# PROCEEDINGS
# OF THE
# NATIONAL SHELLFISHERIES ASSOCIATION

OFFICIAL PUBLICATION OF THE NATIONAL
SHELLFISHERIES ASSOCIATION;
AN ANNUAL JOURNAL DEVOTED TO
SHELLFISHERY BIOLOGY

## VOLUME 65

*Published for the National Shellfisheries Association, Inc. by*
*Waverly Press, Inc., Easton, Maryland*

JUNE 1975

PROCEEDINGS
OF THE
NATIONAL
SHELLFISHERIES
ASSOCIATION

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# ABSTRACTS OF THE TECHNICAL PAPERS PRESENTED AT THE 1975 NSA CONVENTION

## CULTURING AND TRANSPLANTING HATCHERY-SPAWNED QUAHOGS

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Between May and October, 1973, 5.5 million quahogs, averaging 2.0 mm along their anterior-posterior axis, were placed in trays suspended in the water column. By November, the mean survival rate was 52% and the mean size of quahogs delivered in May and June was 6.4 mm. Quahogs subjected to freezing water temperatures and ice during the winter of 1973-74, had a survival rate of 99%. Other hatchery-spawned quahogs with a size range of 8-20 mm were transplanted onto natural bottom. Recovery and survival of shell stock planted at a water temperature of 6°C was greater than that planted at 20°C.

## MOTION PICTURE FILM ENTITLED "PREDATORY BEHAVIOR OF THE BORING SNAIL *UROSALPINX CINEREA*"

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The film depicts the approach, mounting, penetration, and feeding of oysters and mussels by the oyster borer. After a short sequence on fossils, the film shows snail approaching a jar filled with live pumping oysters, climbing the outside against the flow of water, entering the jar and boring oysters. The control jar is ignored. The remainder of the film demonstrates mounting of oysters, selection of boring site, details of shell penetration magnified under an optical system, slow motion pictures of radular activity, and feeding on the flesh inside.

## HOST RESPONSE TO INFECTION WITH *BUCEPHALUS* IN *CRASSOSTREA VIRGINICA*

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Little or no host response to *Bucephalus* infections in *Crassostrea virginica* has been reported by Hopkins (1954) and Cheng and Burton (1965). Mackin and Loesch (1954) reported an intense cellular response to *Bucephalus* sporocysts infected with a haplosporidan hyperparasite. Sprague (1962) mentions a similar response to *Bucephalus* sporocysts infected with a microsporidan hyperparasite. Canzonier (per. comm.) reported leucocytic responses to moribund and dead *Bucephalus* sporocysts.

During a year-long survey (1972-73) of *Bucephalus* infections in a natural population of oysters from the Navesink River (New Jersey), host response was noted in a number of varying infection conditions. One case is of special interest. Young sporocysts in presumably new infections elicited an intense cellular response in 3 of 7 oysters with similar infection intensities. In one case phagocytes were observed within the sporocyst proper.

This response may be due to previous experience with the parasite. It may also be due to environmental conditions which alter the normal host-parasite relationship in this biological system.

## MSX-OYSTER INTERACTIONS: LEUCOCYTE RESPONSE TO *MINCHINIA NELSONI* DISEASE IN *CRASSOSTREA VIRGINICA*

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Several investigators have reported an increase in leucocytes in oysters with the development of *Minchinia nelsoni* (MSX) disease (Myhre, 1967; Farley, 1968). However, there has been no attempt at quantification of this response to date. This presentation represents an attempt to measure leucocyte population changes during MSX disease development.

Two stocks of oysters, one resistant and one susceptible to MSX, were sampled at regular intervals for one year (May 1972–June 1973). Alterations in the total leucocyte populations (TLP) were monitored by counts of leucocytes/oil field (OF), (20 OF/oyster), number of hyaline leucocytes/OF, (10 OF/oyster), and the percentage of MSX plasmodia phagocytized/200/MSX/oyster.

Both stocks showed an increase in the number of leucocytes/OF during the summer of 1972. There was no significant difference between the two stocks. During the winter and spring the number of leucocytes/OF ranged from 10%–30% higher in infected oysters when compared with uninfected oysters in the resistant stock. During the same period the number of leucocytes/OF in infected susceptible oysters ranged from 80%–140% higher than that of uninfected oysters. The number of leucocytes in infected susceptible oysters was significantly higher than that of infected resistant oysters during this time interval.

There was no significant difference in the percentage of MSX plasmodia phagocytized/sample, between the stocks. In both stocks the average percentage of MSX phagocytized was usually less than 10%.

Hyaline leucocytes constitute less than 10% of the TLP in uninfected oysters. Changes in the % hyaline leucocytes/TLP were similar in both stocks. In oysters with gill lesions in the summer there is a slight increase in hyaline leucocytes (10%–20% of the TLP). Hyaline leucocytes total 15%–40% of the TLP in oysters sampled in the winter and spring. In oysters with general infections throughout the year, hyaline leucocytes ranged from 25%–50% of the TLP.

The lack of significant differences in leucocytic responses to MSX between resistant and susceptible oysters verifies earlier speculation from this laboratory that leucocytes probably play a minor role in the resistance mechanism. This suggests that unknown humoral responses may control MSX lesion development in resistant oysters.

## MSX-OYSTER INTERACTIONS: SOME NEW OBSERVATIONS ON *MINCHINIA NELSONI* DISEASE DEVELOPMENT IN STOCKS OF OYSTERS RESISTANT AND SUSCEPTIBLE TO *M. NELSONI*-CAUSED MORTALITY

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Two stocks of oysters (*Crassostrea virginica*), one resistant, one susceptible to *Minchinia nelsoni* (MSX) were sampled at regular intervals for one year (May 1972–June 1973) on the Cape Shore tidal flats, Cape May, New Jersey. Mortality rates were monitored and histological sections of 250 oysters/stock were examined for MSX prevalence/sample, weighted MSX intensity/sample, MSX lesion type/oyster, and MSX plasmodium type/sample.

Patent MSX lesions were observed in both stocks by the first week of July 1972. Three weeks later the susceptible stock reached 100% prevalence and remained high (70%–100%) for the rest of the experimental period. The increase in prevalence in the resistant stock was much slower, reaching 90% six weeks after the initial patent infection was detected. Infection prevalence declined in the stock to 10% by mid-September 1972, rose to 90% over the winter-spring period, and then dropped to 50% in June 1973.

The quantity of MSX lesions in susceptible

oysters was three times that of the resistant stock during the summer of 1972. No significant difference in numbers of lesions between the two stocks was observed during the fall 1972 to spring 1973 period. In June 1973 the numbers of MSX lesions in the resistant stock was significantly lower than that of the susceptible stock.

Gill lesions outnumbered general infections for every sample except one (May, 1973) in the resistant stock. Only three general infections were observed in this stock during the three months following detection of the first patent infection. General infections developed within 4 weeks of the first patent infection detected in the susceptible stock and persisted for the remainder of the experiment.

Within 3 weeks of the first patent infection in the resistant oysters, 70% of the MSX plasmodia observed were either uninucleated or binucleated. These plasmodial types declined to 10% throughout the winter and then increased to 35% in June 1973. Susceptible oysters had an average of 10% uninucleated and binucleated plasmodial types throughout the experiment.

These observations are discussed relative to previous reports from this laboratory and other laboratories involved in monitoring MSX infection in *C. virginica*.

## A FIELD EXPERIMENT TO DETERMINE THE ROLE OF SEDIMENT BOUND HEAVY METALS AND SALINITY REGIME IN THE HEAVY METALS UPTAKE OF OYSTERS

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and

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Hatchery-reared oyster spat were set on panels and deployed to three field environments of varying salinity. Exposure levels at each station were 12″, 36″, and 72″ from the bottom. The stations were sampled at approximately one-month intervals. Collected samples: oysters, settleable solids, suspended sediments and water were analyzed for heavy metals concentrations by A. A. spectrophotometry. The data was treated statistically by analysis of variance and a theoretical relationship of salinity, growth rate, and metal uptake is proposed. Design and fabrication of an apparatus for panel deployment, exposure, and recovery was tested.

## A REPORT ON THE SUCCESSFUL UTILIZATION OF PLASTIC TRAYS FOR THE LARGE SCALE CULTURE OF *C. GIGAS* IN BAJA CALIFORNIA, MEXICO

Richard D. Glenn

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Laboratory produced seed of *C. gigas* have been grown to marketable size in six months or less utilizing plastic trays in an off-bottom culture. This report describes the general physical, chemical and biological factors of the growing area and the design of a continuous production oyster farm.

## AN EXAMPLE OF OYSTER PRODUCTION DECLINE WITH A CHANGE IN THE SALINITY CHARACTERISTICS OF AN ESTUARY, DELAWARE BAY 1800–1973

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The oyster production of Delaware Bay has declined in two vast steps so that three rather striking and declining levels have come to pass. From 1880–1931 inclusive, the average annual production was 14,247,000 pounds a year; from 1932–1957 it was 7,951,000 pounds a year; and from 1959–1970 the average has been 859,000 pounds or 5.9% of the 1880–1931 level. Each stepwise decline occurred 3 years following the diversions of Delaware River water to New York City in 1929 and 1953. These diversions were strongly opposed by many American oyster biologists, particularly Thurlow C. Nelson and his colleagues, acting for the State of New Jersey. The predicted effects of these diversions upon Delaware Bay have come to pass. Doubtless, other fisheries production dependent upon low salinity estuarine waters, such as menhaden, have also been seriously diminished. Questions of equity could arise.

## GROWING OF HATCHERY REARED SPAT IN THE POTOMAC RIVER

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and

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In October and November, 1972, the Virginia Institute of Marine Science, Chesapeake Biological Laboratory and the Potomac River Fisheries Commission planted two acres in the upper Potomac River near Morgantown with cultchless spat. Densities were about 765,000 to 405,000 per half acre. Size ranged from approximately $^1/_2$"–$^3/_4$". To date about half of these spat have survived. Mortalities were associated with unusually low salinities.

## LOCOMOTION AND PHAGOCYTIC BEHAVIOR OF AMEBOCYTES OF THE HARD CLAM, *MERCENARIA MERCENARIA*, AS REVEALED BY TIME-LAPSE CINEMICROGRAPHY

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Two principle types of amebocytes of the hard clam, the large and small granulocytes, were studied with phase-contrast and interference phase-contrast (Nomarski) optics. Cells were taken from blood sinuses of the posterior adductor muscle and placed on clean cover slips in a moist chamber for five minutes. Then the cover slips were fastened to slides by a vaseline seal which prevented desiccation of the preparation during prolonged filming.

Small granulocytes were relatively active and usually flowed in unidirectional patterns. Cells moved by rapid extensions of ectoplasm. Granules of the endoplasm rapidly flowed in the direction of the advancing ectoplasm. Much movement of endoplasmic granules was evident even when cell locomotion temporarily ceased. Mitochondria were highly plastic and usually took the form of large blunt granules and would stretch into long sausage-shaped structures for brief intervals. Cells adhered firmly to the cover slips. This was most evident in the posterior portion of the cell as strands of ectoplasm would stretch, then suddenly release contact with the cover slip in order to "catch up" with the main portion of the cell.

Large granulocytes adhered to cover slips as large, flat cells. Granules appeared as distinct mounds and ridges when viewed with Nomarski interference phase optics. No motion of these cells could be detected by direct viewing. Time-lapse studies at 30 frames per minute (fpm) revealed an extremely active waving motion of the ectoplasmic border of the cell. Although large granulocytes had fewer granules than small granulocytes, all granule types were in constant motion in the endoplasm. Granules were always confined to a small area around the nucleus. This large cell does move at a very slow rate by a sliding motion.

Boiled, washed yeast cells were added to preparations of granulocytes and ensuing phagocytosis was filmed at 40 fpm. Small granulocytes rapidly flowed into a cluster of yeast cells. Ectoplasmic extensions of the granulocytes flowed rapidly in between and around until all the yeasts were incorporated as phagosomes. The latter consisted of a membrane wall that surrounded a clear area in which the yeast cell was contained. Film records revealed as many as eight to ten yeast cells engulfed within a few minutes.

Large granulocytes phagocytized yeast cells by enveloping them with wave-like extensions of the outer ectoplasm.

## INCREASING EARNINGS AND PRODUCTION IN THE OYSTER INDUSTRY OF PRINCE EDWARD ISLAND

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The oyster industry of Prince Edward Island has been an important part of that Province's tradition and economy since the mid-1800's. In recent years, annual oyster production has been 20,000 to 30,000 boxes (a box contains $1^1/_4$ standard U. S. bushels), and, along with fishermen's earnings, is trending downward. About 200 fishermen use hand tongs to harvest most oysters from 100 to 150 acres of public grounds in various estuaries.

The biological and ecological potentials for

increasing both quantity and quality (based on shell shape) of oysters are enormous because (1) adequate spatfall occurs practically every year, (2) growth increments of oysters range from $^3/_4$"–$1^1/_2$" a year, (3) survival rates of oysters are high, (4) at least 200,000 boxes of unharvested stocks of small oysters grow either in deep water or on poor shallow grounds, (5) several million bushels of oyster shells lie in buried deposits, and (6) about 1,000 acres of otherwise barren grounds possess favorable environmental features for producing high quality oysters.

Use of large vessels to transplant both oysters from unharvested stocks and shells from deposits and spread these on barren public grounds is recommended. In 1973 the Province's first oyster vessel, a 43-foot catamaran, was constructed. It transplanted and spread 34,000 boxes of oysters on 68 acres of grounds. Earnings of each fisherman are expected to rise steadily from an annual average of $2,300 and reach about $4,550 after the transplanting program has been underway a few years. Earnings of local buyers and mainland wholesalers should rise significantly. Overall oyster production should more than double. Use of the vessels may not always be required because the newly-established oyster beds will self-perpetuate themselves with regular annual spatfall, even under intense harvesting pressure.

The expenditures to finance the oyster rehabilitation program should be exceeded many times by monetary benefits to the fishermen. Cost-to-benefit ratios should eventually exceed 1:20, as costs involve only the transplanting of existing live oysters and shells.

The author developed and implemented this program during a one-year period, 1972–73, while engaged as oyster consultant to the Provincial Department of Fisheries.

## DECLINE OF *MINCHINIA NELSONI* IN MARYLAND WATERS OF CHESAPEAKE BAY

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Prevalence of *Minchinia nelsoni*, a haplosporidan pathogenic to oysters (*Crassostrea virginica*), steadily decreased from its highest level in 1964 (average of 19.6% prevalence for all areas sampled) until the cessation of regular sampling in 1972. At this time *M. nelsoni* was not observed in oyster tissues from any of the areas sampled. After Bay-wide sampling was discontinued, only oyster tissues from 9 areas in the Manokin River, a southern Maryland, Eastern Shore tributary to Chesapeake Bay, were examined with any regularity. In spite of the reduction in sampling and histological examinations, there are sufficient data to indicate that *M. nelsoni* either disappeared or became inactive at least 2 years prior to Hurricane Agnes. This information does not support the common claims that the storm drove *M. nelsoni* out of the Maryland waters of the Chesapeake Bay.

## PHARMACOLOGY AND CHEMISTRY OF NATURAL GUMS USED AS BINDERS IN FOODS FOR MARICULTURE

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The chemistry and pharmacology of extracts from marine plants such as agar, carrageenan, furcelleran, and alginates are briefly described. these extracts and other products derived from them have many pharmaceutical uses; for example, as anti-coagulants, connective tissue growth enhancers, bulk laxatives, and coagulants. The chemistry of these extracts, the structure of the polysaccharides, and the variations, both physical and chemical, are dependent on the types of plants and the kinds of salts present. The possible effects of these compounds, in the form of binders and capsules, when fed to certain marine animals are discussed.

## COOLING METHODS FOR SOFT-SHELL CLAMS

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Maryland soft clams harvested during the warm summer months are subject to quality

deterioration due to bacterial growth. Cooling of the clams has been suggested as a possible solution.

This investigation was designed to develop data from which rational comparisons of various cooling techniques could be made. Three sources of cooling were investigated: ice, dry ice and mechanical refrigeration. These sources were utilized in several different sizes and types of systems under conditions of both natural and forced circulation. Various types of containers were tested to determine their effect on cooling rate.

Results of the experiments were used to derive cooling curves for various combinations of equipment and cooling source. Ice and dry ice quantities, equipment descriptions and power requirements are discussed.

## THE ROLE OF *SERRATIA MARCESCENS* IN THE COLORATION OF "RED" OYSTERS AND SOFT-SHELL CLAMS

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A study was made to determine the role of a chromogenic strain of *Serratia marcescens*, isolated from shucked osyters, on seasonal red coloration of shellfish. This event reduces the market acceptability of affected shellfish. Experiments were designed to test effects of the organism in live and shucked oysters and soft-shell clams. Live clams and oysters were held overnight in water of varying temperatures containing *Serratia* concentrations of $5.6 \times 10^6$ per ml. The mollusks were then opened and examined for coloration. The live clams were tinged pink in the gills and superficial tissues, but the digestive glands and shell liquor did not differ from unexposed controls. After 5 days refrigeration the shucked clam meats showed a pinkish-orange liquor, had a putrid odor and were of unacceptable quality. Oysters similarly treated showed no coloration and after 5 days refrigeration the meats were still in good condition. It was concluded that *S. marcescens* plays no significant role in the coloration of marketable quality shellfish.

## A LAWSUIT (ENVIRONMENTALLY ORIENTED) BROUGHT AGAINST MECHANICAL CLAM HARVEST IN WASHINGTON STATE WITH A HANKS-TYPE HARVESTER

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Harvest of Eastern soft-shell clams on the intertidal flats of Skagit Bay by a Hanks-type harvester was halted by an injunction issued against the clam harvester and the Washington State Department of Fisheries. Initial basis for the injunction was fear of damage to a high intertidal rush used for food by wild fowl located above the clam beds. Trial was held and the Court ruled that the clam harvest operator had failed to comply with the terms of the Washington State Shorelines Management Act, and that mechanical clam harvest was both a substantial development and dredging in terms of the Washington law. He further ruled that the Department of Fisheries had failed to consider terms of the Washington State Environmental Policy Act of 1971 and that the clam harvest permit was not valid.

This trial and action have potential for major impact on the Washington shellfish industry and it currently appears that it may greatly complicate the management regulation and conduct of shellfish harvest in Washington State.

## NSA PACIFIC COAST SECTION

### GENETIC VARIATION IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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*University of Washington*

The genetic variation that has been investigated is shown by electrophoretic separation of proteins (enzymes) on starch gels. This procedure allows an analysis of single gene differences between individuals and will give an indication of the genetic diversity within and between populations.

Eleven enzyme (protein) systems have been examined at our laboratory reflecting twenty loci; seven loci were monomorphic, eight were polymorphic, and five remain unresolved. The eight polymorphic loci found in the fifteen resolved loci (53.3%) indicates that *C. gigas* is a highly polymorphic species. This high degree of genetic variation within a population should provide material for genetic improvement of the species.

### APPLICATION OF SPANISH MUSSEL CULTURE TECHNIQUES IN PUGET SOUND, WASHINGTON

Linda Chaves and Kenneth K. Chew

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*University of Washington*

Mussel culture in Spain is of the floating raft type which has a high yield per unit area. Manual labor plays a large role though in the growth and preparation of the finished product. Ropes are thinned and harvested manually. Prior to marketing, the mussels must undergo depuration or canning which is not yet fully automated there. Without modification this would not be economically sound in the United States.

A pilot study is being conducted in Puget Sound to determine the biological possibility of a commercial industry. Natural setting, substrate for seed collection, and growth are several of the factors being observed. During the past eight months of the study a major set has occurred at only one station and prior foulding of the ropes has been observed to be a deterrent to successful setting. Of the three types of substrates being used, manila, sinclove, and oyster strings, the sinclove appears to be the most promising.

### PROGRESS IN CENTRAL CALIFORNIA SHELLFISH SEED PRODUCTION

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*Moss Landing, California*

International Shellfish Enterprises, a four year old California mariculture company, is presently expanding facilities to present for sale large quantities of oyster and clam seed to be available in the early spring, 1975. Using hatchery reared seed, International is growing oyster seed, primarily *C. gigas*, to a size of 1″ for use by shellfish growers throughout the world. Clam seed of 3–7 mm will also be available for planting. Production of these large quantities is possible by use of a hatchery and nursery tank farm using the warm water from a Moss Landing power plant.

### THE ASSESSMENT OF SUBTIDAL GEODUCK CLAM POPULATIONS BY VISUAL AND PHOTOGRAPHIC TECHNIQUES

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Since 1967 the Washington State Department of Fisheries has been surveying subtidal clam stocks in Puget Sound with SCUBA divers. Geoduck (*Panope generosa*) stocks are evalutated by visual counts of siphons or siphon holes (shows). The number of geoducks detected by divers using the visual method varies considerably depending on how well the clams "show" when the surveys are made. Preliminary obser-

vations from nine plots showed that the percentage of the true population detected in our visual surveys could vary from as low as 26 to a high of 92. In more detailed studies, the percentage detected was low during the cold winter months and high during spring, summer, and fall, and averaged approximately 40% from year-around monthly samples. The percentage detected in underwater photos is also highly variable, and is subject to similar errors. The montly "showing factors" have been used to refine our diver survey counts and the total Puget Sound subtidal geoduck population estimate. The estimate for the 33,992 acres surveyed thus far is 114,700,000 clams.

Surveys with underwater TV or cameras mounted on sleds, tripods, or other devices which come in contact with the bottom should be done with a knowledge of the effects of mechanical disturbance on the "showing factor". Geoducks at certain times of the year are extremely sensitive to mechanical disturbance and will withdraw their siphons from the substrate surface at the slightest contact of divers or equipment with the bottom. We have successfully surveyed geoducks with underwater TV and have observed large numbers of these clams in water as deep as 240 feet. Mechanical disturbance was kept to a minimum by keeping the camera and all other equipment suspended off bottom on cables.

This study demonstrated that useful survey information on the populations of marine benthic organisms can be obtained with visual or photographic techniques, but the surveys should be done with knwoledge of the percentage which can be detected and the effects of equipment or divers on that percentage.

## GENETIC MANIPULATION AND BREEDING IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

William Hershberger and Kenneth Chew

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*University of Washington*

As part of the Sea Grant program to investigate the summer mortality in Pacific oysters apparently associated with *Vibrio* species of bacteria, a genetics study has been initated to investigate the selective breeding of the oyster for resistance to the disease. Results on single gene differences demonstrate a high degree of genetic variation on which breeding can be based, but no genetic differences have been shown between the various populations which can be correlated to their historical mortality level.

However, laboratory experiments in which oysters were challenged with a mortality-inducing situation and a large portion killed indicated that some individuals with a particular phenotype survived better. Although these results are preliminary, there is an indication that at least one gene can be used to "mark" slightly more resistant oysters. With markers such as this, more resistant individuals can be chosen for a breeding stock to improve the resistance of the total population used for marketing.

Other characteristics desirable to the oyster grower that can also be improved through breeding and the types of genetic manipulation necessary will be discussed.

## PLANTING HATCHERY SPAWNED MANILA CLAMS (*VENERUPIS JAPONICA*) IN PUGET SOUND BEACHES

Chris Jones and Kenneth K. Chew

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*University of Washington*

Hatchery spawned Manila clams averaging 3.7 mm in length were planted in four Puget Sound locations in the spring of 1973 to determine the feasibility of rehabilitating potential clam producing beaches that do not currently have commercial densities of clams. Heavily dug public beaches may also be repopulated in this way. The variables of planting density and tidal height were examined with regard to growth rate and mortality. Plots were planted with densities of 300, 600, 1200, and 2000 clam seed per square meter with an unplanted control plot. Samples were taken at two weeks, thriteen weeks and twenty-five weeks subsequent to planting. Two of the four plots were unsuccessful in that there was almost no recovery of planted clams after thirteen weeks. It is unknown whether the failure was due to movement of the clams or to mortality caused by predation and physical factors. The other two

plots were more promising with recovery ranging up to 140 clams per square meter in December with a size averaging 23 mm. Recovery was higher and the clams were faster growing at the +2 level than at the +4 level. Absolute numbers of clams recovered increased with increasing planting density but the percentage recovered dropped sharply. The possible reasons for this outcome were discussed.

## *CRANGON NIGRICAUDA* AND *CRANGON FRANCISCORUM* IN YAQUINA BAY, OREGON

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The distribution, reproduction, and growth of *Crangon nigricauda* and *Crangon franciscorum* in Yaquina Bay, Oregon are described from the examination of 8,244 *C. nigricauda* and 4,568 *C. franciscorum* collected by beam trawl at bimonthly intervals during Dec. 1970 through Feb. 1972.

The distribution of *C. nigricauda* and *C. franciscorum* is generally related to water temperature and salinity. Both species exhibit a wide tolerance for temperature (5.2–16.5 C for *C. nigricauda*; 5.3–21.5 C for *C. franciscorum*) and salinity (≥19 ppt for *C. nigricauda*; 0.2–34.4 ppt for *C. franciscorum*). Between species, *C. nigricauda* displays a preference for cooler temperature and higher salinity than does *C. franciscorum*. Within species, variations in response to temperature and salinity changes were observed between size and sex groupings.

The spawning season for both species is from Dec. to mid-Aug. *C. franciscorum* and *C. nigraicauda* ovigerous females disappear from the Bay in August and September, respectively. Berried females were collected in waters ranging from 6.8–12.9 C for *C. nigricauda* and 6.8–19.2 C for *C. franciscorum*, and with salinity of ≥25.4 ppt for *C. nigricauda* and ≥14.6 ppt for *C. franciscorum*. Both species exhibit bimodal spawning periods with larger females initiating the spawning season. Fecundity is correlated ($R^2 \geq .91$) with total length (TL), with the mean and range being 4,016 and 2,393–7,000 for *C. nigricauda* and 3,528 and 1,923–4,764 for *C. franciscorum*.

Growth of young was defined by the regression equations: Y = −6.04 + 0.76 (TL) summer and Y = +7.79 + 0.95 (TL) winter for *C. nigricauda*. Only a summer growth rate estimate was attainable for *C. franciscorum*: Y = −25.44 + 1.37 (TL). Both species exhibited differential growth after attaining sexually recognizable sizes, with females being 8–10 mm TL larger than males at maturity. Females apparently live a maximum of 1½ yr and males not more than 1 yr.

## AMINO-ACID REQUIREMENTS OF THE DUNGENESS CRAB

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The amino acid requirements of the Dungeness crab, *Cancer magister*, were investigated with radiometric techniques. Seven crabs were tested in all. Five were injected with glucose-$^{14}$C(U), one with glutamic acid-$^{14}$C(U), and one with phenylalanine-$^{14}$C(U). The following amino acids were determined to be nonessential; alanine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, proline, serine, tyrosine (with phenylalanine present).

The glutamic acid injected crab showed no labeled proline though all others expected to be labeled were. The phenylalanine injected crab showed labeling on glutamic acid, alanine, and tyrosine.

These results are similar to those reported for other arthropods.

## RECENT FINDINGS ON THE SUMMER DISEASES OF PACIFIC OYSTERS

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Although the 1960's was a decade in which significant Pacific oyster mortalities occurred in specific bays along the Pacific Coast, the first four years in 1970 did not demonstrate a similar pattern. Some mortalities did take place during these years, but they were very low in incidence and were considered to be the normal background mortalities due to predation or other

natural causes. During the summer of 1974, there was a general mortality of up to 15–20% in specific areas of Willapa Bay and Rocky Bay in central Puget Sound in late July and August.

Laboratory studies and evaluation of field data have shown that high temperature and high nutrient levels in water are associated with oyster mortality. Under experimental conditions, oysters dying in high temperature water have been shown to carry large numbers of bacteria in the heart, blood, and pericardial fluid. Vibrios (particularly *V. anguillarum*) isolated from dying oysters have been shown to cause mortalities when introduced into healthy oysters under high temperature conditions by either active or passive inoculation. Detectable ammonia levels are present in the dying and dead oysters.

Field studies have shown that total bacterial counts and mesophilic vibrio counts rise with the onset of summer water temperatures. Percent incidence of *V. anguillarum* is higher during summer months also.

The Willapa Bay and Rocky Bay mortalities porvided the first opportunity to test the hypothesis based on laboratory findings that disease and death were due to bacterial infection. Dead or moribund oysters were found to contain bacteria in the heart blood. Apparently healthy oysters from the mortality area were also found to contain bacteria, though healthy oysters usually have sterile heart fluids. $NH_3$ was also detected. Vibrios from these oysters were shown by inoculation experiments to be virulent towards healthy oysters and could be recovered from the heart fluids. The initial findings thus support the bacterial infection hypothesis, and work is continuing to confirm or refute its validity.

## SHELL GROWTH OF BUTTER CLAMS AND LITTLENECK CLAMS NEAR MADRONA BEACH ON CAMANO ISLAND

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A possibly unique abnormality of unknown origin in butter clams (*Saxidomus giganteus*) and littleneck clams (*Protothaca staminea*) from a localized area on Camano Island, Washington is being investigated at the College of Fisheries, University of Washington. The nature of the abnormality is a scalloping or indentation along the ventral margins of the valves which sometimes extend as grooves from the ventral margins of the valves which sometimes extend as grooves from the ventral shell margin to the umbo. Outside of a several hundred foot horizontal distribution of the abnormal clams, few traces of the shell defect are found. The same general area which is inhabited by highest percentages of abnormal clams is also washed by a freshwater seepage which originates at about the two foot tide mark and below. The problem of determining the cause of the abnormality is being approached by comparing the affected area with unaffected areas, mapping the abnormal clam distribution and seepage range, histological examination of clam tissue, examination of clams for trace metals, analysis of properties and contents of the seepage, and a literature search.

## ENERGY EFFICIENCY IN THE PACIFIC OYSTER INDUSTRY[1]

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The energy efficiency of the Pacific oyster industry in Willipa Harbor was estimated by converting all manpower, fuel, vessel, machinery and other inputs of production of a standard firm into kcal and comparing this to kcal in the oyster meat. On an annual basis the ratio of kcal output to kcal of input was estimated to be .21.

This level of efficiency was similar to that of other fishery industries of Washington and to other U. S. agricultural protein production systems, which was surprising since oyster culture requires neither artificial feeding nor traditional fishing. The diesel fuel used in planting, transplanting and harvesting accounted for 85% of the input energy.

[1] Research supported by a National Science Foundation Grant for Undergraduate Research, GY-11242.

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# LIVING MARINE ANIMALS IN A SHIP'S BALLAST WATER

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## ABSTRACT

*Seawater ballast taken aboard in Japan, 1 May, 1973, was sampled when the ship berthed in Australia two weeks later. It contained living copepods, amphipods, ostracods, unidentified crustacean larvae, polychaetes (larvae and adults) and chaetographs. No mollusc larvae were found although most physical-chemical conditions of the ballast water were optimal for larvae of Pacific oysters.*

## INTRODUCTION

Samplings of ballast water in two holds of a ship were made on 15 May, 1973, eight hours after it berthed at Eden in Twofold Bay, New South Wales, Australia (S. Lat. 37°05′; E. Long. 149°55′). It had just completed a 14.5-day passage without cargo from Tagonoura, Japan (N. Lat 35°12′; E. Long. 138°42′). The ship's officers stated that the ballast water was boarded partly in Tagonoura Harbour and partly at sea during the first 4 days of the passage.

## CHARACTERISTICS OF BALLAST WATER

Results of water sampling at the surface and bottom of both ballast holds and at the surface of Twofold Bay adjacent to the ship, are tabulated below (Personal Communication, 1973, Mr. E. A. Scribner, chemist).

## PLANKTON OF BALLAST WATER

At the same time, two bottom-to-surface vertical hauls through the ballast water were made in each hold with a standard cone-type plankton net (length, 1.8 m; mouth diameter, 0.5 m; mesh apertures, 88 u). One 10 m-long, horizontal haul at a depth of 0–3 m was also made in Hold No. 4.

Pocket-magnifier inspection of the fresh catches revealed numerous, swimming organisms; crustaceans being the most conspicuous. Microscopic examination after formalin preservation showed that the crustaceans included planktonic copepods of several species (0.5–3 mm long), amphipods (5 mm long), an ostracod and juvenile stages of various unidentified groups. The most abundant organisms were eyed polychaete larvae that had contracted into spherical forms (0.2 mm diam.). Adult benthic polychaetes (whole and fragments, 1–5 mm long) and chaetognaths (5–8 mm long) were also present in small numbers. The hauls were remarkably uniform as regards total numbers of animals caught and relative abundance of the various types composing the catches.

## DISCUSSION

It is assumed that all these organisms were pumped aboard with the ballast water 10–14 days previous to the samplings. On that assumption the results indicate that conditions in the ballast water (Table 1) were favourable to survival and that the plankters did survive an inter-hemisphere transport through more than 70 degrees of latitude before they were discharged with the ballast water into a new environment.

Presumably most of these organisms could accommodate to the small salinity change (maximum, 1.6 0/00) when discharged into Twofold Bay on 15 May (see Table 1). But the drastic temperature change (approximately 10°) would

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TABLE 1. *Physicol charocteristics of woter samples.*

| Sample Point | Temperature (°C) | Salinity ( ) | Dissolved Oxygen (mg/l) | Dissolved Oxygen (% sat'n.) | (pH) |
|---|---|---|---|---|---|
| *Ballast Water:* | | | | | |
| Hold No. 2 | | | | | |
| surface | 25.6 | 35.1 | 6.5 | 100 | 8.1 |
| bottom (15 m) | 25.4 | 35.3 | 6.5 | 100 | 8.0 |
| Hold No. 4 | | | | | |
| surface | 26.0 | 34.3 | 6.3 | 97 | 8.1 |
| bottom (12 m) | 25.8 | 35.1 | 6.5 | 100 | 8.0 |
| *Twofold Boy:* | | | | | |
| surface | 16.7 | 35.9 | 7.8 | 103 | 8.1 |

reduce their metabolic rates and could well be directly or indirectly lethal to most or all of them. At other seasons and in other harbours the discharge of ballast water might be less shocking to organisms it contains.

The Twofold Bay study supports the often-quoted conjecture of Peters and Panning (1933) that ballast water is a vector for long-dustance dissemination of aquatic organisms and may be responsible for mysterious appearances of exotic species.

Pacific oysters appeared mysteriously in New Zealand recently (Dinamani, 1974) and may have been introduced in this way because, except for salinities, the tabulated data show that ballast water conditions were close to optimum for larvae of that species (Fujiya, 1970; Quayle, 1969).

## ACKNOWLEDGMENTS

This study was assigned jointly to me and my then colleague, Mr. E. A. Scribner (chemist), by the Director of the Fisheries Branch of the New South Wales Chief Secretary's Department in which I was employed in 1972–73. I thank Mr. Scribner for personal communication of data and for helpful discussions of the draft of this paper.

The plankton collections are deposited with Dr. W. B. Malcom, Chief Biologist, New South Wales State Fisheries, Sydney, N.S.W., Australia.

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*Proceedings of the National Shellfisheries Association*
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# CULTCHLESS SETTING OF EUROPEAN OYSTERS, *OSTREA EDULIS*, USING POLISHED MARBLE[1]

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*ABSTRACT*

*Early experiments indicate that polished marble may have ideal characteristics as a substrate in cultchless setting of oysters. It is highly attractive to setting larvae and is hard and smooth enough to permit removal of juveniles without excessive damage. Techniques are described for the setting process.*

Highly polished marble appears to possess ideal characteristics for use in producing cultchless oysters. It is not only attractive to setting larvae but is hard and smooth enough to permit easy removal of spat after metamorphosis. This note reports some encouraging early results using polished marble as a setting substrate.

The idea of using polished marble as a setting substrate occurred to us after observing the behavior of larvae while testing a variety of substrates for use in the cultchless process. First, we observed that apparently mature eyed larvae in 100-gal. polyethylene tanks would delay metamorphosis for several days if no suitable substrate was presented. Only occasionally have we observed a mass setting of larvae on the sides of polyethylene tanks. If, however, a molluscan shell (oyster, sea or bay scallop) was added to the culture, the shell would be blackened with set very rapidly. Molluscan shells, however, are a poor choice of setting substrate because the irregular surfaces make it difficult to remove the juveniles and obtain the cultchless form.

Other substrates were tried, *i.e.*, glass, various plastics, and "Mylar" sheets (Dupuy, 1972). Occasionally, we have obtained some set on "Mylar", but neither "Mylar" or the others are highly attractive to setting of European oysters. It appears that some property associated with a molluscan shell (possibly calcium carbonate) is highly stimulatory to setting larvae. Thus, the ideal setting substrate in the cultchless process would appear to be a calcium carbonate-derived material that is hard and smooth, permitting easy removal of metamorphosed oysters. Marble, which is limestone recrystallized under heat and pressure, would appear to have these characteristics and when mature larvae are exposed to smooth marble, the response is dramatic with heavy sets being achieved in short order.

*Setting procedure with European oysters.*

Larvae ($1 \times 10^6$) are reared to maturity in large (100-gal) polyethylene vats. After the mature larvae have achieved eyespots, a small scallop or oyster shell tied to a string is introduced to monitor the ability of the larvae to set. As soon as the small shell is heavily blackened with set, the entire batch of larvae is transferred to a setting bath. Setting baths should be sufficiently shallow to permit easy addition and removal of cultch surface.

[1] Ira C. Darling Center Contribution No. 90, and was supported by NOAA Sea Grant Project No. 04-5-158-39.

In the setting baths, we attempt to manipulate conditions to obtain a massive set in as short a time as possible. This allows an efficient manipulation of cultch surfaces and maximizes the percentage conversion of larvae to spat. Since it has been demonstrated that adult oyster metabolites and increased temperatures may stimulate setting in American oysters (Veitch and Hidu, 1971; Lutz, *et al.*, 1969), we raise the temperature to 24–26°C and add several gallons of sea water from adult European oyster conditioning baths. Cultured algae are added in liberal amounts. With a young vigorous brood of larvae which have delayed setting, these conditions have produced a heavy set on the marble surfaces within one to several hours. Intensity of setting on the marble slabs is closely monitored to avoid a total blackening of the marble surface which might result in the smothering of metamorphosing spat.

The marble slabs are then moved to separate culture baths at 24°C with adequate algal foods for a 24 to 48 hour period to allow the spat to produce a fan of new juvenile shell growth. Spat are then scraped off with a double-edged razor blade to produce cultchless juveniles. If spat are scraped off too early, before they achieve a small band of juvenile growth, they then appear to have difficulty in metamorphosing in the cultchless form. If they are scraped off too late, then excessive shell damage may result from removal.

We are now using polished marble exclusively in our procurement of cultchless oysters and have much to learn about optimal methods of presentation and removal of oysters. However, with our favorable early results, we feel that others should be aware of marble as a setting substrate and should experiment with it themselves.

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*Proceedings of the National Shellfisheries Association*
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# A GREGARINE-LIKE PARASITE ASSOCIATED WITH PATHOLOGY IN THE DIGESTIVE TRACT OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA*

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## ABSTRACT

*Histological examination of oysters, Crassostrea virginica, from Connecticut and Maryland waters showed that an amoeboid or gregarine-like parasite was associated with seasonal pathology in the digestive tract. Focal sloughing of cells of the columnar epithelium and clear zones, or halos, around individual parasites were observed only in the spring months. The specific pathological response was found during 1967 and 1968 in Connecticut, and in 1970 and 1973 in Maryland. Comparative studies with oysters from each location provided certain immature growth stages which resembled developmental forms of gregarines of the Nematopsis-Porospora group. It is tentatively suggested, that the parasite overwinters in hibernating oysters, undergoes vegetative growth in the spring when oysters resume feeding, and is cleared from the digestive tract in association with a transient host tissue response.*

## INTRODUCTION

The seasonal occurrence of an amoeboid organism in the digestive tract of the American oyster, *Crassostrea virginica*, was first reported by Newman (1971), who briefly described the parasite in shellfish collected from New Haven Harbor, Connecticut. Subsequently, similar or closely related organisms were discovered in oysters collected in April 1970 and 1973 from several tributaries of Chesapeake Bay, Maryland (Sawyer, Newman and Otto, 1973). Comparative studies on oysters from those two sources suggest that the amoeboid organisms are vegetative stages of an unknown species of gregarine. The severe focal cellular response and sloughing of columnar epithelial cells in the infected oysters appears to be similar to pathological conditions discussed by Ball (1951), who described new species of gregarines from marine crustacea. Further observations on the organisms found in *C. virginica* are presented in this report to illustrate probable life-cycle stages other than the amoeboid vegetative form.

Pathological conditions recognized in commercially valuable shellfish include two broad categories: those which are attributable to a specific pathogen and those which are characterized by a specific pathological response for which the causative organism is unknown. Sprague (1971) reviewed the principal pathological conditions recognized in oysters which, although widely recognized, often were characterized by the lack of an identifiable etiologic agent, *viz.*, "Denman Island Disease," "Amber Disease" and others. Sindermann and Rosenfield (1967) published a comprehensive review which summarized the principal shellfish diseases of known etiology, and Sawyer (1966) briefly summarized the taxo-

nomic status of amoeboid organisms from tissue and mantle fluid of *C. virginica*. The present report concerns both a specific pathological response and the documentation of the probable etiologic agent.

## METHODS

Histological sections of 1,337 oysters, *C. virginica*, collected from New Haven Harbor, Connecticut were examined during 1966 and 1967 (Newman, 1971). Monthly samples from rivers and tributaries of Chesapeake Bay, Maryland were similarly examined during 1970 and 1973. Tissue sections were stained with Harris hematoxylin and eosin, Fuelgen reaction with fast-green counter-stain, or the PAS (Schiff) procedure. Microscopic examinations were made of all specimens in each monthly sample and data were analyzed to determine whether seasonal variations were associated with histopathological findings. Water temperature and salinity were recorded at the time of all but one of the collections from Maryland (Table 1).

## RESULTS

Histological examination of oysters collected in Connecticut showed that an amoeboid parasite was present in the gut epithelium of 14 of 1,337 oysters (1%) (Newman, 1971). All infections in Connecticut oysters except for one in February and one in June were detected in the months of March and April. Subsequent observations on oysters collected in Maryland showed that a similar parasite was present only in April 1970 and 1973. The number of infected oysters in Maryland ranged from 1 in 25 to 7 in 25 (Table 1). In all of the infected oysters the parasites were restricted to the columnar epithelium of the digestive tract, always in localized foci, and never beyond the limit of the basement membrane. Oysters sampled in the areas listed in Table 1 were studied throughout the year and their generally healthy condition indicated that significant mortalities were not associated with the seasonal appearance of the parasite.

The smallest recognizable stage of the parasite was a spherical form found in localized areas of the intestinal epithelium (Fig. 1–2). Round forms had a distinct central sphere which stained deep red with the Feulgen reaction and was surrounded by a wide halo after staining with hematoxylin. Another stage resembled an emerging sporozoite (Fig. 3) which may have progressed to a pyriform body (Fig. 4) and developed to a trophozoite (Fig. 5). Trophonts, apparently in syzygy (Fig. 6), had an ovoid deutomerite and the elongate filamentous protomerite. Encysted forms, possibly young gametocytes (Fig. 7) were found infrequently. (See Newman, 1971, Fig. 7, for comparison.)

Specific pathological response in infected oysters was evidenced by sloughing of degenerate cells of the columnar epithelium or the displacement of healthy host tissue by trophonts or spherical forms. Proliferating asexual stages of the parasites were not observed in any of the oysters examined. Life cycle stages of the parasite found in the oysters resembled forms which typically have been illustrated for gregarine parasites of crustaceans and suggest that growth in molluscs possibly was atypical.

## DISCUSSION

Further observations on the occurrence of an apparent gregarine parasite in *C. virginica* are presented to extend its geographical range to

TABLE 1. *Source and prevalence of a gregarine-like parasite in Maryland oysters, crassostrea virginica.*

| Source | Date | Number Positive | Condition | T°/C | Salinity* |
|---|---|---|---|---|---|
| Chester River | Apr 1970 | 7/25 | Healthy | 5.74 | 11.3 |
| Choptank River | Apr 1970 | 5/25** | Healthy | 7.26 | 13.6 |
| Herring Bay | Apr 1970 | 2/26*** | Watery | 7.94 | 5.5 |
| Cedar Point Hollow | Apr 1970 | 1/25**** | Healthy | 4.20 | 13.5 |
| Manokin River | Apr 1973 | 2/25 | Healthy | — | — |

* ppt

** 2 with *Nematopsis ostrearum*

*** 1 with *Nematopsis ostrearum*

**** 19 with *Nematopsis ostrearum*

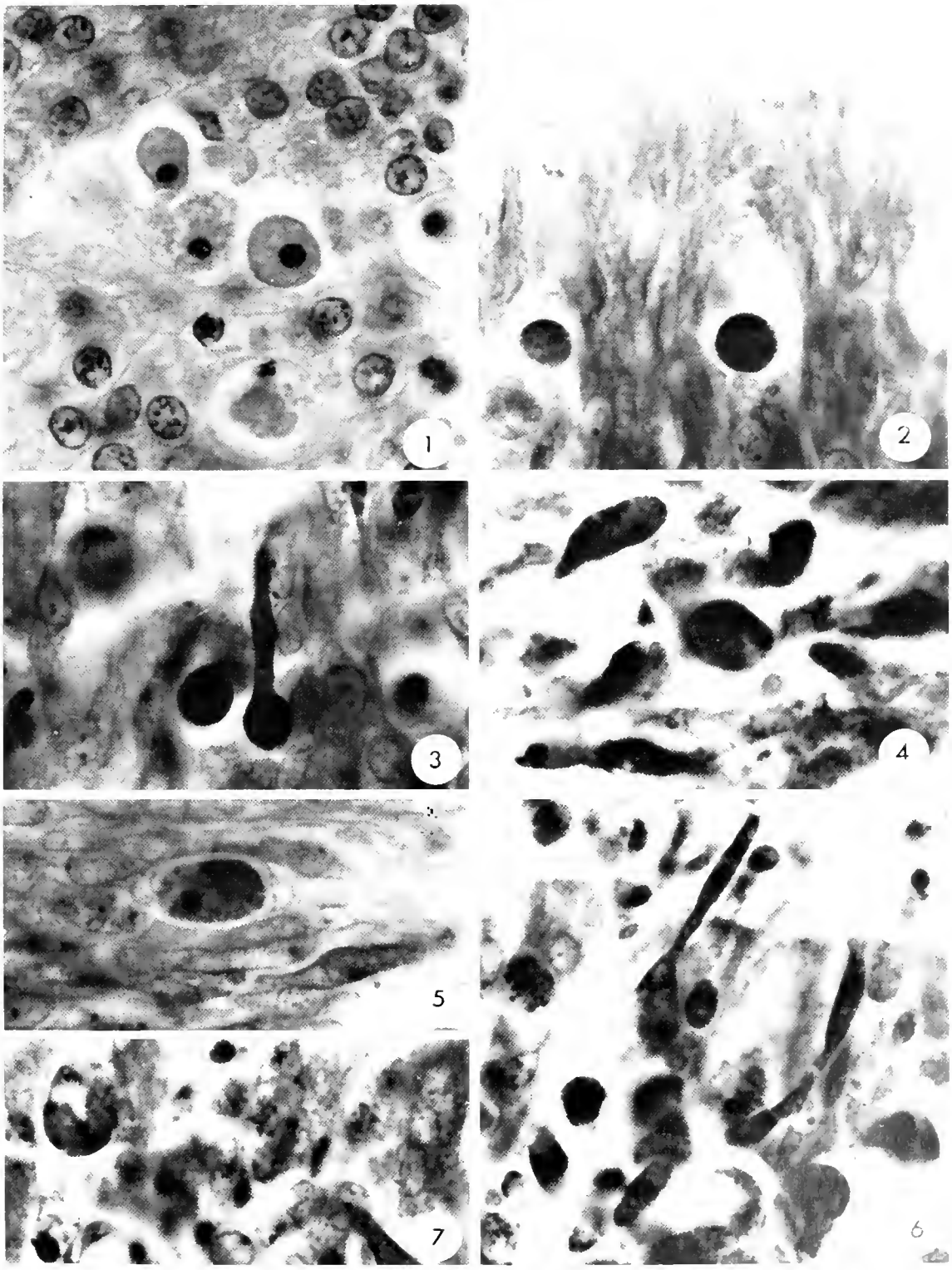


FIG. 1–7. *Photomicrographs of gregarine-like parasites in columnar epithelium of digestive tract of Crassostrea virginica, hematoxylin-eosin stain, ×1400.* FIG. 1–2. *Immature spores. Note host tissue response.* FIG. 3. *Sporozoite leaving spore.* FIG. 4. *Pyriform shape of sporozoite developing to trophozoite or sporont stage.* FIG. 5. *Mature trophont. Note absence of host response.* FIG. 6. *Paired trophonts in syzygy, apparently in process of sloughing into lumen. Note bulbous deutomerite and filamentous protomerite.* FIG. 7. *Encysted forms, probably a gametocyst.*

include Maryland and to amplify the original observations of Newman (1971). At the same time, we propose to redesignate the organism as gregarine-like instead of amoeboid as reported in the earlier publication. It is noteworthy that the pathological condition in the digestive tract was identical in both Connecticut and Maryland oysters. Furthermore, the location of the parasites among sloughing cells of the gut epithelium represented a host response which was similar, although not identical, to an earlier report (Ball, 1951) of intestinal pathology in crabs caused by several new species of *Nematopsis* and *Carcinoectes*. Until new information on the host-parasite relationship in oysters is obtained, we propose tentatively to identify the parasites as gregarines, possibly of the *Nematopsis-Porospora* type.

Mature spores were not detected in tissue sections of gill and palp epithelium of the infected oysters. According to Leger & Duboscq (1913), and to Hatt (1927a, 1927b, 1928), the spores of certain species of *Nematopsis* and of *Porospora* mature in the gills of hosts such as oysters, mussels, chitons, *etc.*, after infection by gymnospores from crustacean hosts. Typical life cycles have been elucidated for *Nematopsis ostrearum* Prytherch (1940), which develops in the oyster and mud crab, *Porospora gigantea* v. Beneden (1869), which develops in the lobster and the common mussel, and others. Typically, infection of the molluscan host is initiated by direct penetration of the gymnospores into cells of the gill epithelium and the pallial lobes of the palps, but not by penetration of the epithelium of the tegument or the digestive tract. In contrast, development in the crustacean host does occur in the digestive tract, beginning with sporulation and progressive development of sporozoites to trophozoites, syngyns, and finally, gametocytes. Photomicrographs of the life-cycle stages present in tissue sections of oysters from Connecticut and Maryland show developing parasites in stages of growth that usually are found in a crustacean host. We do not have a satisfactory explanation for our findings, but we propose that such development is atypical and incomplete. The absence of parasites and degenerative changes in the gut epithelium, except during March and April, suggest that the organisms probably overwinter in hibernating oysters, causing transitory tissue pathology in early spring; then either perish or undergo further development in an unknown second host species. The present stage of our knowledge suggests that atypical growth of the parasites induces transient sloughing of gut epithelium with progressive repair and recovery of the oyster host.

The apparent seasonality of the infection summarized in this report suggests that several interpretations may be proposed on a strictly speculative basis. We have considered the possibility that feeding oysters might have spores or sporocysts passing harmlessly through their digestive tracts during the months in which they feed and grow; such stages not necessarily belonging to species which normally parasitize oysters. Later, when oysters cease feeding during cold seasons, transient organisms might be trapped in the digestive tract when they would reside until feeding activity was resumed in the spring. During the winter interlude, such organisms could be trapped between crypts or villi of the intestine and transported from the lumen to the columnar epithelium by phagocytes. Finally, during the spring months of March and April, when we found active cell sloughing and growth stages of the parasites, transient host response would effectively remove the last vestiges of the overwintering protists. This hypothetical interpretation might account for the presence of immature stages of a gregarine which more appropriately would be expected to reside in the digestive tracts of crustacean hosts. Abortive growth of immature parasites in atypical host animals is well-documented in parasitological literature. Future reports on the same or related host-parasite interactions should extend the geographic range of this disease and perhaps yield new information on developmental stages which we did not observe. It is likely that the tissue response illustrated here for Connecticut and Maryland oysters will be recognized as an entity in much the same way as "Malpeque Bay Disease," "Amber Disease" and other pathologic conditions are diagnosed although the precise nature of the causative agent is uncertain.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the as-

sistance of Mrs. Janet Hammed, Maryland Department of Natural Resources, Oxford, Maryland.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1975*

# ENERGY PARTITIONING IN THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)

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## ABSTRACT

*Research was conducted to develop an energy budget for the American oyster, Crassostrea virginica (Gmelin), in culture. Caloric values of pseudofeces, feces, food ingested, food cleared and food assimilated were determined at three levels of algal (Phaeodactylum tricornutum Bohlin) concentration, $1.0 \times 10^5$, $5.0 \times 10^4$ and $2.5 \times 10^4$ cells·ml$^{-1}$ at 20° and 25°C; approximately 74–148 calories per 12 hours were used by the oysters. Oysters at the lowest food concentration showed the greatest amount of filtration, while oysters at the medium food concentration cleared the most food. In addition, the greatest amount of feces and pseudofeces were produced at the medium food concentration. There was little difference between the amount of energy assimilated at the high and medium concentration. The mean assimilation efficiency obtained from the three food concentrations was 67.6%, and the mean filtration rate (water pumped 6.2 ml·hr$^{-1}$ mg·dry tissue weight$^{-1}$) was consistent with other studies.*

## INTRODUCTION

This research was undertaken to develop an energy budget for the American oyster, *Crassostrea virginica* (Gmelin). An energy budget relates the intake of food by an organism to its subsequent utilization. To develop an energy budget, it requires assessing food intake, rejecta and the amount of energy utilized by the animal. The budget takes the form:

$$C = P + R + F + U \text{ (Crisp, 1971)}$$

where C = Consumption, P = Production, R = Respiration, F = Feces, U = Urine.

Some aspects of an energy budget for *C. virginica* have been studied. Oxygen consumption of *C. virginica* was reviewed by Galtsoff (1964). Walne (1972) determined the influence of current speed, body size, and water temperature on the filtration (water pumped) rates of two species of oysters. Dame (1972) reported for the first time the quantitative examination of growth rates in intertidal oysters and a simultaneous examination of respiration in relation to size and temperature. Tenore and Dunstan (1973) investigated the effects of different concentrations of mixed phytoplankton on the feeding and biodeposition rate of the American oyster to understand bioenergetics of filter-feeding herbivores. The significance of this budget resides in its application to mariculture.

## MATERIALS AND METHODS

### *Water*

Sea water for the oyster experiment was pumped from the Broadkill River, Delaware, at high slack water to assure salinity of 25‰ and low turbidity. This water was filtered through a cloth bag (5 $\mu$) and then vacuum filtered through a "Whatman 40" filter and finally passed through a 0.45 $\mu$ Millipore filter. Background count of particles per ml determined by a Coulter Counter was less than 500 per ml. The

range of pH was 8.0–8.1. Water temperature (20°C ± 0.5) for all experiments was controlled by a constant temperature bath and circulator.

*Algae*

Sea water for culturing algae was pumped into a settling tank at high slack water. After a few days, water was filtered through a 5 $\mu$ bag and "Whatman 40" filter. The water was enriched according to the method of Matthiessen and Toner (1966). After enrichment, a twenty liter carboy (filled to the 16 liter mark) was autoclaved at 18 pounds pressure and 125°C for two hours, cooled and inoculated with two liters of a 14 day old *Phaeodactylum tricornutum* (Bohlin) culture.

Caloric content of the alga was determined by two methods: 1) bomb calorimeter (Parr automatic adiabatic calorimeter), 2) quantitative dichromate oxidation (Standard Methods, 1965; Maciolek, 1962).

The conversion of mg COD/L (as obtained by wet oxidation) to calories per gram of sample follows Maciolek (1962). The conversion factor used was 1 mg $O_2$/L = 3.4 calories/L. The dried alga was ashed to constant weight at 600° in a muffle furnace (Parsons, *et al.*, 1961).

*Oysters*

Oysters were artificially spawned, reared and set in the laboratory. They were then held for 18 months in running sea water from the Broadkill River at ambient temperatures.

Oysters were randomly selected from the laboratory-reared stock and were allowed to acclimate and purge their guts at 20° C for 12 hours prior to each experiment. Each oyster was placed in a three-liter glass jar filled with two liters of filtered sea water. The oysters were placed on a pedestal so that feces would fall to one side of the jar and pseudofeces would fall on the other (Haven and Morales-Alamo, 1965).

Four oysters (approximately 6.8–7.2 cm in height and 27.7–30.9 gm wet weight) were used in each 12-hour experiment. These were supplied one of three levels of algal concentration: $1.0 \times 10^5$ cells/ml (high), $5.0 \times 10^4$ cells/ml (medium), or $2.5 \times 10^4$ cells/ml (low). After an experiment, each oyster was sacrificed and dried to a constant weight in an analytical oven (at 80°C). Thirty experiments were performed.

A Coulter Counter, Model B, was used to count the number of algal cells cleared by the oyster. A one hundred micron aperture tube was used. The method to obtain a specified amount of algal cells and to maintain a constant amount of cells throughout an experiment was described in Langefoss (1973).

Feces and pseudofeces were collected by pipetting immediately upon production and placed in a collecting jar. Desalting was accomplished using a Dow hollow fiber beaker dialyzer Model b HFD-1. Water was passed through the dialyzer for 40 minutes, after which the sample was dried to constant weight at 80° C. The sample was then placed in a Ca $SO_4$ filled dessicator and cooled to room temperature (22–23° C). This weight represents total feces or pseudofeces production for the animal for 12 hours. Caloric determinations of feces and pseudofeces were made by the quantitative dichromate oxidation method (Maciolek, 1962; Standard Methods, 1965).

Pseudofeces production was subtracted from the amount of algal material cleared with the difference as a measure of ingestion. Assimilation was estimated by subtracting fecal production from ingestion estimates. All data were converted to energy units based on calorimetric analysis.

The total number of cells removed was divided by the cells/ml (food concentration) to yield the number of milliliters of water filtered (water pumped). Cell counts were made with a Coulter Counter.

## RESULTS

*Oysters*

Energy partitioning by oysters at three levels of algal concentration, $1.0 \times 10^5$ cell/ml (high), $5.0 \times 10^4$ cell/ml (medium), and $2.5 \times 10^4$ cell/ml (low) is shown in Figure 1. Each mean represents at least 24 oysters. Oysters at the medium food concentration cleared the most food (Fig. 1). In addition, the greatest amount of pseudofeces and feces was produced at the medium food concentration. However, there was little or no difference between the energy assimilated at the high and medium concentration.

The percentage assimilation and amount of filtration ($L \cdot hr^{-1} \cdot$gram dry tissue weight$^{-1}$) is

present in Figure 2. Determination of filtration rates was described in detail by Langefoss (1973). Oysters at low food concentration showed the greatest amount of filtration. However, the rate of filtration and the percentage assimilation were inversely related between the medium and high algal concentration (Fig. 2).

Table 1 summarizes the various aspects of energy partitioning in the oyster. The mean assimilation efficiency obtained from the three food concentrations was 67.6%.

Students' 't' test (Sokal and Rholf, 1969) was performed on data comparing the three food concentration levels with one another for each part of the energy budget (Table 2). The notation A-B, A-C, and B-C was the mean of calories cleared; for example, at a concentration of $1.0 \times 10^5$ cell/ml (A) when compared with calories cleared at concentration $5.0 \times 10^4$ cell/ml (B), etc., there were significant differences for the

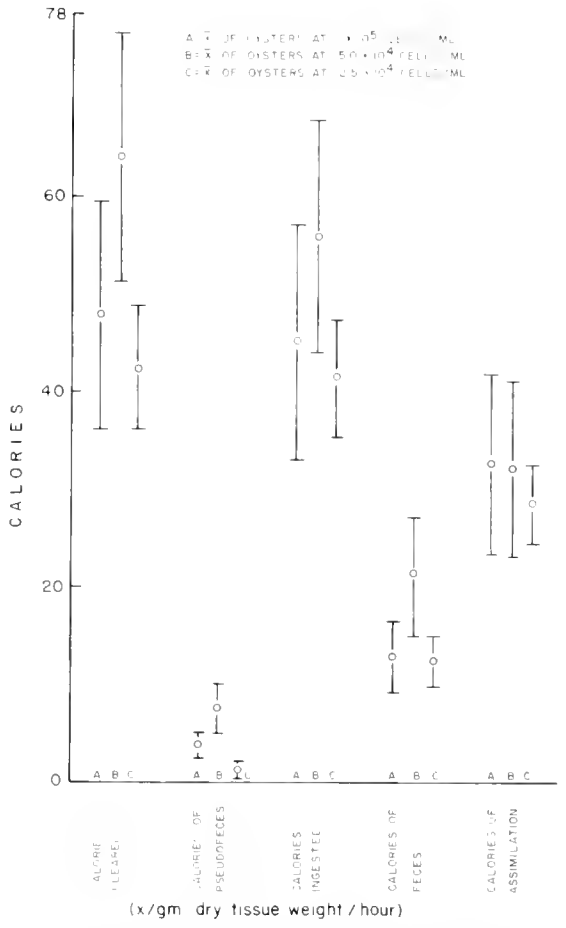


FIG. 1. *Plot of calories cleared, calories ingested, calories of pseudofeces, calories of feces, and calories assimilated. Bars represent the 95% confidence interval. N = 24–30 oysters.*

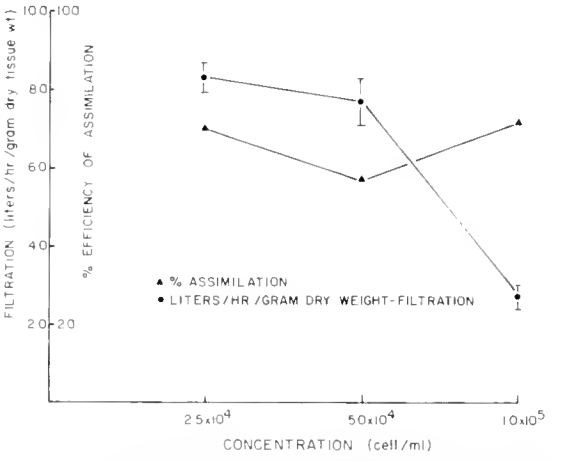


FIG. 2. *Percentage assimilation and filtration rates at three levels of food concentration. Assimilation was calculated by dividing the amount of food retained by the oyster by the amount of food ingested.*

TABLE 1. *Summary of energy partitioning at the three levels of food concentration (±95% confidence interval).*

| Algal Concentration | Mean calories/gram dry weight/hr. | | |
|---|---|---|---|
| | High | Medium | Low |
| Cal. of pseudofeces | 3.91 ± 1.36 | 7.25 ± 2.51 | 1.11 ± .84 |
| Cal. of feces | 13.01 ± 3.61 | 21.25 ± 5.84 | 12.47 ± 2.84 |
| Cal. ingested | 43.99 ± 11.95 | 56.75 ± 12.18 | 41.19 ± 6.08 |
| Cal. cleared | 47.90 ± 12.29 | 64.00 ± 12.84 | 42.30 ± 6.32 |
| Cal. assimilated | 30.98 ± 9.22 | 35.50 ± 8.99 | 28.72 ± 4.1 |
| % efficiency of assimilation | 70.42 ± 5.42 | 62.55 ± 8.42 | 69.73 ± 4.06 |
| Filtration liter·hr$^{-1}$·gm dry tissue wt.$^{-1}$ | 2.78 ± 0.59 | 7.83 ± 2.37 | 8.39 ± 1.24 |
| Dry oyster tissue wt. (grams) | 0.422 | 0.246 | 0.211 |
| Whole oyster wt. (grams) | 29.13 | 30.93 | 27.74 |

TABLE 2. *Comparison of calories at the three food concentration levels.*

| ('t' test) |
|---|
| Total calories cleared: |
| A-B = 1.510 |
| A-C = 0.819 |
| B-C = 2.719* |
| Total calories of pseudofeces: |
| A-B = 2.161* |
| A-C = 2.867** |
| B-C = 4.330** |
| Total calories ingested: |
| A-B = 1.009 |
| A-C = 0.466 |
| B-C = 1.932 |
| Total calories of feces: |
| A-B = 2.153* |
| A-C = 0.192 |
| B-C = 2.455* |
| Total calories of assimilation: |
| A-B = 0.051 |
| A-C = 0.599 |
| B-C = 0.634 |

99* level of confidence
95** level of confidence
A $1.0 \times 10^5$ cells/ml
B $5.0 \times 10^4$ cells/ml
C $2.5 \times 10^4$ cells/ml

calories of pseudofeces produced per level of food concentration. In addition, there were significant differences for the calories cleared and calories of feces produced between medium and low algal concentrations. The same relationship was computed for calories of feces produced between the high and medium algal concentration.

*Algae*

The mean caloric content of *Phaeodactylum tricornutum*, used as food for oysters in other experiments (Davis and Guillard, 1958), was 2200 cal/gram dry weight and 5200 cal/gram ash free dry weight; the latter figure is similar to that of *Nitzschia paradoxa* (Gmel.) Grun. (Paine and Vadas, 1969). Bomb calorimetric values and estimates from wet oxidation analysis were within 5% of each other. The mean ash content ranged from 40–45% of the dry weight.

## DISCUSSION

In general, there was a significant difference between the amounts of pseudofeces produced per food concentration, but not in the amount of calories ingested (Table 2). This suggests that the oyster will take in a limited amount of calories regardless of the concentration of food. This was supported to some extent by the lack of statistical significance between levels of calories assimilated (Table 2).

In the three different levels of food concentration reported here, approximately 74 to 148 calories per 12 hours were assimilated by the oysters. It did not matter whether the oysters were fed $1 \times 10^5$ cells/ml or $5.0 \times 10^4$ cells/ml. However, the low concentration ($2.5 \times 10^4$ cells/ml) resulted in low values for calories cleared and calories assimilated. At the medium concentration ($5.0 \times 10^4$ cells/ml), the oysters exhibited the greatest amount of ingestion. This concentration was most favorable in terms of number of food particles cleared (Fig. 1). In terms of percentage assimilation, the medium ($5.0 \times 10^4$ cells/ml) concentration was least effective (Fig. 2).

At the medium concentration, there was greater production of both pseudofeces and feces; hence, greater waste (Fig. 1). Tenor and Dunstan (1973) reported increasing pseudofeces production with higher food concentrations. Based on the present study, reducing the food concentration by 50% did not reduce calories of assimilation 50%. At the lowest filtration rate, there was increased percentage assimilation (Fig. 2).

The lowest concentration ($2.5 \times 10^4$ cells/ml) produced 85% fewer pseudofeces than the medium concentration. It also produced 4.1% and 41% fewer feces than the high and medium concentration, respectively, while at the same time it produced 19% and 9% calories of assimilation fewer than the medium or high concentration. Tenore and Dunstan (1973) reported percentage assimilation efficiencies (77.4–85.7) higher than those reported here. Since they did not distinguish between feces or pseudofeces, their different technique may explain the high values.

The percentage assimilation was nearly the same in the high and low concentration, indicating greater utilization at these levels (Fig. 2). The filtration rate was affected by the food concentation at the highest level. Filtration rate was markedly increased at the low level of food concentration, suggesting a more acceptable par-

ticle concentration. This was supported by the small amount of pseudofeces production at the low level (Fig. 2). At no time was there evidence of clogging. Food concentration in excess of $2 \times 10^6$ cells/ml would probably be necessary to induce clogging (Loosanoff and Engle, 1947). The mean filtration rates (6.3 $ml \cdot hr^{-1} \cdot mg$ dry tissue $weight^{-1}$) compared favorably with the results (6.6 $ml \cdot hr^{-1}$ mg dry tissue $weight^{-1}$) of Allen (1962).

No significant differences were computed for calories ingested and calories assimilated (Table 2). This suggested that the energy ingested and assimilated was independent of the concentration of food. Perhaps the variation in these figures was too high to detect with the 't' test.

The only significant difference in total energy cleared at the three food levels was between the medium and low concentration. The greatest clearing occurred in the medium concentration (Table 1, Fig. 1). For filtration purposes, the medium concentration was apparently the optimum cell density for oysters since on either side of this concentration the amount of calories cleared decreased noticeably (Fig. 1).

There were significant differences in pseudofeces relative to each level of food density (Table 2). Among the categories involved in energy partitioning, pseudofeces varied most, although this is not evident from Fig. 1. This was influenced by the individual oyster's reaction to the environment and/or its particular physiology at the time of observation.

There were statistically significant differences in the amount of feces produced (Table 2). The medium food concentration produced significantly more feces than the other two concentrations (high and low, Fig. 1). This might be explained by the fact that the oyster was generally more active at this food concentration. The values for feces followed the pattern established in all preceding categories (Fig. 1).

In the energy budget proposed here, no provision was made for nitrogenous excretion products. Hammen, *et al.* (1966) found excretion in oysters as follows: 12.5 $\mu$ moles ammonia/100 gram whole oyster/day, 2.5 $\mu$ moles urea/100 gram whole oyster/day, 1.0 $\mu$ moles amino acids/100 gram whole oyster/day, and 3.2 $\mu$ moles unidentified/100 gram whole oyster/day. What this represented in caloric expenditure on the part of the oyster was unknown. Potts (1967) also stated that nitrogenous wastes are excreted in molluscs, but did not specify the metabolic origin of some of the components. Widdows and Bayne (1971) reported that energy loss through excretion may be an important component of the energy equation in the blue mussel, *Mytilus edulis* Linné.

The energy budget developed herein could be improved with several modifications to the experimental design. The results of researchers using *Phaeodactylum* as an oyster food have been contradictory (Tenore, personal communication). Perhaps another algal species which grows more predictively and has different nutrient properties should be tested. Mixed species cultures were be more representative of conditions in natural coastal environments (Tenore and Dunstan, 1973). However, it would be necessary to determine the caloric content of the experimental species and the relative amount of each species unless they all had the same caloric content. Experiments should be conducted at several temperatures within the temperature range for pumping of oysters per geographic area. The number of food concentration levels should at least be double that used here. This would provide a more accurate basis to determine percentage efficiency of assimilation. Finally, the caloric determination of soluble excretory products should be determined empirically and not by subtraction, which is commonly done.

## ACKNOWLEDGMENTS

This research was made possible by a grant from the NOAA Sea Grant Program. Mr. Robert Malouf was extremely helpful in early plans and discussion of this project. Drs. L. Salisbury and J. Taylor aided in the earlier phases of the work. We would also like to thank Dr. R. Dame who reviewed earlier drafts and provided constructive criticism and Dr. K. Tenore who shared some observations from his research.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# THE ORGANIC CONTENT OF SHELLS AND SOFT TISSUES OF SELECTED ESTUARINE GASTROPODS AND PELECYPODS[1]

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## ABSTRACT

The total organic matter in shells and soft tissues of 3 species of gastropods and 14 species of pelecypods was measured by combustion at 475 ± 5° C. The organisms were collected along the eastern coast of the United States during 1970–1972. The percent organic material in the shells of pelecypods ranged from 12.2 (*Argopecten irradians*) to 71.9% (*Crassostrea virginica*); in soft tissues from 25.5 (*C. virginica*) to 84.3% (*A. irradians*); and in pallial fluid from 2.6 (*C. virginica*) to 9.1% (*Modiolus demissus*). For the gastropods (*Littorina irrorata*, *Ilyanassa obsoleta* and *Urosalpinx cinerea*), the average organic material in the shells, 35.9, and in the soft tissues, 63.7%, were similar for the three species. The percent ash-free dry weight for the shells, soft tissues and pallial fluid of pelecypods averaged 4.5, 79.5 and 30.5%, while that for the shells and soft tissues of gastropods averaged 3.1 and 77.8%. The percent of ash-free dry weights of pelecypods ranged from 1.4 (*A. irradians*) to 21.4% (*Solemya velum*) for shells; 68.1 (*Abra aequalis*) to 93.9% (*Rangia cuneata*) for soft tissues; and 20.0 (*C. virginica*) to 60.2% (*R. cuneata*) for pallial fluid. The percent ash-free dry weight of gastropod shells ranged from 2.7 (*L. irrorata*) to 3.2% (*I. obsoleta*) and for soft tissues from 67.7 (*I. obsoleta*) to 85.1% (*U. cinerea*). The other species of pelecypods that were analyzed were *Argopecten gibbus*, *Mercenaria mercenaria*, *Tagelus plebeius*, *Chione cancellata*, *Mytilus edulis*, *Macoma tenta*, *Tellina versicolor*, and *Corbula contracta*.

Since molluscs are important estuarine herbivores and the organic matrix of their shells rarely is assimilated by carnivores or other organisms, the production of shells represents a drain on the productive capacity of an estuary.

## INTRODUCTION

Many estuarine molluscs are important commercially, as food for man, and ecologically, for their contribution to the production and energy flow of estuarine systems. Williams, Murdoch and Thomas (1968) imply that the importance of the benthos as part of the herbivore link in the marine food chain increases with decreasing water depths and is a significant component in estuarine environments. Thus, live benthic molluscs are an essential segment of the estuarine food web, contributing to the flow of energy and materials to predators, and through feces and excretion, to the detrital, decomposer and producer trophic levels. Upon death, the disintegration of molluscs recyles nutrients into the ecosystem. However, the organic matrix of molluscan shells may constitute a large part of the total organic content of these organisms (Kuenzler 1961; Bernard 1974) and may represent a significant energy pool in the system. If this is the case, permanent burial of shells in the sediment would represent a substantial drainage of

[1] This research was supported through a cooperative agreement between the National Marine Fisheries Service and the U. S. Atomic Energy Commission.

organic production from the system since utilization and breakdown of the organic matrix appears extremely slow.

Numerous fish species feed upon molluscs. These fishes may be able to decalcify some of the shell material, and its organic matrix might serve as a source of energy. This aspect of energy exchange and the whole concept of organic matrix availability in molluscan shells have received little attention.

Since pelecypods and gastropods form a significant part of the biomass and food web in estuarine systems (Thayer, Adams and LaCroix, 1975; Price, Thayer and Montgomery 1974), and since the organic matrix of the shells constitutes a significant portion of this biomass, the invertebrate studies being carried out at the Atlantic Estuarine Fisheries Center includes analyses of the organic matrix of shells (Thayer et al. 1973). This paper presents the results of analyses on the contribution of molluscan shells, meats and, in some cases, pallial fluid, to the total organic content of several estuarine molluscs.

## METHODS

We determined the organic content of 14 species of pelecypods, including one species (*Modiolus demissus*) from four geographic areas and one subspecies (*M. d. granosissima*), and three species of gastropods (Table 1). In most cases, 50 individuals of each species were analyzed separately. Small molluscs, however, were not treated separately. Each individual was scrubbed with a soft brush to remove attached organisms. On some of the larger bivalves, the pallial fluid was collected by draining for approximately two minutes during shucking. The organisms were shucked and separated into shell, meat and pallial fluid components.

Gastropod shells also were scrubbed with a soft brush to remove encrusting organisms, after which the gastropods were frozen at −20° C until analysis. After thawing, the meats of *Ilyanassa* were pulled gently from the shell. The soft tissues of *Littorina* and *Urosalpinx*, however, were removed by air pressure after a small hole had been drilled in the apex of the shell.

The shell and meat of all specimens were blotted on filter paper and wet weights of the three components—shell, meat and pallial fluid—was obtained. These components were dried to a constant weight at 100° C and weighed. The organic content of each component was estimated from loss of weight upon

TABLE 1. *Species, size range, and location of molluscs analyzed for orgonic content of shells and soft tissues.*

| Species | Origin | Size Range (grams) |
|---|---|---|
| Pelecypods | | |
| *Argopecten irradians* | Bogue Sound, N. C. | 8.02–74.02 |
| *Argopecten gibbus* | Offshore, N. C. | 33.65–62.19 |
| *Crassostrea virginica* | Newport River, N. C. | 80.88–170.05 |
| *Mercenaria mercenaria* | Cape Lookout, N. C. | 22.75–137.57 |
| *Tagelus plebeius* | Newport River, N. C. | 0.37–24.23 |
| *Modiolus demissus* | Sandy Hook Bay, N. J. | 0.47–27.71 |
| *Modiolus demissus* | Wachapreague, Va. | 1.49–71.99 |
| *Modiolus demissus* | Cape Lookout, N. C. | 14.46–72.74 |
| *Modiolus demissus* | St. Simons Island, Ga. | 0.48–93.39 |
| *Modiolus demissus granosissima* | Gulf Breeze, Fla. | 2.00–23.23 |
| *Chione cancellata* | Bogue Sound, N. C. | 13.54–28.38 |
| *Mytilus edulis* | Boothbay Harbor, Maine | 3.54–14.36 |
| *Macoma tenta* | Newport River, N. C. | 0.007–0.405 |
| *Solemya velum* | Newport River, N. C. | 0.018–0.254 |
| *Tellina versicolor* | Newport River, N. C. | 0.005–0.162 |
| *Abra aequalis* | Newport River, N. C. | 0.026–0.118 |
| *Corbula contracta* | Newport River, N. C. | 0.018–0.054 |
| *Rangia cuneota* | Neuse River, N. C. | 38.32–89.59 |
| Gastropods | | |
| *Urosalpinx cinerea* | Newport River, N. C. | 0.063–1.739 |
| *Ilyanassa obsoleta* | Newport River, N. C. | 1.135–2.507 |
| *Littorina irrorata* | Newport River, N. C. | 2.106–3.785 |

ashing at 475 ± 5° C for 36 hr. This temperature is sufficient to oxidize organic carbon to carbon dioxide but will not break down carbonate compounds (Paine 1964, 1966).

## RESULTS AND DISCUSSION

The percentages of organic matter in the total dry weight of the shell, meat and pallial fluid of the species analyzed are shown in Table 2. Meats are largely organic matter, ranging from 67.2% organic matter for *Ilyanassa obsoleta* to 93.9% for *Rangia cuneata* and averaging 80.9% for all species. The shell material contained an average 4.3% organic matter and ranged from 1.4% of the shell of *Argopecten irradians* to 21.4% for shells of *Solemya velum*. The pallial fluid was intermediate in organic content, averaging 30.9% and ranged from 20.0% for *Crassostrea virginica* to 60.2% for *Rangia cuneata*. The organic content of shells and meats of pelecypods averaged 4.6% and 79.5%, respectively, and that of gastropods averaged 3.0% and 77.8%, respectively.

Shell and meat organic content appears related to age or size of the organisms, and since a wide range of sizes were used for each species, this relation was in part responsible for the variation observed for each species. The percent organic matter in the shell tended to be greater for small individuals and least for larger organisms, whereas the reverse was true for the meat component. Casual observation also suggested that the smaller the percent organic content of the shell the more brittle is the shell.

There are few data available in the literature on the organic content of the shell component of molluscs. Vinogradov (1953) noted that the organic content of pelecypods generally varies from 0.1–4.0% of the shell and that they generally have more organic matter in their shell than do gastropods. For example, the organic content of the shell of *Pecten varius* reportedly is 0.7% and that of *Mytilus edulis* is 3.9% (Vinogradov 1953); Bernard (1974) reported a shell organic content of 2% for *Crassostrea gigas*. Our data (Table 2) agree with Vinogradov's data on the range of organic content of shell and indicate that the organic content of the shells of the pelecypods we analyzed was greater than that of gastropod shells (4.6% vs. 3.0%). Vinogradov also noted that estuarine and marine molluscs have a higher organic content in their shell than do freshwater molluscs. Kuenzler (1961), studying the energetics of *Modiolus demissus* in a Georgia salt marsh, obtained an average

TABLE 2. *Percent ash-free dry weights for shell, meat and pallial fluid of some estuarine molluscs (± one standard deviation).*

| Species | Shell | Meat | Pallial Fluid |
|---|---|---|---|
| *Argopecten irradians* | 1.37 ± 0.15 | 82.47 ± 1.73 | 35.15 ± 6.10 |
| *Argopecten gibbus* | 1.72 ± 0.02 | 68.68 ± 11.59 | – |
| *Crassostrea virginica* | 3.04 ± 1.16 | 71.87 ± 8.47 | 20.01 ± 4.02 |
| *Mercenaria mercenaria* | 1.90 ± 0.18 | 79.79 ± 4.32 | 26.81 ± 10.47 |
| *Tagelus plebeius* | 2.82 ± 0.34 | 77.80 ± 4.25 | – |
| *Modiolus demissus* (N. J.) | 6.16 ± 0.52 | 84.42 ± 1.98 | 22.57 ± 4.72 |
| *Modiolus demisus* (Va.) | 5.34 ± 0.40 | 84.79 ± 2.84 | 21.78 ± 4.44 |
| *Modiolus demissus* (N. C.) | 4.63 ± 0.23 | 81.22 ± 2.33 | 22.00 ± 2.84 |
| *Modiolus demissus* (Ga.) | 5.43 ± 0.63 | 87.65 ± 3.32 | 25.20 ± 4.73 |
| *Modiolus demissus* (Fla.) *granosissima* | 5.86 ± 0.42 | 86.69 ± 1.56 | 32.69 ± 4.65 |
| *Chione cancellata* | 2.95 ± 0.82 | 79.39 ± 4.18 | 38.26 ± 4.44 |
| *Rangia cuneata* | 2.07 ± 1.82 | 93.86 ± 2.23 | 60.24 ± 7.97 |
| *Mytilus edulis* | 5.32 ± 0.40 | 81.93 ± 3.33 | – |
| *Macoma tenta* | 2.73 | 71.91 | – |
| *Solemya velum* | 21.43 | 82.56 | – |
| *Tellina versicolor* | 2.26 | 73.63 | – |
| *Abra aequalis* | 2.48 | 68.07 | – |
| *Corbula contracta* | 4.35 | 73.42 | – |
| *Urosalpinx cinerea* | 3.10 ± 0.83 | 85.07 ± 3.22 | – |
| *Ilyanassa obsoleta* | 3.23 ± 0.50 | 67.62 ± 6.42 | – |
| *Littorina irrorata* | 2.72 ± 0.31 | 80.74 ± 2.65 | – |

organic content of the shell of 10.6% using a technique similar to the one we employed. Our *M. demissus* data from New Jersey, Virginia, North Carolina and Georgia, and data on *M. demissus granosissima* from Florida, however, indicate an average shell organic content of 5.5% (coefficient of variation of mean values of 7.9%), with a range in the five geographic samples of 6.1% (New Jersey) to 4.6% (North Carolina) (Table 2).

Ash-free dry weights of each component for each species were summed and the percent of the total represented by each component was computed (Table 3). The percent of the total represented by the meat component ranged from 25.5% in *Crassostrea* to 87.0% in *Tagelus* and averaged 62.3% for all species. Organic matter contributed by the shell component ranged from 12.2% for *Argopecten irradians* to 71.9% in *Crassostrea* and averaged 35.4% of the total organic matter. That percent contributed by pallial fluid averaged 4.9% and ranged from 2.6% of the total for *Crassostrea* to 9.1% for *Modiolus demissus* collected from North Carolina. For the three gastropod species analyzed, the meats and shells contributed 64.1% and 35.9%; for pelecypods the mean contribution to the total organic content by these components was 62.0 and 38.0%, respectively.

The contribution by the meat and shell components to the total organic content appeared related to size. The contribution to the total by the shells tended to increase with size whereas the meat contribution decreased with increasing size. For example, in *Tagelus* less than 5.1 g wet weight, the shell contributed 10% of the total and the meat 90%, whereas these components contributed 16% and 84%, respectively, in organisms between 15–25 g; for *Modiolus* (North Carolina) the shell contributed 45% of the total in organisms less than 20 g and 50% in organisms 60–75 g, whereas the meat showed a decreasing contribution with size. A final example of this relation is in *Crassostrea virginica* in which the shells of smaller organisms (less than 90 g) contributed 71% of the total and for organisms greater than 150 g the shell contribution was 85%. These are only trends since there was no statistical significance between size groups.

Data for *Modiolus* (Table 3) were analyzed to determine if geographic location influenced the proportion of the total organics contributed by each of the three components. Analysis of variance of data indicated that there was a signifi-

TABLE 3. *Percent of the total organic material in the shell, meat and pallial fluid of some estuarine molluscs (± one standard deviation).*

| Species | Shell | Meat | Pallial Fluid |
|---|---|---|---|
| *Argopecten irradians* | 12.17 ± 1.18 | 84.27 ± 1.98 | 3.56 ± 1.43 |
| *Argopecten gibbus* | 19.52 ± 3.17 | 80.55 ± 4.09 | – |
| *Crassostrea virginica* | 71.89 ± 9.95 | 25.55 ± 9.87 | 2.56 ± 1.07 |
| *Mercenaria mercenaria* | 38.60 ± 3.83 | 54.29 ± 5.27 | 7.41 ± 2.99 |
| *Tagelus plebeius* | 13.00 ± 1.81 | 87.00 ± 3.36 | – |
| *Modiolus demissus* (N. J.) | 40.23 ± 3.20 | 55.59 ± 3.46 | 4.11 ± 1.59 |
| *Modiolus demissus* (Va.) | 44.54 ± 3.95 | 50.71 ± 3.81 | 4.75 ± 1.64 |
| *Modiolus demissus* (N. C.) | 47.44 ± 2.96 | 43.42 ± 3.94 | 9.14 ± 2.18 |
| *Modiolus demissus* (Ga.) | 40.45 ± 4.45 | 54.02 ± 9.39 | 4.32 ± 1.59 |
| *Modiolus demissus* (Fla.) *granosissimo* | 37.35 ± 4.66 | 57.91 ± 5.13 | 4.74 ± 1.96 |
| *Chione cancellato* | 46.72 ± 7.56 | 49.36 ± 7.34 | 3.99 ± 0.88 |
| *Rangia cuneata* | 45.47 ± 3.94 | 50.93 ± 2.35 | 3.63 ± 1.57 |
| *Mytilus edulis* | 38.79 ± 6.53 | 61.21 ± 6.53 | – |
| *Macoma tenta* | 32.34 | 67.66 | – |
| *Solemya velum* | 25.99 | 74.01 | – |
| *Tellina versicolor* | 26.84 | 73.16 | – |
| *Abra aequalis* | 31.39 | 68.61 | – |
| *Corbula contract* | 22.36 | 77.64 | – |
| *Urosalpinx cinerea* | 37.18 ± 6.31 | 62.81 ± 6.31 | – |
| *Ilyanassa obsoleta* | 37.69 ± 3.68 | 62.32 ± 3.99 | – |
| *Littorina irrorata* | 32.81 ± 3.91 | 67.18 ± 3.91 | – |

cant increase in the contribution by the shells from New Jersey to North Carolina and also from Florida to North Carolina. There also was a significant decrease in the contribution by the meat component from New Jersey to North Carolina and from Florida to North Carolina. The pallial fluid contribution was significantly greater in the North Carolina mussels than from those collected either north or south of that area.

Wilbur (1964) noted that environmental temperature has an influence on the crystal type in molluscan shells. He also stated that the influence of temperature on crystal type is mediated through changes in mantle secretory activity. This will cause changes in the inorganic and organic components of the extrapallial fluid which secretes the organic matrix and secondarily in the organic matrix itself. Thus, the significant geographic differences noted for the contribution by the shell and meat components of *Modiolus* probably are in part the result of environmental differences in the specific areas of collection.

The pH of the gut of many fishes (Barrington 1957; Baptist 1966) is sufficient to hydrolyse proteins and to decalcify some of the shell material from ingested molluscs. Thus, the protein matrix of the shell, especially the periostracum is potentially available to predators of molluscs. Adams (1974) has shown that unidentified pelecypods, *Bittium varium* and juvenile *Argopecten irradians* are fed upon by several fishes inhabiting grass beds in the Beaufort, N. C. area. Pelecypods composed 14% of the annual diet of spot (*Leiostomus xanthurus*), *Bittium varium* composed 83% of the diet of striped burrfish (*Chilomycterus schoepfi*) (Fig. 1) and scallops contributed 25% of the diet of oyster toadfish (*Opsanus tau*). In addition, we have found numerous *Bittium varium* and other gastropods in the gut of pinfish (*Lagodon rhomboides*).

The rate of decomposition of organic matter in mollusc shells deposited in the sediments is probably slow and could represent a substantial sink of organic matter in estuarine environments. We have analyzed old, weathered oyster shells which had lain unburied in an intertidal area; the area used to have an oyster shucking house nearby which ceased to operate 50 years

FIG. 1. *A striped burrfish* (Chilomycterus schoepfi). *The gut (foreground) has been removed and exposed showing gut contents consisting entirely of gastropods.*

ago. The shells had an organic content of 1.2 ± 0.3%, a content which is approximately 40% of that present in living oyster shell (Table 2). Smith and Wright (1962) reported weathered oyster shells which had been dredged from the Gulf of Mexico had an organic content of 0.06%, while Degens and Love (1965) found measurable amounts of amino acids present in Tertiary *Gyraulus trochifermis* shells. Thus, the rate of decomposition of the organic matrix of molluscan shells buried in sediments is extremely slow.

Our data (Table 3) on pelecypods and gastropods indicate that death of these molluscs and burial of the shells would constitute a loss of 12–72% of the organic material present in the organism at any one time. The loss of organic production through shell burial, however, would be considerably less because a substantial portion of the organic matter produced by molluscs dur-

ing their life is in the form of gametes, mucus and excretory products which are expelled from the living organism and not included in our estimate of total organic matter. Bernard (1974) estimated that for a mature *Crassostrea gigas* reef, 0.05% of the potential food available (0.4% of estimated assimilation) goes into shell protein matrix over the year. This protein matrix production was equal to the amount of energy excreted by the reef.

Energy dynamics studies of estuarine and freshwater systems indicate that the majority of molluscs utilize detritus, algae and phytoplankton as energy sources. In return, the soft parts of these organisms and their feces and pseudofeces supply energy and nutrients to both aquatic and terrestrial carnivores and to the microbial and detrital community. Within aquatic systems the soft tissues and biodeposits are recycled rapidly but a different situation occurs for the organic matrix of molluscan shells. Williams and Murdoch (1970) stated that with one important exception all of the organic matter produced in the estuarine ecosystem appears readily available to other trophic levels. The exception is the organic matrix of molluscan shells. There appears to be no information on the ability of organisms, which either ingest shell material or bore into shells, to extract organic material from the shell, but it is potentially available. In addition, burial in the sediment of all or some of the organic matter contained in the shell would represent a substantial drainage of organic matter from the system since recycling of this organic matrix is extremely slow.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# GROWTH OF OYSTERS IN A RECIRCULATING MARICULTURAL SYSTEM[1]

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## ABSTRACT

*Eight groups of oysters* (Crassostrea virginica) *have been cultured for nearly one year post setting in a recirculating maricultural system. Each group, consisting of approximately 200 animals, was fed a different diet. All the diets consisted of algae from monospecific, but not axenic, cultures, and since the water in the maricultural system was recirculated, the oysters had access to only the food which we supplied. We believe this to be the first report of oysters grown for an extensive period of time on defined diets. The experiment is still underway and will continue until the animals have reached a marketable size. Extrapolated growth rates indicate that the fastest growing osyters will reach marketable size in approximately two years after setting. This is much sooner than the reported three to four years to marketable size among wild oysters in Delaware Bay.*

## INTRODUCTION

While oysters are undoubtedly one of the most highly studied of all marine organisms, there is little known concerning their nutritional needs. There is almost no information about the biochemical aspects of their nutritional requirements and only slightly more known of gross food types which will support their growth (Nelson, 1947; Ukeles, 1971). Over the past one hundred years, however, three schools of thought regarding the way in which filter-feeding bivalves obtain their food have evolved: 1) that first proposed by Pütter (1909) wherein dissolved organic material is the main nutritional source for the animals; 2) that first put forth by Petersen and Jensen (1911) where inanimate detritus is the main component; 3) that first supported by Dean (1887) wherein phytoplankton constitute the main food. While bivalve molluscs almost certainly derive some nutritional value from dissolved organic compounds (Collier, 1959) and from detritus (Gavard, 1927), the view that planktonic algae are the main food source of these animals has gained widest acceptance.

There has been a large amount of study of the nutritional value of many species of algae to European, Japanese, and American oyster larvae (Davis, 1950; Davis, 1953; Davis and Chanley, 1956; Davis and Guillard, 1958; Imai and Hatanaka, 1949; Loosanoff and Davis, 1963; Loosanoff et al., 1955; Loosanoff and Marak, 1951; Walne, 1956, 1963, 1965) but very little examination of the value of different algal species to post-larval bivalves (Galtsoff, 1964). In the present paper, we report the results of a study where oysters (*Crassostrea virginica* Gmelin) have been fed defined algal diets for a period of forty-six weeks post setting.

## MATERIALS AND METHODS

### *Hatchery techniques*

Oysters used in these experiments were hatchery-reared. The parents of these animals were collected from the Broadkill River near Lewes, Delaware. Techniques for conditioning

[1] Publication No. 102, College of Marine Studies, University of Delaware.

and spawning these brood stock were those developed by Maurer and Price (1967). Larvae were reared in 400 liter conical tanks where the water was changed daily and the larvae fed a mixture (1:1 ratio) of *Isochrysis galbana* and *Monochrysis lutheri* at a rate of $5 \times 10^7$ cells per liter of culture water per day. Clutchless spat were obtained by a method similar to that of Dupuy (1972).

*Culture System*

Water in the system was largely recirculated but small amounts of new water were introduced daily along with the algal food. There was no control of evaporation in the system and salinity was adjusted periodically through addition of fresh water. Salinity varied from 29‰ to 34‰ over the course of the experiment. Water temperature was controlled by air temperature surrounding the system, and while water in the system was generally near 20°C, occasional equipment failure allowed temperature to fall as low as 16°C and to rise as high as 26°C.

Oysters were held in eight growing tanks situated above an 8000-liter waste treatment apparatus (Fig. 1). Water in each growing tank was recirculated independently and after 24 hours, the water was drained into the waste treatment module and the growing tanks immediately refilled with water from the reservoir.

The waste treatment apparatus consisted of a submerged biological filter, an ultraviolet component, and an activated carbon filter. The apparatus was described in detail by Epifanio et al. (1973).

*Diets*

There were eight groups of 200 animals in the experiment, and each group received a different diet (Table 1). A batch method of feeding was used where the concentration of algal cells in each growing tank was brought to a predetermined level and the oysters allowed to deplete it. The volume of each growing tank was 100 liters, and every group was initially fed $5 \times 10^7$ cells/liter/day. This ration was gradually increased until, at the experiment's end, the oysters were being fed $4 \times 10^8$ cells/liter/day. These rations were determined by periodically measuring the rate of removal of cells from suspension by the oysters. Concentrations were adjusted so that all the cells were removed in twenty-four hours.

Algae were cultured in 200-liter, covered glass tanks at 18° ± 2° using *f*/2 medium (Guillard and Ryther, 1962). Illumination was provided by cool-white fluorescent bulbs with incident radiation upon the sides of the culture vessels at 585 microwatts/cm$^2$ as measured with a Spectra Model PR-1000 Radiometer (flat response from 450–950 nannometers). Water used in the cultures was pumped from Delaware Bay, filtered to remove particles larger than 0.5 micrometers, passed through an activated carbon

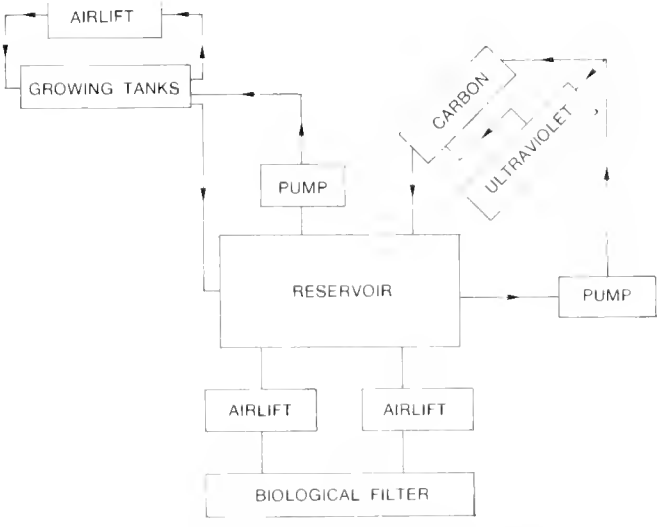


FIG. 1. *Schematic of culture system.*

TABLE 1. *Composition of diets.*

| Diet | Algal Species | Cell Count Ratio |
|---|---|---|
| A | *Phaeodactylum tricornutum* | – |
| B | *Phaeodactylum tricornutum* + *Carteria chuii* | 1:1 |
| C | *Phaeodactylum tricornutum* + *Croomonas salina* | 1:1 |
| D | *Phaeodactylum tricornutum* + *Isochrysis galbana* | 1:1 |
| E | *Phaeodactylum tricornutum* + *Carteria chuii* + *Croomonas salina* | 1:1:1 |
| F | *Phaeodactylum tricornutum* + *Carteria chuii* + *Isochrysis galbana* | 1:1:1 |
| G | *Phaeodactylum tricornutum* + *Croomonas salina* + *Isochrysis galbana* | 1:1:1 |
| H | *Phaeodactylum tricornutum* + *Croomonas salina* + *Carteria chuii* + *Isochrysis galbana* | 1:1:1:1 |

filter, and irradiated with ultraviolet light. A semicontinuous culture technique was employed where the volume of algal suspension used in feeding shellfish was replaced with fresh medium daily. This technique provided a high level of stability in the cultures and a given culture generally could be used for three to four weeks before deteriorating. Algae were counted with a Coulter Counter, Model $Z_B$, fitted with a 100-micrometer aperature tube. Species integrity was assured microscopically.

*Water quality*

Levels of ammonia, nitrites, reactive phosphorous, pH, alkalinity, salinity, and dissolved oxygen were measured in the recirculating system weekly. Temperature was measured daily. Techniques for water quality analysis were described by Srna et al. (1973) and Epifanio et al. (1973).

*Observations*

Shell height (linear distance from umbone to bill) was used as an indicator of growth. Measurements were made to the nearest millimeter with a Vernier caliper.

## RESULTS AND DISCUSSION

The present study is one of the most extensive, long-term examinations of the value of various algal species as foods for post-larval oysters. The use of a recirculating seawater system allowed maintenance of a high level of water quality (Table 2) and assured that the shellfish had access to only the food which we provided them; there was no possibility that the shellfish were obtaining some nutrition from wild phytoplankton or detritus.

It is clear from our results that a diet of *Phaeodactylum tricornutum*, alone, is insufficient to support the growth and survival of *Crassostrea virginica*. Animals fed this diet showed very poor growth, and all had perished after 25 weeks (Fig. 2). This finding corroborates that of Walne (1970) who found *Phaeodactylum tricornutum* a poor food for *Crassostrea gigas*.

Analysis of variance of mean shell height among the experimental groups surviving the full 46 weeks of the experiment indicated a highly significant difference in the size of the oysters in the different groups (Fig. 3, Table 3). A Duncan Multiple Range Test indicated that diets fed Groups E and H supported most rapid growth while the diet fed Group C yielded the

TABLE 2. *Water quality in culture system. Parameters were monitored weekly. None of the parameters approached toxic levels (Epifanio et al., 1975).*

| Variable | Mean Value | S.D. | Range |
|---|---|---|---|
| pH | 7.90 | 0.08 | 7.75–8.05 |
| Dissolved oxygen (mg/liter) | 7.30 | 0.29 | 6.90–7.80 |
| Reactive Phosphorous (micromoles/liter) | 18.98 | 13.27 | 20.00–45.71 |
| Ammonia (micromoles/liter) | 4.84 | 4.10 | 2.0–20.0 |
| Nitrite ion (micromoles/liter) | 0.11 | 0.01 | 0.10–0.15 |

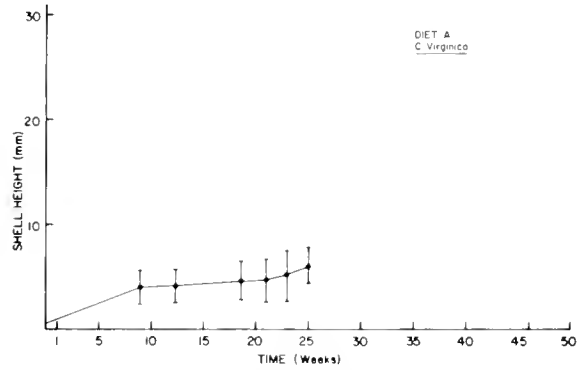


FIG. 2. *Growth of oysters*, Crassostrea virginica *fed* Phaeodactylum tricornutum. *Bars around points are ± one standard deviation.*

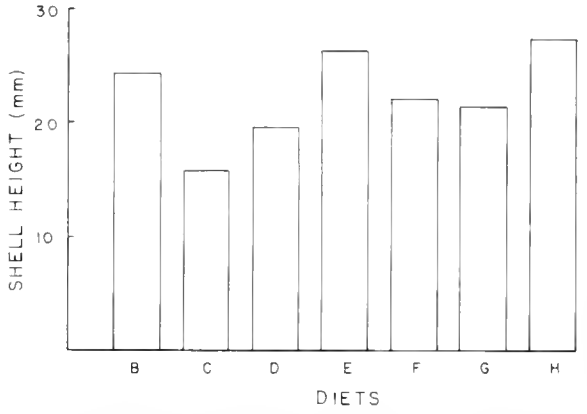


FIG. 3. *Size of oysters in each group after 46 weeks of growth.*

TABLE 3a. *Analysis of variance table for mean shell height in Groups B to H after 46 weeks of growth. Asterik denotes significant F value at 0.01 probability level.*

| | Sum of Squares | d.f. | Mean Squares | F |
|---|---|---|---|---|
| Between groups | 12666.96 | 6.00 | 2111.16 | 72.56* |
| Within groups | 34333.45 | 1180.00 | 29.10 | |
| Total | 47000.41 | 1186.00 | | |

TABLE 3b. *Duncan Multiple Range Test ($\alpha = 0.01$) for differences in mean shell height. Letters refer to experimental groups, and underscoring indicates overlapping ranges of shell height.*

C D F G B E H

← *smaller* → *larger*

smallest animals. Animals in Group B were significantly larger than those in Groups G and F and those in G and F significantly larger than those in Group D.

The algae used in this study were chosen because of their wide taxonomic variety and because they had been shown to have some utility as foods for oysters in other studies (Goodrich et al., 1968). While it is impossible to make any statistical inference concerning the relative food-value of the individual algal species used in the experiment, it can be seen that *Carteria chuii* was a component of the diets fed to both of the fastest growing groups, and it makes up fully half (in terms of numbers of cells) of the composition of Diet B which produced second fastest growth. Since *Phaeodactylum tricornutum*, which makes up the other half of Diet B, is undoubtedly a poor food, it can be inferred that *Carteria chuii* is one of the more important components of the diets tested. This is further substantiated by the fact that *Isochrysis galbana*, when combined with *Phaeodactylum tricornutum*, alone, promoted significantly slower growth than any of the diets containing *Carteria chuii* and that *Croomonas salina*, when combined with *Phaeodactylum tricornutum* alone, produced slower growth than any diet other than the one-part *Phaeodactylum tricornutum* diet.

Animals in the fastest growing group reached a mean shell height of slightly greater than 27 mm after 46 weeks of growth. This compares favorably with growth of oysters in natural waters of the Middle Atlantic region during their first growing season. Shaw (1966) indicated that oysters which set in Broad Creek, Maryland, during the summer of 1960, had reached a mean shell height of about 25 mm by May, 1961, and Beaven (1952) reported that oysters from several Maryland locations in the Chesapeake Bay reached a shell height of about 30 mm by the beginning of their second growing season. Maurer and Aprill (1973) found that hatchery-reared spat grown in off-bottom culture in various rivers in Delaware reached a mean shell height of between 10 and 20 mm (depending on location) by the beginning of their second growing season. It must be pointed out, however, that those animals living in nature grew only during the warmer months of the year while

those in the present study grew at a rather constant rate for the entire 46 weeks. Therefore, while the yearly growth of the oysters in our system was comparable to or better than natural growth, the daily growth during the respective growing seasons was somewhat less than natural growth.

Nevertheless, the results of the present study show that it is unquestionably possible to grow oysters in recirculating seawater systems on a diet consisting solely of cultured phytoplankton. There is no particular reason why this method of culturing oysters must be conducted adjacent to or even near a source of natural seawater. The possibilities for mariculture of oysters at inland sites is certainly not out of the question, and in the least, our techniques allow the maintenance of filter-feeding marine bivalves at inland research facilities.

## ACKNOWLEDGMENTS

The authors wish to thank their colleagues Dr. Richard Srna for providing the water quality analyses and Mr. Gary Pruder for overseeing the design and construction of the culture system. Mr. Dennis Logan, Mr. Andrew Marinucci, and Mr. Robert Flaak fed the animals on weekends while Ms. Christine Turk and Mr. Earl Greenhaugh were responsible for the daily maintenance of the shellfish and algae over the 46 weeks of the experiment. Dr. Ellis Bolton provided light intensity measurements.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# OIL BIOASSAYS WITH THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)[1]

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## ABSTRACT

*Oyster bioassays were conducted to determine the relative toxicity of four test oils and a reference toxin. The oysters* (Crassostrea virginica) *were exposed to oil-water dispersions of two crude and two partially refined petroleum hydrocarbons. The partially refined oils #2 fuel and Venezuela bunker C were found to be more toxic than the two crude oils tested, South Louisiana and Kuwait. Oysters demonstrated greater resistance to test oils than to the reference toxin, dodecyl sodium sulfate. Valve closure by oysters made it difficult to determine percent mortality data in 96-hour or extended studies. Composition of test solutions is compared to calculated values of oil in water and referenced to the relative toxicity demonstrated. Behavior and condition of test animals is discussed in relation to bioassay results.*

## INTRODUCTION

Considerable bioassay work on marine animals has been conducted in the laboratory to determine the relative toxicities of petroleum hydrocarbons, dispersants and other potentially hazardous compounds. These investigations have focused on selected crude or refined petroleum products tested on a wide variety of animals. Many of the studies include discussions on the relative merits of static and flow-through bioassays, behavioral responses of test animals and use of suitable test animals for given regions. Much of this work is detailed and discussed by Anderson (1973).

In an effort to compare laboratory studies to field conditions, recent reviews attempt to relate the composition and behavior of oil in water to both the animals tested and their ecosystems (Nelson-Smith, 1970; 1973; Wilber, 1969; Moore, 1973). Little information, however, has been derived or cited in these studies pertaining to the actual concentration of the exposure medium, particularly in regard to the petroleum hydrocarbons in aqueous phase or the chemical composition of oils tested.

This present work emphasizes the additional need to reference behavior and condition of test animals. Rather than report calculated concentrations of oil in seawater, actual concentrations, composition and relative toxicity of test solutions are cited and relationships between behavior, condition and relative toxicity of the oil-water dispersions discussed. This study was initiated to provide baseline information in a broad program to examine the sublethal effects on estuarine animals, brought about by both environmental alteration and introduction of potentially harmful compounds.

## MATERIALS AND METHODS

Two crude oils, South Louisiana and Kuwait, and two partially refined oils, #2 fuel oil (containing approximately 40% aromatics) and Venezuela bunker C oil (a residual), were utilized. Oils were supplied in 55-gallon barrels by the American Petroleum Institute. Contents were transferred to one-gallon amber glass jars. Be-

[1] This work was partially supported by the American Petroleum Institute, through Letter of Agreement No. 0520-P to Texas A&M University and the Texas A&M Sea Grant College Program through the National Oceanic and Atmospheric Administration, Grant No. 04-3-158-18.

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fore being sealed with aluminum foil liners or Teflon caps, jars were flushed with nitrogen.

American osyters, *Crassostrea virginica*, were collected at natural and artificial reefs in the Galveston Bay system. Primary collection sites were intertidal reefs at Six Mile Road and Eight Mile Road in West Bay, and Morgan's Point Reef in Galveston Bay (Fig. 1). Oysters collected in West Bay ranged from approximately 40–70 mm in shell length, while oysters collected at Morgan's Point ranged from 70–90 mm. West Bay oysters were collected as needed, while oysters from Morgan's Point were held in trays at the Seabrook Laboratory, Texas Parks and Wildlife Department, prior to shipment to the laboratory in College Station.

After shell cleaning, oysters were placed in large, aerated aquaria and maintained at 20‰ salinity and 20°C in the commercial seawater product, Instant Ocean (Aquarium Systems, Inc., Eastland, Ohio). The 10-, 20- and 30-gallon aquaria used as holding tanks were maintained in subdued light, gently aerated and maintained at 20‰ salinity by addition of distilled water. Oysters were used within 7 to 28 days after collection and were not fed.

Preliminary experiments showed that in the laboratory oysters could acclimate within 72 hours to new salinities ranging from 10 to 30‰ (Anderson, 1973). Animals having low condition indices or testing positively for fungal parasites, *Labyrinthomyxa* spp., were discarded. To determine the comparative health of animals tested at various times and from different locations, a standard toxicant (dodecyl sodium sulfate: DSS) was used as a reference during toxicity studies.

The basic procedure used in this bioassay was a modification of LaRoche, Eisler and Tarzwell (1970). One-quart, wide-mouth jars were used as assay containers. Measured amounts of the oils or reference toxin, DSS, were added to the artificial seawater to make 500 ml test solutions. Solutions to be tested were tightly capped with new aluminum foil liners or Teflon liners and shaken for five minutes at approximately 200 cycles per minute on a shaker platform.

One hour after mixing, one oyster was added per jar. Since extremely high concentrations of oil were employed, dispersions were characterized by large numbers of droplets. Solutions were aerated by using disposable pipettes low-

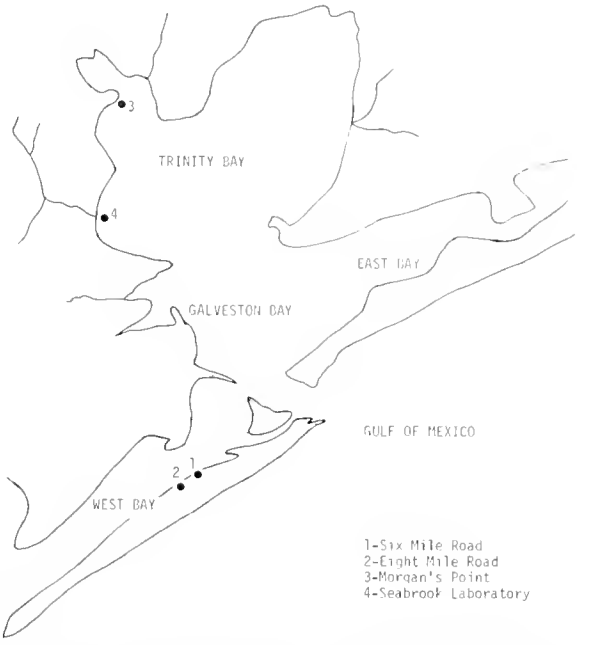


FIG. 1. *Location of oyster sampling and holding stations in the Galveston Bay system.*

ered into the test jars through holes in the lids. When impossible to count the number of bubbles, aeration was decreased. For tests with the reference toxin, vessels were packed with towels around the holes in the lids to contain the foam from the detergent-like material. Test animals were examined every 12 hours. Oysters that were gaped or failed to close their valves after being soundly tapped by a pipet, were removed. Bioassays were considered for termination after 96 hours. However, this proved to be inappropriate because of the oysters' great resistance to test oils and their ability to remain closed. Long-term bioassays, often extending over several months, were then conducted, and the time to first death ($TL_0$), time to 50% mortality ($TL_{50}$) and time to 100% mortality ($TL_{100}$) determined for each group of animals tested.

Petroleum hydrocarbons present in the aqueous phase of the oil-water dispersions were prepared for analysis by infrared and gas chromatographic methods. Aliquots were drawn carefully from below the surface of the oil-water dispersions, with care taken to avoid contamination of the water sample by any surface slick present. A 200 or 400 ml aliquot of each oil-water dispersion was taken for analysis. De-

TABLE 1. *Percent mortality of winter-collected oysters exposed for 96 hours to various concentrations of the four test oils. Percent oil concentration in solution is compared with duration of exposure in hours.*

| | #2 Fuel Exposure Time (hours) | | | | Venezuela Bunker C Exposure Time (hours) | | | | South Louisiana Crude Exposure Time (hours) | | | | Kuwait Crude Exposure Time (hours) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % Oil | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
| .001 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| .005 | 0 | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| .01 | 0 | 0 | 10 | 10 | 0 | 0 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| .05 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| .1 | 0 | 10 | 40 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 |
| 1 | 0 | 20 | 30 | 40 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 10 | 10 | 50 | 50 |
| 10 | 0 | 40 | 40 | 50 | 0 | 10 | 20 | 80 | 20 | 40 | 40 | 80 | 0 | 10 | 50 | 50 |
| 50 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 20 | 0 | 0 | 20 | 60 | 0 | 0 | 0 | 0 |

tailed petroleum hydrocarbon analyses were not conducted, since they were available in this laboratory through bioassay studies with other estuarine species (Neff and Anderson, 1973; Anderson, Neff, Cox, Tatem and Hightower, 1974).

## RESULTS AND DISCUSSION

Reliable $TL_{50}$ data were difficult to obtain in these bioassay experiments because of the oysters' tendency to cease pumping and close their valves, as well as their ability to rapidly depurate petroleum fractions. Results of the 96-hour bioassays with the test oils and reference toxin show the oysters' low sensitivity to these materials (Tables 1 and 2). The reference toxin, DSS, consistently resulted in lower $TL_{50}$ values than the four test oils, while oysters collected in late fall showed greater resistance than summer-collected test animals. In all bioassays, including long-term studies (Table 3), #2 fuel and Venezuela bunker C oils were most toxic, with the two crude oils, South Louisiana and Kuwait, being considerably less toxic. Kuwait crude demonstrated the lowest toxicity of the petroleum hydrocarbons tested (Table 3).

Additional bioassays conducted with oysters collected in winter months revealed a pattern of increased resistance to test oils (Anderson, 1973). Again, this resistance was substantiated by testing with the reference toxin. Winter oysters also proved less sensitive to the standard toxicant than did summer-collected specimens.

The water phase data for this study were generated by Anderson (1973), Neff and Anderson (1973) and Anderson, Neff, Cox, Tatem and Hightower (1974). These investigators reported

TABLE 2. *Percent mortality of oysters collected during different seasons exposed to the reference toxicant, dodecyl sodium sulfate (DSS).*

| | DSS-October 1972 Exposure Time (hours) | | | | DSS-February 1973 Exposure Time (hours) | | | |
|---|---|---|---|---|---|---|---|---|
| % DDS | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
| .001 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| .01 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 |
| .05 | 0 | 0 | 10 | 20 | 0 | 0 | 0 | 0 |
| .1 | 0 | 0 | 20 | 30 | 0 | 0 | 0 | 10 |
| .5 | 0 | 0 | 20 | 20 | 0 | 0 | 10 | 20 |
| 1 | 60 | 80 | 80 | 80 | 0 | 20 | 60 | 60 |
| 5 | 60 | 80 | 100 | 100 | 10 | 10 | 60 | 90 |

TABLE 3. *Results of static bioassays in which oysters were exposed to 1% oil-water dispersions, expressed as $TL_0$ (time of first mortality in days); $TL_{50}$ (time to 50% mortality); $TL_{100}$ (time to 100% mortality).*

| | $TL_0$ (Days) | $TL_{50}$ (Days) | $TL_{100}$ (Days) |
|---|---|---|---|
| Venezuela Bunker C | 5 | 6 | 13 |
| #2 Fuel | 5 | 8 | 14 |
| South Louisiana Crude | 9 | 14 | 18 |
| Kuwait Crude | 20 | 35 | 70 |

that the concentration of total hydrocarbons in oil-water dispersions was a function of the amount of oil added to the bioassay container. They found that the concentration of total hydrocarbons derived from South Louisiana and Kuwait crude oil increased linearly with the amount of oil added. For South Louisiana and Kuwait crude, the amount of oil in the water phase ranged from approximately 16 to 80 ppm for 0.01 to 10% oil added. For Kuwait crude, the range was approximately 19 to 42 ppm for 0.01 to 10% oil added. Using #2 fuel oil, they found

that as the amount of oil added was increased, a corresponding rise did not occur in total hydrocarbon content in the water phase. At 0.1%, the maximum (51 ppm) was reached with slightly lower levels (47 ppm at 1%, 37 ppm at 10%) recorded with the addition of more oil. For #2 fuel oil, the range of oil in the water phase was approximately 14–15 ppm for 0.01 to 10% oil added. Anderson, Neff, Cox, Tatem and Hightower (1974) attributed this phenomenon to increased droplet coalescence at very high oil concentrations. Similar data for the residual oil, Venezuela bunker C, were not available because of the difficulty in working with this extremely viscous product. Detailed composition of test solutions is discussed by Anderson, Neff, Cox, Tatem and Hightower (1974).

As pointed out in literature already cited, the oil-water dispersions were unstable in the bioassay containers. Concentrations of total oil hydrocarbons in the aqueous phase of the oil-water dispersions dropped rapidly during gentle aeration. In analytical work provided to this study and cited in Anderson, Neff, Cox, Tatem and Hightower (1974), generally only 10% of the original hydrocarbons were present in the dispersion after the first 24 hours of aeration. It is difficult then to assess over any length of time, the amount of petroleum hydrocarbons actually in solution. In addition, Anderson (1973) found that oysters depurate petroleum fractions during these static exposures, with amounts of oil in solution constantly changing. Anderson (1973) noted wide individual differences in the test exposures over time, along with the rapid rise of microorganisms in the various dispersions.

This study indicated an increased resistance to toxicants by oysters collected during the late fall and winter. A partial explanation is revealed in the Gulf oyster's life cycle. In summer and early fall, oyster meats are in poor condition; i.e., watery, translucent, thin. Spring spawning, accompanied by loss of food reserves (glycogen), markedly affects quality. Rising summer temperatures increase incidence of infection by pathogens. Animals are further depleted by a second spawning in the fall. As a result, oysters collected in summer and early fall have poor condition indices and often are infected with the highly pathogenic *Labyrinthomyxa* spp. In this study, tests conducted by Dr. William Wardle at the Texas A&M Marine Laboratory in Galveston, indicated negative or very low incidence of *Labyrinthomyxa* spp. in oysters tested.

Experimentation conducted in late fall and winter was carried out with oysters in good to excellent condition (Anderson, 1973). The molluscs were fat with large glycogen reserves. Because of the high quality of the meats and low incidence of disease, these oysters can be expected to be more hardy and resistant. Results show the experimental animals used in winter months to be almost twice as resistant as those employed in the bioassay experiments conducted in summer and early fall. Observations by other investigators on loss of condition associated with summer months support these conclusions (Fingerman and Fairbanks, 1956; Galtsoff, 1964; Roosenburg, 1969; Quick, 1971). As stated by Gardner, Barry and LaRoche (1973), sufficient background data on the test animals must be available to assess bioassay results.

Though static bioassays cannot be expected to replicate field conditions, good baseline data can be drawn, particularly when one references the animal's behavior and health in relation to actual concentrations of the test solutions. Though oysters are difficult to study in bioassays, they are an important shellfish resource which reflect the problems of sessile animals.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# GROWTH OF THE EUROPEAN OYSTER, *OSTREA EDULIS* LINNÉ, IN THE ST. CROIX ARTIFICIAL UPWELLING MARICULTURE SYSTEM AND IN NATURAL WATERS[1]

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## ABSTRACT

*Three populations of the European oysters,* Ostrea edulis *Linné, were grown from 3-mm spat to marketable adults in 12 to 16 months in the artificial upwelling mariculture system on St. Croix, U.S. Virgin Islands. The spat were obtained from Pacific Mariculture, Inc., Pescadero, California. The shellfish were fed three species of diatoms,* Bellerochea spinifera *Harg. and Guill.,* Chaetoceros *cf.* simplex *Ostf. and* Thalassiosira pseudonana *Hasle and Heim. Algal cultures, grown in 45,000-liter pools, were pumped continuously through the shellfish tanks; the salinity was 34.75 to 34.95‰ and water temperature varied between 22° and 29°C during the experiments. Larvae produced and released by one population were reared through setting, but the spat did not complete metamorphosis.*

*For comparison,* O. edulis *were grown in Salt River, a natural inlet on St. Croix. After 82 days, mortality was 100% in the Salt River population. The salinity range was 33.7 to 37.6‰ and the temperature fluctuated between 25° and 32°C.*

## INTRODUCTION

In the St. Croix artificial upwelling mariculture system, nutrient-rich deep water is pumped into ponds on shore, where planktonic diatoms are grown as food for filter-feeding shellfish. The productivity of this system is much higher than that of natural upwelling systems because the deep water is not diluted with nutrient-poor surface water. Another advantage of using deep water in a mariculture system is that it is free of pollutants, parasites, diseases and predators. The St. Croix site was chosen because the ocean reaches a depth of 1,000 meters approximately 1.6 kilometers off shore.

Deep water is pumped continuously from 870 meter depth through three 1,830 meter long, 7.5 centimeter diameter polyethylene pipelines into 45,000 liter concrete pools in which unialgal cultures of planktonic diatoms are grown. The pool cultures are started by inoculating them with starter cultures grown in 200-gal tanks.

[1] This work was supported by NOAA Sea Grant 04-3-158-66 from the U.S. Department of Commerce. Lamont-Doherty Geological Observatory Contribution No. 2381, City University Institute of Oceanography Contribution No. 99.

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The pool cultures are operated continuously for up to 30 days. The growth rate of the algae is regulated by the rate at which nutrients are supplied by the incoming deep water, thus assuring nearly complete utilization of the nutrients in the deep water. The algal cultures in the pools are pumped continuously to shellfish tanks, at metered rates, based on the feeding activity of the animals. The total flow pumped to the shellfish matches the flow of deep water into the algal pools, so that the pool volume remains constant.

Ten species of shellfish have been screened for growth and survival in the St. Croix system. Eight species grew well and reached market size quickly: they are *Ostrea edulis*, *Crassostrea gigas*, *Crassostrea gigas* Kumomoto variety, *Tapes semidecussata*, *Mercenaria campechiensis*, $F_1$ Clam (a cross *M. campechiensis* × *M. mercenaria*), *Argopecten irradians*, and *Pinctada mertensi*.

*Ostrea edulis* Linné was first introduced in the artificial upwelling mariculture system in April 1972. This paper reports the growth of three populations of the European oyster in this system, and the results of comparative growth experiments in two natural environments.

## MATERIALS AND METHODS

In the artificial upwelling mariculture system, sea water was pumped from 870 m depth in the Caribbean Sea through a one-mile-long pipeline into 45,000-liter pools on shore in which continuous unialgal cultures of planktonic diatoms—*Bellerochea spinifera* (clone STX-114), *Chaetoceros simplex* (clone STX-105) or *Thalassiosira pseudonana* (clone 3H)—were grown. The algal cultures ($10^4$ to $10^6$ cells $ml^{-1}$) were pumped continuously into the shellfish tanks at metered rates. The temperature in the tanks varied between 22° and 29°C. Details of the system are given by Baab *et al.* (1973).

In Salt River, a natural inlet on the North Shore of St. Croix, the oysters were grown in trays (positioned below the low tide mark) suspended from a dock. The water depth at the dock was about 2 m. The tidal range in Salt River is 38 cm.

Cultchless, *O. edulis* used in all experiments were obtained from Pacific Mariculture, Inc., and grown in stacked Nestier trays (Division of Vanguard Industries, Inc., Cincinnati, Ohio). Until the spat were greater than 13 mm in diameter they were held in the trays by liners made from 1/16-inch mesh, plastic-covered fiberglass window screening. At four-week intervals, wet weight and length (measured from umbo to posterior margin) were determined on a sample of 100 oysters randomly selected from each of the populations.

## RESULTS AND DISCUSSION

In April 1972, 50,000 3-mm *O. edulis* spat were introduced to the mariculture system. These juveniles were part of an experiment designed to select suitable shellfish species for the artificial upwelling mariculture operations. Figure 1 shows the growth of *O. edulis* over a 16-month period. Total mortality of this population was 19%. After 13 months, the European oysters averaged 75 mm in length and their average weight was 40.7 gm. The oysters were nearly 100 mm in length and averaged 64.1 gm after 16 months. Bardach, Ryther and McLarney (1972) report that *O. edulis* grow to market size of 75 mm in diameter or a total weight of 65 gm in four years in France. Our oysters attained market size diameter in 13 months and reached market size weight in 16 months.

In April 1973, viable larvae (120–140 $\mu$ in length) were collected with a 62-$\mu$ plankton net from the tank containing the *O. edulis*. The larvae were reared in 379-liter polyethylene tanks and fed the same algal diet as the adults. Water in the tanks was changed daily and no antibiotics were added. When the larvae began to set, cultch (*O. edulis* shells) was placed in the tanks. Even though the larvae set, they did not complete metamorphosis and died within a day. Possible causes of this failure of metamorphosis may be of nutritional, environmental or infectious origin or a combination of these factors. The larvae were fed only two species of diatoms; literature on larval feeding studies report that *Isochrysis galbana*, *Monochrysis lutheri*, *Dunaliella euchlora* and *Platymonas* sp. in unialgal cultures and in mixtures are suitable food for bivalve larvae (Davis and Guillard, 1958; Loosanoff and Davis, 1963; Walne, 1963; and Walne, 1966). The temperature in the larval rearing tanks reached 29°–30°C in the afternoons. Loos-

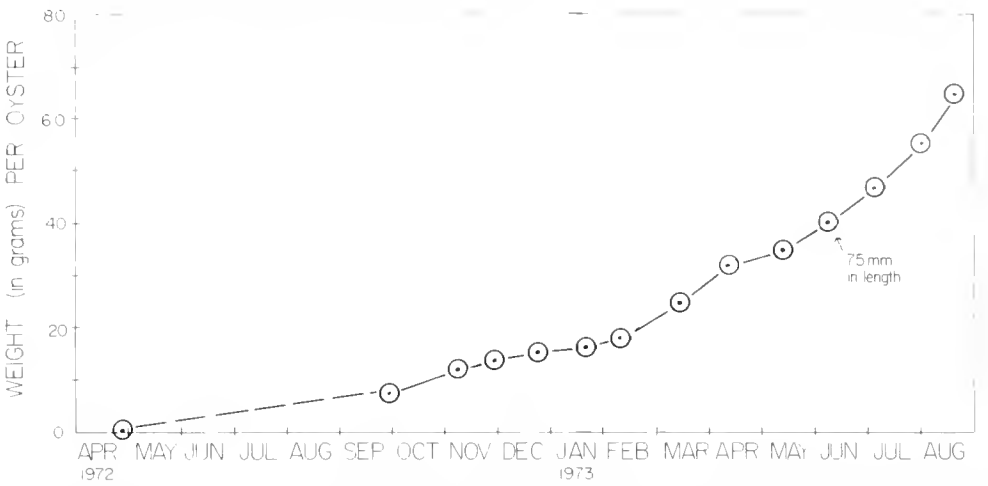


FIG. 1. *Average weight per oyster (including shell) of the first population of* Ostrea edulis *in the artificial upwelling mariculture system, St. Croix, U.S. Virgin Islands.*

anoff and Davis (1963) report that 18°–20°C is optimal for the setting of *O. edulis*. Throughout the larval study, no antibiotics were added to the standing larval culture.

In October 1972, an experiment designed to compare the growth of *O. edulis* (8-mm juveniles) in a controlled environment (the artificial upwelling mariculture operation) to growth in an uncontrolled natural environment (Salt River Inlet, see Table 1) was started. The *O. edulis* in the mariculture operation reached 75 mm in length in 10 months; after 82 days no *O. edulis* survived in the Salt River Inlet population (Fig. 2). Land run-off caused by heavy rains increased the silt content of Salt River Inlet. The oysters were covered with 10–20 mm of silt in their trays; this was believed to be the cause of the 100% mortality. During these rains, the salinity in Salt River Inlet reached a low of 33.7‰. However, fouling by sponges and algae was heavy and predators (crabs and drills, *Murex pomum* Gmelin and *Murex brevifrons* Lamarck) were present.

Mortality for the batch of oysters grown in the controlled environment was 81%. This increased mortality (first experiment was 19%) can be attributed to the saturated NaCl-dip given to this batch to remove infestations of the bryozoan, *Bowerbankia gracilis* Leidy. The shellfish were placed in a saturated salt solution (300 gm NaCl per liter of sea water) immediately after they were removed from the shellfish tanks. After one minute in the vigorously aerated salt dip, the shellfish were air-dried for one hour. On two occasions, however, the oysters were out of water almost an hour before the salt treatment and several days later high mortalities occurred. The total mortality for this experimental batch was 35% if the percent mortality reported is corrected for the deaths caused by the salt treatments. These oysters were checked for possible disease and/or pathogenic bacteria and nothing was found.

In August 1973, an experiment using 3.2-mm

TABLE 1. *Comparison of the environmental factors in the artificial upwelling mariculture operation and in Salt River Inlet.*

| Environmental Conditions | Mariculture Operation | Salt River Inlet |
|---|---|---|
| Temperature (°C) | 22–29 | 25–32 |
| Salinity (‰) | 34.8–34.9 | 33.7–37.6 |
| * Phytoplankton chlorophyll *a* (mg/m$^3$) | 22.4–54.0 | 0.56–1.14 |
| * Particulate matter (mg/liter) | Negligible (<1) | Low during drought; heavy during rainy season |
| Degree of fouling | Light—*Bowerbankia gracilis* Leidy | Heavy—sponges, algae, bryozoans, tube worms, sea squirts |
| Predators | Absent | Crabs and *Murex brevifrons* Lamarck; *Murex pomum* Gmelin |

* Haines, K. C. (unpublished).

spat was begun to substantiate the results of previous experiments on St. Croix and to compare growth in other natural environments—Long Island Sound, Virginia, Florida, and Salt River Inlet. Presently, there are *O. edulis* in only two of these locations—the St. Croix mariculture system and Greenport, Long Island. Oysters sent to the Virginia Institute of Marine Sciences (Michael Castagna, Eastern Shore Laboratory) arrived in very poor condition and died within a week. From Florida State University, R. Winston Menzel reported that the *O. edulis* were in satisfactory condition on arrival but died after being suspended in flowing sea water. After 55 days, none of the *O. edulis* in the Salt River Inlet population survived. Silta-

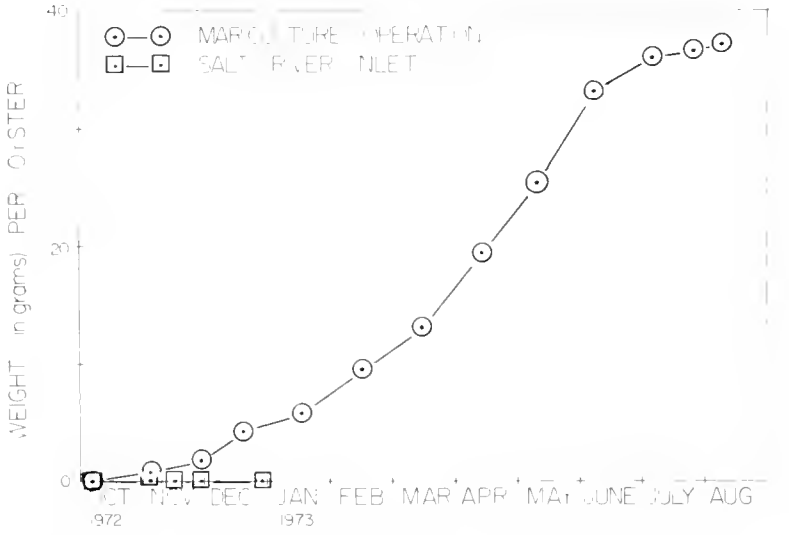


FIG. 2. *Average weight per oyster (including shell) of the second population of* Ostrea edulis *in the artificial upwelling mariculture system compared to growth in Salt River Inlet, St. Croix.*

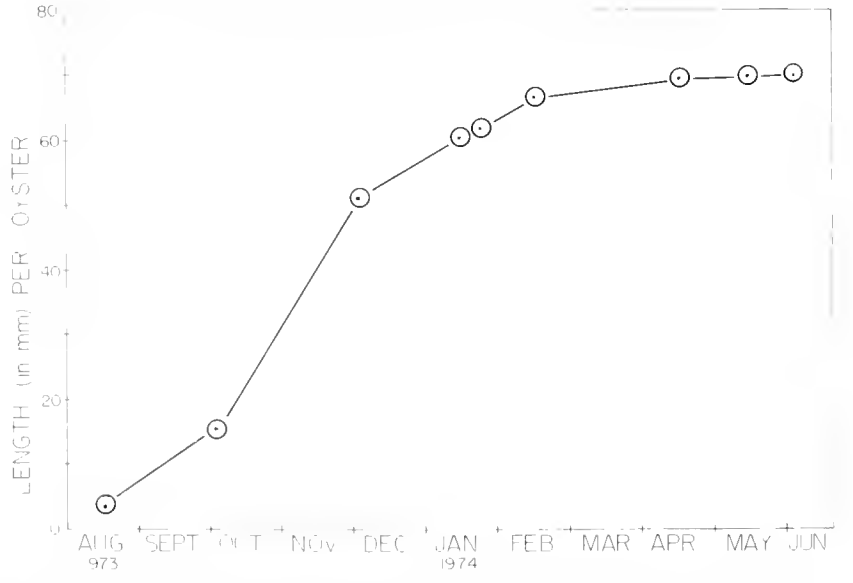


FIG. 3. *Average length of the third population of* Ostrea edulis *in the artificial upwelling mariculture system. The average weight of this population is given in Figure 4.*

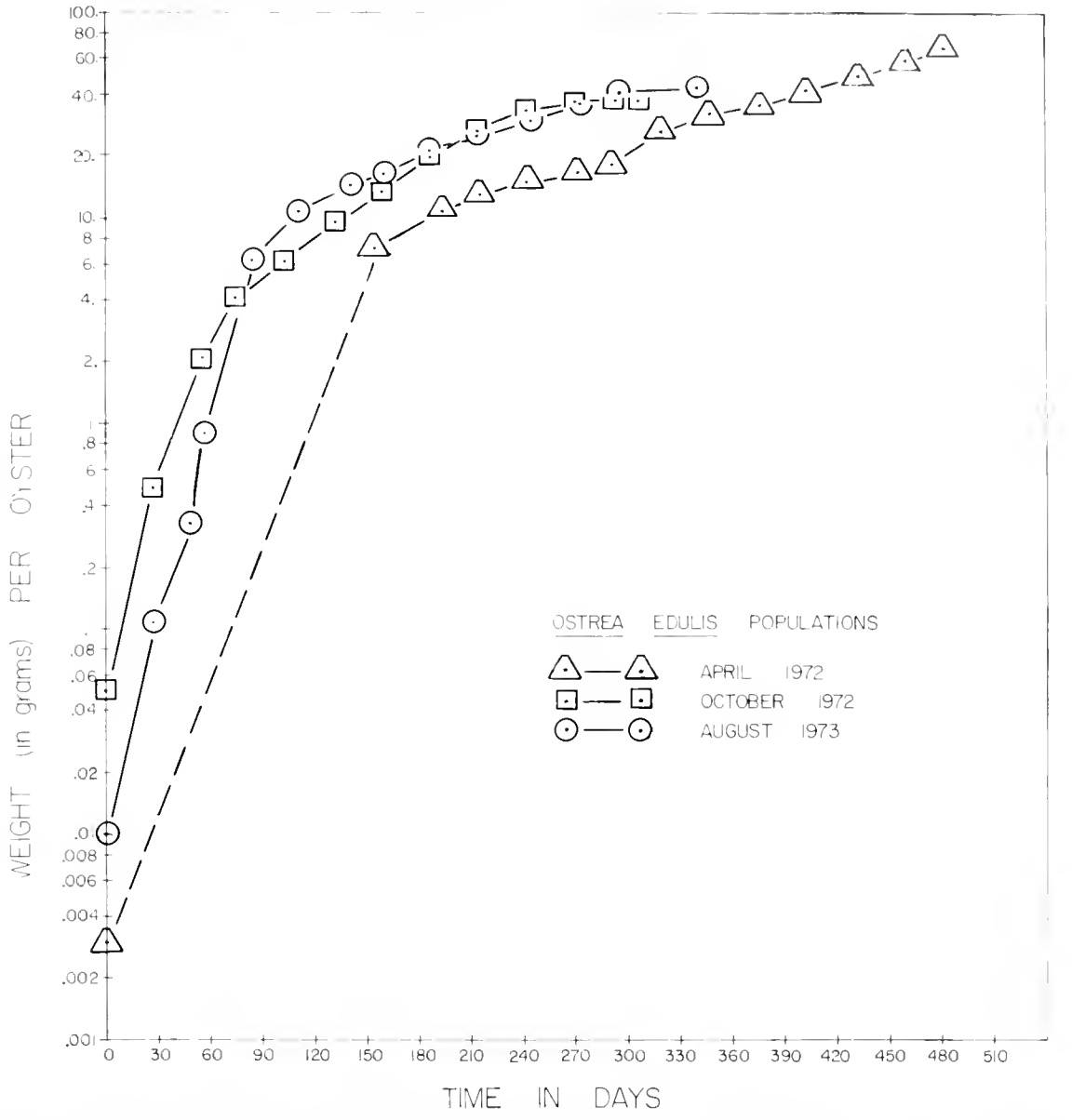


FIG. 4. *Average weight per oyster (including the shell) of three populations of* Ostrea edulis *in the artificial upwelling mariculture system.*

tion was suspected as the cause of death in the Florida and in the Salt River Inlet populations.

In July 1974, the oysters in Greenport, New York (Paul Chanley, Shelter Island Oyster Company) averaged 44 mm; in June 1974, the *O. edulis* in the St. Croix mariculture operation averaged 69 mm in length (Fig. 3). Marketable adults were obtained after 12 months in the mariculture operation; mortality in this experiment was 24.3%.

## CONCLUSION

Due to improved handling techniques for optimization of growth, each successive batch of *O. edulis* grown in the St. Croix system attained market size in a shorter period of time (Fig. 4). The food and oxygen requirements and growth densities were established for the *O. edulis* grown in the St. Croix mariculture system. Densities varied with the size of the oys-

ter—as juveniles, densities greater than 25/ft$^2$ were acceptable; at 40-mm length, 15 to 16/ft$^2$, and at 75-mm length, 7 to 8/ft$^2$. Feeding the oysters three species of diatoms on a rotating schedule appeared to be adequate. Oxygen concentration in the shellfish tanks was kept at 5 ppt or greater.

The excellent growth of *O. edulis* in the artificial upwelling mariculture system can be attributed to the constant food supply, the deep water source relatively free of particulate matter, and the extended growing season.

Planned improvements in the supply of food and management of the oysters in the artificial upwelling system should further reduce the time they require to reach market size.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# OBSERVATIONS ON SPAWNING AND GROWTH OF SUBTIDAL GEODUCKS (*Panope generosa*, Gould)[1]

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## ABSTRACT

*Histological preparations of subtidal geoducks from Puget Sound were examined to determine their annual reproductive cycle. One annual spawning season occurred in spring and early summer. Most clams were in a spawned-out conditions in late summer. Gametogenesis occurred rapidly in the fall and continued into the winter. By early spring, most were mature and ready to spawn.*

*The growth rate of subtidal geoducks was estimated from mark and recovery studies in five locations throughout Puget Sound. These experiments showed that during the first 3 years of life, marked geoducks grew from 20 to 30 mm/year in total shell length. Growth of older marked geoducks was less, and the shell length in the majority over 100 mm did not increase at all.*

*Growth rate based upon length frequency distributions from two locations amounted to about 30 mm/year for the first 3 years. This figure more accurately estimated the true growth rate because of the setback in growth caused by handling in the mark and recovery studies.*

*Geoducks are estimated to reach the average adult size of 158 mm in 10 years and, thereafter, growth is reduced. The average length of geoducks in separate populations varied from 123.8 mm to 171.3 mm in samples taken in 22 locations. The largest clam from a sample of 2,037 was 206 mm. The growth rate probably varies considerably from one clam bed to another.*

*The length-weight curve based on 1,213 pairs of observations can be expressed by the equation: $\log_{10}$ weight (in grams) = $-3.42983 + 2.97281 \log_{10}$ length (in millimeters). The sigmoid age-weight curve shows that the average 10-year-old geoduck weighs about 1,200 grams, and the greatest annual-weight gains occur between the third and seventh years.*

## INTRODUCTION

The geoduck is the largest clam in the Pacific Northwest. They range from Alaska to California, and are very abundant in Puget Sound (Goodwin, 1973), where they are an important sport and commercial clam. They are normally found buried 50–60 cm in a sand-mud substrate. The author has observed them in Puget Sound from the lower intertidal zone to depths of over 60 m. Puget Sound is hydrographically very complex. Dabob Bay and other locations (Fig. 1) stratify during the summer and are warmed by solar radiation to maximum temperatures of over 20 C at the surface. Minimum temperature in the winter can be as low as 6 C. In other areas of Puget Sound where waters are more thoroughly mixed, summer maximums of 15 C or less are common. Geoducks living at 60 m are probably never exposed to water over 10 C. Because of this complexity and the wide vertical

[1] The work reported here was partially financed by the National Marine Fisheries Service, Fisheries Research and Development Act, PL 88-309.

distribution of geoducks, these clams in Puget Sound exist in many temperature regimes.

## SPAWNING

### *Histological methods*

Spawning information was obtained from histological examination of gonads from 124 specimens collected by divers in several locations from July 1968 to August 1969. Sections from transverse slices from the gonads were preserved in Davidsons' fixative and prepared by standard techniques of dehydrating in alcohol, embedding in paraffin, and staining with Harris' hematoxylin and eosin.

### *Sex ratio*

Of the 124 geoducks studied, 58 were females and 66 were males, virtually a 1:1 ratio (Table 1; Fig. 1). None were observed with both sex products. They ranged in size from 65 to 198 mm total shell length. Of the 29 which were less than 120 mm, 15 were females and 14 were males. This information suggests a lack of sex reversal. Anderson (1971) found a ratio of males to females of 51:3 in geoducks shorter than 100 mm, but concluded that they are gonochoristic

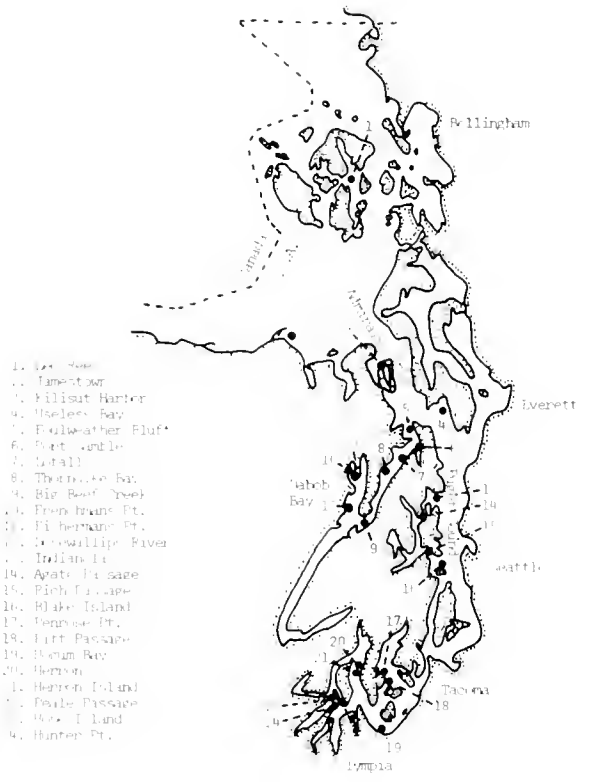


FIG. 1. *Location of sampling stations.*

TABLE 1. *Stage of gonadal development of geoducks in Puget Sound, 1968–1969.*

| Date | Sample location | Water depth (meters) | Number sample | Gonadal condition: Early active Male | Early active Female | Late active Male | Late active Female | Ripe Male | Ripe Female | Partially spent Male | Partially spent Female | Spent Male | Spent Female |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *1968* | | | | | | | | | | | | | |
| July 26 | Rich Passage | 11 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| July 29 | Frenchmans Point | 8–11 | 10 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 1 | 4 |
| Aug. 23 | Frenchmans Point | 11 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 |
| Sept. 20 | Frenchmans Point | 8–11 | 8 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Oct. 15 | Big Beef Creek | 8 | 3 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Oct. 24 | Frenchmans Point | 8–11 | 8 | 1 | 1 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Nov. 22 | Big Beef Creek | 9 | 8 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |
| Nov. 27 | Frenchmans Point | 8–11 | 5 | 0 | 1 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 |
| Dec. 19 | Hope Island | 3 | 7 | 0 | 0 | 1 | 3 | 2 | 1 | 0 | 0 | 0 | 0 |
| Dec. 21 | Agate Passage | 5–6 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dec. 26 | Frenchmans Point | 8–11 | 6 | 0 | 0 | 0 | 0 | 1 | 5 | 0 | 0 | 0 | 0 |
| *1969* | | | | | | | | | | | | | |
| Feb. 28 | Frenchmans Point | 8–11 | 25 | 0 | 0 | 0 | 0 | 10 | 13 | 2 | 0 | 0 | 0 |
| April 30 | Frenchmans Point | 8–11 | 8 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 |
| May 19 | Frenchmans Point | 8–11 | 4 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 |
| May 19 | Big Beef Creek | 5 | 8 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 |
| July 3 | Frenchmans Point | 8–11 | 7 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 0 | 0 |
| July 10 | Leo Reef | 18 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Aug. 7 | Frenchmans Point | 8–11 | 8 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 1 | 2 |

and the ratio was due to males maturing at a smaller size than females.

## STAGE OF GONADAL DEVELOPMENT

The seasonal gonadal changes and spawning cycles have been studied and described for many of the important pelecypods of the East Coast of the United States, including the eastern oyster, *Crassostrea virginica*, Loosanoff (1942), Kennedy and Battle (1964); northern quahog, *Mercenaria mercenaria*, Loosanoff (1937), Porter (1964); softshell clam, *Mya arenaria*, Ropes and Stickney (1965), Shaw (1962), Pfitzenmeyer (1965); coot clam, *Mulinia lateralis*, Calabrese (1970); and the surf clam, *Spisula solidissima*, Ropes (1968).

Similar studies have been conducted on the West Coast pelecypods, such as littleneck clam, *Paphnia staminea*, Quayle (1943); jingle, *Pododesmus cepio*, Leonard, Jr. (1969); mussel, *Mytilus edulis*, Moore and Reish (1969); native oyster, *Ostrea lurida*, Coe (1932); gaper clam, *Tresus capax*, Machell and DeMartini (1971); geoduck, Andersen (1971); manila clam, *Venerupis japonica*, Holland and Chew (1974); and the softshell clam, Porter (1974).

Many different schemes have been used in these papers to describe the various stages of gametogenesis. For the present study, gametogenesis was divided into five stages after Ropes (1968) except the stages "spent" and "resorbing" were combined into one called "spent".

1. *Early active:* Follicles are small and contain early stages of sex cells. Connective tissue is abundant.
2. *Late active:* Follicles are enlarged and contain sex cells of different stages of development. Male follicles contain spermatozoa and early spermatogenic stages. Female follicles contain primary oocytes, many of which are still attached to the follicle walls. Connective tissue is reduced in amount.
3. *Ripe:* Follicles are full size and filled with spermatozoa and oocytes. Connective tissue reduced to thin layer between the follicles.
4. *Partially spent:* Follicles are reduced in size and often partially empty. Connective tissue increasing in amount.
5. *Spent:* Follicles are greatly reduced in size and contain few if any mature sex cells. Connective tissue is greatly increased in amount.

## SEASONAL SEXUAL CYCLE OF FEMALES

Spawning begins in the spring with the major release of ova occurring during June. The clams examined at the end of July 1968 and during the first of July 1969 were either partially or completely spent. Most gonads in this stage contained a few residual ova in the contracted follicles. Connective tissue between the follicles was thickened. Females examined in September were beginning to show active gametogenesis. October and November samples revealed that gametogenesis was proceeding rapidly with the follicles proliferating and invading the connective tissues and with oocytes enlarged and attached to the follicle walls. By January, all females contained follicles with oocytes of varying stages of development, some of which were the mature size.[2] All females appeared to be mature and ready to spawn in April. The gonads were large, distended, and creamy in texture. The follicles were large and filled with ova. The follicle wall was thin and most of the connective tissue between the follicles had disappeared. The connective tissue between the follicles contains inclusions (probably glycogen or lipid) which are abundant during early stages of gametogenesis and are gradually lost as the gonad ripens. These findings are in general agreements with those of Andersen (1971), who stated that geoducks spawn in a single mode from March to July. He found them partially spent as early as January, and completely spent in August.

## SEASONAL SEXUAL CYCLE OF MALES

All males apparently do not follow as concise a seasonal pattern as do the females. Some males with sperm were observed in all months sampled. The majority, however, do follow a pattern similar to that described for females. Andersen (1971) found no difference between females and males in the timing of the annual gonadal cycle. The major release of sperm occurred during May and June, so that by July

[2] Oocytes stripped from clams sampled in June and measured in wet smears were 75 $\mu$. Newly released ova in natural spawnings averaged about 82 $\mu$.

most males were partially or completely spent. The typical male sampled in August has contracted and partially empty follicles. Connective tissue between the follicles is abundant. Six of the 15 males examined in September and October contained mature sperm, and the other nine were in active gametogenesis stages. By April and May, all males examined appeared ripe.

## LABORATORY SPAWNING OF GEODUCKS

Additional information on the geoduck sexual cycle was obtained from 35 spawning experiments conducted in the laboratory. Thermal stimulation was used to initiate natural release of gonadal products in the majority of these experiments. The spawners were held in heated water pumped from Dabob Bay. The majority of these experiments were conducted within a few days after the clams were collected. The short time that the spawners were held in the laboratory before spawning probably had little effect on the annual timing of spawning. Some spawning was accomplished by mechanically stripping the gonads of sperm and ova. Natural laboratory spawning, which produced normal larvae, has been initiated with thermal stimulation as early in the winter as January 10. Geoducks have spawned in water ranging from 8.5 C to 16.0 C, with the majority of successful spawnings occurring in water of 12 C to 14 C. Spawning occurred in the laboratory as late as July 5. Mechanical stripping experiments, which have resulted in normal larval development, were completed as early as November 29, however, none were attempted later than June 11.

Spawning in nature was observed twice by our divers near Frenchmans Point. One occurred on April 20, 1969, in 9–12 m of water at 8–10 C, and the other in 8–9 m of water on July 13, 1969, when the water temperature was not measured. In each case, only a single clam was spawning. The sex products flowed from the excurrent siphon continuously over a period of several minutes.

## GROWTH

### *Annual ring method*

Geoduck shells from many locations in Puget Sound were examined for annual rings. Difficulty was encountered in determining whether or not a ring was an annulus or not. After considerable effort, this method was judged to be unfeasible for geoduck age or growth estimation. Andersen (1971) came to the same conclusion in his study of geoducks from Hood Canal. Tegelberg (1964) questioned the validity of the ring method in determining the growth of razor clams.

### *Mark and recapture method*

Mark and recapture experiments were conducted at various locations in marked plots in substrates of sand and mud mixtures in water depths ranging from 5 m to 14 m calculated from zero tide (Table 2; Fig. 1).

Geoducks were first removed from the plots by divers with small hand-held washout nozzles or venturi suction dredges. The same number of geoducks that were taken from a plot were marked, measured, and planted into the plot. Numbers were ground into the shell in large clams with thick shells, and small clams were marked with waterproof ink. Only the total length (greatest anterior-posterior distance) of the right valve was measured. Large geoducks were planted in individual holes excavated by a venturi dredge. They were planted in the substrate at a depth of about 60 cm, which is the depth at which most adult geoducks live. Small geoducks, being good diggers, were placed in small holes made by the diver's fingers and protected for a few minutes until the clams completely buried themselves. Small wire stakes were placed near each planted geoduck to facilitate future recapture.

Four hundred-ninety geoducks were marked and planted in five separate plots. Of these, 202 were recovered: 107 alive and 95 dead (Table 2). The majority of the mortalities was due to handling. The geoducks in this table were divided into two groups, those that were less than 100 mm and those more than 100 mm total shell length at marking.

The smaller geoducks showed substantial growth. The greatest rate of growth for any single group of clams was 2.8 mm/month, or 33.6 mm/year. These clams, which were planted at Fishermans Point, averaged 48 mm in shell length at marking. The average growth rate for all geoducks less than 100 mm was 1.8 mm/

TABLE 2. *Mark and recapture data of five growth experiments (total shell length in mm).*

| Experiment | Number marked | Number recovered: Alive | Number recovered: Dead | Date planted | Date recovered | Average length of geoducks at marking | Average length change | Range of length change | Greatest length change per month in any individual | Average length change per month |
|---|---|---|---|---|---|---|---|---|---|---|
| *Clams more than 100 mm total shell length at marking* | | | | | | | | | | |
| Big Beef Creek | 79 | 9 | 18 | 11/68 | 11/69 | 141.2 | +1.2 | −3 to +5 | +0.4 | +0.1 |
| Port Gamble A | 81 | 10 | 30 | 12/69 | 2 in 1/71 | 141.0 | −1.5 | −2 to −1 | −0.2 | −0.1 |
| | | | | | 8 in 5/72 | 133.5 | −1.4 | −3 to 0 | −0.1 | 0.0 |
| Agate Pass | 86 | 25 | 27 | 1/70 | 6 in 11/70 | 131.7 | −0.3 | −1 to +4 | +0.4 | 0.0 |
| | | | | | 19 in 5/72 | 133.9 | −1.3 | −7 to +1 | −0.2 | 0.0 |
| Herron Island | 91 | 16 | 17 | 2/70 | 4 in 1/70 | 142.8 | −0.7 | −2 to 0 | −0.2 | −0.1 |
| | | | | | 12 in 7/72 | 125.8 | +1.9 | −5 to +15 | +0.5 | +0.1 |
| *Clams less than 100 mm total shell length at marking* | | | | | | | | | | |
| Big Beef Creek | 5 | 1 | 0 | 11/68 | 11/69 | 81.0 | — | — | — | — |
| Port Gamble A | 19 | 8 | 2 | 12/69 | 1 in 1/71 | 95.0 | 0 | 0 | 0 | 0 |
| | | | | | 7 in 5/72 | 60.4 | +18.2 | +2 to +38 | +1.3 | +0.6 |
| Port Gamble B | 18 | 6 | 0 | 5/70 | 4 in 1/71 | 31.6 | +11.2 | +5.3 to +17.5 | +2.2 | +1.4 |
| | | | | | 2 in 5/72 | 27.5 | +24.5 | +22 to +27 | +1.1 | +1.0 |
| Agate Pass | 11 | 3 | 0 | 1/70 | 5/72 | 54.3 | +43.0 | +5 to +62 | +2.2 | +1.5 |
| Herron Island | 9 | 2 | 1 | 2/70 | 6/72 | 85.5 | +25.5 | +15 to +36 | +1.2 | +0.9 |
| Fishermans Point | 91 | 27 | 0 | 2/71 | 10 in 9/71 | 39.6 | +20.2 | +11 to +39.5 | +5.0 | +2.5 |
| | | | | | 3 in 2/72 | 47.7 | +27.3 | +16 to +33 | +2.8 | +2.3 |
| | | | | | 5 in 12/72 | 48.0 | +61.4 | +54 to +73 | +3.3 | +2.8 |
| | | | | | 2 in 12/73 | 51.0 | +87.0 | +84 to +90 | +2.6 | +2.6 |
| | | | | | 7 in 10/74 | 41.8 | +95.6 | +80 to +115 | +2.7 | +2.2 |
| Total | 490 | 107 | 95 | | | | | | | |

month, or 21.6 mm/year. The greatest rate of growth shown by an individual geoduck was 5 mm/month, or 60 mm/year.

Significant growth did not occur in clams larger than 100 mm. Many of these larger clams showed a loss in total shell length from marking to recovery, apparently due to shell recession. Shell recession was also observed by Andersen, 1971.

The marking process produced a significant setback in growth. The setback caused pronounced checks in the rings of the shells. The shock of mark and recapture was apparently more severe in the larger clams since these clams collectively showed no growth after marking.

TABLE 3. *Relationship between size of geoducks and rate of growth (length in mm).*

| Length at marking | Number of geoducks | Regression intercept | Slope | Predicted increase in length after 52 weeks |
|---|---|---|---|---|
| 16–39.9 | 20 | +9.4 | +0.28 | 23.8 |
| 40–59.9 | 10 | +7.0 | +0.46 | 30.8 |
| 60–79.9 | 4 | +18.2 | +0.04 | 20.2 |
| 80–99.9 | 7 | −2.6 | +0.05 | 0.0 |
| 100–119.9 | 15 | +0.9 | +0.00 | 1.0 |
| 120–139.9 | 6 | +0.6 | −0.01 | −0.2 |
| 140–159.9 | 22 | +1.0 | −0.02 | 0.0 |
| 160–179.9 | 4 | +0.6 | −0.01 | 0.2 |

The relationship between the size of the clams and growth is shown in more detail in Table 3. The data in the table are linear regressions of time from marking to recovery on change of length and include clams from all five locations listed in Table 2. The table shows an inverse relationship of size and rate of growth for marked clams and that small clams are capable of growing about 30 mm/year in shell length.

### *Length frequency distributions*

Length frequency distributions were prepared from data collected from Fishermans Point and the mouth of the Dosewallips River (Fig. 2). The year classes were identified and the length differences between year classes used as a measurement of the yearly growth increment. The samples from Fishermans Point were

obtained by divers who collected all geoducks observed regardless of size. Those from the Dosewallips River flats may be biased for size because they were obtained from sport clam diggers who normally select larger clams.

Fig. 2-A, which depicts geoducks taken at Fishermans Point in December 1969, is typical of an unharvested subtidal population in that it is unimodal with the peak at 165 mm and includes very few small geoducks. Many geoducks were removed from this population for various experiments between December 1969 and April 1970. In May 1970 another sample was taken and at this time a group of small geoducks was present which averaged 30 mm total shell length (Fig. 2-B). Whether or not sampling had stimulated setting was undetermined. The small clams were believed to have set in the spring of 1969 and to be 1 year old.

The 1969 year class was still present in Janu-

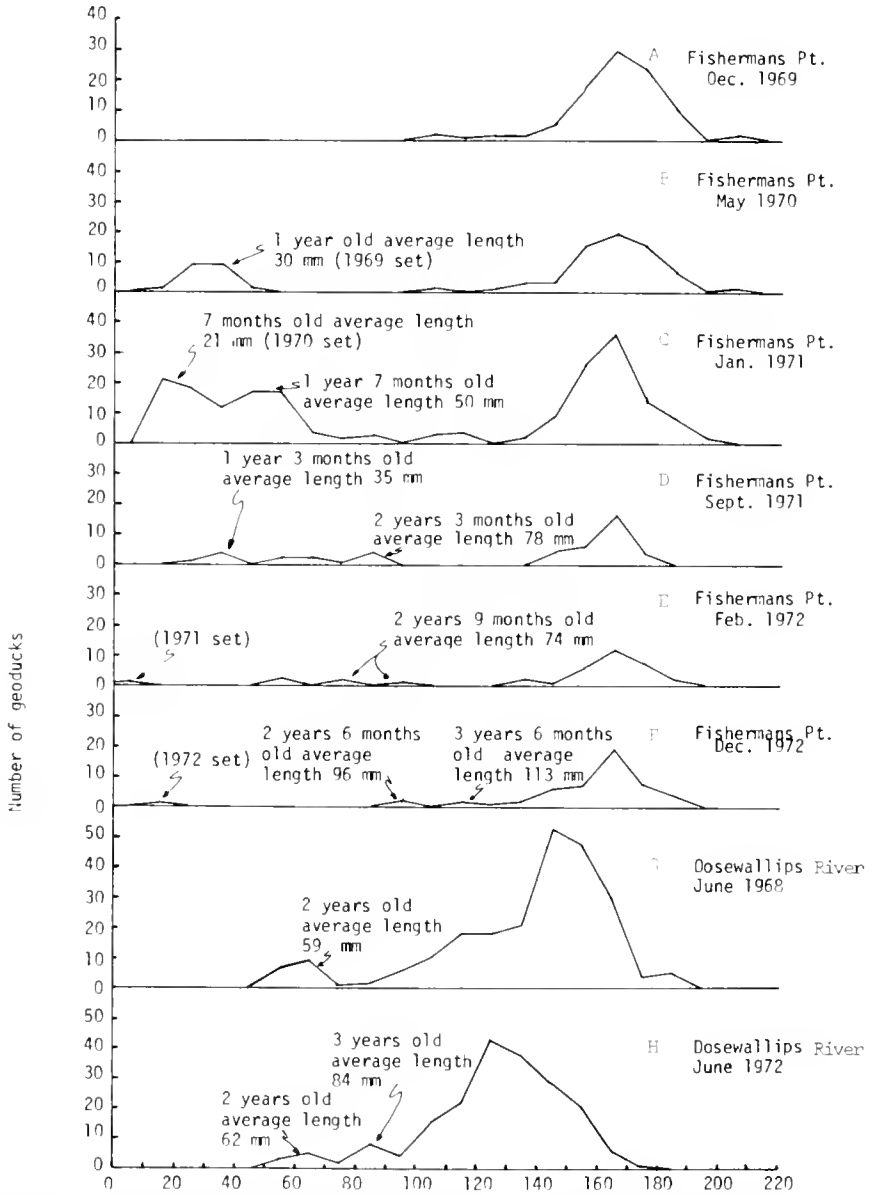


FIG. 2. *Length frequency distributions of geoducks from Fishermans Pt. and Dosewallips (shell length in mm).*

ary 1971 when another sample was taken (Fig. 2-C). They had increased to an average shell length of 50 mm. Some of these clams were marked at this time and replanted, thereby allowing positive identification of the year class at future sampling dates. Another year class was present which resulted from a set in the spring of 1970, and averaged 21 mm total shell length. The large adult clams were still present and unchanged at an average of 165 mm in total shell length.

Both small year classes were present in September 1971, February 1972, and December 1972, as well as the large adult clams which remained unchanged in average total shell length (Fig. 2-D, 2-E, and 2-F).

The Dosewallips River flats were sampled on two occasions, and the geoducks are depicted in Fig. 2-G and 2-H. This population has been intensively harvested by recreational clam diggers for many years. The distance between the peaks in the curves of Fig. 2 indicates that for the first 2 years of life, geoducks grow about 30 mm/year. The average length of the various year classes is shown in Table 4.

TABLE 4. *Shell length of geoducks based on length frequency distribution (length in mm).*

| | Age in years | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Fishermans Point | | | |
| Average length | 33.8 | 62.4 | 96.5 |
| Sample size | 65.0 | 61.0 | 17.0 |
| Dosewallips River | | | |
| Average length | – | 61.0 | 84.0 |
| Sample size | – | 26.0 | 11.0 |
| Average length | 33.8 | 62.0 | 91.6 |

## GROWTH DISCUSSION

The mark and recapture experiments showed that growth in small geoducks was substantial but very slow, if at all, in large geoducks. Growth was found to be set back by the marking and planting procedure. Large clams seemed to be more affected than small ones. Due to the setback, the rate of growth estimated by this method is thought to be slightly less than the true rate. The growth estimate determined from length-frequency distributions probably gave the most accurate results and is a reliable method when enough is known about the population studied to be sure of making accurate judgments of the various year classes in the population.

A growth curve based on the data of Table 4 is shown in Fig. 3. The end point of the curve (158 mm) is the average adult size for geoducks in Puget Sound. The average age was calculated from 22 locations where at least 20 geoducks were taken (Table 5). The largest one-half of the geoducks of each sample was selected and an average shell length calculated. These mean lengths varied from a high of 183.9 mm at Kilisut Harbor to a low of 138.7 mm at Foulweather Bluff, and averaged 157.7 mm.

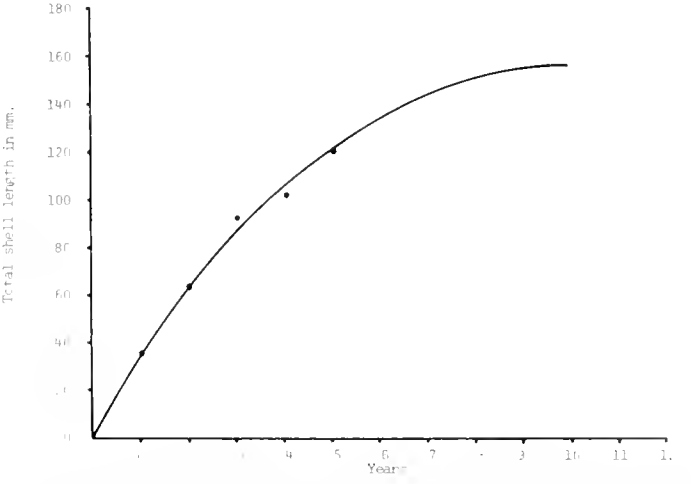


FIG. 3. *Relationship between shell length and age of geoducks.*

TABLE 5. *Average shell length of geoducks from 24 locations in Puget Sound (length in mm).*

| Area | Sample size | Total sample | Largest one-half of geoducks from sample |
|---|---|---|---|
| Jamestown | 71 | 140.1 | 157.4 |
| Kilisut Harbor | 21 | 171.3 | 183.9 |
| Useless Bay | 85 | 134.1 | 146.9 |
| Foulweather Bluff | 95 | 126.8 | 138.7 |
| Port Gamble | 179 | 134.4 | 148.1 |
| Lofall | 25 | 129.8 | 142.9 |
| Thorndike Bay | 58 | 134.6 | 148.8 |
| Big Beef Creek | 77 | 147.6 | 156.9 |
| Frenchmans Point | 40 | 133.8 | 157.7 |
| Fishermans Point | 99 | 163.8 | 175.0 |
| Dosewallips River | 229 | 135.8 | 156.1 |
| Indianola | 89 | 123.8 | 142.5 |
| Agate Passage | 78 | 148.5 | 164.7 |
| Blake Island | 75 | 132.8 | 146.7 |
| Penrose Point | 39 | 154.7 | 167.7 |
| Pitt Passage | 48 | 146.8 | 160.0 |
| Hogum Bay | 32 | 145.5 | 157.1 |
| Herron | 33 | 144.7 | 158.9 |
| Herron Island | 233 | 146.1 | 155.9 |
| Peale Passage | 20 | 140.5 | 157.5 |
| Hope Island | 34 | 171.1 | 181.2 |
| Hunter Point | 32 | 156.4 | 165.4 |
| Total | 1,692 | $\bar{x}$ = 143.8 | $\bar{x}$ = 157.7 |

The time interval needed for the average geoduck to reach 158 mm is unknown. However, an estimate is given in Fig. 3. The data for the first 3 years are mine; and for years 4 and 5, data from Andersen (1971) are used. The curve was extended beyond the fifth year and crosses 158 mm on the shell length axis between 8 and 10 years. The curve is extended beyond the data and is offered only as an estimate of the general growth pattern for subtidal Puget Sound geoducks based on present data. The average size of geoducks varies greatly from one population to another (Table 5), and I would expect that growth rates would also vary considerably from one bed to another.

Andersen (1971) gives geoduck growth increments based on length-frequency histograms for the first 5 years. He developed a von Bertalanffy growth curve which fits the data well for the first 5 years, and then extrapolated the curve beyond the data to 40 years. The curve is similar to Fig. 3 for the first few years, but goes above 200 mm at the end. I believe the curve is too high at the end of the growth phase. Of the 2,037 geoducks taken from numerous locations in Puget Sound from unexploited subtidal stocks,

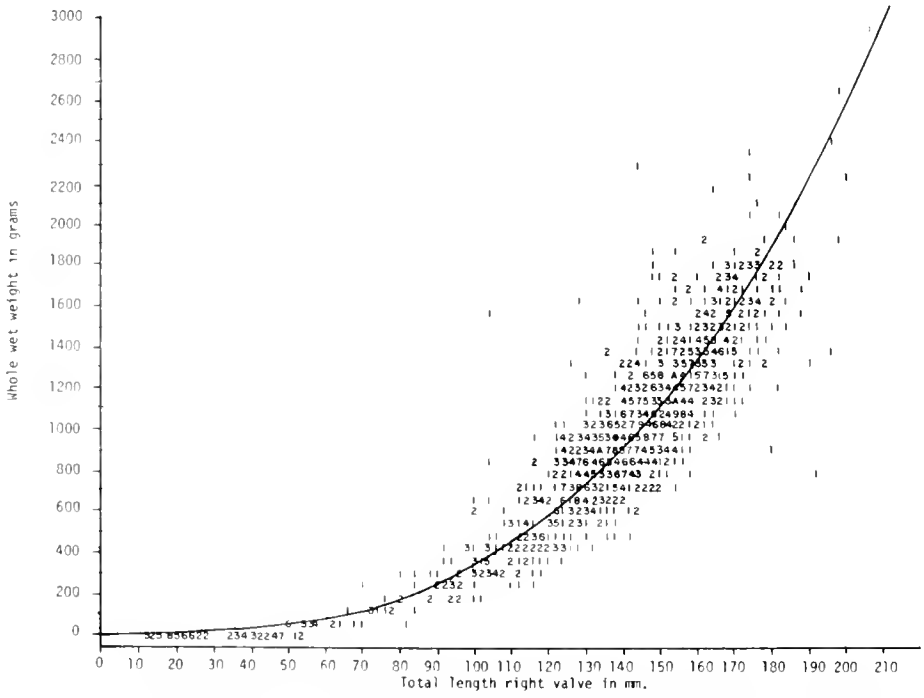


FIG. 4. *Relationship between total shell length and whole wet weight.*

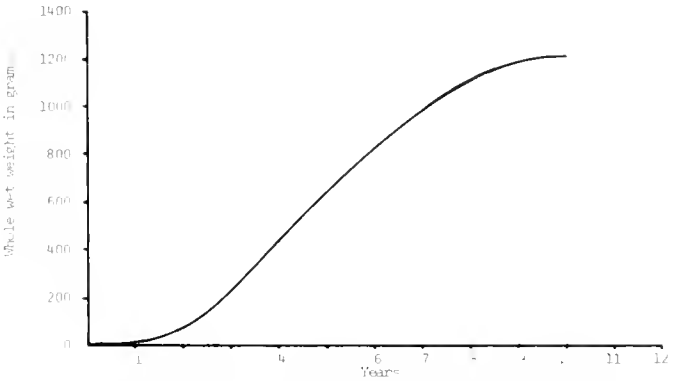


FIG. 5. *Relationship between whole wet weight and age of geoducks.*

only four had been above 200 mm total shell length, the largest of which was 206 mm. The average length was only 142.9 mm.

The relationship between shell length and whole wet weight is shown in Fig. 4. In obtaining the relationship of $\log_{10} W = -3.42983 + 2.97281 \log_{10} L$, 1,213 pairs of observations were used. Due to an oversight, the 66 geoducks of less than 50 mm shell length of Fig. 4 were not included in the equation. Data from the length-weight and age-length curves were combined in Figure 5. The curve shows that the greatest annual weight gain occurs between the third and seventh year of life.

The average whole, freshly-dug geoduck consists of about 56% meat. Meat is defined as all portions of the clam minus the shell and water of the major body cavities.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# CLAM MARICULTURE IN NORTHWEST FLORIDA: FIELD STUDY ON PREDATION[1]

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## ABSTRACT

*Twenty thousand small hatchery reared Mercenaria clams were planted for a period of nine months in a predation experiment at two field locations in Northwest Florida. At each location four 4.5 $M^2$ plots were established, in each of which were planted 2500 clams. Before planting the clams, one plot was prepared with a substrate of pea gravel and one with crushed oyster shell at each location. Two plots at each location received no substrate additive and served as controls. One control plot at each location was covered with a wire cage to exclude predators. Survival was over 50% in the wire covered control plots; less than 1% in the unprotected control plots; slightly more than 2% in the plots with shell and 10% in the plots with gravel. In this area of Florida gravel and crushed shell added to the substrate do not ensure satisfactory survival of small clams.*

## INTRODUCTION

Mariculture of quahog clams (*Mercenaria*) has been advocated for some time (Menzel & Sims, 1961) as certain procedures were developed. Further refinements of techniques, as well as development of new ones, should enable such a venture to become even more attractive and result in clam mariculture becoming a viable reality.

The advancements made so far have been experimental. Hybrids between the northern *M. mercenaria* (L.) and the southern *M. campechiensis* (Gmelin) have better commercial traits than either of the parent species, at least in warmer southern waters. The northern quahog can remain closed, alive and in good condition for a considerable period, which permits the very valuable shell trade of cherrystones and littlenecks, whereas the southern species quickly gapes and spoils when removed from the water. Although the northern clam has an annual growth rate greater in Florida than in more northern waters, the growth rate is not as great as that of the native southern clam. The hybrid between the two species has the storage qualities of the northern parent and the fast growth rate of the southern parent (Menzel, 1962, 1968, 1971, 1974; Menzel and Sims, 1962).

For the past two years we have been investigating $F_1$, $F_2$ and $F_3$ hybrids as well as backcrosses of the two species to determine if certain combinations of crosses have even better growth rates, using original parent species from various areas along the Atlantic and Gulf coasts of the United States.

It is fairly easy to induce spawning and to rear the larvae through to setting stage, and up to 1–2 mm, under the controlled conditions of a

[1] Sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant #04-3-158-43. The U. S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notations that may appear hereon.

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hatchery. At the present time it is more practical to grow clams to marketable size in open waters where they can feed on naturally occurring food, than to rear them under the controlled conditions in a closed system, where the costs of space, equipment, food water quality and labor may be prohibitive.[1]

We have never been able to obtain growth rates in the laboratory comparable to those in the field. Since we are interested in the fastest growth rate and maturity possible, we plant clams in open water as soon as possible. We rear small clams (1–2 mm at planting) in the field, but encounter excessive mortalities, mainly from crab predation, unless protected. Experimentally we control predation by using sand-filled containers, covered with plastic window screening, over which we place open-ended wire cages (1.2 cm mesh), pressed into the bottom. We have successfully grown larger (15 mm at planting) clams within fences of wire and netting of 1.2 cm mesh), pressed into the bottom. We have successfully grown larger (15 mm at planting) clams within fences of wire and netting of 1.2 cm mesh. With our present techniques, the costs of rearing clams to 15 mm before planting in the field, are excessive from a commercial standpoint. We need a low-cost method whereby small clams (1–2 mm or less) can be planted, protected and reared in open water.

Castagna, Mason and Briggs (1970) reported that "clams as small as sand grains" were planted in Virginia waters with excellent results. They planted clams in a "protective" bottom prepared with a substrate (gravel, slag or shell). This paper reports on our attempts to use this technique in Florida waters.

## METHODS AND MATERIALS

In the spring of 1972, in the course of our experiments on selection and hybridization, we reared excesses of clams. These clams were kept in holding tanks (1 × 1 × 0.65 M) supplied with continuously pumped seawater from the adjacent bay at our Ed Ball Marine Laboratory, Turkey Point, Florida. No substrate was provided in the tanks and the accumulated silt was flushed out at intervals. The clams grew, but not at a spectacular rate. In the fall of 1972, the clams were used in a "protective substrate" experiment at Turkey Point and at Alligator Harbor, the site of the former marine laboratory, Florida State University.

In both locations clams were planted in a firm bottom, several centimeters below mean low water. Substrate samples from the two locations were analyzed for sand, silt, clay, organics and carbonate content. The range of salinities at both locations was 25–35‰, with an average ca. 30‰ at Alligator Harbor and 28‰ at Turkey Point.

The clams had different parents for plantings in the several plots and the pedigrees were different for the two locations. All were backcrosses. Those planted at Alligator Harbor were the progeny of an $F_1$ female (wild female *M. campechiensis* × wild male *M. mercenaria texana*) crossed with a wild male *M. campechiensis*. The pedigree for those planted at Turkey Point was the same for the male parent (wild *M. campechiensis*) but the female parent was an $F_1$ hybrid of wild female *M. mercenaria* × wild male *M. campechiensis*. The size range when planted (4–20 mm) ranged mostly from 7 to 10 mm long. The clams were removed from the tanks, allowed to dry and sprayed with red enamel paint, prior to planting, to distinguish them from any that might be recruited from the wild population. After the paint had dried the clams were returned to the tanks for a period before planting. The treatment produced no mortality and the color is still readily visible after nearly two years on the remaining surviving clams.

Wooden frames of 5 cm × 10 cm lumber, having inside dimensions of 1.5 M × 3 M were constructed and four of these frames were placed at each location, parallel to each other about a meter apart. The frames were partially buried in the bottom and secured with stakes. At each location two of the plots were used as controls with no substrate added. One of the plots at each location was planted with ca. 5 cm of pea gravel and one with ca. 5 cm of crushed oyster shell. In each plot of 4.5 $M^2$, 2500 marked clams were hand-scattered as evenly as possible

[1] The school of Marine Science, University of Delaware is now operating a pilot closed system facility for rearing mollusks.

at a low spring tide. At each location one of the control plots was covered with an open bottom, 1.2 cm mesh plastic-coated wire cage, which was pressed into the bottom 5–7.5 cm. The top surface of the cage was 7.5–10 cm above the bottom.

The plantings at each location were examined at intervals and at the termination of the experiment the entire area of each plot was dug carefully and the clams recovered.

## RESULTS

The bottom substrate analysis showed no significant differences at the 5% level (Student t test) in sand and silt at the two locations. Percentage sand ranged from 91–99. There were significant differences (Student t test at 5% level) in clay (mean 0.6% at Alligator Harbor, 3.5% at Turkey Point), organics (mean 0.5% Alligator Harbor, 1.5% Turkey Point) and carbonates (mean 1.2% Alligator Harbor, 7.1% Turkey Point). These higher values at Turkey Point may have been due to the presence of a four-year old spoil deposit of sand, clay and limestone ca. 100 meters west and extensive seagrass beds ca. 25 meters south of the location.

Table 1 summarizes the plantings at the two locations and the survival under each treatment. The clams at the time of termination were ca. 1.5 years old and had been exposed to the open environment ca. 0.8 years. The survival was very low in the plots containing gravel and shell, only slightly better than the control plots with no protection at all. Pea gravel did provide better protection than shell. The best survival was under the cages.

Various known predators were observed on the plots at the two locations, and on occasions were observed feeding on the planted clams.

TABLE 1. *Clam mortality field experiment.*
Plots of 4.5 square meters, each with 2500 marked clams.
Planted November 1972; examined September 1973.
Location I – Alligator Harbor; Location II – Turkey Point.

| Substrate Type | LOCATION I Survival # | I % | II Survival # | II % | I & II Survival # | I & II % |
|---|---|---|---|---|---|---|
| Control | 1 | 0.1 | 31 | 1.0 | 32 | 0.6 |
| Shell | 0 | 0.0 | 109 | 4.8 | 109 | 2.2 |
| Gravel | 18 | 0.6 | 487 | 19.5 | 505 | 10.1 |
| Control (wire cage) | 901 | 36.0 | 2035 | 81.4 | 2936 | 58.7 |

The predators appeared to be more numerous at Alligator Harbor, but no quantitative data are available. The predators include lightening whelks, *Busycon contrarium* (Conrad), moon snails, *Polinices duplicatus* (Say), blue crabs, *Callinectes sapidus* (Rathbun), and stone crabs, *Menippe mercenaria* (Say). Feeding depressions created by sting rays, *Dasyatis* spp. and butterfly rays, *Gymnura micrura* (Bloch & Schneider), were seen in the plots. Many broken as well as intact, empty shells were recovered when the experiment was terminated, but their numbers were not recorded nor were attempts made to determine the probable cause of mortality.

## DISCUSSION AND CONCLUSIONS

Although clam survival under the wire cage control plots at Turkey Point is considered to be commercially satisfactory, the survival at Alligator Harbor was lower than usually obtained from other observations with caged enclosures. Perhaps the relatively low survival can be explained by predators gaining entry. No routine inspections were made to be certain that the cage was firmly entrenched in the bottom. On one occasion a corner of the cage was found to be out of the bottom.

Survival in protective substrates was several fold greater than in control plots with no predator protection, but unacceptably low for commercial operations. It is assumed that the mortality resulted from predation and not from some other factor(s). There is little or no current at either location that might aid clam migration, besides the abundance of dead marked shells many showing evidence of predation, belied this as a factor in the recovery of live clams. There is a remote possibility that gravel and shell hash caused mortality. A control for assessment of this possibility was not included in the tests, i.e. no graveled or shell planted plots were covered with wire cages.

The different survival rates obtained in Virginia and Florida, using similar substrate additives, could be explained in several ways. First, particle size and amount of protective substrate may have been different. Second, we have a plethora of predators in Florida, more so than in Virginia, and our milder winter season permits activity of predators almost year round.

In our experiments the sizes of clams, when planted, was much greater than the sand-grain size reported in Virginia. This larger size should have lessened the clams' vulnerability to predators and the survival rate should have been higher than that obtained for Virginia waters.

In conclusion, for commercial mariculture, survival of planted clams must be at a profitable level, which we judge to be not less than 50%. Based on our observations in Florida, adequate survival cannot be obtained with protective substrate additives such as gravel and shell. Fencing, with constant attention to make certain that predators do not gain entry, does give good survival. This method is expensive and cheaper and effective methods must be found before clam mariculture will become financially rewarding.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# SCALE-UP OF FOOD UTILIZATION BY THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)

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## ABSTRACT

*An approach for studying scale-up of food utilization by shellfish is presented. The approach is based on the fact that food concentration is a primary variable controlling food removal and growth of filter-feeders.*

*Twenty raceways were constructed each approximating a tubular reactor by a series of ten stirred-tank reactors. Each section contained ten second-year oysters. Each raceway received water at a different combination of flow rate and relative food concentration. The relative concentrations were formulated by mixing unfiltered seawater with filtered seawater.*

*The oysters were allowed to grow relatively undisturbed for a period of 121 days. Analysis of the weight growth data indicate that scale-up of a filter feeding-system is possible using space time as a scaling factor. It is also demonstrated that higher conversions occur with lower concentrations. However, a much larger raceway is required to obtain the same amount of growth.*

## NOMENCLATURE

| | |
|---|---|
| a, b, c, d | Molal ratios, dimensionless |
| A | Flow rate of food, grams/day |
| $A_0$ | Flow rate of food at beginning of a raceway, grams/day |
| [A] | Relative concentration of food, dimensionless |
| $[A]_0$ | Relative concentration at beginning of a raceway, dimensionless |
| B | Cummulative rate growth, grams/day |
| C | Waste production rate, grams/day |
| F | Water flow rate, grams/day |
| FCHT | Filtered water constant head tower |
| FCT | Flow control tower |
| k, k′, $k_1$, $k_2$ | Rate constants, same units as r |
| $\ell$ | Liter |
| N | Number of animals |
| PCHT | Primary constant head tower |
| r | Rate of reaction, grams/animal-day |
| t | Time |
| UCHT | Unfiltered water constant head tower |
| V | Volume of reactor |
| $x_B$ | Conversion ratio, grams growth/grams food |
| $\alpha$, $\beta$, $\gamma$, $\delta$ | Order of reaction, dimensionless |

## INTRODUCTION

One problem common to all types of animal culture is that of finding a suitable food material and a satisfactory way of distributing this

food to individual animals. Filter-feeders, oysters in particular, are not exempt from this problem. This paper does not concern itself with the oyster's specific needs of nutrition or the exact mechanisms by which the oyster uses this food. Rather, we are concerned with the engineering variables which must be used to design an oyster-growing system that will efficiently utilize the available food found in raw seawater.

## CONCEPTS OF FEEDING

The first principle of food utilization is that the food leaving a system must be less than the food entering the system. In the case of filter-feeders, for which the food is suspended in water, this principle can be restated to say that the food concentration leaving the system must be less than that entering the system.

Obviously, almost any feeding method for filter-feeders will reduce the food concentration. However, for discussion purposes the methods can be categorized into three basic types, which were adapted from Smith (1970): (a) the batch system, (b) the stirred-tank system, and (c) the tubular-flow system or raceway. These types are schematically illustrated in Fig. 1. In types *b* and *c* the food concentration at any particular point within the filter-feeding system remains constant with time and therefore is in a steady state. In type *a*, food concentration varies with time and therefore is in an unsteady state.

The batch system depends on unsteady state to lower the food concentration. With this method the filter-feeder chamber is filled with water-borne food. After a certain amount of time the chamber is drained and filled with another batch of water. The water, when drained, will have a lower food concentration than when added, thus adhering to the principle mentioned in the first paragraph above. Therefore, two basic engineering variables must be evaluated for the batch system. These are the initial food concentration and the time between water changes.

The first of the steady state systems, *b*, is the stirred-tank system. With this system water-borne food enters and leaves the chamber continuously. Inside, the water is mixed perfectly so that all the filter-feeders receive the water at the same concentration. Notice that the animals

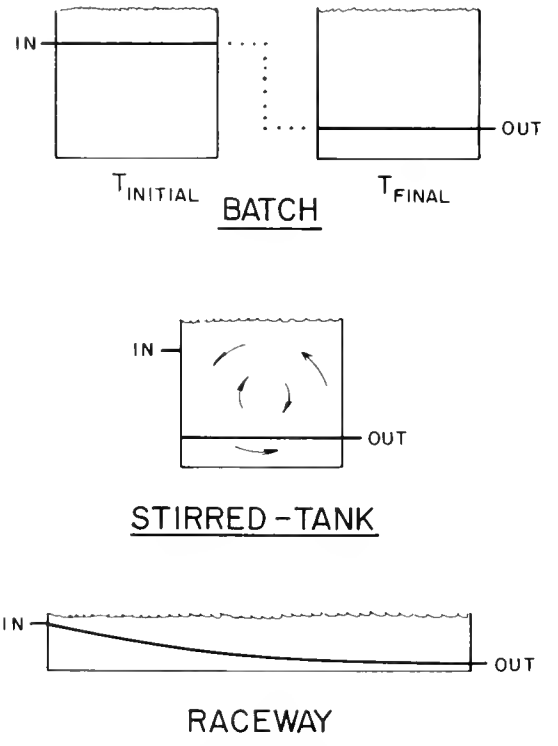



FIG. 1. *Food concentration profiles for the three basic types of feeding systems.*

will be feeding on water with the same concentration as that leaving the chamber and this concentration is lower than the concentration entering the chamber.

The basic engineering variables which have to be considered with the stirred-tank system are flow rate and initial food concentration of the water entering the system. The disadvantage to this type of system is obvious. If the concentration of food in the system is at a level which can be utilized efficiently, then the food concentration leaving the system is also at this highly utilizable level and therefore food is being wasted. However, the food concentration may be lowered further by running the water through a series of stirred-tank reactors, and in this case the series of reactors approximate a raceway.

The system selected for this study is the raceway system, *c*. In this type of system the water flows down a raceway containing filter-feeders. Ideally, each incremental unit of water flows at a constant rate through the raceway. The food concentration decreases as the water gets fur-

ther down the raceway. In the raceway system, as with the stirred-tank system, the basic engineering parameters to evaluate are food concentration and flow rate of water entering the system. This system has the advantage of being able to utilize food efficiently, but also has the disadvantage of nonuniform growth down the raceway. Nonuniform growth can be partially remedied by periodic reversal of flow direction.

In comparing these three types of systems it becomes obvious that the question of food utilization is not an easy one. Given a specific supply of food to be used to feed a specific group of filter-feeders, this supply could be used in at least three very basic ways, each way giving different growth rates. Hence, when evaluating growth data, one must be careful to consider what feeding system was used. This necessity was also pointed out by Johnson *et al.* (1968).

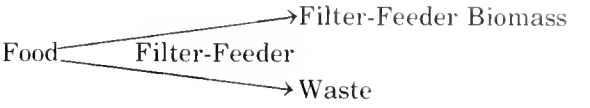
Among the first researchers to mathematically relate food uptake to food concentration for bivalve mollusks were Fox *et al.* (1937). Fox hypothesized that the instantaneous rate of food uptake was directly proportional to the concentration of the food. For a batch system this theory yields a linear relationship between the natural logarithm of concentration and time. Fox's experiments with suspended $CaCO_3$ and mussels substantiate the theory very well.

Most bivalve feeding research has been done with batch-type systems. However, Matthiessen and Toner (1966) concluded that a more satisfactory arrangement would involve continually flowing water, and Ryther (1972) further suggested "a long rectilinear raceway" to remove food efficiently from the water.

Pruder *et al.* (1974) studied this type of system using a vertical rector containing oysters. They concluded that the number of cells removed per unit time per oyster is a function of the quantity of cells already cleared and is independent of the algal cell concentration. One very serious objection to their work is that the experiment only lasted 24 hours. Their data indicate that 24 hours was not enough time for the system to come to equilibrium, much less obtain data at steady-state. As a result his experimental apparatus was not a tubular flow reactor as proposed but some combination of a tubular flow reactor and a batch reactor.

## MODEL DEVELOPMENT

The growth of filter-feeders is a very complex chemical process. This study will simplify the situation by approximating this process by two simple reactions:




This is to say that food, in the presence of a filter-feeder, is converted to the biomass of that filter-feeder plus waste products. The use of this model derived from chemical kinetics can be partially justified by considering the following points:

1. The rate of a chemical reaction is affected by the concentration of the reactant. Smith (1970).

1a. The rate of food uptake by a filter-feeder is a function of the food concentration. Fox (1937).

1b. The rate of growth of a filter-feeder is a function of the food concentration. Matthiessen and Toner (1966).

2. The presence of a catalyst is required for some chemical reactions to occur.

2a. The presence of a filter-feeder is required for the conversion of food to filter-feeder biomass.

Because of the similarity of this model to a chemical reaction, it seem reasonable to suppose that chemical kinetics techniques could be used to model filter-feeder growth, food concentration, and waste concentration.

The rate of reaction for a reaction which can occur only in the presence of a catalyst may be defined, for a raceway system, as:

$$r = \frac{dP}{dN} \qquad (1)$$

where

r = rate of reaction
dP = incremental change in amount of a reactant or product per unit time
dN = incremental amount of catalyst (in this case a pseudo-catalyst, the number of filter-feeders)

Early workers in chemical kinetics found that simple relations existed between rates of reac-

tion and concentration of reactants as cited in Smith. Consider the reaction

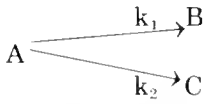
$$a\,A + b\,B \underset{k'}{\overset{k}{\rightleftarrows}} c\,C + d\,D$$

where the capital letters represent the reactants and products and the lower case letters represent the appropriate molal ratios. The rate of reaction can be expressed as

$$r = k[A]^{\alpha}[B]^{\beta} - k'[C]^{\gamma}[D]^{\delta}$$

where r is the rate of reaction, k and k′ are the rate constants, and $\alpha$, $\beta$, $\gamma$, and $\delta$ are the orders of the reaction with respect to the concentrations [A], [B], [C], and [D].

One type of reaction system which may be used to model filter-feeder growth is the simultaneous complex system. A complex system is one in which more than one reaction occurs. The simultaneous complex reaction can be represented:




where A is the reactant and B and C are the products, the ratio of which depends on the rate constants $k_1$ and $k_2$. In the filter-feeding system discussed in this paper, A represents the food in the water, B represents that portion which is used in growth by the filter-feeder, and C represents that portion which is expelled from the filter-feeder as waste.

The rates of reaction are given by

$$r_A = \frac{dA}{dN} = -k_1[A]^{\alpha} - k_2[A]^{\beta} \qquad (2a)$$

which is the rate of food consumption;

$$r_B = \frac{dB}{dN} = k_1[A]^{\alpha} \qquad (2b)$$

which is the rate of filter-feeder growth;

$$r_C = \frac{dC}{dN} = k_2[A]^{\beta} \qquad (2c)$$

which is the rate of waste formation.

However, this biological reaction is autocatalytic. In this case an autocatalytic reaction is one in which the product of the reaction serves as a catalyst to the reaction. In other words, the increase in size of the filter-feeder due to its growth increases its ability to remove food from the water and grow.

The exact relationship between the size of the filter-feeder and its ability to convert food to biomass is not known. If it is assumed that over a short period of time this increase in ability is negligible, then Equations 2a, b, c may be used without modification.

After the rate equations have been established it still remains to model the entire raceway to determine how well the model works. A mass balance over a differential mass of filter-feeder will provide the following relation:

Biomass entering element + Biomass grown in element − Biomass leaving element
= Accumulation of biomass (3)

For non-motile filter-feeders this becomes:

$$0 + r_B\,\Delta N\,\Delta t - 0 = A_0\,\Delta x_B\,\Delta t \qquad (4a)$$

hence

$$r_B\,\Delta N = A_0\,\Delta x_B \qquad (4b)$$

where $A_0$ is the flow rate of food into the system ($A_0$ is the product of the water flow rate, F, and the initial relative concentration, $[A]_0$), and $x_B$ is the conversion fraction of food to growth.

$$dx_B = \frac{1}{A_0} r_B\,dN \qquad (4c)$$

$$x_B = \frac{1}{A_0}\int_0^N r_B\,dN \qquad (4d)$$

but $x_B = \dfrac{B}{A_0}$ and $r_B = k_1[A]^{\alpha}$,

$$B = \int_0^N k_1[A]^{\alpha}\,dN \qquad (5)$$

Because [A] is a complicated function of N, stepwise numerical integration is used to obtain the solution to this equation; that is at each step n,

$$[A]_n = [A]_{n-1} - (k_1[A]_{n-1}^{\alpha} + k_2[A]_{n-1}^{\beta})\frac{\Delta N}{F} \qquad (6)$$

The cumulative growth rate B (Equation 5) can be plotted against N/F and the resulting

curves compared with experimental values.

Stated in simple terms, Equations 5 and 6 show that N/F is the required ratio of the number of filter-feeders to water flow rate necessary to obtain B growth with an $[A]_0$ initial concentration of food in the water, using a raceway.

## TESTING THE CONCEPT

It is, of course, necessary to evaluate the parameters $\alpha$, $\beta$, $k_1$, and $k_2$ in the mathematical model so that it can be used to design a feeding system. These parameters were extracted from growth data taken from experimental raceways. Each raceway was fed with water at a certain food concentration and flow rate, the two basic engineering variables for a tubular reactor.

The system parameters will vary with the species and age of animals, the type of food utilized, the water temperature, salinity, pH, etc. Hence, it was necessary to choose a specific system in order to analyze the parameters. The system chosen employed second-year oysters, *Crassostrea virginica* (Gmelin), and natural water from the Wareham River in Southeastern Massachusetts. The water and food parameters were those natural to the river.

For this study, natural water has advantages over alternate sources of food such as unialgal, mixed unialgal, enriched natural, or artificial foods. Two of these advantages are:

1. Natural water is the most widely used source of oyster food. No other source of food has been shown to permit normal growth for extended periods of time.
2. It is a very reliable and easily obtainable source of food.

The most disconcerting thing about using natural water is the difficulty of determining the exact amount of food in it. There is simply no practical way to compare size, number, and food value for the different types of plankton and arrive at a number representing the exact food concentration of a particular sample of seawater for oysters. For this reason the food concentrations were assigned values relative to the food concentrations of natural seawater, although it was recognized that the absolute concentration did vary with time.

Recall that two main engineering parameters control growth in a tubular reactor or raceway. These are flow rate and initial food concentration. For the experiment, flow rate was varied by simply metering different amounts of water to each raceway. Food concentration must be varied between raceways in such a way that the quality of the food remains the same and only the concentration is lowered. The technique chosen was that of mixing filtered seawater with the unfiltered seawater. A stream containing a 1.0 liter/minute flow of filtered water and and a 1.0 liter/minute flow of filtered water was taken to have a relative food concentration of 0.5.

One additional complication is created by the use of natural water. Since the proposed model is based on food concentration as a primary independent variable, it is necessary to know the concentration profile down the raceway. But the concentration at various points down the raceway cannot be measured for basically the same reason that the food concentration in the original natural seawater cannot be measured. In addition, there are other problems. For example, what correction factor should be applied for plankton which are expired, partially digested, wrapped in the mucus-like pseudofeces, or clumped together in fecal material? These problems would seriously restrict accurate evaluation of food concentration even for a unialgal culture.

For these reasons, the concentration is measured indirectly by first determining the growth-food concentration relationship. The concentration at any point can then be inferred by measuring the growth at that point down the raceway.

The design of the tubular reactor or raceway is also an important consideration. In an experimental setup the water flow down the raceway may be small enough that the pumping motion of the oysters creates considerable local upstream movement of the water. This upstream movement conflicts with the theory of a tubular reactor and must be avoided. To avoid this situation in the experimental raceways, each raceway was divided by several sets of baffles. Hence, the raceway actually approximates a tubular reactor by a series of ten stirred-tank reactors, the oysters doing the stirring. A reverse flow would probably not occur in a large operation because the increased flow rate neces-

sary to feed a large number of oysters would dwarf the water flow caused by oyster pumping.

## THE EXPERIMENTAL APPARATUS

The experiment was conducted at the University of Massachusetts Aquacultural Engineering Laboratory next to the Wareham River in Wareham, Massachusetts. Oysters were grown in 20 raceways, each with a different combination of food concentration and flow rate. Each raceway contained 100 oysters for a total of 2000 oysters. The support system to maintain the raceways consisted of water supply, filtering, flow control, and drainage facilities. A line diagram of the entire experimental apparatus may be seen in Fig. 2.

Water for the experiment was pumped into the laboratory from the Wareham River. That portion which was to be filtered flowed into a slow sand filter (Fig. 3). The sand filter was contained in a 2.4 x 2.4 x 1.2 meter deep stainless steel tank coated with epoxy paint on the inside. Six meters of 3.8 cm. ($1^1/_2$ in.) perforated

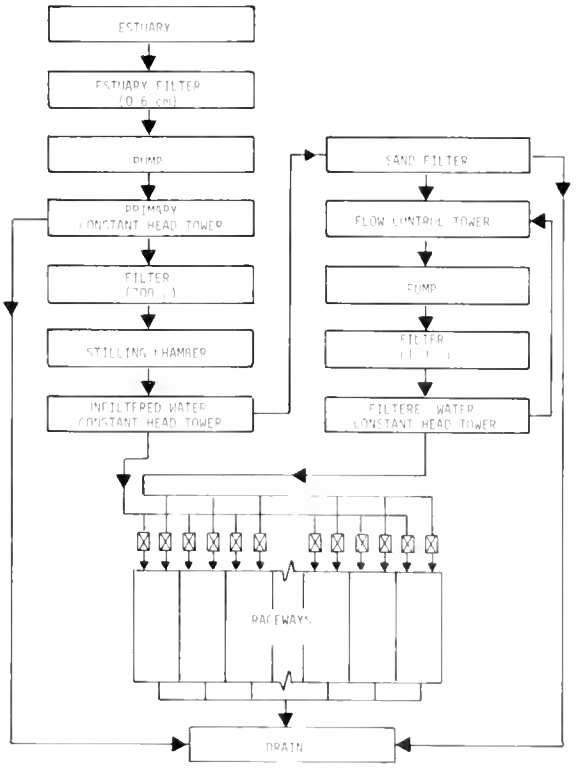



FIG. 2. *Line diagram of the experimental apparatus.*

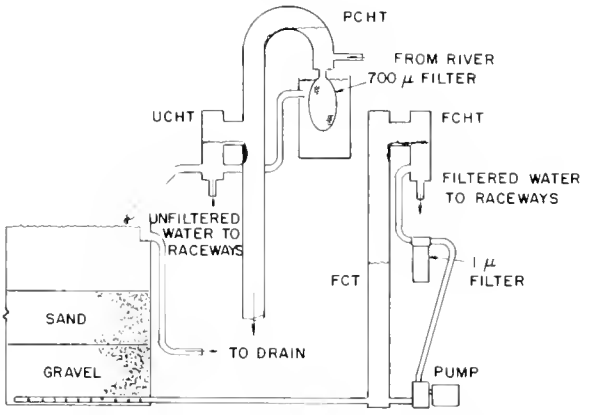



FIG. 3. *Constant head towers and filtering systems.*

polyethylene pipe was placed on the bottom to aid in water distribution. The tank was filled with pea gravel to a depth of 0.4 meter. Fine mortar sand was placed over the gravel to an additional depth of 0.4 meter. The filter was initially filled with water from the bottom to avoid air locks within the sand. After the water had reached a level higher than the sand, water was added from the top and drained from the bottom, which was the normal mode of operation.

In about one week, the top of the sand would become covered with so much material filtered out of the water that the sand would not filter the required 0.2 liter/second. At this time the water inlet would be stoppered and the water level allowed to fall to just below the sand level, so that the top one to two centimeters of sand could be shoveled off. The filter was then filled with water from the bottom and allowed to continue normal operation. Additional sand was added to the filter every four to six weeks.

To complete the filtering process, water was pumped from the sand filter through a nominal 1.0-micron filter (Fig. 3). The filter cartridge was orlon line wound around a perforated PVC center. This cartridge was not rechargeable and had to be replaced every three to five days. After water was filtered through this part of the system, it presumably contained no food.

The water flowed from constant head towers to the distribution pipes, which were constructed of CPVC. Each pipe had a hole drilled over the mixing chamber of each raceway. Short

lengths of Tygon tubing were fitted into the holes to carry the water into the raceways. Hoffman clamps on the tubing were used to regulate the flow. This method of flow control worked satisfactorily on the filtered water but the tubing often clogged with the unfiltered water and had to be cleaned frequently.

The raceway complex itself (Fig. 4) was constructed of fir lumber, fir plywood, and glass. All 20 raceways were constructed on a single 102 x 204 cm. (4 x 8 ft.) piece of plywood. Each raceway was divided into 12 sections by small panes of glass. The water flowed from one section under a pane of glass, up between the two panes, and over the second pane into the next section. The first and last sections were used as mixing and discharge chambers, respectively. The raceways were covered with a piece of plywood with a black polyethylene film skirt to avoid algae growth within the raceways.

## OPERATING PARAMETERS

The primary operating parameters for the system were the flow rate and food concentration supplied to the system. The flow rate is simply the total of the unfiltered and filtered

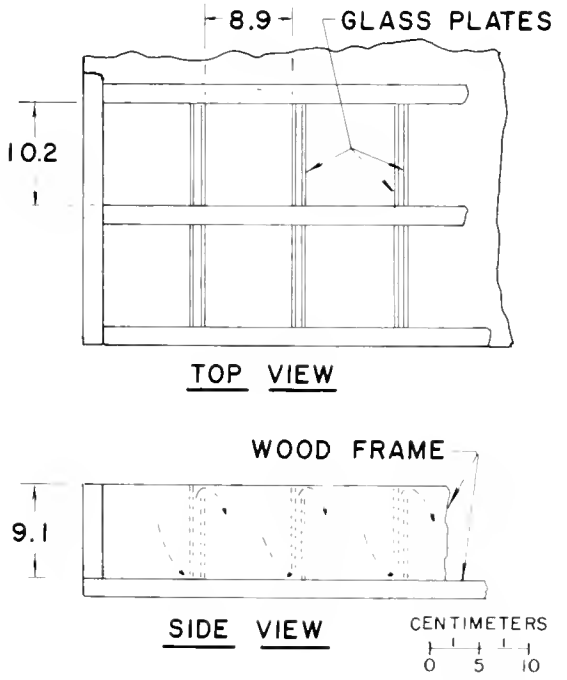



FIG. 4. *Sketch of a section of the raceway complex.*

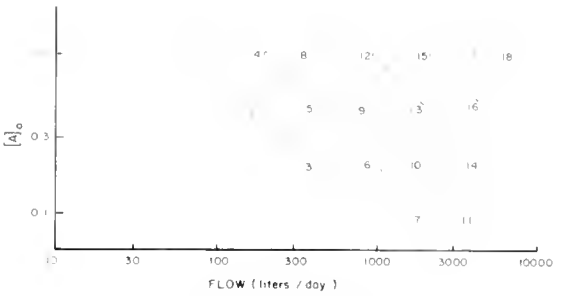



FIG. 5. *Raceway flow rates and initial concentrations.*

water flowing into each raceway. Taking the concentration of food in the unfiltered water to be 1.0 and the concentration of filtered water to be zero, the concentration of the water supplied to each raceway is

$$[A]_o = \frac{\text{unfiltered flow}}{\text{unfiltered flow} + \text{filtered flow}}$$

The flow rates and food concentration for each raceway are indicated by the points on the graph in Fig. 5. The number beside each point represents the number assigned to each raceway. The points were so arranged that diagonal lines connecting the points would be lines of equal amounts of food. Raceways 19 and 20 received a zero concentration of food at flow rates of 982 and 2099 liters/day, respectively. These controls would help determine whether all the food was indeed removed from the filtered water.

## COLLECTION OF GROWTH DATA

The oysters used for testing had been collected as spat in net bags on scallop shells in 1971. These animals were grown in net bags from March, 1972, until March, 1973, and were separated as individuals during the spring of 1973. On April 28, they were cleaned with a brush and barnacles were removed. The oysters were weighed and separated into size groups.

On April 29, oysters were placed in the raceways. Oysters were chosen from each size group so that each of ten raceway sections had ten oysters with approximately the same size distribution and with as small a size range as possible. The oysters from each section were removed and weighed as a group. The weight range was 177 to 213 grams.

On August 27, each group of ten oysters was removed from the raceway and cleaned with a brush. The glass plate baffles were also removed for cleaning, and the raceways were scraped clean and flushed out. Baffles and oysters were returned to the raceways.

On August 28, 121 days after the start of the experiment, each group of ten oysters was removed and weighed.

## ANALYSIS OF DATA

The weight growth rate of the oysters in each section of each raceway was determined by subtracting the initial weight of the group from the final weight and dividing by the number of elapsed days.

Growth in raceways 1 through 7 was confined to the first very few sections. Under this circumstance it is difficult to justify that the raceway was approximating a tubular reactor. For this reason raceways 1 through 7 were not used in the analysis of the data.

Recall that raceways 19 and 20 received only filtered water with presumably all the food removed. Not surprisingly,the growth was negative for nearly every section down the raceway. This indicates that the food level was indeed very low. However, the curves also show a very slight gradation in growth indicating that there was a very small amount of food in the water.

The theory developed earlier mandates that a zero concentration yield a zero growth. The data indicate that a zero concentration yields a negative growth. Presumably, this loss in weight provided the food necessary to maintain vital body functions. Since a similar amount of nutrients would be required by all the other groups of oysters in the experiment, they would also utilize a certain amount of food without exhibiting any growth. To correct for this condition, the weight loss per section was averaged for raceways 19 and 20, yielding a loss of seven grams per section. This amount was added to the growth in every section of each raceway.

The resulting growths were then numerically integrated down each raceway to yield the cumulative growth curves some of which are indicated by the data points in Fig. 6. In making the growth integral curve for each raceway, integration was discontinued at the point on each curve where net negative growth occurred. This

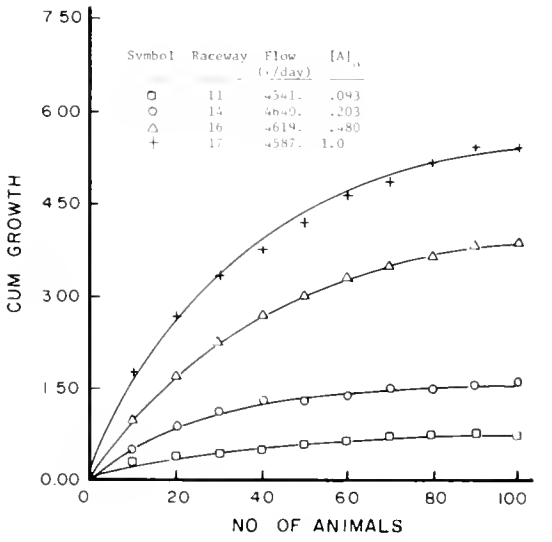



FIG. 6. *Cumulative growth (grams/day) of oysters versus number of animals.*

negative growth was usually concurrent with a large proportion of expired oysters in the raceway section. The data curves for each raceway were fitted to an equation of the form

$$B = b_1 - b_2 \exp(-b_3 N) \tag{7}$$

where,

B = cumulative growth rate
N = number of animals
$b_1, b_2, b_3$ = constants.

The least squares criterion was used. The resulting curve for each raceway is used to connect the data points in Fig. 6. The equation form was chosen because of its ability to fit the data points.

By taking the first derivative of Equation (7) the following relationship is found:

$$\frac{dB}{dN} = b_2 b_3 \exp(-b_3 N) \tag{8}$$

Recall from the earlier model development that

$$\frac{dB}{dN} = k_1 [A]^{\alpha} \tag{2b}$$

hence,

$$\frac{dB}{dN} = k_1 [A]^{\alpha} = b_2 b_3 \exp(-b_3 N) \tag{9a}$$

and,

$$[A] = \left[\frac{b_2 b_3}{k_1} \exp(-b_3 N)\right]^{1/\alpha} \qquad (9b)$$

Differentiating,

$$\frac{d[A]}{dN} = \frac{-b_3}{\alpha}\left[\frac{b_2 b_3}{k_1} \exp(-b_3 N)\right]^{1/\alpha} \qquad (10)$$

Since $dA = Fd[A]$,

$$\frac{dA}{dN} = \frac{-Fb_3}{\alpha}\left[\frac{b_2 b_3}{k_1} \exp(-b_3 N)\right]^{1/\alpha} \qquad (11)$$

Recall again from earlier model development that

$$\frac{dA}{dN} = -k_1[A]^{\alpha} - k_2[A]^{\beta} \qquad (2a)$$

hence,

$$\frac{dA}{dN} = -k_1[A]^{\alpha} - k_2[A]^{\beta} = \frac{-Fb_3}{\alpha}\left[\frac{b_2 b_3}{k_1} \exp(-b_3 N)\right]^{1/\alpha} \qquad (12)$$

Equations (9a) and (12) must be solved to obtain values for the constants $k_1$, $k_2$, $\alpha$, and $\beta$. The only place where the food concentration, [A], is known is at the boundary, i.e. the beginning of the raceway before any food is removed, i.e. $N = 0$. Simplifying Equation (9a) to the case where $N = 0$,

$$\left(\frac{dB}{dN}\right)_{N=0} = k_1[A]_0^{\alpha} = b_2 b_3. \qquad (13)$$

Plotting the product of $b_2$ and $b_3$ versus the initial concentration for the respective raceways resulted in the points shown in Fig. 7. The points were fitted to a curve of the form $k_1[A]^{\alpha}$ using a weighted least squares criterion. The resultant values of $k_1$ and $\alpha$ were 0.1837 gram per animal per day and 0.8488 respectively.

Equation (12) reduces to

$$\left(\frac{dA}{dN}\right)_{N=0} = -k_1[A]_0^{\alpha} - k_2[A]_0^{\beta} = \frac{-Fb_3}{\alpha}\left[\frac{b_2 b_3}{k_1}\right]^{1/\alpha} \qquad (14)$$

where $N = 0$. Again, a weighted least squares criterion was used in obtaining values for $k_2$ and $\beta$. The values obtained for $k_2$ and $\beta$ were 1.407 x

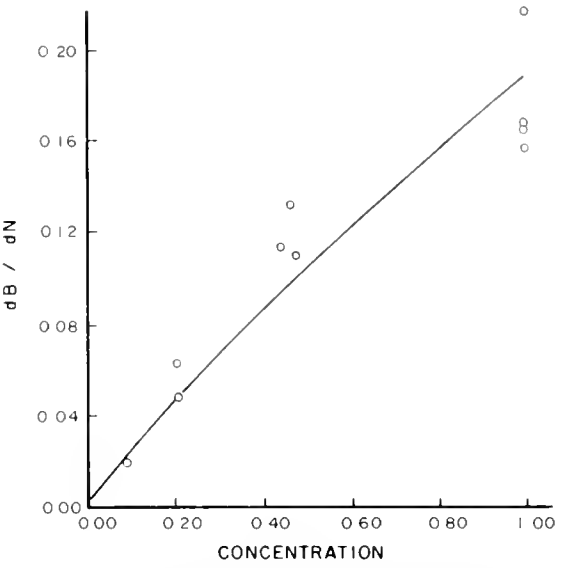



FIG. 7. *Growth rate (grams/animal-day) versus relative food concentration.*

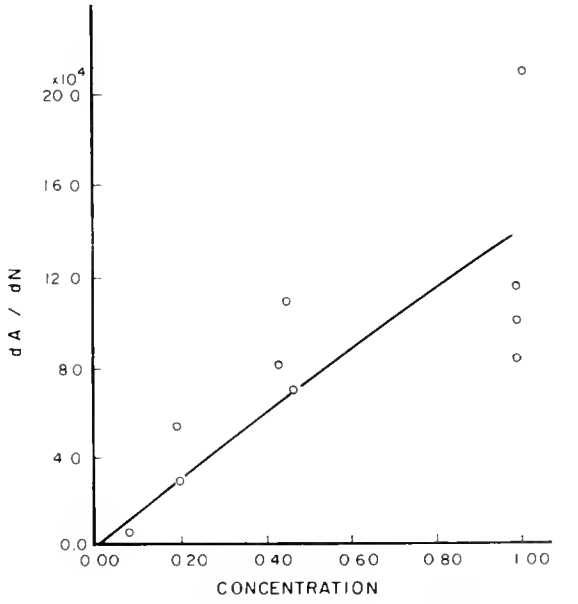



FIG. 8. *Food removal rate versus relative food concentration.*

$10^5$ grams per animal per day and .9125 respectively. The experimental values of $(dA/dN)_{N=0}$ were plotted against their initial concentration in Fig. 8.

Note that the relationship between the rate of removal of food, dA/dN, and the concentration is nearly linear as shown in Fig. 8. This is in

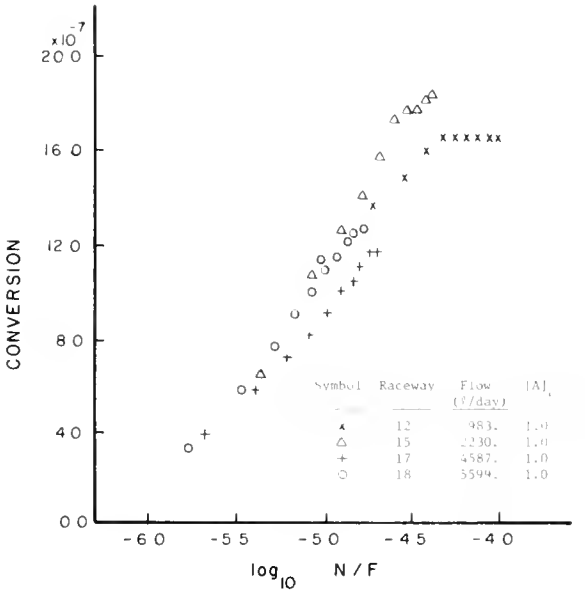



FIG. 9. *Conversion versus logarithm of space time (animal-days/gram) at equal initial concentrations.*

agreement with the work of ZoBell (1937) when he demonstrated that the relationship was linear for a batch reactor system.

A further comparison of the experimental data and the calculated values is given in the next section.

## RESULTS AND DISCUSSION OF RESULTS

A basic question investigated was that of scale-up. That is, can space time, N/F, be used as the primary scaling parameter in this biological system? Recall that in the model N is the number of oysters over which the water has flowed and F is the flow rate. Systems which are directly scalable should have identical conversions at the same space time. Recall the conversion $x_B$ is the fraction of the food which has been converted to filter-feeder biomass. Directly scalable systems in this study would be raceways with the same initial concentration of food, $[A]_o$.

Fig. 9 shows the data for the raceways where $[A]_o = 1.0$. These data show that engineering scale-up can be accomplished on filter-feeders using space time as a scaling factor. This fact is independent of the question of whether the data fit the mathematical model proposed in this paper, and has much greater practical importance.

Fig. 9 also demonstrates that the growth rate is not a strong function of velocity at least in the velocity range studied. The arrangement of the points for a particular space time is not in the order of increasing or decreasing flow rates as would be expected if growth were a function of velocity.

The next question to be answered is how well the chemical kinetics assumptions which were made approximate the actual growth in the raceways. To answer this the model was used to generate conversion data using a step by step numerical technique. The resulting curve is shown in Fig. 10. Note that the model best approximates raceway 18 (see Fig. 11). This would be expected since raceway 18 had the highest flow rate. With a higher flow rate, each section of the raceway represents a smaller portion of space time; hence the raceway more nearly resembles a tubular reactor. It is important to realize that the model curve was not made to fit the data points in Fig. 9, but rather was generated from constants obtained from Fig. 7 and 8. Fig. 10 is a check to determine if the model is reasonable.

The implications of the kinetics of filter-feeding may be better understood by using the model to make a graphical parametric analysis

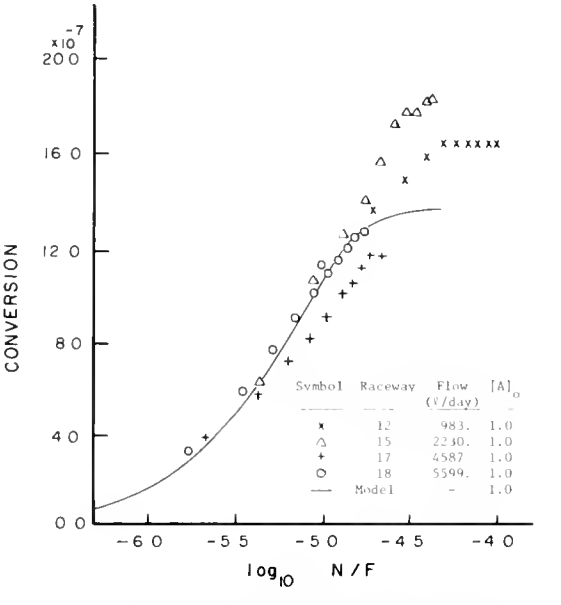



FIG. 10. *Conversion versus logarithm of space time (animal-days/gram) at equal initial concentrations.*

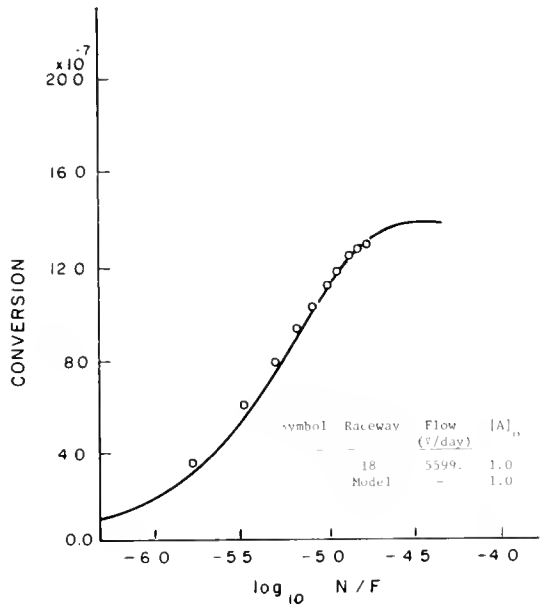



FIG. 11. *Conversion versus logarithm of space time (animal-days/gram) for raceway 18.*

of the growth of the oysters. Fig. 12 contains the conversion versus space-time relationship taken at several concentrations. This graph could be used to design a filter-feeding system for any food concentration (in the range studied) and amount of animals. It can be seen that at all space times the lower concentrations give slightly better food conversion. It might be mistakenly inferred from Fig. 12 that it is best to feed lower concentrations. Fig. 12 shows that for a system with a specified N and F, a lower initial concentration of food results in a slightly higher percentage of the food, $F[A]_0$, being converted to filter-feeder biomass. However, the absolute amount of food converted, $x_B F[A]_0$, will be much higher with a higher initial concentration of food.

Fig. 13 shows the calculated growth rate of the oysters down the raceway. In each curve the total amount of food is held constant and the flow rates and concentrations are varied to meet this criterion. Keep in mind that the low concentration flow rate is ten times the high concentration flow rate. Notice that much higher growth rates occur with the higher concentrations at the beginning of the raceway. The lower concentrations achieve a larger growth rate toward the end of the raceway.

A more dramatic characterization of the implications of this growth pattern may be seen in Fig. 14. These curves are simply integrations of the curves in Fig. 13. It may be seen that the total amount of growth in the raceway is higher for lower concentrations. The point is that lower concentrations require much longer raceways to achieve the same amount of growth.

In an economic optimization situation where

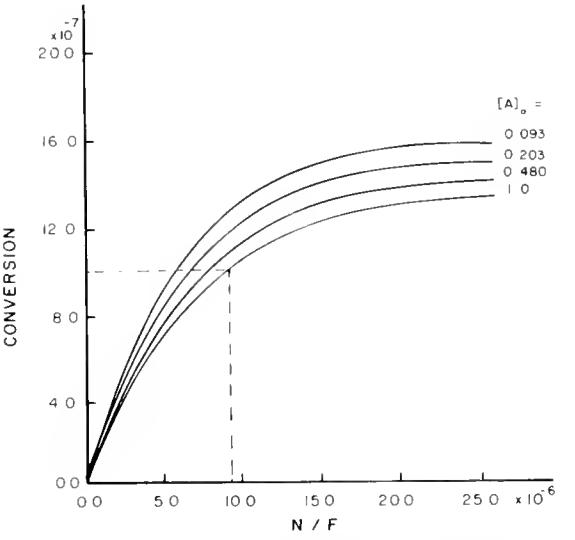



FIG. 12. *Conversion versus space time (animal-days/gram) at various initial concentrations.*

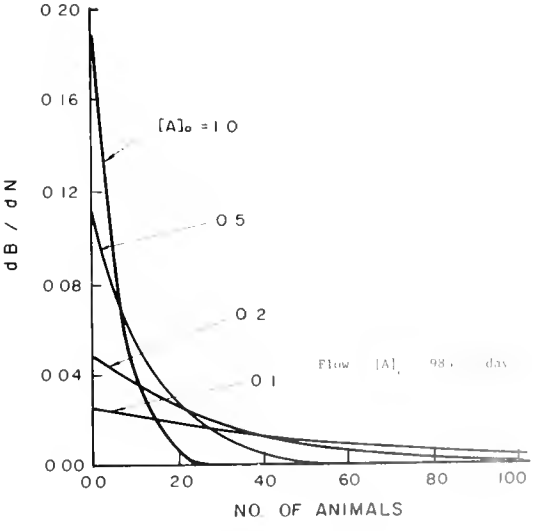



FIG. 13. *Growth rate (grams/animals-day) versus number of animals.*

food, raceway length, and flow rate all represent capital outlays, the cost of the higher flow rate and longer raceways must be weighed against the benefits of higher conversion to arrive at an optimum design. The optimal raceway length for an extensive system in which a river provides the water flow would undoubtedly be different from the optimal raceway length for an intensive system in which the water must be pumped out of the river.

## EXAMPLE

Perhaps the best way to obtain a working understanding of some of the concepts presented herein is to work a simple example problem. The problem is to determine the flow parameters necessary to provide food for 25,000 oysters (100 bushels at market time) and the growth rate at these conditions. Assume that the oysters are second-year oysters weighing approximately 20 grams each since the data are strictly applicable only to this size animal and to the seawater-food conditions of this study.

The first step is to determine the initial relative concentration of food in the water. Fig. 12 indicates that a slightly higher conversion is possible with lower concentrations. However, Fig. 14 demonstrates that a much larger and therefore more expensive system is required to obtain this conversion. Therefore, choose the higher relative concentration, $[A]_o = 1.0$.

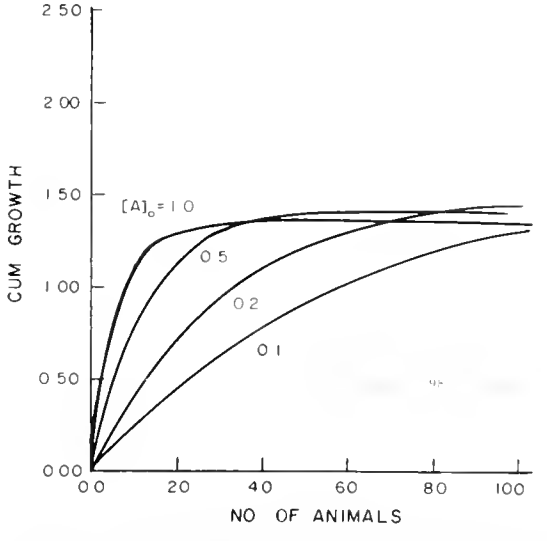



FIG. 14. *Cumulative growth (grams/day) versus number of animals.*

Assume that an economic analysis of the cost of the food, the cost of pumping water, the cost of the raceway, etc. and the value of the product, yields an optimum point on the space-time-conversion curve where $N/F = 9.0 \times 10^{-6}$ animal-days per gram and conversion = $10. \times 10^{7}$ (see Fig. 12).

The flow rate may then be determined,

$$\frac{N}{F} = \frac{2.5 \times 10^{4}}{F} \text{ animals}$$

$$= 9.0 \times 10^{-6} \frac{\text{animal-days}}{\text{gram}}$$

$$F = \frac{2.5 \times 10^{4}}{9.0 \times 10^{-6}} = 2.78 \times 10^{9} \text{ grams/day}$$

$$= 2.78 \times 10^{6} \text{ liters/day}$$

Finally, the growth of the oysters is calculated:

Growth = Flow x initial concentration x conversion

$= (2.78 \times 10^{9})\ (1.0)\ (10. \times 10^{7})$

= 2,780 grams/day,

or an average of

$$= \frac{2{,}780}{25{,}000} = .111 \text{ gram/animal/day.}$$

This amount represents gross growth and must be corrected to account for food used in metabolic processes. Recall that this correction is 7.0 grams per 10 oysters per 121 days.

$$\frac{7.0}{(10.)\ (121.)} = .0058 \text{ gram/animal/day}$$

then,

Net growth = 0.111 − 0.0058 = .105 gram/animal/day.

As a second sample, it is interesting to make a crude approximation of the pumping costs required to grow a bushel of oysters to adult size assuming that water-borne food is pumped from an estuary. It appears reasonable to assume that the oyster is able to maintain the same conversion ratio used in the previous example until it reaches market size. Also assume that a marketable oyster weighs 50 grams and that the seawater must be pumped against a three-meter head.

Growth = Flow × initial concentration × conversion

$$\frac{50 \text{ grams}}{\text{oyster}} \frac{250 \text{ oysters}}{\text{bu.}} = (\text{Flow}) \times (1.0) \times (10. \times 10^{-7})$$

$$\text{Flow} = \frac{1.25 \times 10^{10} \text{ grams}}{\text{bu.}} = 1.25 \times 10^{7} \text{ liters/bu.}$$

$$\frac{1.25(10^{7}) \text{liters}}{\text{bu.}} \frac{\text{kg}}{1.0 \text{ liter}} \frac{3.0 \text{ m.}}{1} \frac{\text{kwh}}{3.67(10^{5}) \text{kg-m.}} \frac{\$.03}{\text{kwh}} = \$3.03/\text{bu.}$$

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# OUT-BAY CULTURE OF BIVALVE MOLLUSCS[1]

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ABSTRACT

*A relatively new technique in oyster farming called "Out-bay Culture" is proposed. Its advantages and problems are discussed. Data are presented indicating the probable success of this technique. The technique provides necessary control over the operation. In the future oyster aquaculture will call for a formulated ration. Out-bay culture is speculative at the present but may develop into a commercial reality.*

Oyster culture has traditionally been accomplished in estuaries. In the early stages, the bottom was used both intertidally and subtidally. More recently, the entire water column has been used by hanging oysters from floating devices or by fixed off-bottom culture employing either strings or trays.

Bottom culture is restricted to acceptable bottom types and current conditions as well as water quality. Many traditional oyster grounds are near cities or industry where the water may be polluted and unacceptable for culture. Water column cultures are also restricted by current conditions, storms and other water uses. Rafts or stationary devices may interfere with commerce, boating, water skiing, fishing, etc. Good oyster sites are difficult to find and maintain.

Oyster aquaculture, as practiced, is extensive. One has little control over environmental variables such as temperature, salinity, and water flow. Intensive culture brings large numbers of animals confined in relatively close quarters with some control over environmental parameters. A ration is also involved. Out-bay culture is the term used in this report for controlled oyster culture in an intensive way. It is hardly more than an idea at present, but competition for space in estuaries and water quality will make this concept progressively more attractive.

Out-bay culture requires rearing oysters in tanks, ponds or some similar contained water volume with control of water flow, retention time, depth, and the opportunity to treat the water if necessary. The advantages are freedom from storm and tidal action, as well as salinity control during the rainy season. Some of the unresolved problems are stocking rate, water flow per unit of biomass, and exchange rate or retention time. The understanding and optimizing of these variables will lead to maximizing the yield from a given sized container.

The next step is the development of a ration. At present we can feed larvae and small spat with culture algae and get reasonably consistent results. However, we are a long way from "feed lot" oysters. To really get estuaries into oyster protein production a ration is needed to supplement or replace natural food.

To illustrate what might happen in pond culture we conducted a pilot exercise. This was not a controlled experiment. Only one tank was available and we sought to find out the water volume-oyster relationship under a precise set of conditions.

[1] This work is a result of research sponsored by the Oregon State University Sea Grant College Program, supported by NOAA Office of Sea Grant, Dept. of Commerce, under Grant #04-5-158-2. Technical Paper No. 4108, Oregon Agricultural Experiment Station.

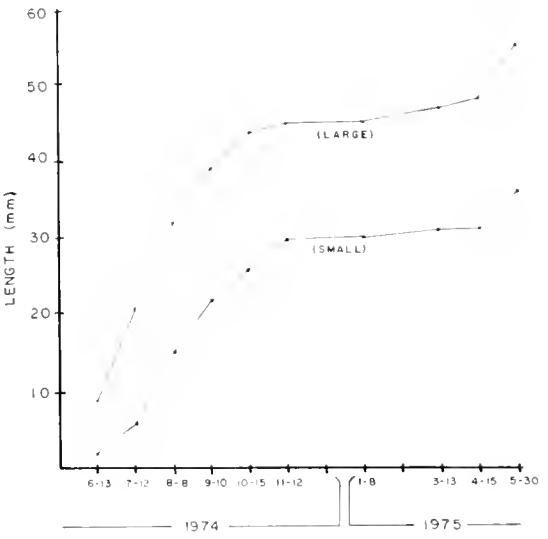


FIG. 1. *Growth curve in mm of large and small oyster spat (Crassostrea gigas) from June 1974 to June 1975.*

The tank measured 17′ by 30′ with a water depth of 4 feet. The water volume was about 15,000 gal. We hung 112 strings of 7 shells each, or a total of 784 shells with a total of about 4,400 spat of *Crassostrea gigas* in the tank. The seed was of two sizes, one averaging 2 mm and the other 8.9 mm. The water flow was 10 gal per minute. This flow filled the tank in about 24 hr. At this flow rate, it was expected that the growth of the oysters would cease in 3 or 4 months, giving us an idea of the flow-oyster mass relationship under these conditions.

Oysters were measured on a monthly basis. A total of 1,500 randomly selected oysters were measured, half from the large seed and half from the small seed. The tank was drained to facilitate measuring the oysters. All the shells were rinsed and the sides and bottom of the tank were cleaned before the tank was refilled.

Fig. 1 shows the length of the two-sized oysters over a year's time. Inspection of the curve shows that growth proceeded through the fall months. A slow or non-growth period was noted during the winter. Growth resumed the following spring. Table 1 gives the mean size of spat. The 10 gal per minute flow allowed about 13 l per oyster per day or about 0.5 l per oyster per hour. It was felt that the incoming water could not provide enough food for the growth noted. Thus, there must have been significant production of phytoplankton in the pond which provided more food than the oysters required. It seems that the tank was not overstocked as anticipated.

TABLE 1. *Mean size in mm of large and small oyster seed (Crassostrea gigas) grown for one year in out-bay culture.*

| Date | Small | Large |
|---|---|---|
| June 13, 1974 | 2.0 | 8.9 |
| July 12, 1974 | 6.8 | 21.2 |
| August 8, 1974 | 15.4 | 31.8 |
| September 10, 1974 | 21.9 | 38.9 |
| October 15, 1974 | 26.2 | 43.6 |
| November 12, 1974 | 29.6 | 44.9 |
| January 8, 1975 | 29.8 | 45.2 |
| March 13, 1975 | 31.2 | 46.7 |
| April 15, 1975 | 31.1 | 47.9 |
| May 30, 1975 | 36.0 | 55.2 |

Although no rigorous conclusions can be drawn from this exercise and the results noted are only valid for this one set of conditions, the observations are encouraging. In the future we will examine some of the variables influencing the system's productivity. The contributions, if any, from the growth on the sides and bottom of the tank, and the flow-oyster-water retention relationship to achieve maximum protein production are being investigated. We also will use out-bay culture to test alternate rations, when they come along.

Advantages of this culture method are mechanical control, the opportunity to treat the water if necessary, and, in Oregon, to open new estuaries for oyster culture. Some of our estuaries have too much fresh water in the winter months. With out-bay culture, pumping would cease at this time and acceptable salinities could be maintained. Finally, when rations are developed and "feed lot" culture becomes economical, out-bay culture could be the culture method for oyster protein production.

*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# COLIFORM BACTERIA LEVELS CORRELATED WITH THE TIDAL CYCLE OF FEEDING AND DIGESTION IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*) CULTURED IN DEEP BAY, HONG KONG

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## ABSTRACT

*Over two 24 hour periods in the winter and summer of 1974 experiments have been undertaken upon the Pacific oyster (Crassostrea gigas) in Deep Bay, Hong Kong to determine the effect, if any, of the tide and the sequence of feeding and digestion in the oyster upon coliform bacteria levels.*

*The results clearly show that the digestive process in the oyster, as revealed by the times of reformation and dissolution of the crystalline style is closely related to the tide; the animals feeding at the time of high tide and digestion being completed during low tide. Such a pattern fits in well with current concepts of the processes of feeding and digestion in the Bivalvia.*

*In winter, pollution levels were generally low and no tidal pattern of contamination of the oysters was observable. In summer, however, when the monsoonal rains flush out the polluted streams of Hong Kong into Deep Bay and the Pearl River (into which Deep Bay empties) is in flood, pollution levels increase and a distinct tidal pattern of contamination is apparent resulting from the differential feeding levels by the oysters at the times of low and high tide. These results show that isolated tests upon contaminated oysters have little or no value either on a tidal or a seasonal basis, and that only a comprehensive monitoring programme can explain wide ranging coliform counts.*

## INTRODUCTION

Hong Kong's rural New Territories comprising a land area of some 370 square miles supports a human population of over 1 million and a pig and poultry population estimated at some 400,000 and 6 million respectively and for which on the agricultural side at least no means of effluent disposal is available. Similarly human effluents receive only primary screening before being discharged into the sea. Streams and watercourses passing through agricultural areas are grossly polluted and Hong Kong's coastal waters are similarly degraded.

To the north west, Deep Bay located on the Hong Kong-Chinese border supports a Pacific oyster (*Crassostrea gigas* Thunberg 1793) industry utilising a primitive method of bottom laying (Morton, 1975). Such oysters are typically polluted especially in the summer months when the rain bearing S.E. Monsoon flushes out the streams and rivers into the bay and also causes a flooding of the Pearl river, draining much of southern China and into which Deep Bay opens (Fig. 1) (Leung *et al.*, 1975). High coliform counts in the region of the Pearl River characteristically result from this flooding (Watson and Watson, 1971). In Deep Bay too, pollution levels are high (Leung *et al.*, 1975).

Wood (1969) showed how oysters and mussels collected at different states of the tide possessed variable numbers of faecal coliform bacteria (i.e. *Escherischia coli*) with a peak occurring at the time of low tide. Recent research by Morton (1973) has shown how in a wide range of littoral bivalves, noteably the European oyster *Ostrea*

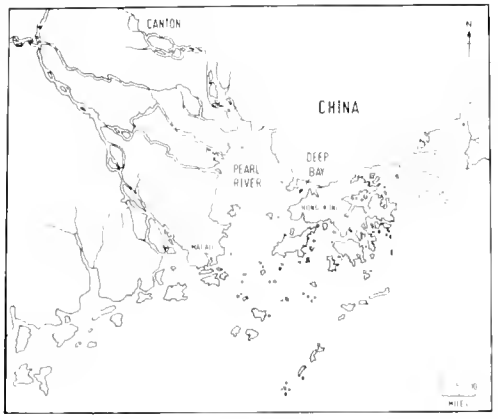


FIG. 1. *A map of Hong Kong showing the position of the Pearl River and Deep Bay, where the commercial oyster beds are located and where these experiments were undertaken.*

*edulis* (Morton, 1971) the processes of feeding and digestion are closely correlated with the state of the tide.

In Deep Bay where the oysters are known to be significantly polluted it was decided to repeat the experiments of Wood (1969) using *Crassostrea gigas* and, if proven true, to correlate such findings with changes in water quality and the feeding behaviour of the oysters over two tidal cycles in both winter and summer. The results of these investigations are reported here.

## MATERIALS AND METHODS

In February 1974 and in July 1974 four oysters were placed in each of 24 wire baskets and suspended in the water from the side of a pier at Tsim Bei Tsui that extends onto the mud flats of Deep Bay. The baskets were so arranged that they rested on the surface muds at approximately mid tide level and were covered and exposed by the tide in time with the commercial oysters growing close by.

The oysters were established 2 days prior to the experiments being carried out in order for them to acclimatise.

During the experimental periods water samples were collected every hour and the temperature, salinity and pH recorded. Four oysters were also collected every hour and were scrubbed clean in distilled water, washed a second time and then opened, using a sterile European oyster knife, through the ligament. The pH of the mantle fluids was measured using a Radiometer pH meter 27 fitted with microcapillary electrodes. The crystalline style was then removed and measured along its greatest length and width to the nearest 0.5 mm. The volume of the style was calculated as the volume of a cone. Each oyster was then measured volumetrically, mixed with an equal volume of 0.2 M phosphate buffered saline (pH 7.0) and homogenised at top speed for 30 seconds in an MSE homogenizer. The samples were submitted sequentially to presumptive (PCC) and confirmed (CCC) coliform counts for *Escherischia coli* according to the protocol of the American Public Health Association (1970). Tubes showing acid and gas production after 24 or 48 hours were regarded as presumptively positive for faecal coliforms; the confirmed count was made by inoculating samples from the positive tubes into brilliant green bile broth and tryptone water for acid/gas and indole production, respectively, at 44°C for 24 hours to exclude irregular and other coliform organisms. The coliform density of the oyster expressed as "Most Probable Number" (MPN) per 100 ml was computed from Standard tables (American Public Health Association, 1970) to give an average hourly MPN/ml of tissue.

## RESULTS

### *Hydrology*

*The tides.* The tides in Hong Kong are semi-diurnal at the time of springs and diurnal at neaps. Moreover a diurnal inequality exists with respect to the seasons so that a higher high tide and a lower low tide occurs during the daylight hours in summer and during the night in winter (Fig. 2).

*Temperature.* During February the water temperature was seen to remain relatively stable at some 18–21°C with only a diurnal variation of 1–2°C; the waters warming up in the afternoon and cooling at night. During the summer, however, i.e. July, the water temperature was much higher (25–26°C) and with a similar diurnal variation. A dramatic fall in temperature was seen at the time of the low tide which occurred in the late evening possibly resulting from a greater influence of cool fresh waters flowing over the muds and shallow bay waters that had been heated up during the day.

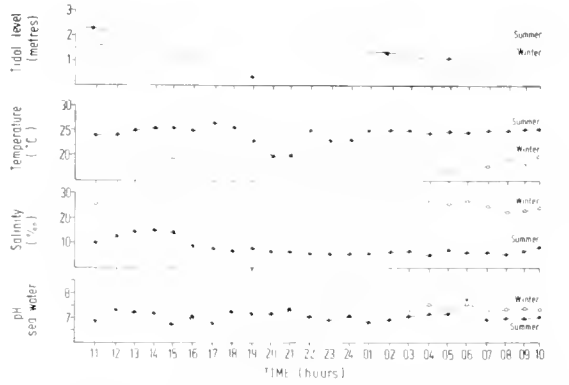


FIG. 2. *Hydrological changes taking place in the waters of Deep Bay over the two, 24 hour experimental periods.*

*Salinity*. During February the salinity of the Deep Bay waters remained consistently high (between 25–29‰) as the fresh water influence, created by the arrival of the S.E. Monsoon in summer, was seasonally reduced. At the time of the lower low tide in the early morning a number of smaller salinity values were recorded, presumably as the fresh water outflow temporarily exerted itself. During July, however, a different picture presented itself and the salinity was some 20‰ lower as the seasonal rains flooded the rivers and streams draining into the bay. Only at the time of the higher high tide that occurred in the early morning were a number of higher salinity values recorded presumably as more oceanic water masses were pushed into the bay with the incursion of the tide.

*pH*. In July the pH of the waters of Deep Bay remained relatively stable over the 24 hour period and were consistently lower than those seen in the winter. In February, moreover, marked fluctuations occurred correlated with the state of the tide. During both high tides higher pH values were recorded possibly indicating a greater incursion of more oceanic waters at this time though this was only relatable to salinity with respect to the daylight high tide.

*The oysters*

*pH of the mantle fluids*. The pattern of changes seen in the pH of the mantle fluids closely followed that seen in the waters of Deep Bay. This trend was best seen in winter when peaks in the pH of the mantle fluids occurred typically one hour later than similar peaks seen in the waters of Deep Bay (Fig. 3). In winter also lower pH values were recorded especially at the time of the low tide which occurred during the evening. In summer much smaller variations in pH occurred, though still following the trend seen in the waters of Deep Bay.

*The crystalline style*. The crystalline style of intertidal bivalves e.g. *Cardium edule* and *Ostrea edulis* (Morton, 1970; 1971) has been shown to typically dissolve on the ebbing tide and reform on the flowing tide. The dissolution of the style is primarily affected by the release of fragmentation spherules into the stomach from the digestive diverticula (Morton, 1973) though, as

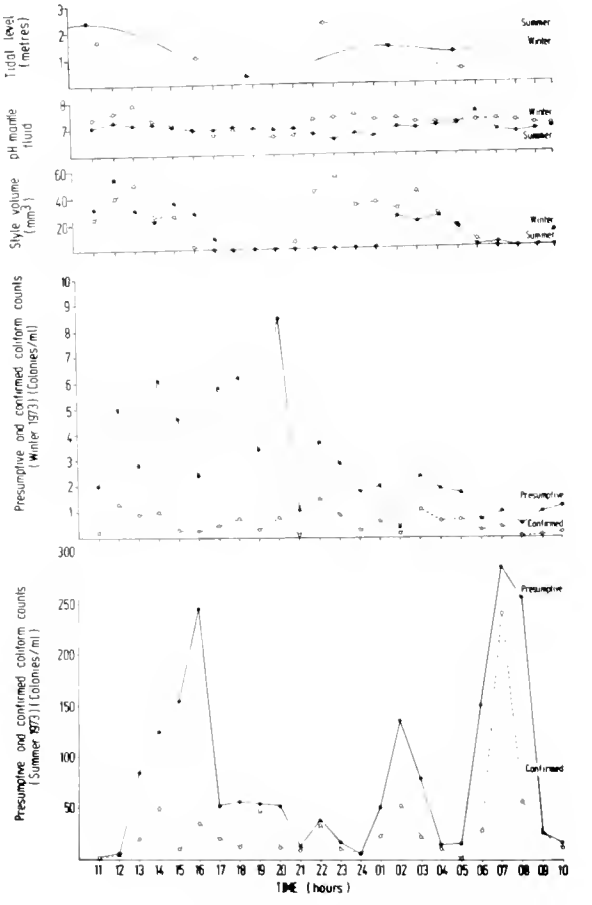


FIG. 3. *A correlation between the state of the tide, the pH of the mantle fluids and style volume of the oyster and the levels of presumptive and confirmed coliform counts in the oyster over the two 24 hour experimental periods.*

will be discussed later, the arrival of food in the stomach also causes an initial, but not complete, dissolution of the style.

In winter, i.e. in February, the crystalline style of *Crassostrea gigas* retained its firm, rod like structure for a longer time over the period of the high water that occurred during the night. The style was rod-like for a much shorter period during the reduced tide of the daylight hours. During both periods of low tide, however, the style dissolved completely.

The reverse situation was seen in summer with the style remaining firm longer during the extended period of high water that occurred during the daylight hours. At night it was a solid rod for some four hours only; possessing even then a volume only half that recorded at night. Again during both low tides the style dissolved completely.

During the period in both winter and summer when the style was the largest i.e. during the time of the highest high tides, a slight dissolution occurred in the middle of this period, but the presumably reforming style was able to overcome this minor disillutory effect caused, it is thought, by the arrival of food in the stomach, and to retain its structure until dissolved completely later by the arrival in the stomach of fragmentation spherules from the digestive diverticula. A similar pattern of formation and dissolution is seen in the European oyster *Ostrea edulis* (Morton, 1971).

*Microbiology*

*Seasonal variations*. A marked seasonal variation was encountered in both the presumptive and confirmed coliform counts from the homogenised oysters.

During the winter, i.e. in February, presumptive and confirmed coliform counts never exceeded 10 and 1 MPN/ml. respectively indicating that at this time (if British hygiene standards of less than 2 *E. coli*/ml. of tissue are applied) the oysters are relatively clean.

In summer, however, the counts for presumptive and confirmed coliforms rose to maxima of 285 and 240 MPN/ml. respectively. Estimates for the two series of tests are comparable and suggest that the majority of the faecal bacteria present in the oyster are *E. coli* and thus of human origin. Similar results have been obtained on a monthly basis by Leung *et al* (1975) who also correlated a rise in contamination levels with a flushing out of the watercourses and streams (typically serving as sewers) into Deep Bay. These results and the earlier results of Leung *et al* (1975) demonstrate that the Deep Bay oysters are contaminated, particularly in summer.

*Tidal variations*. In winter given the generally low levels of pollution no significant variation in the levels of contamination could be detected with respect to tidal changes. This was especially true of the confirmed coliform counts. Presumptive coliform counts were generally high around the time of the low tide that occurred in the afternoon but not during the lower low tide in the early morning.

In summer, however, a more obvious pattern was revealed especially with regard to the presumptive coliform counts where a distinct tidal pattern emerged with peaks of bacterial abundance occurring, as first noted for *Ostrea edulis* and *Mytilus edulis* by Wood (1969), at the times of low tide. The pattern of changes in the confirmed coliform counts (i.e. *E. coli*) of *Crassostrea gigas* generally followed the trend seen with respect to presumptive coliform counts, but were much more erratic.

## DISCUSSION

Deep Bay drains a number of small streams and rivers and in turn, forms an integral part of the much larger Pearl River.

The hydrology of the bay reflects Hong Kong's compromised climate of cool dry winters and hot, wet summers so that the water has a low temperature and is of high salinity in winter and a high temperature and of low salinity in summer. A greater range of temperatures have been recorded from Deep Bay than elsewhere in Hong Kong (Morton and Wu, 1975) because the shallow waters of the bay are warmed up higher and cooled down lower.

Tidal changes in the hydrology of the bay also influence water quality so that, for example, in winter the incursion of more oceanic waters into the bay at the time of high tide affected the pH. This was not so obvious in summer.

The pH of the mantle fluids of *Crassostrea gigas* generally followed the changes seen in the pH of the water, though lower values were re-

corded at the time of low tide, indicating that the shell valves were shut and that carbon dioxide levels were built up in the mantle fluids at this time. The volume of the crystalline style of *C. gigas* also altered in accordance with the tide, becoming firm and rod like at high tide and dissolving with each low tide. Bernard (1973) has similarly shown that in Canada, the style of *C. gigas* dissolves every tidal cycle. Complete dissolution, affected by the release of fragmentation spherules from the digestive diverticula when intracellular digestion had taken place occurred at the time of low tide though a slight dissolution mid way through the high tide period when the style was forming is thought to result from the arrival of food in the stomach. Similar patterns of feeding and digestion occur in the European oyster *Ostrea edulis* (Morton, 1971) and demonstrate the close relationship that exists between the animal and the environment.

High presumptive coliform counts from the oysters occurred in the summer and have been shown to coincide with the time of each low tide; winter values being considered too low to statistically demonstrate such a trend. A similar pattern has been reported upon by Wood (1969) for *Ostrea edulis*.

With respect to the tide, the pH of the mantle fluids, the style volume and the coliform counts it can be seen that there is a time lag in, what is in effect, the sequence in the feeding and digestive processes of the oyster. Thus the pH of the mantle fluids rose a few hours after high tide, the style was well formed later still and a peak in coliform counts was recorded somewhat later at the time of low tide. The oysters, located at mid tide level, would still be feeding at half ebbing tide, indeed on the falling tide the concentration of food in the oyster should be maximal. These results do show that on the ebbing tide, the concentration of bacteria has risen to a high level and can only further be concentrated (but not added to) by the organs of feeding and digestion so that actual numbers can rise no further. Subsequent low levels of bacteria probably result from the digestion of the bacteria by the oysters and the cleansing of the mantle fluids and intestine of pseudofaeces and faeces respectively with the returning tide.

A number of important conclusions can be drawn from the results of this investigation. Oyster contamination is likely to be highest at the time of low tide and it would seem sensible to crop the oysters (at least in Hong Kong) at the time of high tide when contamination is likely to be less. The high levels of contamination of the oysters in summer would also suggest that either the beds should be closed at this time or that the oysters should be cleansed prior to resale—a practice not yet undertaken in Hong Kong. Furthermore these results demonstrate that single or isolated tests for coliform bacteria in shellfish have little or no value. A comprehensive monitoring programme should test not only on a seasonal basis but also, as this study has shown, over a tidal regime. Finally this study has demonstrated the intimate relationship that exists between the oyster and the environment, especially with regard to the tides, and how contamination levels (resulting from the oysters feeding in polluted waters) are similarly bound up with this pattern.

## ACKNOWLEDGEMENTS

The authors are grateful to the Commissioner of Police, The Royal Hong Kong Police Force, for permission to use over the experimental periods the facilities of Tsim Bei Tsui Police Post in Deep Bay and to Tim Garland and Rudolph Wu who helped with the running of the experiments.

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*Proceedings of the National Shellfisheries Association*
*Volume 65 - 1976*

# CHANGES IN THE TOTAL PROTEIN, LIPID, CARBOHYDRATE, AND EXTRACELLULAR BODY FLUID FREE AMINO ACIDS OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*, DURING STARVATION

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A study was conducted concerning the response of the oyster *Crassostrea gigas*, to prolonged starvation. At the end of the starvation period there was a dry weight loss amounting to 61.0% of the pre-starved value. There was an increase in the % water content from 82.0 to 89.7. All major food reserves were extensively utilized. Lipid was found to be the most important energy reserve, assuming total oxidation. The digestive gland and mantle were the primary sources from which energy reserves were drawn during the early stages of starvation. The gills and adductor muscle exhibited the least depletion of reserves. The gonad exhibited a relatively low level of catabolic activity during the early stages of starvation. Signs of sexual maturity were evident at the middle of the starvation period. The degree of maturity was considerably less than that occurring under natural conditions during the same period. During the last 50 days of starvation the gonadal reserves were extensively catabolized. The free amino acid concentration in the hemolymph showed a considerable decrease.

## INTRODUCTION

The ability of oysters to resist starvation is well known. Gillespie, Ingle, and Havens (1964) found that the oyster *Crassostrea virginica* could live up to 390 days without any apparent source of food. Pora, Wittenberger and Portilla (1969) found that individuals of the species *C. rhizophorae*, maintained under anaerobic conditions for two weeks, showed little change in their lipid, glycogen or nitrogenous constituents, whereas individuals maintained under conditions of normal oxygenation consumed about 40% of their lipid and glycogen and 6% of their organic nitrogen. Studies by Millar and Scott (1967) with the larva of *Ostrea edulis* indicated that during periods of starvation the larva utilized lipid the most, carbohydrate the least, and protein utilization was intermediate. This reflected the fact that the lipid content of the larva was 2–4 times that of carbohydrate, whereas in the adult, carbohydrate content was 3–4 times that of lipid. Studies of starvation in other molluscs indicate a great variation in the extent of utilization of the various body constituents.

The objectives of this study were to:

1) Determine the change in the total protein, lipid, carbohydrate, and body fluid-free amino acids, of *Crassostrea gigas* during starvation.
2) Develop some insights into the mechanisms by which the oyster survives prolonged periods of starvation.

## MATERIAL AND METHODS

### *Experimental Design*

59 oysters of the 1970 year class were collected from North Humboldt Bay, California in mid-March 1972. The oysters were cleaned of all mud and fouling organisms and then placed in an insulated fiberglass tank containing 500 l of sterilized and filtered sea water. Every two weeks the tank water was changed and the tank scrubbed with a dilute chlorine solution. Partic-

ulate matter was removed by filtering the tank water to remove all matter of $2\mu$ in diameter and greater. Prior to filtering, the water was passed through a UV sterilizer. The water was recirculated in the tank and continuously passed through two spun-glass and activated charcoal filters. Temperature control was accomplished by use of a series of coiled 2 cm inner-diameter tubes placed off the bottom of the tank through which cool ocean water was continuously circulated. Temperature was maintained at 13.5C ± 2C. Salinity was adjusted to $25^{0}/_{00}$–$28^{0}/_{00}$ by use of aged tap water. The tank water was maintained near oxygen saturation by use of compressed air.

The following environmental factors were monitored: (i) temperature-daily; (ii) salinity-bi-weekly; (iii) pH-bi-monthly; (iv) oxygen saturation-bi-monthly.

*Analytical Procedure*

The initial weight was determined by inserting a wood wedge between the valves of ventilating oysters, removing the oysters from the tank, draining the intervalvar fluid, air drying the valves, and then weighing the drained oysters to the nearest gram. The starvation period lasted 175 days. A sample of five oysters was removed from the tank every 25 days for the first 125 days. A sample of nine oysters was removed at the end of the starvation period. A total of seven samples were analyzed. Upon removal from the tank the hinge ligaments were cut, valves pried open sufficiently to drain the intervalvar fluid, the valves air dried, and then each oyster weighed. After weighing the drained oyster the adductor muscle was severed, the body removed and placed in a petri dish, and the valves weighed. The oyster body was dissected into adductor muscle, mantle, gills and palps, digestive gland, and gonad (the style sac, intestines and rectum were pooled with the gonad). Care was taken to collect all the extracellular body fluid which was subsequently filtered through Whatman no. 1 filter paper. Each body component was pooled, weighed, freeze-dried, and then reweighed. Pooling of the body components prevented any measurement of variation within the samples. The freeze-dried body components, including the body fluid, were homogenized and then stored in capped vials at −15C until ready for analysis.

Each pooled body component was analyzed colorimetrically for total protein, total carbohydrate and total lipid. The body fluids from the 25, 75, and 175 day samples were analyzed for their free amino acid composition.

*Total protein.* A sample of each body component was homogenized with a glass tissue grinder in doubly distilled water. Cold 15% trichloroacetic acid was added to the homogenate. The homogenate was centrifuged, supernatant discarded, and the precipitate dissolved in 1N NaOH. An aliquot was then treated following the method of Lowry, *et al.* (1951) using bovine serum albumin as a standard.

*Total carbohydrate.* A sample of each body component was homogenized with a glass tissue grinder in a 2:1 (v/v) chloroform-methanol solution. The homogenate was centrifuged and the supernatant discarded. The precipitate was air dried, pulverized with a glass stirring rod, and then digested in 10% trichloroacetic acid at 95 C. An aliquot of the supernatant was diluted and then treated following the method of Dubois, *et al.* (1956) using glucose as a standard.

*Total lipid.* Lipids were extracted by the method of Folch, Lees, and Sloane-Stanley (1957) and subsequently analyzed by the method of Marsh and Weinstein (1966) using tripalmitin as a standard.

*Chromatography of body fluid free amino acids.* Amino acid analysis was done by gas-liquid chromatography with a Varian Aerograph model 1860 gas chromatograph. The freeze-dried body fluid was homogenized with a glass tissue grinder in doubly-distilled water followed by deproteinization and ion-exchange cleanup by the method of Gehrke, *et al.* (1968). Subsequently the samples were prepared by the method of Roach and Gehrke (1969) and then chromatographed on a column packed with stabilized grade ethylene glycol adipate (EGA). The body fluid chromatograms were compared with chromatograms of a standard amino acid mixture.

## RESULTS

The first observed effect of starvation was the disappearance of the greenish-brown chloroform soluble pigment of the digestive gland. Its dis-

appearance was probably due to the fact that the oysters had stopped feeding on algae rich in carotenoids. At the end of the starvation period the oysters appeared extremely emaciated and were characterized by a change in body coloration from creamy-white to greyish-tan. The mantle, originally thick and creamy in appearance, became very watery and thin to the point of translucence. The interior of the valves exhibited regressive lines of shell layering which indicated a shrinkage of the mantle edges from the valve edges. The first sign of gonadal maturation appeared at 75 days. The sex was determined by examining a gonadal smear. At 125 days four of the five oysters in the sample were sexed. At 175 days all of the oysters showed signs of gonadal atrophy. Only four of the oysters from the 175 day sample of nine oysters could be sexed. There was no indication in the tank or in the filters that the oysters had spawned during the starvation period. There was a total of 18 mortalities; all of which occurred during the first 100 days of starvation. The cause of the mortalities was uncertain, although excessive handling could have been a factor.

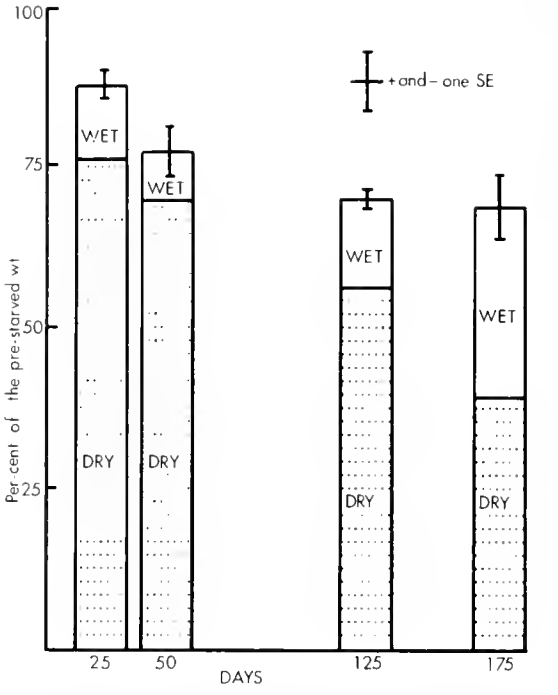


FIG. 1. *Wet and dry weight expressed as a percent of the pre-starved wet and dry weight.*

Due to mortalities and difficulties in determining the initial weight of some oysters it was impossible to determine the weight loss of all oysters. The wet weight loss was determined for the 25, 50 125 and 175 day samples (Fig. 1). At the end of the starvation period the wet weight was 68% of the original weight, a wet weight loss of 32%. There was an increase in the average water content from 82.0% to 89.7% (Table 1). The dry weight at the end of the starvation period was 39% of the original dry weight (Fig. 1), a dry weight loss of 61%. The digestive gland, gonad, and mantle showed the greatest dry weight loss. The adductor muscle, and gills and palps showed the least (Table 2).

In general the % protein of the whole body increased while carbohydrate decreased during starvation. The % lipid of the whole body exhibited little change (Table 1). By the end of the starvation period only the adductor muscle did not show a considerable increase in the % protein. Similarly it was the only body component not showing a considerable decrease in % carbohydrate. The gonad and the adductor muscle were the only components showing considerable increase in the % lipid. Considering the % protein, carbohydrate and lipid of the time zero sample as the pre-starved values, I calculated the utilization of protein, carbohydrate and lipid of the whole body in terms of dry weight, expressed as % of the pre-starved value:

$$U = 100 - \frac{(X_f)}{(X_o)} \cdot (DW).$$

U is the utilization expressed as a % of the pre-starved value; $X_f$ is the % substrate in the starved sample; $X_o$ is the % substrate in the pre-starved sample; DW is the dry weight of the 25, 50, 125 and 175 day samples expressed as a % of the original weight. Carbohydrate was utilized the most, protein the least, and lipid utilization was intermediate (Table 3). Following a similar procedure, the utilization of protein, carbohydrate and lipid was calculated for each body component. These calculations were done exclusive of the weight of the body fluid. At the end of the starvation period all body components except the adductor muscle exhibited the same pattern as exhibited by the whole body with carbohydrate being the most utilized, protein

TABLE 1. *Change in the gross biochemical constituents and water content of the oyster body components during starvation.*

| Days | Component | Relative composition[a] | Protein[b] | Carbohydrate | Lipid | Percent water |
|---|---|---|---|---|---|---|
| 0 | Adductor | .12 | 668 | 62 | 49 | 76.4 |
| | Gonad | .24 | 284 | 346 | 186 | 73.8 |
| | Gills | .10 | 358 | 219 | 150 | 86.6 |
| | Mantle | .29 | 291 | 284 | 162 | 77.9 |
| | Dig. gld. | .25 | 300 | 299 | 187 | 60.8 |
| | Body fluid | | | | | 95.6 |
| | Whole body[c] | | 343 | 269 | 159 | 82.0 |
| 25 | Adductor | .14 | 651 | 76 | 52 | 75.5 |
| | Gonad | .29 | 332 | 319 | 219 | 73.4 |
| | Gills | .16 | 378 | 236 | 164 | 79.9 |
| | Mantle | .29 | 286 | 358 | 180 | 77.3 |
| | Dig. gld. | .12 | 341 | 304 | 174 | 72.9 |
| | Body fluid | | | | | 96.4 |
| | Whole Body | | 372 | 281 | 170 | 84.5 |
| 50 | Adductor | .16 | 615 | 60 | 55 | 76.6 |
| | Gonad | .23 | 363 | 211 | 218 | 77.6 |
| | Gills | .19 | 344 | 183 | 162 | 79.9 |
| | Mantle | .30 | 320 | 261 | 171 | 81.5 |
| | Dig. gld. | .14 | 358 | 215 | 185 | 74.2 |
| | Body fluid | | | | | 96.0 |
| | Whole body | | 381 | 200 | 166 | 86.2 |
| 75 | Adductor | .14 | 617 | 60 | 55 | 76.6 |
| | Gonad | .23 | 363 | 211 | 218 | 77.6 |
| | Gills | .19 | 344 | 183 | 162 | 79.9 |
| | Mantle | .30 | 320 | 261 | 171 | 81.5 |
| | Dig. gld. | .14 | 358 | 215 | 185 | 74.2 |
| | Body fluid | | | | | 96.0 |
| | Whole body | | 381 | 200 | 166 | 86.2 |
| 100 | Adductor | .12 | 638 | 67 | 52 | 78.9 |
| | Gonad | .31 | 446 | 179 | 203 | 79.4 |
| | Gills | .14 | 370 | 168 | 163 | 83.5 |
| | Mantle | .31 | 297 | 292 | 183 | 82.3 |
| | Dig. gld. | .11 | 401 | 200 | 186 | 77.7 |
| | Body fluid | | | | | 96.8 |
| | Whole body | | 403 | 200 | 169 | 86.8 |
| 125 | Adductor | .14 | 649 | 76 | 64 | 79.4 |
| | Gonad | .30 | 451 | 158 | 241 | 79.4 |
| | Gills | .15 | 396 | 182 | 158 | 82.4 |
| | Mantle | .31 | 307 | 281 | 183 | 81.3 |
| | Dig. gld. | .10 | 440 | 176 | 183 | 78.2 |
| | Body fluid | | | | | 96.2 |
| | Whole body | | 425 | 190 | 178 | 85.4 |
| 175 | Adductor | .16 | 671 | 69 | 65 | 79.7 |
| | Gonad | .20 | 402 | 155 | 235 | 81.9 |
| | Gills | .18 | 428 | 153 | 155 | 84.3 |
| | Mantle | .34 | 366 | 227 | 177 | 83.8 |
| | Dig. gld. | .13 | 414 | 165 | 174 | 80.0 |
| | Body fluid | | | | | 96.5 |
| | Whole body | | 443 | 168 | 168 | 89.7 |

[a] Less dry wt. of body fluids.
[b] mg/g dry wt.; all samples analyzed in triplicate.
[c] Calculated by totaling the products of column 3, and columns 4, 5, and 6 respectively.

TABLE 2. *Dry Weight of each body component expressed as a per-cent of pre-starved value.*

| Days | Adductor | Gonad | Gills | Mantle | Dig. gld |
|---|---|---|---|---|---|
| 0 | – | – | – | – | – |
| 25 | 89 | 91 | 117 | 75 | 36 |
| 50 | 90 | 84 | 108 | 62 | 36 |
| 125 | 66 | 70 | 84 | 60 | 23 |
| 175 | 46 | 29 | 62 | 41 | 18 |

TABLE 3. *Utilization of protein, carbohydrate and lipid of the whole body expressed as a per-cent of the pre-starved value.*

| Days | Protein | Carbohydrate | Lipid |
|---|---|---|---|
| 0 | – | – | – |
| 25 | 19 | 21 | 20 |
| 50 | 20 | 37 | 30 |
| 125 | 30 | 60 | 36 |
| 175 | 55 | 78 | 63 |

the least, and lipid intermediate. In the adductor muscle, protein was the most utilized, lipid the least and carbohydrate was intermediate (Table 4).

Fifteen free amino acids were detected in the extracellular body fluid (Table 5). Glycine, alanine, proline, glutamic acid and aspartic acid comprised greater than 70% of each sample. With the exception of a high concentration of tyrosine in the 25 day sample, tyrosine, ornithine, serine, leucine, threonine, and lysine were present in moderate concentrations while valine, phenylalanine, methionine and hydroxyproline were present in amounts less than 0.01 mg/100 ml. All free amino acids in the body fluid decreased except aspartic acid, which showed an increase amounting to 9% of the 25 day value.

## DISCUSSION

After the first 50 days of starvation the wet weight loss began to level off. However, the dry weight loss continued to increase. The dry weight loss and a general increase in the state of hydration of the body components occurred concomitantly. The hydration of the body fluid remained constant throughout the starvation period. This seems to indicate that cell volume was maintained. The adductor muscle and gills exhibited the least dry weight loss. These two organs are the most important in maintaining

TABLE 4. *Utilization of protein (P), carbohydrate (C), lipid (L) of each body component expressed as a per-cent of the pre-starved value.*

| | Adductor | | | Gonad | | | Gills | | | Mantle | | | Dig. gld. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days | P | C | L | P | C | L | P | C | L | P | C | L | P | C | L |
| 25 | 11 | 6 | 8 | +7 | 16 | 0 | +2 | +20 | +33 | 26 | 0 | 10 | 59 | 65 | 70 |
| 50 | 19 | 16 | 7 | +17 | 33 | 0 | +11 | +12 | +18 | 38 | 33 | 31 | 63 | 63 | 67 |
| 125 | 36 | 21 | 16 | 9 | 59 | 7 | 22 | 29 | 20 | 37 | 42 | 33 | 66 | 88 | 80 |
| 175 | 53 | 50 | 39 | 68 | 87 | 63 | 25 | 55 | 36 | 49 | 67 | 54 | 76 | 90 | 83 |

[a] Symbols: + =substrate concentration greater than pre-starved value.

TABLE 5. *Free amino acid composition of the extracellular body fluids.*

| | Concentration[a] | | |
|---|---|---|---|
| Amino acid | 25[b] | 75 | 175 |
| Ala | 6.416 | 4.819 | 2.775 |
| Val | 0.161 | 0.073 | 0.044 |
| Gly | 6.833 | 3.552 | 2.802 |
| Leu | 1.035 | 0.433 | 0.515 |
| Pro | 5.921 | 4.000 | 1.918 |
| Thr | 0.951 | 0.690 | 0.320 |
| Ser | 1.263 | 1.611 | 0.746 |
| Met | +[c] | + | + |
| HyPro | + | + | + |
| Phe | 0.095 | + | 0.036 |
| Asp | 2.359 | 2.425 | 2.570 |
| Glu | 4.700 | 4.150 | 3.357 |
| Tyr | 3.422 | 0.686 | 0.289 |
| Orn | 1.506 | 1.442 | 0.493 |
| Lys | 0.930 | 0.404 | 0.324 |
| Total | 35.592 | 24.285 | 16.189 |

[a] In mg/100ml.
[b] Days of starvation.
[c] Present in amounts less than 0.01 mg/100 ml.

TABLE 6. *Gonadal development; starved vs. non-starved.*

| | Per-cent[a] | |
|---|---|---|
| Days | Starved | Non-starved |
| 0 | – | 24 |
| 75 | 23 | 49 |
| 175 | 20 | 52 |

[a] Per-cent gonad of total dry weight of sample (less extracellular body fluid).

the structural and biochemical integrity of the oyster. It is natural that they should show the least evidence of destructive metabolism. For the first 125 days, the mantle and digestive gland showed the greatest dry weight loss. These two organs are generally considered the major storage organs of the oyster. The digestive gland showed the most rapid dry weight loss. The early weight loss of the digestive gland was probably accentuated by the presence of undigested food in the stomach of the oysters taken as the pre-starved sample. There was considerable evidence of active shell layering in the starved oysters. As mentioned previously one of the visible effects of starvation was the shrinkage of the mantle from the valve periphery and the consequent layering of new shell material. The gonad showed relatively little weight loss during the early stages of starvation. It was during this period that maturation of the gonad was occurring. Evidently the ability of the gonad to ripen was not totally deterred by the extreme conditions of starvation. The degree to which gonadal maturation occurred was considerably less than that occurring under natural conditions at the same period. Oysters were taken from North Humboldt Bay from the same site from which the starved specimens were originally collected. These oysters were dissected and treated in the same manner as the starved oysters. Comparable data on gonadal development were thus obtained. Gonadal development was considerably retarded in the starved oysters (Table 6).

During the early stages of starvation the protein content of the gonad increased and the lipid content remained constant (Table 4). This was somewhat different from the response of the other body components, (with the exception of the gills) which exhibited decreases in all of their energy reserves. The large decrease in the carbohydrate reserve of the gonad concomitant with the increase in protein suggests that the material and energy for gonadal maturation was drawn directly from the carbohydrate reserve of the gonad.

Carbohydrate was the most metabolized reserve in terms of grams utilized. If the utilized amounts of carbohydrate, protein, and lipid were completely oxidized to carbon dioxide and

water, then the energy provided by each substrate is derivable. Lipids were the most important substrate in terms of total energy (Table 7). Oysters are usually considered to have a carbohydrate-oriented metabolism because of their high glycogen content. The data presented indicates that during starvation lipid and protein are the major energy reserves.

One of the physiological effects of starvation in molluscs is the decrease in oxygen consumption which implies a decrease in the metabolic activity of the starving organism with time. It is possible to calculate the change in energy requirements during starvation assuming complete oxidation of energy reserves. The total energy requirements decreased substantially for the first 125 days after which a dramatic increase in energy requirements occurred (Table 7). During the last 50 days there was a large increase in utilization of lipid; the major contributor being the gonad. It is known that the eggs of oysters are rich in lipid. The rate of dry weight loss was increased for every body component, except the digestive gland, during the last 50 days relative to the values for 125 days (Table 8). Possibly during the late stages of starvation there is a decrease in the efficiency of the metabolic pathways, accounting for the apparent increase in the energy requirements during the final sample period. It is quite possible that incomplete oxidation of protein and lipid is occurring.

Decreases in the free amino acid content of the extracellular and intracellular body fluids of stressed bivalves seem to be common (Jeffries, 1972; Feng, Khairallah and Canzonier, 1970). Stressed bivalves show a decrease in the free amino acid pools of both intracellular and extracellular fluids. It is interesting to compare the response of the free amino acid pools of stressed bivalves with that of bivalves subjected to salinity changes. Lynch and Wood (1966) found that with decreasing environmental salinity there was a concomitant decrease in the free amino acid pool of the adductor muscle of *C. virginica*. Some of the free amino acids showed less response than others. Aspartic acid exhibited little response to change in salinity. The data of Feng et al. (1970) and Jeffries (1972) indicate that aspartic acid did not decrease with stress but actually increased. In my study aspartic acid was the only free amino acid to show an increase during starvation. Apparently whether oysters are exposed to stress by environmental pollution, parasitism, starvation or decreased salinity the free amino acid pools are generally affected in the same way with the major exception of the non-protein amino acid taurine. I did not measure taurine, but in the studies by Jeffries (1972) and Feng, *et al.* (1970) taurine was measured and found to increase with stress. In general, bivalves subjected to decreasing salinity show a decrease in taurine content (Schoffeniels and Gilles, 1972).

It is possible to speculate on what the similarity between the salinity induced change and that due to starvation means. When a euryhaline mollusc is placed in a medium of low salinity, the extracellular fluid tends to adjust and to reflect the osmotic pressure of the external medium. The intracellular osmotic pressure reflects that of the extracellular fluid (Schoffeniels and Gilles, 1972). In marine molluscs free amino acids are present in high concentrations relative to vertebrates. These free amino acids serve as solutes for raising the osmotic pressure of body fluids and especially that of the intracellular fluids (Campbell and Bishop, 1970). When subjected to dilute media the tissues lose osmotic components, especially amino acids, and

TABLE 7. *Energy metabolism of starved oysters.*

| Days | Kcal[a] protein | Kcal carbohydrate | Kcal lipid | Kcal total |
|---|---|---|---|---|
| 0–25 | .011 | .012 | .013 | .036 |
| 25–50 | .010 | .001 | .010 | .021 |
| 50–125 | .005 | .003 | .001 | .009 |
| 125–175 | .009 | .015 | .016 | .040 |

[a] Kcal consumed/(T) ($W_m$); T = sample interval in days, $W_m$ = median dry weight between interval in grams.

TABLE 8. *Weight loss of each body component between sample intervals.*[a]

| | 0–25 | 25–50 | 50–125 | 125–175 |
|---|---|---|---|---|
| Adductor muscle | 60 | + | 60 | 100 |
| Gonad | 80 | 110 | 70 | 430 |
| Gills | +[b] | 60 | 50 | 90 |
| Mantle | 330 | 220 | 10 | 380 |
| Digestive gld. | 760 | – | 60 | 60 |

[a] Weight loss is $\mu$g/day/$W_m$ between each interval; $W_m$ = median dry weight between interval in grams.
[b] Symbols: + = increase in dry weight, – = no weight change.

inorganic ions, and assume osmotic pressures comparable to that of the media. In some cases the decreases in osmotic pressure of intracellular fluids may be due to increased tissue hydration (Campbell and Bishop, 1970), but normally changes in cell volume of euryhaline invertebrates are slight (Gilles, 1969). In crustaceans the concentration of intracellular free amino acids is regulated partly by a mechanism involving changes in the permeability of the cell membrane and partly by modification of the pathways normally responsible for the metabolism of amino acids (Gilles, 1969). The modification of these pathways is believed to be due in part to changes in intracellular ionic concentration. It is quite likely that this same mechanism is present in marine molluscs (Schoffeniels and Gilles, 1972).

In this study the decrease in the extracellular free amino acids probably reflected an intracellular decrease. This postulated intracellular decrease could be due to the increased hydration of the cells due to the depletion of polymeric reserves, the alterations in the pathways of amino acid metabolism. It is possible that this intracellular decrease could be partly compensated for by an increased concentration of taurine.

A study to determine the effects of starvation on the extracellular and intracellular non-protein amino acids would seem to be warranted.

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