initial rate of synthesis of the blocked form of the protein increases more rapidly, in comparison with the other form, then declines to an induced rate common to both. Comparison of the observed pattern of accumulation of the two proteins with that predicted from the rates of synthesis indicated that the blocked form is either more rapidly degraded or transported out of the gills. The blocking moiety may impart properties to the protein that facilitates this differential behavior.

TEST FOR EFFECTS OF EUTROPHICATION ON THE TOXICITY OF COPPER TO THE BLUE MUSSEL, *MYTILUS EDULIS*. **Gregory A. Tracey,** Science Applications International Corporation, c/o EPA, Narragansett, RI 02882.

The effects of eutrophication on the toxicity of copper (Cu) to the blue mussel, *Mytilus edulis*, was tested using outdoor, 13,000 L experimental marine ecosystems (mesocosms). Mussels were held in exposure systems which received overflow water from mesocosms. A single addition of $CuSO_4$ to each of three mesocosms elevated background Cu by 40 $\mu g\ L^{-1}$. Eutrophic conditions within mesocosms were created by daily nutrient additions over a 16-fold concentration range. Because of flushing and other losses, total Cu exposure concentrations were initially high but then exponentially declined to near background levels (4 $\mu g\ L^{-1}$) within 28–32 days. While total and dissolved Cu concentrations were similar among treatments over time, particulate-bound Cu concentrations were higher in treatments of lower nutrient enrichment.

Bioenergetics measurements were made in the laboratory under standardized conditions in order to elucidate persistent treatment effects. After 4 days exposure, reduced clearance rates and reduced assimilation efficiencies of test algae were observed in mussels from treatments which had higher particulate Cu. After 32 days exposure, reduced assimilation efficiencies were noted in all Cu treatments in comparison to the control (no nutrients or copper added). Trends observed in other bioenergetic parameters (clearance, respiration and excretion rates) were less pronounced, but also indicated a net reduction in the energy available for growth ("scope for growth"). These responses could not otherwise be explained by differences in other environmental parameters (dissolved oxygen, total suspended matter, chlorophyll *a*, phytoplankton composition). These results suggested that eutrophication may modify the availability of biologically active Cu species. In addition, Cu uptake through food may have been a toxicologically significant route of exposure.

BROWN CELLS OF OYSTERS AS A POLLUTION INDICATOR. **Paul P. Yevich,*** SAIC, 27 Tarzwell Drive, Narragansett, RI 02882; **Gerald A. Zaroogian,** ERLN-EPA, 27 Tarzwell Drive, Narragansett, RI 02882.

The role of the brown cells in oysters has not been determined. Brown cells in healthy oysters are found in the connective tissue, mainly around sinusoids, in the lining of the pericardial cavity and the muscle bundles of the auricle. The brown cell is a connective tissue cell whose cytoplasm may contain small light yellowish brown globules or large reddish brown globules which may occupy the entire cytoplasm of the cell. Histopathologic studies were conducted on oysters (*Crassostrea virginica*) collected from 4 sites along the Pawcatuck River, R.I. during spring, summer, fall and winter. Regardless of collection site, animals that showed histopathologic changes also showed changes in the brown cells. An increase in the number of brown cells per microscopic field was accompanied by an increase in size and color of the globules. This was especially noted around foci of inflammation, degeneration and necrosis. Oysters collected from polluted sites showed as increase in number and size of the brown cells in comparison of those collected from non-polluted sites. By observing the condition of the brown cells, it was possible to determine whether or not the animals had been exposed to a chemical or biological stress. We are of the opinion that the brown cells in oysters act as a detoxifying mechanism.

FUNCTION OF BROWN CELLS IN *CRASSOSTREA VIRGINICA* AND *MERCENARIA MERCENARIA*. **Gerald E. Zaroogian,*** U.S. Environmental Protection Agency, Narragansett, R.I. 02882; **Paul P. Yevich,** S.A.I.C. Marine Sciences Branch, Narragansett, R.I. 02882.

Brown cells of *Crassostrea virginica* and *Mercenaria mercenaria* are involved in the excretory process. Toluidine blue (soluble dye) accumulated in brown cells after dye injection into the foot muscle and addition to brown cell isolates. In comparison, carmine red (particulate dye) did not accumulate under the same conditions. FITC-labelled bovine albumen accumulated in brown cells when introduced *in vivo* and to cell isolates. Kinetic studies indicated uptake of FITC-albumen occurred within 2 hrs. when added to cell isolates and within 24 hrs. after injection into the foot muscle. As much as 50% of Ni and 30% of Cd added were

detected in brown cells 15 minutes after introduction to cell isolates. Accumulation increased linearly with solute concentration and stopped when equilibrium was reached between intracellular and extracellular Ni and Cd concentrations. Brown cell isolates were separated into three fractions on a discontinuous Percoll gradient. Assays with lysates from each fraction indicated the presence of acid phosphatase, glutathione reductase and lysozyme. Lysozyme and acid phosphatase are good markers for lysosomes, therefore, their presence in brown vesicles suggested that these vesicles are secondary lysosomes. Data indicate that the larger brown vesicles appeared to be the result of fusion of smaller vesicles. It appeared that brown cells function in accumulation and detoxification of inorganic and organic compounds.

RED TIDE

PYRODINIUM BAHAMENSE* AND PSP ALONG THE PACIFIC COAST OF CENTRAL AMERICA. Eugenia Canahui, LUCAM, Ministry of Health, Guatemala; **Fernando Rosales Loessener,** Ministry of Agriculture, Guatemala; **Lee Miller, Ellen W. King,** and **S. Hall,** Food and Drug Administration, HFF-423, 200 C Street SW, Washington, DC 20204.

In the summer of 1987, an outbreak of PSP due to a bloom of *Pyrodinium bahamense* on the Pacific coast of Guatemala left 26 people dead. This was the first known occurrence of PSP in this area. In the fall of 1989, another bloom of *P. bahamense* occurred, apparently affecting the entire west coast of Central America to varying degrees and leading to very high levels of toxicity in shellfish. However, prompt action by the government of Guatemala prevented an outbreak of illness that otherwise would likely have been even more serious than the first.

DINOPHYSOID DINOFLAGELLATES RESPONSIBLE FOR DIARRHEIC SHELLFISH POISONING IN EASTERN NORTH AMERICA: TOXICITY, SYSTEMATICS, AND BIOGEOGRAPHIC ASPECTS. Allan Cembella* and **Richard Larocque,** Maurice Lamontagne Institute, Dept. of Fisheries and Oceans, Mont Joli, Quebec, Canada G5H 3Z4; **Michael Quilliam** and **Steven Pleasance,** Atlantic Research Laboratory, Halifax, Canada B3H 3Z1.

The gastrointestinal disease known as diarrheic shellfish poisoning (DSP) has been frequently associated with the consumption of shellfish contaminated by certain species of dinoflagellates. Along the east coast of the United States, *Dinophysis* spp. have been occasionally identified as the causative organisms of DSP, and suspected cases of intoxication may also have occurred in Canada. However, the presence of particular DSP toxin components has never previously been confirmed in dinoflagellates nor toxic shellfish from North American waters.

In the present study, the seasonal dynamics of blooms and taxonomic affinities of dinophysoid species were investigated in the Bay of Gaspé, along the western coast of the Gulf of St. Lawrence. Okadaic acid, one of the major DSP toxin components, was identified in natural dinoflagellate populations harvested during late summer by net hauls and size fractionation. This toxin was detected in a preliminary screening of dinoflagellate extracts with an enzyme-linked immunological assay (ELISA), and was subsequently quantified by high-performance liquid chromatographic (HPLC) separation of the toxin components, followed by fluorescence detection. Spectral identification of this compound was also achieved by a novel ion-sray mass-spectrometry technique. The application of such methods to the analysis of DSP toxins in dinoflagellates from diverse locations and in toxic shellfish will be addressed.

IMPACTS OF THE 1987–88 NORTH CAROLINA RED TIDE. Patricia K. Fowler,* North Carolina Department of Environment, Health and Natural Resources, Division of Environmental Health, Shellfish Sanitation Branch, P.O. Box 769, Morehead City, NC 28557; **Patricia A. Tester,** National Marine Fisheries Service, NOAA Southeast Fisheries Center, Beaufort Lab, Beaufort, NC 28516.

The first recorded occurrence of *Ptychodiscus brevis* (formerly *Gymnodinium breve*) along the North Carolina coast caused approximately 365,000 acres of approved shellfish harvesting waters to be closed and impacted approximately 50% of the oyster and 90% of the clam harvesting areas in the State. In addition, there were significant scallop mortalities reported for some areas. The dollar value of the North Carolina shellfish resource was reduced by almost 50% from the previous season and the economic loss to the coastal community was estimated conservatively at $24 million.

By the time *P. brevis* was identified from North Carolina coastal waters (2 November 1987), there were 6 million cells/liter in nearshore waters. Harvesting of oysters, which had started in all parts of the State on 15 October, was halted immediately. Harvesting of clams was also delayed until waters reopened between 19 February and 6 May 1988.

There were 48 confirmed illnesses from consumption of shellfish contaminated by brevetoxins during this red tide event. Thirty-five cases occurred before the first ban on shellfish harvesting (2 November 1987) and all but six cases occurred between 27 October and 5 November. One other case occurred in December and was traced to shellfish harvested on 31 October and frozen until the time of the meal.

***GYMNODINIUM CATENATUM:* A RECENTLY DISCOVERED CAUSE OF PARALYTIC SHELLFISH POISONING. Gregory Gaines,** 2001 N. Adams St. #903, Arlington, VA 22201.

Gymnodinium catenatum, a photosynthetic, unarmored, chain-forming dinoflagellate, has caused PSP in Spain, the Pacific coast of Mexico and in Tasmania. It is the only unarmored dinoflagellate known to produce these toxins, and so represents a problem in justifying the use of toxins as classificatory characters. In addition, most of the toxins present in this species are N-sulfocarbamoyl derivatives, which are poorly detected by the standard mouse bioassay.

A major issue surrounding this species is its distribution. In Tasmania and Spain, where it was previously unknown, some researchers have suggested that it may have been introduced recently, perhaps by human activities such as transport in the ballast water of cargo ships. On the other hand, *G. catenatum* was first described in 1943. Since then, it has been reported from the eastern and western Pacific, the eastern and western Atlantic, and the Mediterranean. This wide distribution, together with the known ability of this species to form durable resting cysts, suggests to the contrary. That is, it suggests that the recent toxic outbreaks are not due to recent transport, but rather to episodes of synergism between ecological variables, resulting in optimal local conditions for dense growth of already resident but sparse or encysted populations.

MONITORING PROGRAM FOR DSP IN DUTCH SHELLFISH GROWING AREAS. P. Hagel, Netherlands Institute for Fishery Investigations, P.O. Box 68, 1970 AB IJmuiden, The Netherlands.

The worldwide presence of shellfish toxins makes it necessary to take appropriate measures to protect public health and to prevent the introduction of toxic shellfish into commerce.

The Netherlands Institute for Fishery Investigations is responsible for the sanitary shellfish control in The Netherlands. Within this control there exists a monitoring program for the detection of diarrhetic shellfish poison (DSP) and the identification of DSP-toxins producing phytoplankton species, e.g. the dinoflagellate *Dinophysis acuminata*.

For the determination of DSP in shellfish a rat-bioassey is used, in which white rats are fed with the hepatopancreas of the shellfish to be analysed. The refusal of hepatopancreas and the consistency of the faeces produced are used for a rating system for the presence of DSP-toxins. Although this rat-bioassay is very relevant to the potential consumers of shellfish products, no information is produced on the specific DSP-toxins present.

Phytoplankton counts in the shellfish growing areas and in the adjacent coastal waters are used to forecast the development of DSP-poisoning and the time necessary for depuration of the affected shellfish areas. Depending on the water temperature it takes one week up to several weeks, after the disappearance of the toxic phytoplankton, for the shellfish to lose it's toxicity.

During the presence of DSP-toxins the harvesting of shellfish from an affected area is prohibited. Depending on the water temperature, two to three weeks after the disappearance of the toxins the harvesting of shellfish is allowed to be resumed again.

RECENT DEVELOPMENTS IN DETECTION METHODS FOR SHELLFISH TOXINS. S. Hall,* E. W. King, and **L. Miller,** Food and Drug Administration, HFF-423, 200 C Street SW, Washington, DC 20204; **E. Canahui,** LUCAM, Ministry of Health, Guatemala; **D. Price,** California State Department of Health Services, Environmental Health Division, Santa Rosa, California 95404; **D. Gann,** California State Department of Health Services, Division of Laboratories, Sanitation and Radiation Laboratory, Berkeley, California 94704; **J. Hurst,** State of Maine Department of Marine Resources, West Boothbay Harbor, Maine 04575.

Effective monitoring programs for shellfish toxicity require efficient detection methods. Work is being conducted both on the improvement of the mouse bioassay and the development and evaluation of alternative methods.

PARALYTIC SHELLFISH POISONING TOXINS AS A CHEMICAL DEFENSE IN BUTTER CLAMS: THE EVIDENCE. Rikk G. Kvitek, Department of Zoology, University of Washington, Seattle, WA 98195.

Results from a series of studies investigating 1) the relative resistance of bivalve species to saxitoxin (STX), 2) the influence of STX contaminated prey on the feeding behaviors of sea gulls (*Larus glaucenscens*) and sea otters (*Enhydra lutris*), and 3) the geographic distribution of sea otters with respect to butter clam (*Saxidomus giganteus*) toxicity and abundance, strongly suggest that butter clams are able to utilize dinoflagellate toxins as a highly effective deterrent to predation. Neurons from the butter clam and its congener the Washington clam (*S. nuttalli*) were shown to be 10–100 times more resistant to saxitoxin (STX) than those from four co-occurring infaunal bivalves (*Mya arenaria, Mya truncata, Tresus capax, Protothaca staminea*).

Captive sea otters fed live butter clams ad libitum, either reduced their prey capture and consumption rates, or discarded the highly toxic siphons, and gills with attached kidneys and paricardial glands when switched from very low toxicity (37 ± 9 μg STX/100 g) to highly toxic (226 ± 96 μg STX/100 g) prey. Paralytic shellfish poisoning symptoms were observed only in the otter calculated to have consumed the most STX (154 μg kg^{-1} d^{-1}), and all subjects were released in good health. These findings suggest that sea otters are not immune to paralytic shellfish poisoning toxins (PSPT), but that they are able to detect and avoid consumption of lethal amounts of toxic prey.

A comparison of sea otter distribution with that of butter clam toxicity in southeast Alaska, suggests that the sequestering of PSPT likely limits the distribution of sea otters to the exposed outer coast where toxic prey is rare. Finally, benthic surveys

throughout Alaska show that dense butter clam populations can persist only in the absence of sea otter predation, demonstrating the probable impact of PSPT as a chemical defense.

***ALEXANDRIUM* SP., *GYMNODINIUM CATENATUM*, AND PSP IN VENEZUELA. A. La Barbera** and **Gisela Estrella,** Estacion Experimental Sucre, FONAIAP, Cumana, Venezuela; **Lee Miller, Ellen W. King,** and **S. Hall,** Food and Drug Administration, HFF-423, 200 C Street SW, Washington, DC 20204.

The north coast of Venezuela is a very productive region with a vigorous and developing shellfish industry based on both cultured and wild stocks. Unfortunately, this region also occasionally gives rise to dense blooms of toxigenic phytoplankton which can render the shellfish quite toxic and have caused human illness and death, and serious disruptions in the shellfish industry. A monitoring program has been established to deal with the problem, based on mouse bioassay of periodic and lot samples. Both *Gymnodinium catenatum* and various *Alexandrium* species have been observed, and contribute to toxicity.

PARALYTIC SHELLFISH POISONING (PSP) IN OFFSHORE WATERS OF THE NORTHEAST. Robert J. Learson and **Christopher Martin,** National Marine Fisheries Service, Gloucester Laboratory, Emerson Avenue, Gloucester, MA 01930.

Paralytic Shellfish Poisoning (PSP) occurs annually during the summer months along the eastern coast of North America from Cape Cod to Canada.

The National Shellfish Sanitation Program which is operated cooperatively between the FDA and shellfish producing states routinely monitors PSP in inshore waters. PSP blooms in offshore waters are not routinely monitored and have not been considered to be a public health problem. During July and August of 1989 significant toxic blooms of PSP were experienced in the Gulf of Maine and under the NSSP protocol softshell clams, mussels, and quahog fisheries were periodically closed. In July 1989 Canadian Fisheries and Oceans reported PSP in scallops on Georges Bank and shut down the roe-on-scallop fishery. In August the State of Massachusetts determined that surf clams harvested from southern Georges Bank contained high levels of PSP (300–500 micrograms/100 grams). This resulted in a closure of the offshore surf clam fishery. Results of further monitoring of shellfish in offshore waters are reported. The future implications of offshore biotoxin outbreaks are discussed.

UPTAKE AND DEPURATION OF PSP TOXINS FROM THE RED TIDE DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE* BY *MERCENARIA MERCENARIA*. Jihyun Lee,* and **V. M. Bricelj,** Marine Sciences Research Center, State University of New York, Stony Brook, N.Y. 11794; **A. D. Cembella,** Maurice Lamontagne Institute, 850 route de la mer, P.O. Box 1000, Mont-Joli, Quebec, Canada G5H 3Z4.

Laboratory experiments were conducted to investigate the influence of dinoflagellate cell toxicity on PSP (Paralytic Shellfish Poisoning) toxin uptake and depuration by *Mercenaria mercenaria*, previously reported to remain non-toxic during red tides. Hard clams were exposed to two strains of *Alexandrium fundyense* of high and low toxicity (GtCA29 and GtLI22; 101 and 5 pg Saxitoxin eq $cell^{-1}$ respectively) over ca. 2 weeks, and then allowed to depurate for another 3 weeks. Clams readily ingested and absorbed a monospecific diet of strain GtLI, but only ingested strain GtCA when offered in combination with a known good algal food source. A toxin saturation level of 10.5×10^3 μg Saxitoxin eq 100 g^{-1}, 131× above the U.S. regulatory limit, was attained within 13 days of exposure to strain GtCA. Toxin levels dropped to 40% of the maximum through gut evacuation, within 24h of depuration, but remained 10× above the regulatory limit by the end of depuration. During detoxification of clams fed with strain GtCA, analysis of toxin composition by HPLC showed a gradual enrichment in saxitoxin, concomitant with a decrease in gonyautoxins I&IV. This study thus provides the first experimental evidence of PSP intoxication by *M. mercenaria*.

RATIONALE FOR TESTING FOR P.S.P. BY A PRIMARY PRODUCER OF SHELLFISH. Link Murray, Blue Gold Sea Farms, Ltd. P.O. Box G392, New Bedford, MA 02740.

A small primary producer of shellfish (mostly mussels) decided two years ago to conduct in-house tests for PSP in America and for DSP in Ireland. The reasons were: 1) safety for customers; 2) efficiency for operations; 3) need for standards superceding state and national boundaries. The added benefits have been: 1) the development of a laboratory position; 2) ability to direct harvesting effort on an hourly basis; 3) free consulting from visiting experts and officials; 4) intellectual stimulation and improved morale; 5) insurance against arbitrary regulations or errors of external laboratories; 6) cash savings from absence of recall either in America or in Europe. The costs of the program have been minimal. Despite insufficient data for rigorous verification, the program has led to some predictive ability by the staff.

With experience, the company would recommend that any supplier of shellfish wishing to comply with the primary regulation, "Thou shalt not Kill," test their product for PSP. The process is simple and education is available. The best source for PSP and other quality programs is the FDA (both Shellfish and Health Service) because they combine theoretical with applied knowledge. Other good sources of help are universities and lab staffs of other companies.

TOXIC DINOFLAGELLATE BLOOMS AND PARALYTIC SHELLFISH POISONING IN CALIFORNIA, 1927–1989. Douglas W. Price, Shellfish Program Coordinator, Environ-

mental Management Branch, California Department of Health Services; **Kenneth W. Kizer,** Director, California Department of Health Services.

Paralytic shellfish poisoning (PSP) has been a public health concern in California for over 60 years. Since 1927, when PSP was made a reportable disease, 510 cases, including 32 deaths, have been recorded in the State. Expansion of the shellfishing industry and growing numbers of people involved in sport shellfishing activities in recent years have heightened concern by public health officials in PSP prevention, and prompted a review of available information on PSP incidents and toxic dinoflagellate blooms in California. This paper presents information on the seasonal occurrence, geographic distribution, and types of shellfish involved in PSP illnesses in California. In addition, data from the coastal shellfish monitoring program are used to describe the frequency, intensity, seasonal occurrence, geography, and dynamics of toxic dinoflagellate blooms along the California coast. Also noted are differences in toxin levels in mussels, oysters, and other types of shellfish exposed to the same bloom conditions. Toxic bloom characteristics and changes in commercial and sport shellfishing activities are discussed in relation to PSP risks and the State's PSP prevention program. The development of that program and an assessment of its effectiveness over the past 60 years are included.

THE EFFECTS OF TOXIC ALGAE ON THE BEHAVIOR AND PHYSIOLOGY OF BIVALVE MOLLUSCS. Sandra E. Shumway,* Department of Marine Resources, West Boothbay Harbor, Maine 04575 and Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575; **Terry L. Cucci,** Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575.

It has long been believed that toxic dinoflagellates and other toxic algal species have little or no effect on the well-being of marine molluscs. Despite some data to the contrary, many authors have perpetuated the myth that these algal species are harmless to the molluscs. Studies in our laboratory during the past 5 years have indicated that toxic dinoflagellates can elicit various responses by the host molluscs. Responses are species-specific and have been shown to vary according to geographic locations. Responses to the presence of the toxic dinoflagellate, *Alexandrium tamarensis*, include shell-valve closure and or siphon retraction (*Mya arenaria, Mytilus edulis, Geukensia demissa*), cessation of feeding (*Mercenaria mercenaria*), increased rates of particle selection (*Ostrea edulis*), production of mucus (*M. edulis* from Spain and Rhode Island, *Placopecten magellanicus, G. demissa*), mortalities (*M. edulis* from Spain); transient cardiac inhibition (*M. arenaria*), long term decrease in heart rate (*O. edulis*), varied inhibition of cardiac activity (*G. demissa, M. edulis*); inhibition of byssus production (*M. edulis* and *G. demissa*); increased rates of oxygen consumption (*M. arenaria*, some *M. edulis*), decreased rates of oxygen consumption (*P. magellanicus, Spisula solidissima*) and mortalities (*M. edulis* from Spain). *Modiolus modiolus* has thus far exhibited no response to the presence of *A. tamarensis*. These data are compared with other molluscan species' responses to the presence of toxic algal species.

CURRENT DEVELOPMENTS IN MONITORING PROGRAMS FOR SHELLFISH TOXICITY: A GOVERNMENT PERSPECTIVE. Ira J. Somerset, Food and Drug Administration, Northeast Region, 1 Montvale Avenue, Stoneham, Massachusetts 02180.

Both consumer safety and the economic well-being of the seafood industry depend on programs that detect and intercept toxic shellfish before they get to the consumer. When these programs are successful and sustain consumer confidence in the safety of the product, the entire seafood industry benefits. Failure which leads to human illness can have serious economic impacts on the seafood industry as a whole. As the understanding of the distribution and nature of shellfish toxicity evolves, the structure of the monitoring programs is being refined to ensure that they protect public health so that consumer confidence will be maintained and the industry will prosper.

RED TIDE IN NORTH CAROLINA: A CASE STUDY IN TRANSPORT, DISTRIBUTION & PERSISTENCE. Patricia A. Tester,* National Marine Fisheries Service, NOAA Southeast Fisheries Center, Beaufort Lab., Beaufort, NC 28516; **Patricia K. Fowler,** North Carolina Department of Environment, Health and Natural Resources, Division of Environmental Health, Shellfish Sanitation Branch, P.O. Box 769, Morehead City, NC 28557.

Ptychodiscus brevis (formerly *Gymnodinium breve*) was identified (6×10^6 cells l^{-1}) from water samples taken off the North Carolina coast on 2 November 1987. This was the first recorded occurrence of *P. brevis* north of Florida and extended the range of this toxic, subtropical dinoflagellate over 800 km northward. Before the end of this bloom 3.5 months later, there were 48 cases of neurotoxic shellfish poisoning reported in humans and over 1,480 km^2 of shellfish harvesting waters were closed during prime harvesting season. The economic loss to the coastal community was conservatively estimated at $25 million.

P. brevis blooms are common along the Gulf coast of Florida and the transport of cells to the east coast of Florida has been documented. We suggest the Florida Current-Gulf Stream system transported *P. brevis* northward to the coast of North Carolina in October 1987. Approximately 30 days after a bloom was reported off Tampa-Charlotte Harbor, FL (10–29 Sept. 1987) a strong shoreward incursion of Gulf Stream water onto the North Carolina coast was detected. Using satellite images of sea surface temperature we substantiated the timing and shoreward movement of a parcel of Gulf Stream water as well as the stability of this feature

on the North Carolina continental shelf during the *P. brevis* bloom. Both alongshore wind speed and direction were also important factors in the distribution of this bloom.

DOMOIC ACID, A NEW SHELLFISH TOXIN: THE CANADIAN EXPERIENCE. J. L. C. Wright, National Research Council of Canada, 1411 Oxford St., Halifax, Nova Scotia B3H 3Z1.

During the latter half of 1987, human intoxication was reported in Canada following ingestion of cultivated mussels from a localised area of Prince Edward Island. Affected people displayed symptoms including vomiting, seizures, disorientation and memory loss. Four people died. Known toxins, pollutants and heavy metals were quickly ruled out as being responsible. Chemical investigations into the nature of the toxin were undertaken in this laboratory. Using two separate bioassay-guided fractionation schemes, the toxicity in the shellfish was discovered to be associated with the neurotoxin domoic acid. The concentration of domoic acid in mussels ranged from 300–900 ug/g shellfish tissue.

Domoic acid was originally isolated some thirty years ago from the red macroalga *Chondria armata*. In this case the source of the neurotoxin was eventually traced to a massive bloom (*ca* 10^7 cells/litre) of the pennate diatom *Nitzschia pungens* forma *multiseries* that occurred in several estuaries used for mussel culture. Studies in our laboratory have shown that non-axenic cultures of *N. pungens* produce domoic acid after the culture has entered the stationary phase.

This is the first known instance of human poisoning by domoic acid and the first involvement of a diatom in shellfish toxicity. The implications for aquaculture of filter-feeding molluscs is not yet defined but they are potentially significant.

CLAM BIOLOGY AND CULTURE

POND CULTURE OF HARD CLAMS. C. B. Battey* and **J. J. Manzi,** Marine Resources Research Institute, Charleston, SC 29412.

Pond culture of shrimp is a rapidly growing aquaculture industry in the southeast. The growing season for shrimp in the southeast extends from spring through fall and the ponds are left vacant over the winter. The economics of shrimp farming in the southeast could be improved by utilizing the ponds in this off-season for culture of other species. Studies over the last few years have demonstrated the potential of utilizing these ponds for culture of hard clams. Ponds can be used for nursery culture of small clams, grow-out of larger seed, and conditioning of broodstock. Problems encountered in previous years included difficulties in maintaining phytoplankton blooms in the ponds, excessive growth of macroalgae, and high mortality with very small seed. This study investigated two types of direct pond culture: on-bottom and off-bottom culture of 7mm seed. In addition, pond water was pumped to a portable upwelling nursery in which smaller seed (3mm) were stocked at varying densities. An adjacent pond was managed to induce intense phytoplankton blooms and used for water exchange with the culture pond. Growth and survival were compared between the on-bottom and off-bottom cultures and between densities in upwelling units. Results were compared with studies done in previous years.

USE OF CONCENTRATED BACTERIA TO ENHANCE SURVIVAL OF EARLY POST SET *MERCENARIA MERCENARIA* IN A FLOWING SEAWATER NURSERY. Michael Castagna,* Mark Luckenbach, and **Patricia Kelley,** College of William and Mary, School of Marine Science, Virginia Institute of Marine Science, Wachapreague, VA 23480.

Commercially available concentrated nitrifying bacterial mixtures were added to a flowing seawater clam nursery system. Clams receiving the bacterial additive had significantly better survival than control groups. There was no measurable difference in the amount of ammonia, nitrite, or nitrate between treated and untreated trays. Possible reasons for the improved survival are discussed.

PERIODICITY OF GROWTH LINES IN LARVAL AND POSTLARVAL SHELLS OF *MERCENARIA MERCENARIA*. Serena Cenni* and **Robert M. Cerrato,** Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794; **Scott E. Siddall,** Kenyon College, Department of Biology, Gambier, OH 43022.

This study provides information on the temporal significance of microgrowth lines in larvae and early juvenile hard clams (*Mercenaria mercenaria*). To determine the periodicity, and if the nature of the periodicity is either intrinsic or extrinsic, hard clam larvae and postlarvae were reared in the lab under different experimental light regimes. All other potential sources of external cues were either controlled or monitored. In one experiment, larval and postlarval cultures were reared in light of gradually varying photoperiod and intensity matched to the natural light regime at the time of the experiment. In the second experiment, larvae and postlarvae were kept under constant dark conditions. Postlarvae raised under constant dark conditions were transferred into the field where they were subjected to the full range of natural, external cues.

Microgrowth lines in larvae from both light regimes appear to occur with a frequency of about four lines per day. In larvae grown under constant dark conditions, microgrowth lines are regularly spaced, suggesting a periodic internal rhythm. Microgrowth patterns in larvae from natural photoperiod cultures are more irregular in spacing. In postlarvae from both light regimes, the fre-

quency of microgrowth line production decreases to 1–2 per day. Postlarvae reared in the dark and later transferred to the field show a very obvious color change but no obvious change in the frequency of line production. The relationship between larval and postlarval microgrowth lines and the diurnal cycle is discussed.

TISSUE PRODUCTION, PRODUCTIVITY AND TURNOVER OF HARD CLAMS, *MERCENARIA MERCENARIA*, AT DIFFERENT DENSITIES AND TIDAL LOCATIONS. **Arnold G. Eversole*** and **Joy G. Goodsell,** Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson, SC 29634-0362; **Peter J. Eldridge,** Charleston Laboratory, NOAA, NMFS, P.O. Box 12607, Charleston, SC 29412.

Hard clams (SL = 13 mm) were planted in protected trays at densities of 290, 869 and 1,159/m^2 in subtidal and intertidal locations. Clams representative of the size distribution of each treatment were sampled 21 times during a 3-year grow-out period. Shell lengths (SL in mm) and wet weights of shucked meats (TWW in mg) were used to derive: TWW = 1136.9 − 131.3 SL + 4.4 SL^2; r^2 = 0.94. Calculated tissue production (g/m^2) and productivity (g/m^2/day) estimates were significantly highest in those trays held at the subtidal location and planted at 1,159/m^2. Clams at 290/m^2 reached market size (SL = 44 mm) approximately 100 and 285 days earlier than clams at 869 and 1,159/m^2, respectively. However, production estimates using variable times to market size indicated that clams grown at the intermediate density (869/m^2) had the highest tissue production rate. Efficiency of tissue production (turnover) was highest at 290/m^2 and lowest at 1,159/m^2. These findings will be discussed in relation to the current methodology used for stocking clams in field grow-out operations.

CHARACTERIZATION OF HARD CLAM (*MERCENARIA MERCENARIA*) HABITATS IN THE EASTERN GREAT SOUTH BAY, NEW YORK. **Jeffrey Kassner,*** Town of Brookhaven, Medford, N.Y. 11763; **Robert M. Cerrato,** Marine Sciences Research Center, State University of New York, Stony Brook, N.Y. 11794.

Annual hard clam (*Mercenaria mercenaria*) census data for the eastern Great South Bay, N.Y. from 1986 to 1989 suggests the existence of discrete and stable areas of high and low hard clam abundance. For analysis, these areas have been defined as contiguous census stations with hard clam densities above and below the mean hard clam census density, respectively. Comparison of population characteristics of a high and a low hard clam density area revealed no differences in the ontogenetic growth or the abundance of harvestable size hard clams but the abundance of subharvest size hard clams was an order of magnitude greater and the recruitment to the fishery was 7 times greater in the high density area.

To characterize and contrast physical-chemical and biological characteristics in high and low hard clam density areas, sediment-profile photographs were taken and analyzed at 89 stations along 11 transects traversing high and low density areas using the REMOTS (*R*emote *E*cological *M*onitoring *o*f *t*he *S*eafloor) system. The spatial pattern of hard clam abundance appears related to 4 of the 20 parameters REMOTS measures: sediment surface relief (SSR), depth to RPD (RPD), sediment grain size (SGS), and sediment compactness (SC). Overall, the 41 high hard clam density stations had a greater SGS, rougher SSR, shallower RPD and greater SC compared to the 48 low density stations. Along transects, hard clam abundance was directly related to SC but not consistently with SSR, RPD, or SGS. Qualitative analysis of the photographs shows shell fragments to be generally present at high hard clam density stations, but not at low density stations. The sediment-profile photographs suggest high hard clam abundance is associated with reduced biogenic reworking of the sediment, presence of shell fragments, and/or greater bottom stability.

EFFECTS OF HURRICANE HUGO ON CLAM CULTURE ACTIVITIES IN COASTAL SOUTH CAROLINA. **J. J. Manzi,* C. B. Battey,** and **N. H. Hadley,** Marine Resources Research Institute, Charleston, SC 29412.

Most clam culture activities in South Carolina are concentrated in the Charleston vicinity and therefore were heavily impacted by Hurricane Hugo. The majority of the damage resulted from storm surge rather than high winds. The storm struck at high tide and storm surge ranged from 6–14 feet in Charleston Harbor to as much as 21 feet north of Charleston. The land-based nursery system and the water distribution system for the shellfish hatchery at the Marine Resources Research Institute were completely destroyed. In the field, intertidal units were displaced as much as ¼ mile from their original locations. Many units were never recovered, having apparently been moved into deeper water. Although many clams were lost from damaged cages, there did not appear to be substantial mortality in the units which were recovered. There was wide variation in the extent of damage, depending on the location of the grow-out areas and the orientation of water channels relative to the direction of the storm surge. Post-storm recovery was hampered by extensive damage to docks and equipment and by navigational difficulties resulting from loss of channel markers and the prevalence of debris in the waterways.

PREDATION OF JUVENILE SOFT SHELL-CLAM (*MYA ARENARIA*) BY JUVENILE DUNGENESS CRAB (*CANCER MAGISTER*). **Raul Palacios*** and **David A. Armstrong,** School of Fisheries, University of Washington, Seattle, WA 98195.

Juvenile Dungeness crab (*Cancer magister*) were allowed to forage in the laboratory on buried juvenile soft-shell clams (*Mya arenaria*). Equal numbers of two size classes of clams (6–12,

12–18 mm shell length) were offered to isolated crab in the 3d instar (I3, 12.0–16.8 mm carapace width, CW), 4th instar (I4, 16.9–22.3 mm CW) and 5th instar (I5, 22.4–28.0 mm CW) intermolt stages.

No "size-refuge" was found within the range of clams offered. All crab were able to crush or chip the edge of shells to prey on clams from the larger group, but the numbers of smaller clams preyed upon was significantly higher than expected had the crab been eating randomly from each group. This difference was even more significant when predation rates were calculated as biomass (clam dry weight) consumed in each size class. Size-specific consumption suggest prey selection to minimize handling time.

Consumption rates, both in numbers and biomass, increased with crab size: I3 crab consumed an average of 1.0 clam · day^{-1} (7.3 mg clam · day^{-1}), I4 crab 2.1 clam · day^{-1} (18.5 mg · day^{-1}) and I5 crab 6.0 clam · day^{-1} (52.9 mg · day^{-1}). Weight-specific consumption rates (biomass ingested as percent of crab weight) were similar for I3 and I4 (8.3% and 8.1%, respectively) but increased to 13.6% for I5.

Given typical densities of 5 to 10 crab · m^{-2} in certain habitats of intertidal flats in Grays Harbor estuary, WA, through the summer months, the settling crab population can consume 5 to 60 clam · m^{-2} · day^{-1}. Clam density in spring (May) shortly after recruitment is about 60 · m^{-2} which can get to zero by July. Calculations above suggest that 0+ crab are capable of decimating a new year class of *Mya* over small spatial scales.

FACTORS WHICH INFLUENCE THE GROWTH OF THE HARD-CLAM *MERCENARIA MERCENARIA* IN SMALL-BOAT MARINAS. Robert B. Rheault,* Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882; **Michael A. Rice,** Department of Fisheries and Aquaculture Science, University Rhode Island, South Kingston, RI 02879.

Growth studies were initiated in 1988 and 1989 using *Mercenaria mercenaria* seed suspended in predator proof cages under floating docks in three small-boat marinas and a "clean water" control site. The goals were to evaluate the potential for utilizing these unique environments for the nursery culture of shellfish seed for subsequent growout in certified waters, and to quantify the relative influence of various environmental variables on growth and survival. Weekly measurements of temperature, salinity, total seston, particulate organic matter (POM), chlorophyll-a concentration and fecal coliform were compared with semi-weekly growth rate determinations.

Growth and survival were excellent at all sites. The regression of growth rate versus particulate organic matter concentration had the highest correlation coefficient ($r = 0.7227$) followed by total seston ($r = 0.5547$) and chl-a ($r = 0.4520$). Multiple regression with temperature improved the r value in all cases ($r = 0.7495$, 0.7067, and 0.6784 respectively).

GENETICS/POLYPLOIDY

MITOCHONDRIAL DNA ANALYSIS OF NATIVE AND SELECTIVELY BRED CHESAPEAKE BAY OYSTERS, *CRASSOSTREA VIRGINICA*. Bonnie L. Brown,* Chesapeake Scientific Investigations Foundation, Richmond, VA 23230; **Kennedy T. Paynter,** Chesapeake Bay Institute, Johns Hopkins University, Shady Side, MD 20764.

A major focus of Chesapeake Bay oyster culture research is on selective breeding to produce rapidly growing, disease resistant animals. The present study examined both nuclear and mitochondrial DNA genotypes of native and selectively bred groups of Chesapeake Bay oysters in order to establish genetic markers useful in making comparisons between such groups and to provide direct information regarding origins of locally selected strains. Native oysters were sampled from a natural oyster bar at the northern fringe of the *C. virginica*'s zoogeographical range in the Chesapeake Bay near the mouth of Fairlee Creek, from a natural bar in the mid-Chesapeake reach near Tolley Pt., MD and from Horsehead Pt. in the James River, VA representing the southern Chesapeake region. A population of oysters selectively bred for fast growth was also analyzed. These oysters were reportedly 8–10 generations removed from native Chesapeake Bay oysters.

Native MD oysters were highly variable. Horsehead Pt. oysters were characterized by low variability. The selected population was least variable which was expected due to small effective population sizes. Each oyster population sampled contained rare genotypes not found in any other population. In addition, the selected population did not exhibit the common genotype found in native animals and contained several unique genotypes not found in the survey of native genotypes. This suggests that the selected animals have been genetically isolated from the native populations for many generations. At least two explanations can account for the high frequency of unique genotypes in selected animals. The unique genotypes could have been present in the initial wild sample from which the founding oysters were derived and increased in frequency due to genetic drift. Alternatively, they may have evolved *in situ* during the selective breeding process.

EFFECTIVE POPULATION SIZE FOR SHELLFISH BROODSTOCK MANAGEMENT: CONFLICTS BETWEEN THEORY AND PRACTICE. David Bushek* and **Standish K. Allen,** Rutgers Shellfish Research Laboratory, New Jersey Agricultural Experiment Station, Port Norris, NJ 08349.

The goal of a shellfish hatchery is to produce uniform, high quality spat. Initially, domestication of the species and strong selection for obvious, commercially important traits (e.g., fast growth, disease resistance) will tend to decrease genetic diversity through the loss of alleles. In the long term, continued selection will improve the stocks performance under more specific condi-

tions, but this is limited by the amount of genetic diversity present.

Much has been written about the importance of maintaining genetic diversity in domesticated agricultural species. This is no less important for hatchery produced shellfish, but the biology of shellfish creates unique problems. Population genetic theory demonstrates that loss of genetic diversity is inversely related to effective population size (N.). Several factors, sex ratio and family size in particular, can be used to maximize N., but practical methods that are sensitive to the commercial realities of shellfish production have not been developed.

Using data from the Rutgers Cape Shore Hatchery, this paper will first examine the practical problems of maintaining a large N., then describe a practical method of repeated spawning for increasing N., without slowing down the selection process or dramatically increasing labor.

HETEROSIS AND HETEROZYGOTE DEFICIENCIES IN *MULINIA LATERALIS*. **Kristin M. Churchill*** and **Patrick M. Gaffney,** College of Marine Studies, University of Delaware, Lewes, DE 19958.

Allozyme surveys often report heterozygote deficiencies and a correlation between multiple locus heterozygosity and shell length in natural population of marine bivalves. Hypotheses offered to explain both phenomena include aneuploidy, null alleles, molecular imprinting, inbreeding, population mixing, and selection. In natural populations, none of these hypotheses can be unambiguously eliminated due to lack of information about genetic, environmental, and demographic factors, whereas in laboratory matings, inbreeding and population mixing can be eliminated.

In this study, we investigate origins of heterosis and heterozygote deficiencies using the coot clam *Mulinia lateralis* as a laboratory model. Use of offspring from a factorial mating design with known parentage enables us to discriminate among the various hypotheses.

To determine whether environmental variation acts as a selective pressure, half of the progeny from each single-pair mating was placed in an environment with a constant salinity of 30 0/00, while the other half was placed in an environment with the salinity fluctuating between 15 0/00 and 30 0/00. Stress of adjusting to fluctuating salinity can enhance differences among genotypes.

Electrophoretic data for parents and offspring in a factorial mating of five males and four females (20 families) were used to test the hypotheses of null alleles, spontaneous aneuploidy, molecular imprinting, as well as genotype-specific mortality and growth rate.

HYBRIDIZATION, TRIPLOIDY AND SALINITY EFFECTS ON CROSSES WITH *CRASSOSTREA GIGAS* AND *CRASSOSTREA VIRGINICA*. **Sandra L. Downing,** School of Fisheries, WH-10, University of Washington, Seattle, WA 98195.

With pollution and disease decimating the populations of *Crassostrea virginica*, a hybrid with the hardier *Crassostrea gigas* could be one answer for revitalizing the industry. During the spring of 1988, a complete factorial design was used to produce monospecific and interspecific, diploid and triploid oysters. To induce triploidy, newly fertilized eggs were treated with cytochalasin B from 20–35 min after insemination at 25C. Salinity (18 to 30 ppt) was tested to determine its effects on triploid induction and rearing in general.

Survival was slightly lower in the monospecific treated groups than in the untreated diploid crosses. With one exception, monospecific crosses outsurvived interspecific groups. *C. gigas* sperm fertilized *C. virginica* eggs, but in the reciprocal cross, fertilization was less successful. Consequently, survival to 48 hours was <1% in the GV and GGV crosses. Similar to published results, there was a large die-off around day 7 for groups with *C. virginica* as the maternal parent. Generally these effects on survival could be overcome by rearing many larvae. As with earlier hybrid triploid work, poor sperm quality lead to induction percentages below 20%, even in the monospecific crosses. Otherwise, percentages of above 60% were induced including the GGV cross.

When broodstock was conditioned at 28 ppt, larvae did not survive when treated at 18 ppt. Survival was affected at 20 ppt, especially in the triploid groups, for *C. gigas*, but not for *C. virginica*. In the first trial, 100% triploidy was induced in the 20VVV group while only 84% in the 30 ppt group. When this was repeated using *C. gigas*, 100% triploidy was induced in both groups.

EFFECTS OF CULLING AND TEMPERATURE ON FAMILY CONTRIBUTION IN HATCHERY REARED PACIFIC OYSTERS. **H. L. Franklin*** and **S. L. Downing,** University of Washington, WH-10, Seattle, WA 98195.

The commercial oyster industry in the Pacific Northwest relies heavily on hatchery-produced seed. Although hatcheries use large numbers of parents to maintain genetic diversity, it has not been determined whether artificial hatchery conditions selectively reduce this number. Several commercial practices may be selecting for faster, more uniformly growing larvae, but at the same time may be affecting the number of parents contributing progeny to the next generation. Culling is one such practice in which smaller, slow growing larvae are removed at each water change. Another practice that may be exerting selective pressure is using different larval rearing temperatures.

Experiments were run to test the combined effects of culling and temperature, and the effect of culling alone. Full-sib families were reared at three temperatures (20, 25, and 30°C) with culled and nonculled treatments at each temperature. Larval results indi-

cate that groups reared at 30°C had 50% fewer survivors to straight hinge than groups reared at 20 and 25°C. Starch gel electrophoresis was used to identify family markers and to determine overall family contribution to each treatment group. Unequal contributions from all families are used as indicators of selection. Preliminary results indicate that the 30°C groups are predominantly represented by two families. In the 20 and 25°C groups, certain families are also disproportionately represented, but the families are different than the ones making a larger contribution to the 30°C groups. In the second experiment spat counts indicate that the culled treatments have fewer spat set per shell than the non-culled treatments (53.3 and 71.1 spat/shell, respectively).

ANEUPLOID PACIFIC OYSTER LARVAE PRODUCED BY TREATING WITH CYTOCHALASIN B DURING MEIOSIS I. Ximing Guo, School of Fisheries, University of Washington, Seattle, WA 98195; **Ken Cooper,*** Coast Oyster Co., Quilcene, WA 98376; **W. Hershberger,** School of Fisheries, University of Washington, Seattle, WA 98195.

Polyploid Pacific oysters, *Crassostrea gigas,* are produced by treating fertilized eggs with cytochalasin B (CB) during meiosis. Polyploidy results by the drug CB preventing polymerization of actin filaments, which indirectly affects the normal segregation pattern of chromosomes. The resulting ploidy depends on how chromosomes are segregated into the first (PBI) or second (PBII) polar bodies.

In a series of experiments we examined the segregation of chromosomes and resulting ploidy in relation to the timing of CB treatment during meiosis. Ploidy of embryos and larvae were determined by flow cytometry and direct counts of chromosomes. The segregation pattern of chromosomes during meiosis was observed in samples stained with an acetic acid-orcein stain.

Fertilized eggs treated with CB at 25°C are: 1) 2N, 3N or 4N if the treatment affects PB I; 2) 3N if the treatment affects PB II; and 3) 5N if the treatment affects both PB I and PB II. Further analysis by direct counting of chromosomes showed that greater than 60 percent of the polyploids produced by blocking PBI were aneuploid, whereas the number of aneuploids occurring when PBII was blocked was not significantly greater than in diploid controls.

Direct staining of the chromosomes during meiosis showed an unusual configuration of maternal chromosomes following blocking of PBI, where the 20 maternal dyads form three division planes oriented in a "tripolar" configuration. We suggest that the tripolar segregation of chromosomes results in a high proportion of aneuploids, as evidenced by the occurrence of increased numbers of embryos with 25 and 36 chromosomes. This type of segregation can also result in an abnormal tetraploid. We further found no evidence to suggest that triploids produced following treatments meant to block PBI are different than meiosis II triploids.

USE OF OFFSPRING GENOTYPES TO DETERMINE "BEST" PARENTS IN A MASS SPAWNING OF HARD CLAMS. Nancy H. Hadley,* Marine Resources Research Institute, Charleston, SC 29412; **R. T. Dillon, Jr.,** Biology Department, College of Charleston, Charleston, SC 29424.

South Carolina wildstock clams were mass spawned in two separate experiments, each involving 150 individuals. In one experiment 11 males and 12 females spawned. In the other, 38 males and 21 females spawned. The clams were spawned in individual containers but the gametes were pooled prior to fertilization. The offspring populations were sampled at 2 years of age to determine individual genotypes at 6 enzyme loci. Offspring were segregated by size and approximately 60 of the largest and 60 of the smallest were subjected to electrophoresis. Tissue samples were collected from the original spawners to determine genotypes of the parents. A computer program was developed to determine the probability of each offspring resulting from each possible parental combination. A few of the parental clams were lost, and as a result some offspring could not be assigned to any parental combinations. One parent had no possible offspring in the two year old population. The resulting data was used to determine the best parents, i.e. those which had a greater probability of producing offspring which survived and were large at two years of age. These parents will be respawned in an attempt to reduce size variation in the offspring.

ALLOZYME SURVEY OF THE POPULATION STRUCTURE OF *CRASSOSTREA VIRGINICA* INHABITING LAGUNA MADRE, TEXAS AND ADJACENT BAY SYSTEMS. T. L. King and **J. D. Gray,*** Perry R. Bass Marine Fisheries Research St., Texas Parks and Wildlife Dept., Star Route Box 385, Palacios, TX 77465.

Crassostrea virginica ranges from Nova Scotia to the Yucatan Peninsula, Mexico. Biochemical evidence to date suggests the occurrence of four racially distinct populations of *C. virginica* within this range: the Canadian, U.S. Atlantic, U.S. Gulf, and Bay of Campeche. In addition an "unusual" population in Laguna Madre, Texas exhibiting a major transition in genetic structure between Corpus Christi Bay and the lower Laguna Madre has been reported. The purpose of this study was to elucidate the population structure of *C. virginica* inhabiting the Laguna Madre system and adjacent bays.

Allelic variation at 26 putative gene loci was surveyed among 10 geographic populations of *C. virginica* from San Antonio Bay to South Bay, Texas. Heterogeneity of allele frequencies was observed in 9 of the 19 polymorphic loci surveyed. Cluster analysis of Roger's genetic similarity estimates and Wright's F-statistics suggested the presence of a high degree of population subdivision among the populations surveyed. Estimates of the number of migrants between populations suggested little gene flow occurs be-

tween bay systems north of the upper Laguna Madre and the lower Laguna Madre.

Our results indicate two races of *C. virginica* occupy the Texas coast with the upper Laguna Madre serving as a transition zone. It is unknown if the *C. virginica* race occupying the lower Laguna Madre is unique to the Texas coast or if this population represents the northern most range of the Bay of Campeche race.

GENETICS OF TRANSPLANTED BAY SCALLOPS IN LONG ISLAND WATERS: EVIDENCE FOR SELECTIVE MORTALITY. Maureen K. Krause, Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794-5245.

Long Island populations of the bay scallop, *Argopecten irradians*, suffered disastrous recruitment failures and mortalities due to the "brown tide" events of 1985 and 1986. In an attempt to rejuvenate the fishery, hatchery-produced seed were transplanted into Long Island waters on several occasions. The degree of genetic differentiation between introduced and native stock and the implications for survival and reproductive success were investigated using electrophoretic markers.

Large and significant differences in allele frequencies between transplanted and native populations were consistently observed at five out of six polymorphic loci. Transplanted scallops sampled approximately one year after introduction show large shifts in allele frequencies in the direction of native scallop population frequencies. This evidence for selective mortality is discussed in relation to the management of the bay scallop fishery as well as to the broader concerns over the transplantation of shellfish.

Preliminary results indicate a substantial contribution from the 1988 transplants to the successful natural recruitment in the fall of 1989. Ongoing population genetics studies of these recruits will also be presented.

RECOMMENDATIONS FOR COMMERCIAL PRODUCTION OF TRIPLOID OYSTERS. Greg M. Shatkin* and **Standish K. Allen,** Rutgers Shellfish Research Laboratory, P.O. Box 687, Port Norris, New Jersey 08349.

Cytochalasin B (CB) was used to induce triploidy in *Crassostrea virginica* at 25 and 28°C. Fertilized eggs were pooled and exposed to CB at concentrations of 1.0 mg CB/liter for three 10-minute intervals and 0.5 mg CB/l for two 15-minute intervals beginning at 10 minutes post-fertilization. In addition, a commercial spawn was exposed to CB using our recommended conditions. The spat were grown to straight hinge under conditions which simulated commercial culture. The entire experiment was conducted twice.

At 48 hours, all larvae exposed to CB treatments at 25°C displayed higher survival than those treated at 28°C. Survival rates after 48 hours (daily attrition) were similar for all treated groups, whether treated at 25 or 28°C. As seen in other studies, mortalities due to treatment with CB were expressed during the first two days after treatment and, subsequently, larval survival was comparable in all treatments.

Different treatments produced varying proportions of triploids. But variation within treatments was also high in some cases. For example, eggs treated at 10 minutes post-fertilization for 10 minutes with 1.0 mg CB/l at 25 and 28°C ranged in triploid yield from 7 to 100% and 13 to 93%, respectively. The best treatment was 0.5 mg CB/l for 15 minutes at 25 minutes post-fertilization at 25°C, which yielded triploids at a mean of 96% with little variation between replications. It was this "recommended treatment" which produced 100% triploids when tested in a commercial spawn.

VIBRIO

VIBRIO VULNIFICUS* IN POST-HARVEST SHELLSTOCK AND PROCESSED GULF COAST OYSTERS. David W. Cook and **Angela D. Ruple,** Gulf Coast Research Laboratory, Ocean Springs, MS 39564.

Cases of primary septicemia attributed to *Vibrio vulnificus* usually occur between April and October and are generally associated with oysters consumed from the half-shell rather than oysters purchased as a shucked product. Our studies have found that the levels of *V. vulnificus* in post-harvest shellstock oysters vary directly with the water temperature in the harvest area, and that temperature abuse during shellstock transport may lead to multiplication of *V. vulnificus*. Levels of *V. vulnificus* in shell oysters collected from processing plants during winter and early spring months were low and often undetectable. During summer months *V. vulnificus* levels frequently exceeded 110,000 MPN/g.

Processing of oysters which included shucking and washing had little effect on the levels of *V. vulnificus* in oysters. Iced storage of shucked oyster meats reduced the level of culturable *V. vulnificus* by greater than 99%.

***VIBRIO* SPECIES OF THE U.S. WEST COAST. Charles A. Kaysner,** Seafood Products Research Center, Food and Drug Administration, Bothell, WA 98041.

Species of the Genus *Vibrio* have been of interest to the FDA during the past two decades. Most recently, the survival of *V. vulnificus* in harvested oysters, the emergence of a unique biosserovar of *V. parahaemolyticus* on the West Coast and septicemia caused by *V. cholerae* non-01 has been investigated by the SPRC.

While many human illnesses caused by *V. vulnificus* have occurred in the Gulf Coast region and have resulted from consumption of oysters (*Crassostrea virginica*) containing this organism and harvested from Gulf Coast waters, several illnesses have been reported in California, with oysters implicated traced to sources along the Gulf Coast. In our studies, *V. vulnificus* survived in both

C. virginica and *C. gigas* (the Pacific oyster) for at least one week after injection of live cells into both shellstock and commercially shucked product stored at 10°C and on ice (0.5°C). The pathogen also survived at least 14 days in *C. virginica* stored at 2°C after uptake of cells by shellstock maintained in laboratory tanks. These data support the evidence of illnesses contracted from consumption of raw oysters harvested from waters containing *V. vulnificus* and shipped to various parts of the U.S.

A unique bioserovar of *V. parahaemolyticus* has been identified as a predominant strain isolated from patients in CA. This urease positive (Uh+) 04:K12 serogroup was determined by epidemiological evidence to be in some cases from shellfish harvested from West Coast waters. We found a high percentage of Uh+ strains in the total *V. parahaemolyticus* population in one oyster growing estuary in the State of WA. The Uh+ 04:K12 serotype was previously isolated from this estuary after 5 illnesses were reported by individuals after consumption of oysters harvested there. The 58% incidence of Uh+ strains in this estuary is much higher than that of 6% determined for two other estuaries.

An unusual case of *V. cholerae* septic shock occurred recently in WA. The non-toxigenic, non-01 strain isolated from the patient's blood and spinal fluid caused lethality in mice with low LD_{50} values similar to those observed for virulent *V. vulnificus* strains. The patient was compromised and died before a food history could be obtained. Secondary information did not completely rule out a food vector. No wounds were observed which could have been an additional route of infection. Biochemically identical non-toxigenic, non-01 *V. cholerae* strains were isolated from estuarine sediments near the residence of the patient although oysters and water examined from the area contained a different biotype. This suggests the source of the organism from the sediment, but the route of infection still remains unknown. This is the first case of septic shock caused by *V. cholerae* in the State of WA.

VIBRIO VULNIFICUS—A NEW MONSTER OF THE DEEP? A REVIEW OF CLINICAL AND EPIDEMIOLOGIC FEATURES OF INFECTION IN HUMANS. J. Glenn Morris, Jr., Division of Geographic Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201.

Vibrio vulnificus is a recently identified halophilic marine Vibrio that has been implicated as a cause of primary septicemia, serious wound infections, and, possibly, gastroenteritis in humans. Primary septicemia (septicemia without a known focus of infection) occurs in persons with underlying liver disease or who are immunocompromised. One third of patients present in shock or become hypotensive within 12 hours of hospital admission. Over 50% of persons with primary septicemia die; mortality rates exceed 90% for patients with hypotension. Septicemia has been significantly associated with consumption of raw oysters, and it is likely that infection is acquired through the gastrointestinal tract by eating oysters containing the organism. Wound infections are associated with exposure to estuarine water or shellfish. Infections may be mild and self-limited, or may progress to severe cellulitis and myositis. While mortality due to wound infections is usually confined to high risk groups, serious infections can occur in normal, healthy hosts. *V. vulnificus* has also been isolated from stool samples from persons with gastroenteritis; its role as a gastrointestinal pathogen remains to be defined.

V. vulnificus is clearly a significant human pathogen, capable of causing high rates of mortality among susceptible hosts. Further studies are needed to determine how the risk of infection with this organism can best be reduced.

THE VIABLE BUT NON-RECOVERABLE STATE IN _VIBRIO VULNIFICUS_: A NEW CAUSE FOR CONCERN? James D. Oliver, Department of Biology, University of North Carolina at Charlotte, Charlotte, NC 28223.

Infections with *V. vulnificus* occur primarily in the warm summer months. It had been assumed that *V. vulnificus* cells died in seawater during the cold months, and that this die-off could explain the seasonality of infections. It now appears, however, that this apparent loss of cell viability is actually due to the entry of *V. vulnificus* into a "viable but non-culturable" state. During this low water temperature-induced state, the cells are no longer culturable by standard procedures and only more elaborate methodologies can demonstrate their existence. An obvious implication of such cells in oysters is that routine examinations for their presence may be negative, while viable and potentially virulent cells may be present in high numbers. Until recently, it was unknown whether pathogens in such a non-culturable state were able to produce infection. It has recently been reported, however, that nonrecoverable cells of *V. cholerae* are able to produce symptoms consistent with cholera. Thus, it appears that the presence not only of culturable *V. vulnificus* in oysters, but of nonrecoverable cells, may present a public health concern. This paper will summarize our studies on the non-recoverable state of *V. vulnificus*, and on the possible role such cells may play in the epidemiology of *V. vulnificus* infections.

DEPURATION OF VIBRIOS FROM FLORIDA SHELLFISH. G. E. Rodrick* and K. Schneider, Department of Food Sciences & Human Nutrition, University of Florida, Gainesville, FL 32611.

Depuration is the process of purification whereby shellfish are placed in disinfected, recirculating seawater and allowed to actively filter-feed. Both environmentally and artificially infected oysters and clams were subjected to depuration in a pilot scale system using either ultraviolet light or ozone as a disinfectant. Extensive reductions in fecal coliforms, *Vibrio vulnificus* and

non-01 *Vibrio cholerae* were achieved in seawater; however, less reduction of microorganisms was observed in the shellfish meats using ultraviolet light. Laboratory infected shellfish showed significantly higher reductions when compared to environmentally infected shellfish. This suggests the presence of a persistent microbiology flora for which ultraviolet depuration may be ineffective. (Supported in part by Florida Sea Grant DOC/NA-86AA-DSG068.)

RAPID IDENTIFICATION OF *VIBRIO VULNIFICUS* FROM OYSTER SHELLFISH. R. J. Siebeling* and **J. G. Simonson,** Department of Microbiology, Louisiana State University, Baton Rouge, LA 70803.

Detection of human pathogens, such as *V. vulnificus*, in shellfish by rapid, specific and inexpensive methods is paramount to the shellfish industry and the public health. Currently several approaches are being investigated. Serological reagents and gene probes are presently under field trials to test their efficiency and sensitivity. Two approaches which utilize serological reagents to detect vibrio pathogens have been examined. First, latex beads and *Staphylococcus* cells armed with monoclonal antibody (MAb) specific for species-specific flagellar (H) antigens agglutinate, in the slide test, *V. vulnificus* specific dipstick, was designed with the objective to detect this pathogen in shellfish homogenate within one working day. Affinity purified anti-*V. vulnificus* MAb, covalently linked to the dipstick membrane, is employed to capture and immobilize the vibrio onto the dipstick (capture step). The immobilized *V. vulnificus* cells are next exposed to a MAb-biotin-avidin alkaline phosphatase conjugate (detection step) and the dipstick is developed when exposed to the substrate which produces a blue color on the dipstick. The total working time is six to eight hours. The dipstick will detect 10^6 *V. vulnificus* cells per ml in a 6 h enrichment broth, seeded with 100 cells. The dipstick is currently being tested with oyster homogenate enrichment cultures.

THE ECOLOGY OF *VIBRIO VULNIFICUS* IN *CRASSOSTREA VIRGINICA*. Mark L. Tamplin, Food and Drug Administration, Fishery Research Branch, Dauphin Island, AL 36528.

Vibrio vulnificus is part of the natural microflora of estuarine environments and can be an important opportunistic pathogen of humans who ingest raw oysters (*Crassostrea virginica*) harvested from Gulf of Mexico waters. The environmental parameters and mechanisms involved in uptake, retention, and elimination of *V. vulnificus* by oysters are not understood. The present studies investigated the effects of physicochemical and biological parameters on the presence of *V. vulnificus* in oyster shellstocks and in pure cultures. In natural populations of oysters, the digestive tract contained the highest concentrations of *V. vulnificus*, followed by adductor muscle, mantle, gills and hemolymph. Depuration methods did not reduce *V. vulnificus* in whole oysters, but changed the distribution in specific tissues, with sequential increases in adductor muscle, mantle and gills. High levels of *V. vulnificus* were observed when water temperatures ranged from 20°C to 30°C. Low concentrations of *V. vulnificus* were isolated from sediment and oysters and were not detected in seawater when water temperatures were below 15°C. Measurements of *V. vulnificus* in field samples of seawater, ranging from 1 ppt to 35 ppt salinity, showed highest concentrations at 5 ppt. In addition, *V. vulnificus* was associated with a diverse group of plankton species, primarily benthic. These results indicated that *V. vulnificus* is part of the commensal flora of oysters, and that this association is directly influenced by temperature, salinity, and particulate matter.

STATUS AND TRENDS

"DERMO" AND THE SOUTHERN OSCILLATION?? Julie D. Gauthier,* Eric N. Powell, Elizabeth A. Wilson, and **James M. Brooks,** Oceanography Department, Texas A&M University, College Station, TX 77843.

A four year Gulf-wide study (1986–1989 Status and Trends Program, NOAA) (Laguna Madre, Texas to the Florida Everglades) has shown that prevalence (PI) and intensity (WI) of the oyster parasite *Perkinsus marinus* are strongly correlated with long-range weather patterns, including those possibly associated with the 1987 El Nino/Southern Oscillation (ENSO) event. Previous studies have indicated that temperature and salinity control the impact of this disease on oyster populations in the Gulf of Mexico, but until recently have been limited to small-scale, short-term hydrographic conditions.

In this study, PI and WI showed significantly comparable responses in oyster populations over spatial scales as large as 1300km, and sites located at similar latitudes on both sides of the Gulf displayed similar disease patterns. Concurrent, parallel changes in disease incidence on spatial scales as large as this can only be explained by long-term and large-scale climatic variations in temperature and rainfall patterns. ENSO-related temperature and precipitation anomalies have been described from the Gulf of Mexico and Southern United States. Data from previous ENSO events (1875–1980) show coherent negative departures in temperature and positive departures in precipitation across the Gulf. In this study, lower temperatures and subsequent weakening of disease, especially at low latitude sites, occurred in 1987 during the last ENSO event. The 1988 North American drought, which has also been attributed to teleconnections between large-scale weather patterns associated with the Southern Oscillation, may explain the 1988–89 trend towards higher PI and WI in higher latitude sites, particularly in the northeastern Gulf.

THE IMPLICATIONS TO THE TAXONOMY OF *MYTILUS* OF HISTOPATHOLOGICAL OBSERVATIONS OF *MYTILUS EDULIS* COLLECTED FOR THE MUSSEL WATCH PROGRAM. Robert E. Hillman, Battelle Memorial Institute, Duxbury Operations, Duxbury, MA 02332.

Recent electrophoretic studies of populations of mussels purported to be *Mytilus edulis* indicated that *M. edulis* does not exist on the west coast of the United States (J. H. McDonald and R. K. Koehn, Mar. Biol. 99, 111–118, 1988). Rather, populations of mussels in southern California were very similar to *M. galloprovincialis*, probably accidentally introduced into southern California; populations from Oregon and Alaska were similar to *M. trossulus*, indigenous to the Baltic Sea and parts of eastern Canada. In central and northern California, *M. galloprovincialis* and *M. trossulus* and their hybrids co-occur.

Two types of histopathological observations of mussels collected for the Mussel Watch program indicate that at least the southern California mussels may indeed be a species of *Mytilus* other than *edulis*. Measurements of cell and nuclear diameters of disseminated sarcoma cells in mussels collected from San Pedro Harbor and Marina del Rey in southern California were significantly larger than the cell and nuclear diameters of sarcoma cells from mussels collected from Long Island Sound on the east coast, and Yaquina Bay and the Puget Sound and Straits of Juan de Fuca areas on the west coast. In addition, the occurrence of *Steinhausia mytilovum* in mussels from Marina del Rey established a new geographic record for the microsporan species. Previously, *S. mytilovum* had been reported only occasionally in populations of *M. edulis* from Maryland north to Long Island Sound, and in 10 percent of *M. galloprovincialis* from the Gulf of Naples.

Based on the histopathological differences, it is reasonable to assume the possibility that the southern California mussels are actually *M. galloprovincialis*, accidentally introduced to southern California, and that *S. mytilovum* was accidentally introduced with the mussels.

BENZO[A]PYRENE METABOLISM IN OYSTERS FROM FLORIDA SITES IN NOAA'S STATUS AND TRENDS MONITORING PROGRAM. S. J. McDonald,* T. L. Wade, M. C. Kennicutt II, and **J. M. Brooks,** Geochemical and Environmental Research Group, College Station, TX 77845; **E. N. Powell,** Department of Oceanography, Texas A&M University, College Station, TX 77843; **R. W. Estabrook,** Department of Biochemistry, Southwestern Medical School at Dallas, Dallas, TX 75235.

Molluscs are known to accumulate organic contaminates from their environment. Considerable interest has been generated in developing biochemical indices which respond to only one type of environmental contaminant and that are useful in monitoring programs to indicate stress. One such possibility is the mixed-function oxidase benzo[a]pyrene hydroxylase (BPH) associated with cytochrome P-450 and which has been shown to metabolize benzo[a]pyrene in a variety of organisms to more polar, excretable derivatives. *Crassotrea virginica* collected from sites in NOAA's Status and Trends monitoring program during 1989 were assayed for BPH and cytochrome P-450 reductase activities. Specimens were collected from four sites in Florida; two considered "clean" and two considered "contaminated" based on aromatic hydrocarbon body burden values from three previous monitoring years. Previous studies have demonstrated low BPH activities in oysters. BPH activities were reevaluated because of recent advances in assay techniques and the unique, extensive data base on these sites. BPH activities were measured using fluorometric techniques and were consistently low with a maximum value of 3 $pmol^{-1}$ min^{-1} mg protein. Cytochrome P-450 reductase activities ranged from 8.5 to 9.6 $nmol^{-1}min^{-1}$ mg protein. The use of the classical BPH inhibitor 7,8 benzoflavone (BNF) in BPH assays produced unusual results in that BPH activity was not inhibited or in some instances actually stimulated. Low concentrations of BNF can stimulate some forms of cytochrome P-450 while inhibiting others. These results indicate that oysters may have cytochrome P-450 properties not observed in some other bivalve species.

TEN-YEAR TRENDS IN CHEMICAL CONTAMINATION IN MUSSELS AND OYSTERS. Thomas P. O'Connor,* and **Gunnar G. Lauenstein,** NOAA NS&T Program, NOAA N/OMA32, Rockville, MD 20852.

Since 1986 the National Status and Trends (NS&T) Program within the National Oceanic Atmospheric Administration (NOAA) has been conducting the Mussel Watch Project. That Project, based on using mussels and oysters as sentinels of chemical contamination, collects and analyzes mollusks at about 200 coastal and estuarine sites around the United States. Data from collections made in 1986, 1987, and 1988, have been used to define the status and trends of chemical contamination (NOAA, 1989). Ten years earlier, in 1976, 1977, and 1978, there was another nationwide Mussel Watch Program that also collected and analyzed mollusks at about 100 sites. Concentrations of six elements, silver (Ag), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn), have been measured in both programs and 50 sites are common to both.

Comparisons between the two sets of three-year data sets reveal a national decrease in lead concentrations. Cadmium concentrations are now significantly lower at 11 of the common site-pairs and significantly higher at only one. Copper concentrations, on the other hand, are higher now in mussels (not oysters) and, because the earlier program collected mussels in summer while the later did so in winter, may reflect seasonal rather than decadal differences. The results for nickel and zinc generally showed little change but there was some tendency for nickel changes to parallel those of copper. Silver showed as many decreases as increases but the increases tended to be in mussels along the West Coast.

DDT RESIDUES IN THE U.S. GULF OF MEXICO OYSTERS: REVIEW AND PERSPECTIVE. **Jose L. Sericano,* Terry L. Wade,** and **James M. Brooks,** Geochemical and Environmental Research Group, 10 S. Graham Rd., Department of Oceanography, Texas A&M University, College Station, Texas 77845.

DDT is a chlorinated pesticide that has been widely used throughout the world from 1945 to early 1970's when environmental persistence and concerns about human health led to its ban in the United States as well as in many other developed Nations. DDT and its metabolites has been included in the suite of compounds being studied by NOAA's National Status and Trends (NS&T) program, which is designed to monitor the current status and temporal trends of selected organic and inorganic contaminants in U.S. coastal areas. The DDT concentrations in oyster tissues obtained during the first three years of the NOAA's NS&T program for the Gulf of Mexico are discussed. From 1986 to 1988, DDT and its metabolites were analyzed in 479 oyster samples. Concentrations ranged over two orders of magnitude. DDT and its metabolites were the most abundant chlorinated pesticide measured during this study. DDT represented the major fraction. The geographic distribution of sites in the northern Gulf of Mexico having high or low total DDT concentrations is well defined. Based on the three years of NS&T data, there were only a few sites that had statistically significant monotonic changes in concentration with time. However, a general decrease in total DDT concentrations in oysters from the northern Gulf of Mexico coastal and estuarine environments is evident when comparing the NS&T data to historical Gulf of Mexico data. The rate of DDT disappearance, as monitored by Gulf of Mexico oysters, is consistent with its decline in other ecosystems.

THE EVOLUTION OF PCB ANALYSES FOR THE NOAA MUSSEL WATCH PROGRAM AND OVERVIEW OF PCB LEVELS IN BIVALVES FROM 1989 MUSSEL WATCH FIELD SEASON. **William G. Steinhauer,* Carole S. Peven,** and **Paul D. Boehm,**[a] Battelle Ocean Sciences, 397 Washington Street, Duxbury, MA 02332.

An important parameter measured in the NOAA Mussel Watch Program is the PCB content of bivalves and marine sediments. The measurement was altered in 1988. For the first two years of the program, PCBs were quantified as level of chlorination by measuring individual chromatographic peaks corresponding to known retention times of standard solutions. The level of chlorination of resolvable PCB peaks were determined by GC/MS analysis of commercial PCB formulations. Since 1988, eighteen distinct PCB congeners have been measured. In order to compare data, a correlation between the two measurements has been determined. Because PCBs have been reported as Aroclor formulations in much of the historical record for shellfish and sediments, a correlation between the current NOAA method relying on high resolution capillary chromatography for individual PCB congener analysis, and traditional packed column gas chromatography for Aroclor formulation identification and analysis (EPA Method 608) has also been determined. The utility of the NOAA method for analysis of environmental samples is discussed. PCB levels in bivalves collected during the 1989 field season on the U.S. east and west coasts are presented.

FINDINGS OF TRIBUTYLTIN IN EAST AND WEST COAST BIVALVES: 1988–1989. **Allen D. Uhler,* Gregory S. Durell,** and **William G. Steinhauer,** Battelle Ocean Sciences, 397 Washington Street, Duxbury, MA 02332.

Tributyltin (TBT) has been used as a biocide in numerous antifouling paint formulations for more than 15 years. TBT, once released into the aquatic environment, is readily accumulated by a variety of organisms, including bivalves. Compelling laboratory and field evidence for detrimental effects of TBT to marine and estuarine organisms has led to vigorous State and Federal legislative action that has severely restricted the use of TBT-based antifoulant paints. However, the efficacy of such legislation can only be measured by direct evidence of changes in environmental levels of TBT in the aquatic environment.

Beginning in 1988, the NOAA National Status and Trends Mussel Watch Program began measuring TBT in East and West Coast bivalves. The objectives of the TBT monitoring program are to (1) determine the distribution of TBT levels in bivalves on a broad regional basis, and (2) determine if environmental levels of TBT are changing with time as a result of the recent State and Federal restrictions on the use of TBT antifoulant paints.

This paper presents the results of a 1989 survey of the distribution of TBT and its' related degradation products dibutyltin (DBT) and monobutyltin (MBT) in bivalves from 74 East Coast (Maine to Florida) and 47 West Coast (Washington to S. California) sampling stations. Sampling locations with very high levels of TBT are identified. A comparison of the 1989 survey data with results from a limited 1988 survey (25 total stations) is also presented.

NOAA STATUS AND TRENDS GULF OF MEXICO MUSSEL WATCH PROGRAM: THE FIRST FOUR YEARS. **Terry L. Wade,** Geochemical and Environmental Research Group, Department of Oceanography, Texas A&M University, 10 S. Graham Rd., College Station, TX 77845.

Oysters have been employed as bioindicator organisms to characterize the current status and long-term trends for 12 trace elements and 57 organic contaminants from 67 Gulf of Mexico sampling sites. Sampling sites are distributed throughout the Gulf of Mexico, away from known point sources of input, and are sampled yearly to provide geographical description of the chronic con-

[a]Arthur D. Little, 25 Acorn Park, Cambridge, MA 02140.

taminant loading of the entire Gulf. Three stations at each site are analyzed individually in order to assess the natural intra-site variability so that significant changes can be detected. Extensive intercomparison exercises assure the comparability of analytical measurements with companion studies on the East and West coasts. The first four years of data for the Gulf of Mexico represents over 40,000 individual data points. The highlights of this extensive, high quality data set will be discussed. The general trend from this large data set is contaminant concentrations that show no significant changes during the four-year sampling period. There are, however, certain sites that have experienced significant changes in contaminant concentration over the last four-year sampling period, including monotonic increases and decreases. Generally, the concentration of the various contaminants do not show any significant relationship to each other. This is probably due to different input sources. Higher concentrations of most contaminants are associated with proximity to large urban areas. Two areas that appear to be exceptions to this generalization, St. Andrew Bay, FL and Choctawhatchee Bay, FL, will be discussed in more detail.

THE DISTRIBUTION OF CHEMICAL CONTAMINANTS IN GULF COAST OYSTER POPULATIONS: RELATIONSHIP TO DISEASE AND CLIMATE CHANGE. E. A. Wilson,* R. J. Taylor, T. L. Wade, B. J. Presley, J. M. Brooks, E. N. Powell, and **J. D. Gauthier,** Department of Oceanography, Texas A&M University, College Station, TX 77843.

Pollutant body burden and the presence of disease in bivalve populations are often used to describe the changing quality of the surrounding environment. NOAA's Status and Trends Program ("Mussel Watch") is an environmental monitoring program designed to monitor changes in environmental quality along the Atlantic, Pacific and Gulf coasts of the United States by measuring levels of chemical contaminants in fish, bivalves and sediments and identifying biological responses to these contaminants. As part of this program, pollutant body burden of trace metals and polyaromatic hydrocarbons (PAH's) was measured in oysters (*Crassostrea virginica*) collected from up to 71 sites along the Gulf coast from Brownsville, Texas to the Florida Everglades. The biological component of the study included determining the prevalence and intensity of infection by the endoparasitic protozoan *Perkinsus marinus* in these oyster populations. Sampling was conducted yearly from 1986 to 1989.

Pollutant body burden of total PAH's and selected trace metals (silver, arsenic, cadmium, copper and zinc) was analyzed with respect to *P. marinus* prevalence and infection intensity. Significant changes in concentration and spatial distribution of PAH's and trace metals occurred from year to year throughout the study. The spatial and temporal pattern of changes in pollutant body burden throughout the Gulf was similar to that observed in changes in prevalence and intensity of *P. marinus* suggesting either a direct link between the presence of chemical contaminants in the environment and prevalence and intensity of disease, or that both respond similarly to environmental change. Changes in pollutant body burden over the 4 year period also indicate a strong relationship to large-scale, long-term climatic changes occurring throughout the Gulf region. These long-term climate changes may be as important in determining the distribution of contaminants and disease as the presence of point sources of pollution.

SETTLEMENT AND RECRUITMENT

OYSTER SPAT RECRUITMENT: A MENACE TO OYSTER CULTURE IN COASTAL GEORGIA. M. Paige Adams,* Randal L. Walker, and **Peter B. Heffernan,** Shellfish Research Laboratory, University of Georgia Marine Extension Service, P.O. Box 13687, Savannah, Georgia 31416-0687.

The plethora of oyster spat produced during the spawning season coupled with the lack of suitable setting substrates is a major impediment to oyster, *Crassostrea virginica*, mariculture in the coastal waters of Georgia. These factors produce clusters of long narrow oysters which are of little commercial value. This study was designed to evaluate site of deployment and bag rotation as antifouling (oyster spat) methods to be used in conjunction with oyster bag culture. In addition to monitoring spatfall per oyster, growth and survival rates of cultured oysters were recorded.

Replicate bags (n = 3), each containing 75 oysters, were deployed subtidally (on the river bottom and off the bottom on a trestle) and intertidally (on the bottom). Bags were rotated either seasonally, once monthly, or twice monthly. Results show that oyster spat settlement was significantly higher on oysters placed subtidally off bottom than on those oysters placed on intertidal or subtidal bottoms. Growth was significantly greater for oysters grown subtidally off bottom. Subtidal bottom oysters exhibited greater growth than those in intertidal once and twice monthly rotated bags, but their growth was not significantly different from oysters in intertidal bottom seasonally rotated bags. There were no significant differences in growth between any rotation treatments placed intertidally on the bottom. Considerable overlap occurred between survival rates of all treatments, but subtidal bottom treatments tended to have the lowest survival rates. Results showed that bag rotation had no effect on spat settlement, growth, or survival rates of the cultured oysters. Recommendations for producing single oysters in coastal Georgia are discussed.

EFFECTS OF LOCATION AND TYPE OF SUBSTRATUM, AND ADULT STOCK ON BAY SCALLOP, *ARGOPECTEN IRRADIANS*, RECRUITMENT. W. G. Ambrose, Jr., Institute for Coastal and Marine Resources and Department of Bi-

ology, East Carolina University, Greenville, NC 27858; **C. H. Peterson,** Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, N.C. 28516.

Settlement preference of the bay scallop, *Argopecten irradians*, for artificial substrata was tested in the laboratory and field. In the laboratory, significantly more larvae settled on 3 mm clear mesh screen compared to 4 mm black mesh. When these two substrata along with 3 additional materials (artificial turf, burlap, 5 mm black mesh) were offered, settlement was greatest on turf, which had the highest surface area, and there was no significant difference in settlement between the 3 mm clear and 4 mm black meshes. Field results were similar to those in the laboratory with the greatest recruitment in the field on substrata with the highest surface area.

Effects of substratum position in the water column, presence of seagrass and concentration of spawners on recruitment were also tested in the field. Spat collectors located near the bottom collected significantly more spat than those located 1 m higher in the water column or in grass beds. Recruitment was greatest in areas nearest the highest concentrations of adult scallops.

BIOTIC AND ABIOTIC FACTORS INFLUENCING THE RECRUITMENT OF SOFT-SHELL CLAMS, *MYA ARENARIA* L., TO SOFT-BOTTOM INTERTIDAL AREAS IN DOWNEAST MAINE, USA. Brian F. Beal, University of Maine at Machias, 5 O'Brien Avenue, Machias, Maine 04654.

Soft-shell clams, *Mya arenaria* L., historically comprise the second most important public marine fishery along the coast of Maine each year. During the past seven years statewide landings have decreased 66% from 6.7 million pounds in 1982 to 2.3 million pounds in 1988. Concomitantly, there has been an even larger decrease in landings in the easternmost county where 40% to 50% of all clams harvested along the coast are taken (82% decline from 3.6 million pounds in 1982 to 0.65 million pounds in 1988). Lack of a successful recruitment to most intertidal areas over the past ten years, especially in the Downeast area, has accompanied these dramatic declines in landings. Understanding the mechanisms that influence recruitment patterns of this commercially important bivalve may enable shellfish managers to directly control the recruitment success of a given year class and enhance the standing stock. During an 18-week field experiment (13 May to 7 September 1984) on a mudflat in Jonesboro, Maine, I assessed the relative importance of six separate sediment types and the organisms that colonized those sediments on the recruitment rate of individuals of *M. arenaria*.

Sediments were placed in metal coffee cans (0.02 m^2) each of which had adequate drainage holes. Containers were dug into the flat so that a 1-inch lip protruded up through the mud and were placed in a six by ten matrix with one meter distances between rows and columns. Ten replicates of each sediment type was used. The treatments were as follows: 1) azoic mud from the intertidal flat where the experiment was conducted, 2) azoic mud plus four adults of *M. arenaria* (65 to 90 mm in shell length), 3) crushed shells of *M. arenaria* which had been shucked on 12 May 1984, 4) crushed shells of *M. arenaria* which had been shucked and weathered approximately one year, 5) poorly sorted gravel obtained from a roadside gravel pit located in Jonesboro, Maine, and 6) equal volumes of gravel (as described above) and sawdust from northern white cedar (*Thuja occidentalis* L.). Five replicates of each treatment were sampled on 5 July 1984 and the remaining were sampled on 7 September 1984. Samples were washed through a 0.5 mm sieve and the macrofauna in each identified and enumerated.

After eight weeks only three individuals of *Mya arenaria* were found in the thirty samples. All were located in the gravel treatment. After 18 weeks there were significant differences ($P < 0.001$) in the number of *M. arenaria* that had recruited to the various experimental sediment treatments. The gravel sediments attracted significantly more clams than any other treatment ($\bar{x}$ = 31.6 individuals/container ± 2.1 SE) while the mixture of sawdust and gravel attracted the next highest density of recruits ($\bar{x}$ = 15.2 individuals/container ± 6.6 SE). There were no significant ($P < 0.05$) differences in the recruitment and subsequent survival success of individuals of *Mya* in any of the remaining treatments ($\bar{x}$ = 3.15 ± 0.77 SE) which suggests that adult densities of 200/m^2 or fewer have little effect on juvenile recruitment success.

No clear patterns linking this recruitment scenario with the abundance of resident and colonizing infauna was observed. For example, there were no significant ($P < 0.05$) differences in the density of adult or juveniles of the predatory polychaete *Nereis virens* between the gravel and other treatments. Corophium volutator, a tube-building amphipod thought to be important as a biotic disturber, was present in high densities ($\bar{x}$ = 272.2 to 642.6 individuals/container) in all four non-shell treatments. These results suggest that previously accepted models employing simple biotic mechanisms to explain recruitment patterns of intertidal marine bivalves are not sufficiently adequate and must be modified to include physical parameters such as sedimentary dynamics.

ENDOGENOUS CONTROL OF OYSTER SETTLEMENT AND METAMORPHOSIS. S. L. Coon,* D. B. Bonar, and **R. M. Weiner,** University of Maryland, College Park, MD 20742 and Center of Marine Biotechnology, Baltimore, MD 21202.

The ability to manipulate oyster settlement behavior and metamorphic development independently provides important insights into the natural endogenous processes which control the complex transition from a swimming larva to a sessile juvenile. These control processes include a dopaminergic behavioral (settlement) pathway and an adrenergic morphogenetic (metamorphosis) pathway.

When a larva encounters an environmental cue which initiates

settlement, the endogenous response is mediated by dopamine. This response can be mimicked by compounds which stimulate dopamine receptors (SKF82526), increase dopamine release (tyramine), or increase dopamine levels in the larva (L-DOPA). Dopamine stimulation initiates settlement but does not induce metamorphosis, indicating the need for additional environmental cues to initiate morphogenetic changes. Initiation of settlement in oyster larvae prior to exposing them to cultch increases subsequent attachment and metamorphosis.

Oyster metamorphosis is mediated by endogenous norepinephrine (or epinephrine) interacting with alpha-1-adrenergic receptors. This response can be mimicked by compounds which stimulate alpha-1-adrenergic receptors. Exposure of competent oyster larvae to norepinephrine or epinephrine induces metamorphosis, without settlement behavior, resulting in unattached spat.

Our current understanding of these endogenous processes allow the formulation and testing of critical hypotheses regarding their relationship to natural environmental cues such as bacterial films or other oysters.

ANNUAL AND SPATIAL PATTERNS OF OYSTER (*CRASSOSTREA VIRGINICA*) SPAT SETTLEMENT IN DELAWARE BAY, 1954–1989. Stephen R. Fegley,* Donald E. Kunkle, Harold H. Haskin, and **John N. Kraeuter,** Rutgers Shellfish Research Laboratory, P.O. Box 687, Port Norris, NJ 08349.

For the past 35 years, summer and early fall oyster (*Crassostrea virginica*) spat settlement was sampled approximately weekly over a wide region of Delaware Bay. The number of stations sampled varied from year to year from a low of 18 to a high of 50. The stations range from near the bay mouth to the upbay limit of oysters and encompass all of the significant oyster grounds present on the New Jersey side of the bay. At each station, spat were collected on 20 clean, oyster valves held within wire mesh (1″ × 1″) bags suspended approximately 1 m above the bottom.

We will present the annual means (and associated variations) in spat settlement at each of the stations. Using these data we examine the relationship, both within and across years, between station location within Delaware Bay and physical factors (for example, Delaware River flow discharge or large scale Delaware Bay hydrodynamic features) and biological factors (such as the intensity of MSX, *Haplosporidium nelsoni*, epizootics or oyster larval abundance). We will also compare our results to similar data gathered in other estuaries where *Crassostrea virginica* occurs.

NJAES Publ. #K-32403-2-89 supported by State of New Jersey Dept. of Envr. Prot. funds.

PRODUCTION AND ROLE OF AMMONIA, AN INDUCER OF SETTLEMENT OF VELIGER LARVAE OF OYSTERS. William K. Fitt and **Douglas E. Haymans,** Department of Zoology, University of Georgia, Athens, Georgia 30602; **Steven L. Coon,** Department of Zoology, University of Maryland, College Park, Md. 20742.

Laboratory experiments with ammonium chloride have shown ammonia (NH_3) to be an inducer of settlement behavior of veligers of oysters in the genus *Crassostrea*. In spite of the fact that most animals and bacteria produce ammonia as a by-product of protein catabolism, natural levels of dissolved ammonia in seawater are typically low. We confirm this observation in Georgia salt marshes, but found increasing concentrations of ammonia/ammonium in proximity to the substrate. High concentrations (>200 μM) have been documented from oyster beds in salt marshes. Eyed veligers exposed to oyster-conditioned seawater responded only to seawater containing >100 μM ammonia/ammonium, suggesting that: 1) ammonia is a natural cue, 2) as with other invertebrate larvae, veligers of oysters can be induced to settle by an adult-produced cue, and 3) a live and productive oyster bed, with its associated bacteria and assemblage of other invertebrates, has the potential of providing both settlement cues and appropriate substrate for veliger larvae.

SETTLEMENT AND RECRUITMENT OF *MERCENARIA MERCENARIA* IN LONG ISLAND SOUND, CONNECTICUT. Ronald Goldberg,* and **James C. Widman,** National Marine Fisheries Service, Northeast Fisheries Center, Milford Laboratory, Milford, CT 06460.

Several approaches have been employed to quantify temporal and spatial patterns of settlement of the hard clam, *Mercenaria*. Sediment filled boxes used as settlement collectors were deployed at 3 sites in Long Island Sound; Greenwich, Milford, and Stonington. The greatest number of set occurred at Greenwich and Milford. Settlement occurred from June to November at Greenwich and Milford, but only from August through November in Stonington. To assess the survival of young recruits, hatchery reared 14mm clams were deployed within quadrats at the 3 stations. Survival was greatest at Milford and is attributed to protection from predators by the presence of broken shell litter on the seabed. To determine recruitment patterns on a decadal time scale, a resource survey and age analysis of shells from existing populations were conducted. Population density of adult clams was 1.89 clams m^2 at Greenwich, compared to 0.74 m^2 at Milford and 0.1 m^2 at Stonington. Age-length plots and Von Bertalanffy growth curves indicate density-dependent population growth at Greenwich. A ten year gap of recruitment found in the Greenwich population is associated with a period of intense fishing activity that may have had a negative impact on settlement. Information from the settlement, early life-stage survival, and shell aging analyses is discussed in terms of hard clam population dynamics in Long Island Sound.

SIMULATING THE POPULATION DYNAMICS OF DISEASED OYSTER POPULATIONS: SHOULD BROOD STOCK BE CONSERVED? **E. Hofmann,*** Department of Oceanography, Old Dominion University, Norfolk, VA 23529; **E. Powell, E. Wilson,** Department of Oceanography, Texas A&M University, College Station, TX 77843; **S. Ray,** Department of Biology, Texas A&M University at Galveston, Galveston, TX 77550.

Oysters in the Gulf of Mexico are infected by the endoparasitic protozoan, *Perkinsus marinus*, and the ectoparasitic snail, *Boonea impressa*, each of which reduces oyster growth rate and reproductive potential by removing assimilated carbon from the oyster. Additionally, *P. marinus* is an important source of mortality in adult oysters and the snail further reduces oyster growth rate by interfering with the oyster feeding and by enhancing *P. marinus* infection intensity. To assess the ecological/commercial impact of these two parasites, a mathematical model describing the interactions between the oysters and the two parasites was developed. The model is formulated in terms of equivalent energy units (joules m^{-2}) and includes processes that result in oyster growth, reproduction, and energy loss to the snails and *P. marinus*. Consequently, the model also includes the processes involved in snail and *P. marinus* growth and the factors influencing the transmission of both parasites between healthy and infected oysters. Parameterization of the processes in the model is based upon data for Gulf coast oysters. The model comprises a system of coupled ordinary differential equations describing the time-dependent behavior of healthy and infected oyster populations and the transmission of both parasites between them. Model simulations are used to determine the conditions under which a stable population of oysters can be obtained with a given level of parasite infection.

SIMULATING THE POPULATION DYNAMICS OF DISEASED OYSTER POPULATIONS: ENVIRONMENTAL VARIATION AND DISEASE. **J. Klinck,*** Department of Oceanography, Old Dominion University, Norfolk, VA 23529; **E. Powell, J. Gauthier, K-S. Choi,** Department of Oceanography; **D. Lewis,** Department of Veterinary Microbiology and Parasitology, Texas A&M University, College Station, TX 77843.

Oyster in the Gulf of Mexico are infected by two parasites: the endoparasitic protozoan, *Perkinsus marinus*, and the ectoparasitic snail, *Boonea impressa*. The level of infection of both is influenced by environmental factors such as temperature and salinity. To investigate the effect of these environmental factors on infection intensity, a mathematical model was constructed consisting of a system of ordinary differential equations describing the interactions among the oysters and the two parasites. Oyster and parasite processes are formulated as functions of temperature and salinity, where appropriate. Oyster processes are also formulated in terms of the available food supply. Simulations of the time-dependent behavior of average oyster populations infected by both parasites were performed for a range of temperature, salinity and food concentrations encountered in Gulf coast waters using monthly-averaged values for the environmental factors. A series of simulations were run to investigate the effect of colder-than-average and warmer-than-average temperatures on the level of parasitism in oyster populations and variations in temperature and rainfall gradients typical of long-term changes in climate in the Gulf. Model runs showed that the timing and duration of climatic changes can be quite important in determining the final level of *P. marinus* infection in Gulf coast oyster populations.

RECRUITMENT OF SCALLOPS IN THE MID ATLANTIC BIGHT: IS VERTICAL RELIEF IMPORTANT? **Roger Mann,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Settlement of scallops in shallow environments is often associated with vertical relief or structures. Settlement of *Argopecten irradians* on *Zostera marina* is such an example. On the inner continental shelf, where *Placopecten magellanicus* exists in commercially viable numbers, such structure is not immediately evident; however, unexpected observations of *Placopecten* settlement on the exterior of a defaunated sediment box experiment, deployed by submersible at 50 m depth off Cape May, NJ in a flat, hard bottom environment, stimulates reconsideration of the importance of vertical relief. A discussion of scales of relief important to flow disturbance and videotape illustrating bottom types found on the inner continental shelf will be used to lead a speculative discussion on how and where scallops might settle at these depths.

RECRUITMENT OF HARD CLAMS, *MERCENARIA* SPP., IN THE INDIAN RIVER LAGOON, FLORIDA: REPEATED FAILURE OR SUCCESSFUL STRATEGY? **Dan C. Marelli,* William S. Arnold, Paige A. Gill,** and **Carita A. Lund,** Florida Department of Natural Resources, Florida Marine Research Institute, 100 Eighth Avenue S.E., St. Petersburg, FL 33701-5095.

Rapid increases in commercial landings of *Mercenaria* spp. from the Indian River lagoon, Florida, in the early 1980s stimulated a research program to examine aspects of the population dynamics and ecology of these bivalves. One facet of this research has involved studying recruitment of *Mercenaria* spp. and its relationship to hydrographic, meteorological, and environmental parameters. Results demonstrate that recruitment rates vary spatially within the lagoon (on meso and mega scales), and that recruitment rates have varied both seasonally and annually.

The level of recruitment we have observed during 1986–1989 has not been sufficient to support a fishery at the level seen in the early 1980s. The broad "failure" of *Mercenaria* recruitment in the lagoon may be a general feature of the Indian River population(s). It is probable that massive *Mercenaria* recruitment suc-

cesses are dependent upon natural climactic events that occur at irregular intervals. Typical levels of recruitment, while considered inadequate by many fishermen, seem to be consistent enough to sustain the population. "Normal" recruitment is spatially variable and probably controlled by settlement patterns and by fluctuations in effects of localized biological disturbances.

SPATIAL SETTLEMENT PATTERNS OF OYSTERS IN SOUTH CAROLINA. William K. Michener,* Andrew H. Barnard, William H. Jefferson, and **Paul D. Kenny,** Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, SC 29208; **James W. Brunt,** Department of Biology, University of New Mexico, Albuquerque, NM 87131.

Total annual oyster (*Crassostrea virginica*) settlement was examined at three sites along a salinity gradient in the mesohaline Wando River Estuary, SC and at 99 sites representing a wide array of salinity and hydrodynamic characteristics in the euhaline North Inlet Estuary, SC. Size and height above the substrate were measured for all live barnacles and oysters at all sites. Intertidal zonation of barnacle species was not observed, except for *Chthyamalus fragilis,* which occurred only at mid to upper levels in the intertidal zone. Settlement and spatial allocation by barnacles and oysters differed at all spatial scales (inter-estuary, inter-reef, tidal level, and height above substrate). Large scale oyster and barnacle settlement patterns were related to hydrographic patterns. Oyster settlement intensity was highest in the low intertidal, although submergence time did not solely account for observed settlement patterns.

A series of short-term experiments were performed annually from 1986–1989 to further elucidate the factors affecting spatial settlement patterns. Settlement intensity was not directly related to late-stage larval abundance in the water column, but was demonstrated to be affected by orientation of the substrate, and sampling frequency. The importance of temporal and spatial scale in sampling population phenomena is discussed and new methods for monitoring shellfish recruitment are presented.

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE MAXIMIZATION OF SPAT SETTLEMENT IN THE GIANT SCALLOP, *PLACOPECTEN MAGELLANICUS*. G. Jay Parsons* and **John C. Roff,** Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1; **Michael J. Dadswell,** Department of Biology, Acadia University, Wolfville, N.S. B0P 1X0.

The giant scallop (*Placopecten magellanicus*) is a commercially important species both in Canada and the U.S., yet few studies have dealt with its early life history or recruitment processes. In an effort to develop predictors of successful recruitment, we examined the relationship of several environmental factors (larval occurrence, substrate conditioning, and potential predators) to maxima in spat settlement. The temporal distribution of larvae in Passamaquoddy Bay, N.B. revealed a pattern of three pulses, in which the occurrence of new veligers was correlated with tidal periodicity. Using artificial substrates we have demonstrated that the microscale influence of a biogenic film was greater than substrate texture on the intensity of spat settlement. Finally, from monitoring the number of newly settled starfish and scallops, a concordance between peak settlement times was observed, with the starfish maxima occurring about three weeks prior to that of scallops.

The application of these results with the manipulation of spat collecting techniques will allow for an enhanced procurement of scallops for aquaculture purposes. Furthermore, with a technique now developed for ageing spat, it should be possible to construct a detailed pattern of settlement and determine if a correlation exists between spawning and settlement periodicities.

MONITORING THE INITIAL RECRUITMENT PATTERNS OF *CRASSOSTREA VIRGINICA* (GMELIN) SPAT ALONG A TIDAL GRADIENT. G. Curtis Roegner, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

The survival of newly settled oyster spat at various tidal heights (aerial exposure levels) was monitored by image analysis. Hatchery-reared oyster larvae were allowed to settle on ceramic plates, and the plates were photographed to record the number of spat present and assigned to tidal treatments. At weekly intervals the plates were rephotographed, and a time series for each plate was created. Percent survival over time was determined by comparing the number of spat present at each time interval with the number of settlers. Thus settlement and recruitment were clearly distinguishable.

The mortality which occurred during the first week dominated the resulting recruitment patterns. The intertidal treatments (above 10% emersed) were strongly affected by physical factors, especially temperature-induced desiccation, and suffered complete mortalities during periods of high aerial temperatures. Physical stressors below the 10% emersion level were less significant: at these treatment levels, initial mortality appears to have been mainly a result of post-metamorphic stress. No initial mean percent survival exceeded 36%, and mean mortality rates declined over the next month. The accurate determination of settlement is thus highly dependant on sampling period.

DISPERSION AND DISTRIBUTION OF OYSTER LARVAE IN CARAQUET BAY, NEW BRUNSWICK. Thomas W. Sephton,* Department of Fisheries and Oceans, Science Branch, P.O. Box 5030, Moncton, N.B., Canada, E1C 9B6; **David A. Booth,** Department of Fisheries and Oceans, Institute Maurice-Lamontagne, Mont Joli, Quebec, G5H 3Z4.

The northernmost commercially exploited population of eastern oyster, *Crassostrea virginica,* is located in Caraquet Bay,

N.B. (latitude 47°50′) in the southern Gulf of St. Lawrence. Following its decimation by Malpeque disease from 1950–1960, a substantially smaller population reestablished itself and remains the most productive public fishing ground in the province. The population is self sustaining even though the tidal prism of this shallow bay (average depth 2m) is about 30% of the total volume. In light of rather harsh physical attributes of the Bay, an intensive field project was conducted to examine the dispersion and distribution of oyster larvae in Caraquet Bay in 1988.

Physical oceanographic research examined the water column structure, Lagrangian displacement using drifting buoys, water circulation patterns using current meters and water exchange rates of the Bay during the 5 week larval period. At the same time, plankton was sampled (integrated pumped sample) daily and then twice daily (as spatfall approached) at 12 stations in and outside the Bay to examine larval development, timing of spatfall and the quantitative spatial distribution of larvae. Results showed that larval development was normal but the horizontal distribution of larvae changed over time in the Bay in relation to the overall water circulation patterns. The location of the optimal spat (seed) collection sites (for local enhancement projects), and the larval export and retention mechanisms of the Bay are discussed in view of the results of the study.

SPATIAL AND TEMPORAL DISTRIBUTION OF BIVALVE LARVAE IN OYSTER BAY, LONG ISLAND, NEW YORK. Scott E. Siddall,* Department of Biology, Kenyon College, Gambier, Ohio 43022; **Serena Cenni,** Marine Sciences Research Center, SUNY, Stony Brook, New York, 11794.

The harvest of natural populations of oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*) has been a vital economic activity in Oyster Bay, an shallow embayment on Long Island's (New York, USA) north shore. In recent years, bivalve culture has played an increasingly significant role in shellfish landings in the Bay. The local shellfish management program includes spawner relays, sanctuaries and selective closures. The objective of this study was to evaluate the effectiveness of some of these management activities by describing the spatial and temporal distribution of bivalve larvae and postlarvae before, during and after peak spawning periods.

Quantitative benthic surveys were used to describe the distribution of adult oysters and hard clams (sources of larvae) and bivalve seed (fate of larvae) before and after the peak of spawning (June through September, 1988). Hard clams were examined for gonadal development. Each week throughout this period, quantitative plankton samples were collected at 20–25 stations within the Bay. Experimental substrates were deployed at selected locations to estimate the character of bivalve settlement. Chlorophyll-a measurements were made using a fluormeter.

As many as 500,000 prodissoconch-II larvae per cubic meter were encountered. Chlorophyll-a, taken as an estimate of food present in the water column, averaged 7.04 μg/liter. Tidal creeks leading into the Bay were important sources of larvae. There was little evidence that substantial numbers of larvae were transported out of the Bay. While these descriptive results tend to validate aspects of the shellfish management program, the overall cost of this intensive sampling program probably exceeds the value of the information to managers.

REDUCED OYSTER RECRUITMENT IN A RIVER WITH RESTRICTED TIDAL FLUSHING. Timothy C. Visel,* Sea Grant Marine Advisory Program, The University of Connecticut at Avery Point, Groton, CT 06340; **Robert E. De Goursey,** Marine Sciences Institute, The University of Connecticut at Avery Point, Groton, CT 06340; **Peter J. Auster,** National Undersea Research Center, The University of Connecticut at Avery Point, Groton, CT 06340.

The Pataguanset River in East Lyme, Connecticut, historically supported a natural oyster bed that has recently declined in productivity. A series of surveys of the river (1985–1988) identified one natural bed comprised of large adult oysters (10 cm to 18.7 cm shell ht.) and few juveniles (<4.6 cm shell ht). The reintroduction of an oyster fishery would quickly deplete this resource without substantial recruitment of seed oysters. Three attempts to restore the oyster setting capacity of the bed by cultch planting and shell base cultivation were unsuccessful. No new seed oysters were observed. Direct underwater observations confirmed heavy silting of newly planted shell cultch, preventing the setting of oysters. Further examination of the lower Pataguanset River near a railroad causeway revealed a historic oyster bed buried under approximately 1 meter of organic sediment. The construction of the railroad causeway reduced the overall width of the river from over 1,000 meters to approximately 15 meters. Effects of the causeway including increased siltation and reduced salinities due to restricted tidal flushing, have negatively impacted the population dynamics of the natural beds. Ideally, tidal flow should be restored. However, management under the current hydrologic regime should include hydraulic cultivation and intensive shell base maintenance in order to enhance oyster productivity.

MOLECULAR CUES OF *CRASSOSTREA* SET THAT ARE SYNTHESIZED BY BACTERIA. R. Weiner,* M. Walch, C. Fuqua, D. Sledjeski, L. Dagasan, and **S. Coon,** University of Maryland, College Park, Maryland and Center of Marine Biotechnology, Baltimore, Maryland.

In the natural environment, oyster larvae undergo a stereotypical sequence of actions before they become sessile and metamorphose. It has long been known that certain bacterial biofilms are beneficial to oyster set. We have isolated and characterized important specific chemical cues from bacteria. Ammonia initiates swim search behavior above the biofilm. Products of tyrosinase activity may cue swim and crawl search behavior just above and

on the biofilm. Using a cosmid system in *Escherichia coli*, we have cloned and expressed the tyrosinase gene of *Alteromonas colwelliana*, a bacterium which is extremely beneficial to oyster set. The DNA fragment encoding tyrosinase was subcloned in a pUC19 vector, sequenced and reduced to 1.2 KB. The gene was expressed, in vitro, using an S_{30} system and found to encode a 42,000 MW protein, which correlated with the size of its DNA open reading frame. The products of the tyrosinase were identified after fractionation in a C-18 reverse phase HPLC column, by electrochemical detection of di- and trihydroxy phenyl products. An atypical reaction product was isolated, 5-hydroxy dihydroxy phenylalanine (5-TOPA), which was a strong inducer of swim-search behavior.

During swim/crawl-search, *Crassostrea* larvae sample the substratum. If it has appropriate features, the larvae cement down. We have examined the biofilms of marine bacteria, particularly *A. colwelliana*, to test the hypothesis that a specific bacterial product cues the ultimate decision to become sessile. One strategy was to present purified exopolysaccharide (EPS) and other surface bacterial components, such as lipopolysaccharide (LPS), to competent larvae. The other was to block determinants of these molecules. Lectins and monoclonal antibodies to formalinized whole cells, purified EPS and outer membrane components were used for this purpose. Neither the lectins nor monoclonal antibodies blocked the ability of *A. colwelliana* films to cue set. From this and other evidence we theorize that an integral biofilm determinant does not cue set but rather that it could be some molecule (e.g. 5-TOPA) that is bound by EPS.

TRANSPORT OF WATERBORNE CHEMICALS AND LARVAL SETTLEMENT. R. K. Zimmer-Faust and **S. G. Morgan,** Marine Environmental Sciences Consortium and Department of Biology, University of Alabama, Dauphin Island, AL 36528; **S. Macintyre,** Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

We recently have begun to examine the effects of hydrodynamics and waterborne substances that are released from benthic sources on the settlement behavior of oyster larvae. In the field, we will deploy arrays of electromagnetic current meters and warm-bead thermistor probes to measure friction velocities and other physical properties of environments where oyster larvae typically settle. A system is being developed that uses micro-electrodes to record the dispersal of water-soluble tracers in natural habitats over spatial scales as small as 10 microns and over temporal scales as short as 5 milliseconds. These measurements will allow us to determine instantaneous fluxes of waterborne tracers in micro-environments adjacent to seabeds. Laboratory experiments will initially test for effects of waterborne compounds that are released by adult and juvenile oysters or bacterial films on the swimming and settlement behaviors of oyster larvae. Later experiments will be conducted in flumes that are equipped with microprocessors, which will enable us to control the distributions of chemical solutions that are introduced in laminar and turbulent flows.

SHELL DISEASE IN MARINE CRUSTACEANS

ETIOLOGY AND PATHOLOGY OF SHELL DISEASE. Robert A. Bullis, University of Pennsylvania, Laboratory for Marine Animal Health, Marine Biological Laboratory, Woods Hole, MA 02543.

The exoskeleton of crustaceans consists of four layers. The epicuticle is made up of lipids or proteins and covers the three inner chitinous layers—the exocuticle, which is calcified and contains proteins and pigments, the calcified endocuticle, and the innermost layer, the noncalcified endocuticle.

Shell erosions result when these layers come under enzymatic attack by microorganisms such as bacteria of the genera *Vibrio*, *Aeromonas*, and *Flavobacter* or fungi of the genus *Fusarium*. Microbial invasion may also occur through setal pores or hypodermal ducts. Insult to the epicuticle may result from natural abrasions, traumatic injury, or by the direct action of enzymes produced by microorganisms. Proliferation of chitinolytic microorganisms begins when the epicuticle is breached, exposing the underlying chitinous layers. As the disease progresses, these layers are sequentially invaded, eventually producing the pitting and erosion characteristic of the disease.

Host responses to invasion by microorganisms include deposition of melanin (antimicrobial defense) and increased chitin formation (shell repair). Molting can rid the host of the diseased exoskeleton except in advanced cases.

Physiological disturbances due to overcrowding, poor water quality, unhygienic holding conditions, or possibly pollutants can cause an imbalance in normal chitin repair mechanisms. Decreased chitin formation and mineral deposition coupled with impaired immune function can cause the balance to shift in favor of the microorganisms. Calcified areas eventually become friable and perforate, exposing underlying soft tissues and forming the "ulcerative" stage of this disease.

SHELL DISEASE AMONG THE BLUE CRAB POPULATION OF PAMLICO SOUND, NORTH CAROLINA. David W. Engel,* National Marine Fisheries Service, Beaufort Laboratory, Beaufort, NC 28516; **Edward J. Noga,** North Carolina State University, School of Veterinary Medicine, Raleigh, NC 27606.

Shell disease among the blue crab, *Callinectes sapidus*, population of the Pamlico River in eastern North Carolina has become a concern to both commercial fishermen and resource managers. This particular form of chitinoclastic disease is extremely aggres-

sive, and unlike other forms of shell disease, involves only the carapace of affected crabs. The disease is characterized by the sloughing of large areas of the calcified carapace, and a majority of the cases involve the lateral spines. In some cases the spines are completely eroded exposing the gills which also appear to be in a degenerative condition. The disease is most common among older crabs, terminal molt females and large males, but diseased juvenile crabs also have been collected showing the typical carapace lesions. To determine the etiology of the disease we are investigating the mechanisms by which blue crabs are able to maintain shell integrity against the endemic microbial flora normally occurring on the shell. The objective of the investigation is to determine the mechanisms of the nonspecific immune response of the blue crab, and to test the activity of the isolated antimicrobial compounds against contaminants identified to occur in the Pamlico River. Utilizing a quantitative microbial assay system, powerful bacteriocidal compounds have been demonstrated to exist in the hemolymph of both healthy and diseased crabs. In addition, chromatographic separation of the hemolymph proteins has shown that at least one of the active proteins has a molecular weight of 40 to 60K daltons. The investigation to characterize and identify the proteins is continuing. It also should be noted that crabs with similar lesions have been collected from areas north and south of the Pamlico River in North Carolina and from two locations in Florida, St. Johns River and Biscayne Bay.

SHELL DISEASE IN AMERICAN LOBSTER OFF THE MASSACHUSETTS COAST. Bruce T. Estrella, Massachusetts Division of Marine Fisheries, Sandwich, MA 02563.

The incidence of shell disease in American lobster was described for Massachusetts coastal waters. Shoal, relatively warm semi-enclosed areas with large organic loads exhibited a greater prevalence than deeper and colder open-water environments. Disease incidence was highest in the larger size groups indicating a possible inverse relationship with molt frequency. Hard-shelled lobster were more symptomatic than paper-shelled lobster.

A LOBSTER SHELL DISEASE SURVEY OF MAINE'S LOBSTER DEALERS AND POUND OWNERS. Rodman G. Getchell, Maine Department of Marine Resources, West Boothbay Harbor, ME 04575.

Maine's lobster dealers and pound owners were surveyed in order to document whether shell disease is a significant problem and what practical measures are commonly employed to control its spread. The survey was conducted in the form of a questionnaire mailed to industry members, followed up by telephone interviews and onsite consultations. A summary of the results will be presented that describes the scope of the disease outbreaks and the preventative steps that operators of lobster holding facilities in Maine take to prevent its spread.

SHELL DISEASE AND GILL BLACKENING IN THE ROCK CRAB, *CANCER IRRORATUS*. Thomas K. Sawyer, Rescon Associates, Inc., Box 206—Turtle Cove, Royal Oak, MD 21662.

Shell disease and/or blackening is known to occur naturally in many species of freshwater or marine crustaceans. Erosion, pitting, or perforation of the exoskeleton is known to be caused by a variety of bacteria and fungi. Gill blackening, however, may be caused by the accumulation of noxious organic mud and silt between adjacent gill lamellae as well as by tissue responses to biological and chemical agents. Blackening caused by microorganisms or chemicals usually is limited to discrete foci and cause black spots to form in otherwise normal-appearing tissue. In contrast, smothering of the gills by sediment or fouling organisms may lead to blackening of 50% or more of the gills.

Extensive studies in the New York Bight apex have shown that sediments contaminated with barge-delivered wastes often have the appearance of black mud and silt, and an odor characteristic of hydrogen sulfide or petrochemicals. Rock crabs collected from contaminated sites were found to have a higher prevalence of shell or gill disease than those collected from sites not affected by ocean disposal practices. Further studies are needed to determine whether microorganisms associated with shell disease in contaminated ecosystems are the same as those in laboratory-held, or offshore populations.

SHELL DISEASE IN MARINE CRUSTACEANS—A CONCEPTUAL APPROACH. Carl J. Sindermann, Oxford Laboratory, National Marine Fisheries Service, Oxford, MD 21654.

Shell disease of marine crustaceans, recognized early in this century as a problem in impounded populations, has been investigated more recently because of its possible association with degraded habitats and because of its potential role in marine aquaculture. Understanding of this microbially-induced disease condition has progressed to a point where tentative hypothesis may be proposed: (1) Chitin deposition is an important defense mechanism in Crustacea; (2) Shell disease is an external indication of metabolic disturbance or trauma, compounded by the activity of chitinoclastic microorganisms; (3) Shell disease is intimately associated with success or failure of processes of external defense and wound repair in crustaceans; (4) Shell disease may be less important in species with short-life spans than in longer-lived species; (5) Shell disease may occur in particularly high prevalences in offshore deep water crustaceans; (6) Pollutants (or other stressors) may foster the development and increase the severity of shell disease; (7) Shell disease is a controllable condition in captive or cultured crustacean populations.

Evidence to support these hypotheses varies in "robustness" but a conceptual base for understanding the significance of shell disease in marine crustaceans seems to be emerging.

PREVALENCE AND SEVERITY OF SHELL DISEASE AMONG DEEP-SEA RED CRABS OF THE MIDDLE ATLANTIC BIGHT IN RELATION TO OCEAN SEWAGE SLUDGE DUMPING. Randall R. Young, Waste Management Institute, Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000.

The extent and severity of shell disease among Middle Atlantic Bight deep-sea red crabs (*Geryon quinquedens*) from six offshore sites of varying distance from the 106-Mile Sewage Sludge Dumpsite were assessed by evaluating each individual according to predetermined rating criteria. Additional specimens dating to the late nineteenth century and maintained in the Smithsonian Institution crustacean collection were also examined and rated in the same manner. Chemical analyses were conducted to assess the body burdens of sewage-related contaminants in some red crab samples.

Overall disease prevalences among sampled crabs ranged from 86% to 100%, with 13% to 30% rated as moderately to severely diseased. Disease prevalences among Smithsonian Institution samples varied from 69% to 100%. Appearance of the shell disease ranged from very small black spots to large grey to black patches covering a substantial portion of the carapace, often arranged in a bilaterally symmetric pattern. There was a positive correlation between animal size and disease severity. Variations in disease severity were not associated with proximity to the sewage sludge dumpsite.

ARCTICA

EFFECTS OF INTRASPECIFIC DENSITY ON THE GROWTH OF *ARCTICA ISLANDICA* LINNE INSIDE FIELD ENCLOSURES LOCATED IN EASTERN MAINE, USA. Brian F. Beal* and **M. G. Kraus,** University of Maine at Machias, 5 O'Brien Avenue, Machias, Maine 04654.

Ocean quahogs, *Arctica islandica* Linne, have become an important fishery in Downeast Maine during the past four years. For example, from 1986 to 1988 annual landings increased nearly 700% from 136,000 pounds to 989,000 pounds, while dockside revenues rose dramatically so that by 1988 the catch was valued at $1.84 million. Previous investigations of *A. islandica* conducted at deep oceanic sites near Long Island, N.Y., the Middle Atlantic Bight, and southern Georges Bank have shown directly and indirectly that growth rates over a variety of sizes are extremely slow and variable.

To determine if similar growth patterns exist within the heavily harvested quahog populations in the Machias Bay area of the Downeast Maine coast (Lat. 44°35′N; 67°26′W), we individually marked and measured 2,325 quahogs ranging in shell length (SL) from 36.8 mm to 65.5 mm ($\bar{x}$ SL = 48.7 mm ± 0.07 SE) that had been collected from the commercial catch and transplanted them between 12 and 30 September 1985 to forty-five 30.5 cm^3 plots at a depth of 20 m in well-sorted fine sand sediments using SCUBA. Quahogs used in the transplant experiment were divided into 3 densities (12, 30, and 60 individuals/plot) based on quantitative *in situ* sampling performed within several commercial beds using a Smith-MacIntyre sampler. In this factorially designed test, five replicates of each density treatment were added to one of three cage types (open enclosures, complete enclosures, and enclosures with a partial top). Cages were excavated after 1 year and the growth of each survivor assessed.

A linear growth function explained significantly more of the variation in annual growth than either a logarithmic, parabolic, or Gompertz model. Average annual growth, independent of density or cagetype effects, for the 1,844 survivors was only 1.04 mm. Increasing local density from 12 to 30 animals/plot (130 to 323/m^2) did not significantly ($P < 0.05$) affect either growth rate or relative growth; however, relative growth of quahogs at the highest density (60 animals/plot or 645/m^2) was significantly depressed by a factor of 1.2 ($P < 0.0001$) when compared to animals at either of the two lower densities. The influence of density was not consistent across all enclosure types as relative growth was enhanced by a factor of 1.34 inside complete cages containing quahogs at the lowest density compared to growth inside the other two enclosure types at that same density.

Enclosure type also influenced the growth of individuals of *Arctica islandica* as animals inside complete cages grew faster than individuals located within either open enclosures or partially covered enclosures. Presumably, competition for food is the mechanism that resulted in this effect. These results suggest that a large proportion of the individual variability of growth within and between size classes of wild quahogs can be explained by local abundance patterns and bottom topographical features.

SEASONAL CONDITION OF *ARCTICA ISLANDICA* IN THE MID-ATLANTIC BIGHT. Lowell W. Fritz, Institute of Marine and Coastal Studies, Rutgers University, Port Norris, NJ 08349.

Samples (n = 40) of the ocean quahog, *Arctica islandica*, were obtained monthly from a commercial processor between November 1988 and October 1989. The following measurements were obtained on each individual: shell length (SL), height, width, dry weight and internal volume (SV); somatic tissue dry (SDW) and wet weight (SWW); visceral tissue dry (VDW) and wet weight (VWW); total tissue dry (TDW) and wet weight (TWW); and sex (by gonad biopsy). Condition of individual quahogs was calculated in the following ways, with separate calculations for females, males and the total sample each month: CSV = TDW/SV, BI = VDW/TDW and the calculated SDW, VDW, TDW and TWW for a standard-sized animal (SL = 95 mm) based on semi-log regressions.

There was little seasonal trend evident in CSV and BI for females, males and the total sample each month. Similarly, calculated SDW, VDW, TDW and TWW each month revealed no significant seasonal variation. Data suggest that site-specific differences in growth may have obscured any seasonal trends in condition.

Males and females varied similarly in condition and calculated tissue weight throughout the year, but males had greater CSV in spring and summer and higher BI throughout the year than females. Females outnumbered males 56% to 44% and had greater SL within each month and across all samples; no hermaphrodites were found. Morphometric differences between males and females are discussed.

NJAES Publication No. K-32001-7-89 supported by Fisheries and Aquaculture TEX Center of Rutgers University and state funds.

GROWTH RATE OF *ARCTICA ISLANDICA* LINNE: A COMPARISON OF WILD AND LABORATORY-REARED INDIVIDUALS. **M. Gayle Kraus*** and **Brian F. Beal,** University of Maine at Machias, 5 O'Brien Avenue, Machias, ME 04654; **Samuel R. Chapman,** Darling Marine Center, Walpole, ME 04575.

Ocean quahogs are considered a long-lived, slow-growing species. Ages of individuals between 65 mm and 110 mm in shell length collected from southern Georges Bank to Long Island, N.Y. have been estimated at 30 to 221 years. Quahogs collected from commercial beds near Machias Bay (Lat. 44°35′N; Long. 67°26′W) in Downeast, Maine show similar, slow growth rates. Past attempts to artificially increase the rate of growth of this species inside sediment-free containers indicated low growth potential suggesting that the ocean quahog is an unlikely or even unsuitable candidate for aquaculture.

To determine the growth potential for *A. islandica* in sediment-filled trays, we individually marked and measured fifty wild quahogs ($\bar{x}$ = 9.6 mm ± 0.29 SE estimated at between 2 and 5 years old), placed them in shallow (13 cm deep) trays in a constant-flow seawater system at the Darling Marine Center (located on the Damariscotta River in Walpole, Maine) for two years beginning in December, 1987. Quahogs were always kept at ambient river temperatures and did not receive any food supplements during the experiment. Quahogs were sampled (excavated from the containers and measured to the nearest 0.1 mm) on the yearly anniversary and also during March, June, September, and December, 1989. Average shell length during September, 1989 for 49 of the 50 survivors was 45.0 mm ± 0.59 SE. Using growth rate data (age determined by standard acetate peel method) for specimens collected from the Machias Bay area, we estimate a wild individual of similar length to be approximately 29 years old. Therefore, in 1.75 years, we have compressed the growth of these animals by nearly 25 years compared with growth in their natural habitats.

This dramatic increase in the growth rate of cultured quahogs compared with those in the wild suggests that competition for food and/or disturbance (both physical and biotic) limits growth rates in natural populations. These results indicate that this species has the potential of being cultivated in shallow-water sites protected from predators.

GROWTH PATTERNS WITHIN THE SHELL OF THE OCEAN QUAHOG, *ARCTICA ISLANDICA:* A REVIEW AND RECENT OBSERVATIONS. **Richard A. Lutz*** and **Lowell W. Fritz,** Institute of Marine and Coastal Sciences, NJAES, Rutgers University, New Brunswick, NJ 08903; **Joseph A. Dobarro,** New Jersey Department of Environmental Protection, Rutgers Shellfish Research Laboratory, Port Norris, NJ 08349; **Alden Stickney,** Maine Department of Marine Resources, Boothbay, Harbor, ME 04575; **Michael Castagna,** Virginia Institute of Marine Science, Wachapreague, VA 23480.

A number of studies reported within the literature over the past decade have suggested that certain structural patterns within the entirely aragonitic shell of the ocean quahog, *Arctica islandica,* may reflect annual cycles of growth. Within the inner shell layer, these "annual" patterns of growth are characterized by alternating sublayers of simple prismatic and fine to irregular complex crossed lamellar microstructures; within the outer shell layer, simple prismatic or granular microstructural growth horizons alternate with regions of homogeneous microstructure. In each layer, the prismatic or prismatic/granular regions are manifested as distinct "lines" on acetate replicas of polished and etched radial shell sections when viewed under an optical microscope at a magnification of approximately 40×. With the underlying assumption that such lines are forming with an annual periodicity, age estimates as high as 225 years have been reported for this species.

Recent analyses of radially-sectioned shells of known-age specimens of *Arctica islandica:* (1) spawned under laboratory conditions in September 1980; (2) subsequently reared in mesh containers suspended from fixed and floating platforms in Gulf of Maine waters; (3) and sacrificed between July 1988 and August 1989 (final shell lengths ranging from 47.5 to 68.4 mm) did not reveal an unambiguous one-to-one correspondence between the number of years elapsed since spawning and the number of simple prismatic or granular microstructural regions in either the outer or inner shell layer. Additional analyses of radially-sectioned shells (final shell lengths = 24.4, 26.6, and 33.3 mm) of three known-age specimens (spawned in May 1984) of this species transplanted in June 1987 to a site in 45 m of water off the coast of New Jersey did not reveal an unambiguous one-to-one correspondence between internal shell growth patterns and the number of years elapsed since spawning or transplantation (specimens sacrificed in

July 1988). It is suggested that caution should be exercised in utilizing microstructural patterns within the shell of *Arctica islandica* for obtaining unambiguous age estimates of specimens of this species from at least certain environments.

NJAES Publication K-32001-9-89 supported by state funds, various NOAA Sea Grants, Borden Company, and the Fisheries and Aquaculture Technology Extension Center.

LARVAL ECOLOGY OF *ARCTICA ISLANDICA* ON THE INNER CONTINENTAL SHELF OF THE EASTERN UNITED STATES. Roger Mann, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Arctica islandica occupies a wide latitudinal and bathymetric range on the inner continental shelf of the eastern United States. The inshore limit of distribution limit appears to be limited by the 16C bottom isotherm in the summer months. Adults exhibit some burrowing activity but laboratory observations suggest they are rather sedentary. This behaviour suggests that larval settlement occurs throughout the adult distribution range (i.e. immigration of post settlement stages is relatively unimportant). But where do the larvae come from? *Arctica islandica* larvae are long lived and the Middle Atlantic Bight, at least, exhibits a residual bottom flow. This presentation reviews current knowledge of seasonal net flow on the eastern continental shelf and incorporates data on larval development rate to speculate on possible distances of *Arctica islandica* larval dispersion on the inner continental shelf.

POPULATION AND FISHERY DYNAMICS OF OCEAN QUAHOG IN THE MIDDLE-ATLANTIC BIGHT, 1976–1990. S. A. Murawski,* F. M. Serchuk, J. S. Idoine, and **J. W. Ropes,**[1] National Marine Fisheries Service, Northeast Fisheries Center, Woods Hole, Massachusetts 02543.

An intensive fishery for the ocean quahog, *Arctica islandica*, developed in the Middle Atlantic region, beginning in 1976, in response to declining populations of surf clam, *Spisula solidissima*. Management of the stock has been predicated on the recognition of the very limited productivity potential of the resource (slow growth and poor recruitment rates). It was recognized early on that harvest rates greater than a few percent of the extant stock would result in rapid depletion of the accumulated stock, particularly in areas nearest the traditional ports and shucking facilities. Since the inception of the fishery, vessel logbooks have been required of all fishery participants, documenting the catch, effort and fishing location of individual fishing trips. In this study the areal extent and catch rates of the fishery over time are evaluated, in relation to the spatial distribution and abundance of the region-wide stock. In particular we focus on the harvest rates in relation to the relative abundance of various stock components.

A critical element for the long-term viability of the fishery is recruitment to areas that have been intensively harvested. Research vessel surveys of the region-wide quahog resource are reviewed, and alternative sampling strategies for evaluating the potential for new recruitment in areas subjected to harvest are evaluated.

Growth rate information is updated for ocean quahog, based on data collected from a marking experiment initiated in 1978. Previously unpublished growth increment data from recaptured animals, as well as length frequency data from the marking site off Long Island New York are used to re-evaluate growth equations originally proposed for the ocean quahog resource in that area.

Finally, we review research requirements necessary to support the long-term management of the ocean quahog and surf clam resources of the region, and evaluate the relative priority of proposed research, in light of likely management scenarios for the stocks.

INTRODUCTIONS AND TRANSFERS OF MOLLUSKS: RISK CONSIDERATIONS AND IMPLICATIONS

GENETIC ASPECTS OF INTRODUCTION AND TRANSFER OF MOLLUSCS. Standish K. Allen,* Rutgers Shellfish Research Laboratory, New Jersey Agricultural Experiment Station, Port Norris, NJ 08349; **Patrick M. Gaffney,** College of Marine Studies, University of Delaware, Lewes, DE 19958.

Transfer of molluses is a concern in situations where endemic stocks have a distinct, adaptive genetic structure. Because of their typically planktonic larval stage, many molluses tend to maintain genetic uniformity across broad geographic regions. In addition, many commercial species have been the objects of repeated intentional transfers for more than a century. Transfers among these populations pose little genetic risk.

Introductions pose significant genetic concerns for both introduced and endemic populations, depending on the degree of reproductive isolation existing between them. When interbreeding occurs, the fate of the hybrid progeny is determined by their overall performance (i.e., viability and fertility). If hybrids are less fit, natural selection strengthens reproductive isolation between the two populations, reducing the potential for genetic exchange. Transient loss of gametes by the native population to inviable hybrid combinations entails little risk if the number of introduced animals is small compared to the natives. However, loss of gametes by the natives is more serious if the introduced population size increases by subsequent introductions or by breeding. If

[1]Deceased

hybrids enjoy equal or greater fitness, they may persist and allow exchange of genes between introduced and endemic populations. Because natural selection acts continually to eliminate less favorable genotypes, the result of introgressive hybridization may be improved fitness (but loss of genetic distinctness) of both populations. The concept of "gene pool contamination" is more applicable to unique isolated populations than to many marine molluscs.

Genetic considerations regarding the introduced population include the size and geographic origin of the founder population. Transfers and introductions of genetically manipulated molluscs (e.g., polyploids or hybrids) are of less concern because of reduced fertility.

THE INTRODUCED MARINE AND ESTUARINE MOLLUSKS OF NORTH AMERICA: AN END-OF-THE-CENTURY PERSPECTIVE ON FOUR CENTURIES OF HUMAN-MEDIATED INTRODUCTIONS. James T. Carlton, Maritime Studies Program, Williams College—Mystic Seaport, Mystic, CT 06355.

Four centuries of human-mediated introductions of marine and estuarine mollusks from Europe, Japan, and the Indo-Pacific to North America have resulted in strikingly different invasion patterns: the Pacific coast, with nearly 40 non-native species, has perhaps four times the number of introductions as the Atlantic coast (10 or fewer), which in turn, has twice the number of introduced mollusks as does the Gulf coast (5 or fewer). Hypotheses to account for these biogeographical-ecological differences in the susceptibility and/or resistance of these regions to molluscan invasions are reviewed. The introduced mollusks on each coast that have proven to be most significant in ecological, social, or economic terms are identified. The potential for future invasions, the risks involved, and means to reduce these risks, are considered in light of the recent spectacular successes of the Asian clam *Potamocorbula* in San Francisco Bay and the European zebra mussel *Dreissena* in the Great Lakes. There is little doubt that what Charles Elton identified in 1958 as the molluscan "chess game" will continue into the 1990s.

THE POLITICAL PROCESS AS IT RELATES TO SHELLFISH. Lee R. Crockett* and **James McCallum,*** U.S. House of Representatives, Committee on Merchant Marine and Fisheries, 1334 Longworth HOB, Washington, D.C. 20515.

Certain activities associated with shellfish resources and their habitats have become prominent and in many cases controversial in recent years. This prominence has resulted in a multiplicity of conflicting constituency views and pressures that are often brought to bear on Federal legislators for their consideration and possible action. The force for political action depends on: 1) the size of the constituency advocating action; 2) the quality and persuasiveness of the constituency's arguments; 3) the unity and commitment of the constituency; and 4) the political climate that exists at the time. At times solution, or at least resolution, to constituency problems or demands is easily accomplished through the political process. However, at other times the problems are so intractable that only marginally acceptable, or "stop gap," solutions can be reached with great difficulty.

Examples of the Federal political process to be discussed include: 1) oyster disease research; 2) department or agency roles in aquaculture; 3) continuous seafood inspections; 4) ballast water introductions; and 5) ocean pollution and monitoring.

PATHOGENS, PARASITES, AND PREDATORS OF MOLLUSKS: SPREAD AND CONTROL. Susan E. Ford, Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

Commercially important mollusks have been moved from one location to another around the world for centuries. Predators and competitors have often been moved at the same time, have survived and colonized the new location, and have caused serious economic problems. Evidence for transmission of disease-causing organisms in the same manner is less clear. It is strongest in cases where pathogens are known to be transmitted directly between individuals of the host species. Examples are parasites of the genus *Perkinsus*, and the oyster parasite *Bonamia ostrea*. On the other hand, circumstantial evidence only links the parasites *Marteilia* spp. and *Haplosporidium* spp. to movement of host oysters, and neither of these has been shown to be transmitted directly from oyster to oyster.

Government restrictions have limited, but not entirely prevented, the movement of infected animals. Causes include lack of knowledge about possible pathogens in molluscs intended for transport, inadequate detection methods for known pathogens, and indifference or carelessness on the part of industry members and resource managers.

The growth of molluscan aquaculture forecasts an increasing demand for long distance shipment of both broodstock and seed. Regulations must be realistic and consistent to gain acceptance by the industry and to be enforceable. Diagnostic techniques must be made less costly and more rapid, and diagnostic services must be readily available. Reliable and cost-effective methods for eliminating parasites from infected molluscs should be explored. Finally, the basic biology of disease-causing organisms and their interactions with molluscan hosts must be understood to a much greater degree than they are today.

This is NJAES Publication No. K-32405-2-89, supported by State funds.

MOLLUSCAN SHELLFISH INTRODUCTIONS—CONCERNS OF STATES. Mike Hickey*, Massachusetts Division of Marine Fisheries; **John W. Hurst, Jr.,** Maine Department of Marine Resources.

Introductions of indigenous and non-indigenous mollusks into a marine environment for commercial and scientific purposes have been practiced since the beginning of shellfish aquaculture. Increasing demand for mollusks of commercial value encourages these introductions for a variety of economic and biological reasons resulting in a multitude of potential problems from associated diseases, pests, predators and competitors. This paper discusses the pro's and con's of such introductions, problems of management and strategies currently employed to prevent potential adverse effect associated with introductions.

ANOTHER OYSTER FOR THE CHESAPEAKE BAY? A DISCUSSION OF HABITAT CONSIDERATIONS WHEN SELECTING SPECIES FOR INTRODUCTION. Roger Mann, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Protocols for consideration of species introductions have been developed by the International Council for the Exploration of the Seas (ICES), the European Inland Fisheries Advisory Commission (EIFAC) and the American Fisheries Society (AFS). All three protocols stress the importance of "matching" the donor and recipient environments. If environmental and biological data bases are adequate (and they rarely are) the degree of "match" can be used to predict potential impact, in terms of short term growth and survival and long term reproductive success, of an introduction. The depressed state of the Chesapeake Bay oyster resource has stimulated discussion of use of another oyster in the bay to improve water quality, reestablish a degenerating component of the benthic community, and provide an alternative commercial crop. This "case study" will be used as a working example to illustrate the complexity of the habitat consideration process in introduction procedures.

SOCIAL AND CULTURAL DIMENSIONS OF THE MOVEMENT OF MOLLUSCS. Bonnie J. McCay, Department of Human Ecology, Cook College, Rutgers University, P.O. Box 231, New Brunswick, New Jersey 08903.

Cases of the introduction and movement of molluscan species are reviewed in relationship to two social questions. One is the social impact of new industries based on once-exotic species, or how families, communities, and lifestyles may be affected by such change; the case to be reviewed is that of the introduction of *Crassostrea gigas* into Zeeland (Netherlands) oystering. Another is cooperation between shell-fishermen, scientists, and others in shellfish rehabilitation programs. The case reviewed is that of Mercenaria mercenaria spawner transplants in New Jersey (US). My recommendations include improving the nature and extent of shell-fishermen participation in science and management policy, implementation, and evaluation.

LAWS AND REGULATIONS RELATIVE TO INTRODUCTION AND TRANSFERS OF SHELLFISH. Mr. James Peaco, Attorney, Inspection Services Division, National Marine Fisheries Service, Washington, DC; **Frederick G. Kern,** National Marine Fisheries Service, Northeast Fisheries Center, Oxford Laboratory, Oxford, Maryland.

The Department Secretaries of Commerce, Interior, Agriculture, and Treasury are currently empowered to promulgate rules and regulations pertaining to the introduction and transfers of molluscan species. Congress and the President have mandated Federal agencies, under the Fish and Wildlife Coordination Act, to conserve and protect the marine resources of the United States. The Secretaries also have the authority under the "Lacey Act" to "promulgate rules and regulations pertaining to importation of injurious wildlife, including a listing of such injurious species, which prohibit their import except under a rigid permit system . . .". Recognizing the need for a Federal policy to restrict the indiscriminate introduction of and export of exotic species, President Jimmy Carter signed Executive Order 11987 instructing Federal agencies "to the extent permitted by law, restrict the introduction of exotic species . . . ." The order also encourages states, local governments and private citizens to prevent the introductions of exotic species into natural ecosystems of the United States. In addition to examples and explanations as to how the Lacey Act impinges on molluscan introductions and transports, other pertinent Federal and State laws, regulations, and regulatory proposals will be discussed in the context of resource and habit safety.

THE ECONOMICS OF MOLLUSC INTRODUCTION AND TRANSFER: HISTORY AND FUTURE OF PRIVATE AND PUBLIC DECISIONS. Ivar E. Strand,* Department of Agricultural and Resource Economics, University of Maryland, College Park, MD 20742; **Eileen A. Lavan,** Marine-Estaurine-Environmental-Sciences Program, University of Maryland, College Park, MD 20742.

The introduction and transfer of molluscs depends on a variety of forces to generate sufficient economic returns to stimulate interest by either private firms or public agencies. This paper will examine historical events surrounding the successful introduction or transfer of oysters, abalone and mussels to identify past forces which have stimulated the activity. It will also speculate on how current economic trends will influence future introduction and transfer activity.

We will also examine the economics of risk in private and public decisions concerning the introduction and transfer of molluscs. In particular, the decision criteria for private firms (who run the risk of disease introduction and quality degradation) will be contrasted with the conservative "safety first" posture of public officials. Alternative decision criteria and management strategies will be explored.

INTRODUCTION AND TRANSFER OF MOLLUSCS IN THE NORTHEAST PACIFIC. L. J. Wiegardt,* Wiegardt Brothers, Ocean Park, Washington, 98640; **N. Bourne,** Pacific Biological Station, Nanaimo, B.C. Canada. V9R 5K6.

Several species of molluscs have been introduced to the west coast of North America either intentionally or unintentionally. In addition there has been and continues to be widespread transfer of molluscs between different areas along the coast. Results of these introductions and transfers have been variable. The most famous introduction was the Pacific oyster, *Crassostrea gigas*, which now supports a large industry in the northeast Pacific. The manila clams, *Tapes philippinarum*, which was introduced with the Pacific oyster also supports a multimillion dollar industry. However, deleterious organisms were introduced with Pacific oysters and a brief review of the advantages and disadvantages of the introduction is given. The advent of hatcheries with quarantine facilities now makes it possible to introduce exotics or transfer molluscs from area to area on a large scale with minimal danger of introducing pests, parasites or diseases with them. The need for further introductions of molluscan species and transfer of species to other areas of the northeast Pacific is reviewed. Mechanisms controlling introductions and transfers of molluscs (and other organisms) are discussed. The general conclusion is that any further introductions should be carefully reviewed and controlled and regulations tightened to control movement of molluscs into or throughout the northeast Pacific.

PARASITES AND DISEASE

EXTENT OF CASTRATION OF PRAWNS (*PANDALUS PLATYCEROS*) BY *SYLON* (CRUSTACEA: RHIZOCEPHALA). Susan M. Bower* and **James A. Boutillier,** Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada, V9R 5K6.

Pandalus platyceros is one of two species of shrimp for which ovigerous females injected by *Sylon hippolytes* are reported. The purpose of this study was to examine the effect of *Sylon* on the gonad of all sexual stages of this protandric hermaphrodite. The mean dry gonad weight of 106 infected preovigerous female *P. platyceros* from the central coast of British Columbia was less than that of uninfected prawns (n = 167). The extent of reduction in gonad weight was correlated with development of the parasite. At early stages of infection (parasite only visible internally), the mean gonad weight was reduced by 75% while at later stages (parasite or resulting scar visible externally), the gonad was about 91% of normal mean gonad weight. Prawns which survived the infection (only melanized roots visible internally) showed signs of recovery in mean gonad weight to about 50% that of uninfected prawns. Histologically, 20% of 105 infected younger prawns (juvenile, male, and transition) had no appreciable aberration in gonad morphology. The remaining 80% were abnormal in gonad morphology and/or secondary sex characteristics (shape of second pleopods). Abnormal gonad morphology (castration) was always manifested as retardation of gonad development with degeneration of some gametes in 57% of the cases. The mechanism of castration appeared to be indirect and may have resulted from hormonal control of the prawn by *Sylon* and/or attributable to interference with the general nutrition of the host (i.e. competition for available nutrients).

SUSCEPTIBILITY OF MSX-RESISTANT STRAINS OF THE EASTERN OYSTER AND OF THE JAPANESE OYSTER TO *PERKINSUS MARINUS*. Eugene M. Burreson,* Judith A. Meyers, Roger Mann, and **Bruce J. Barber,** School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Pt. VA 23062.

Efforts to revitalize the Chesapeake Bay oyster resource involve the development of disease-resistant strains of *Crassostrea virginica* and/or the potential use of genetically manipulated strains of *C. gigas*. Progeny from three strains, Delaware Bay natives, Delaware Bay MSX-selected (six generations), and Mobjack Bay natives (Chesapeake Bay), have been exposed to MSX and *Perkinsus* for two years; progeny from three other strains, Chesapeake Bay MSX-selected (six generations), lower James River natives, and upper James River natives (susceptible controls) have been exposed to MSX and *Perkinsus* for one year. All strains have low levels of MSX, but most have high levels of *Perkinsus* and high mortality. For example, after two years of exposure the Delaware Bay MSX-selected strain has a *Perkinsus* prevalence of 96% and a total mortality of 45%. The lower James River strain has the lowest levels of both diseases, but it has only been exposed for one year. It appears that resistance in *C. virginica* is not a generalized response, but is specific for the selected parasite.

Diploid and triploid *C. virginica* and *C. gigas* are being exposed to *Perkinsus* under controlled conditions in the laboratory. Preliminary results will be presented.

COMPARISON OF OYSTER DEFENSE MECHANISMS FOR MSX-RESISTANT AND -SUSCEPTIBLE STOCKS HELD IN CHESAPEAKE BAY. Marnita M. Chintala,* University of Maryland, Horn Point Environmental Laboratory, Cambridge, MD 21613; **William S. Fisher,** University of Texas Medical Branch, Marine Biomedical Institute, Galveston TX 77550.

An oyster stock from Delaware Bay, laboratory-selected for resistance to MSX disease at Rutgers University, was placed in the Maryland portion of Chesapeake Bay (Deal Island) and compared to a local MSX-susceptible stock for one year (1988–89). Measurements of hemocyte activities revealed no differences in hemocyte capacity to spread to an ameboid shape, salinity regu-

late or locomote *in vitro*. The susceptible stock exhibited higher serum protein concentrations and higher lysozyme concentrations during the spring and summer. Serum lectins, as measured by bacterial agglutination, decreased for both stocks during the year, but lectin titers were greater for the resistant stock during the summer. Results from other studies reinforce the differences observed in lectin titers. Serum lectins may act in a defensive capacity by opsonizing nonself material that enters the hemolymph. Histological examination of oysters showed 0% prevalence of MSX for the resistant stocks and 60% prevalence (with 40% systemic infection) for the susceptible stocks. Diagnostic tests for infection *Perkinsus marinus* (Dermo disease) found both resistant (58% prevalence) and susceptible (67% prevalence) stocks infected with the disease. It appears then, that defense mechanisms responsible for resistance to MSX disease do not substantially increase resistance to Dermo disease.

CYTOLOGY AND TIME-LAPSE CINEMATOGRAPHY OF HEMOCYTES OF THE SOFT-SHELL CLAM, *MYA ARENARIA*. **Albert F. Eble, Missy Reside,** and **Lori Auletta,** Trenton State College, Dept. Biology CN4700, Trenton, NJ 08650-4700.

Three cytotypes have been observed: Cytotype I has a cell area of 69.5 μm^2, a nuclear area of 5.5 μm^2 and an ecto-endoplasmic ratio of 0.65; it is highly motile. Cytotype II has a cell area of 258.9 μm^2, a nuclear area of 13.6 μm^2 and an ecto-endoplasmic ratio of 2.2; it is weakly motile and aggregates *in vitro:* cells will fuse to form masses with 4–6 nuclei. Cytotype III has a cell area of 256.5 μm^2, a nuclear area of 23.2 μm^2 and an ecto-endoplasmic ratio of 3.2; it shows little motility.

Three granule types were recognized: A—circular with a diameter of 0.5 μm and seems to be a primary lysosome; B—highly plastic with a range of 0.4–2.0 μm and appears to be a secondary lysosome; C—circular with a diameter of 1.0 μm and is a lipid-storage granule.

When viewed with time-lapse cinematography at 36× normal, all cell types exhibit much membrane ruffling; distinct centrosomes are visible in Cytotypes I and II as well as much intracellular motion of cell organelles.

***IN VITRO* RECOGNITION AND PHAGOCYTOSIS OF THE OYSTER PATHOGEN MSX.** **Susan E. Ford,* Sheila A. Kanaley,** and **Kathryn A. Ashton-Alcox,** Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

Phagocytosis is usually considered the main response of mollusks to foreign material. We have employed an *in vitro* phagocytosis assay to explore and quantify recognition and response of host (*Crassostrea virginica*) and non-host hemocytes to plasmodial stages of the parasitic protozoan MSX (*Haplosporidium nelsoni*). In this assay, MSX is collected from the hemolymph of infected oysters, enriched by "panning" (a process that takes advantage of differences between parasites and hemocytes in their ability to adhere to surfaces), and added to monolayers of hemocytes. Parasites, hemocytes, or both, may be treated to alter their physiological state during their encounter with other cells. Phagocytosis is determined by microscopic examination.

In most trials, fewer than 5% of plasmodia were phagocytosed by granular hemocytes of *C. virginica* from a natural stock in Delaware Bay that shows some resistance to the parasite. However, when plasmodia were subjected to low-salinity shock, nearly all those that subsequently took up the vital strain trypan blue (a measure of non-viability), were phagocytosed. This supports earlier histological observations that oyster hemocytes do not phagocytose live MSX, but do engulf dead parasites. Hemocytes from the Japanese oyster, *C. gigas*, which is thought to be resistant to MSX, did not attack parasites in this assay. Hemocytes from the ribbed mussel, *Geukensia demissa*, however, rapidly ingested them.

The assay appears an ideal model system to investigate the mechanisms underlying recognition and phagocytosis of MSX (or lack of it) because results were so unequivocal. When it occurred, phagocytosis was rapid and nearly 100%. Absence of phagocytosis was equally clear. The evidence so far points to the development of resistance to MSX in *C. virginica* without evoking the phagocytic response believed to be the primary defense mechanism in molluscs. This is NJAES Publication No. K-32405-1-89, supported by State funds.

LOW SALINITY CONTROL OF *HAPLOSPORIDIUM NELSONI* (MSX). **Harold H. Haskin,*** and **Susan E. Ford,** Rutgers Shellfish Research Laboratory, P.O. Box 687, Port Norris, N.J. 08349.

It has long been recognized that exposure of MSX-infected oysters to lowered salinities may "purge" them of the parasite. In vitro studies have shown that MSX may be destroyed within minutes at salinities below 10. Influences of ever-varying ambient salinities and seasonal changes in temperature are not well understood. Modest studies as described here are needed for guidance to growers who consider use of lowered salinities to reduce losses.

Results of three relevant experiments are summarized here: (1) In a laboratory experiment at spring temperatures, 4 groups of oysters from an ambient salinity of 24 were exposed to various lower salinities down to 6. Equilibration to salinities below 10 was expedited by exposure of oysters to successively lower salinities rather than by a single step. (2) In a late winter field experiment oysters from an ambient salinity of 17.5 were exposed at two locations to tidally fluctuating river salinities ranging from 2.9 to 15.2. With temperatures at 2.8–3.8°C hemolymph in all oyster samples ranged between salinities of 10.9 and 11.8. With rising temperatures, later, hemolymph salinities dropped to the ambient salinity. (3) August survivors of very MSX-susceptible yearlings at Cape Shore, Delaware Bay, were transferred to lower river salinities for 3 weeks and then returned to the Cape Shore. A growth

spurt in 2½ weeks, compared with the stunted controls that had remained at the Cape Shore, was most impressive.

Small scale studies such as these promise guidance to those in the industry inclined to "purge" MSX from their oysters. NJAES Publ. #K-32405-4-89

THE EFFECT OF SALINITY AND *PERKINSUS MARINUS* INFECTION ON SOME IMMUNOLOGICAL PARAMETERS OF THE OYSTER *CRASSOSTREA VIRGINICA*. **Jerome F. La Peyre,* Fu-Lin E. Chu,** and **Lisa M. Ragone,** School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Various immunological parameters were measured in *Perkinsus marinus* infected eastern oysters (*Crassostrea virginica*) after exposure to 4 salinities (6, 9, 12 and 20 ppt) for 4, 6 and 8 weeks. Total and differential hemocyte counts, hemocyte phagocytosis and hemagglutinin, lysozyme and total protein of cell-free hemolymph were determined in individual oysters ($n = 15$) at each of the above salinities and time periods. Mean total hemocyte counts at 6, 9, 12 and 20 ppt were respectively 1.43 ± 1.03, 1.7 ± 0.99, 2.39 ± 1.74 and 2.67 ± 1.91 million cells per ml. Total hemocyte count was determined to be weakly correlated with both salinity ($r = 0.299$, $p = 0.0001$, $n = 180$) and *P. marinus* intensity ($r = 0.235$, $p = 0.004$, $n = 180$). No relationship could be shown with cell-free hemolymph hemagglutinin and total protein with either salinity or intensity of *P. marinus* infection. Results on phagocytosis of *Escherichia coli* by hemocyte will also be presented.

GROWTH, MORTALITY, MSX INFECTION AND YIELD OF INTERTIDALLY GROWN *CRASSOSTREA VIRGINICA*, **D. T. J. Littlewood,* R. N. Wargo,** and **J. N. Kraeuter,** Shellfish Research Lab., Cook College, Rutgers University, P.O. Box 687, Port Norris, NJ 08349.

Oysters (*Crassostrea virginica*) bred for resistance to the protozoan parasite MSX (*Haplosporidium nelsoni*) were grown at five intertidal levels (28–60% aerial exposure [AE]) for 218 days on tidal flats in the Delaware Bay. Mortality, growth, condition index and yield of marketable oysters (>70 mm shell height) were inversely proportional to AE. The number of *Polydora* casts on the inside of the valves decreased with greater AE (0 infections above 50% AE).

Less than 20% of oysters at each intertidal level were infected with MSX at the end of the trial and there was no statistically significant difference in infection rates between levels.

We conclude that the incidence of MSX infection or intensity is unaffected by intertidal height (within 28–60% AE) amongst disease resistant oysters. A reduction in feeding time and fouling may explain the relationship between high survival and poor growth in oysters experiencing longer AE.

NJAES Pub. No. K-32404-3-89 supported by Fisheries & Aquaculture Tech. Ext. Cent. and State funds.

CLASSIFICATION OF THE HAPLOSPORIDIIDAE. **Elizabeth R. McGovern*** and **Eugene M. Burreson,** School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Parasites in the family Haplosporidiidae, which includes important oyster diseases, are separated into two genera, *Haplosporidium* and *Minchinia*. Species of both genera have spores with an orifice closed by a hinged operculum and ornamentation consisting of structures variously described by different authors as wrappings, ribbons, threads, filaments or tails.

Haplosporidan spore ornamentation should be placed in three major categories: spore wall filaments, epispore cytoplasm tails and wrappings. Filaments, as found on spores of *H. parisi*, are composed of wall material and are formed as the spore wall is forming. Epispore cytoplasm tails are more ephemeral and may be shed after spores are released from the host as in spores of *Minchinia* sp. infecting *Teredo* spp. Wrappings, exemplified by spores of *H. costale*, are formed in epispore cytoplasm and adhere to the spore wall following lysis of the cytoplasm. Additionally, several species have been described as lacking ornamentation.

At present, species possessing spore wall filaments and those ornamented by wrappings are placed in the genus *Haplosporidium*. Species with epispore cytoplasm tails and species with unornamented spores are assigned to *Minchinia*. Generic assignments should be based on the description of the type species of each genus; however, the type species of the genus *Haplosporidium*, *H. scolopli* has not been studied with electron microscopy and the origin of its epispore extensions is uncertain. Further research into the composition of wrappings is necessary to determine if they are more similar to the ornamentation of *H. scolopli* or to *M. chitonis*, the type species of *Minchinia*.

To standardize generic assignments of species in the Haplosporidiidae, spores must be studied at various stages of maturity with both SEM and TEM. Furthermore, terms used to describe spore ornamentation need to be more clearly defined.

THE ROLE OF OYSTER SCAVENGERS IN THE SPREAD OF THE OYSTER DISEASE *PERKINSUS MARINUS*. **Judith A. Meyers,*** and **Eugene M. Burreson,** School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The role of oyster scavengers in the transmission of the oyster disease *Perkinsus marinus* is examined. Several species known to feed on dying oysters were fed pieces of tissue from oysters infected with *P. marinus*. Fecal pellets were subsequently collected and cultured in fluid thioglycollate media for four days. Staining with Lugol's iodine and microscopic examination revealed *P. marinus* hypnospores in the feces of the following species, *Cal-*

linectes sapidus, *Panopeus herbstii*, *Eurypanopeus depressus* and *Gobiosoma* sp. Other species are being examined. Viability of *P. marinus* cells was tested using trypan blue. In all cases *P. marinus* was found to survive passage through the digestive tracts of scavengers. In a related series of experiments fish and crabs fed infected oyster tissue were placed in aquaria with 20 live, disease free oysters. The oysters will be sacrificed after 10 weeks of exposure and examined for *P. marinus*. Results will be presented.

ISONEMA-LIKE FLAGELLATES (PROTOZOA: MASTIGOPHORA) AS POTENTIALLY OPPORTUNISTIC PATHOGENS OF BIVALVE MOLLUSCS. Thomas A. Nerad,* American Type Culture Collection, Rockville, MD 20852; **Christopher F. Dungan,** Maryland Dept. of Natural Resources, Oxford, MD 21654; **Thomas K. Sawyer,** Rescon Assoc., Box 206-Turtle Cove, Royal Oak, MD 21662.

Hemolymph from the American oyster, *Crassostrea virginica*, inoculated into thioglycollate broth, yielded a unique flagellate in up to 20% of the oysters sampled. Sterility test showed that bacterial and fungal contaminants sometimes were present, suggesting that the flagellates may have been present in the mantle fluid or on surface tissue rather than in the hemolymph.

Studies with the light microscope showed that the flagellates belonged to the genus *Rhynchopus*, a genus closely related to *Isonema*. Further studies employing electron microscopy and isoenzyme electrophoresis revealed that the new isolate was closely related to strains of *Rhynchopus* previously isolated from water samples from Bermuda, Solomons, MD, and a marine aquarium in Rockville, MD. The flagellates are being maintained axenically for future testing to see if they may act as opportunistic pathogens to *C. virginica*. Kent, Elston, Nerad, and Sawyer (J. Invert. Pathol., *50:* 221–225, 1987), have described an *Isonema*-like flagellate associated with fatal infections in larval Geoduck clams, *Panope abrupta*. New studies are planned to determine whether the flagellates cause disease on a seasonal basis, or under conditions of poor water quality.

THE EFFECT OF LOW SALINITY EXPOSURE ON *PERKINSUS MARINUS* INFECTIONS IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*. Lisa M. Ragone,* and **Eugene M. Burreson,** School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

In recent years *Perkinsus marinus*, a pathogen of the eastern oyster, *Crassostrea virginica*, has spread throughout Virginia's estuaries and become prevalent in low saninity areas previously believed to be disease free. Such widespread distribution of the parasite poses a serious threat to the oyster industry and increases the need to gain a better understanding of the relationship between host, parasite, and salinity. In order to define the effect of low salinity exposure on *P. marinus*, a laboratory experiment was conducted in which oysters parasitized by *P. marinus* were exposed to 4 salinity treatments (20, 12, 9 and 6 ppt) for a period of 8 weeks. Infection prevalence and intensity was assessed in samples (n = 25) drawn from each treatment group after exposure of 2, 4, 6 and 8 weeks and oyster mortality was determined daily. The pathogen persisted throughout the course of the experiment at all salinities tested; however, development of *P. marinus* infections to lethal levels was delayed in oysters maintained at 12, 9 and 6 ppt. Total oyster mortality was significantly reduced in oysters exposed to 9 and 6 ppt. The resulting total mortalities at 20, 12, 9 and 6 ppt were respectively 27.8, 30.9, 14.7 and 13.6 percent. Although *P. marinus* appears to be able to tolerate low salinity (12–6 ppt) it is apparently less virulent at salinities less than 9 ppt.

PRODUCTION MODELLING

DEVELOPMENT OF A SIMULATION MODEL OF A CULTURED MUSSEL (*MYTILUS EDULIS*) POPULATION: POTENTIAL AND LIMITATIONS. Michael Brylinsky,* Acadia Centre for Estuarine Research, Acadia University, Wolfville, Nova Scotia, Canada, BOP 1XO.

In an attempt to elucidate the complex relationships existing between the physical and biological processes that control the growth, spawning and mortality of cultured mussels, a computer simulation model was developed. The model is driven by temperature and particulate inorganic and organic matter concentration, and outputs shell and meat growth rates and spawning times. The model has been validated against several independent data sets and appears to have considerable predictive ability. The potentials and limitations of the model will be discussed in terms of its usefulness for addressing problems such as site selection, carrying capacity, summer mortality and the environmental impact of mussel culture.

A MODEL TO DETERMINE OPTIMUM SEEDING DENSITIES FOR BLUE MUSSELS, *MYTILUS EDULIS*, ON BOTTOM CULTURE LEASE SITES IN MAINE. Daniel E. Campbell,* Department of Marine Resources, West Boothbay Harbor, Maine 04575; **Carter R. Newell,** Great Eastern Mussel Farms Inc., Tenants Harbor, Maine 04860.

The bottom culture of blue mussels, *Mytilus edulis*, is a new industry along the Maine coast which has generated the need for more scientific and technical information upon which production and management decisions can be based. We have developed a model that can be used to estimate the mussel seeding densities that will result in optimum production of mussel meat and shell on various lease sites along the Maine coast. The critical processes associated with food and feeding of mussels on the lease site are the import of food onto the the lease site, the transfer of food from surface waters to the bottom boundary layer, and the mussel feeding process. We made four simplifying assumptions as follows: (1) Food from all sources is aggregated into a single state

variable; (2) No allowance is made for on site carbon production; (3) Negative effects of high seston concentrations on mussel feeding were not considered; (4) Mussel reproduction is not separated from growth.

Food supply to the mussels is controlled by the flux of food onto the lease site and the transfer of food into the bottom boundary layer through turbulent mixing. The horizontal flux of food onto the site is determined by the tidal exchange volume and the food concentration difference between water on the site and water offshore. Flux of food into the boundary layer is a function of the average tidal current velocity and the bottom roughness. Bottom roughness in turn is a function of mussel length. This model was able to reproduce growth rates of meat and shell and shell lengths found on one of the Great Eastern Mussel Farm lease sites. Sensitivity analysis of the model showed that a 50% increase in food supply caused a 63% increase in meat weight, a 39% increase in shell volume and a 75% increase in the POM flux to the mussels. Various mussel seeding densities were evaluated in the model which resulted in accurate prediction of the mussel size and shell volume reductions that were observed at very high seeding densities. Over the range of mussel densities from 300 to 4500 m^{-2} the average weight of an individual mussel varied by 82% while mussel length varied by only 22%. We conclude that mussel seeding density is a critical and controllable factor determining meat and volume yield which if properly adjusted on individual lease sites can result in substantial additional profits for the mussel bottom culture industry.

ECOSYSTEM DYNAMICS AND BIVALVE CULTURE. **Richard F. Dame,** University of S. Carolina, Coastal Carolina College, Conway, S.C. 29526.

Dense bivalve systems have the potential to influence phytoplankton concentration through filtration and excretion. These organisms are coupled in a grazing loop which can enhance productivity. Because direct estimates of oyster and mussel bed feeding and excretion rates are often an order of magnitude higher than scaled-up values computed from laboratory rates, a total ecosystem approach to carrying capacity is urged.

ENVIRONMENTAL PROCESSES AND MUSSEL PRODUCTION IN A LAGOON SYSTEM FROM THE GULF OF ST. LAWRENCE (QUÉBEC). **P. Mayzaud,* P. Souchu,** and **S. Roy,** INRS-Océanologie, 310 des Ursulines, Rimouski, (Québec), G5L 3A1, CANADA.

Over the past decade, the lagoon system of the Magdalen Islands has been the object of a developing mussel aquaculture industry. Contrary to other northern temperate sites of mussel culture where natural fertilization occurs by nutrient input from river discharge, upwellings or anthropogenic sources, the Magdalen Islands lagoon displays oligotrophic characteristics with a lack of dissolve inorganic nitrogen. From spring to fall, the primary productivity of the system depends essentially on regeneration processes from the pool of dissolve organic matter. The overall limited depth gives to the sediment the major role in the recycling of the particular organic matter which is most active under the sites of mussel production during the maximum of the water temperature (mid-August/early September). Throughout the period surveyed (spring–summer–early fall, 1987–1989) rainfalls appear to be a major source of inorganic nitrogen. Primary production and chlorophyll or carbon standing stock are relatively high and typical of regenerated production (small cells <15 μm) with maximum values late summer (August–September). Mussel energy budgets for 1 year and 2 years age classes showed periods of negative scope for growth during the reproductive season (June–July) and one week after the temperature maximum (late August–September). The results of these budgets are discussed in terms of ability of the lagoon to sustain high yields.

USE OF THE BOSS (BENTHIC ORGANIC SESTON SAMPLER) TO INVESTIGATE THE DEPLETION OF PHYTOPLANKTON ABOVE A MUSSEL BED IN MAINE. **Carter R. Newell,*** Great Eastern Mussel Farms, Inc. (GEM), Tenants Harbor, Maine 04860; **D. K. Muschenheim,** Bedford Institute of Oceanography, Dartmouth, Nova Scotia B2Y4A2; **D. A. Murphy,** GEM.

Field profiles of seston were taken upstream and over a mussel bed at two shallow (3–5 m depth) mussel grow-out sites in Maine using the BOSS (Benthic Organic Seston Sampler). The device consists of a ship deployed tripod in which is suspended a carriage holding two vertical arrays of spring loaded 50 ml syringes. The carriage pivots freely, allowing it to passively orient into the current. A time release mechanism allows for the dissipation of resuspended sediment at the tripod's landing, after which 10 undisturbed samples are taken in duplicate from 5 cm off the bottom to 50 cm. Samples were analyzed for chlorophyll a, particulate carbon and nitrogen, cell concentration and biovolume.

At both a low (2 cm sec-1) and moderate (9 cm sec-1) current site, upstream profiles showed higher cell concentrations (predominantly diatoms) at the 5 cm height than at 10, 15, 25, 50 cm heights or at the surface. When compared with samples upstream, phytoplankton concentrations at 5 cm were 3 to 5 fold less over the mussel bed. Field measurements of the consumption of phytoplankton by mussels at each site and profiles using the BOSS are used to generate a simple hydrographic model of the flux of seston to the mussel bed. The results indicate that algal sinking rates may significantly influence spatial phytoplankton concentrations above dense populations of suspension feeding bivalves.

THE CENTRAL ROLE OF SHELLFISH IN THE SIMULATION MODEL OOSTERSCHELDE ECOSYSTEM. **H. Scholten*** and **P. M. J. Herman,** Delta Institute for Hydrobiolo-

gical Research, Vieratrt 28, Yerseke N.L.—NL. **O. Klepper** and **A. C. Smaal.** Tidal Waters Division, PO Box 8039, 4330 EA Middelberg, Netherlands.

The Oosterschelde estuary (S.W. Netherlands) is a relatively unpolluted mesotrophic ecosystem with high biotic diversity and high primary and secondary production. Mussel culture and cockle fishery are the major human influences in the area, both in terms of effect on the ecosystem and in economic sense.

The simulation model SMOES (= Simulation Model Oosterschelde EcoSystem) describes the main carbon and nutrient flows in this estuary. The model calculates food (phytoplankton, suspended detritus) availability for mussels and cockles. The calculation of primary production requires inclusion of nutrient (N, Si) cycles; the competition between mussels and other grazers requires modelling zooplankton, cockles and other bottom organisms.

Mussel and cockle biomasses are no state-variables, but forcing functions in the model, because the biomasses of these organisms are mainly determined by man (mussel culture and cockle fishery). Special attention has been paid to model sensitivity, parameter estimation and uncertainty in the model predictions.

Since 1986 a permeable storm-surge barrier reduces the import of solid substances from the North Sea and the fresh water discharges on the estuary considerably. SMOES has been used to predict the effect of these changes on the Oosterschelde ecosystem. Another application as a research tool was the investigation of the effect of suspension feeders on ecosystem stability with SMOES.

For management purposes, SMOES predicted the effects of a reduced nitrogen load of the river Rhine, the influence of alternative management of the adjacent brackish water lake Veere and the impact of a varying mussel biomass on the Oosterschelde ecosystem.

THE FUNCTIONAL ROLE OF MUSSELS IN THE OOSTERSCHELDE ESTUARY. A. C. Smaal,* and **W. Vonck,** Tidal Waters Division, P.O. Box 8039, 4330 EA Middelburg, NL; **T. C. Prins,** Delta Institute for Hydrobiological Research, Yerseka, NL.

The Oosterschelde estuary (SW Netherlands) is extensively used for the cultivation of the mussel *Mytilus edulis* on bottom plots. The mussel population comprises ca. 45% (13.2 g ADW/m^2) of the total benthic suspension feeder biomass.

To estimate the impact of the mussels on the ecosystem, a number of studies have been conducted. Results are included in the SMOES model. Uptake and release rates of particulate and dissolved material have been measured throughout the year under ambient conditions in the laboratory, and have been compared with the field situation by applying a 10 m long plexiglass tunnel. It is shown that the mussel population has a capacity of filtering the whole waterbody every 4–5 days, which results in a large flux of material towards the bottom. A large part of the seston is resuspended again. Chlorophyll shows a net flux towards the mussel population. Moreover, there is a net release of inorganic nutrients. The mussel beds are an important site of nutrient regeneration.

This is significant for the Oosterschelde estuary, because the completion of a coastal engineering project in 1987 resulted in less light attenuation, reduced nutrient loadings and increased residence time of the waterbody. As a consequence, nutrient availability now determine phytoplankton dynamics. Regeneration of Si and N by musselbeds might stimulate primary production, while filtration reduces algal biomass, resulting in an increased turnover of phytoplankton. So, in estimating the carrying capacity of an estuary for benthic suspension feeders these types of feedback mechanisms have to be taken into account.

DEVELOPMENT OF MUSSEL BIOMASS ON CULTURE PLOTS IN THE EASTERN SCHELDT (NETHERLANDS) AS A FUNCTION OF GROWTH, MORTALITY AND FISHERIES. M. R. Van Stralen,* R. Dijkema, J. Bol and **C. Brand,** Netherlands institute for fishery investigations, Field laboratory, P.O. Box 77, 4400 AB, Yerseke, Netherlands.

To protect the South-Western part of the Netherlands from flooding, in the mouth of the Eastern Scheldt estuary a storm surge barrier was completed in 1987. Due to this barrier and two additional dams, situated more inland, the tidal amplitude and current velocities have been reduced by 10% and 30% respectively. Studies have been undertaken into the consequences of these changes for the ecosystem and for the bottom culture of mussels. A dynamic simulation model has been developed to integrate the results of the different field studies and to serve as a predictive management tool.

In this model changes in mussel biomass are incorporated as a forcing function. As mussels are important as consumers of phytoplankton and for the regeneration of nutrients, the results of model runs depend strongly on the accuracy of the time series of mussel biomass used. Since the ecosystem model does not cover local processes on culture plots, data on mussel growth, mortality and the impact of seeding and harvesting on culture plots are studied in relation to local environmental and zootechnical conditions.

Factors causing mortality seem to be determining for the quantity of mussels produced, while market value depends on the growth and meat yield of the landed mussels. Due to mortality, the mussel biomass harvested is about equal and in 1989 even lower than the amount of halfgrown- and seed mussels seeded in spring. In the post-barrier situation, an increase of siltation on culture plots and changes in water exchange between tidal channels and the production areas increased mortality and reduced growth rates. These changes and their consequences for model calculations and for the prospects of mussel culture in the Eastern Scheldt are discussed in this paper.

FEEDING BY BIVALVES

OMNIVOROUS FEEDING BY *CRASSOSTREA VIRGINICA* LARVAE: CONSUMPTION OF NATURALLY OCCURRING PHYTOPLANKTON, PROTOZOA AND BACTERIA. **Brad S. Baldwin,* Roger I. E. Newell,** and **Tom W. Jones,** Horn Point Environmental Laboratories, University of Maryland, Box 775, Cambridge, MD 21613.

In the natural environment planktotrophic bivalve larvae must acquire adequate nutrition in order to support proper growth, development and metamorphosis to the benthic life stage. While previous studies have elucidated many aspects of larval feeding behavior and energetics, little is known concerning the natural diets of bivalve larvae. Here we describe the ability of veliger larvae of the American oyster, *Crassostrea virginica*, to consume both autotropic (14C-bicarbonate labeled) and heterotrophic (3H-thymidine labeled) food organisms found in natural plankton assemblages in Chesapeake Bay. We have found that large umbo stage larvae can ingest particles ranging from 0.2 to 30 μm, including both phytoplankton and heterotrophic bacteria and protozoa. Our results suggest that oyster larvae can utilize the diverse food types typical of many coastal systems.

SUSPENSION FEEDING IN BIVALVES: OVERVIEW OF A TURBID SUBJECT. **Peter G. Beninger,** Centre d'études et de recherches sur l'environnement et Département de biologie, Université de Moncton, Moncton, N.B., Canada, E1A 3E9.

Although the characteristics of suspension feeding have been extensively studied in bivalves, the underlying mechanisms are not yet known with certainty. The feeding phenomena are not easily observed directly without disturbing the phenomena themselves, whereas indirect studies are unable to provide information on the sites and processes involved. Radically different models have been proposed for particle capture, selection, and ingestion. Through a synthesis of the literature and the results of ongoing anatomical research, a paradigm is proposed to account for particle fate from the incurrent flow to the stomach. Several points in this paradigm require further research in order to maintain or eliminate alternative possibilities.

EFFECTS OF TOXIC DINOFLAGELLATES ON FEEDING AND MORTALITY IN JUVENILE BIVALVES: COMPARISON OF SIX COMMERCIALLY IMPORTANT SPECIES. **Michael P. Lesser,* Sandra E. Shumway, Janeen Barter,** and **Tina Paseno,** Bigelow Laboratory for Ocean Sciences, and Department of Marine Resources Research Laboratory, McKown Point, West Boothbay Harbor, ME., 04575.

Of the many environmental concerns facing aquaculturists, exposure to shellfish to blooms of toxic dinoflagellates can be a sudden and catastrophic economic loss. Although a number of studies have begun to elucidate the effects ot toxic dinoflagellates on adult shellfish, little has been done with the fast-growing juvenile phase. Many commercial species are dependent upon a grow-out phase where they are exposed to natural seston. Juvenile phase shellfish might be expected to exhibit different sensitivities and responses when exposed to toxic dinoflagellates during the grow-out phase.

We have begun an integrated study on the effects of toxic dinoflagellates on juvenile shellfish by examining rates of mortality, and effects on feeding in the following commercially important species; *Mytilus edulis, Argopecten irradians, Mercenaria mercenaria, Crossostrea virginica, Ostrea edulis,* and *Spisula solidissima*. Our initial experiments have shown that juveniles of the species listed above actively feed and have no mortalities when exposed to bloom concentrations of *Protogonyaulax tamarensis* in combination with natural seston. We present additional experimental data relevant to answering questions on the feeding and mortality of these species when exposed to bloom concentrations of *Protogonyaulax tamarensis*, and *Gyrodinium aureolum*.

EFFECTS OF FOOD QUALITY ON FEEDING BEHAVIOR OF THE BLUE MUSSEL, *MYTILUS EDULIS*. **Gregory A. Tracey,** Science Applications International Corporation, % EPA, Narragansett, RI 02882.

Recent studies of the blue mussel, *Mytilus edulis*, in nutrient-enriched mesocosms and in Long Island embayments suggest that feeding by this bivalve varies depending on both food quality and food availability. The observed behavior suggests a strategy in which ingestion is endogenously controlled to maintain, rather than maximize, phytoplankton consumption. The behavioral que upon which feeding rate appears to be based is the degree of "dilution" of available phytoplankton by non-phytoplankton particles. The extent to which this behavior exists in other species is not known, but may partly explain observations of reduced bivalve feeding on ambient seston during algal "bloom" conditions. However, this strategy was ineffective (i.e. growth was reduced) when food quality varied due to novel changes in seston composition, including 1) a non-algal diet of high particulate organic content and 2) the shift in phytoplankton composition to a noxious algal species. The implications of these results in regard to the evolution of the bivalve feeding strategy will be discussed.

FEEDING AND GROWTH OF *MERCENARIA MERCENARIA* SUBJECT TO WAVE-SUSPENDED BOTTOM SEDIMENTS. **Elizabeth J. Turner*** and **Douglas C. Miller,** College of Marine Studies, University of Delaware, Lewes, DE 19958 (302) 645-4284.

Many bivalve species such as *Mercenaria mercenaria* live in areas where sediment suspension by wind-generated waves is common. A series of experiments was carried out in an oscillatory water tunnel to simulate a summer storm event, and to quantify feeding behavior and growth before, during and after the simulated storm. Juvenile *M. mercenaria* were subjected to 55×10^6

cells C-ISO/liter in gentle wave conditions (peak oscillatory velocity = 7 cm/s, suspended sediment concentration <10 gm/l) and in high velocity conditions (>20 cm/s) with associated suspended sediment levels exceeding 190 mg/l at a height of 3 cm above the bed. Growth, measured by analysis of microgrowth lines in the shell, was found to decrease from 200 μm/day during calm conditions to 130 μm/day during simulated storm events. Observations of clams feeding under waves showed that pseudofecal production increased in frequency from 0.6 ejections/hr to 4.7 ejections/hr during storms, and that clams situated on ripple crests produced significantly more pseudofeces than those in ripple troughs. This indicates that, although more organic material is present in suspension during storms (as measured by AFDW), *Mercenaria* is expending energy to clear the gills, and a reduction in net growth occurs.

CHEMICAL MEDIATION OF THE FEEDING BEHAVIOR OF BIVALVES. J. Evan Ward,* Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF A1C 5S7; **Nancy M. Targett,** College of Marine Studies, University of Delaware, Lewes, DE 19958.

The feeding behavior of bivalves is a dynamic process, influenced by both chemical and physical changes in the environment. Bivalves are sensitive to a variety of inorganic and organic substances dissolved in sea water, including naturally produced compounds from microalgae. We have shown that dissolved and adsorbed microalgal ectocrines can influence the filtration and selection of particles by the blue mussel *Mytilus edulis*. Response of mussels to these ectocrines depends upon microalgal species, and concentration of metabolite used. In addition, preliminary results using the hard clam *Mercenaria mercenaria* indicate that interspecific differences in response to ectocrines from the same microalgal species are possible.

Extraction techniques have been developed by us to remove and isolate active microalgal ectocrines from culture filtrates. Inhibitory ectocrines from one species of microalga, *Heterosigma akashiwo*, are concentrated in a non-polar fraction, and are active at concentrations comparable to those in laboratory cultures. Using these techniques, we are currently identifying specific natural chemical signals that cue bivalve feeding behavior.

Based on this research, we suggest that: (1) bivalves rely on microalgal ectocrines to gather information about the complex mixture of particles in the seston, and (2) ectocrines on the surface of, or in a boundary layer surrounding microalgal cells are more important in mediating bivalve feeding than dissolved ectocrines.

OYSTERS

ONE HUNDRED YEARS LATER: AN INTERTIDAL OYSTER RESOURCE COMPARISON. William D. Anderson* and **Willis J. Keith,** South Carolina Marine Resources Center, Charleston, S.C. 29412.

Beginning in late December 1890, the United States Fish Commission steamer *Fish Hawk* completed a three month resource assessment of *Crassostrea virginica* beds and bottom areas suitable for oyster cultivation in South Carolina. Approximately 775 acres of natural oysters were located and delineated on U.S. Coast and Geodetic Survey charts. Survey results also suggested that oyster productivity could be greatly enhanced by constructing tidal ponds similar to European culture methods.

A cartographic comparison of the *Fish Hawk*'s 1890/91 survey is made with the State's recently completed intertidal oyster resource asesssment. Analyses of natural resource changes are demonstrated with a geographic information system. Finally, a characterization of the industry in 1890 is contrasted to its current status.

REVITALIZING A NORTHERN GULF FISHERY: DETERMINATION OF THE COST VERSUS BENEFITS FOR RELAYING OYSTERS. David D. Burrage* and **Benedict D. Posadas,** Sea Grant Advisory Service, 2710 Beach Boulevard, Suite 1-E, Biloxi, MS 39531.

The Northern Gulf Coast oyster industry has experienced severe declines in production over the past two decades. Loss of oyster grounds to siltation, extreme salinity fluctuations, and sanitary closures have reduced oyster landings.

Relaying oysters from restricted to approved waters may aid this fishery. Calculating the costs and benefits of relaying oysters will allow an economic assessment of such efforts. We collected economic and technical data on dredging, transport and planting of oyster seed, and on harvesting oysters for a pilot relay program. Over 5,300 bbl (1 MS bbl = 3 sacks; 1 bbl = 0.17 m^3) of oysters were relayed during a 12-week period (May–August, 1989). Subsequent monthly sampling of the relayed oysters suggest a 30% or better recovery rate.

The average cost of dredging and transporting the oyster seed from the closed oyster grounds to the relaying site was approximately $6.26/bbl planted. Contracted planting boats added around $1.49/bbl of oyster seed planted. The total cost incurred in relaying oysters amounted to $7.75/bbl planted or $25.81/bbl harvested (30% yield). The cost of harvesting, yields and dockside prices of planted oysters will be determined after the opening of the Mississippi season in late November, 1989. The price during the 1989–90 season is expected to be $22–$26/sack (0.06 m^3) or $66–$78/bbl.

METABOLISM OF SATURATED AND UNSATURATED FATTY ACIDS IN ADULT OYSTERS (*CRASSOSTREA VIRGINICA*). Fu-Lin E. Chu, Virginia Institute of Marine Science, School of Marine Science, The College of William and Mary, Cloucester Point, VA 23062.

This study investigated the incorporation and metabolism of

^{14}C-labeled saturated and unsaturated fatty acids in adult eastern oysters (*Crassostrea virginica*) and the influence of temperature on these processes. The incorporation of injected palmitic (16:0) and linolenic (18:3ω3) acids was higher in cold- (3–7°C) than warm- (22–25°C) acclimated oysters; linoleic acid (18:2ω6), incorporation was equivalent in both cold- and warm-acclimated oysters. Temperature influenced the relative distribution of radiolabeled fatty acids in the neutral and polar lipid fractions. In both cold- and warm-acclimated oysters the proportion of all three fatty acids in the neutral lipids was always equal to or greater than that in the polar lipids, but as acclimated temperatrue increased, the level of labeled fatty acids in polar fraction increased. In oysters which were previously acclimated in cold water for 2 months, ^{14}C-labeled 18:2ω6 and 18:3ω3 were primarily in polar fraction. Oxidation of incorporated fatty acids was much higher in warm- than cold-acclimated oysters. Radiolabeled 16:0 and 18:0 were detected in lipid extracts from oysters administered with radiolabeled 18:2ω6 or 18:3ω3 indicating de novo synthesis of these saturated acids. Elongation of 18:2ω6 was more extensive than that of 18:3ω3 and 16:0, but elongation activities were low. Desaturation of 18:2ω6 was found only in one of the warm-acclimated oysters; no desaturation of 16:0 or 18:3ω3 was observed in either cold- or warm-acclimated oysters.

USE OF A MARK-RECAPTURE TECHNIQUE TO ASSESS CRAB-ATTRIBUTABLE MORTALITY RATES OF SUBTIDAL JUVENILE OYSTERS, *CRASSOSTREA VIRGINIA*. **David B. Eggleston,** School of Marine Science, The College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

A field mark-recapture study assessed the relative effects of season, oyster shell-height, and oyster density on total and crab-attributable mortality rates of juvenile oysters (10–45 mm shell height; SH) within two subtidal oyster reefs in the lower Chesapeake Bay. Predation by crabs was distinguished from other mortality sources by the presence of chipped or cracked valve margins, puncture holes within the umbo region, crushing of the umbo region, and complete crushing of the valves. Field results were compared with previous laboratory studies of mud crabs and blue crabs feeding upon juvenile oysters as a function of temperature, oyster shell-height, and oyster density.

Comparison of two methods for predicting cumulative mortality rates from crabs and unknown sources suggest that this mark-recapture technique accurately assessed temporal survivorship of juvenile oysters. Field and laboratory predation rates were positively correlated with oyster and density and temperature, with 15°C being the critical temperature below which predation rates were significantly depressed. Juvenile oysters achieved a size refuge from crab predation at ca. 29 mm SH in the laboratory and field. These results suggest that an outplanting strategy of oysters >29 mm SH (attached to oyster-shell cultch) during the fall, when water temperatures reach ca. 15°C, would minimize losses due to crab predation.

SUITABILITY OF FLY ASH-CEMENT AGGREGATE FOR OYSTER CULTCH. **Jurij Homziak*** and **Patricia Simm,** Coastal Research and Extension Center, Mississippi State University, Biloxi, MS 39531; **Lloyd W. Bennett,** Mississippi State University School of Veterinary Medicine, Mississippi State, MS 39762; **Ron Herring,** Mississippi Power Company, Gulfport, MS 39507.

Pellets of fly ash-cement aggregate (7½% cement) may be an alternative oyster cultch. We evaluated pellet stability, potential for leaching and contamination of oysters by heavy metals from the ash. With a mean compressive strength of 2.10×10^5 kg/m^2 and no significant differences in size frequency distributions of pellets compared before and after 30 d in immersion in seawater, the aggregate appears stable in the marine environment.

Aquarium leachate studies over 30 days detected order of magnitude greater concentrations of chromium ($\bar{x}$ = 59.9 – 1348.0 ppb) and significantly higher levels of selenium ($\bar{x}$ = 1.3 – 7.6 ppb) in ash aggregate aquaria compared to water only or clam shell controls. Selenium levels did not exceed public water supply standards. Analysis to determine proportions of tri- and hexavalent chromium is underway. Manganese concentrations in treatment and clamshell control aquaria were occasionally significantly greater than in water only controls. Analysis of metal concentrations in oyster tissues is in progress.

ANALYSIS OF THE GULF REGION OYSTER FISHERY. **Walter R. Keithly,*** Center for Wetland Resources, Louisiana State University, Baton Rouge, LA 70803; **Ronald J. Dugas,** The Louisiana Department of Wildlife and Fisheries, 400 Royal St., New Orleans, LA 70130.

Significant changes have occurred in the U.S. oyster industry during the past three decades. Production, for example, has declined significantly, especially in recent years. Processing activities, with the demise of the canning industry, are radically different than those observed in previous decades. Imports, once a relatively small component of total U.S. oyster supply, now dominate it. Finally, the regional shares of U.S. oyster production, in terms of both poundage and value, have been altered significantly in recent years. These changing shares represent changes in both regional production and prices.

The purpose of this paper is to outline some of the changes noted above while concentrating on the Gulf Region. Specifically, the paper addresses: (a) historical changes in U.S. oyster production, expressed in terms of poundage and value, and relative changes in comparable figures at the Gulf Region level, (b) changes in processing activities at the national and Gulf Region level, and (c) the rate and composition of oyster imports by country of origin, and potential impacts from these imports.

Where feasible, analysis of the aforementioned issues is provided at the state level in the Gulf Region.

Changes occurring in the oyster industry are wide-spread and the appropriate management agencies must react to them accordingly in setting management objectives. The purpose of this paper is to provide information to assist management agencies in this role, especially at the Gulf Region level.

AN ECONOMIC ANALYSIS OF RETURNED OYSTER LEASES: THE LOUISIANA EXPERIENCE. Walter R. Keithly* and **Kenneth J. Roberts,** Center for Wetland Resources, Louisiana State University, Baton Rouge, LA 70803; **Ronald J. Dugas,** The Louisiana Department of Wildlife and Fisheries, 400 Royal St., New Orleans, LA 70130.

Recent events in the domestic oyster industry have placed Louisiana in the forefront of the nation's oyster production. Chesapeake production problems coupled with increased leased acreage in Louisiana, which provides the basis for some 70% to 80% of its annual harvest, has altered historical oyster production activities. Almost a third of the national oyster production during the 1986–88 period was Louisiana based compared to less than a quarter during 1981–85 and less than 20% during 1976–80.

The increasing relevance of Louisiana's oyster industry coincides with one of uncertainty. Acreage leased by the state, though increasing, is also being returned to the state in record proportions. Such a situation suggests that all acreage is not equally productive and that economic returns from some acreage do not cover the annual leasing fee of two-dollars per acre.

Leases returned to the state since the mid 1970's due to non-renewal or failure to pay the appropriate leasing fees, have been made available to the public via a public, voice based, bidding procedure. Returned leases were evaluated to determine: (a) association with particular water bodies, (b) history of renewal and transfers, and (c) acreage of leases, perhaps indicating inefficient sizes. Results of this evaluation are presented in the paper. Also, the paper provides a discussion of the bidding process and outcome including: (a) auction price per lease, (b) price per acre, (c) those receiving bids, and (d) whether or not the successful bidder owns other leases.

THE EFFECTS OF FEED WATER FLOW RATE ON THE GROWTH OF AQUACULTURED *CRASSOSTREA VIRGINICA* IN HAWAII. Chee-Yin Lam and **Jaw-Kai Wang,*** Agricultural Engineering Department, University of Hawaii, Honolulu, Hawaii 96822.

Selected nursed oysters (*Crassostrea virginica*) with an initial average weight of 3.59 gram were grown in vertical suspension in a tank which was divided into three chambers. The oysters were divided into three groups, glued onto strips of polyvinyl chloride and suspended in the three chambers. They were fed commercial shrimp pond effluent at the following flow rates: 4.00 (high), 1.33 (medium), and 0.45 (low) liters/day/gram. After 209 days, the final average weights of the oysters were 66.43, 53.67, and 40.65 grams. The average recorded dissolved oxygen concentrations of the effluent of the three chambers were 7.0, 6.0, and 5.2 ppm, respectively. The survival rates were 90.56, 73.33, and 52.12 percent and the percentages of oysters 55 grams or larger on the harvesting day were 91.67, 76.69, and 8.33 percent for the high, medium, and low feed water flow rates. Up to an average weight of about 20 grams, there was no significant difference between the growth rates of the oysters receiving 4.00 liters/day/gram and those receiving 1.33 liters/day/gram. All of the oysters grew rapidly during a period of increased temperature and decreased salinity towards the end of the grow-out period.

THE ADVANTAGES OF MAKING A SOCIAL ASSESSMENT OF A SHELLFISH COMMUNITY BEFORE DEVELOPING A SHELLFISH CULTURE PROGRAM. Clyde L. MacKenzie, Jr., Sandy Hook Marine Laboratory, Northeast Fisheries Center, Highlands, NJ 07732.

Recent advances in hatchery culture of oysters and hard clams have increased production of these shellfish in small areas, mostly by private companies. However, shellfish production remains sluggish in most areas of the U.S. east coast. A reason for slow development may be that projects proposed for developing shellfish culture may not suit many communities, especially those supported by public fisheries. Social assessment of communities are needed to determine the types of projects best suited for them.

Social assessments include interviewing local fisherman, lay people and politicians to identify community needs and the cultural setting. People typically uninvolved become involved, impacts ignored become identified and assessed, involvement becomes more focused and issue centered and community support for projects is general. Assessments are also needed of shellfish beds and resources. Examples of successes and failures from Prince Edward Island, Long Island Sound and Martha's Vineyard will be given.

PRODUCTION COSTS ASSOCIATED WITH PLAYING OYSTER ROULETTE IN BARATARIA BAY, LOUISIANA: A SEED BEDDING AND HARVESTING ENDEAVOR. Earl J. Melancon,* Department of Biological Sciences, Nicholls State University, Thibodaux, Louisiana 70310; **Richard Condrey,** Coastal Fisheries Institute, Center for Wetland Resources, Louisiana State University, Baton Rouge, LA 70803.

We documented production costs for 17 bedding leases in lower Barataria Bay using eight different captains and their vessels. Production costs were influenced by the distance the fishermen had to travel to obtain their seed oysters for bedding, the distance of the beds from the selling dock, the influence of Bay temperature and salinity on oyster predation and sack yield per

lease, and the number of sacks per day the purchasing sheds could handle.

Average daily expenses were 16 percent higher while bedding than while harvesting for sale. This difference was due to the fuel consumed by the vessels while bedding seed and to higher galley expenses. Total variable expenses (bedding + harvesting) were separated into each expense category's contribution: labor for two deckhands (60%), general maintenance of vessel and engine (17%), vessel fuel, oil and grease (13%), galley supplies (9%), and butane and ice (1%). Fixed costs increased expenses by 35% and included vessel depreciation, state fees and insurance.

The costs of harvesting a sack of oysters from a bedding reef are dependent on lease yield and the average daily expenses to operate. Accordingly, as yield increased the costs of harvesting a sack decreased. For example, a lease yield ratio of 1.1:1 (number of sacks harvested:number of sacks bedded) will reduce the average costs to harvest a sack of oysters by 83% when compared to a lease yield ratio of 0.1:1.

Seed supplies on the state reefs have been declining steadily and some fishermen have explored other avenues for seed, including hatcheries and planting clam shell on private reefs as cultch. The information we have obtained will help fishermen and state managers in comparing costs associated with alternative sources of seed.

ESTIMATION OF SURFACE AREA OF SHELLS OF THE OYSTER *CRASSOSTREA VIRGINICA* USING ALUMINUM FOIL MOLDS OF THE SHELL SURFACE. Reinaldo Morales-Alamo, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Surface area of oyster shells was estimated from an aluminum foil mold of the inner and outer surfaces. This estimate was similar to another estimate believed to approximate very closely the true surface area of the shell; the latter estimate was obtained from the integrated sum of the surface area of individual segments of equal width (usually 1 cm) from the same shell using the Trapezoidal Rule. Correlation analysis of these two estimates resulted in r^2 values between 0.984 and 0.997 for the inner and outer surfaces of left and right valves. The 95% confidence interval for regression lines of foil-method area on segment-sum integration area at values of the latter between 40 and 60 cm^2 were between 5 and 12% of the mean value on the regression line. The area within an outline drawing of the shell margin was regressed on the foil-method area estimate and the resulting regression line values evaluated as an alternate method for rapid estimation of the average surface area of each shell in a sample from the same population. 95% confidence intervals around the areas predicted from the shell outline area were small enough to permit use of this rapid method in comparisons of numbers of spat per unit area between shell samples from different locations or time periods.

ALTERNATIVE CULTCH MATERIALS: PHYSIOLOGY AND GROWTH OF *CRASSOSTREA VIRGINICA* GROWN ON STABILIZED COAL ASH. Karolyn M. Mueller, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Coal ash, stabilized with cement, has been proposed as an alternative cultch material for finfish and shellfish reefs. One potential problem is that coal ash contains significant quantities of trace and toxic metals. Stabilized coal ash has been shown to be an acceptable settlement substratum for oyster larvae, but effects on physiology and growth have not been determined. This study was designed to assess the growth of oysters, *Crassostrea virginica*, on stabilized coal ash as compared to growth on oyster shell cultch. Oysters grown for six months on coal ash and shell cultch were harvested and analyzed for general and biochemical condition indices, and for determination of trace and toxic metal concentrations in tissues. Ash-grown oysters were significantly larger than shell-grown oysters, yet all oysters were of the same condition. Ash-grown oysters also had a significantly greater amount of inorganic material per unit surface area than did shell-grown oysters; both groups had similar amounts of organic material per unit surface area. Protein and carbohydrate concentrations were not significantly different for ash-grown vs. shell-grown oyster; lipid concentrations were variable within and between groups and no size or weight relationship could be determined. Of the six metals determined, iron was the only element that differed significantly between ash-grown and shell-grown oysters and was greater in shell-grown oysters. All six element concentrations were within the reported ranges for oyster tissue. These results indicate that while there are growth differences between ash-grown and shell-grown oysters, the ash-grown oysters grow at least as well as oysters grown on shell.

THE EFFECT OF BIOSYNTHETIC TROUT GROWTH HORMONE ON OYSTER GROWTH. Kennedy T. Paynter,* Chesapeake Bay Institute, Johns Hopkins University, Shady Side, MD; **Y. L. Tang** and **T. T. Chen,** Center of Marine Biotechnology, University of Maryland, Baltimore, MD.

We are studying the growth promoting effect of rainbow trout biosynthetic growth hormone (GH) on oysters. The complementary DNA (cDNA) of trout GH was cloned and introduced into *E. coli* for large scale production of the polypeptide. GH inclusion bodies were isolated from *E. coli* cells, dissolved in a 5 M guanidine hydrochloride solution, and the denatured GH polypeptide was renatured by dialysis against 50 mM ammonium bicarbonate buffer (pH 10.0). Groups of juvenile oysters (8 mm avg shell height) were incubated in various concentrations (10^{-7} M, 10^{-8} M, 10^{-9} M and untreated control) of GH for 5 hrs once per week at room temperature. After incubation in the hormone the animals were returned to a single upwelling tray divided into quadrants in the wet lab where they were maintained until the next treatment. Shell height was recorded each week. After three weeks, the

groups which received 10^{-7} M and 10^{-8} M GH treatments were significantly longer (shell height) than the control or 10^{-9} M GH treatment group ($P < 0.05$). After five weeks the animals were sacrificed and shell height, total weight, shell weight, wet tissue weight, dry tissue weight, and condition index were measured. Significant differences ($P < 0.05$) were observed between the two highest treatment groups and the control group in shell height, shell weight, dry tissue weight, and condition index. These results complement the findings of others who have shown growth promoting effects of vertebrate growth hormone on other molluscs. The impact of these results on our understanding of bivalve growth will be addressed, and the promise of biosynthetic growth hormone for shellfish aquaculture will be discussed.

ENHANCING LOUISIANA'S OYSTER RESOURCES THROUGH SHELL PLANTING. William S. Perret,* Ronald J. Dugas, and **Mark F. Chatry,** Louisiana Department of Wildlife and Fisheries, 400 Royal Street, New Orleans, LA 70130.

In recent years, Louisiana's oyster production has averaged over 12 million pounds of oyster meat annually with a dockside value exceeding $25 million dollars. The state's oyster producing area is divided into public seed grounds and private bedding grounds. The basic organization of the industry is for the state to supply the seed on the public grounds, and for the private lease holders to transfer the seed to their leases for growth to market size.

Since 1926 the state has planted over 1 million yards^3 of cultch material. While reef oyster shell and steam plant shell have been used, the preferred cultch material since the mid-1960's has been clamshell (*Rangia cuneata*).

Sites for these shell plants are selected by studying bottom conditions and sediment types, turbidity, current patterns, salinity, water temperature, and historical catch from the area. Additionally, oyster fishermen have aided greatly by providing background information on the areas, and actually assisted in selecting the final sites.

It has been found that for every boat load of seed oysters, one to three inches in height, taken from Louisiana shell plants and bedded in September of one year, will yield two to four boat loads of marketable oysters, three to five inches in height, by April of the next year. Thus, it is obvious that the planting of clam shell on the public seed grounds is a management tool that is vital and cost-effective to the oyster industry.

DATING OYSTER SHELL AGE BY THE RATE OF DECOMPOSITION OF THE ORGANIC MATRIX. E. Powell,* Department of Oceanography, Texas A&M University, College Station, TX 77843; **J. King,** Jefferson Patterson Park and Museum, St. Leonard, MD 20685.

Dating archaeological sites may require dating time-since-death of molluscan shells. Calibration curves relating protein matrix decomposition to shell age were developed using oyster shells from documented historic and C^{14}-dated prehistoric shell middens located in the Patuxent River area of Chesapeake Bay. A procedure for estimating time-since-death over at least the last 1500 yr is described using protein-bound and free aspartate, serine, glutamate, alanine and glycine. Loss of shell protein could consistently be modeled by the sum of two first-order reactions. The need for two first-order rate constants suggests that a portion of the protein pool breaks down much more rapidly than the remainder. Most of the glycine, alanine and serine is in that pool. Appearance of free amino acid (FAA) could be adequately modeled in all 5 cases as the sum of 4 first-order reactions, two formative reactions using the previous rate constants and two loss terms. Much less FAA was present than produced by matrix breakdown, indicating significant FAA decomposition or diffusional loss. Diffusional loss is the most likely process. The compartmentation found for the protein-bound amino acids coincided with that found for the FAA pool, indicating a physical separation of the two compartments. Compartmentation may result from the positional and chemical differences between the soluble and insoluble matrix which produce one pool characterized by rapid protein breakdown and diffusional loss of FAA and another characterized by slow protein breakdown and slow diffusional loss.

SURVIVAL, CONDITION, AND GLYCOGEN AND SUCCINATE LEVELS IN OYSTERS, *CRASSOSTREA GIGAS*, DURING AND AFTER PROLONGED AIR STORAGE. Matthias N. L. Seaman, Institut für Meereskunde, Düsternbrooker Weg 20, 2300 Kiel, F.R.G.

Pacific oysters, *Crassostrea gigas*, with average weights of 11 g and 72 g were held in PVC boxes at temperatures of 0°C and 7°C for 20 weeks, from Nov. 1988 to March 1989. In each case, some oysters were held in air only, and others were sprinkled with water. The survivors were reimmersed in the sea after overwintering, and monitored until Sept. 1989.

Of the oysters sprinkled at 7°C, 80% survived in the 11 g and 52% survived in the 72 g group, and mortality after reimmersion was negligible. In the oysters sprinkled at 0°C, overwintering survival was 8% and 27%, respectively, but mortality was total within one week after reimmersion. The oysters held without irrigation suffered total mortality by the end of the storage period.

Succinate levels show that the oysters held without irrigation switched to anaerobic metabolism; oysters sprinkled with water metabolized anaerobically during the first few weeks of storage, but later reverted to aerobic metabolism. Condition index and glycogen levels in the experimental oysters were generally lower than in controls overwintered in the Baltic Sea, even after six months of reimmersion; however, the differenees between individual oysters within the groups were much higher.

Overall survival was lower than expected, and may partly be attributable to the experimental set-up. The fair survival rates in

two of the groups and their performance after reimmersion, however show that air storage is possible for several months if adequate conditions are provided, which confirms results on *C. virginica*.

GENERAL BIOLOGY

INFLUENCE OF A FISH PEN ON THE LOCAL LOBSTER HARVEST IN THE WESKEAG RIVER, MAINE. Robert C. Bayer,* George W. Kupelian, Deanna L. Prince and **Cheryl D. Waltz,** Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469; **Ralph Hamill,** Weskeag Fisheries, South Thomaston, Maine 04858.

This study was conducted to determine the effects of the presence of a small fish farm on the local lobster fishery. Lobstermen had reported an increase in catch near the pen the first year the pen was in place. This study measured the number of lobsters caught in two zones; the inside zone marked by buoys 100 yards from the pen, and the outside zone the next 100 yards away from the pen. There were approximately 3,000 rainbow trout in the pen fed twice daily. Fishermen recorded their catch from the two zones on a data sheet. There were 644 trap hauls during the study. Traps from the inside zone produced an average of 0.46 (SE = 0.06) legal size (≥83 mm carapace length) lobsters per trap haul compared with 0.36 (SE = 0.05) legal size lobsters per trap haul in the outside zone. A paired t-test indicated this was significant ($P < 0.05$). There was no significant difference in numbers of short lobsters (<83 mm carapace length) trapped in each zone ($P < 0.05$).

Divers made monthly observations and video recordings of the bottom under and around the pen. Examination of the video recordings revealed that lobsters turned featureless mud bottom into habitat. The lobsters initially made open craters which they further excavated into deep burrows. The lobsters were apparently attracted to the pen area, perhaps by chemosensory stimuli from solubles coming from feces or uneaten feed, or by the shade afforded by the cage.

TRANSFERRING OYSTER HATCHERY TECHNOLOGY: CAN THE EAST COAST LEARN FROM THE WEST COAST? Richard E. Bohn, Cooperative Extension Service, University of Maryland, Leonardtown, MD 20650.

Oyster hatcheries, and the aquaculture systems which have resulted from their success, dominate the industry on the West Coast and are reaching national markets with their products. A combination of aggressive research and development, a compliant regulatory atmosphere, and the biological necessities of a non-native oyster have resulted in a flourishing and stable oyster growing industry from Alaska to California. Other areas of the country find their powerful commercial fisheries, faltering natural resources, and strict regulatory approaches hindering the development of a similar growth. Valuable lessons which led to the success of the West Coast industry, and their application to other parts of the country, are presented. Variations in the biology of the Pacific oyster (*Crassostrea gigas*) and the American oyster (*Crassostrea virginica*), and their implications in culture methods are also discussed.

EFFECTS OF HYPOXIA ON RESPIRATION IN BLUE CRABS, *CALLINECTES SAPIDUS*. P. L. Defur,* Environmental Defense Fund, Richmond, VA 23219; **C. P. Mangum,** College of William and Mary, Williamsburg, VA 23185.

This research was part of the effort to understand and manage hypoxic and anoxic events in the Chesapeake Bay and tributaries. Blue crabs were exposed to low oxygen (30% saturation = 50 mmHg) in the laboratory (25°C; 15‰) for 5–25 da. to assess short term and long term responses. Initially, there were increases in blood flow and water flow over the gills, lasting 3–4 days. By 7 da., there was evidence that these responses had subsided, metabolism had been adjusted, and a change in the oxygen carrying protein, hemocyanin, had been initiated. By 23–25 da. hypoxia, three dramatic changes had taken place. The first was an alteration of the structure of the hemocyanin, involving a change in the ratio of subunits. The second was an increase in levels of blood Ca^{++}, urate and lactate; all improve the function of hemocyanin in hypoxia. The third was a loss of mitochondria from the muscle cells in the swimming appendage. Blue crabs seem capable of adjustments permitting survival of long periods of hypoxia, but these require at least 7 days. The change in hemocyanin may not be rapidly reversible upon return to normoxia, prolonging the period during which the crabs are affected by hypoxia. Supported by grants from Virginia Sea Grant (to PLD) and NSF (to CPM).

USE OF A COMPUTERIZED MONITORING AND CONTROL SYSTEM IN A SHELLFISH HATCHERY/NURSERY. Paul R. Hadley, The Hadley Company, 1214 Grimsley Drive, Charleston, SC 29412.

A computerized monitoring and control system was designed and installed in a shellfish hatchery/nursery adjacent to Charleston Harbor, South Carolina. The system utilized a personal computer and a programmable logic controller to read multiple probes and turn on/off relays. A variety of probes were tested for monitoring various parameters, including water temperature, air temperature, water pressure, salinity, dissolved oxygen, pH and fluorescence. The system was used continuously for 9 months to monitor water temperature in 5 locations, air temperature in 2 locations, salinity, and pH. The system was also used to control water temperature in two tanks by activating a heat exchanger. Conditions falling outside modifiable set-points generated visual and/or audible alarms in the hatchery and a voice-synthesizing telephone dialer reported problems during non-working hours. The system generated histor-

ical printouts of all data which allowed determination of trends and timing of problems which occurred when operators were not on site. The system saved data on diskette at 30 minute intervals. This data could be retrieved into databases or spreadsheets for manipulation. Data was used to generate reports of daily temperature and salinity for dissemination to about 30 interested parties in state and federal agencies.

AERATION OF LOBSTER POUNDS. Daniel S. Hagopian and **John G. Riley,*** Bio-Resource Engineering Department, University of Maine, Orono, ME 04469.

In Maine, New Brunswick, and Nova Scotia, the American lobster (*Homarus Americanus*) is over wintered in dammed-off tidally flushed embayments called lobster pounds. The lobsters are fed to increase their weight and held until the market price rises in the spring. In late summer and early fall when sea water temperature are at their highest, low dissolved oxygen (d.o.) levels occur in the pounds, particularly at slack tide and can result in sometimes catastrophic losses. This study was conducted to understand the dissolved oxygen cycle in a lobster pound and to identify the opportunity for the application of mechanical aeration techniques to lobster pounds. Tests, conducted in the fall of 1988, in a fully stocked pound in Stonington, Maine showed that the dissolved oxygen level in the bottom waters follows a cycle which is set by the tide. It is highest at high tide and lowest just before the pound is flooded by the incoming tide. Other factors which strongly influence the need for aeration are the water temperature, the height of the tide, lobster respiration, and wind speed. The results also show clearly that supplemental aeration is necessary from September through the beginning of December in order to avoid stressfully low d.o. levels. At first, continuous aeration is required, while later in the holding season aeration is only needed for a few hours before the minimum d.o. level is reached. Just over 3 horsepower per acre are required at peak demand. Tests of different mechanical aerators reveal that several small electrically powered surface and sub-surface aerators are capable of delivering a uniformly high d.o. level to the bottom water of the lobster pound. Bottom aeration systems are not appropriate for this application. The benefit of mechanical aeration (in terms of decreased mortality) more than offset the capital and operating costs. There is also the potential for increased profits through earlier stocking and increased stocking density.

THE USE OF SALT DIPS TO REDUCE COLIFORM LEVELS IN SOFT SHELL CLAMS. W. Pete Jensen,* Eric B. May and **Keith L. Lockwood,** Maryland Department of Natural Resources, Tidewater Administration, Fisheries Division, Tawes State Office Building, Annapolis, MD 21401.

Soft shell clams represent a significant fisheries in the Chesapeake Bay. As with many estuarine systems, the Chesapeake Bay is subject to on-shore development placing a burden on adjacent waters and sediments such as the introduction of bacteria from agriculture or urban runoffs. Such on-shore activity can increase coliform levels in sediments and the resident clams. During the summer months coliform levels become elevated to levels that exceed Interstate standards. Beginning in 1989, Maryland required the use of ice or refrigeration to maintain coliforms at levels present prior to harvesting. Such methods are seen as cumbersome and expensive.

Preliminary studies by the Fisheries Division have shown that salt dips of 5%, 10%, and 15% sodium chloride for 10 or 20 minutes will reduce fecal coliform levels. These results are consistent with the existing literature on growth requirements for coliforms. It is clear from the results that salt dips effectively reduce coliforms of concern to human health.

The advantage of this practice would be to provide an alternative to refrigeration or icing. The results, potential applications, and methods of integrating with the existing procedures for harvesting will be discussed in this paper.

ENVIRONMENTAL ASSESSMENT OF OYSTER SHELL DREDGING IN THE UPPER CHESAPEAKE BAY. Christopher C. Judy, Maryland Department of Natural Resources, Tawes State Office Building, Annapolis, MD 21401.

The Maryland Department of Natural Resources conducts an annual oyster repletion program which depends on the planting of oyster shell cultch to provide habitat for oyster settlement. The shells are obtained by hydraulically dredging large, buried shell deposits at sites in the upper Chesapeake Bay. Oyster shell dredging has been conducted since 1960. In 1986 an environmental assessment of the effects of oyster shell dredging was initiated. It investigated changes in bottom topography, water quality, benthic community structure, and fish usage of the dredging areas. Dredged areas were compared to undredged areas.

INGESTION AND DIGESTION OF OYSTER (*CRASSOSTREA VIRGINICA*) LARVAE BY GELATINOUS ZOOPLANKTON. Victor S. Kennedy* and **Jennifer Purcell,** Horn Point Environmental Laboratories, University of Maryland, Cambridge, MD 21613; **David Cargo,** Chesapeake Biological Laboratory, University of Maryland, Solomons, MD 20688.

We studied ingestion and digestion of oyster larvae by the ctenophore *Mnemiopsis leidyi*, and three life history stages of the sea nettle *Chrysaora quinquecirrha*. The ctenophore ingested and digested trochophores and nearly all veligers offered, rejecting few. Digestion was rapid. Sea nettle ephyrae and medusae ingested and digested trochophores, but rejected most veligers. Medusae ingested and digested that shucked meat of newly settled spat, suggesting that the presence of the shell led them to reject veligers. The benthic scyphistomae stage of the sea nettle ingested veligers, but digestion was relatively slow and took up to 24 h to be completed.

Sea nettle medusae prey on ctenophores in summer in Chesapeake Bay, lowering their numbers gently. Thus, although the pelagic stage of the sea nettle may prey on trochophores, it protects veligers from ctenophore predation. The benthic stage of the sea nettle is an oyster predator, but its small size and slow digestion may make it of minor significance.

EVALUATION OF *SALMONELLA TYPHIMURIUM* WG49 HOST ASSAY METHOD FOR ENUMERATION OF MALE-SPECIFIC COLIPHAGES IN AN ESTUARINE ENVIRONMENT. Martha W. Rhodes and **Howard Kator,*** School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

An assay method (Havelaar, A. H. and W. M. Hogeboom, J. Appl. Bacteriol. 1984, 56:439–447) for enumeration of male-specific coliphages in sewage was evaluated in a Virginia subestuary subject to nonpoint pollution sources including fecal inputs from livestock. Phages were enumerated using the bacterial host *S. typhimurium* WG49 modified to produce *Escherichia coli* sex pili. WG49 has been reported to detect male-specific ribonucleic acid (FRNA) coliphages in sewage with little interference from somatic salmonella phages.

FRNA phages and fecal coliforms were enumerated from water and sediment samples collected seasonally from the estuary and feeder streams and microbiological densities related to selected environmental parameters. Mean phage densities on WG49 ranged from <1 to 5×10^{-1} 100 ml^{-1} water and $<10^2$ to 7×10^3 100 g^{-1} dry sediment. Examination of 300 purified phage isolates showed 99% were RNAase resistant, 97% were lytic on the female parent salmonella strain (WG45), 4% were lytic on male *E. coli* and none were lytic on female *E. coli*. Parallel enumeration of samples on WG45 and WG49 yielded equal or greater phage densities on the former host. Selected phages were also lytic for field isolates from four of 10 salmonella serovars but nonlytic for fecal coliform isolates. The significance of somatic phage recovery by the *S. typhimurium* WG49 host in an estuary lacking a point source of sewage is discussed.

PROTOZOANS, FUNGI, AND BACTERIA AS INDICATORS OF COASTAL CONTAMINATION RELATED TO OCEAN WASTE DISPOSAL PRACTICES. Thomas K. Sawyer,* Rescon Associates, Inc., Royal Oak, MD 21662; **Elmer E. Davis,** American Type Culture Collection, Rockville, MD 20852.

Microorganisms of terrestrial origin are excellent indicators of water and sediment contamination in marine ecosystems. Well-known species of viruses, bacteria, and protozoans have been recovered and identified from ocean waste disposal sites, sewage outfalls, and shellfish producing waters, and serve as useful indicators of the progressive deterioration of aquatic ecosystems, or the recovery of sites where environmental quality has improved. EPA Region III has carried out studies on sediment quality near sewage outfalls at Bethany Beach, DE, Ocean City, MD, and Virginia Beach, VA, using enteric bacteria and freshwater or soil protozoans (Amoebida: Acanthamoebidae) as indicators for the spread of sewage discharges away from the outfall pipes. In 1989, efforts were made to culture and identify several fungi that had appeared routinely on culture plates in previous years. Preliminary studies have shown that an acellular slime mold, *Physarum gyrosum*, and three genera of other fungi, *Fusarium* sp., *Alternaria*, and *Cladosporium*, were present in one of the sediment samples taken near the Ocean City outfall. Our investigation suggests that further studies on fungi may provide useful information on the dispersal and/or persistence of microbial contaminants in coastal and offshore marine sediments.

FISH-OYSTER POLYCULTURE IN WARM WATER MARINE PONDS. M. Shpigel,* Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel; **J. J. Lee** and **B. Soohoo,** City College, CUNY, New York, NY.

An integrated fish-oyster polyculture system was examined at IOLR, Eilat, Israel. Sea water from the Gulf of Eilat (Red Sea) was pumped to three marine fish ponds stocked with gilthead bream (*Sparus aurata*) which were fed a 35–40% protein diet. Inefficient diet utilization combined with intense solar radiation produced dense phytoplankton blooms and associated extreme fluctuations in oxygen and pH values. Oysters (*Crassostrea gigas*) were used to improve water quality by filtering the excess phytoplankton. Two systems were compared. One used recirculation with a single PVC lined fish tank and oyster tank. In the second system the effluent from three fish ponds drained to a common earthen settling pond and then passed to the oysters. Oxygen, temperature, salinity, ammonia and particulate organic matter was similar in both systems; however, oysters grew faster in the second system where phytoplankton diversity was higher and concentration more stable.

POSTERS

SCANNING ELECTRON MICROSCOPY AND X-RAY ANALYSIS OF SHELL DISEASE LESIONS IN THE AMERICAN LOBSTER. Robert C. Bayer,* Deanna L. Prince, Cheryl D. Waltz and **Alan R. Corey,** Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469; **Rodman G. Getchell,** Maine Department of Marine Resources, West Boothbay Harbor, ME 04575.

Preliminary studies of shell disease in *Homarus americanus* were made using lobsters obtained from Nova Scotia bearing exoskeletal lesions. Lesions were observed using scanning electron microscopy (SEM) and X-ray analysis.

SEM showed an absence of epicuticle where lesions occurred.

Damage to the procuticle was evident as well. Several species of chitinoclastic bacteria of various morphologies were observed within the lesions. Magnifications of approximately 5100× revealed a cluster of *Aerococcus viridans*, the bacterium that causes gaffkemia, on the shell near a diseased area. At the same magnification, microscopic cracks were found in uninfected regions, possibly allowing for the invasion of future pathogens.

X-ray analysis of the samples showed an absence of calcium and phosphorous in the infected area. The shell contained these elements in areas where lesions were not present.

In an attempt to produce samples for further study, a bacterial culture was isolated from a lesion on an infected lobster. This culture of *Pseudomonas* spp. was swabbed on the abraded shell of a healthy lobster. The lobster developed shell lesions within eight weeks.

FRNA BACTERIOPHAGES AS INDICATORS OF FECAL POLLUTION IN AN ESTUARINE ENVIRONMENT. David M. Boyd,* Howard Kator and **M. Rhodes,** School of Marine Science, Gloucester Point, VA 23062.

A number of alternate microbial indicators of fecal pollution in receiving waters have been proposed. One of these, the male-specific coliphage containing ribonucleic acid (FRNA), has not been evaluated in shellfish growing waters. The occurrence of FRNA phage in point source impacted water samples may be a better measure of pathogenic viral persistence than traditional coliform indicators. Accordingly, water and sediment samples were obtained from a tidal creek of the Ware River, Virginia, which receives treated effluent from a sewage treatment plant. Samples were assayed for male-specific phage using a method (Havelaar and Hogeboom. J. Appl. Bacteriol. 1984, 56:439–447) designed to enumerate FRNA phage lytic for *Escherichia coli*. Samples were collected along a salinity gradient over a six month period to evaluate the seasonal and spatial distribution of FRNA phage. Parallel plating on male and female host strains and subculturing and purification of isolated plaques were used for confirmation of FRNA phage and to evaluate the sensitivity of the assay to non-FRNA phage. Fecal coliform densities and selected physical/chemical parameters were also measured and compared with phage titer and occurrence. These results and the applicability of this assay method as an indicator of water quality in an estuarine watercourse impacted by a point source of sewage pollution will be discussed.

A SEASONAL AND SPATIAL STUDY OF THE UPTAKE, SEQUESTERING AND TRANSFORMATION OF PARALYTIC SHELLFISH TOXINS BY THE GIANT SCALLOP, *PLACOPECTEN MAGELLANICUS*. Allan D. Cembella, Maurice Lamontagne Institute, Department of Fisheries and Oceans, 850 route de la Mer, Mont-Joli, Quebec, Canada G5H 3Z4; **Sandra E. Shumway,** Department of Marine Resources, West Boothbay Harbor, Maine 04575, USA.

The giant scallop, *Placopecten magellanicus*, is known to accumulate toxins associated with the toxic dinoflagellates, *Alexandrium* spp. Our previous studies, based on mouse bioassay results, have indicated that these toxins are not evenly distributed between the tissues and that the scallops remain toxic for extended periods of time. It has also been noted that animals from deep water (180 m) are more toxic, and for longer periods of time, than their inshore counterparts (20 m). In the present study, scallops were collected at regular intervals from inshore and offshore locations and individual tissues were analyzed for the presence of toxins using high-performance liquid chromatography (HPLC). Toxins were present in all tissues examined, including low levels in adductor muscles during some sampling periods. Gonadal tissue contained consistently low levels of toxins, with gonyautoxins (GTX_2 and GTX_3) present as the predominant components. Digestive glands and mantles were both highly toxic throughout the sampling period, although digestive glands were usually more toxic than mantles. Differences between the two populations and possible transformations of toxins involving the conversion of gonyautoxins and neosaxitoxin to saxitoxin will be discussed.

SEASONAL AND MICROGROWTH LINE PATTERNS IN THE CHONDROPHORE OF *MYA ARENARIA*. Robert M. Cerrato,* and **Heather V. E. Wallace,** Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794.

Soft-shell clams, *Mya arenaria*, were collected bimonthly during 1986–87 and monthly during 1989 at an intertidal site in Stony Brook Harbor, Long Island, New York. Thin sections of the chondrophore ground to about 250 microns show a distinct seasonal pattern. During May–June, a thin translucent band, most likely associated with spawning, is formed. This feature is followed by a seasonal pattern consisting of a dark region forming in spring, a light region in summer, and a second dark region in fall–winter.

In sections ground to about 150 microns, microgrowth lines are evident. These also show a seasonal pattern with wide, regularly spaced lines in spring, thin but regular lines in summer, and irregularly appearing lines forming in fall–winter. Microgrowth line patterns appear to be as complex as those found in *Mercenaria mercenaria* and include subdaily, tidal cycle patterns during periods of rapid growth. Coupled with an allometric relationship between shell size and chondrophore length, reconstruction of shell growth is possible.

DETECTION OF *VIBRIO VULNIFICUS* IN ENVIRONMENTAL SAMPLES USING GAS CHROMATOGRAPHY. Charles P. Davis,* Suzanne Barth, Karen B. Williams, and

Mary L. Rutter, Texas Department of Health, Austin, TX 78756.

Vibrio vulnificus has become important in recent years as the cause of an often fatal septicemia in susceptible patients having consumed raw or insufficiently cooked seafood. It is most often associated with raw oysters. Those people primarily at risk are those with a history of alcoholism, diabetes, and immunodeficiency, but it can even infect people with low gastric acid.

The current method for identifying these organisms in environmental samples involves biochemical testing that can take up to two weeks for a most probable number determination of *Vibrio vulnificus* levels. Gas chromatography coupled with a computer can be used to identify these organisms within three days of receipt in the laboratory.

We analyzed over 500 isolates found in oysters harvested from the Gulf of Mexico. Isolates were grown on Thiosulfate Citrate Bile Sucrose agar (TCBS). Gas chromatography, compared to the traditional biochemical method, showed a per isolate sensitivity of 97% and specificity of 90% for *Vibrio vulnificus*. In addition, gas chromatography has proven to be more cost efficient than the traditional biochemical method.

BIOMINERALIZATION OF BARITE BY *CORBICULA FLUMINEA*. Lowell W. Fritz, Institute of Marine and Coastal Sciences, Rutgers University, Port Norris, NJ 08349; **Greg Ferrence,** Indiana University of Pennsylvania, Indiana, PA 15705; **Timothy R. Jacobsen** and **Richard A. Lutz,** Rutgers Univ.

Barite crystal rosettes were discovered on the inner depositional surface of the inner complex crossed-lamellar shell layer of specimens of the Asiatic clam *Corbicula fluminea*, collected live from populations in the Maurice River, NJ. Crystal composition was verified by energy dispersive x-ray spectroscopy of intact crystals on shells and within shell fragments. Crystal mineralogy was determined by x-ray diffraction analyses of the heavy fraction of ground shells.

A barium exposure experiment was conducted using 200 clams collected from the Delaware River, where no barite rosettes had been previously observed. The Delaware River has approximately half the level of dissolved Ba as the Maurice River (50 μg/l). Organisms exposed to the two highest Ba concentrations (100 and 500 μg/l added to Maurice River water) formed barite crystals across the entire inner shell surface (lateral tooth; inside and outside the pallial line). No barite rosettes were observed on the inner shell surface of organisms in the time = 0 sample, after 4 weeks in well-water, nor on the shell exterior of any experimental or control specimen. Results suggest that Ba uptake was proportional to the level of exposure to dissolved Ba, and that Ba was eliminated from tissues into the extrapallial fluid and shell, where it crystallized as the non-biologically active mineral, barite.

New Jersey Agricultural Experiment Station Publication No. K-27204-1-89 supported by state funds and the NJ Department of Environmental Protection, Office of Science and Research.

CHARACTERIZATION OF PILI OF *VIBRIO VULNIFICUS* BY ELECTRON MICROSCOPY AND ELECTROPHORESIS. Rita M. Gander,* and **Sujata K. Patel,** Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas 75235.

In support of efforts to screen oysters for potentially harmful contaminating organisms, the goal of this research is to identify markers of *Vibrio vulnificus* which may be associated with initiation of infections in man. Pili from five clinical and two environmental strains of *V. vulnificus* were characterized by electron microscopy (EM) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). EM examination of whole cells from each of seven strains showed thin, rigid, filamentous structures extending from cell surface. *V. vulnificus* produced the highest number of piliated cells during the stationary growth phase when grown in broth at 30°C with shaking. Purified pili from the seven isolates were analyzed by SDS-PAGE. Two subunit patterns were observed; pili with subunit molecular weights of 36.3- and 34.7-kDa or 36.3- and 32.9-kDa. Purified pili from VA918, a selected test strain, was compared to preparations of polar flagella (core) and peritrichous flagella from the same strain. EM examination demonstrated an average pilus width of 6.3 nm compared to 15 nm and 16.5 nm widths for the polar and peritrichous flagella, respectively. In addition, SDS-PAGE analysis of purified preparations from VA918 revealed major subunits of 26.3- and 32.9-kDa for pili, 43.6-, 41.7-, and 35.5-kDa for polar flagella, and 41.7- and 40.7-kDa for peritrichous flagella. These data suggest that pili of *V. vulnificus* are morphologically and biochemically distinct from polar and peritrichous flagella. In addition, purified pili from *V. vulnificus* strains appear to share a common 36.3-kDa subunit.

REPRODUCTIVE BIOLOGY OF THE WHELK *BUCCINUM UNDATUM* IN THE NORTHWEST GULF OF ST-LAWRENCE. L. Gendron,* Maurice-Lamontagne Institute, C.P. 1000, Mont-Joli (Quebec) G5H 3Z4.

The reproductive biology of the whelk *Buccinum undatum* was investigated in order to determine a minimum catchable size based upon the size of sexual maturity. Because of the size of the geographic area concerned, and its heterogeneity in oceanographic characteristics and in fishing pressure, variability of the size of sexual maturity was also examined.

Samples were obtained in spring of 1988 and 1989, before the beginning of reproduction, from 8 sites, either by SCUBA diving or from commercial trap fishing. Sexual maturity of males was determined morphometrically, assuming that males with a ratio of penis length to shell height ≥ 0.50 were mature. Maturity of females was determined from the gonado-somatic index (GSI), ex-

pressed as a function of eviscerated body weight. Females with a GSI ≥0.06 were considered mature. The percentage of mature animals present in each size class of 5 mm (shell height), from a minimum of 40 mm to a maximum of 120 mm was then computed, for each sex and site seperately.

In most sites, a decrease in the percentage of mature animals at larger sizes was observed, indicating the occurrence of reproductive senility. The relationship appeared rather dome-shaped and was fitted by a polynomial (2nd degree) regression, rather than by the usual logistic curve. Size at 50% sexual maturity and size at which reproductive senility occurred were compared for all sites and related to age (determined by counting the rings on the operculum), and on-site standing densities.

THE CHICK ASSAY FOR PSP: AN ALTERNATIVE TO THE MOUSE BIOASSAY USEFUL IN RURAL AREAS. **M. N. Gomaa** and **S. Hall,** Food and Drug Administration, HFF-423, 200 C Street Sw, Washington, DC 20204; **J. A. Doerr,** Poultry Science Department, College of Agriculture, University of Maryland, College Park, Maryland 20742.

Shellfish are frequently produced and PSP occurs in locations far from laboratories where PSP assays are typically conducted. When it is impractical to ship samples from these locations to the central laboratory for assay, it is desirable to set up a field laboratory to conduct bioassays but in such a location it may be difficult to obtain mice to perform the standard bioassay. Such a situation prevails in the Philippines, where PSP has become a severe problem in recent years. However, chickens are typically raised in the rural villages and chicks are readily available on a regular basis. We decided to investigate the use of chicks as assay animals and found that they were a practical alternative for such situations. Sensitivity was equal to or greater than the mouse bioassay and precision was adequate for use in shellfish toxicity monitoring. It is therefore possible for shellfish growers in remote locations to set up low cost monitoring programs to check their products.

SPATIAL AND TEMPORAL DIFFERENCES IN THE ECOPHYSIOLOGY OF BIVALVES FROM POLLUTED AND UN-POLLUTED AREAS OF THE DUTCH DELTA AREA. **Herman Hummel, Roelof H. Bogaards, Lein De Wolf** and **Willem-Jan Goossen,** Delta Institute for Hydrobiological Research, Vierstraat 28, 4401 EA Yerseke, The Netherlands.

In the polluted estuary Westerschelde, and the relatively clean sea-arm Oosterschelde and brackish lake Grevelingen differences in the condition, growth, biochemical constitution, reproduction, concentration of PCBs and heavy metals, and the genetics (isoenzymes) of adult specimens (2 years or older) of the bivalves *Macoma balthica* and *Mytilus edulis* were assessed monthly during 1 year, and related to environmental factors.

In the (polluted) Westerschelde estuary the concentration copper in the animals and in the sediment were positively correlated. At one station in the (relatively un-polluted) Oosterschelde, a surprisingly high concentration copper was found in *Macoma*, but not in the sediment. At this station low values for growth and condition were found. The concentration cadmium in the sediment increased with decreasing salinity. The concentration cadmium in animals and sediment were negatively related. Probably, the bioavailability of cadmium increases at increasing salinity.

Highest PCB concentrations were found upstream in the Westerschelde. A strong decrease in PCB content during spring was not due to elimination of PCBs from the animal, but mussels may shed a substantial part of their PCBs by means of spawning of gametes. In contrast to this, an increase could be due to accumulation of PCBs from the water (100% in 2 to 3 months).

An ordination analyses showed that seasonal (temporal) changes in the ordinated characteristics of *Macoma* at the several sampling stations are primarily determined by changes in temperature and/or chlorophyll a, whereas (spatial) differences between the stations are governed by differences in salinity. Moreover, the FAA stress-parameters, i.e. the Taurine/Glycine ratio and Serine + Threonine sum, are negatively correlated and primarily determined by changes in chlorophyll a and temperature, but not by salinity as is the total FAA concentration.

PRELIMINARY COLLABORATIVE STUDY OF THE HPLC METHOD FOR PSP TOXINS. **James M. Hungerford** and **Marleen M. Wekell,** Food and Drug Administration, Seafood Products Research Center, Bothell, WA; **Sherwood Hall,** Center for Food Safety and Applied Nutrition, Washington, D.C.

Paralytic shellfish poisoning (PSP) results from the ingestion of neurotoxins bioaccumulated in shellfish following blooms of Alexandrium.

Among the promising instrumental methods for detecting PSP toxins is a high pressure, liquid chromatographic (HPLC) method developed by FDA. The method provides a toxin profile and is more sensitive than the mouse bioassay currently used. Sophisticated instrumentation is required, and the method involves careful control of several experimental variables. A pre-collaborative study has been initiated to assess equipment requirements and method ruggedness. Preliminary results are presented and other approaches to screening for PSP toxins are discussed.

LIFE HISTORY OF THE SMALL PATAGONIAN OCTOPUS, *OCTOPUS TEHUELCHUS* d'ORBIGNY. **Oscar Osvaldo Iribarne,** CQS-HR20, University of Washington, Seattle, WA 98195.

Although this species has been reported from shallow waters down to 90 m depth, knowledge is almost entirely based on intertidal samples. In this study both intertidal and subtidal samples were taken during 1982–1987, in northern San Matias Gulf (41°S, 63°30'W). This is a large egged (eggs: 9–12 mm long ×3–5 mm wide, stack of 4–6 mm long) and small-sized (up to

150 g) octopus. Egg laying occurs between autumn and winter. Embryonic development takes about 4 months (water temperature: 4°C to 19°C). Large hatchlings ($\overline{DML}$: 6.64 mm, $\overline{TL}$: 14.23 mm, $\overline{TW}$: 0.139 g) emerge over spring and early summer, and development is direct. Maximum size is reached after 17 to 18 months; mating takes place in summer. Females reduce their feeding activity when they reach maturity, and cease eating while brooding. Mean life-span is two years, but some individuals (mostly females) may live up to three years. Females approaching the beginning of their brooding period move to the subtidal zone. There males outnumber females until the end of summer; then females (mostly brooders) outnumber males. In the intertidal zone sex-ratio was 1:1 from December to late March, but in April males outnumber females.

These life history traits will be discussed in relation to environmental conditions prevailing in the San Matias Gulf.

SEAFOOD SAFETY ELECTRONIC BULLETIN BOARD. **Susan H. Kuenstner,** New England Fisheries Development Association, 280 Northern Avenue, Boston, MA 02210.

The New England Fisheries Development Association has completed an electronic bulletin board which addresses shellfish (and finfish) contaminants. The bulletin board is geared towards scientists studying seafood contaminants, public health officials and members of the seafood industry. It is accessible with a computer, modem, communications software and a SCIENCEnet mailbox. The bulletin board contains background information and reference lists for each of the major seafood contaminants. Contaminants currently covered on the board of interest to shellfish biologists include: Toxins (ASP, DSP, NSP, PSP), Bacteria (6 *Vibrio* species, *Listeria monocytogenes*) and Viruses (hepatitis A, Norwalk virus, poliovirus). Another important feature on the bulletin board is the forum, the section which allows interaction among users. The forum is a convenient way to communicate with other scientists, request information, stay up-to-date, give advice or brainstorm.

IDENTIFICATION OF *VIBRIO VULNIFICUS* BY CELLULAR FATTY ACID COMPOSITION. **Warren L. Landry** and **Charles N. Roderick,** FDA, Dallas, TX 75204.

Gas-Liquid Chromatography was used to analyze cellular fatty acid profiles of 304 *Vibrio vulnificus* isolates and to develop a computer generated library for the Hewlett-Packard (HP) 5898A Microbial Identification System (MIS) as a means of rapid identification. The purpose of this project was to find an identification procedure which could rapidly differentiate between the different Vibrio species. This library entry was compared to the library included with the HP MIs for *Vibrio vulnificus*.

Of the 304 strains examined 291 were environmental isolates and 13 were from a clinical origin. All *Vibrio vulnificus* isolates were confirmed by gene probe analysis.

Validation for *Vibrio vulnificus* entry in the Landry Library was 97.4% as compared to 67% with the HP library.

PRODUCTIVITY OF THE GIANT SCALLOP (*PLACOPECTEN MAGELLANICUS*) MAINTAINED IN SUSPENDED CAGES, FOR THE PURPOSE OF AQUACULTURE, IN PORT AU PORT BAY, NEWFOUNDLAND, CANADA. **Marc Lanteigne** and **Leslie-Anne Davidson,** Department of Fisheries and Oceans, Science Branch, Gulf Fisheries Centre, Box 5030, Moncton, New Brunswick, Canada, E1C 9B6.

In Atlantic Canada, a growing number of aquaculturists are trying to cultivate the native giant scallop using Japanese techniques. This interest has created a demand for scientific studies to get a better understanding of the biology of the species in the farming environment.

A study, to evaluate the productivity of the Giant Scallop, was initiated in the spring of 1989, on the western coast of Newfoundland. Twenty (20) months old scallops were divided in groups of 25, 50 and 100 scallops. Each group was placed in a standard circular Japanese pearl net and suspended on sub-surface long lines (2 meters below the surface) for two (2) to 14 weeks. Two (2) samples of each group were taken every two (2) weeks and processed for production measurements. Temperature, salinity, chlorophyll and seston measurements were also taken weekly at the rearing site.

The results of the first year study are presented and compared with the information found in the literature. The information is also used to present some preliminary economic scenarios on the feasibility of cultivating the giant scallop species suspended cages.

USE OF SHALLOW WATER INSHORE HABITATS OFF GRAND MANAN, BAY OF FUNDY, CANADA, BY MATURE AMERICAN LOBSTERS, *HOMARUS AMERICANUS*. **Peter Lawton,*** and **David A. Robichaud,** Fisheries and Oceans, Biological Station, St. Andrews, N.B., E0G 2X0, Canada.

Based on SCUBA-diving surveys and lobster trap-sampling during August and September, 1982–83, Campbell (Can. J. Fish. Aquat. Sci., in press) described seasonal aggregations of berried (ovigerous) female lobsters, *Homarus americanus*, in shallow water (1–22 m depth) within Flagg Cove, Grand Manan.

Subsequent to these surveys, a salmonid aquaculture site was approved within Flagg Cove. In response to expansion of aquaculture activity in spring 1989, and concern expressed by traditional fishing interests, SCUBA divers re-surveyed lobster populations occupying Flagg Cove during September 1989. Dives were also conducted at several other locations around Grand Manan. Both the size range, and density (within 100 m^2 line transects) of berried females encountered at Flagg Cove were comparable between years. Lobsters were discovered in abundance in shallow

waters near Seal Cove, southern Grand Manan; however, there were significantly fewer berried females at this site.

Further seasonal monitoring of mature lobster utilization of shallow water embayments off Grand Manan is planned for 1990. Problems associated with objectively assessing the importance of particular "breeding" sites, and determining cause-effect relationships between aquaculture development (and/or other anthropogenic impacts) and changes in the numbers and proportion of berried female lobsters utilizing such sites, are discussed.

RECENT OCCURRENCE OF PARALYTIC SHELLFISH POISONING (PSP) TOXINS FROM THE NORTH WESTERN COASTS OF FRANCE. Martial LeDoux* and **J. Maro Fremy,*** CNEVA/LCHA, 43 rue de Dantaig, F-75015 Paris; **Elizabeth Nezan,** IFREMER, 13 rue de Kérose, F-29110 CONCARNEAU; **Evelyne Erard,** IFREMER, BP 70, F-20283 BREST.

During the past few years, dense blooms of the dinoflagellate *Alexandrium minutum* Halim were observed along French coasts; during July 1985 in Vilaine Bay (6×10^6 cells per liter) and during August 1988 in the river Aber Wrac'h (2.3×10^8 cells per liter). For the last case, Gonyautoxine (GTX) were identified in mussels and oysters. During July 1989, a fleeting bloom of an *Alexandrium* sp was observed in Morlaix Bay, Brittany, France, the maximum of cell density was 3×10^6 cells per liter. Mussels, oysters and cockles were contaminated by paralytic shellfish poisons. Toxin production was demonstrated by mouse bio-assay and HPLC (as described by Sullivan). The *Alexandrium* sp did not seem to be very toxic; oysters and cockles did not contain high levels of toxins and became quickly safe. Mussels presented higher toxic levels and remained toxic two weeks after the disappearance of the bloom. Toxin profiles of the wild strain, temporary kist and shellfish were studied by HPLC: GTX2 and GTX3 were the major toxins found in both dinoflagellate and shellfish extracts. When any shellfish contamination did not occur during the 1988 bloom, the action level of 80 ug per 100 g were reached during the 1988 and 1989 blooms. But no PSP case in human was reported because preventive measures were rapidly taken to avoid toxic shellfish consumption.

HERITABILITY, GENETIC CORRELATION, AND GENOTYPE-ENVIRONMENT INTERACTION OF LARVAL AND JUVENILE GROWTH RATE IN THE COOT CLAM, *MULINIA LATERALIS*. Adam N. Ludwig, University of Delaware, College of Marine Studies, 700 Pilottown Road, Lewes, DE 19958.

Based on a need for improvements in commercially important traits in hatchery reared bivalves, a study of the quantitative genetics of larval and juvenile growth rates was performed using the Coot Clam (*Mulinia lateralis*) as a model system. The goal of this study is to estimate heritabilities for larval and juvenile growth rate, any genetic correlation between larval and juvenile growth, and any genotype-environment interaction for juvenile growth in *M. lateralis*.

Clams were spawned by way of thermal stimulation and mated in a factorial design. The larvae were cultured using the methods of Calabrese and Rhodes (1974). Using measures of larval shell area at four day intervals, the heritability of larval growth was estimated. After settlement, juveniles from the same families were grown in separate water tables. The only environmental difference between the tables was salinity. This was accomplished by fluctuating the salinity in one of the tables. These different environments resulted in mean performance differences of the various families across environments. From measurements of juvenile shell area in both environments, the genotype-environment interaction was analyzed graphically, statistically, and by way of genetic correlation. In addition, from the juvenile growth data, estimates of the heritability of juvenile growth and genetic correlation between larval and juvenile growth were calculated.

WATER QUALITIES ASSOCIATED WITH RAPID OYSTER GROWTH. Michael E. Mallonee, and **Kennedy T. Paynter,** Chesapeake Bay Institute, The Johns Hopkins University, Baltimore, MD.

Extremely rapid growth has been observed in raft cultured oysters in the Chesapeake Bay. In previous studies, animals raised in floating rafts in a shallow tidal creek grew at an average rate of 15 mm/month during their first growing season. Although genetic influences on growth were demonstrated in that study, the very high growth rates in all of the animals suggested that the environment was exceptionally conducive to oyster growth. In an effort to learn more about the relationships between genetics, environment, and growth, we began a series of oyster growth experiments in which animals of the same cohort were raised in different regions of the Chesapeake Bay. Water quality and oyster growth were measured biweekly at these sites. Water qualities measured included salinity, pH, temperature, size-fractionated chlorophyll a levels, chlorophyll b contents, and total suspended solids. Growth was measured as an increase in shell height. Condition indices and mortalities were also determined.

Five sites were chosen based on environmental diversity, security and availability. Average chlorophyll a levels between sites ranged from 8 µg/l to 25 µg/l and average salinities from 8.0‰ to 18.0‰. Growth rates differed significantly between sites and were positively correlated with both chlorophyll a and salinity. The potential of raft oyster culture in the Chesapeake Bay will be discussed, and the importance of assessing water qualities when determining the potential of growout sites will be addressed.

BODY BURDEN AND TISSUE ALLOCATION OF SAXITOXIN IN TWO CLAM SPECIES. Roger Mann and **Julia S. Rainer,*** Virginia Institute of Marine Science, College of Wil-

liam and Mary, Gloucester Point, VA 23062; **Sherwood Hall**, Department of Seafood Toxin Research, Food and Drug Administration, 200 C Street, Washington, D.C. 20204.

Mercenaria mercenaria and *Mya arenaria* were fed various concentrations of saxitoxin producing dinoflagellates over 48 to 72 hour time periods. Body burden, toxin accumulation rates and toxin tissue allocation were determined by sacrificing animals at 12 hour intervals and assay, by HPLC, of whole wet tissue or body parts (gill and mantle, adductor, viscera). Ingestion of concentrated dinoflagellates resulted in narcotization of extended siphons but not of pumping activity in both species. Translocation of toxin into the adductor muscle was seen as soon as 12 hours after initiation of feeding in *Mya*.

CONTAMINANT LEVELS IN OYSTERS FROM THE CHESAPEAKE BAY: 1981–1985. D. L. Murphy, Maryland Department of the Environment, Baltimore, Maryland 21224, USA.

On a twice yearly basis, tissue samples of the oyster, *Crassostrea virginica,* are collected by the Maryland Department of the Environment, and analyzed for metal and organochlorine pesticide contamination. The results of this activity for the five year period from 1981 through 1985, are presented here. No contaminants measured in any shellstock samples exceeded those currently considered acceptable for human consumption. Mean mercury levels in Maryland oysters were found to have declined from 1981 through 1985. Cadmium and copper were measured at significantly elevated levels in oysters from oyster beds in the upper Patuxent River. The distribution of detectible levels of lead in Maryland shellstock has increased from 1981 through 1985. Chlordane was found to be the most widespread of the few organochlorine pesticides detected; highest levels were found primarily in urban watersheds.

POLYNUCLEAR AROMATIC HYDROCARBON LEVELS IN SHELLFISH, OVERVIEW OF 1989 MUSSEL WATCH FIELD SEASON. Carole S. Peven* and **William G. Steinhauer,** Battelle Ocean Sciences, 397 Washington Street, Duxbury, MA 02332.

Concentrations of 19 polynuclear aromatic hydrocarbons (PAH) and 4 alkyl-substituted PAH were measured in bivalves on the U.S. east coast and west coasts. The sum of the measured PAH and substituted PAH ranged from 0.09 to 5.1 μg/g on the east coast and from 0.1 to 5.6 μg/g on the west coast. The highest mean total PAH accumulations in east coast bivalves were measured in mussels from the Upper Bay in the Hudson-Raritan Estuary. Mussels from the Lower Bay in the Hudson-Raritan Estuary also contained relatively high PAH levels. PAH concentrations in bivalves were generally lower for site south of the metropolitan New York/New Jersey area, with exceptions being oysters collected near the Chincoteague Inlet, North Carolina, and near Fort Johnson, South Carolina. The highest PAH concentrations on the west coast were measured in mussels from Elliot Bay, Washington. Other Puget Sound sites also displayed relatively high targeted PAH levels. Other west coast sites displaying relatively high PAH levels were San Diego Bay and San Pedro Harbor. Relative amounts of petrogenic vs pyrogenic PAH are estimated from source ratios.

CALCULATION OF CONDITION INDEX IN OYSTERS. Julia S. Rainer, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The condition index of 2.5–3 inch oysters from a single reef in the James River, VA was calculated using four different methods. Oysters were sampled over a six week period in the summer of 1987. Three methods utilize a ratio of dry meat weight to shell cavity volume. The variation in these method lies in the estimation of shell cavity volume. The fourth method is gravimetric and expresses condition as a ratio of dry meat weight to dry shell weight.

ESTIMATION OF OYSTER STANDING STOCK USING SCUBA. Julia S. Rainer* and **Roger Mann,** Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Dredge collections from oyster reefs give only a qualitative estimate of oyster abundance. Quantitative bottom collections were made using SCUBA from fixed size quadrats, placed randomly on a LORAN based grid at twelve locations, on Horse Head Bar in the James River, VA in the fall of 1988. We present data on spatial variability in oyster standing stock and size distribution, and discuss the values and limitations of intensive sampling for management of oyster resources.

OYSTERS AND TOXIC ALGAL BLOOMS: ARE THEY IMMUNE? Sandra E. Shumway, Janeen Barter and **Sally Sherman-Caswell,** Department of Marine Resources, West Boothbay Harbor, Maine 04575.

A literature survey has indicated that various species of oysters exhibit very low levels of toxicity when exposed to toxic algal blooms. A study was begun in 1988 when oysters (*Crassostrea virginica* and *Ostrea edulis*) were suspended in cages with mussels, *Mytilus edulis*, in Boothbay Harbor, Maine. Samples were taken of all species on a bi-weekly basis to assess the level of toxicity due to accumulation of the toxic dinoflagellate, *Alexandrium* (*Protogonyaulax*) *tamarensis*. Oysters showed only slight levels of toxicity. Further, oysters did not become toxic until approximately 2 weeks after the toxin appeared in *Mytilus*. Both oysters and mussels released the accumulated toxin within 2–4 weeks after peak toxicity levels were reached. Comparison of these data with those from the literature indicate that there is a general trend for oysters to accumulate less toxin than mussels

when simultaneously exposed to toxic algae. There is also some indication that oysters can rid themselves of the toxins more quickly than many other species of molluscs.

SPAWNING AND SPAT SETTLEMENT OF THE CATARINA SCALLOP *ARGOPECTEN CIRCULARIS* IN BAHIA MAGDALENA, B.C.S., MEXICO. **Arturo Tripp-Quezada,** Centro Interdisciplinario, de Ciencias Marinas, Apartado Postal #592, La Paz, B.C.S., Mexico.

The populations of *Argopecten circularis* (Sowerby, 1835) in coastal water of Baja California Sur have been overfished, so the commercial extraction of this resource is no longer appealing.

In order to increase scallop production, aquaculture techniques and repopulation of previously productive areas using scallop spat caught at sea, have been promoted.

The object of this study was to determine what parameters were useful in planning activities of extensive cultivation based on culture of *A. circularis* in Bahia Magdalena. The following aspects were considered:

1. Spawning season
2. Selection of sites to obtain scallop spat
3. Determination of the efficiency of three types of spat collectors.

The results of this study show that in Bahia Magdalena, the best season to collect scallop spat is in winter time; the best sites of collection are Santa Elena and San Vicente; and the most efficient spat collector was onion bags filled with plastic mesh.

GROWTH AND SURVIVAL OF HARD CLAMS AT VARIOUS DENSITIES IN THREE LONG ISLAND SOUND LOCATIONS. **James C. Widman** and **Ronald Goldberg,** National Marine Fisheries Service, Northeast Fisheries Center, Milford University, Milford, Connecticut 06460.

Hatchery-reared clams, *Mercenaria mercenaria*, were held in partially buried, 0.6 × 0.6 × 0.25 m vinyl-coated wire-mesh cages at 3 sites in Long Island Sound. These were grown at densities of 500, 1000, and 3000/m^2, in triplicate, at a depth of 5 m mean low water. At one site, cages were deployed with clams at 5 additional densities ranging from 100–5000/m^2. Clams grew from an initial mean-shell height of 11.5 mm in April 1987 to a range of 17.4–24.1 mm by November 1987. Density was not a significant factor affecting growth at any site, although there was a significant difference between cages at each site. Clams grown in Greenwich were larger than those grown in Milford or Stonington. Survival was not affected by density, although the cages did not completely exclude predators. Survival was lower at the Stonington site. At each site, 4 additional cages holding clams at 500/m^2 were randomly positioned away from the main cage grid. There was no significant difference in the growth of these clams and those grown in the main cage grid.

[1]Fellow of COFAA

THE NATIONAL SHELLFISHERIES ASSOCIATION

The National Shellfisheries Association (NSA) is an international organization of scientists, management officials and members of industry that is deeply concerned and dedicated to the formulation of ideas and promotion of knowledge pertinent to the biology, ecology, production, economics and management of shellfish resourses. The Association has a membership of more than 900 from all parts of the USA, Canada and 18 other nations; the Association strongly encourages graduate students' membership and participation.

WHAT DOES IT DO?

—Sponsors an annual scientific conference.
—Publishes the peer-reviewed *Journal of Shellfish Research*.
—Produces a Quarterly Newsletter.
—Interacts with other associations and industry.

WHAT CAN IT DO FOR YOU?

—You will meet kindred scientists, managers and industry officials at annual meetings.
—You will get peer review through presentation of papers at the annual meeting.
—If you are young, you will benefit from the experience of your elders.
—If you are an elder, you will be rejuvenated by the fresh ideas of youth.
—If you are a student, you will make most useful contacts for your job search.
—If you are a potential employer, you will meet promising young people.
—You will receive a scientific journal containing important research articles.
—You will receive a Quarterly Newsletter providing information on the Association and its activities, a book review section, information on other societies and their meetings, a job placement section, etc.

HOW TO JOIN

—Fill out and mail a copy of the application blank below. The dues are 30 US $ per year ($20 for students) and that includes the *Journal* and the Newsletter!

NATIONAL SHELLFISHERIES ASSOCIATION—APPLICATION FOR MEMBERSHIP
(NEW MEMBERS ONLY)

Name: _____ For the calendar year: _____ Date: _____
Mailing address: _____
_____
_____
Institutional affiliation, if any: _____
Shellfishery interests: _____

Regular or student membership: _____
Student members only—advisor's signature REQUIRED: _____

Make cheques (*MUST* be drawn on a US bank) or international postal money orders for $30 ($20 for students with advisor's signature) payable to the National Shellfisheries Association and send to Dr. Tom Soniat, Dept of Biology, University of New Orleans, New Orleans, Louisiana 70148 USA.

INFORMATION FOR CONTRIBUTORS TO THE *JOURNAL OF SHELLFISH RESEARCH*

Original papers dealing with all aspects of shellfish research will be considered for publication. Manuscripts will be judged by the editors or other competent reviewers, or both, on the basis of originality, content, merit, clarity of presentation, and interpretations. Each paper should be carefully prepared in the style followed in Volume 3, Number 1, of the *Journal of Shellfish Research* (1983) before submission to the Editor. Papers published or to be published in other journals are not acceptable.

Title, Short Title, Key Words, and Abstract: The title of the paper should be kept as short as possible. Please include a "short running title" of not more than 48 characters including space between words, and approximately seven (7) key words or less. Each manuscript must be accompanied by a concise, informative abstract, giving the main results of the research reported. The abstract will be published at the beginning of the paper. No separate summary should be included.

Text: Manuscripts must be typed double-spaced throughout one side of the paper, leaving ample margins, with the pages numbered consecutively. Scientific names of species should be underlined and, when first mentioned in the text, should be followed by the authority.

Abbreviations, Style, Numbers: Authors should follow the style recommended by the fourth edition (1978) of the *Council of Biology Editors [CBE] Style Manual*, distributed by the American Institute of Biological Sciences. All linear measurements, weights, and volumes should be given in metric units.

Tables: Tables, numbered in Arabic, should be on separate pages with a concise title at the top.

Illustrations: Line drawing should be in black ink and planned so that important details will be clear after reduction to page size or less. No drawing should be so large that it must be reduced to less than one third of its original size. Photographs and line drawings preferably should be prepared so they can be reduced to a size no greater than 17.3 cm × 22.7 cm, and should be planned either to occupy the full width of 17.3 cm or the width of one column, 8.4 cm. Photographs should be glossy with good contrast and should be prepared so they can be reproduced without reduction. Originals of graphic materials (i.e., line drawings) are preferred and will be returned to the author. Each illustration should have the author's name, short paper title, and figure number on the back. Figure legends should be typed on separate sheets and numbered in Arabic.

No color illustrations will be accepted unless the author is prepared to cover the cost of associated reproduction and printing.

References Cited: References should be listed alphabetically at the end of the paper. Abbreviations in this section should be those recommended in the *American Standard for Periodical Title Abbreviations*, available through the American National Standard Institute, 1430 Broadway, New York, NY 10018. For appropriate citation format, see examples at the end of papers in Volume 3, Number 1, of the *Journal of Shellfish Research* or refer to Chapter 3, pages 51–60 of the *CBE Style Manual*.

Page Charges: Authors or their institutions will be charged $50.00 per printed page. If illustrations and/or tables make up more than one third of the total number of pages, there will be a charge of $30.00 for each page of this material (calculated on the actual amount of page space taken up), regardless of the total length of the article. All page charges are subject to change without notice.

Proofs: Page proofs are sent to the corresponding author and must be corrected and returned within seven days. Alterations other than corrections of printer's errors will be charged to the author(s).

Reprints: Reprints of published papers are available at cost to the authors. Information regarding ordering reprints will be available from The Sheridan Press at the time of printing.

Cover Photographs: Particularly appropriate photographs may be submitted for consideration for use on the cover of the *Journal of Shellfish Research*. Black and white photographs, if utilized, are printed at no cost. Color illustrations may be submitted but all costs associated with reproduction and printing of such illustrations must be covered by the submitter.

Corresponding: An original and two copies of each manuscript submitted for publication consideration should be sent to the Editor, Dr. Sandra E. Shumway, Department of Marine Resources, and Bigelow Laboratory for Ocean Science, West Boothbay Harbor, Maine 04575.

Phone: 207-633-5572

FAX: 207-633-7109

JOURNAL OF SHELLFISH RESEARCH

Vol. 8, No. 2 December 1989

CONTENTS

Cover Photo: *Geukensia demissa* in saltmarsh. Photo courtesy of Peter Heffernan.

MBL WHOI LIBRARY
WH 1AA9 -