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INHIBITION OF SHELL GROWTH IN THE PRESENCE OF MERCURY AND SUBSEQUENT RECOVERY OF JUVENILE OYSTERS[1]

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ABSTRACT

Juvenile oysters (Crassostrea virginica) *were given static exposure for 12 hours each day to mercuric acetate added at 100 ppb or 10 ppb mercury for 47 days. Shell growth was measured as the increase in height (distance from hinge to posterior margin). Inhibition in shell growth was used as an indicator of physiological stress. After 47 days, shell growth was reduced by 77% for the 100 ppb group and by 33% for the 10 ppb mercury group compared to controls. Oysters in seawater for a 162-day depuration period demonstrated shell growth rates comparable to controls within 34 days (100 ppb) and 20 days (10 ppb).*

INTRODUCTION

Estuarine and coastal waters have become the repositories for numerous effluents of both industrial and agricultural activities. Mercury is one of the most toxic of these materials and increased mercury concentrations in seawater are reflected by increased mercury concentrations in coastal marine organisms (Klein and Goldberg, 1970). Mercury stress might, therefore, be expected to mediate major physiological adjustments by marine and estuarine species.

Published studies of the effects of mercury on bivalves have been limited primarily to determinations of whole body mercury residues (Kurland *et al.*, 1960; Craig, 1967; Kopfler, 1974). Residue studies conducted over extensive periods have provided data on the rate of accumulation and removal of mercury from mollusk tissues (Seymour and Nelson, 1971; Cunningham and Tripp, 1973; Cunningham and Tripp, 1975a). Unlu *et al.* (1970) determined mercury concentrations in various tissues of *Tapes descussatus;* mercury was accumulated directly from seawater or from ingestion of contaminated algae. Cunningham and Tripp (1975b) performed a similar experiment on *Crassostrea virginica* to monitor changes in the tissue distribution of mercury during an accumulation and depuration period. One disadvantage of most residue studies is that experimental organisms must be sacrificed periodically rather than being monitored continuously. Thus, little is known of the details of physiological stress imposed on organisms.

Butler *et al.* (1960) demonstrated that shell growth in juvenile oysters could be employed as a sensitive method for the continuous monitoring of physiological stress occurring in bivalves exposed to various concentrations of pesticides. The method of Butler *et al.* (1960) was used in studies by Shuster and Pringle (1969) of cadmium, chromium, zinc, and copper; by Lowe *et al.* (1971) of DDT, toxaphene, and parathion; and by Lowe *et al.* (1972) of the polychlorinated biphenyl compound Aroclor 1254. These authors however,

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monitored only shell growth inhibition during the exposure period and did not measure the time required for resumption of normal shell growth during a depuration period as originally suggested by Butler *et al.* (1960). This could be important in determining long term effects of various toxicants on populations and whether recovery of exposed individuals to a healthy physiological state is possible.

This study was initiated to determine the inhibitory effects of mercury on shell growth, and the time required for the juveniles to recover and resume normal shell deposition when held in ambient seawater.

MATERIALS AND METHODS

Experimental Stock

Juvenile *Crassostrea virginica* were reared from larvae produced in artificial spawning experiments at the University of Delaware's Shellfish Laboratory, Lewes, Delaware. All juveniles were 15 months old at the initiation of the study on July 10, 1971, and ranged in height (distance from hinge to posterior margin) from 3.66 to 6.20 centimeters. This height corresponds to the size range recommended for shell growth experiments (Butler, 1965). Oysters were cleaned, and the outer shell was allowed to air dry so that each individual could be permanently marked with an identification number using waterproof ink. The posterior shell margins were then filed to remove all new shell growth, and height measurements were recorded. Thirty-six oysters were thus marked, and were randomly divided into three groups: 100 parts per billion (ppb) mercury, 10 ppb mercury, and controls.

Each experimental group was placed in a separate 230-liter tank receiving unfiltered Broadkill River water (daily salinity range 33 to 17 o/oo; DeWitt, 1971). Oysters obtained all their food from the seawater supply. The shell growth study initiated on July 10 was made in conjunction with a mercury residue study begun on July 1 and previously reported by Cunningham and Tripp (1973).

Preparation of Experimental Environment

A stock solution of mercury was prepared containing 0.30 grams of mercuric acetate, $Hg(CH_3COOH)_2$, dissolved in one liter of distilled water. Dilutions were in parts per billion mercury rather than in parts per billion mercuric acetate. The experimental dilutions (100 ppb and 10 ppb mercury) were chosen to correspond to concentrations found in the Delaware River basin which ranged from trace amounts to a maximum of 90 ppb mercury (Delaware River Basin Commission, 1970).

Each experimental day was divided into two 12-hour periods. During the 12-hour feeding period (8 a.m. to 8 p.m.), unfiltered seawater was pumped directly into each experimental tank at a rate of 2.8 liters per minute. A constant volume of 190 liters of seawater was maintained in each tank during this period and aeration was provided. Mean summer water temperature was 25C ± 2C.

During the 12-hour mercury exposure period (8 p.m. to 8 a.m.), the seawater input was stopped and a constant volume of 190 liters of aerated water was maintained. To this constant volume, stock mercury solution was added to yield 100 ppb or 10 ppb mercury. No mercury additions were made to the control tank. In the morning, the mercury-contaminated water was removed, fresh seawater was introduced, and continued to flow for the duration of the 12-hour day-time feeding period. This alternating 12-hour cycle of mercury exposure was maintained for 47 days, from July 10 through August 26, and was employed to reduce the volume of mercury-contaminated effluent which would have been produced by a 24-hour flowing system.

At the end of the mercury exposure period, a 162-day depuration period was initiated to determine whether shell growth comparable to controls could be resumed in juveniles previously exposed to mercury. For this portion of the experiment, ambient seawater was pumped into each tank at the rate of 2.8 liters per minute. Water temperature ranged from 25C to 0C during the cleansing period, (August 26, 1971 to February 5, 1972).

Measurement of Shell Growth

During the mercury exposure period, juveniles were removed from the experimental environments after 5, 15, 20, 35, and 47 days. Height measurements were made on each individual to determine the amount of newly deposited shell. After each measurement, juveniles were again filed and measured. The filing procedure prescribed

by Butler *et al.* (1960) stimulates further shell production and evens the irregular margin of the elongated shell that forms during the normal growth process.

During the 162-day depuration period, juveniles were removed from the experimental environments after 20, 34, 62, and 162 days. The same procedures for measuring shell growth and filing used in the exposure period were employed for this portion of the experiment.

All shell growth data were evaluated to the $P < 0.05$ level of confidence using an analysis of variance coupled with a Duncan Multiple Range test. A detailed summary of the procedure for ranking the means and computing the Duncan Multiple Range (DMR) values is given in Steel and Torrie (1960).

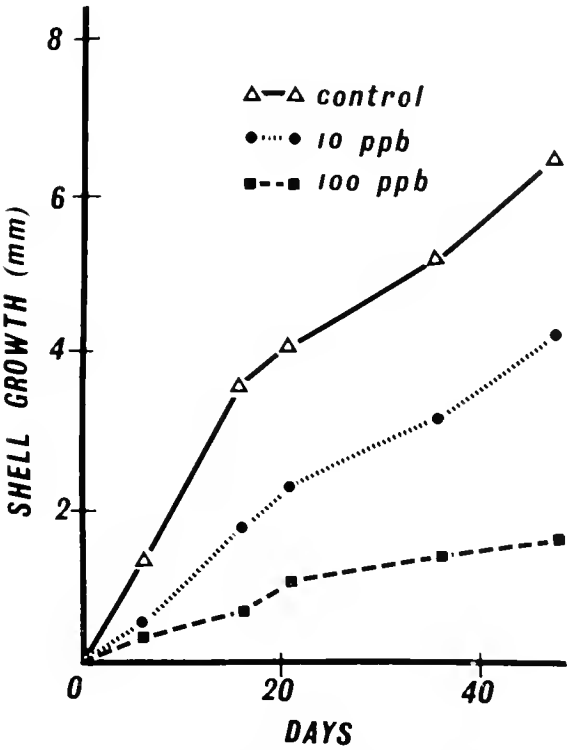

Fig. 1. *Cumulative shell growth in juvenile oysters during a 47-day mercury exposure period.*

RESULTS

Mercury Exposure Period

Juvenile oysters exposed to 100 ppb and 10 ppb mercury-contaminated seawater exhibited significant inhibition in shell growth after only 5 days of exposure (Table 1). After 47 days exposure, the mean cumulative shell production in the control, 10 ppb and 100 ppb groups was 6.53 mm, 4.37 mm, and 1.50 mm, respectively. Cumulative shell growth during the exposure period was reduced by 77% in the 100 ppb group and by 33% in the 10 ppb group (Fig. 1).

Depuration Period

After 20 days of cleansing in ambient seawater (the time of earliest measurement), there was no significant difference in mean shell growth between the control and 10 ppb group. The rate of recovery was slower in the 100 ppb group, however, and only after 34 days did that group return to the normal rate of shell deposition (Fig. 2). A recession in shell deposition observed in all 3 groups after September 30 (day 34) may have been due to the seasonal decline in ambient water temperature. Seasonal recession in shell growth in oysters was observed by Butler *et al.* (1960).

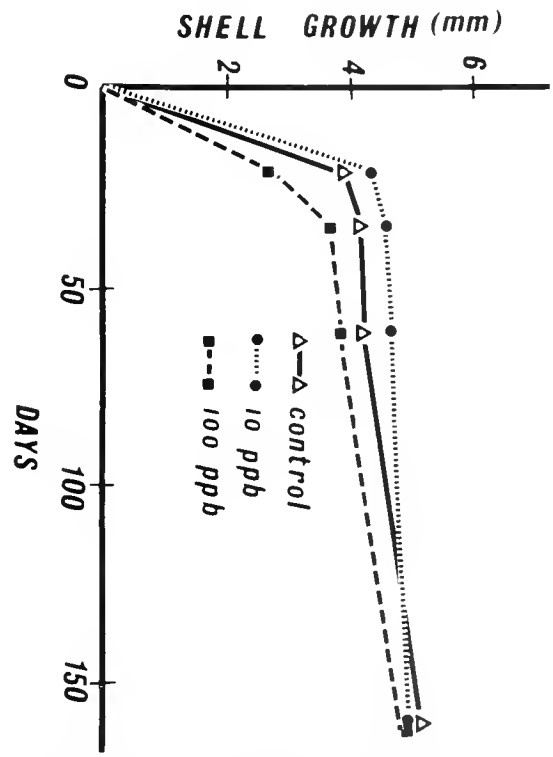

Fig. 2. *Cumulative shell growth in juvenile oysters during a 162-day depuration period.*

TABLE 1. *Cumulative shell growth (mm) in juvenile oysters,* Crassostrea virginica *during a mercury exposure period and subsequent depuration period.*

		MERCURY EXPOSURE PERIOD				
		MEAN SHELL GROWTH (mm ± 1 S.D.)				
Date 1971	*Days*	*Control*	*10 ppb*	*100 ppb*	*F test*	DMR[a]
July 10	0	—	—	—	—	ABC
July 15	5	1.13±0.26	0.47±0.16	0.36±0.14	4.28	A BC
July 25	15	3.73±0.62	1.82±0.42	0.69±0.19	11.11*	A BC
July 30	20	4.12±0.61	2.20±0.52	1.03±0.29	9.56*	A BC
August 14	35	5.31±0.77	3.26±0.56	1.33±0.39	10.74*	A B C
August 26	47	6.53±0.78	4.37±0.70	1.50±0.40	13.46*	A B C
		DEPURATION PERIOD				
August 26	0	—	—	—	—	
September 15	20	3.98±0.48	4.34±0.72	2.68±0.32	2.93	AB C
September 30	34	4.18±0.43	4.61±0.72	3.79±0.41	0.53	ABC
October 28	62	4.19±0.46	4.64±0.71	3.87±0.42	0.47	ABC
February 5	162	5.17±0.65	5.00±0.68	5.00±0.57	0.02	ABC

a Duncan Multiple Range (DMR) Analysis: Any two means (A = Control, B = 10 ppb, C = 100 ppb) not underscored by the same line are significantly different ($P<0.05$ level of confidence) (Steel and Torrie, 1960).
*$P<0.05$

DISCUSSION

In biological systems, mercury can act as an inhibitor by combining reversibly with the sufhydryl groups of cysteine residues that are essential for the catalytic activity of some enzymes (Barron *et al.*, 1948; Lehninger, 1970). This biochemical view of mercury inhibition in enzyme systems may be exemplified on the whole organism level by shell inhibition in juvenile oysters. Mercury ions may be inactivating enzymes in several metabolic pathways required in the shell deposition process. Reversal of the inhibitory effect is demonstrated during the depuration period by rapid recovery of mercury exposed individuals to shell production rates comparable to those of unexposed controls. In earlier experiments, adult oysters maintained in mercury-contaminated seawater (100 ppb and 10 ppb) exhibited a large decline in mercury tissue residues after a cleansing period in ambient seawater (Cunningham and Tripp, 1973; 1975a), and juveniles would be expected to exhibit a similar decline.

Shellfish concentrate heavy metal ions in their tissues to many times the concentrations present in seawater without exhibiting detectable detrimental effects. Pringle *et al.*(1968) measured this concentration effect (biological enrichment factor) for 8 metals in the oyster, *Crassostrea virginica.* Values ranged from 2,900 for manganese to 226,000 for cadmium. Mercury residues in adult oysters of 27,950 ppb (for a 10 ppb exposure group) did not cause mortality greater than that on controls (6%), but 50% mortality was recorded (for a 100 ppb exposure group) when the average mercury tissue residue was 140,710 ppb (Cunningham and Tripp, 1973). Thus, mortality is a poor criterion for assessing the detrimental effects of mercury accumulation in oysters. Quicker, more precise measurement of reversible inhibitory effects of toxicants can be obtained using Butler *et al.*(1960) shell growth technique. Shell growth inhibition and recovery studies should be used to augment residue experiments and could be more widely used to test water quality.

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THE REPRODUCTIVE CYCLE OF THE SUNRAY VENUS CLAM *Macrocallista nimbosa* (LIGHTFOOT 1786)

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ABSTRACT

The annual reproductive cycle is described for the sunray venus clam, Macrocallista nimbosa *(Lightfoot). Clams used in the study were collected from waters adjacent to Blacks Island in St. Joseph Bay, Florida. The reproductive activity was determined by histological examination of gonadal sections, by monitoring variation in the glycogen content of the tissues, and by variation in strip spawning potentiality.*

Results from the histological study indicate that spawning during 1974 began in July for males and continued through December, with peak spawning occurring during November. Females began spawning in August and continued throughout November, reaching peak spawning activity during October and November.

The reproductive activity was reflected in the seasonal variation in glycogen content, with greatest glycogen storage occurring during the winter, averaging 15%, then reaching an annual low value of 3.8% in July, with a slight rise in August and September and a drop to 5.1% during October, returning to high winter values again in December.

Clams were strip-spawned throughout the year, but viable larvae were obtained only during October and November.

INTRODUCTION

The clam fishery in Florida has been dominated by three venerid species, the northern quahog, *Mercenaria mercenaria (L.)*; the southern quahog, *M. campechiensis* (Gmelin); and the sunray venus clam, *Macrocallista nimbosa* (Lightfoot) (Godcharles and Jaap, 1973). The fishery for the sunray venus clam was initiated in February 1967 near Port St. Joe, Florida, but at present, the fishery is inactive because insufficient numbers of clams are available. The present study was undertaken to provide basic information on the biology of this potential mariculture organism.

This paper presents the first published description of the reproductive cycle of the sunray venus clam. Earlier investigators used a variety of research methods, either singly or in combination to determine the reproductive activity in bivalves. Three methods were used in this study: (1) histological examination of gonadal sections; (2) variation in glycogen content of tissue; and (3) variation in strip-spawning potentiality. This study compares and contrasts the suitability of these methods in ascertaining the seasonal reproductive state of *M. nimbosa*.

MATERIALS AND METHODS

The study began in January and was completed in December 1974. The clams were collected in waters 300 meters north of Blacks Island in St. Joseph Bay, Florida in depths ranging from 1.0 to 1.5 meters at mean low tide. Collections for the histological study were made once a month except

for the months of July and August when two collections were made per month. The sample size ranged from 10 to 23 clams per month.

Within 24 hours of collection, a 10-mm cube of gonadal tissue was removed from the mid-lateral portion of the visceral mass of each clam and preserved in Bouin's fixative. After 7 days in Bouin's fixative, the tissues were transferred to 50% ethanol and held for further histological processing. Fixed gonadal tissues were dehydrated in alcohol, cleared in xylene, and embedded in paraffin. The embedded tissue was then sectioned at 8 microns, mounted on a slide, stained in Erlich's hematoxylin, and counterstained with alcoholic eosin.

Each slide was examined microscopically and a random sample of 20 alveoli per slide was assigned to a category of gonad condition. The histological study of the reproductive cycle is based upon examination of 4,000 alveoli. The cyclic reproductive process is divided into 5 phases of gonad condition which apply to both sexes: early active, late active, ripe, partially spawned, and spent. These 5 phases and their distinguishing characteristics are used by various investigators, e.g., Ropes and Stickney (1965) for *Mya arenaria*, Ropes (1968) for *Spisula solidissima*, Cain (1972) for *Rangia cuneata*, and Holland and Chew (1974) for *Venerupis japonica*. There is no sharp distinction between phases and the categories are convenient rather than natural (Ropes, 1968).

The method used in this study of examining 20 alveoli per slide or individual is different from the methods of other investigators who assign each clam or slide to one of the 5 phases. A new procedure was used for the sunray venus clam because one clam may contain alveoli in several different phases, and to assign the individual clam to just one phase would have masked the other phases present. It is also believed that maturation of alveoli from one phase to another can occur within a few days time, and that individual clams do not empty all of their gametes at one spawning, so it is informative to express the proportion of alveoli in the various phases.

The five phases of female gonad condition and their distinguishing characteristics are described:

(1) *Early Active Phase.* Ovogonia occur at the periphery and within the alveolar walls. Follicle cells frequently occur within the alveolus.

(2) *Late Active Phase.* Alveoli in the late active phase contain a large number of elongated stalked ovocytes, whose free ends protrude into the alveolar lumen and whose bases attach to the alveolar wall. The large, stained ovocyte nucleus is a conspicuous characteristic of this phase and basophilic nucleoli are present. The basement membrane is thin.

(3) *Ripe Phase.* The alveolus is termed ripe when the number of ova free within the lumina exceeds the number of attached ovocytes (Cain, 1972). The attached ovocytes resemble those in the late active phase, except that in the ripe phase, amphinucleoli are present. Ripe alveoli are filled with ova and ovocytes and appear crowded together.

(4) *Partially Spawned Phase.* A few ovocytes are still attached to the thickened alveolar walls; a few residual ripe ova may remain in the alveolar lumen.

(5) *Spent Phase.* Alveoli are usually empty of ripe ovocytes; those that are present are usually undergoing cytolysis. The alveolar walls are thickened and oogonia are often present. The spaces between alveoli are filled with mesenchyme.

The stages of male gonad condition are described:

(1). *Early Active Phase.* Alveolar walls are thickened and contain darkly stained spermatogonia. Primary spermatocytes are found at the alveolar periphery and begin to proliferate toward the lumen. Follicle cells may fill the alveoli. Alveoli contain nutritive phagocytes (Loonsanoff, 1937).

(2). *Late Active Phase.* This phase is characterized by the proliferation and maturation of spermatocytes. The spermatocytes are uniformly shaped cells which at the initiation of maturation are found near the alveolar periphery, but as development progresses, migrate toward the alveoli centers. Later in the active phase the spermatocytes elongate and are arranged in radially aligned columns. A central lumen within the alveolus is formed. A small number of spermatozoa may be within the lumen.

(3). *Ripe Phase.* With the development of tails the spermatids are transformed into spermatozoa. Spermatozoa are arranged in radial columns in the alveoli with their tails oriented toward the lumen.

Later in this phase, masses of free spermatozoa fill the alveolar lumen.

(4). *Partially Spawned Phase.* Spermatozoa are present within alveolar centers, but are less numerous than in the ripe phase. A thin band of spermatogonia and primary spermatocytes may be found along the basal membrane before the alveolus is empty of spermatozoa.

(5). *Spent Phase.* Alveoli in the spent phase contain few or no spermatozoa and the lumina are open.

The glycogen content of clam tissue was determined by the colorimetric phenol method (Westenhouse, 1968). Clams used for glycogen analysis were processed within 24 hours of collection. Five clams ranging from 120 to 130 mm in length were analyzed each month for their glycogen content. The glycogen content for each month is a mean of three replicate determinations.

The strip-spawning potential was checked biweekly during the first 5 months of 1974, then checked at weekly intervals for the remainder of the year. The gametes were procured by methods described by Loosanoff and Davis (1963). During each test the gametes from 5 different females and 5 different males were pooled, and half of the gamete solution received 15 ml 0.1N NH_4OH treatment to dissolve the ovum germinal vescicle. Three hours after the addition of sperm to the egg suspension, the eggs were microscopically examined for evidence of cleavage. If cleavage did occur, the cultures were continued and examined 48 hours after fertilization.

RESULTS AND DISCUSSION

Histological Study

Histological examination of the 1974 female reproductive cycle revealed a single annual spawning period which began in August and continued through November with peak spawning activity occurring during the fall months of October and November (Fig. 1).

All five gonadal phases were present throughout the winter and spring months, January to May, with little change occurring in the proportion of the phases throughout this period.

Females contained at least some ripe alveoli nine months of the year, January through September, with ripeness peaking during the summer months of June and July, 52 and 54% respectively, and then declining to very few or no ripe alveoli during October through December.

Females contained alveoli in partially spawned and spent phases throughout the year. There was

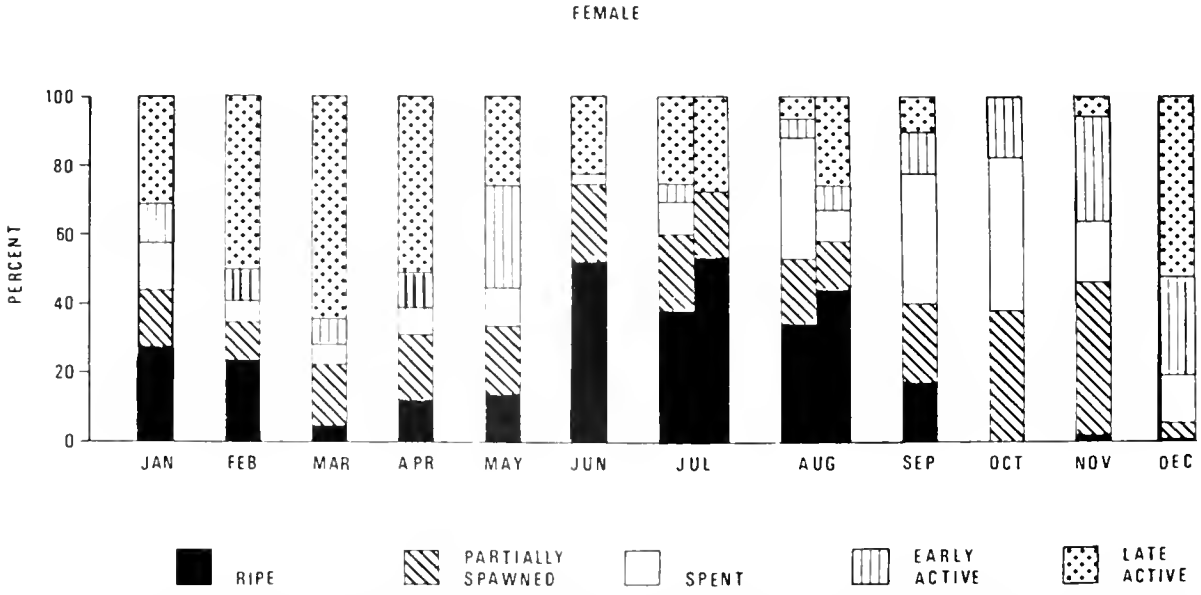

FIG. 1. *The female reproductive cycle of the sunray venus clam,* Macrocallista nimbosa *during 1974 from St. Joseph Bay, Florida. The length of each shaded area represents the percent frequency of alveoli in each reproductive phase.*

little change in the proportion of these two phases during the first seven months of the year. During this time alveoli in partially spawned and spent phases represented 25% of the total alveoli.

In August, there was a sharp increase to 54% in the proportion of alveoli in the partially spawned and spent phases. This proportion increased further in September to 61% and reached an annual maximum of 82% in October, remaining high at 62% in November. There was a sharp decline in spawning activities to the annual minimum of 18% alveoli in the partially spawned and spent phases by the first week of December.

Active phases, which include early active and late active, were present throughout the year. During the first five months of the year alveoli in the active phase represented a high percentage, averaging 58% of the total. During the summer and early fall this proportion was reduced, reaching a yearly minimum in August. The proportion of alveoli in active phases increased in the fall, reaching an annual maximum of 81% in early December.

Males likewise exhibited a single annual spawning period (Fig. 2). Some males contained ripe alveoli throughout the year, averaging 40% for the first five months, then sharply increasing to the annual maximum of 98% in June. Beginning in July and August the percentage of ripe alveoli steadily declined throughout the fall and reached the annual minimum of 3% in December.

There was little change in the proportion of alveoli in the partially spawned phase throughout the first five months of the year. Alveoli in the partially spawned phase were absent during June, but then reappeared and were present throughout the remaining months of the year with alveoli in the partially spawned and spent phases reaching maximum annual values of 68 and 86% during November and December, respectively.

Alveoli in the spent phase first appeared in May and June and were present for the remaining months of the year.

Except for the month of June, active phases were present throughout the annual cycle. There was little change in the proportion of active phases during the winter and spring months, as well as during September and October. The percentage of alveoli in active phases decreased as fall progressed and reached a low in December.

This histological study indicates that the sunray venus clam is a fall spawner, with females beginning spawning in August and continuing through November, with greatest spawning activity occurring during October and November. Males begin spawning activities earlier, starting in July

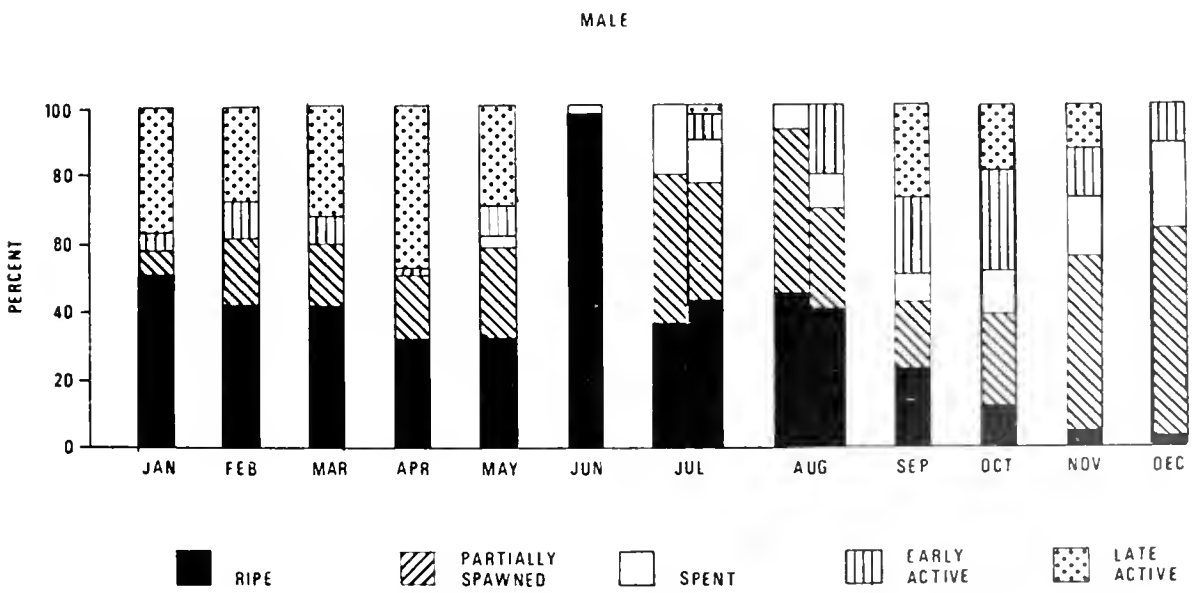

FIG. 2. *The male reproductive cycle of the sunray venus clam during 1974.*

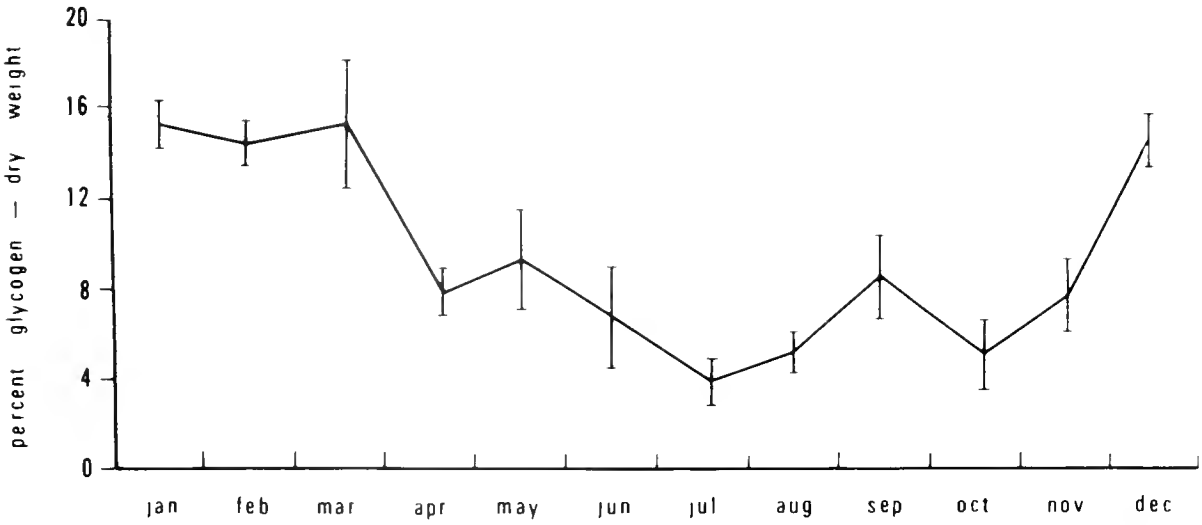

FIG. 3. *The seasonal variation in glycogen content (on a dry weight basis) for the sunray venus clam. The vertical bars indicate 95% confidence intervals.*

and continuing through early December, with greatest activity occurring from October to early December.

Glycogen Analysis

The greatest storage of glycogen in the tissues of the sunray clam occurred during the winter months of January through March (Fig. 3). The annual maximum value of 15.2% occurred in both January and March. A sharp decline in glycogen content during April continued throughout the months of May and June, reaching low values of 3.8 and 5.2% during the summer months of July and August respectively. There was a transient rise in glycogen content during September. Glycogen levels returned in October to a value of 5.1%, reminiscent of July and August. Glycogen

TABLE 1. *The annual strip spawning potential of* M. nimbosa. *The numbers in parentheses indicate the number of trials in which ova development was successful.*

Month	Number of Trials	Ammonium Hydroxide Treatment		No Treatment	
		Development To		Development To	
		Early Gastrula Or Less	Straight-hinge Stage	Early Gastrula Or Less	Straight-hinge Stage
Jan.	2	—	—	—	—
Feb.	2	—	—	—	—
Mar.	2	+(1)	—	—	—
Apr.	2	—	—	—	—
May	2	+(1)	—	—	—
Jun.	4	+(1)	—	—	—
Jul.	4	—	—	—	—
Aug.	4	—	—	—	—
Sep.	4	+(2)	—	—	—
Oct.	4	+(3)	+(3)	+(3)	+(3)
Nov.	4	+(4)	+(4)	+(4)	+(4)
Dec.	4	+(1)	—	—	—

content rose slightly in November and then sharply in December to 14.4%, a value comparable with the high winter values of January through March, thus demonstrating a definite annual glycogen cycle for *M. nimbosa* during 1974.

The seasonal changes in the glycogen content of *M. nimbosa* showed a definite cycle related to the gonadal development and spawning activities.

During the winter months, January through March, the glycogen values were at the annual high and little change occurred in the proportion of the five gonadal phases. In June, the histological study revealed a rapid proliferation of gametes and a corresponding low value of glycogen which decreased to an annual minimum in July at at time when spawning began in males. The correlation between low glycogen values and spawning continued throughout November. Glycogen content showed a small rise in September which was reflected in the histological study in that a corresponding decrease in alveoli in the spawned phases occurred. By December, spawning had ceased and an increase in glycogen approaching the winter values was observed in December.

Strip-Spawning Potential

The annual strip-spawning potential is represented in Table 1. Development of gametes to and beyond the straight-hinge stage occurred only in those experiments conducted during the last three weeks of October and all of November. During this period normal development occurred in both those egg suspensions treated and not treated with ammonium hydroxide. In strip-spawning experiments performed at other times of the year, eggs showed limited development only in those gamete suspensions receiving ammonium hydroxide treatment. In experiments conducted on March 14, May 3, and June 16, a few eggs developed only as far as the 8-cell stage. In experiments conducted September 13, 21, and December 3, several eggs developed to the early gastrula stage.

It is likely that the effect of the ammonium hydroxide is to break down the egg's germinal vesicle so that fertilization may occur (Loosanoff and Davis, 1963). The incomplete development of those eggs may indicate that the eggs were not mature even though sperm addition caused initiation of cleavage.

The results of this study indicate that strip-spawning is successful only during certain times of the year. Comparison of the strip-spawning and the histological study results indicate that strip spawning is not successful throughout the entire period that an animal appears to be in a histologically-ripe or partially-spawned phase, but is confined to a narrower period than one would infer from a histological or glycogen study. The results of the study of strip-spawning potential provides further evidence that *M. nimbosa* in northwest Florida are fall spawners.

ACKNOWLEDGEMENTS

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GROWTH AND MORTALITY RATES OF HATCHERY SEED CLAMS, *MERCENARIA MERCENARIA*, IN PROTECTED TRAYS IN WATERS OF SOUTH CAROLINA

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ABSTRACT

Seed hard clams, Mercenaria mercenaria, *were planted in trays at densities of 290, 580, and 869/m² in three widely separated intertidal areas in South Carolina. Survival of clams was similar at each site although clams at Clark Sound experienced a lower survival rate. Growth of clams planted at Clark Sound and Albergottie Creek was significantly higher than those at Bull Bay. Growth occurred throughout the year with the best growth experienced in summer and fall.*

INTRODUCTION

The hard clam, *Mercenaria mercenaria* (Linne), is present in many estuaries of South Carolina, but has never supported a large commercial fishery because of poor market conditions and a lack of knowledge concerning the extent of the clam resource. However, the potential for hard clams in South Carolina appears promising and the Division of Marine Resources of the South Carolina Wildlife and Marine Resources Department has undertaken several activities designed to increase clam production and improve clam management practices in South Carolina (Gracy, 1974). This report describes one project on growth and survival of hatchery seed clams held in protected trays in the intertidal zone.

Investigators have reported on growth of *M. mercenaria* in Virginia, North Carolina, Georgia, and Florida (Haven and Andrews, 1957; Loesch and Haven, 1973; Chestnut, Fahy and Porter, 1957; Godwin, 1968; Menzel, 1963; Menzel and Sims, 1964), but no published work exists for South Carolina. Moreover, most experiments cited used clams reared in Milford, Connecticut, and growth patterns observed may not represent those of native clams. The present experiment was conducted (1) to determine relative growth patterns and survival rates of a southern stock of *M. mercenaria* planted in South Carolina; (2) to explore the effect of density upon growth and survival; and (3) to serve as preliminary project for future studies. The value of the last objective quickly became apparent because it proved to be quite difficult to find suitable sites for experiments of this type. This was due to intertidal zones that were quite steep and limited in area, or exposed to strong wave action, currents or both. Also, crabs

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proved to be more formidable predators than expected.

MATERIALS AND METHODS

Hatchery seed obtained from Coastal Zone Resources of North Carolina was chosen because a regular supply existed. Also, clams from North Carolina probably are well adapted to the range of environmental conditions that occur in estuaries of South Carolina.

Seed was planted in oyster trays (119 x 58 x 14 cm) to protect it from predation by the blue crab, *Callinectes sapidus* (Rathbun); the stone crab, *Menippe mercenaria* (Say); and other crabs, particularly of the family Xanthidae.

The metal framed trays were lined with one-quarter inch (about 6 mm) galvanized hardware cloth, and fiberglass insect screens were placed in the bottom to retain substrate. The trays were then filled with substrate (sand or sand with shell) to a depth of approximately 10 cm. Clams were planted in the substrate. The tray was then covered by a one-quarter inch galvanized hardware cloth and wired shut. This arrangement reduced predation by crabs, but was not entirely successful.

Clams were placed intertidally in 3 widely separated areas of South Carolina: viz., Clark Sound approximately 10 km south of Charleston (Lat. 32° 43′ 00″ N Long. 79° 56′ 32″ W); in Albergottie Creek in Beaufort county about 110 km south of Charleston (Lat. 32° 26′ 56″ N Long. 80° 43′ 12″ W); and in Bull Bay (Lat. 32° 55′ 48″ N Long. 79° 35′ 09″ W), which is approximately 30 km north of Charleston (Fig. 1). Clams were planted at 3 densities (2 replicates each): 200 (290/m²), 400 (580/m²), and 600 (869/m²) per tray. Clams that died were not replaced. Initial size of clams was determined by measuring a sample of 400 clams. All clams were counted in each tray every 3 months and a sample of 100 clams from each density was measured to the nearest 0.1 mm to determine total anterior-posterior length. In March, 1974, sixty clams were measured at Bull Bay for each density. After clams were counted and measured, new substrate was added to the trays and the clams were placed in the new substrate.

Clams were out of the water approximately 2 or 3 hours in a 12-hour tidal cycle at the Albergottie Creek and Clark Sound sites, whereas, at Bull Bay, clams were out of water about 4 or 5 hours. Clams were planted September 28, 1973; October 25, 1973; and December 20, 1973 at Clark Sound,

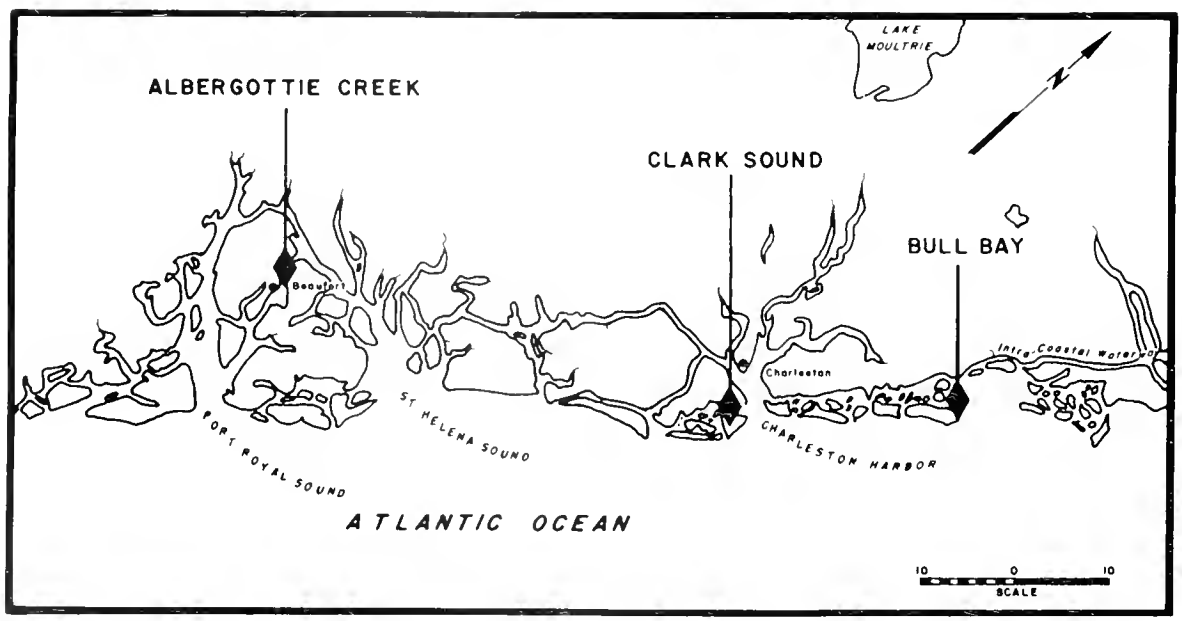

FIG. 1. *Location of sites where seed clams,* Mercenaria mercenaria, *were planted in South Carolina.*

TABLE 1. *Particle size analysis. Percent weight was calculated from the weighted means of five samples taken from each location (Means weighted by weight of samples).*

Location	*Shell* (>.063 mm) % weight	*Sand* (>.063 mm) % weight	*Silt* (.063-.004 mm) % weight	*Clay* (<.004 mm) % weight
Clark Sound	2.2	86.8	6.4	4.6
Bull Bay	15.4	78.0	4.0	2.7
Albergottie Creek	0.7	75.5	13.9	9.9

TABLE 2. *Survival of hard clam seed,* Mercenaria mercenaria, *planted in protected trays in estuaries of South Carolina (Trays of similar density combined).*

Number per square meter	Original number in trays 1973	Survivors March 1974	Survivors June 1974	Survivors September 1974	Survivors December 1974	Survivors March 1975	Survivors June 1975
Bull Bay							
290	400	306	277	250	204	201	196
580	800	589	415	389	358	354	347
869	1200	975	863	740	711	697	689
Total	2400	1870	1555	1379	1273	1252	1232
Total survival rate		.779	.648	.575	.530	.522	.513
Interval survival rate		.779	.832	.887	.923	.984	.984
Clark Sound							
290	400	294	251	233	230	230	224
580	800	445	349	271	265	264	208
869	1200	881	795	676	660	657	650
Total	2400	1620	1395	1180	1155	1149	1082
Total survival rate		.675	.581	.492	.481	.479	.451
Interval survival rate		.675	.861	.846	.979	.995	.942
Albergottie Creek							
290	400	313	202	194	151	145	133
580	800	718	640	598	533	524	492
869	1200	1003	836	809	597	587	570
Total	2400	2034	1678	1601	1281	1256	1195
Total survival rate		.848	.699	.667	.534	.523	.498
Interval survival rate		.848	.825	.954	.800	.981	.951

Bull Bay, and Albergottie Creek, respectively.[1]

Five sediment samples were taken from each experimental site. Samples were sorted into sand, silt, and clay fractions (Wentworth Scale) by using U.S.A. standard testing sieve No. 230 and the pipette method described by Krumbein and Pettijohn (1938). Shell weight is the weight difference in the sand fraction after all carbonate has been disolved by 4 molar hydochloric acid. Weighted means of shell, sand, silt, and clay fractions were calculated for each site in order to obtain an estimate of the average sediment compostition of substrate used in trays.

Statistical analyses were conducted utilizing the Statistical Analysis System (SAS).

[1] Clams planted at Bull Bay and Clark Sound were measured initially; 4 and 5 months after planting, respectively; and at 3 month intervals thereafter.

RESULTS AND DISCUSSION

Sediment Analysis

Results of sediment analysis are presented in Table 1. Results were plotted on the sand-silt-clay triangle diagram proposed by Shepard (1954). Substrates at all sites can be characterized as sand. Bull Bay had the greatest fraction of shell and Albergottie Creek had the largest fraction of silt.

Survival of Clams

Table 2 gives the number of survivors (trays of similar density combined) for the 3 experimental sites. Survival was highest at Bull Bay which had the greatest fraction of shell in the substrate. Castagna (1970) reported that the use of shell ag-

TABLE 3. *Results of two-way analysis of variance test on survival rates.*

Source of Variation	d.f.	F value	Probability > F	Transformed Means	
Location	2	3.21	0.0488	Bull Bay	0.669
Density	2	2.56	0.0871	Albergottie Creek	0.634
Block	5	11.26	0.0001	Clark Sound	0.564
Error	44				

TABLE 4. *Growth of hard clam seed,* Mercenaria mercenaria, *planted in protected trays in estuaries of South Carolina by location and density.*

Number Per Square meter	Original Mean size	Mean size in mm March 1974	Mean size in mm June 1974	Mean size in mm September 1974	Mean size in mm December 1974	Mean size in mm March 1975	Mean size in mm June 1975
Bull Bay							
290	12.30	13.91	16.45	19.56	23.44	24.56	30.38
580	12.30	13.78	15.73	19.62	22.44	24.77	29.90
869	12.30	14.77	16.05	18.53	21.30	24.80	28.33
Weighted mean size	12.30	14.08	16.04	19.02	21.96	24.75	29.10
Absolute increase in mm		1.78	1.96	2.98	2.94	2.79	4.35
Interval percent increase		14.47	13.92	18.58	15.46	12.70	17.58
Total percent increase		14.47	30.41	54.63	78.54	101.22	136.59
Clark Sound							
290	13.60	17.29	24.78	33.51	38.47	41.81	46.34
580	13.60	17.35	23.71	33.96	38.47	43.37	45.43
869	13.60	18.39	23.44	33.37	37.85	39.49	44.05
Weighted mean size	13.60	17.90	23.75	33.53	38.12	40.91	44.79
Absolute increase in mm		4.30	5.85	9.78	4.59	2.79	3.88
Interval percent increase		31.62	32.68	41.18	13.69	7.32	9.48
Total percent increase		31.62	74.63	146.54	180.29	200.81	229.34
Albergottie Creek							
290	12.40	16.07	24.65	31.96	35.50	41.36	44.17
580	12.40	16.17	25.59	33.31	36.77	41.66	44.25
869	12.40	15.78	24.98	33.16	36.29	40.84	43.82
Weighted mean size	12.40	15.96	25.17	33.07	36.40	41.24	44.04
Absolute increase in mm		3.56	9.21	7.90	3.33	4.84	2.80
Interval percent increase		28.71	57.71	31.39	10.07	13.30	6.79
Total percent increase	28.71	28.71	102.98	166.69	193.55	232.58	255.16

gregates reduced predation on seed hard clams. As expected, survival of clams increased with increased size.

A two-way Analysis of Variance (ANOVA) was conducted to determine differences in survival rates. Survival rates for each time period (Block) were calculated for each location (Treatment A) and density (Treatment B). The variance was normalized by an arcsin transformation (Table 3).

Differences in survival rates among blocks were highly significant ($P<0.01$), differences among locations were significant at the 5% level ($P=0.048$), and there were no significant differences between planting densities.

Duncan's multiple range test was employed on the transformed data to determine differences in survival among locations. The only significant difference was that between Bull Bay and Clark Sound.

An examination of the Clark Sound data revealed that one tray had a damaged cover in September, 1974. It is hypothesized that high mortality produced by crabs entering the exposed tray at this time caused the lower survival rate observed at Clark Sound. In essence, predation by crabs appeared to cause most of the mortality observed and the authors do not believe that the crab predation rate varied significantly among locations.

Survival of clams in this experiment was less than that reported by others (Haven and Andrews, 1957; Chestnut, 1952; Menzel and Sims, 1964; Carriker, 1956; Gustafson, 1955). This difference appeared to be due (1) to the smaller size of clams utilized and (2) to failure of the trays to fully protect clams from predation by crabs. Predation by crabs was indicated by presence of shell fragments within trays, particularly during the earlier part of the experiment.

Table 2 shows that survival of clams at all sites was relatively high after June, 1974. The weighted average size of clams at that time varied between 16.0 mm and 25.2 mm (Table 4).

Survival between December, 1974, and March, 1975, was the highest observed in the experiment. This may have been due to the reduced level of activity of crabs during the cooler months.

Growth of Clams

Mean growth rates of clams at different densities at the same site did not appear to vary appreciably (Fig. 2). However, clams planted in Clark Sound and Albergottie Creek grew nearly twice as rapidly as clams at Bull Bay. The following procedures were employed to determine if significant differences existed. Since a visual examination of Figure 2 indicated that growth in shell length had been linear from March, 1974, to June, 1975, linear regressions (shell length vs. time) for each tray were computed to determine slopes (growth rate). The slope for the growth rate for each tray was found to be significantly dif-

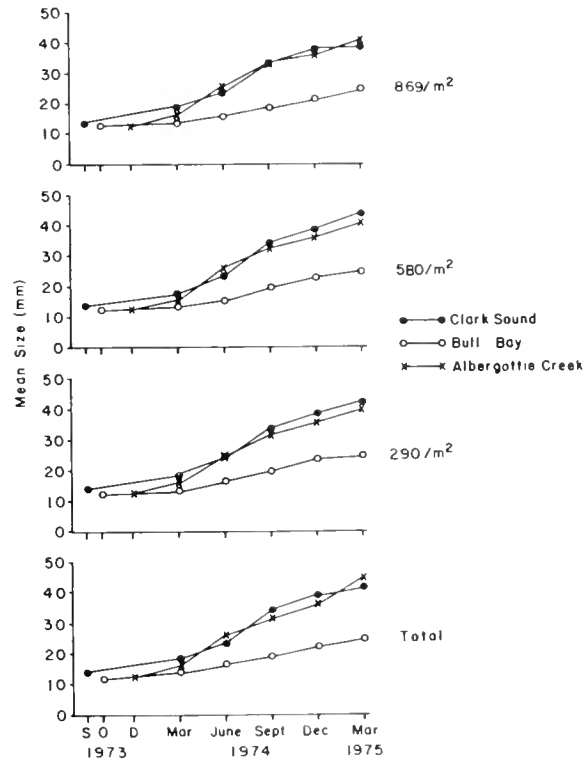

FIG. 2. *Growth of seed hard clams,* Mercenaria mercenaria, *planted in protected trays in three locations in South Carolina by planting density.*

ferent from zero and the average correlation coefficient (R^2) for all trays was 0.77. Average slopes are given in Table 5. A two-way analysis of variance on the slopes for each tray was conducted to determine if differences in growth existed. The results are given in Table 5. A difference in growth clearly existed among locations, but not among densities or the density-location (D x L) interaction. As suggested in Figure 2 Clams at Bull Bay grew significantly less than those at either

Clark Sound or Albergottie Creek. Although growth did not vary significantly among densities, the average rate of growth for each tray did show a downward trend with increased density. The average monthly growth rate for Bull Bay, Clark Sound and Albergottie Creek was 0.8 mm, 1.5 mm, and 1.8 mm, respectively. The most obvious difference in sites was the position of trays in the intertidal zone. Clams at Bull Bay may have grown slower because they were exposed longer than at other locations. Added exposure meant that clams had less time to feed and were exposed to more extreme changes in temperatures than clams at other sites.

Linear increments in growth appeared to be inversely proportional to initial length as reported by Belding (1912) and Pratt and Campbell (1956).

The highest growth rate for any interval occurred between June and September, 1974, at Bull Bay and Clark Sound and from March to June, 1974, at Albergottie Creek (Table 4). The lowest growth rate for any interval occurred between December, 1974, and March, 1975, at Bull Bay and Clark Sound and between March and June, 1975, at Albergottie Creek.

Clams at Clark Sound and Albergottie Creek grew rapidly between planting in the fall of 1973 and September, 1974, and attained average sizes of 33.5 mm and 33.1 mm, respectively. Linear growth between September, 1974, and June, 1975, was reduced at these sites. Conversely, clams at Bull Bay, which had an average size of 19.0 mm in September, continued to experience about the same rate of growth. The variation in growth between sites suggests that the initial size of clams has an important effect upon linear growth. This effect may mask a seasonal effect, particularly when a southern area experiences relatively mild winters as did South Carolina in the past two years.

An examination of daily water temperatures collected at the Marine Resources Center located near the mouth of Charleston Harbor revealed that water temperature never dropped below 10C during the past two winters. The mildness of the past two winters together with the size of clams at planting appears to explain why clams grew throughout the year.

This experiment also suggested that clam growth should not be compared from area to area unless they are of equivalent size or size range (Loesch and Haven, 1973), planted at the same time of year, and adapted to local water temperatures. For instance, Menzel (1963, 1964)

TABLE 5. *Regression coefficients of hard clam,* Mercenaria mercenaria, *seed, planted in protected trays in estuaries of South Carolina by location and density.*

	Density in clams			
Location	290/m²	580/m²	869/m²	Average Slope
Clark Sound	5.66	6.28	5.59	5.34
	5.49	4.91	4.10	
Bull Bay	3.49	3.80	2.71	3.20
	3.11	3.02	3.06	
Albergottie Creek	5.26	4.84	4.86	5.18
	5.66	5.32	5.16	
Average Slope	4.78	4.70	4.25	

Results of Analysis of Variance on Regression Coefficients of Trays

Source of Variation	*d.f*	*Sum of Squares*	*Mean Square*	*F*
Total	17	21.12		
Density	2	0.98	0.49	1.63
Location	2	17.10	8.55	28.50**
D × L	4	0.30	0.07	0.23
Error	9	2.74	0.30	

reported that seed hard clams *Mercenaria mercenaria*, experienced their best growth in spring and fall with little growth observed in the summer months. In that series of experiments clams were reared at Milford, Connecticut, and then planted in Alligator Harbor, Florida. Because Connecticut clams are adapted to colder temperatures, it is not surprising to witness their slower growth during summer in Florida. In contrast, clams reared in North Carolina and planted in South Carolina grew rapidly during the summer, with clams in Clark Sound and Bull Bay experiencing their greatest incremental growth rate between June and September, 1974.

Growth occurred throughout the year, but was somewhat reduced during the colder months. Similar results were reported for clams reared in North Carolina and planted in Georgia (Godwin, 1968).

Effect of Density Upon Growth

Densities at the end of the experiment due to natural mortality were generally less than or equal to 325/m^2. However, two trays had clam densities of approximately 650/m^2. Density did not affect the growth rate in this experiment. Similar results were reported by Godwin (1968) and Gustafson (1955). Although growth of clams less than 45 mm in size was not affected by densities as great as 650/m^2, one can not assume that this is true for larger clams.

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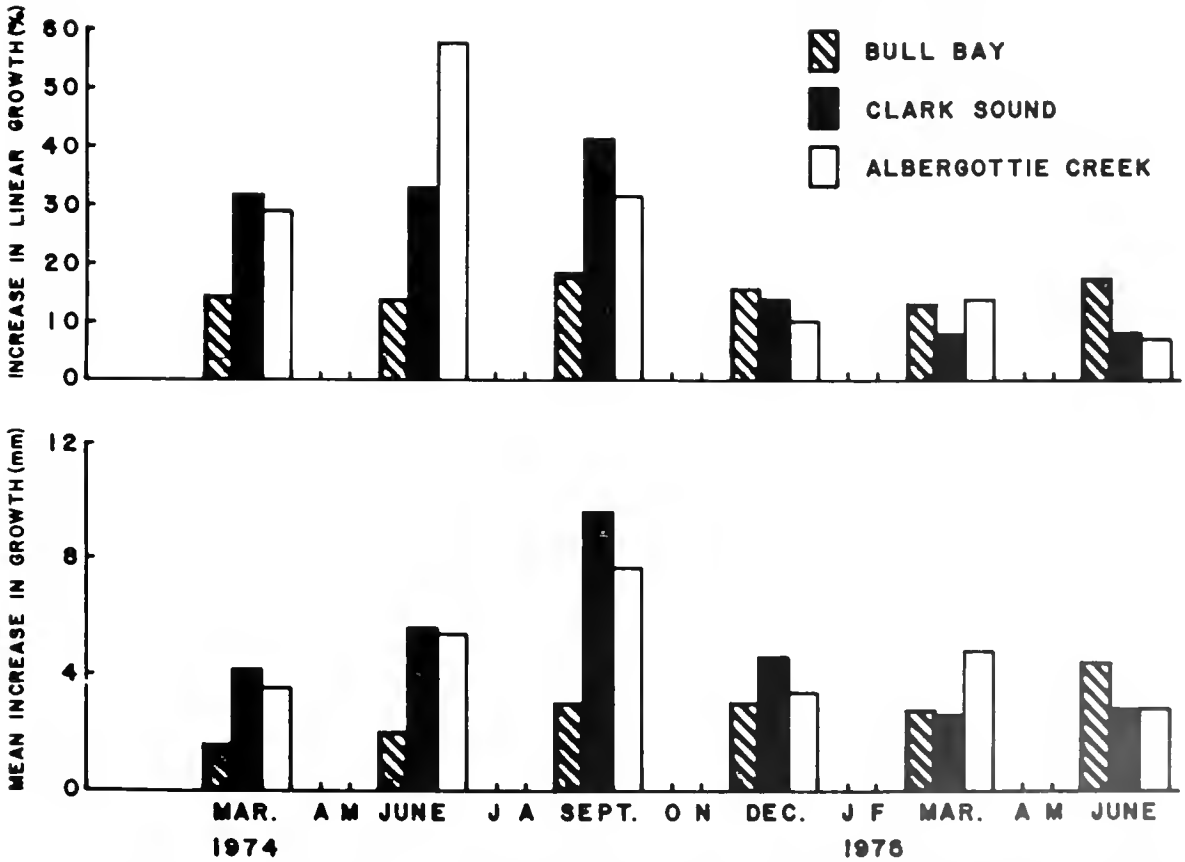

FIG. 3. *Mean absolute increase in mm and percent increase in linear growth by three month periods for seed clams,*Mercenaria mercenaria, *planted intertidally in protected trays at three locations in South Carolina, all trays combined.*

in computer analysis of data, and Mrs. Lourene Rigsbee for typing the manuscript. Thanks are also due to Mssrs. Victor Burrell, Paul Sandifer, and Raymond Rhodes, who reviewed the manuscript and made helpful suggestions.

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GROWTH AND SIZE-WEIGHT RELATIONSHIPS OF THE PINKNECK CLAM *SPISULA POLYNYMA*, IN HARTNEY BAY, PRINCE WILLIAM SOUND, ALASKA[1],[2]

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ABSTRACT

Pinkneck clams, Spisula polynyma, *from Hartney Bay, Prince William Sound, Alaska, were examined. Three samples, a total of 298 specimens, were used to determine the growth history of 16 year classes by the annular method. Length-weight relationships are considered. Dry meat weight (solids) averaged 20.2%.*

INTRODUCTION

The pinkneck or redneck clam, *Spisula polynyma*,[3] is a large bivalve found in intertidal and subtidal Alaskan waters (Chamberlin and Stearns, 1963). It has been reported from Point Barrow to the Strait of Juan de Fuca, and generally occurs in medium grade sediments (Chamberlin and Stearns, 1963). Intertidally it is often found in association with razor (*Siliqua patula*) and butter (*Saxidomus gigantea*) clams (Feder and Paul, unpublished). The extent of this resource in Alaska is unknown; however, the authors have observed populations with commercial potential in the Cordova region of Prince William Sound, Alaska. Kessler and Hitz (1970) reported good subtidal catches in Icy Straits, Southeastern Alaska. On the Atlantic coast of the United States the closely related surf clam, *Spisula solidissima*, is harvested subtidally. The meats are canned and made into chowder or specialty products (Yancey and Welch, 1968). There are a number of papers dealing with the basic biology and fishery potential of surf clams along the Atlantic coast of the United States (see Yancy and Welch, 1968 for bibliography), but with the exception of a geographic study of *S. polynyma* that contains Alaskan distributional information (Chamberlin and Stearns, 1963) no published work on the pinkneck clam from the Pacific coast of North America is available. The purpose of this investigation was to examine growth, size-weight relationships and commercial potential of *S. polynyma* from Prince William Sound, Alaska.

METHODS

Specimens of *Spisula polynyma* were collected intertidally at low tide by digging on sandflats in Hartney Bay, Prince William Sound (Fig. 1). Collections were made February 17, 1973, May 19, 1973, and July 21, 1974. Age was determined for the clams by counting annuli, a series of closely-spaced concentric growth lines which are the result of slow winter shell growth, (Paul and Feder, 1973; Weymouth *et al.*, 1931). The growth history of the specimens was determined by measuring the shell length at each annulus after removing the periostracum with a 10% acid solu-

1 Contribution No. 263, Institute of Marine Science, University of Alaska.

2 This project was conducted with funds provided by the University of Alaska's Sea Grant program (Grant No. 04-3-158-41), NOAA Office of Sea Grant, Department of Commerce.

3 *Spisula alaskana* is a synonomy. (Abbott, 1974)

tion. Shells with badly abraded surfaces were discarded (2% of the 305 clams collected).

The size-weight relationships were examined. The adductor muscles of all specimens collected were severed and the free water in the mantle cavity allowed to drain. The clams were then shucked, the shells weighed and the differences between the whole-live-weight (drained) and the shell-weight recorded as wet-meat-weight. Individual meats were dried to a constant weight at 80°C for dry-weight determinations.

Weights were obtained with a Mettler balance Type P 120. Plots, regression lines, and regression equations were determined and plotted by an IBM 360 Computer. The Gauss-Jordan method was used in the solution of all normal equations (Cooley and Lohnes, 1962; Ostle, 1954).

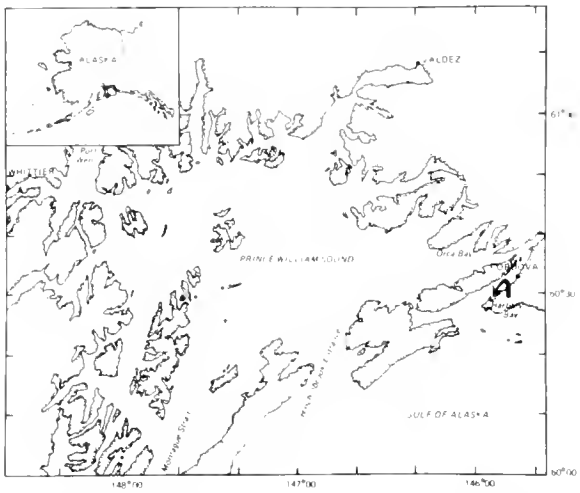

FIG. 1. *Map of Prince William Sound, Alaska; location of the Hartney Bay sandflat sampled for* Spisula polynyma.

RESULTS

Growth

A growth curve for *Spisula polynyma* from Hartney Bay is presented in Figure 2 (also see Table 1). The oldest and largest individual encountered was 16 years old and had a shell length of 151 mm.

Size-Weight Relationships

The equations describing the relationship between length and total weight (drained), length and wet-meat and dry-meat-weight are presented in Table 2. The former two relationships are plotted in Figure 3. Dry-meat weight (solids) was found to average 18.4, 21.9 and 20.3% for the February, May and July collections respectively (Table 2). Figure 4 shows the relationship between dry- weight and shell-length. The average shell

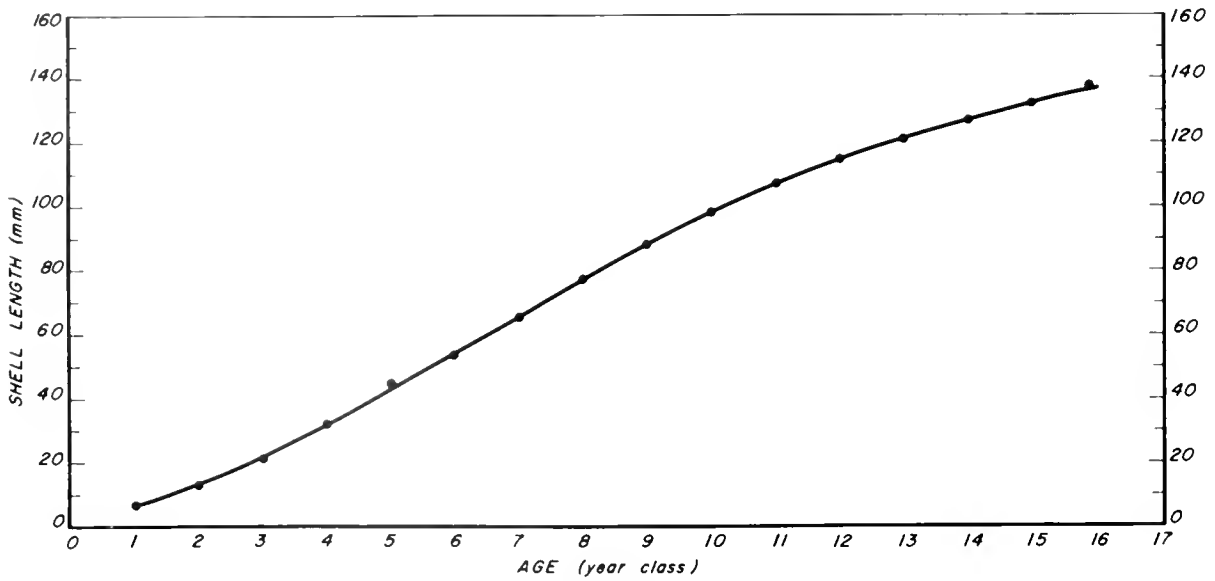

FIG. 2. *The relationship between shell length (mm) and age of* Spisula polynyma *from a sandflat in Hartney Bay, Prince William Sound, Alaska.*

TABLE 1. *Mean shell length ($\bar{L}$ = mm) at each annulus for* Spisula polynyma *from Hartney Bay, Prince William Sound, Alaska. N = the number of annuli measured; S.D. = standard deviation.*

Annulus Number	17 February 1973			19 May 1973			21 July 1974		
	L	N	S.D.	L	N	S.D.	L	N	S.D.
1	7	40	1.0	8	101	1.6	8	157	1.3
2	13	40	2.2	13	101	2.0	14	148	2.6
3	21	40	2.0	20	101	2.5	24	148	5.3
4	31	40	3.1	29	101	3.6	36	144	5.3
5	41	40	3.6	39	101	4.1	49	142	6.5
6	52	40	5.1	49	101	4.3	69	142	7.7
7	63	40	6.0	60	101	4.6	74	142	8.2
8	74	40	6.4	72	99	4.7	85	140	8.5
9	85	40	6.8	83	97	5.1	96	131	8.2
10	94	39	6.8	94	93	4.8	107	119	8.2
11	103	38	7.1	104	87	5.0	116	100	7.8
12	111	34	6.1	112	83	5.0	123	69	7.8
13	118	28	5.6	119	74	4.2	128	38	8.4
14	123	21	5.4	124	55	4.1	133	20	9.1
15	127	8	6.1	131	23	4.6	140	3	9.2
16	136	2	—	142	7	6.1	—	0	—

TABLE 2. *Equations for size-weight relationships of* Spisula polynyma *from Hartney Bay, Prince William Sound. Equations derived from curves in Figures 2, 3 and 4. SD = standard deviation; N = number of individuals.*

Size-weight Relationship	February 17/73 (n = 40)	May 19/73 (n = 101)	July 21/74† (n = 142)	All 3 Collections (n = 283)
Total weight	$\left(\frac{\text{Length}}{26.9}\right)^{3.6}$	$\left(\frac{\text{Length}}{25.9}\right)^{3.5}$	$\left(\frac{\text{Length}}{27.9}\right)^{3.7}$	$\left(\frac{\text{Length}}{27.0}\right)^{3.6}$††
Wet-meat weight	$\left(\frac{\text{Length}}{50.4}\right)^{5.4}$	$\left(\frac{\text{Length}}{28.4}\right)^{3.3}$	$\left(\frac{\text{Length}}{31.3}\right)^{3.5}$	$\left(\frac{\text{Length}}{33.6}\right)^{3.7}$††
Dry-meat weight	$\left(\frac{\text{Length}}{59.0}\right)^{4.2}$	$\left(\frac{\text{Length}}{44.7}\right)^{3.4}$	$\left(\frac{\text{Length}}{44.7}\right)^{3.4}$	$\left(\frac{\text{Length}}{50.5}\right)^{3.7}$†††
Percent solids*	18.4%	21.9%	20.3%	20.2%
S.D.	2.1%	1.5%	2.8%	—
Meat wet-meat weight	103.4gr	116.9gr	125.1gr	—
Mean shell length	118mm	116mm	137mm	—

*$\frac{\text{Dry-Meat Weight}}{\text{Wet-Meat Weight}} \times 100$

† 15 one-year-old clams were not included in the size-weight relationships tabulated for this month

†† See Figure 3

††† See Figure 4

length and mean wet-meat weights for each collection was 118 mm, 103.4 gm; 116 mm, 116.9 gm; ; and 137 mm, 125.1 gm respectively (Table 2).

DISCUSSION

The annular method of aging is reliable for most Prince William Sound clams because of a strong seasonality of growth (R. Baxter in Haven, 1971). Intertidal beaches in Prince William Sound are subject to freezing during low tides in January and February, and under such conditions *Spisula polynyma* forms a distinct winter annulus (also see Paul and Feder, 1973; Feder and Paul, 1974a; Weymouth *et al.*, 1931 for discussions on annulus formation in the clams *Protothaca staminea*, *Mya arenaria* and *Siliqua patula* in Prince William Sound).

Chamberlin and Stearns (1963) report *S. polynyma* to be a long-lived slow-growing clam. They provide two approximations of size and age: 50 mm at 6 years of age; 100 mm at 14 or more years of age. However, they do not indicate where these specimens were collected or how many individuals were examined. Their first value is similar to that found in our study for 6-year old clams; Hartney Bay clams average approximately 120 mm at 14 years of age. *Spisula solidissima* reaches 100 mm in 5 years off central New Jersey and in 8 years off Massachusetts (Yancey and Welch, 1968).

Spisula polynyma is a large clam with individual meats weighing up to 250 grams (0.6 pounds). The 18.4, 21.9 and 20.3% (mean = 20.2%) solids determined in the three collections are close to the 21.4% reported for *S. solidissima* by Ropes (1970).

The commercial demand for hard-shell clams along the Pacific coast of the United States is excellent, and production does not meet demand (Glude, 1974). Significant quantities of hard-shell clams are imported from British Columbia, Canada (Glude, 1974). Currently there is little commercial harvesting of clams in Alaska; however, the state has a potential multimillion dollar clam industry based primarily on razor clams (*Siliqua patula*) (Feder and Paul, 1974b; Orth *et al.*: in press, Rearden, 1974). No abundance estimations are available for Alaskan pinkneck clams; therefore, it is not possible to estimate the value of this resource. However, *Spisula polynyma* is found in association with razor and butter clams and could be simultaneously harvested along with them, thereby further increasing the potential clam harvest in Alaska. Proper management would be required for the pinkneck because of slow growth.

ACKNOWLEDGEMENTS

We thank Merle Hanson for locating the pinkneck clam beds in Hartney Bay and for his

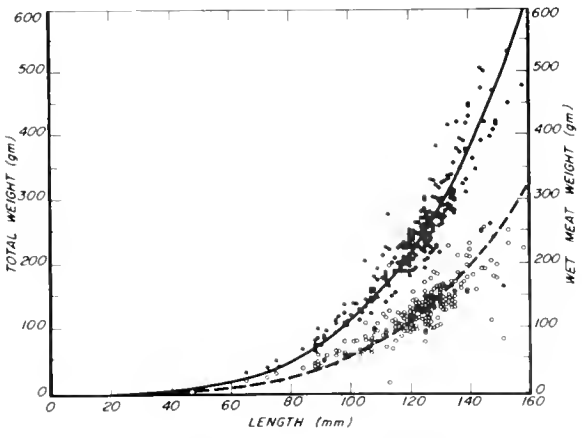

FIG. 3. *The relationship of clam length to total and wet-meat-weight for* Spisula polynyma *collected from a sandflat in Hartney Bay, Prince William Sound, Alaska.*

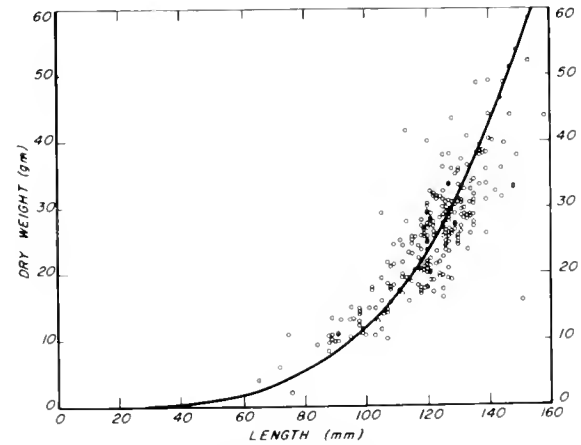

FIG. 4. *The relationship of clam length to dry weight for specimens of* Spisula polynyma *collected on a sandflat in Hartney Bay, Prince William Sound, Alaska.*

general assistance in collection activities. We also asknowledge Tim and Susan Feder for field assistance; George J. Mueller for taxonomic assistance; Rosemary Hobson for computer programming aid and Helen Stockholm for editing.

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AGE, GROWTH, AND RECRUITMENT OF THE BUTTER CLAM, *SAXIDOMUS GIGANTEA*, ON PORPOISE ISLAND, SOUTHEAST ALASKA[1]

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ABSTRACT

Butter clams, Saxidomus gigantea, *from Porpoise Island, southeast Alaska, were examined. Growth was determined for 1,069 specimens by the annular method. On Porpoise Island, butter clams reach a harvestable size of 65 mm in eight to nine years. Recruitment in nine 1 m² sample areas was examined. The number of individuals annually recruited in the population was variable.*

INTRODUCTION

Saxidomus gigantea (Deshayes, 1839), the butter clam, is one of the most common clams in Alaska (Abbott, 1974), and formerly supported an important industry in southeast Alaska. This industry began in 1930 with an initial catch of 25,000 pounds and continued until 1942 with no appreciable expansion. Wartime demand gave the industry impetus to increase production and by 1946 five southeastern Alaskan canneries were producing a pack valued at $170,000 (Orth *et al.*, in press). The clam fishery was of special importance to resident Alaskans because it was a winter operation offering employment and income during an otherwise slack season (Orth *et al.*, in press). However, the presence of a toxin (Paralytic Shellfish Poison) in the canned product led to the decline and ultimate collapse of the butter clam industry in southeast Alaska.

Currently there are many beaches in southeastern Alaska where clams are relatively free of Paralytic Shellfish Poison. The development of a rapid chemical assay for detecting the toxin should aid in the identification of these areas, and enhance the probability for the successful development of a new clam industry in Alaska (R. Neve, Institute of Marine Science, Univ. of Alaska, Pers. Comm.)

Information on growth of butter clams exists for British Columbia, Canada (Fraser and Smith, 1928; Quayle and Bourne, 1972), but no growth data is available from Alaska waters. The purpose of this investigation was to compliment the existing data base by examining age and growth relations of the butter clam in the Juneau region of southeastern Alaska. The material collected also provided information on recruitment.

METHODS

Collections of *Saxidomus gigantea* were made on 23 and 24 May 1975, on Porpoise Island, a small island in the northern portion of southeastern Alaska (Latitude 58°19.'7, Longitude 135°27.'3) about 40 air miles from the city of Juneau.

Nine sample areas, each 1 m², were established on the beach between the tidal heights of −0.3 m and −1.0 m, since intertidal butter clams are generally encountered between these tidal heights.

[1] Contribution No. 266, Institute of Marine Science, University of Alaska.

Within each sample area, the sediment was removed to a depth of 5 cm. This sediment was washed through a series of screens, the smallest mesh being 1.5 x 1.5 mm, and examined for young butter clams. Larger clams were collected by continued digging to a depth of 30 cm within each sample area. Few small individuals were encountered in the quantitative plots, so additional specimens were collected by random digging.

All shells were examined under a 2x lens and shells with badly abraded surfaces were discarded (11% of the clams randomly collected). Age was determined for the 1069 remaining clams by counting annuli, a series of closely spaced concentric growth lines which are the result of slow winter shell growth. The measurements taken on all clams was the greatest length at the last annulus.

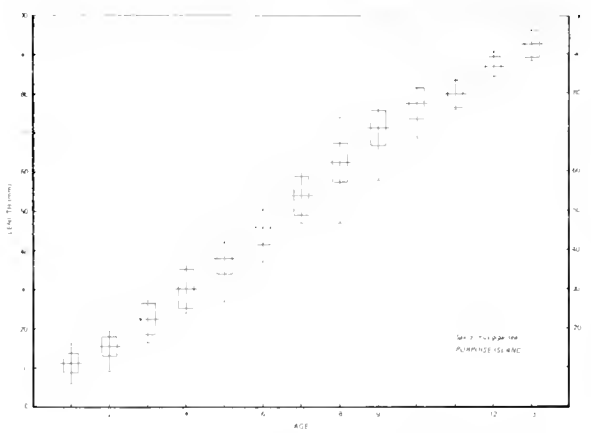

FIG. 1. *The relationship between shell length (mm) and age of* Saxidomus gigantea, *on Porpoise Island, Southeast Alaska. Mean length is denoted by the horizontal line, standard deviation by the box and range by the vertical line.*

RESULTS

Butter clams reach a size of 64 mm in eight to nine years on Porpoise Island (Table 1; Fig. 1). The oldest clam examined was 15 years old and 100 mm long (Table 1). The mean shell lengths for the various age classes are included in Table 1. A growth curve for *Saxidomus gigantea* from Porpoise Island is presented in Figure 1.

The validity of the annular aging method was examined with a standard one-way analysis of variance, utilizing the individual shell lengths within each age class as a basis for comparison (Snedecor, 1956). The calculated F ratio indicated that age classes, as defined by shell lengths, are statistically distinguishable ($P=0.01$). However, aging individuals older than 11 or 12 years becomes progressively more difficult.

The number of *S. gigantea* from each year class was found to be variable in the quantitative plots (Fig. 2).

DISCUSSION

The time needed for *Saxidomus gigantea* to grow to a harvestable size on Porpoise Island is slightly longer than that reported for British Columbia. In the latter area, butter clams reach a length of 65 mm in five to six years, and are harvested at this size (Quayle and Bourne, 1972).

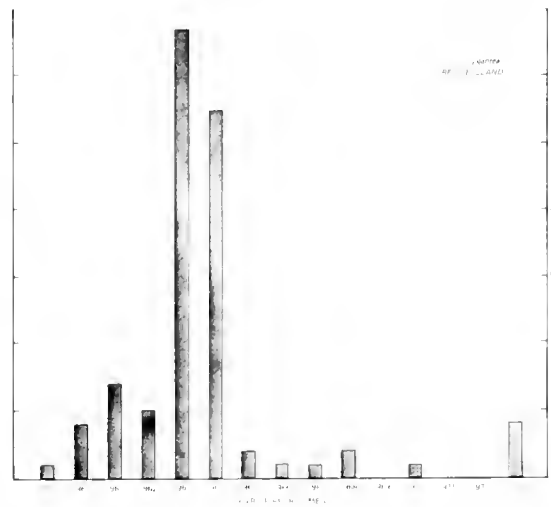

FIG 2. *The total number of* Saxidomus gigantea *by year of recruitment from nine 1.0m² plots on Porpoise Island, Southeast Alaska.*

Quayle and Bourne (1972) report that populations of butter clams in British Columbia fail to spawn in some years with the resultant irregular seedings responsible for fluctuations in adult populations. This also appears to be true for Alaska (Feder and Paul, unpublished). Inhibited spawning is probably related to low water temperature (Amos, 1966).

Currently, British Columbia is the largest source of butter clams and much of this production is sold in the United States (Glude, 1974). The

primary reason for the importation of Canadian butter clam (Glude, 1974) is that current clam production in the United States Pacific Northwest does not meet the demand (Glude, 1974). In the future, Alaska butter clams could become an important additional source of supply to meet growing demands.

TABLE 1. *Average size and age of 1069* Saxidomus gigantea *collected on Porpoise Island, Southeast Alaska.*
N = number of clams; ML = mean shell length; SD = standard deviation; R = range.

Year Class (Age of Clams)	N	ML (mm)	SD (mm)	R (mm)
0	79	5.1	1.4	3.0 - 8.5
1	134	11.2	2.5	6.0 - 16.4
2	109	15.4	2.5	9.0 - 22.5
3	104	22.5	4.1	16.7 - 30.5
4	88	30.3	5.1	24.2 - 38.0
5	98	38.2	3.9	27.0 - 47.0
6	82	45.9	4.5	37.0 - 55.0
7	85	54.1	4.9	42.0 - 64.0
8	72	62.6	5.1	47.0 - 74.0
9	75	71.3	4.6	58.0 - 81.5
10	68	77.4	4.0	68.7 - 87.0
11	40	82.5	3.6	76.0 - 88.2
12	22	86.9	2.5	82.0 - 91.0
13	8	92.8	3.5	88.5 - 98.0
14	4	96.0	2.5	93.6 - 99.5
15	1	100.0	—	—

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EFFECTS OF DIET AND TEMPERATURE ON GROWTH AND MORTALITY OF THE BLUE CRAB, *CALLINECTES SAPIDUS*, MAINTAINED IN A RECIRCULATING CULTURE SYSTEM.

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ABSTRACT

Blue crab growth parameters were measured over a sixty-day period in a recirculating culture system, with each crab in physical isolation. Dependent variables were molt interval, increase in carapace width per molt, percent molt and mortality. No consistant growth differences were detected in animals fed diets ranging from 26 to 75% protein content. A temperature of 30°C generally increased molt frequency and percent of animals molting compared to a temperature of 20°C. Increased temperature appears to depress cuticle expansion and to decrease mortality.

INTRODUCTION

Recirculating maricultural systems have received increased attention in recent years (Epifanio *et al.*, 1973; Chanley and Terry, 1974). Winget *et al.* (1973) repolted the construction of such a system using the blue crab, *Callinectes sapidus* (Rathbun) as a test species. This experimental system was designed to allow simultaneous control of several environmental variables, and the present paper presents data concerning the effect of temperature and diet on growth and mortality of blue crabs held in the system.

MATERIALS AND METHODS

Each culture system consisted of a biological filter made of crushed oyster shell; a fiberglass-coated, plywood reservoir tank; fiberglass-coated plywood growing tanks; temperature-control apparatus; and automated flourescent lighting. The growth tanks were partitioned so that each crab was separated from its neighbors and provided with flowing water. Water used to fill the system was pumped from Delaware Bay, filtered to remove particles larger than 5μm and adjusted to 20‰*S* by addition of fresh water. Water quality was not monitored in the system, but the water was completely replaced every two weeks. Photoperiod was maintained at 16 hours light and 8 hours dark.

The experiment was of a 2 x 4 factorial design and initiated with 35 animals in each cell. Temperature was either 20°C or 30°C and diet either control or one of three formulated preparations. The control diet consisted of 60.5% *Menidia menidia* (which was 80% water), 1.5% each of casein, starch and agar, and 35% tap water. The dry weight, protein concentration in this diet was 74.9 ± 4.5% SD as determined by a Hewlett- Packard C-H-N analyzer. Formulated diets consisted of 17.5% dry feeds (Table 1), 1.5% agar and 81% water. Total water content of all diets was 83-84%. (The dry feeds contained 2-3% water.) Water and agar were mixed, heated, and poured

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into a pan containing the other ingredients. After cooling, the food was sliced into blocks. Since preliminary trials indicated that three to four grams per day represented a maximum ingestion rate, crabs were offered three grams per day, six days a week.

TABLE 1. *Ingredients of formulated diets as a percentage of dry weight.*

	DIET		
INGREDIENTS	A	B	C
Ground Yellow Corn	40.00		
Soybean Meal, 50%		20.00	
Herring Fish Meal		20.00	
Casein			40.00
Crab Meal			
Fish Meal	11.25	11.25	11.25
Soybean Meal	18.75	18.75	18.75
Brewer's Dried Yeast	3.75	3.75	3.75
Fish Solubles	3.75	3.75	3.75
Ground Yellow Corn	17.03	17.03	17.03
Dehydrated Alfalfa	.75	.75	.75
Dried Whey	1.87	1.87	1.87
Soybean Oil	1.50	1.50	1.50
Ground Limestone	.75	.75	.75
Iodized Salt	.19	.19	.19
Methionine	.11	.11	.11
Vitamin and Trace Metal Premix	.30	.30	.30
Total Weight	100.00	100.00	100.00
Calculated Proximate Composition			
Protein	26.00	46.00	62.00
Fat	4.20	3.90	3.30
Fiber	2.16	2.28	1.35
Calcium	.80	.90	.80
Phosphorus	.51	1.09	.50
Ash	4.50	7.50	4.50
MET Energy (cal/g)	1457.00	1397.00	1565.00

Male crabs were collected from Indian River Bay, Delaware, during May and June when ambient water temperatures were between 15°C and 20°C. The mean carapace width of these animals was 5.9 cm, and an analysis of variance (Sokal and Rohlf, 1965) showed no significant difference ($p \leq 0.05$) in mean carapace width among eight experimental groups used in the experiment. Upon arrival in the laboratory, each animal was placed in the system at 20°C or 30°C and fed ground silverside, *Menidia menidia*, until ecdysis after which it was considered an experimental animal. No crabs with damaged or missing appendages were used in the experiment.

The experiment lasted 60 days and dependent variables were: 1) percent of animals that molted within 60 days after introduction to the experimental situation, 2) intermolt period in days, 3) percent increase in carapace width after ecdysis, and 4) percent mortality. Analyses of variance were performed on the data for mean percent increase in carapace width and mean molt interval. All statistical inferences were made at the 0.05 probability level. Since the eight groups within the experiment were not replicated, no statistical

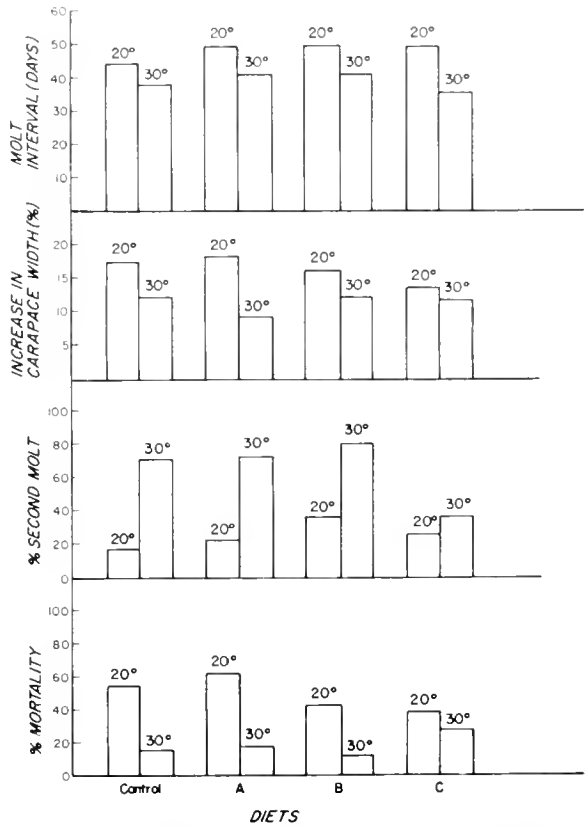

FIG. 1. *Temperature and dietary relationships with parameters of growth.*
Diet A = 26% protein
Diet B = 46% protein
Diet C = 62% protein

treatment was possible for percent mortality and percent second molt.

RESULTS

Temperature and dietary relationships with the dependent variables are shown in Figure 1. F values from analyses of variance are presented in Table 2. Dietary protein content did not consistently affect any of the growth parameters. Diets A and C were identical except for a 40% difference in ground corn or casein content. Diet C (62% protein) appeared to reduce mortality at 20°C but increase it slightly at 30°C. Diet C also reduced percent molt at 30°C but not at 20°C. Replacement of casein with a different source of protein (soybean and herring fish meal in Diet B) did not greatly influence growth patterns.

The 30°C temperature regime significantly decreased molt interval in the experiment and decreased mortality considerably. Also, the percentage of animals molting a second time was generally higher at 30°C. Thus warm water favored three of the growth parameters. On the other hand, 30°C water significantly reduced carapace growth at ecdysis in all three experiments.

DISCUSSION

Diet

A noteworthy result of this experiment was a lack of consistent dietary effect. We were primarily concerned with elucidating a range of protein concentrations which would indicate optimal growth, but were unable to do so at protein concentrations from 26 to 75%. Although it is possible that optimal protein values were masked by interaction with other variables, it appears that continuing nutritional studies with *Callinectes*, should, at least initially, use diets containing 26% protein or less in establishing economically optimal feeds. These results compare favorably with those of Andrews *et al.*, (1972), who suggested that a dietary protein level of 28% was optimal for juvenile penaeid shrimp. However, the findings of Zein-Eldin and McGaffey (1975) indicate that optimum protein concentrations should be re-evaluated as other aspects of the diet are improved. They reported a distinct 52% optimum in one penaeid diet, but found an optimum 32% protein content when combined with very different non-protein components in another diet.

A number of authors (Renaud, 1949; Schwabe, *et al.*, 1952; Scheer, 1959; Heath and Barnes, 1970; Rouse, 1972) have presented evidence to indicate that reptantian crustaceans store food reserves, including protein, during periods of alimentation (intermolt-early premolt) and deplete them during inanition (late premolt-postmolt). It is possible that reserves stored while animals were still in the field could have influenced the results of the present study, but it seems more probable that reserves augmented during feeding would be largely used up during the following postmolt and that material stored in the field would have its major effect during the first laboratory postmolt period and a relatively minor influence thereafter. The hypothesis has not been tested, however, and future nutritional experimentation should probably be performed on animals which have molted several times in the laboratory under controlled diets.

Temperature

Changes in water temperature have been shown to influence survival, molt frequency, and increases in linear and gravimetric dimension among decapod crustaceans (Zein-Eldin and Griffith, 1966; Huges *et al.* 1975), but optimal temperatures vary with different growth parameters. Although Ford *et al.* (1975) found slight enhancement of survival and an increase in molt frequency and linear dimension in *Homarus americanus* between 19°C

TABLE 2. *F values from 2-way analyses of variance of results. Experiments - an asterisk (*) denotes significant F values at the 5% probability level.*

Dependent Variable	Temperature (T)	Diet (D)	T × D
Increase in carapace width	30.66*	0.99	2.52
Molt Interval	17.20*	1.21	0.52

and 22°C, Zein-Eldin and Griffith reported that maximum linear and gravimetric growth in penaeid shrimp occured near 30°C. We have noted somewhat better survival and more frequent and higher percentage of molting at 30°C compared to 20°C. Carapace growth, on the other hand, was uniformly greater at 20°C. Considering the above complications, it may be best to determine optimum growth temperature by using total protein or dry weight production from a given number of animals as a basis of measurement.

Temperature also had a pronounced effect upon mortality. Mortality in groups cultured at 20°C ranged from 37.5 to 62.5% while mortality in the 30°C groups ranged from 12.5 to 27.5%. The reasons for higher mortality at 20°C are not clear as this temperature is certainly not extreme for the species.

Conclusions

Growth observed in this study generally did not compare well with that observed in natural waters. Tagatz (1968) reported that *C. sapidus*, held from April to November in live cars in St. Johns River, Florida, and fed an unspecified species of fish, increased carapace width by 25 percent per molt in all size classes. Temperatures ranged from 14-32C ($\overline{X}$ = 27C). Others (Gray and Newcombe, 1938) described similar growth rates in blue crabs held in live cars in Chesapeake Bay. Increase in carapace width was not this great in our experiment (Fig. 1). Molt intervals were also longer in our laboratory experiments. Tagatz reported a mean interval of 17 ± 6.1 days for animals of the same size range as those used in the present experiment. This indicates faster growth in a more natural situation than achieved here. The work of Kanazawa *et al.* (1970) and Sick *et al.* (1972) indicated a similar problem with penaeid shrimp. Neal (1973), in a discussion of aquaculture research with penaeid species, concluded that no one has been able to produce a defined diet which compares favorably in terms of growth with more natural diets.

ACKNOWLEDGMENTS

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GROWTH OF PACIFIC OYSTERS *CRASSOSTREA GIGAS* AND RELATED FOULING PROBLEMS UNDER TRAY CULTURE AT SEABECK BAY, WASHINGTON[1] [2]

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ABSTRACT

Pacific oysters (Crassostrea gigas) *were grown in Nestier trays under two different sets of conditions at Seabeck Bay on Hood Canal, Washington. One group of oysters was placed in trays that were suspended from a floating dock and were submerged at all times. The other group of oysters was placed in trays that were set out in the intertidal zone at the +2 foot tide level where they were exposed to the air for some portion of each day. Growth and fouling data were collected monthly for each set of trays.*

Fouling was very pronounced on the dock trays and less on the beach trays. Growth patterns for the two different stations were also different. The oysters suspended from the dock grew well during the early months of the year, then ceased to grow in April or May, due to excessive fouling of the trays. The oysters on the beach showed no growth from January to April. Growth for these oysters started in April or May and continued throughout the summer well into the fall.

Data from this study point out the importance of fouling organisms to this type of oyster culture and the different growth rates that can be obtained by placing the trays under different conditions at the same site.

INTRODUCTION

The most popular method for growing Pacific oysters in Japan is hanging culture, using either rafts or long lines (Furukawa, 1971). On the Pacific coast of the United States, this species of oyster is usually grown directly on the beds and harvested by tonging, hand picking, mechanical harvesting, or drag dredging. However, some growers have contemplated the use of tray culture as another means of raising commercial crops of Pacific oysters. This has been especially true with the development of oyster hatcheries along the Pacific coast, making cultchless seed readily available.

The concept of oyster tray culture is not new. It has been utilized for many years in European countries, Australia, and the Eastern United States. Trays of oysters can be hung from long lines, suspended from a dock or other floating structure, where they are kept constantly immersed in sea water, or they can be placed in the intertidal zone.

Although the advantages of tray culture are well known, little information is available as to the types and seasonal trends of fouling organisms occurring under this type of culture in Washington waters. Thus the present study was initiated in Seabeck Bay to better understand fouling pro-

1 Contribution No 443 College of Fisheries, University of Washington

2 This study was supported in part by the Sea Grant Program under the National Oceanic and Atmospheric Administration, U. S. Department of Commerce

blems when oysters are grown under two different conditions. The two conditions are: (1) trays of oysters hung from a floating dock and submerged under water continuously, and (2) trays of oysters placed at the+2 foot tide level in the intertidal zone where they are exposed to air during low tides. Further, the growth of oysters under both conditions was monitored throughout this study.

Although we recognize that the site used for this study may not be representative of the potential oyster tray culture sites in Washington, the results presented are useful in that they compare growth rates of oysters grown in trays at the same site under different conditions and provide information on the importance of fouling organisms in tray culture.

MATERIALS AND METHODS

Site Description

This study was conducted at Pecks Harbor Marina at Seabeck Bay on Hood Canal. One experimental station was located on the southern side of a marina pier where the trays containing oysters were suspended about one meter below the water surface at all times from a floating dock. A second experimental site was located on the beach at the northern side of the pier at the +2 foot tide level.

The oysters in this study were grown in plastic Nestier trays ½ meter on a side and about 8 cm. deep. Small holes in the sides and bottom of the tray allowed for water circulation.

Three stacks of four trays were used at each station. The bottom three trays in each stack contained oysters, while the top tray acted as a lid to keep the oysters from being washed out. Each stack was secured at opposite corners with nylon cord. The stacks of dock trays were fastened together with polypropylene line and suspended from the dock. The trays on the beach were at first held in place by setting two concrete blocks on top of each stack. In the winter the trays were wrapped with polypropylene line and tied to metal pipes driven into the beach.

Test Animals

The oysters used in this study were purchased from the Lummi Island Oyster hatchery at a size of 2-4 cm in shell length. To simulate a commercial density, groups of 200 oysters were separated out at random from the original lot and each of these groups was placed in a separate tray. Ten oysters from each group were marked with dots of nail polish in a three-color coding system that made it possible to identify individual oysters. Two of the marked oysters were placed in each corner of a tray, and two in the center.

Data Collection

Data were collected from the trays on a monthly basis. Growth measurements were taken from the ten marked oysters in each tray and were expressed as the product of their length and width, as defined by Quayle (1969). The increases in this product, or the actual shell area, were used as a measurement of the oysters' growth, much in the same manner as Butler (1953) used his "G" factor to compare the growth rates of different stocks of oysters.

Fouling was defined as any macroscopic organism that attached itself to the outside of the trays or was found inside the trays in this study. These organisms were documented by visual observation and by 35 mm slides taken using the Mayers Underwater Photogrammetric System (MUPS). Representative specimens of organisms not identifiable by sight were stored in 10% formalin and taken to the laboratory for identification. Fouling organisms were not removed from the trays except when they actually preyed on the oysters and so jeopardized the growth study. This meant that the entire stacks of trays could be used like the test blocks in Coe and Allen (1937) and Graham and Gay's (1945) classic fouling studies. Carlisle, Turner, and Ebert (1964) also performed similar studies on fouling communities on artificial reefs.

All of the beach trays were lost in December, 1973, during a severe storm. Thus it was necessary to move some of the dock trays to the beach station. These trays were chosen at random from the original dock trays. The oysters were them remarked with nail polish and measured before placing them at the beach station. The fouling on these trays was not taken off, but as can be seen from the data in Table 2, these animals did not survive at the beach station.

Over 2,000 additional oysters from the Lummi Island Hatchery were added to the study in May, 1974. These oysters were also 2 to 4 cm in length

and were divided into groups, marked and measured in the same manner as the original oysters had been. Trays were stacked together as before and placed at the dock and beach stations.

RESULTS AND DISCUSSION

Fouling Study

The monthly occurrences of the different fouling organisms found on the trays suspended from the dock and those set out on the beach are shown in Tables 1 and 2. A comparison of these tables points out some obvious differences in types and amounts of fouling organisms present at the two stations. The dock trays had a much more diverse fauna and flora of fouling organisms than did the beach trays. Also, fouling of the dock trays was fairly constant; except for a winter die-off, organisms that were found on the dock trays one month were usually found there the next month. This was not true of the beach trays where the fouling organisms present varied considerably from month to month.

The dock trays represented a fairly stable or constant environment. The trays were submerged at all times (except during sampling) and were hanging at a constant depth. The layer of water between the trays and the surface acted as a buffer, protecting the oysters and trays from quick changes in temperature and salinity. By the end of the study the dock trays had developed entire communities of sessile or slow moving organisms. The first organisms to appear on the dock trays were sponges, bryozoans and solitary and colonial tunicates. These animals were present throughout most of the study and covered large areas on all of the trays.

Another important fouling organism found on the dock trays was the mussel (*Mytilus edulis*).

Table 1. *Monthly occurrence of fouling organisms at the dock station, Sept. 1973 to Nov. 1974.*

	TIME IN MONTHS														
	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
Kelp									X	X	X	X		X	
Sponges	X	X	X	X						X	X	X	X	X	X
Sea Anemone (*Metridium*)		X	X	X						X	X	X	X	X	X
Flatworm (*Notoplana*)											X	X	X	X	X
Polychaetes (*Halosydna*)		X	X							X	X	X	X	X	X
(*Serpula*)							X	X	X	X	X	X	X	X	X
(*Nereis*)									X	X	X	X	X	X	
Barnacles (*Balanus*)									X	X	X	X	X	X	X
Amphipods - Gammaridae		X	X				X		X	X		X	X		
- Caprellidae	X	X	X					X	X		X	X	X	X	X
Shrimp - Hippolytidae				X				X		X	X	X	X	X	X
Crabs (*Cancer oregonensis*)							X	X	X	X	X	X	X	X	X
(*C. productus*)													X	X	X
Limpets (*Acmaea*)										X	X	X			
Nudibranchs (*Hermissenda*)		X	X				X	X	X	X	X	X			
(*Archidoris*)			X				X						X	X	X
(*Diaulula*)												X	X	X	
Mussels (*Mytilus*)					X	X	X	X	X	X	X	X	X	X	X
Scallops (*Pecten*)							X			X	X	X	X	X	X
Jingle (*Pododesmus*)							X	X	X	X	X	X	X	X	X
Clams (*Entodesmus*)											X	X	X		
Bryozoans	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Sea cucumbers (*Parastichopus*)	X	X								X	X	X	X	X	X
Sea urchins (*Strongylocentrotus*)															X
Tunicates - Solitary	X	X	X	X						X	X	X	X	X	X
- Colonial	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

The first mussel set on the trays occured in December, 1973. As the mussels grew they covered the trays and oysters with their byssal threads. By April, the trays were so tightly woven together by byssal threads that they were hard to separate. Oysters inside the trays became tied into place and had to be pried out for growth measurements.

Three nudibranch species were found on the dock trays with some regularity. The most common of the three was *Hermissenda crassicornis*. Also found were *Diaulula sandigensis* and *Archidoris montereyensis*. The crabs *Cancer productus* and *C. oregonensis* were found quite often inside the dock trays. These animals had to be removed from the trays when they began to chip and open the oyster shells. While some of the other fouling organisms competed with the oysters for food and space, these crabs were the only fouling organisms that actually preyed on the oysters at the dock station.

Fouling on the beach trays was much less constant than that on the dock trays. This was because the trays on the beach offered a more severe environment than did those hanging from the dock. The lowest tides, which gave the longest exposure to the air occurred at night in winter and during the day in summer. Oysters and fouling organisms in these trays were also subject to rapid salinity changes during rainstorms and considerable wave action during high winds.

All of these factors taken together discouraged many of the sessile fouling organisms found with such abundance in the dock trays. Most of the organisms associated with the fouling community found on the beach trays were mobile to some ex-

Table 2. *Monthly occurence of fouling organisms at the beach station, Sept. 1973 to Nov. 1974.*

	TIME IN MONTHS														
	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
Sponges	X	X	X												
Anemones										X	X	X	X		
Barnacles *(Balanus)*								X	X	X	X	X	X	X	X
Amphipods - Gammaridae								X	X	X	X	X	X	X	X
Shrimp - Hippolytidae															
Crabs *(Cancer productus)*								X	X	X	X	X	X	X	X
(Epialtus)		X					X	X	X	X	X	X	X	X	X
Hermit crabs *(Pagurus)*	X		X				X	X	X	X	X				
Snails *(Thais)*			X				X	X	X	X	X	X	X	X	X
(Searlesia)							X			X					
Mussels *(Mytilus)*										X	X	X	X	X	X
Starfish *(Evasterias)*			X				X	X							
Gunnels *(Apodicthys)*		X	X					X	X	X	X	X	X	X	X
(Pholis)		X	X					X	X	X	X	X	X	X	X

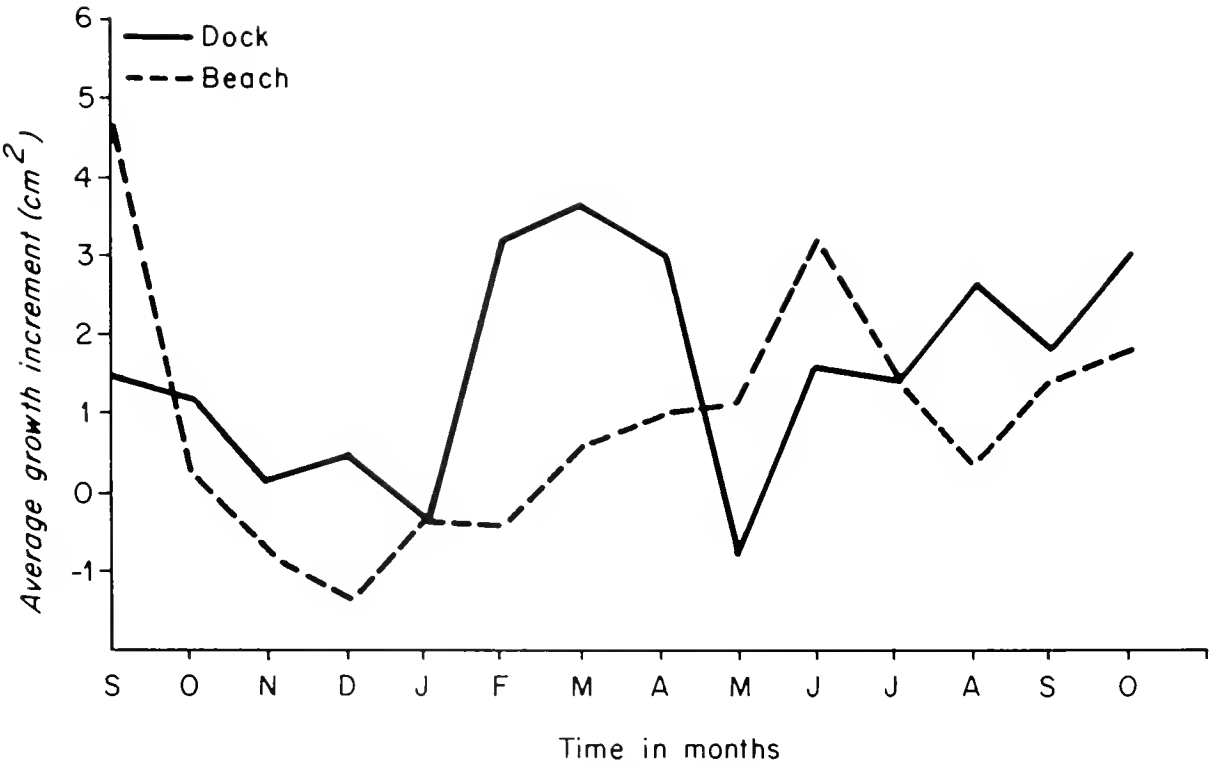

FIG. 1. *Average monthly growth increments for dock and beach oysters, September, 1973 to October, 1974.*

tent. This helped them find shelter when the trays were exposed to particularly harsh weather conditions.

The most common fouling-associated organism found on the beach trays was the drill *Thais lamellosa*. These snails were almost always found on the outside of the trays and posed no threat to the oysters inside. Hermit crabs (*Pagurus*) were common in and on the beach trays. Kelp crabs (*Epialtus productus*) were common on the outside of the trays and were occasionally found inside.

The bottom tray of each beach stack was set directly on the substrate. It occasionally became covered with a thin layer of silt and this attracted different fouling-associated organisms. The most common of these were gunnels (*Pholis* and *Apodichthys*) and spironticarid shrimp.

The only destructive fouling-associated organism found on the beach trays was the starfish *Evasterias*.Although these animals were usually found on the outside of the trays, a few did manage to get inside, probably when they were small larvae. Those that did so were removed when noticed.

Growth Studies

Figure 1 shows the mean monthly growth increments of the dock and beach oysters. Growth increment was computed as the actual increase in shell area from one month to the next, or:

$$\Delta A = A_2 - A_1$$

where A_1 is the shell area at the end of one month, while A_2 is the shell area at the end of the next month. Growth increments were used instead of actual size measurements when looking at the growth of oysters throughout the entire study because of the fact that the beach oysters were lost in December, 1973, and replaced with oysters from the dock trays. Thus, although the actual size of these new beach oysters in January would not have been meaningful, it was possible to compute their actual increase in shell area each month. As can be seen from the figure, the growth pat-

terns of the dock and beach oysters were quite different. A three factor analysis of variance test was conducted on these data, testing for differences in growth increment between the dock and beach stations, the different tray levels within a stack, and the different months of the year. There was a significant difference between the growth increments of the oysters at the two different stations ($F = 3.24$, $p = 0.75$) and during different months of the year ($F = 2.74$, $p = 0.75$). The interaction between these two factors was also significant ($F = 2.13$, $p = .026$).

The analysis described above contained two different age groups of oysters (those present at the beginning of the study and those added in May 1974). Since oysters of different ages grow at different rates (Loosanoff, 1947) the data were also analyzed by age group. This analysis was conducted on the original dock oysters in December, 1973. This was just before the dock trays were split into two groups, one of which was to replace the lost beach oysters. The test showed no differences in size between the trays of oysters left at the dock station and those moved to the beach ($F = .46$, Critical value at $p = .05 = 4.07$). At the end of the study in November, 1974, another analysis of variance test was conducted on the size of the oysters in these dock and beach trays. This test also showed no significant difference in size between the oysters at the two stations ($F = .61$, Critical value at $p = .05 = 5.14$). Although these two groups of oysters grew to the same final size, their patterns of growth were quite different. This is shown in Figure 2, where the average monthly size of the oysters at the two different stations is plotted along with the average monthly water temperature at the site. As can be seen from the figure, the oysters in the dock trays started to grow in February-March and continued to show obvious increases in shell area until April or May. After that their growth leveled off and they decreased slightly in size (probably due to chippage during handling) until August, when they slowly began to grow again. The beach oysters, on the other hand, showed almost no growth from January until April or May. After that they grew well until August. Growth then leveled off until October, when a particularly large amount of shell was added.

A close look at this figure raises several questions. First, why were the dock oysters able to grow during this early period and the beach oysters not able to? And, finally, why did the dock oysters stop growing in April or May, which is normally one of the best growing periods for oysters?

The early growth of the dock oysters took place partly during a period when the water temperature was fairly low (8 to 11C). Winter hibernation, or cessation of growth, has been reported at temperatures below 10 to 11C in British Columbia by Quayle (1969) and in Washington waters by Chew (1961). However, it is not known whether this is caused simply by a slowing of the oysters' pumping mechanism, a lack of food in the colder waters, or a combination

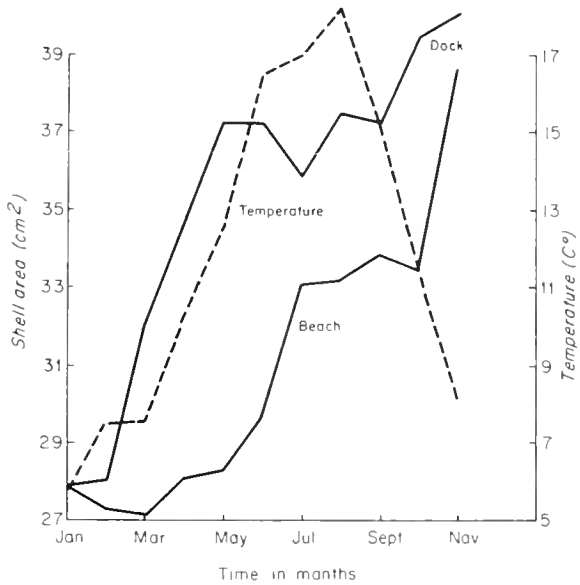

FIG 2. *Average monthly water temperature and average monthly size of original dock and beach oysters, January to November, 1974.*

of the two. It is known that oysters are capable of pumping some water at temperatures below 10C. In this case, the fact that the oysters were actually growing when the mean monthly water temperature was only 8C shows that they must have been pumping water and that there must have been food in the water at this time. An observation that supports this idea is that mussels that set on the trays at the dock station in December, 1973, also showed noticeable growth during the early months of 1974.

Assuming that there was food available in the water during this period, the question becomes why the beach oysters did not show a similar pattern of rapid growth during this period. This is probably because the beach oysters were subjected to a much more harsh and variable environment, particularly during the winter months. Several hard freezes occurred at Seabeck Bay during the winter of 1973-4. Also, several storms followed the one in December that swept away the original batch of beach oysters. These storms had a greater effect on the more exposed beach oysters than on those suspended from the dock. Aside from the temperature changes that accompanied the storm, heavy rains often occurred, subjecting the beach oysters to rapid changes in salinity. Probably the most important factor caused by the winter storms was wave action. Storm waves tossed the oysters around inside the beach trays, often piling them in one corner, chipping fragile growing edges of the oysters, and leaving less room for water circulation and oyster growth. Although the beach oysters probably grew slowly during this period, the large amount of shell chippage masked such growth.

The question remains as to why the dock oysters stopped growing in April or May. Excessive fouling of the dock trays was the probable reason. The mussels that set on the trays in December were getting larger by this time and were beginning to clog up the holes in the trays, restricting water circulation. Kerswill (1949) reports on how much a lack of adequate circulation can slow the growth of oysters and other bivalves. This condition worsened as the summer progressed and was augmented by the fact that the mussels had set most heavily around the sides of the holes. Since mussels are filter feeders like oysters, it is probable that they also competed with the oysters for food items. Also, because the mussels were largely on the outsides of the trays, they had first chance at filtering the water before it went inside the trays to the oysters. However, mussels were not the only fouling organisms on the dock trays. As the water temperature grew warmer, the tunicates, sponges, and bryozoans began to cover more of the tray surface.

The beach trays, on the other hand, did not suffer from these problems. They were exposed to the air for a part of each day, and while this did not stop the oysters from growing it did discourage most of the kinds of fouling that seemed to cause problems on the dock trays.

The oysters in the new trays (those added to the study in May, 1974) were analyzed in a similar manner to those in the original trays. An analysis of variance test conducted on the initial size of the oysters showed no difference between the dock and beach groups ($F_{.} = 3.49$, Critical value at $p = .05 = 7.71$). However, at the end of the study, the mean size of the beach oysters was quite a bit smaller (19.48 cm^2) than that of the dock oysters. The average monthly sizes of the dock and beach oysters in these new trays are plotted in Figure 3. It should be noted that the new dock oysters grew well during the period when the original dock oysters had stopped growing. This is because the new trays were placed in the water late enough to miss many of the spring sets of fouling organisms. This figure also shows that without extreme fouling to contend with the dock and beach oysters had very similar growth patterns during this period. The dock oysters were probably able to grow faster during this period than those on the beach simply because they were exposed to the water for more hours each day and so had more time to feed.

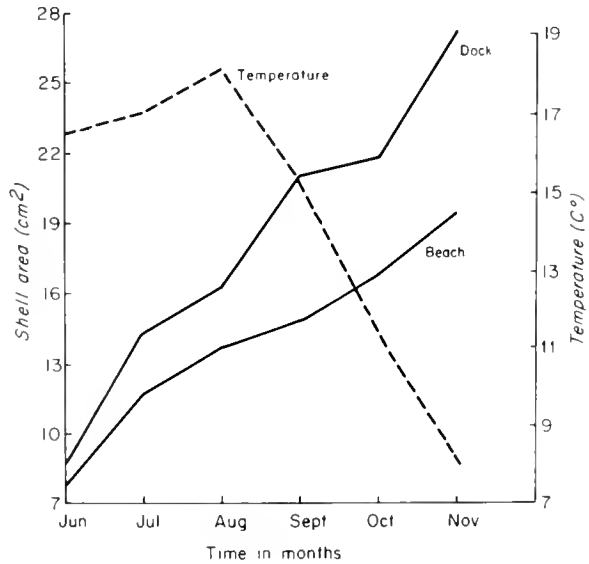

FIG. 3. *Average monthly water temperature and average monthly size of new dock and beach oysters, June to November, 1974.*

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INVESTIGATION OF PRACTICAL MEANS OF DISTINGUISHING *MYA ARENARIA* AND *HIATELLA* SP. LARVAE IN PLANKTON SAMPLES

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ABSTRACT

Bivalve larvae (veligers) of the soft shell clam, Mya arenaria, *and a rocky substrate dwelling clam,* Hiatella *sp., were obtained from induced spawning of adults in the laboratory, and also from live plankton collections made in the vicinity of Hampton Beach, New Hampshire (42°54′ N. Lat., 70°49′ W. Long.).* Mya arenaria *were raised to the settling stage. Morphology of developing larvae of both species was described and photographically illustrated. Distinguishing characteristics of shape, size and coloration were found to be difficult to apply in planktonic studies over much of the period of larval development covered. With experience, however, the investigators could make correct identification of umboned* Mya arenaria *with less than 20% error, aided in part by seasonal differences in peak abundance of larvae of the two species. Obscurity of taxonomy of the genus* Hiatella *is discussed.*

INTRODUCTION

Study of larval distribution of a species often requires processing of a large volume of plankton samples from which the investigators must be able to readily identify the species, usually using shell characteristics as they appear with the organism on its side under the microscope. Photographic illustrations of Sullivan (1948) indicate that a close resemblance exists between shell characteristics of soft shell clam, *Mya arenaria,* larvae and *Hiatella* sp. larvae (specific identity obscure, Yonge, 1971). Larvae of both species are seasonally abundant in plankton samples from coastal Maine and New Hampshire waters (personal observation); hence, a need is apparent for further investigation of the chances of misidentification.

On the western side of the North Atlantic Ocean, definitive bivalve larval identification work including *M. arenaria* larvae (Loosanoff and Davis, 1963; Loosanoff *et al.*, 1966; Chanley and Andrews, 1971) has been conducted south of the Gulf of Maine area where the occurrence of *Hiatella* sp. larvae in neritic plankton communities has not been reported. Sullivan (1948) is the only North American study which includes interspecific comparison of *M. arenaria* and *Hiatella* sp., however the larvae described were obtained from plankton collections only; none were reared from known parents in the laboratory. Moreover, the straight hinge stage of development of *Hiatella* sp. was not described or depicted by Sullivan (1948). Larvae of *Hiatella* have been described from European waters by Odhner (1914), Lebour (1938) and Rees (1950), and both *M. arenaria* and *Hiatella* spp by Jorgensen (1946). Because of limited coverage of developmental stages, and

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possible taxonomic distinctions, the European studies may be of little practical use in distinguishing between planktonic *M. arenaria* and *Hiatella* sp. larvae in North American coastal waters.

METHODS AND MATERIALS

Larvae Reared from Laboratory Spawned Adults

Approximately 1 ml of 0.1 N ammonium hydroxide was injected directly into the gonad of ripe female clams (as suggested by Stickney, 1964) obtained locally from flats in Hampton Harbor, New Hampshire. Males were induced to spawn by this same technique. Released ova were separated from large debris in the spawning bowl by passing the water through a 73μm mesh screen. The ova were then collected on a 41μm mesh screen. Recovered ova were washed into 600 ml pyrex beakers and the volume brought to 500 ml using seawater which was filtered through a 5μm filter bag and allowed to stand for about one week. To ensure fertilization, hypodermically extracted sperm were added to the egg cultures as well as sperm from males induced to spawn by ammonium hydroxide injection.

Fertilized egg cultures were held on a flowing seawater table in which temperatures fluctuated between 16C and 19C. After about twenty-four hours, swimming trocophore larvae were decanted and undeveloped eggs discarded. Larval densities of approximately one to two trochophores per milliliter were maintained in the cultures.

Ova of *Hiatella* sp. were obtained with relatively little effort. Adult animals, obtained in June and July, 1975, from rocky substrate off Hampton Beach, New Hampshire, at a depth of approximately 15 to 20 meters, were initially held in cool (10-13C) running seawater. Spawning resulted when the animals were placed in filtered seawater in glass bowls and allowed to warm to between 16C and 21C. With from six to twenty individuals per bowl, it was not unusual to observe sequential spawning of several animals. Procedures for egg recovery, insemination and establishing larval cultures, were the same as for *M. arenaria* except that lower rearing temperatures (6C to 13C) were used.

To suppress bacterial growth, very dilute solutions of penicillin G (1667 units per mg) and streptomycin sulfate were added to all cultures. The solutions were prepared by dissolving 2 to 4 mg of each of the antibiotics in a single beaker containing 100 ml to 5μm filtered seawater. Approximately 5 ml of the antibiotic solution was added to each 500 ml culture upon initiation and thereafter with each water change. All containers used in connection with larval culture were rinsed in very hot tap water and allowed to air dry. No soaps or detergents were used to clean any materials coming in contact with the larvae.

To exchange the culture medium and monitor larval development, animals were recovered from the old medium three times weekly using either 41 or 73μm mesh screens, depending on organism body size and amount of small debris present. Debris and shells of dead animals were also removed mechanically using microprobes and micropipettes. Animals were washed from the screens into small circular counting dishes and then transferred to a depression slide for measurement and photographing.

Measurements were made using either a dissecting microscope (100 x) or compound microscope (100 and 400 x) with calibrated eyepiece reticles. Successive measurements of shell lengths and widths of the developing larvae were plotted graphically and analysed by linear regression to determine if any species-specific length/width relationships could be discerned. Interspecific differences in hinge length of larvae in the early developmental (straight hinge) stage were compared using Student's t-test and Mann-Whitney U-test (Snedecor and Cochran, 1967). Black and white photographs were taken of selected individuals in various stage of development using a "Polaroid" Land Instrument Camera (Model ED 10) mounted on a compound microscope.

From early July, 1975, until experiments terminated in September, 1975, the principal food source for the larvae culture was a mixture of *Phaeodactylum tricornutum*, *Isochrysis galbana* and *Monochrysis lutheri* each obtained in monoculture from the National Marine Fisheries Service Laboratories at Milford, Connecticut. The three species were maintained both in mixed and monoculture on F/2 medium (Guillard and Ryther, 1962), except that ammonium chloride was omitted from the salt preparation as recom-

mended by Loosanoff and Davis (1963) for *I. galbana* culture. Seawater used to make up the algal culture was vacuum filtered through a glass fiber filter (Whatmann GF/C) to remove organic residue and bacterial cells. Glassware used in algal culture was heated to over 100C in an air drying oven for one hour or more.

Algae were fed to the larvae following each thrice-weekly change of their culture media. The amount of mixed algal culture added varied depending on: 1) the number of bivalve larvae in the culture; 2) visually determined cell density in the food culture; and 3) amount of unconsumed algae in the larval culture beakers prior to changing the medium.

Larvae Reared from Plankton Collections

Plankton tows were conducted in coastal waters of New Hampshire near Hampton Beach, using a 73μm mesh net with a 0.5 diameter opening. Bivalve larvae were separated from larger plankton and debris by passing the sample through 505μm netting. The larvae, along with other smaller plankton were then recovered on a 41μm mesh screen. Bivalve larvae of the desired species were isolated from other planktonic organisms by swirling them to the center of a watch glass and isolating them with probes made from a single strand of camel's hair. Only one isolation of *Hiatella* sp. from plankton samples was carried out, on 2 July 1975, when the density of this species in the plankton was about at its seasonal peak. Larvae identified as *M. arenaria* were isolated from two samples taken weekly from mid-August to mid-September.

Once isolated, the animals were enumerated and placed in 600 ml pyrex beakers containing 500 ml of 5μm filtered seawater. In examining, feeding and changing the culture media the same procedure as described above for laboratory spawned larvae was followed. With each thrice-weekly examination, approximately 100 larvae in the *M. arenaria* cultures were re-examined and identified to determine the extent to which species other than *M. arenaria* had been introduced into the cultures.

RESULTS

Spawning Success

In all, there were four attempts to spawn *M. arenaria* (on 14 and 31 July, 7 and 13 August) by injecting ammonium hydroxide. All succeeded in producing fertilized ova which subsequently developed into umboned larvae, although some of the spawning produced only one or two hundred eggs from up to six female clams. The most prolific production of ova (approximately 1000 eggs from six females) occurred on 31 July 1975. Condition of the gonads and occurrence of large numbers of *M. arenaria* larvae in coastal New Hampshire plankton collections indicated that late July to early August was the peak of the 1975 spawning season.

Four attempts to spawn *Hiatella* sp. (on 19 June and 17, 18 and 24 July) resulted in the release of ova. However, only two spawnings (19 June and 24 July) subsequently produced straight hinge larvae. Only the *Hiatella* sp. culture spawned 24 July was maintained until the larvae had attained the umboned stage. Failures of the earlier cultures were attributed to lack of availability of the proper algal food in June, and malfunction of temperature control of the seawater system during a period of unusally hot weather in July.

Morphometric Observations on Larvae from Induced Spawnings

Comparison of shell length and width measurements in *M. arenaria* (Fig. 1) with *Hiatella* sp. (Fig. 2) showed that length-width relationships

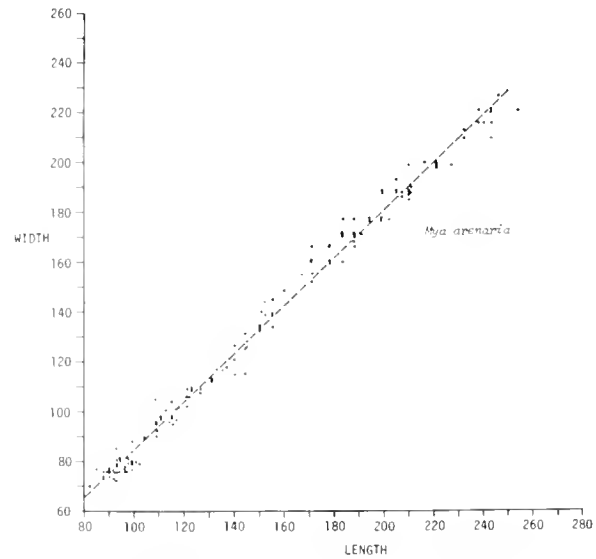


FIG. 1. *Length vs. width relationship in* Mya arenaria *larvae.*

were virtually identical (Fig. 3). In both species larvae were from 80 to 96μm long when the first larval shell (D-shaped or "Straight-hinge") was fully formed.

Mean hinge length in straight-hinge larvae was significantly shorter in *M. arenaria* (P<.01) but overlap in length distribution was considerable (Fig. 4). One characteristic, apparent in many cases, distinguished straight hinge *Hiatella* sp. larvae (shell length 85 to 124μm) and could be seen at magnifications of 100 - 120 x. This was an angular and upswept appearance of the straight hinge line, particulary noticeable at the junction of the hinge and posterior margin. This characteristic

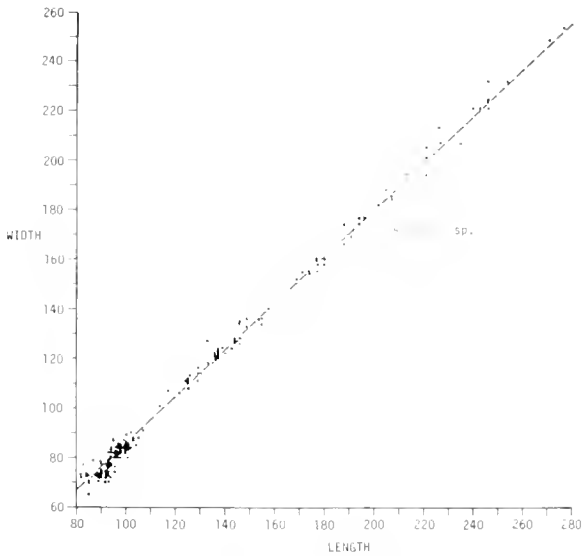


FIG. 2 *Length vs. width relationship in* Hiatella *sp. larvae.*

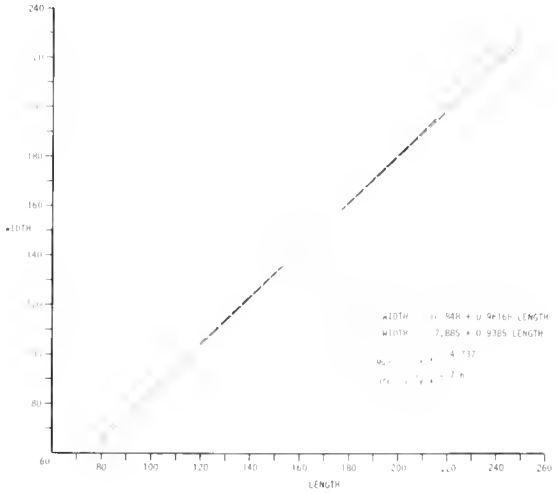


FIG. 3. *Comparison of length vs. width relationship in* Mya arenaria *and* Hiatella *sp.*

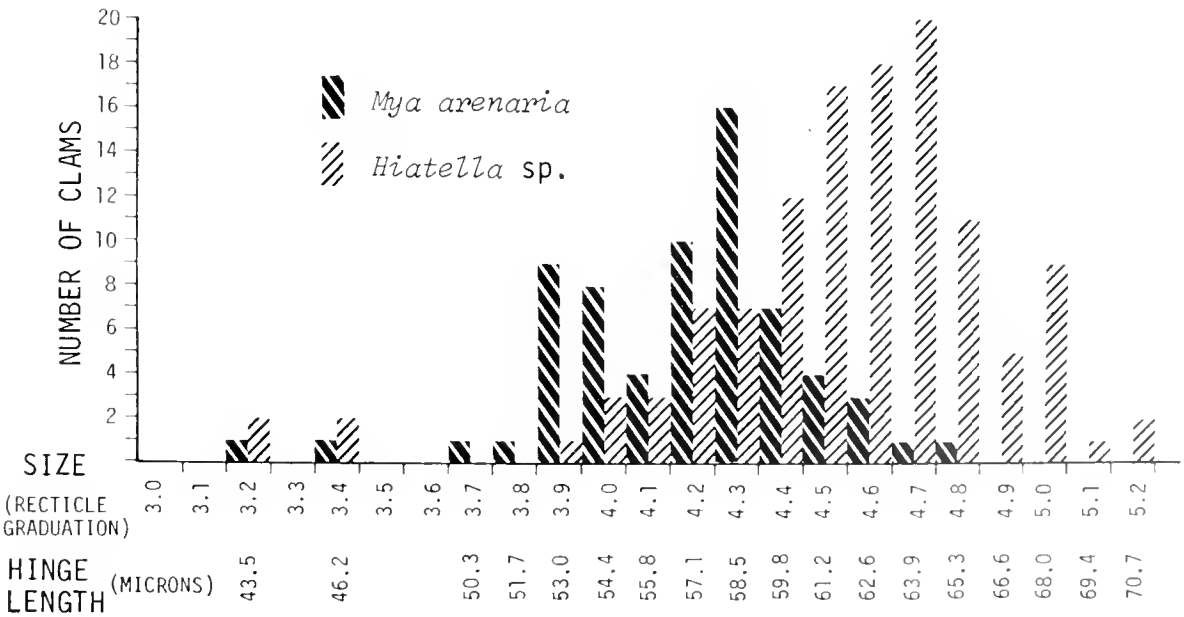


FIG. 4. *Frequency distribution of hinge lengths of* Mya arenaria *and* Hiatella *sp. straight-hinge larvae.*

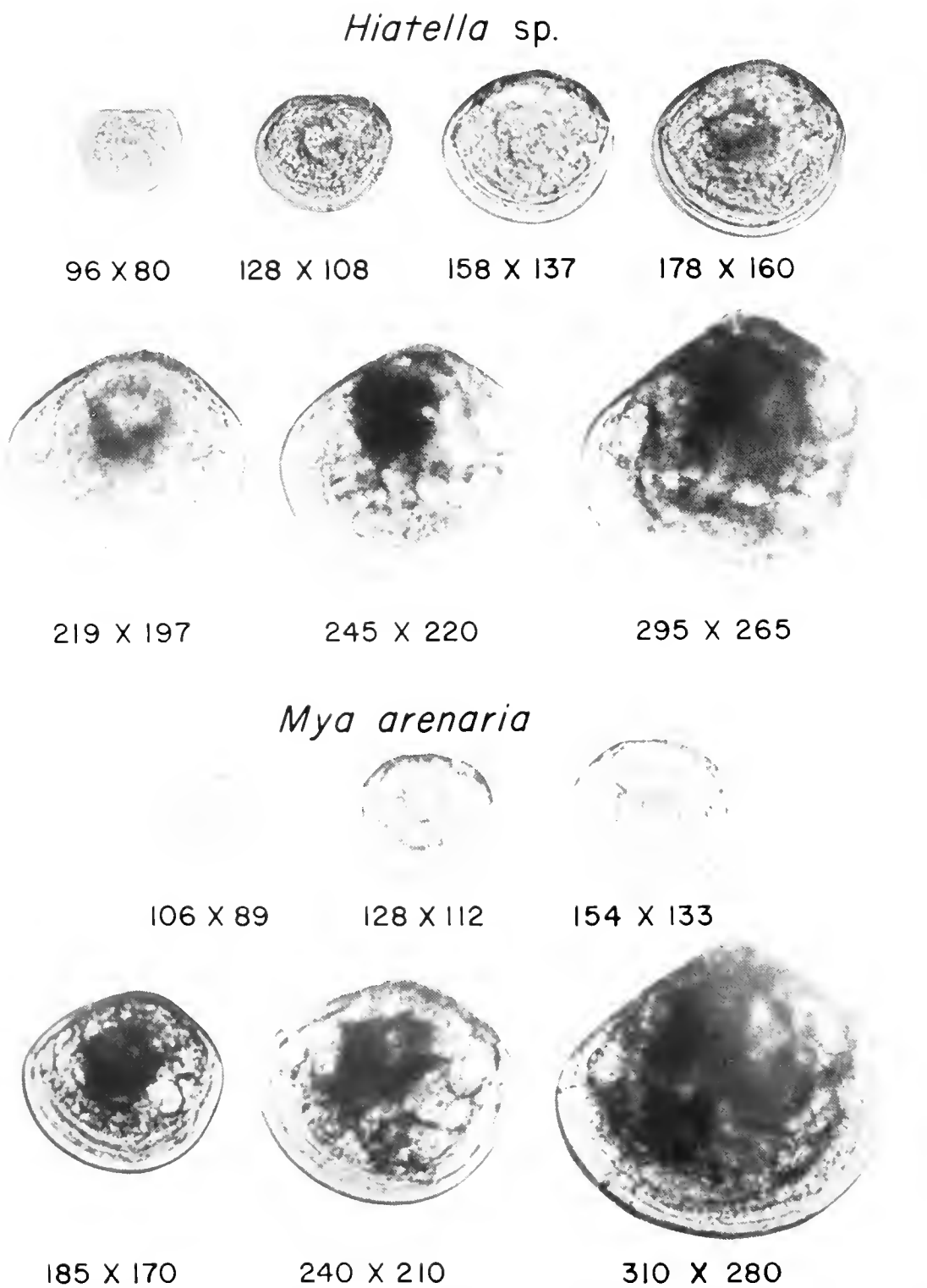
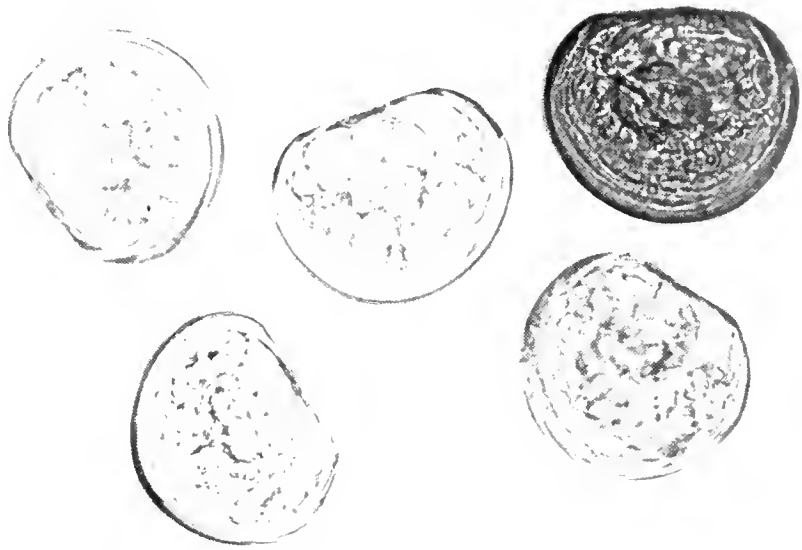
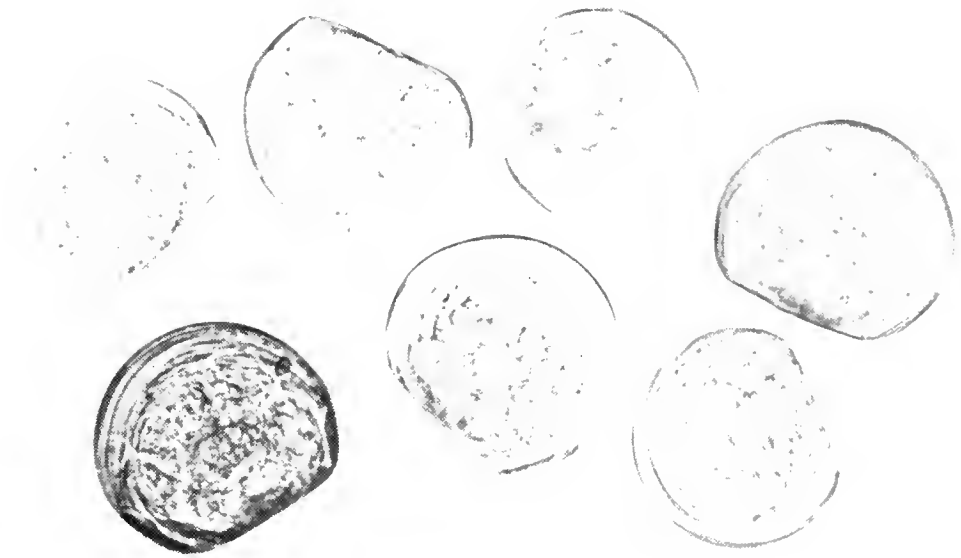


FIG. 5. *Photomicrographs of* Mya arenaria *and* Hiatella *sp. larvae. Length and height measurements given in micrometers. Anterior end of each larva is to the left.*



A.



B.

FIG. 6. *Photomicrographs of 2 to 3 day old straight hinge larvae approximately 95-105 mm long. A.* Mya arenaria *B.* Hiatella *sp.*

gave straight hinge *Hiatella* sp. larvae a "ship's bow" appearance, unlike that of *M. arenaria* larvae (Figs. 5 and 6).

With subsequent growth and development, the straight hinge line in both species appeared to lengthen little if at all. Instead, the valves of the shell lengthened and broadened only from the hinge, giving rise to sloping "shoulders" (Fig. 5). When the larvae were approximately 115μm long, umbones were first observed on each valve, immediately below the straight hinge. As the umbones grew dorsally, the profile of the straight hinge gradually became more and more obscured. Beyond a total length of 125μm, it was usually difficult to obtain accurate measurements of hinge length in either species. At a length of approximately 155μm, the sides of the umbones seemed to merge with the shoulders of the valve to present a profile with a continuous slope from umbone peak to the anterior and posterior margins (Fig. 5). In this report, larvae in which umbones break up the straight hinge profile, but are not yet continuous with valve margins are said to be in "transition" between the straight hinge and umboned stages of development. Both straight hinge and transition phases of development were of relatively short duration, each lasting only a few days (Tables 1 and 2). The umboned stage was the longest of the planktonic larval stages, lasting at least two weeks in the case of the *M. arenaria* culture begun on 31 July, and much longer in the case of the *Hiatella* sp. culture started on 24 July and kept at 6 to 13C under refrigerated conditions.

As Figure 5 suggests, developmental stages of *M. arenaria* and *Hiatella* sp., other than early stright hinge (Fig. 6), were observed to be practically indistinguishable in terms of shape or size characteristics. This situation continued almost until *M. arenaria* approached metamorphosis (i.e., when swimming function of velum was lost). Accompanying metamorphosis in *M. arenaria were a number of conspicuous morphological changes, including: (1) anterior tilting of the umbone, (2) prominent display of gill apparatus, and (3) darkening of the shell (possibly due to thickening) (Fig. 7).*

Mya arenaria larvae reared from eggs in the laboratory, were observed to undergo metamorphosis at a shell length as short as 235μm, although 250 to 270μm was the more usual size

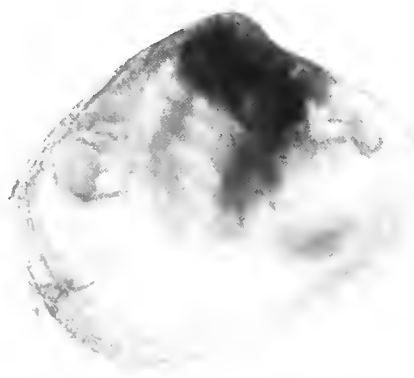


FIG. 7. *Photomicrograph of* Mya arenaria *post larva approximately 700 mm long.*

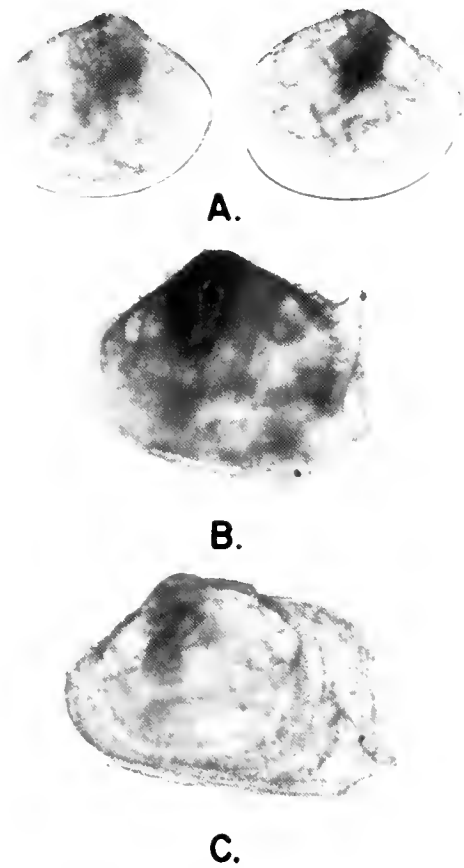


FIG. 8. *Photomicrographs of* Hiatella *sp. taken from plankton samples. A. Pediveligers approximately 360 mm long. B. Post larva approximately 450 mm long showing early development of spines. C. Spineless Post larva approximately 530 mm long.*

TABLE 1. *SUMMARY OF CULTURE RESULTS*, MYA ARENARIA *LARVAE OBTAINED FROM PLANKTON*

DATE OF FIELD COLLECTION	CALCULATED MEAN DENSITY OF *MYA* IN FIELD (m^{-3})	LIFE STAGES AT TIME OF COLLECTION	OBSERVED PERCENTAGE OF CONTAMINATION BY OTHER SPECIES														CULTURE TERMINATION DATE
			27 AUG	29 AUG	1 SEP	3 SEP	5 SEP	8 SEP	10 SEP	12 SEP	15 SEP	17 SEP	19 SEP	22 SEP	24 SEP	26 SEP	
15 Aug.	very high, not enumerated	straight hinge, 100-130μ	9	9	8	11	4	15	11	15	9						17 Sep
15 Aug	20	umboned, 160-210μ	18	8	8	9	10	8	18	16	15						17 Sep
21 Aug	2830	umboned, 170-220μ	22	18	8	14	9	10	19	10	12						17 Sep
26 Aug	560	umboned, 210-270μ	10	18	16	16	21	14	30	18	21						17 Sep
2 Sep	90	umboned, 150-290μ					74	68	67	64	69	71	69				19 Sep
9 Sep	140	umboned, 160-310μ								$\simeq$1	<1	$\simeq$1	<1	<1			
16 Sep	100	umboned, 150-260μ											<1	<1	<1	<1	30 Sep

range at which metamorphosis occurred. Furthermore, *M. arenaria* larvae up to 310μm in length were occasionally observed still swimming freely using the velum. *Hiatella* sp. larvae, obtained from spawning adults on 24 July, showed no sign of undergoing metamorphosis or losing ability to swim during the period 5 to 30 September when up to 50 individuals, between 240 and 340μm shell length, were under observation.

Culture of Larvae from Plankton

Fifty-nine *Hiatella* sp. larvae were isolated from plankton samples on 2 July 1975. These were at a more advanced stage of development (Fig. 8A) than were the 68 day old laboratory spawned *Hiatella* sp. that survived from 24 July to 30 September. The more advanced umboned larvae from the plankton were easily recognizable among bivalve veligers of a variety of species due to both a distinctive shape and coloration. The largest planktonic individuals (approaching 400μm in length) displayed a streak of pink apparently in the mantle tissue, paralleling the ventral shell margin. Two of the individuals isolated from the plankton on 2 July survived until 14 July, by which time they had developed shell spines (Fig. 8B) considered to be diagnostic of *H. arctica* (Abbott, 1974).

Isolation of *M. arenaria* from plankton samples was simplified by familiarity gained from rearing larvae from known parents, and also by sequential occurrence of natural spawning during the summer. Growth and maturation of isolated larvae into what were unquestionably soft shell clams (Table 1) showed that misidentification was normally a minor problem. There was only one serious exception, which was the 2 September isolation. This was carried out after *M. arenaria* larval abundance had declined from a seasonal peak and when larvae of the horse mussel, *Modiolus modiolus*, had begun to dominate the local bivalve larvae population. A subsequent refinement in isolation technique, entailing use of a more finely drawn micropipet to extract isolated *M. arenaria* from samples, resulted in a marked improvement over the "purity" of isolate cultures achieved in the midst of peak *M. arenaria* abundance. In retrospect, initial good results achieved using the cruder isolation technique appeared to have relied heavily on the naturally large propor-

TABLE 2. *COMPARISON OF SHELL LENGTH OBSERVATIONS IN LARVAL* MYA ARENARIA *WITH OBSERVATIONS OF PREVIOUS AUTHORS*

SOURCE	SMALLEST LARVAE OBSERVED (μm)	SMALLEST METAMORPHOSED LARVAE (μm)	LARGEST FREE SWIMMING LARVAE (μm)
Laboratory spawning (1975)	78	235	310
Plankton (1975)	~80*	240	330
Loosanoff and Davis (1963)	86	165	228
Sullivan (1948)	105	——	250
Jorgensen (1946)	82	200	320
Yoshida (1938)	——	240	300
Stafford (1912)	76	——	——

* Few initial measurements taken

tional representation of *M. arenaria* in the August collections.

In all instances, *M. arenaria* isolate cultures were maintained until the majority of individuals in each culture had undergone metamorphosis. Appearances of metamorphosed *M. arenaria* supported earlier evaluations of identity (Table 1). As with the larvae reared from known parents, the smallest plankton-obtained larvae observed to settle and undergo metamorphosis were approximately 240μm long. Occasionally, individuals initially between 280 and 330μm long were isolated from plankton samples. Many of these large larvae set as post larvae "spat" after two or three days in culture.

Rarely were any on the non-*M. arenaria* larvae in the plankton isolates determined or suspected to be *Hiatella* sp. Easily recognized late umboned *Hiatella* sp. were infrequent in plankton samples in August or early September, whereas they were common in mid-June and early July samples. Identities of the contaminant species were not confirmed other than by comparing present observations with photographs by Sullivan (1948) and Chanley and Andrews (1971).

From these comparisons *Spisula solidissima*, *Macoma* sp. and *Cerastoderma pinnulatum* appear to be among species reared with *M. arenaria* and could conceivably be mistaken for early-stage *M. arenaria* larvae. Another tentatively identified group including: *Mytilus edulis*, *Modiolus modiolus*, *Placopecten magellanicus*, *Ensis directus*, *Siliqua costata* and *Gemma gemma* (post larvae brought up into the plankton by water currents) have shapes and accompanying character differences that could not be easily mistaken for *M. arenaria* and were most likely inadvertantly introduced into culture as contaminants.

DISCUSSION

With regard to distinguishing larvae of *M. arenaria* from *Hiatella* sp., the present study determined that identification characters, useful in the examination of large numbers of bivalve larvae from plankton, were lacking through the larval life stages between late straight hinge (shell length of approximately 125μm) and late umbone (shell length of approximately 180-200μm). Early straight hinge *Hiatella* sp. were distinctive because of the "ship's bow" appearance of the ends of the hinge line. The straight hinge stage appeared to be very brief in the case of laboratory reared larvae: 3 to 8 days for *M. arenaria*, approximately 11 days for *Hiatella* sp. Late umbone *Hiatella* sp. larvae (shell length greater than 240μm) were distinctive in color and shape from all other bivalve larvae in plankton samples.

Easily recognized, late planktonic stage *Hiatella* sp. larvae were rare in plankton samples taken in August, 1975, suggesting that misidentification of *Hiatella* as *M. arenaria* would be negligible during that period. This was subsequently confirmed by rearing larvae, initially identified as *M. arenaria*, to metamorphosis (spat). In the Hampton Beach area, the abundance of *Hiatella* is low in late summer when *M. arenaria* is most abundant. Since spawning sequences were temporarally distant, discrimination of *M. arenaria* larvae (smaller than 240μm) from larvae of other species, particularly *Hiatella* sp. was aided as much by differences in relative abundance as by the investigators' ability to distinguish morphological differences.

With live plankton samples, coloration and arrangement of pigment was used to aid in the segregation of *M. arenaria* larvae from those of other bivalves. Earlier authors (Jorgensen, 1946; Sullivan, 1948; and Loosanoff and Davis, 1963) proposed the use of pigment characteristics for identification of bivalve larvae from plankton samples. Although there was disagreement as to which were diagnostic, there was agreement that these characteristics become more useful as the larvae mature; straight hinge larvae generally lack pigment.

Color, in particular, was observed to vary from culture to culture, and larvae obtained from plankton varied in color during the time they were in culture. On the other hand, *M. arenaria* larvae freshly obtained from plankton, consistently displayed deep brown coloration of the digestive gland as described by Sullivan (1948). This feature was conspicuous against a background of the nearly colorless body of early umboned larvae, and was increasingly accompanied by brown-black or black markings of the mantle and/or shell as the larvae grew older. In old *M. arenaria* larvae broken lines of black pigment paralleling the ventral shell margin (an identifying character proposed by Loosanoff and Davis, 1963) persisted even after prolonged culture and, therefore, might be

considered a dependable characteristic. However, *Hiatella* larvae which had not been studied by Loosanoff and Davis (1963) also display a similar characteristic.

Our observations on size of *M. arenaria* at onset of metamorphosis support observations by Yoshida (1938), Jorgensen (1946), Sullivan (1948) rather than those of Loosanoff and Davis (1963). Table 2 summarizes morphometric findings of authors cited above, as well as those of Stafford (1912) and compares their observations, with ours. Our rearing temperatures (16-19C) were lower than those used by Loosanoff and Davis (1963) (19-24C). If earlier authors also worked at cooler temperatures than Loosanoff and Davis (1963), then the larger size at metamorphosis could be explained by cold induced delay of maturation.

The *Hiatella* larvae we reared appeared to be identical with those of *Saxicava (=Hiatella) arctica* depicted by Sullivan (1948), but do not fit descriptions by certain European authors (Odhner, 1914; Lebour, 1938; Jorgensen, 1946; and Rees, 1950). Of the two likely European congeners: *H. arctica* and *H. striata (=H. gallicana =H. rugosa), H. arctica* appeared to be less like the North American *Hiatella* larvae described here and in Sullivan (1948). This larval unlikeness may have led Rees (1950) to declare that Sullivan (1948) had erred in identifying her larvae as *H. arctica.* Nevertheless, both Sullivan's photographic plates and present observations show development of shell spines on post larvae reared from plankton collections. According to Yonge (1971) spines on the shell were "formerly" regarded as diagnostic of *H. arctica.* Abbott (1974) uses this characteristic to distinguish post larvae of *H. arctica* and *H. striata.* Hunter (1949) stated that these spines could easily be lost by the boring or "nestling" habit of the settled juveniles.

Abbott (1974) also maintains (as originally stated by Lebour, 1938) that *H. striata* spawns in winter, while *H. arctica* spawns in summer. This suggests that the species we have dealt with is *H. arctica.* However, Abbott also describes eggs of *H. arctica* as red, and the eggs of *H. striata* as very light in color. Our observations of numerous spawnings showed the egg color of *Hiatella* sp. to be white, or at most, ivory in color, with no reddish hue. Furthermore, we observed a specimen of the spineless form of post larval *Hiatella* in a live plankton sample taken on 26 August (Fig. 8C).

We agree with Yonge (1971) that taxonomic relationships of *H. arctica* and *H. striata (=H. gallicana)* are "unusually obscure". Perhaps the discovery that the *Hiatella* we have dealt with can be spawned with relative ease will stimulate further investigation of taxonomic questions concerning the genus *Hiatella* from the western North Atlantic coast.

While the task of readily distinguishing *M. arenaria* from *Hiatella* is difficult without disarticulating the valves and examining the shell ultrastructure, it has been demonstrated that correct identification is likely, given adequate experience. The risk of misidentification becomes larger at the life stage where the straight-hinge line is obscured by developing umbones (at approximately 125μm) and decreases after the umbones are fully continuous with the shoulders of the valve margins. For very early straight hinge larvae and for larvae with well developed umbones, the identification error was found to be generally less than 20%.

ACKNOWLEDGEMENTS

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OBSERVATIONS OF *CRASSOSTREA VIRGINICA* CULTURED IN THE HEATED EFFLUENT AND DISCHARGED RADIONUCLIDES OF A NUCLEAR POWER REACTOR

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ABSTRACT

*American oysters (*Crassostrea virginica*) were rafted for 26 months at four sites in the effluent waters near Maine Yankee Nuclear Power Reactor in Montsweag Bay and at a control site in the adjacent Damariscotta River. In an evaluation of the thermal effluent for aquaculture, comparisons are made among the sites of the effects of heated effluent on oyster growth and condition, and the uptake and retention of gamma-ray emitting radionuclides. Growth and uptake of radionuclides were observed to be accelerated at the warmer water sites.*

Observed variations in concentrations of gamma-ray emitting radionuclides in the biological component of this study are compared with a pulse driven relaxator model and an existing concentration factor model. Results show that although the concentration factor model is adequate for simple laboratory studies, the pulse driven relaxator model is necessary to describe both the amplitude and time variation observed in this field study. Both experimental results and calculations for ^{58}Co and ^{54}Mn are presented.

INTRODUCTION

Our work since 1973 has been directed toward evaluating the potential use of the thermal effluent and waters surrounding the Maine Yankee Atomic Power Station located at Bailey Point on Montsweag Bay, Wiscasset, Maine, for the culture of the American oyster, *Crassostrea virginica*. In this study we have examined two major aspects of the potential use of thermal effluents in aquaculture. First, growth and quality of the oysters, and second, retention in the oysters of gamma-ray emitting radionuclides released into the environment by the power plant.

In the natural marine environment of Maine it appears possible to produce marketable oysters in a period of two to three years by the use of raft culture techniques. Favorable conditions for growth (temperature and algal food supply) have been found to exist between June and November and growth rates of oysters cultured in Maine appear to be equal or superior to those measured in traditional growing areas such as the Chesapeake Bay and the Gulf of Mexico (Packie, Hidu and Richmond, in manuscript). There are about six months (June to November) of optimal food and temperatures for oyster growth; the spring months (February to May) are not favorable for oyster performance. The latter months are limited by the low ambient temperature (below 8°C) which prevents oysters from taking advantage of the adequate food supply which is present in many areas (Galtsoff, 1964).

The marine environment as found along the cost of Maine is characterized by broad seasonal temperature fluctuations. The use of thermal effluents in aquaculture could prove advantageous

here by providing the temperature elevations above ambient necessary to allow oysters to utilize the existing algal food supply in the early spring. The growing season would thereby be extended and the time to market would be reduced.

The increasing demand for electricity by our civilization and the consequent construction of additional generating facilities in coastal areas will dramatically increase the number of thermal releases available for application in marine aquaculture systems. If present governmental planning is implemented a large part of this future generating capacity possibly will be nuclear.

Nuclear generating facilities are of particular interest because of the use of large volumes of water for cooling of these plants, is generally compatible with the biological requirements for accelerated gametogenesis and growth rates, and the extension of the growing season of many commercially valuable marine species.

The use of heated effluent from power plants for the culture of marine organisms has been discussed at length by many authors: Nash (1968), Burns (1969), Coutant (1970), Mather and Stewart (1970), Strawn (1970), Yarosh (1972), Huguenin and Ryther (1974), and others. Most of the projects utilizing thermal effluents in aquaculture have been of a commercial nature and the results of these efforts, because of their proprietary nature, are not readily available in the literature (Huguenin and Ryther, 1974).

We have determined annual physiological cycles of glycogen storage, percent total solids, shell growth, and the uptake and depuration of gamma-ray emitting radionuclides in American oysters (*C. virginica*) cultured in the effluent of the Maine Yankee nuclear power reactor. Three other points in Montsweag Bay and a control site in the Damariscotta River have also been examined.

Relevant studies at other locations have been undertaken with oysters by Jeffries and Preston (1969), Seymour (1966), Naidu and Seymour (1969), Wolfe (1970), Lowman, Rice and Richards (1971).

Studies of accumulation and depuration have been undertaken for numerous radionuclides in many marine organisms (Lowman, *et al.*, 1971) ^{58}Co in the mussel *Mytilus edulis* (Shimizu, *et al.*, 1971), ^{137}Cs and ^{60}Co in the marine clam *Mya arenaria* (Harrison, 1973) and ^{137}Cs and ^{60}Co in *Crassostrea gigas* (Cranmore and Harrison, 1975).

We have quantitatively measured the uptake and depuration of several radionuclides in the American oyster (*C. virginica*). As a result, we propose a mathematical model of the variation of gamma-ray emitting radionuclides in live oysters. The model considers the dynamics of both the biological and physical processes which control the aquacultural potential in the estuarine system studied.

METHODS

Site Locations

The Maine Yankee Atomic Power Company at Wiscasset, Maine, is powered by a pressurized water reactor and is rated at 855 MW. The plant is cooled by passing up to 960 cubic feet of water per second from Montsweag Bay over its condensers and discharging the warmed water into Bailey Cove, which empties into Montsweag Bay. Oyster tray stations were located in the intake channel (S-1), directly in the outflow effluent (S-2), above the effluent point in Bailey Cove (S-3) and below the effluent point of Long Ledge in Montsweag Bay (S-4) (Fig. 1). The control site was located in

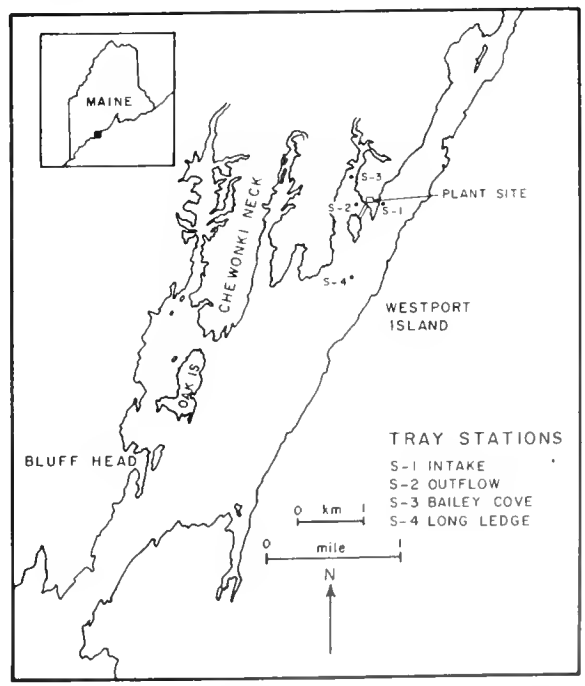

FIG. 1. *Map of Montsweag Bay showing location of tray stations.*

an adjacent estuary at the marine laboratory on the Damariscotta River.

Native American oysters, *C. virginica*, used in this study were obtained from a bed in the Piscataqua River. One hundred fifty oysters to be examined for glycogen content and percent total solids were distributed between two trays at each of the experimental sites and the control site. Additionally, 24 oysters were placed in a separate compartment of one tray at each site to be measured monthly in a longitudinal study of the accumulation of radionuclides.

Environment

Environmental factors which have been shown to influence the growth of shellfish (Galtsoff, 1964), were monitored. A Beckman field salinometer (Model RS5-3) was used to measure salinity and temperature every two weeks during high water at all stations. For one year, water samples were taken every other week at high water at the tray stations to evaluate the food available for the oysters. These samples were used in the determination of chlorophylls and particulate oxidizable carbon (Strickland and Parsons, 1965)*.

Biological

To determine the growth and quality of the oysters used in the study, a monthly random sample of 12 oysters was collected from the field sites for one year. They were held in the laboratory in water collected at the field sites. Fouling organisms were removed. Each oyster was blotted dry, weighed, and measured. All measurements (height, length, and width) were of the maximum dimensions of any parameter. New shell growth was measured on the right and left valves at the location of maximum growth. The larger value was used in calculating the average shell growth of the 12 oysters at a given station. The oyster from each station which most nearly approached the average was selected from each group and photographed.

To determine the condition of the oyster meats the oysters were shucked taking care not to pierce the meat. The meats were allowed to drain for one minute on a plastic mesh and then weighed. All 12 oyster meats from a station were then homogenized. Glycogen was extracted from the homogenized meats according to the method of Burklew (1971). This method employs the digestion of 5 gram aliquots of the homogenized oyster tissue in hot NaOH (30%), followed by the precipitation of glycogen with ethanol (95%). The precipitate is hydrolized with concentrated HCL, and the glucose present is determined by the addition of anthrone dissolved in concentrated H_2SO_4. The color change is measured spectrophotometrically and the milligrams of glucose present calculated from a standard curve.

The percent total solids of oyster tissue has also been widely used to determine oyster condition (Shaw, Tubiash and Barker, 1967). In this study percent solids are calculated from an average of the dry weight of three 5-gram aliquots of the homogenate using the formula:

$$\text{Percent solids} = \frac{\text{dry weights of meats}}{\text{wet weights of meats}} \times 100$$

Radionuclides

In order to detect the possible accumulation of gamma-ray emitting radionuclides, the groups of 24 oysters were taken from their respective stations, scrubbed with a stiff brush to remove fouling organisms, and transported to the environmental radioactivity laboratory at the University of Maine Department of Physics. The outflow station (S-2) was sampled every month. The control (SC), intake (S-1), Bailey Cove (S-3) and Long Ledge (S-4) stations were sampled every other month.

Approximately 1 kilogram of live oysters (selected at random) from each of the groups of 24 was counted for 5000 seconds. The resulting data was computer processed (IBM 360/370) using the Compton continuum subtraction method (Covall, 1959). After counting, the oysters were returned to their original locations to be measured in the following months.

The gamma-ray measurements were carried out using a Ge(Li) detector (Ortec) with 2400 lb low background lead shield. The pulses were amplified

* Additional information on possible available food was taken from Maine Yankee Atomic Power Company Semi-Annual Environmental Surveillance Reports #2-6, (1973-1975) McAlice, "Net phytoplankton and Microzooplankton" and Crippen & Lindsay, "Entrainment Studies".

with a spectroscopy amplifier (Ortec 452) and the bias was provided by a high voltage supply (Ortec 459). The pulses were processed by a multichannel analyzer (Northern Scientific NS-700) with 2048 memory channels and outputted using a teletype to produce a list and a punched tape. The numbers of counts determined were converted from counts/minute into disintegrations per second by using the efficiency determination for the same geometry. Both branching ratios and the variation of efficiency with energy were taken into account. From disintegrations per second, the number of picocuries/gram was determined for each of the gamma-ray peaks which exceeded a statistical criterion. Picocuries/gram of those radionuclides in our library of branching ratios were computed automatically, and new or unidentified peaks were processed by hand calculations.

The efficiency versus energy curve was determined by placing several standard sources (Environmental Protection Agency Analytic Quality Control Laboratory, Las Vegas, Nevada) in a solution of demineralized distilled water. The solution was placed in a 1.0 liter Nalgene cylindrical bottle (which was our standard geometry), and measured. The peaks from the standard sources were analyzed in the same way as the peaks from the unknown. The graphical analysis of the efficiency versus energy was plotted on log-log paper and tested for linearity, and consistency. The results of these periodic calibrations were used to update the computer program. New branching ratios were entered as required. A typical spectrum of gamma-rays is shown in Figure 2.

Model Theory

Constant concentration theories have been suggested in the laboratory studies by Polycarpov (1960), Ruzic (1972), and Davis and Foster (1972). In such theories, the radionuclide concentration in the oysters, C_o, is related to the radionuclide concentration in the sea water C_w, by concentration factor K.

$$C_o = K C_w$$

This factor K becomes larger with time until it reaches an equilibrium value, if the concentration C_w may be found by dividing the released

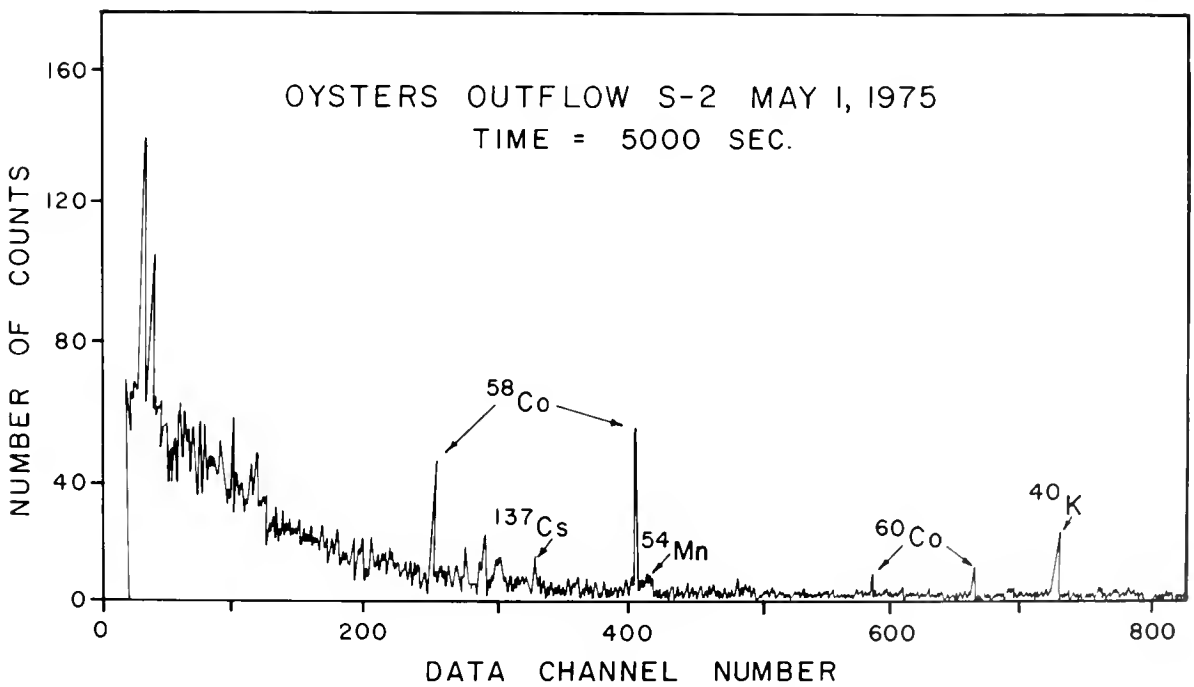

FIG. 2. *Typical Gamma-ray Spectrum: Data channel number on the horizontal axis (energy in keV equals data channel number × 2) and number of counts on the vertical axis (number of counts/5000 sec).*

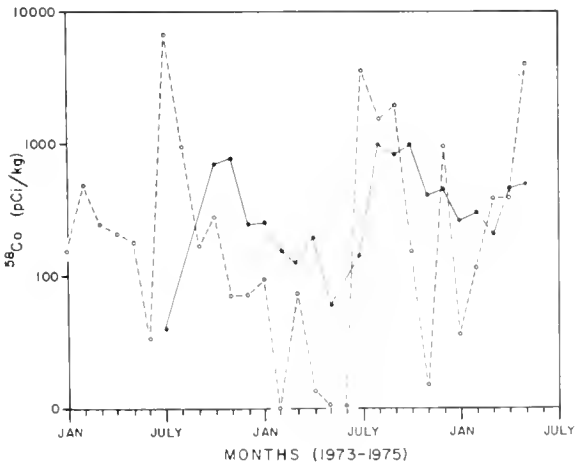

FIG. 3. *Comparison of experimental results for uptake of* ^{58}Co *at outflow, S-2, (-•-•-•-), and predicted values based on the specific concentration theory (--o--o--o--).*

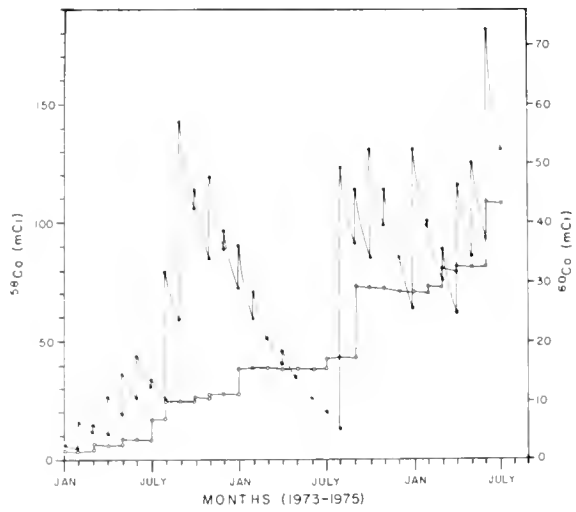

FIG. 4. *Results of pulsed relaxator theory for* ^{58}Co *(-•-•-•-) and* ^{60}Co *(-o-o-o-).*

radioisotope in curies, f_i, by the volume of water used for the release V_w.

$$C_w = \frac{f_i}{V_w}$$

Using this equation in a dynamic situation, as in the case of reactor releases, will give values of K which are less than the equilibrium value for K as found in a laboratory situation. Thus, calculations using laboratory values of K in the case of reactor releases inaccurately estimates the radionuclide concentration (Fig. 3).

To develop a dynamic model of variations in the uptake and depuration of radionuclides by the oysters, the release rates of radionuclides by the reactor was used as the driving source of a multimode pulsed relaxator system. The resulting differential equation may be solved by integration to give exact solutions if appropriate simplifying assumptions are made. These assumptions are that the reactor releases monthly by injecting the nuclides into the estuary in a short time (several hours). The nuclides are then accumulated by the oysters, and are slowly reduced by radioactive decay and by biological cycling, depuration, in the oysters. Initially we assumed that the oysters had constant depuration over the entire year. Later these assumptions were modified to include: a) variation in nuclear reactor plant operations (shut-downs, plant discharge rate, power output, etc.), b) oyster biological parameters (growth rate, glycogen content), and c) estuarial parameters (temperature, salinity, current velocities, standing crop, etc.). As our understanding has improved we have found that by using this radionuclide uptake model, predictions can be made to establish an optimum release pattern for the reactor in order to minimize oyster uptake of radionuclides.

Model

The uptake of radionuclides may be described by a first order linear differential equation:

$$\frac{dN}{dt} = -\lambda N + R(t), \qquad 1)$$

where $\frac{dN}{dt}$ is the increase in atoms of a radionuclide, λN is the rate of loss due to radioactive decay, and R(t) is the rate of introduction of radionuclide from an external source (i.e., the nuclear reactor release schedule). Depuration may be included by creating an "effective lambda" (the sum of the radioactive decay constant and a biological decay constant).

The solution to equation 1 may be written:

$$N = e^{-\lambda t} \int e^{\lambda t} R(t)dt + ce^{-\lambda t} \qquad 2)$$

We assume that releases of radionuclides are made in a sequence of m times (t_1, t_2, t_3.....t_m), and

the amount of nuclide released is given by a function f(t) which for times greater than or equal to t_1, but less than t_2, is given by $f_1(t - t_1)$, and for times greater than or equal to t_2, but less than t_3, by $f_2(t - t_2)$, and so on up to times greater than t_m. The fraction of the radionuclide which is released by the reactor and is retained by the oysters is given by U, so that for the accumulation N(t) we have:

$$N(t)' = e^{-\lambda t}\int_{0}^{t_1+\in} e^{\lambda t} f_1 \delta(t - t_1)\, U dt + \cdots$$

$$+e^{-\lambda t}\int_{t_{m-1}+\in}^{t_m+\in} e^{\lambda t} f_m \delta(t - t_m)\, U dt + ce^{-\lambda t}$$

Assuming U is a constant ratio for retention at all times, we can construct a table of solutions for the intervals between the release times.

$0 \leqslant t \leqslant t_1 \quad N(t) = ce^{-\lambda t}$

$t_1 \leqslant t \leqslant t_2 \quad N(t) = Uf_1 e^{-\lambda(t - t_1)} + ce^{-\lambda t}$

$t_2 \leqslant t \leqslant t_3 \quad N(t) = Uf_2 e^{-\lambda(t - t_2)} + Uf_1 e^{-\lambda(t - t_1)} + ce^{-\lambda t}$

These equations may be interpreted as exponential decay of radionuclides from one release-time until the next release-time. Sudden increases occur at each release-time. Graphs of two typical cases are shown in Figure 4 for a half life for ^{58}Co comparable to two release intervals, and a half life for ^{60}Co comparable to sixty release intervals.

The values for the effective lambda may be determined by using the known values for physical decay, and by either measuring the biological decay constant, or by reference to results in the literature for the biological decay of the particular species involved.

RESULTS AND DISCUSSION

Model Use

Use of this model enables prediction of the accumulation of radionuclides by oysters (Fig. 5). Curve A results only when a physical decay constant (corresponding to a half life of 70 days for ^{58}Co) is used. Curve B employs this physical decay constant and a biological decay constant. The biological decay constant for ^{58}Co corresponds to a half life of 35 days. Curve C is the measured radioactivity at the outflow site, S-2. One can see

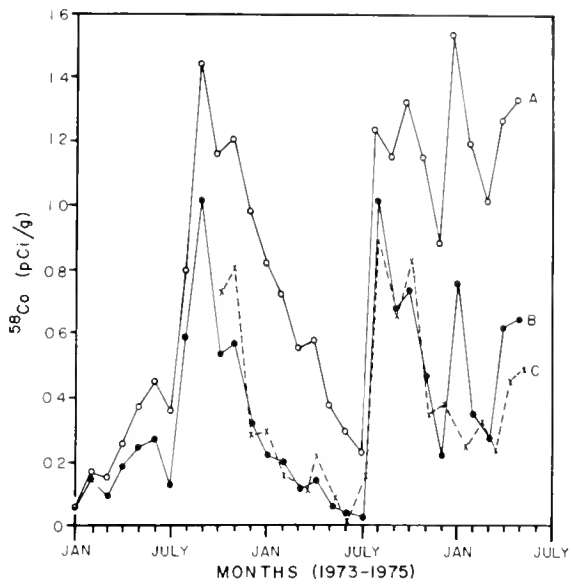

FIG. 5. *Pulsed relaxatory theory using: A. Only physical decay constant (-o-o-o-).*
B. Theory using physical decay constant plus biological decay constant (-•-•-•-).
C. Experimental results (--x--x--x--).

how the theoretical prediction is brought in line with the measured values when both physical and biological mechanisms are combined to form the effective decay constant, lambda. Determining the value of the effective lambda in this way, one can then scale the theoretical predictions to the experimental measurements to determine the value of U for each site. The process of determining the effective lambda and U may be iterated to improve the initial estimation of these values.

The value obtained for U at a particular site is determined by the proximity of that site to the point of radionuclide releases, and the biological and physical oceanography of the site. Factors like temperature, salinity, currents, dissolved oxygen, standing crop and species metabolism play various roles in the determination of U. Thus U should differ from site to site and is a function of season. We have found that reasonable agreement with experimental results is obtained for the simple assumptions that U is constant with time, and that U decreases with distance from the release point measured along current flow paths in the estuary.

The values of the biological decay constant are species and radionuclide specific and are a function of season. The time variations in the biological decay constant are caused by factors similar to those which cause variations of U. However, reasonable agreement between theory and experiment can again be realized with a constant average value for this parameter. U values for ^{58}Co are a function of distance from the discharge point (Fig. 6).

Control Station

Oysters placed at the control site (April, 1973) in the Damariscotta River first exhibited shell growth in May as the temperature at this site rose above 8°C (Fig. 7). Gametogenesis occured during June and July as indicated by a decrease in glycogen, increase in percent total solids and a characteristic decrease in the rate of shell growth. Shell growth resumed in August but ceased to be measurable in September. This is correlated with a decrease in available food since the standing crop of phytoplankton dropped out during this period. Seasonal fluctuation in temperature has been used to determine periods of feeding, reproduction, and shell growth in given localities (Galtsoff, 1964). Since above 8°C oysters in Maine appear to be actively feeding and growing, and temperatures at this site were above 8°C until November, the cessation of shell growth observed in September is thought to be a function of food available to the oysters. The following spring, with adequate food available in April and water temperatures rising to 8°C, shell growth resumed. This cycle of growth has continued.

The results of measurements made to detect the accumulation of radionuclides by control oysters showed that radionuclides of reactor origin were below minimum detectable levels in oysters at this site.

Outflow Station

In the attempt to evaluate the effect of the thermal effluent, oysters were placed directly in the outflow (S-2) in April of 1973. Shell growth was evident after two weeks of exposure to elevated temperatures found in these waters. There was also an immediate drop in glycogen, and an in-

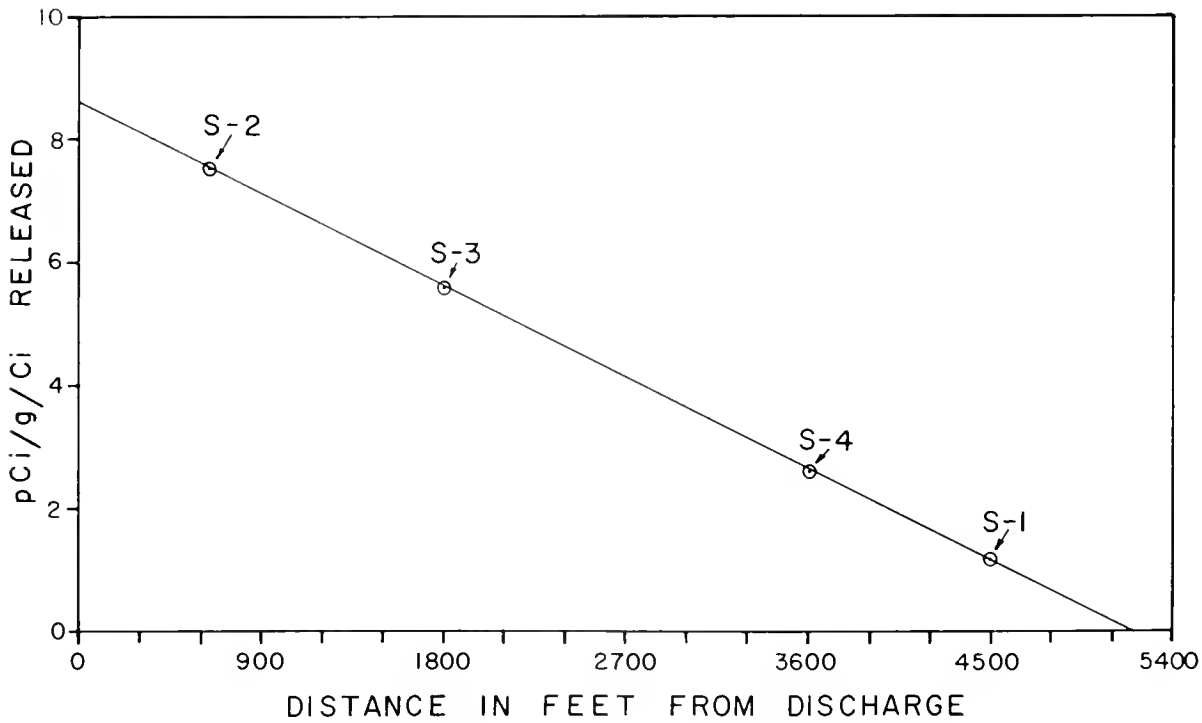

FIG. 6. *Ratio of retention, U, versus distance from the discharge point measured along most direct water route.*

crease of total solids both of which were correlated with gametogenesis. During May the rate of shell growth decreased as oysters at this station spawned. In June, 1973, shell growth resumed and continued through October, 1973. Except for brief plant shutdowns, the water temperature remained elevated throughout the winter. Cessation of shell growth during the fall corresponds well with a decrease in available food. Shell growth resumed in March, 1974, probably due to a spring bloom of phytoplankton and elevated temperatures found at this station. In the Spring of 1975, shell growth started in February, one month in advance of when it had occurred in 1974. Again this is attributable to a spring bloom of phytoplankton and elevated temperature (Fig. 8A).

The radioactive uptake and loss as predicted by theory is seen to be in good agreement with the measurements at this station (Fig. 8B). However, three types of deviation from predictions by the theory are observed. These deviations occur in the months: a) January, 1974, and December, 1975; b) January, 1975; and c) March, 1974, and February, 1975.

In January 1974, and December, 1975, we observed increases in measured radioactivity above that which was predicted by theory. We have no exact explanation for these increases in the retention of ^{58}Co, but it is interesting to note both deviations occur after cessation of shell growth and during a period of steadily decreasing temperatures ($\Delta T \sim 10°C$). The regularity of these deviations point to a seasonal physiological mechanism as controlling these events.

Significantly less radioactivity than predicted by the theory was observed in January, 1975. In addition to cessation of shell growth, the removal of the causeway at the north end of the bay increased cold water circulation, causing temperatures at this site to approach as low as 8°C. The associated changes in oyster metabolism (decrease or cessation of activity) likely caused a decrease in the rate of uptake of cobalt by about a factor of three.

The deviations from theory which occur in April, 1974, and February, 1975, are higher levels than predicted and correlate well with periods of initial shell growth. Since a major portion of ^{58}Co has been found in the shell, it is reasonable that the resumption of shell growth would be marked

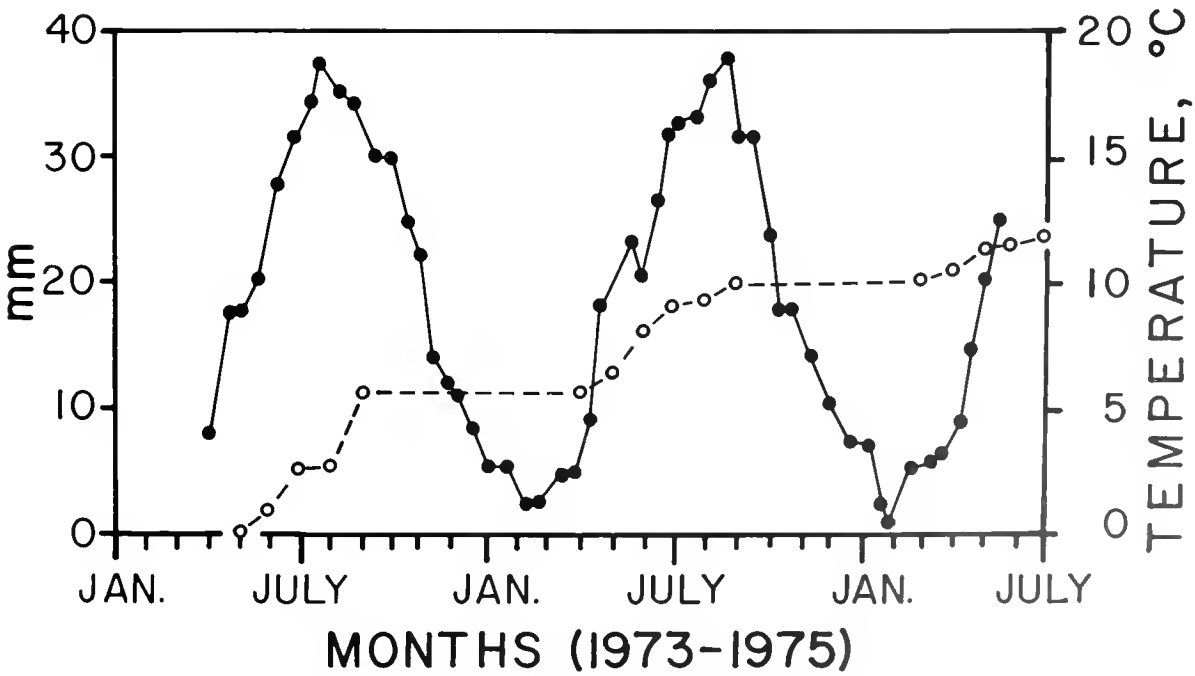

FIG. 7. *Cumulative oyster shell growth (--o--o--o--) in mm. and temperature (-•-•-•-) in °C at control site (SC).*

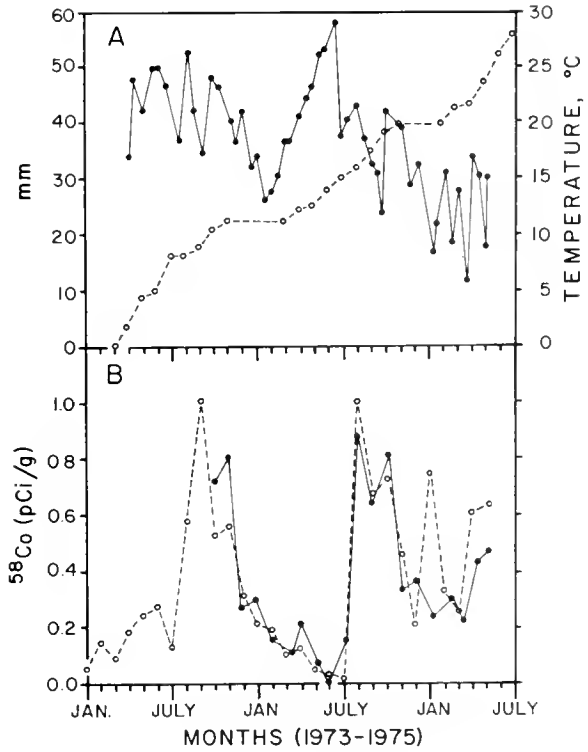

FIG. 8. *A. Cumulative oyster shell growth (--o--o--o--) in mm. and temperature (-•-•-•-) in °C at the outflow site (S-2).*
B. Predicted levels of ^{58}Co using pulsed relaxator theory (--o--o--o--) and measured levels (-•-•-•-) at the outflow site (S-2).

by an increase in the measured levels of ^{58}Co.

The variations in the amounts of ^{58}Co measured in oysters at S-2 during 1974 and 1975 are compared in greater detail in Figure 9. Peaks A and B both occur in the last month of shell growth. A rapid decline to points C and D is observed during both years. This is followed by a modest increase and then a decline to points E and F. Up to points E and F, the similarity of rate accumulation and loss in two different years point to an annual physiological cycle which controls this process. This is substantiated by the decrease shown from F to G in 1974. The slope of this line is due essentially to radioactivity decay. This is masked in 1975, points E to H, by the uptake associated with the resumption of shellfish growth, and is similar to that which occurred (G to I) in the spring of 1974.

Another radionuclide observed in oysters at the

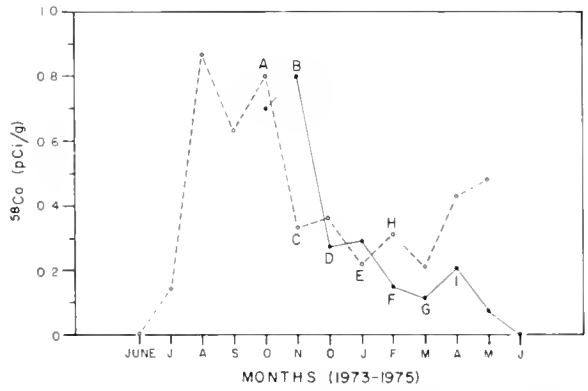

FIG. 9 *Comparison of radionuclide (^{58}Co) uptake by oysters at the outflow station during 1973-74 (-•-•-•-) and 1974-75 (--o--o--o--).*

outflow (S-2) was ^{54}Mn. Figure 10 illustrates a comparison between measured values of radioactivity in oysters at S-2 (for ^{54}Mn) and theoretical prediction. For ^{54}Mn the effective decay constant is 6.3×10^{-3} days^{-1} corresponding to an effective half life of 110 days.

Upper Cove Station

Oysters were placed at the Upper Cove station, S-3, in April of 1973. Gametogenesis was evident in April, 1973, and shell growth first became evident in May, 1973. Oysters in the June, 1973, sample had spawned and were recovering glycogen. Shell growth continued until November, 1973, (Fig. 11A). The general features of the graph depicting the accumulation of radionuclides (Fig.

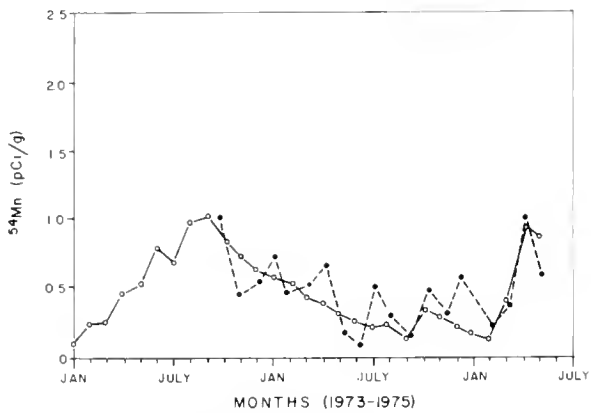

FIG. 10. *Theory (-o-o-o-) and measured levels (--•--•--•--) for the uptake of ^{54}Mn at the outflow site (S-2).*

11B) are similar to those found for oysters at the outflow (S-2) station (Fig. 8B). There is no doubt that some resolution is lost due to the less frequent sampling at the S-3 site (every other month).

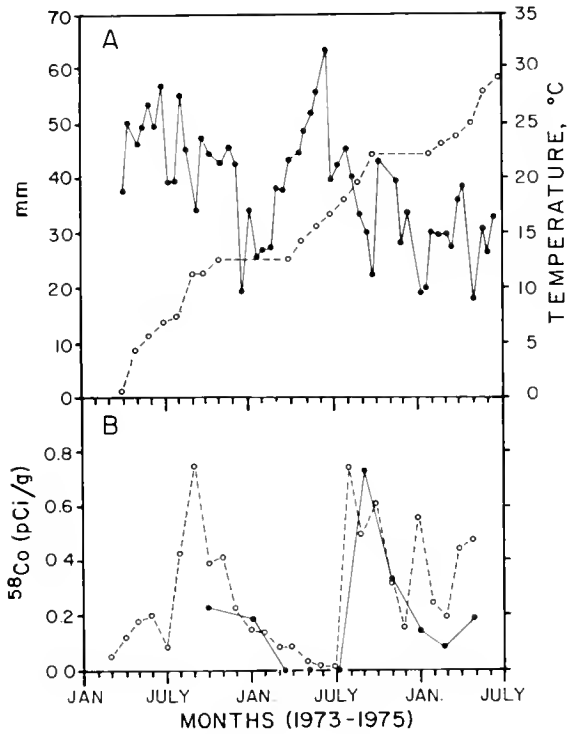

FIG. 11. *A. Cumulative oyster shell growth (--o--o--o--) in mm. and temperature (-•-•-•-) in °C at the Upper Cove site (S-3).*
B. Predicted levels of ^{58}Co using pulsed relaxator theory (--o--o--o--) and measured levels (-•-•-•-) at the Upper Cove site (S-3).

Other Stations

The general features of oyster growth and the uptake and depuration of radionuclides at the intake (S-1) and Long Ledge (S-4) (Figs. 12 and 13), are observed to be similar to those found at outflow (S-2) and Upper Cove (S-3). The fact that they are lower by a factor of five at the intake (S-1) and by a factor of three at Long Ledge (S-4) is related to their distance from the discharge point, the thermal differences associated with these sites, and the consequent variations in oyster metabolism (Fig. 14) and exposure to radionuclides. For a comparison of the accumulation of ^{58}Co by oysters at all sites see Figure 15.

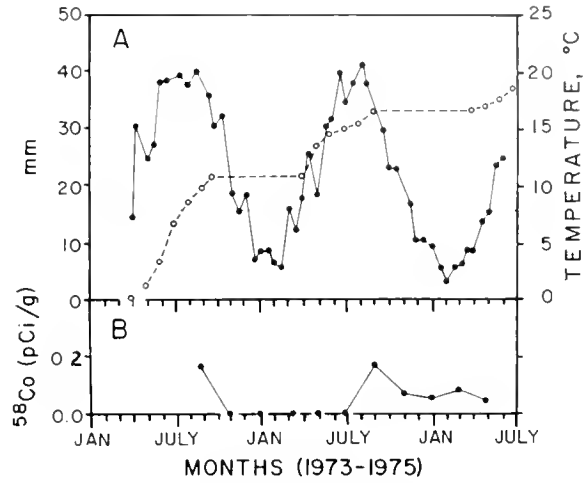

FIG. 12. *A. Cumulative oyster shell growth (--o--o--o--) in mm. and temperature (-•-•-•-) in °C at the intake site (S-1).*
B. Measured levels of ^{58}Co at the intake site (S-1).

The magnitude of cumulative shell growth, in descending order, was the Upper Cove site (S-3), 58.0 mm SD 8.29; the outflow site (S-2), 55.2 mm SD 6.13; the intake site (S-1), 32.0 mm SD 5.16; the Long Ledge site (S-4), 34.0 mm SD 4.33; and the control site (SC), 23.4 mm SD 2.26, respectively. Oysters at all sites in Montsweag Bay showed significantly (.05 level) greater cumulative

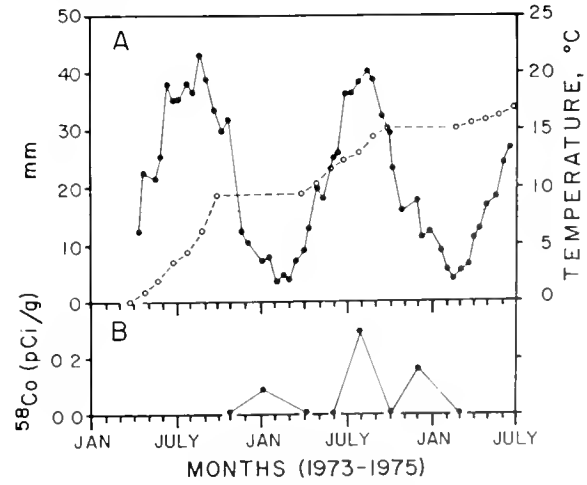

FIG. 13. *A. Cumulative oyster shell growth (--o--o--o--) in mm. and temperature (-•-•-•-) in °C at the Long Ledge site (S-4).*
B. Measured levels of ^{58}Co at the Long Ledge site (S-4).

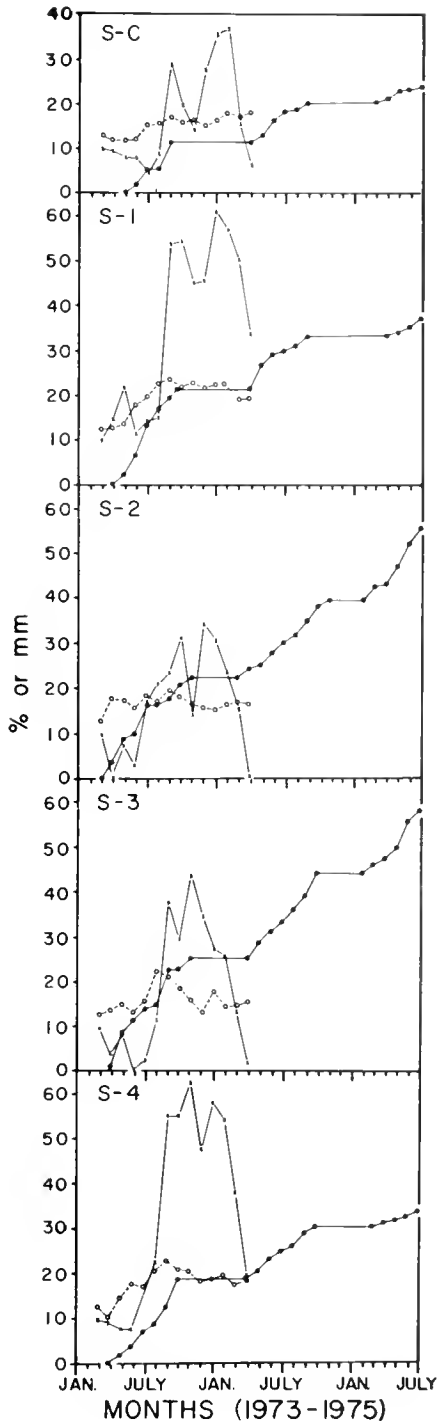

FIG. 14. *Oyster performance at all stations. Cumulative shell growth (-•-•-•-) in mm., glycogen (-x-x-x-) as percent dry weight, percent total solids (--o--o--o--).*

shell growth when compared to controls. Cumulative shell growth was not significantly (.05 level) different at the two warm sites (S-2 and S-3), when compared with each other nor were the cooler water sites (S-1 and S-4) significantly (.05 level) different from each other. There was a significant (.05 level) difference between the cumulative shell growth of oysters at the warm water sites (S-2, S-3) and those at the cooler sites (S-1, S-4) (Fig. 17). Oysters of excellent market quality were observed during the fall and through the winter of 1973-74 at both the intake (S-1) and Long Ledge (S-4) sites. Mortality at all stations was less than

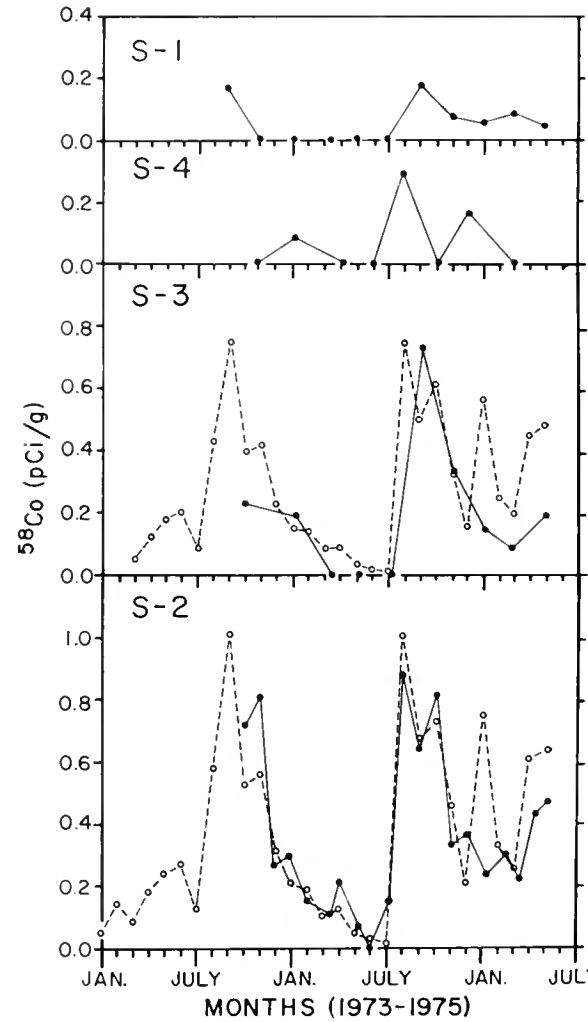

FIG. 15. *Measured uptake of ^{58}Co by oysters at all stations (-•-•-•-). Predicted levels at outflow (S-2) and Upper Cove (S-3) using pulsed relaxator theory (--o--o--o--).*

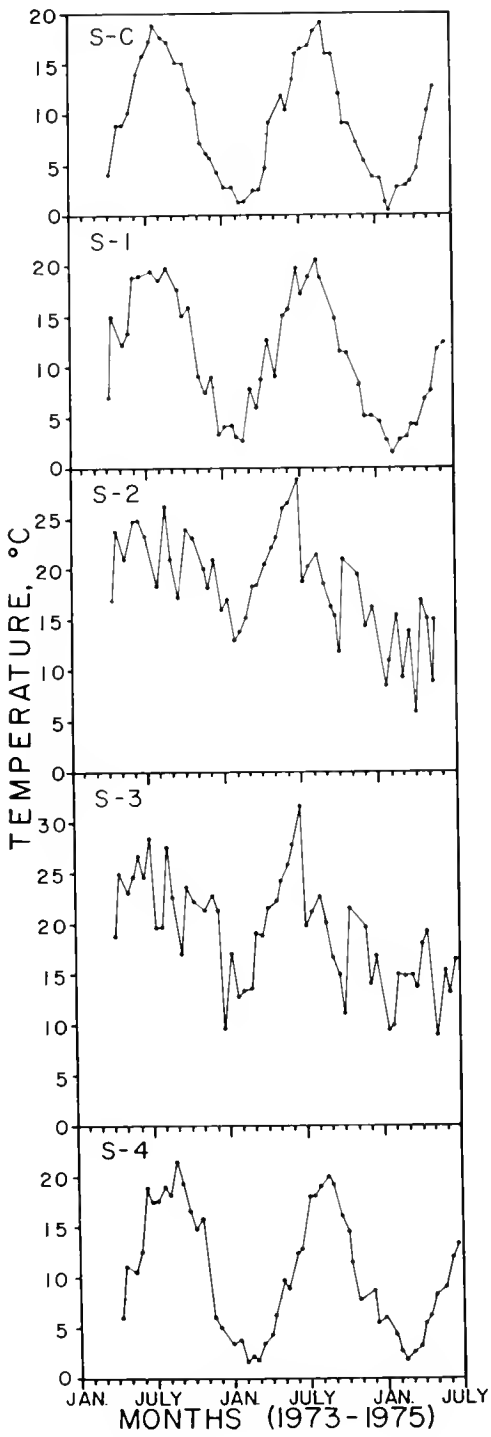

FIG. 16. *Temperatures in °C recorded twice a month at each station. Dotted horizontal line at 8°C represents lower temperature limit of oyster growth.*

5% and there was no significant difference between mortalities when calculated at the .05 level. The meats of the oysters at the Upper Cove site (S-3) and the outflow (S-2) were comparable to controls (Fig. 14).

CONCLUSIONS

The data gathered in this study suggest that a limiting biological factor in optimizing a system which utilizes the waste heat from power plants, is the availability of food for the oysters. The thermal addition of the power plant provides a sufficient increase in temperature (Fig. 16) to allow the oysters located in the warmer sites to take advantage of the increased food available in the early spring. The growing season terminates by November as the available food decreases in the fall even though temperatures remained sufficiently elevated to permit growth.

An additional limiting factor in the use of the warm effluent water is the proliferation of the marine worm *Polydora ligni* and *P. websteri*, which adversely affected the commercial value of oysters from the warmer water sites (Fig. 18).

In examining the potential for the use of thermal discharges for the culture of *Crassostrea virginica*, we have developed a theory of uptake and depuration of radionuclides that is sufficiently descriptive (using the two parameters, λ the effective decay

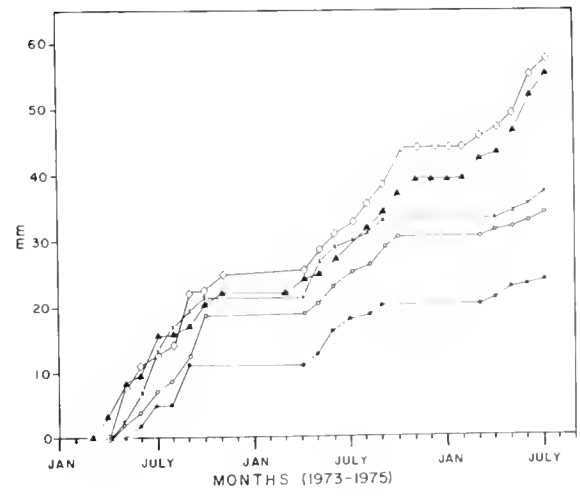

FIG. 17. *Comparison of cumulative oyster shell growth at the control site, SC, (-•-•-•-), intake, S-1, (-x-x-x-), outflow, S-2, (-▲-▲-▲-), Upper Cove, S-3, (-◇-◇-◇-), and Long Ledge, S-4, (-o-o-o-).*

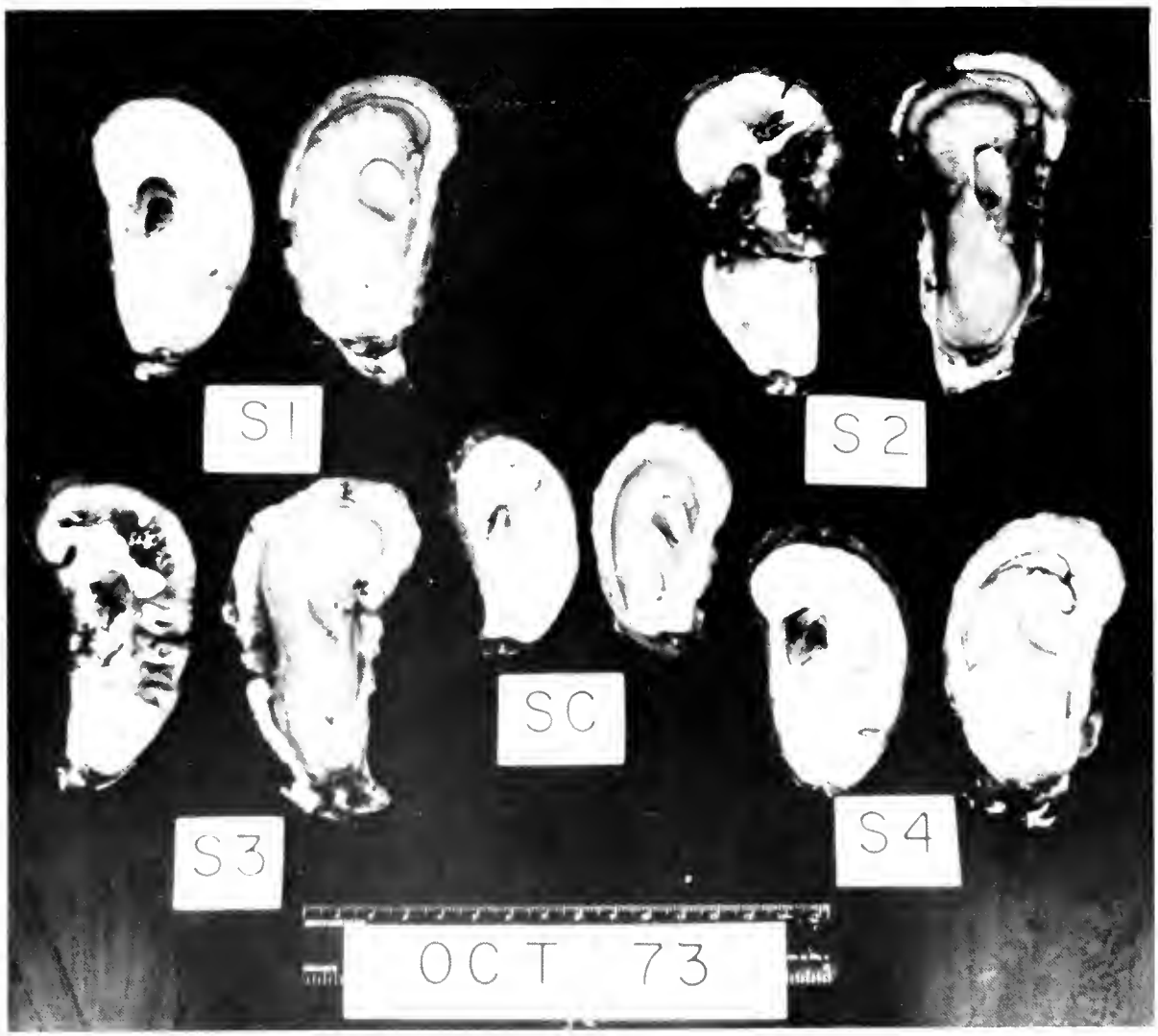

FIG. 18. *Photograph of oysters from all stations showing infestations of* Polydora *at warm water sites. (Black and white print from Kodachrome slide.)*

constant, and U the ratio of retention), to observe variations caused by specific biological processes.

We have found in this study that the concentration factor model does not adequately describe the dynamic nature of the uptake and loss of radionuclides for the case in which the source of these nuclides varies with time. The model described here, which utilizes the release schedule of an atomic power plant to describe the true variation of the source of radionuclides, is in good agreement with values measured in oysters located at various distances from the discharge point.

The practical feature of the theory is its predictive nature. Based upon the seasonal variations in shellfish physiology one can suggest an optimal release schedule that will minimize the concentration of radionuclides in oysters.

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PURIFICATION OF BASKET-HELD PACIFIC OYSTERS IN THE NATURAL ENVIRONMENT

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ABSTRACT

This study demonstrates the practicality of the purification of coliform bacteria contaminated Pacific oysters held in wire-mesh containers. Environmental parameters exert little influence upon the process and oysters reached equilibrium with local conditions within a maximum 48 hours. The technique is suggested as an economical alternative to traditional relaying, or as an adjunct to depuration plant cleansing.

INTRODUCTION

The majority of shellfish producing areas of the world are either nearshore or estuarine and frequently subject to the deleterious effects of human activity. The most serious of these problems is bacterial contamination. Bacterial pathogens are of primary epidemiological interest, but difficulties of recovery of sporadic or rare organisms renders their enumeration technically difficult, so sanitary criteria are based upon the abundant coliform group, a direct indicator of pollution by mammalian or avian faeces. All studies demonstrate that these organisms when ingested by molluscan shellfish, behave in a way representative of other bacteria, and probably also of viruses (Hoff and Becker 1969). A decline in the coliform group, and particularly *Escherichia coli*, is parallelled by reduction in other ingested microorganisms.

Sewage contaminated molluscs, when transferred to clean water, undergo a rapid reduction of coliform organisms. It has long been an established commercial practice to relay shellfish from polluted to clean areas some weeks prior to market distribution. An expansion of this natural cleansing is the development of facilities for artificial purification (generally termed "depuration"). Contaminated shellfish are placed in tanks or lagoons supplied with bacteriologically clean water. Economics of operation of such a plant dictate a maximum cleansing period of around 72 hours, but this may not be sufficient for grossly polluted shellfish. A preliminary basket cleansing period could bring the shellfish within acceptable limits for total cleansing in 24 to 48 hours sojourn in the depuration plant, without a great increase in labour or general costs.

Traditional relaying operations are hampered everywhere by the lack of adjacent foreshores suitable for purification. This procedure is also expensive as it involves double handling, and in zones constrained by tidal conditions, may withdraw manpower more profitably directed to harvesting. Use of baskets substantially reduces costs as individual shellfish need not be handled. Sites may be selected on water quality alone, as baskets may be placed on rocky foreshores, or supported by racks on excessively soft beaches. Baskets may also be suspended from rafts. Baskets may be so placed on the beach, or attached to floated lines, so recovery can be made at any time

convenient to depuration plant operation, irrespective of tidal conditions.

Flexibility of purification site selection renders it likely that at least a small region of suitable water will be available within a reasonable distance from rearing and market sites, and could be so designated and protected by governmental action. In some situations, depending upon the species of mollusc involved, the level of pollution and the quality of the cleansing water, basket purification will be sufficient to ensure a safe product for market. This approach will be of interest to developing countries, where expensive and complex depuration plants do not furnish the answer to local problems. Basket purification will also be of interest to those regions with depuration facilities, by ensuring a reasonable purification time and also as a means of maintaining continuity and uniformity of supply.

MATERIALS AND METHODS

Pacific oysters, *Crassostrea gigas* (Thunberg), gathered from x small area of a grossly polluted bed were loaded into baskets and trahsferred to the mid-tide level of a foreshore with low coliform incidence. The baskets, fabricated from galvanized iron mesh (62 x 62 x 30 cm), held approximately 55 kilos of oysters and were easily handled by two people using hooked carrying poles (Fig. 1).

Eight baskets were used for each experiment, timed to coincide with average seasonal temperatures and bacterial levels over a 2-year period. Each basket was sampled randomly by removal of 12 oysters, at 0, 24, and 48 hour intervals, at the same time three 12-oyster samples were taken from local oyster stock, to provide a base-line for the evaluation of coliform MPN changes.

Most Probable Numbers were obtained using

FIG. 1. *Photograph showing baskets loaded with oysters and handling poles used to lift baskets into boat.*

the standard five-tube geometrical dilution method with Brilliant Bile Green Broth and E.C. Elevated Temperature Media after inoculation into Lauryl Tryptose Broth. Differential tests were undertaken periodically with the IMViC series of reactions to confirm the positive elevated temperature tubes. Results were based upon E. coli, but the general coliform situation was also monitored as a confirmation of results.

Preliminary experiments were made to estimate MPN variability of sewage polluted oysters, the effect of basket loading, uniformity of bacterial reduction in the upper, mid, and lower levels of the baskets, and the intrinsic statistical reliability of the standard MPN method. These data were used to achieve an experimental design that considered replication, randomization, effect of population depletion, and variability in bacterial accumulation and depletion.

The roles of temperature, salinity and turbidity

TABLE 1. *Coliform and* E. coli *MPNs for transferred and local Pacific oysters.*

				Transferred				Local			
				Coliform		*E. Coli*		Coliform		*E. coli*	
			temp	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
December	28	1967	7.5	1,970	1,246	559	661	630	325	12	0.7
	29		7.0	1,378	927	81	159	700	381	14	2
	30		7.0	611	382	11	10	490	198	7	0.7
January	8	1968	5.0	9,687	8,110	1,530	1,200	260	29	68	17
	9		4.5	760	325	107	46	240	29	45	35
	10		5.2	306	132	56	23	330	—	45	7
March	20	1968	3.5	3,662	2,353	351	264	950	551	14	3
	21		3.5	1,227	601	116	39	940	440	12	1
	22		4.0	860	608	25	25	940	212	14	2
July	8	1968	21.0	1,000	576	243	141	330	282	<1.8	—
	9		19.0	285	48	25	26	290	99	<1.8	—
	10		20.0	311	80	3	3	330	156	<1.8	—
December	14	1968	7.0	77,000	59,500	6,950	6,457	1,800	425	170	43
	15		6.0	5,616	6,849	370	250	1,700	370	130	61
	16		6.2	1,613	1,650	181	105	1,700	142	130	57
March	11	1969	1.0	30,125	56,232	4,837	4,887	1,300	720	170	16
	12		3.0	760	325	107	46	790	425	68	32
	13		1.5	416	178	136	125	540	70	70	3
May	13	1969	17.0	1,330	778	372	259	280	85	<1.8	—
	14		17.6	249	154	41	40	130	113	7	1.4
	15		18.0	159	26	16	8	130	98	9	2.0
August	11	1969	20.0	128	32	63	33	78	37	3	1
	12		19.0	121	22	7	5	80	17	<1.8	—
	13		19.5	80	33	6	5	78	0	<1.8	—
February	6	1970	6.0	215,000	38,800	186,000	41,300	1,325	670	700	440
	7		7.5	4,050	3,260	397	230	1,300	283	210	29
	8		7.0	1,380	575	270	250	1,400	425	210	169
June	17	1974	18.0	5,825	3,667	663	453	230	29	<1.8	—
	18		19.5	443	340	40	44	210	—	2	—
	19		18.0	220	70	2	0	230	15	<1.8	—

in the cleansing process were monitored in 350 litre fibreglass aquariums using filtered, ultraviolet irradiated sea water and naturally sewage contaminated oysters. Various turbidity levels were obtained by addition of weighted suspensions of kaolinite.

MPN Variability and Experimental Design

The Most Probable Number concept as first used by McCrady (1915) was limited to populations randomly distributed in a liquid medium. A large literature demonstrates the low accuracy of the multiple tube fermentation method, especially when applied to oyster meats. Allen (1932) showed that if the relative probable error is to be 5%, a minimum 1000 tubes were required for each dilution; and with five tubes per dilution, Halvorson and Ziegler (1933) reported the MPN, at 95% confidence level, between 260% above and 70% below the true count. This unreliability makes data interpretation with a few samples open to wide error.

Twenty 12-oyster samples collected from a small quadrat on the polluted beach in late spring yielded a mean coliform MPN 1160 and 480 for faecal coliforms, with standard deviations of 790 and 350 respectively. This spread required a minimum 40 samples to reproduce results at 95% level of confidence. Laboratory capacity allowed only eight containers and the local oysters to be sampled at one time. With this limited sampling, interpretation is subjective, but by pooling the counts of the eight daily samples, mean trends may be compared.

Sampling of groups of oysters from the lower, mid, and upper levels of the loaded baskets showed no statistically valid differences between samples. It was concluded that bacterial reduction occurred with equal efficiency in oysters from the middle and lower parts of the baskets. However, in the actual determinations, each 12-oyster sample was taken randomly.

Baskets loaded with 5, 10, 20, and 40 kilos of oysters demonstrated MPN's within the range of fully loaded baskets, so the approximately 3% reduction in basket population from sampling did not significantly affect the results.

Originally it was intended to conduct the experiments using baskets loaded to different levels, in a partially hierarchal factorial design, but we were unable to process an adequate number of samples. However, standard loading and simple comparison of the experimental MPNs to the local, adequately support the feasibility of container purification. Early design concepts and data on the reliability of bacterial estimation using the MPN tube method for oyster meats are given in Quayle and Bernard (1968).

RESULTS

Coliform and *E. coli* Most Probable Numbers and Standard Deviations for pooled results for experimental and local oysters are listed in Table 1. An average 90% of the original bacterial load had been eliminated within 24 hours, with only a fur-

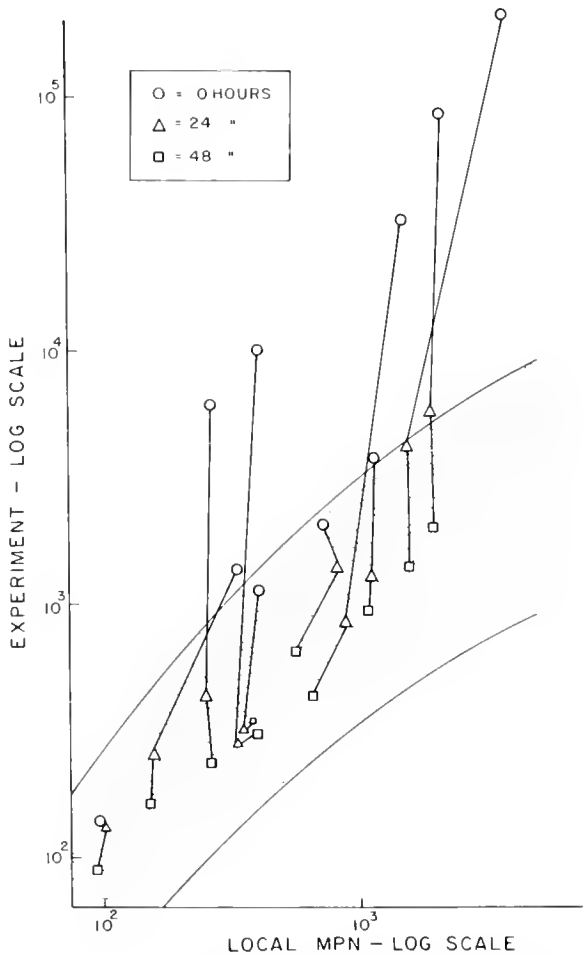

FIG. 2. *General coliform Most Probable Numbers for transferred oysters. Stippled area represents 95% confidence limits for local oysters.*

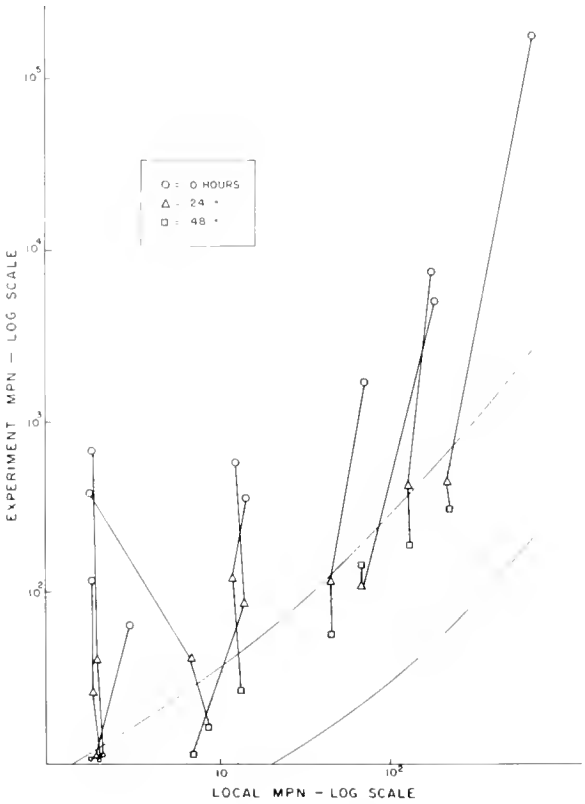

FIG. 3. E. coli *Most Probable Numbers for transferred oysters. Stippled area represents 95% confidence limits for local oysters.*

ther 8% in the next day. Exceptions are observable for those oysters with a polluted MPN close to that of the purification site. The counteracting effect of the ambient MPN n akes interpretation difficult, this may be eliminated by considering the polluted MPN's as a function of the mean local MPN. These are graphed in Yigure 2 for general coliforms and Figure 3 for *E. coli*.

Figure 4 shows the *E. coli* MPNs for groups of oysters held at various temperatures. Each point is the geometric mean of four replicate lots of 12-oyster with an original MPN 480. The 24- and 48-hour curves are virtually identical, with a marginal improvement in bacterial elimination at higher temperature.

Effect of salinity at 10 C in the normal range, is graphed in Figure 5. Each point represents the geometrical mean of four 12-oyster samples with an original *E. coli* MPN 940. The 48-hour curve is below the 24-hour one and the point spread is wider, indicating a further small bacterial reduction in the second day. Both curves show a greater decline at 30‰ and above.

A wide range of turbidity was selected, with an upper limit of 500 mg/l, more than 10 times naturally encountered suspended loads. Figure 6 graphs the declining *E. coli* MPNs at 10 C from an original 730. Each point represents the geometric mean of four 12-oyster samples. There is wide

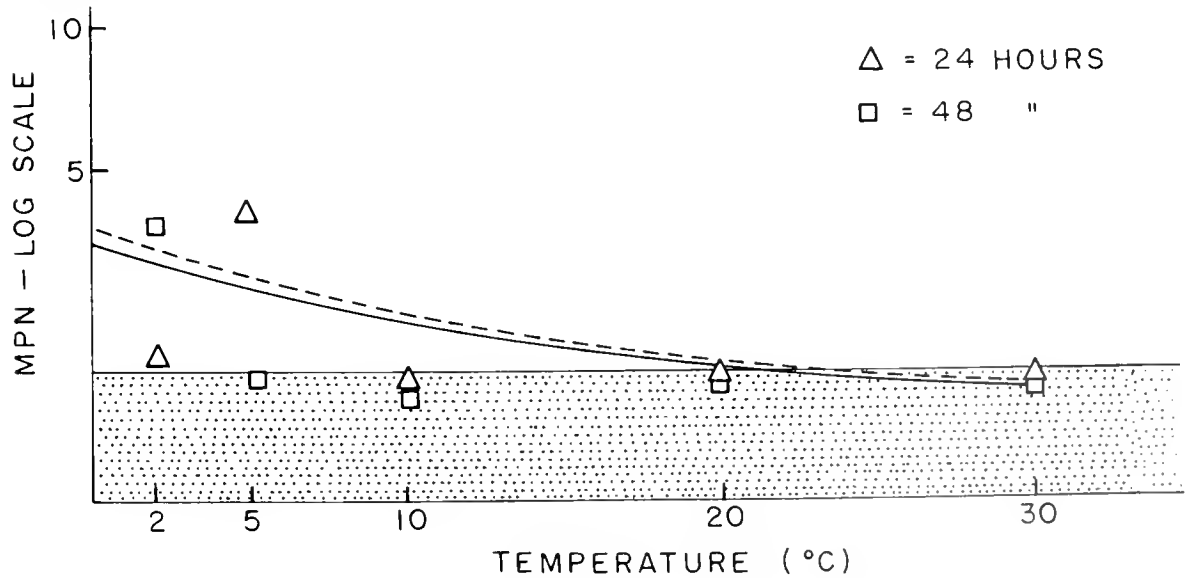

FIG. 4. *Influence of temperature on elimination of* E. coli *in naturally contaminated Pacific oysters. Stippled area below sensitivity of assay.*

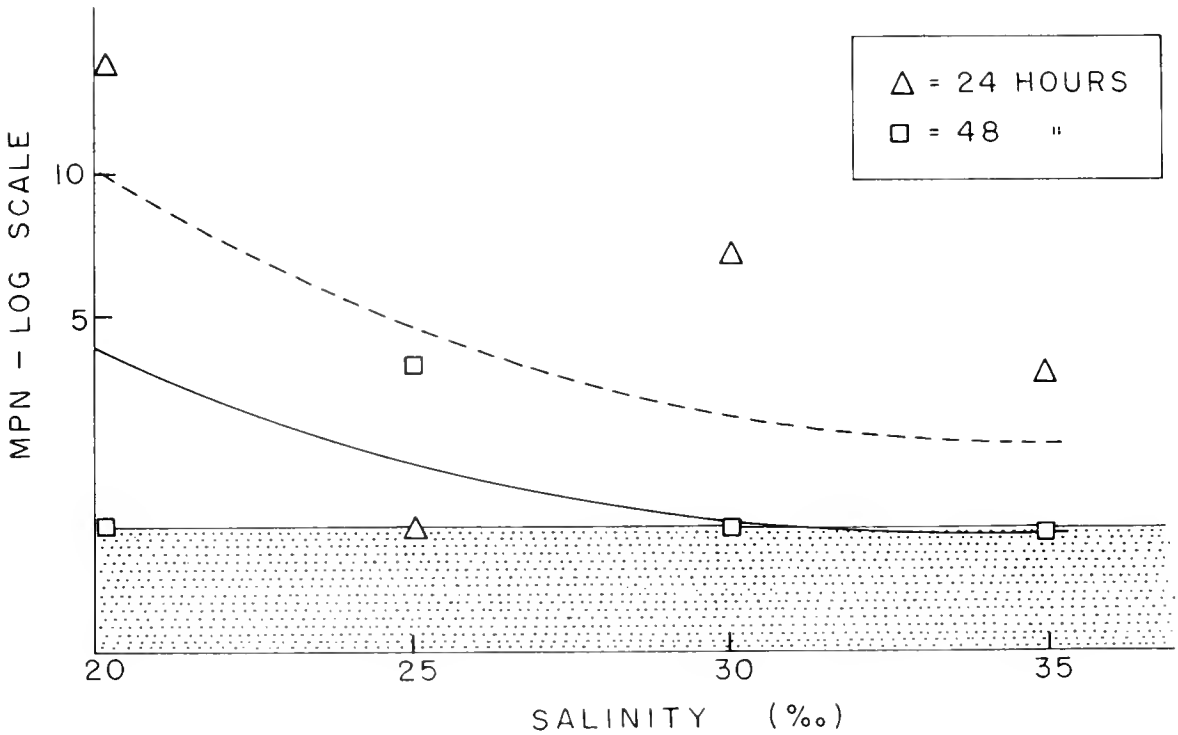

FIG. 5. *Influence of salinity on elimination of* E. coli *in naturally contaminated Pacific oysters. Stippled area below sensitivity of assay.*

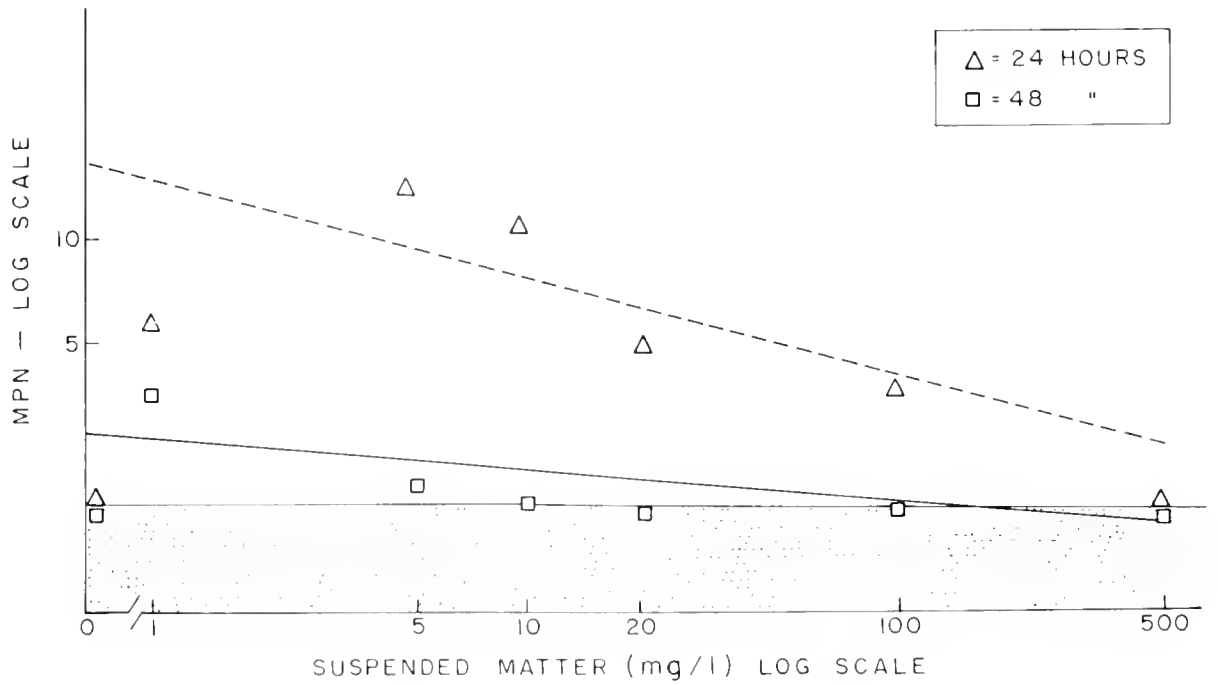

FIG. 6. *Influence of turbidity on elimination of* E. coli *in naturally contaminated Pacific oysters. Stippled area below sensitivity of assay.*

dispersion in the 24-hour points, a greater regularity and slight decrease in the 48-hour points.

DISCUSSION

It is probable that more time is required to eliminate large numbers of microorganisms, but even grossly contaminated (circa 186,000 *E. coli* MPN) reached equilibrium with locxl levels in 24 hours. Less definite results were recorded for oysters with an MPN level close to the local. In this situation the cleansing shellfish will also ingest new bacteria while eliminating those in the gut. Final results are probably temperature dependent, as filtering activity and ingestion are more readily increased by a temperature rise than is the digestive process (Bernard MS).

It is apparent that 24-hour sampling intervals are too long to determine the effect of environmvntal parameters upon bacterial elimination, but it has been adequately established that, under Canadian Pacific conditions, 48 hours are suffgcient for contaminated oysters to reach equilibrium with their transfer site.

Temperature has an effect upon the speed with which material is passed through the digestive tract, and low temperatures (<4C for the Pacific oyster) may inhibit the digestive enzymes and phagocytes of the stomach and midgut (Bernard MS), but these effects would only be apparent in a shorter timeframe. Pacific oysters are able to function in a wide range of salinities, but require a period of adaptation. The marginally better elimination at 30‰ and 35‰ may be attributed to the fact that the subjects of this experiment were collected from 32‰ water.

The amount of suspended matter in the water appears to have little effect upon elimination, and a modest suspended load may indeed help purification by promoting passage of contents through the digestive tract.

It is probable that our results are applicable to other species of shellfish as they closely agree with Arcisz and Kelly (1955) for *Mya arenaria*, Dodgson (1928) for*Mytilus edulis*, and Heffernan and Cabelli (1970) for*Mercenaria mercenaria*. It is likely that acceptable cleansing will occur under a wide range of climatic conditions, and pathogens will be adequately eliminated to ensure a safe product.

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A MATHEMATICAL APPROACH TO DEPURATION

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ABSTRACT

Two equations can be written to describe the change in bacterial concentrations in the shellfish and the water in depuration units. Analytical solutions of these equations for special conditions and numerical solutions for general conditions indicate that there will always be an initial, rapid decrease in bacteria in the oysters during depuration. With suitable flow rates and loading rates, the die off will be exponential and several orders of magnitude reduction can be achieved within 72 hours. Both very low flow rates and very low loading rates increase the residence time of water in the tank, and therefore depuration will occur slowly after about 24 hours. In addition, the bacterial levels at 72 hours may be quite high for the case of very low flow rates. Further improvements and verification of the model are desired, but use of the model can aid in the design and operation of depuration plants now.

INTRODUCTION

Although researchers over the years have investigated and described the various biological functions and environmental factors which are important to the process of depuration, to the author's knowledge, very little work has been done to incorporate these findings into a unified theory of depuration. As a first step in that process, the present study attempts to describe depuration from a phenomenological point of view. The author is aware that this approach neglects many of the physiological aspects of the process and that the model system under consideration is a highly simplified version of the real world situation. Furthermore, no attempt was made to duplicate the results of depuration experiments. Rather the purpose of this study is to determine whether or not the simple model can simulate depuration in general. It is the author's opinion that the mathematical analysis can lead to a better understanding of the interactions which occur and provide a means of evaluating the hydraulic design of flow systems for depuration units. It is hoped that future work can expand the model to incorporate more parameters and to make the model more realistic. But the first need is to demonstrate that the model is a sound one.

EQUATIONS OF DEPURATION

Two equations are needed to describe the total depuration process: one for the shellfish and one for the water in the tank. The first equation tells how the number of bacteria in a shellfish varies with time, and the second equation accounts for changes in the concentration of bacteria in the water. These equations are:

$$dE/dt = -k\,E + p\,f\,c \qquad (1)$$

$$dc/dt = (+k\,E - p\,f\,c - q\,c)\,N/V \qquad (2)$$

The first equation says that the time rate of change of E, the number of bacteria per shellfish, is equal to a fixed percentage, k, of the bacteria present (negative because they are excreted) and a fixed percentage, f, of the bacteria in the water, c, which is pumped through the shellfish at the volumetric rate, p. The second equation says that

[1] VIMS Contribution No. 748

the time rate of change of the concentration of bacteria in the water is equal to those excreted by the shellfish minus those filtered out by the shellfish and those lost from the system. Note that it is assumed that the incoming water has been completely disinfected so there is no term for incoming bacteria. Also since q is the flow of water through the tank *per shellfish*, this term, as well as the other two, must be multiplied by N, the number of shellfish in the tank, and divided by V, the volume of water in the tank to put everything in terms of concentration. All terms in the equations, brief descriptions, units and typical values are listed in Table 1.

A better understanding of these equations can be gained if specific cases are considered. For this study, the depuration of fecal coliforms by the eastern oysters during summer conditions will be used. Assumptions made to relate numbers and volumes of oysters, and other factors are given in Table 2. With a few exceptions, these values have been taken from the Public Health Service publication entitled "Depuration Plant Design" (Furfari, 1966). It should be noted that the equations are written for "ideal" oysters whose behavior matches the average values of a set of real oysters. For the purposes of this study, this idealization does not limit the usefulness of the equations.

TABLE 1. *Symbols Used in Depuration Equations*

Term	Description	Unit
E	number of coliforms per shellfish	$\frac{\text{MPN}}{\text{oyster}}$
c	number of coliforms per volume of water (concentration)	$\frac{\text{MPN}}{\text{100 ml}}$
t	time	hour
k	decay rate	1/hour
f	filtering factor	1
q	flow of water through tank per shell fish (specific flow rate)	$\frac{\text{liters}}{\text{hour-oyster}}$
N	number of shellfish in tank	oysters
V	volume of water in tank	liters
t_{res}	residence time of water	hour

TABLE 2: *Environmental and Behavioral Constants*

1 bushel	=	225 oysters
1 oyster	weighs	25 grams
Temperature	=	20-25 °C
Pumping rate	=	10 liter/hour
Specific flow rate	=	$\frac{\text{1 gallon}}{\text{minute-bushel}} = \frac{\text{1 liter}}{\text{hour-oyster}}$
Decay rate	=	0.17/hour
Filtering factor	=	0.005

During the initial stages of depuration, the bacterial concentration in the water will be zero or very small. This situation could also arise if the flow around the oyster completely removed all fecal matter. For these cases, equation (1) reduces to

$$dE/dt = -k\,E \qquad (3)$$

for which the solution is $E = E_o e^{-kt}$. In other

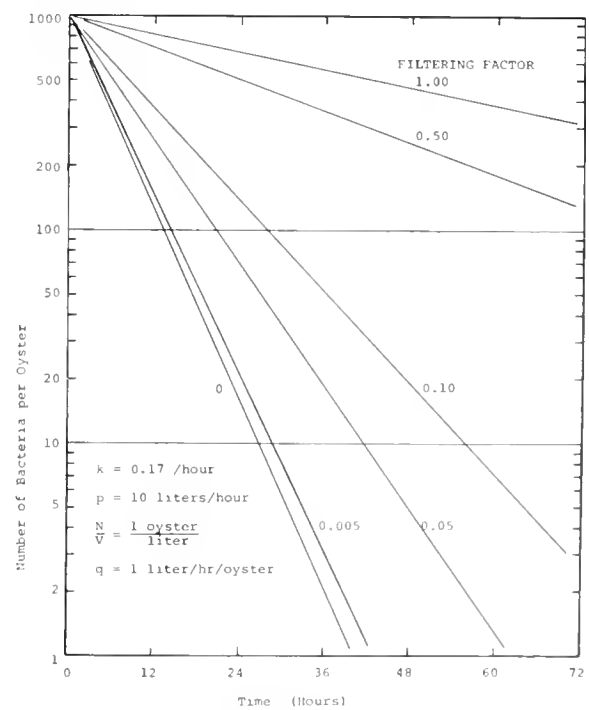

FIG. 1. *Oyster Depuration for Various Filtering Factors.*

words, when there is no feed back of bacteria to the oyster, the decline in bacterial levels within the oysters will be exponential. Thus one could determine the value of k by conducting experiments in which the ambient concentrations are kept very, very low. For this study, k has been assumed equal to 0.17 per hour, or in other words, every hour 17% of all bacteria in the oyster are voided to the water. This means that concentrations will be reduced to one tenth of the original concentration in 13.5 hours, and to 1% of the original value in 27 hours. (Fig. 1).

When natural waters have a relatively constant bacterial level, an equilibrium is reached such that the number of bacteria excreted by an oyster equals the number ingested from the water it pumps. That is, $dE/dt = 0$ and

$$k E_e = pfc_e \qquad (4)$$

or

$$\frac{E_e}{c_e} = \frac{p f}{k} \qquad (5)$$

Equation 5 says that the equilibrium concentration factor (E/c) is equal to the product of the pumping rate, p, and the filtering factor, f, divided by the decay rate, k. This relationship appears to be sound physiologically, because studies have shown that when the water temperature increases from 10C to 20C, the pumping rate and the concentration ratio both increase. (Furfari, 1966). If we assume that this relationship does hold, then equation (5) presents a means of determining the filtering factor, f. If there is an ambient bacterial concentration of 330 MPN/100 ml (or 3300 MPN/liter) then the bacterial concentration in the oyster for summer conditions and Virginia growing areas will be around 4000 MPN/100 grams. (Reference to Hope & Wiley, 1961 in Furfari). If an average oyster weighs 25 grams, then E will be 1000 MPN/oyster. Thus the concentration ratio as defined above is about 0.3 and the filtering factor is 0.005. In other words, the oyster ingests 0.5% of the coliforms in the water which it pumps. Pumping rate has been assumed to be 10 liters per hour.

If all factors other than E and C are assumed to be constant with respect to time, then several analytical methods of solution are available. For this study, the coupled equations (1) and (2) were transformed to finite difference form and programmed on a Hewlett Packard 9800 desk top calculator. The time interval for integration was 0.1 hour. Die off curves for several values of the filtering factor are shown in Figure 1. It is interesting to note that for the assumed flow rate and biomass to volume ratio, the decay of bacteria in the oyster is always exponential. Also depuration occurs, albeit slowly, even if 100% of the bacteria are filtered by the oyster from the water it pumps. For the rest of this study, it will be assumed that $k = 0.17$ per hour, $p = 10$ liters per hour and $f = 0.005$. From a mathematical point of view, the curves shown in Figure 1 appear to be reasonable, but certainly there is need for verification of these biological coefficients. Equation (2) describes the changes in concentration levels in the water in the depuration tank. It includes the two factors which are amenable to control by the designer and operator of a depuration plant: N/V, the biomass to volume ratio or the loading rate, and q, the specific flow rate. The effect of these factors can be illustrated by an analysis similar to that done for Equation (1).

During the initial phase of depuration, E is very large and c is small. Thus the term, kE, is the dominant one and c will increase rapidly. Typically the maximum value for c is attained in the first or second hour of depuration, and it decreases slowly there after. However, E declines rapidly and the kE term quickly becomes so small that it can be neglected during the later stages of depuration. Since the specific flow rate, q, is 1 liter per hour and pf is only 0.05 liters/hour, Equation (2) can be approximated by

$$dc/dt = \frac{Nqc}{V} = (1/t_{res})\, c \qquad (6)$$

where t_{res} = the mean residence time for a parcel of water flowing through the tank, defined as the quotient of the water volume and the total flow rate, which in turn is equal to the specific flow rate times the number of oysters.

$$t_{res} = V/Q = V/Nq \qquad (7)$$

The solution to equation (6) will contain the term $e^{-t/t_{res}}$. In other words, the rate of change of the concentration in the tank will be a function of the residence time of the water.

It should also be noted that if there is no growth

of bacteria and if there is some finite flow of water through the tank, there will be a continual loss of bacteria from the system. Thus the only equilibrium which can occur is when c =0. The rate of decay for low flow rates and large residence times will be very slow, so that depuration will not occur over a practicable period of time. One might criticize the model for not including a term for the growth of bacteria since fecal pellets and other detritus can provide a suitable medium for growth to occur. However, any growth of bacteria in the tank will slow down and interfere with the depuration process. Thus, the condition of no growth is the one which is desired and operating procedures should be designed to remove the biodeposits at frequent intervals.

OPERATIONAL CONTROLS

The two factors which are directly under the control of a depuration plant operator are the flow of water through the tank and the loading rate. Several model runs were made to examine the behavior of the system to variation in these two factors. In Figure 2, the die off curves for a range of specific flow rates are shown. If the specific flow rate is very small, as illustrated by the curve for 0.01 liters/hour/oyster, there is an initial period of rapid decay, followed by an intermediate transtition period and a final slow decay. In the initial stages the die off will be exponential with the decay rate equal to k. In the final stages the die off will be exponential as well, but the decay rate will be smaller and equal to $1/t_{res}$. Since q is small, the residence time is large and the inverse of the residence time will be small too. It is important to note that in addition to the slow die off, the reduction during a 72 hour period is very small. Less than an order of magnitude reduction occurs during this period.

For a specific flow rate of 1 liter/hr/oyster, the reduction in decay rate is much less pronounced but still present. The bacterial level is reduced to less than 1% of the initial count within 60 hours. As the flow rate is increased further, there are additional increases in the decay rate. But above 10 liters/hr/oyster only marginal increases in depuration rate are achieved for large increases in flow rate.

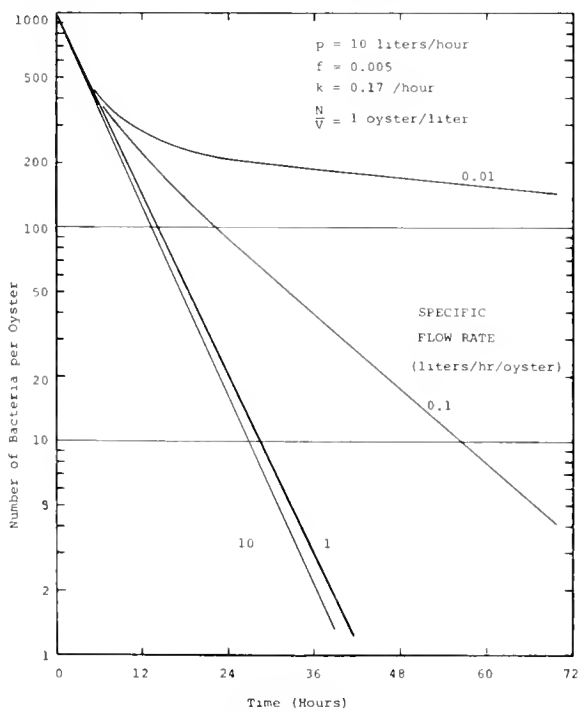

FIG. 2. *Oyster Depuration for Various Specific Flow Rates.*

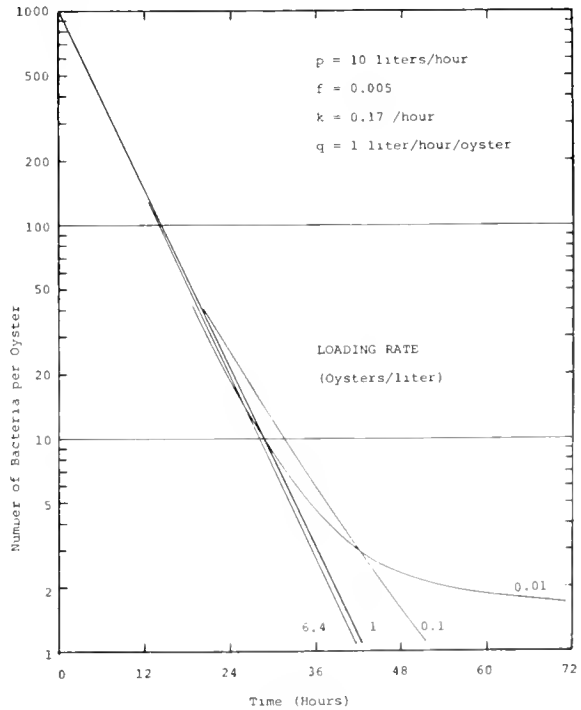

FIG. 3. *Oyster Depuration for Various Loading Rates.*

The loading rate shows variations somewhat similar to those for the specific flow rate. The maximum value which can be achieved is a bushel of oysters in a bushel of water or 6.4 oysters/liter of water. Only a modest variation in depuration is seen if the loading rate is reduced to 0.1 oysters/liter. If the loading rate is reduced by an additional order of magnitude to 0.01 oysters/liter, the final stage of depuration occurs at a slow rate. For this situation, the bacterial levels are reduced to less than 1% of the original value within 48 hours before the reduction in decay rate takes effect.

The two cases of low specific flow rate and low loading rate are related in that each has a long residence time. In each case an initial period of rapid die off will be followed by a period of very slow depuration. With extremely low flow rates, bacterial levels in the water can be high, whereas for low loading rates, a large volume of water is available to dilute the bacteria given off by the oysters. For example, when $q = 0.01$ liter/hr/oyster and $N/V = 1$ oyster/liter, the water concentration at 24 hours is 65 MPN/100 ml (Fig. 2). But when $q = 1$ liter/hr/oyster and $N/V = 0.01$ oyster/liter the bacterial level in the water is only 0.8 MPN/100 ml at 24 hours (Fig. 3). For both cases, the bacteria in the water will be removed from the tank slowly, but the higher levels present with low flow rates will have the effect of maintaining higher levels in the oysters.

DISCUSSION

The sample runs show several features of the depuration process which have a bearing on the operation of a depuration plant. First, there is an initial rapid decay in bacterial levels no matter what flow rate and loading rate are used. Therefore, one possible means of achieving suitable reductions in coliform counts is to hold the oysters with no through flow. At periodic intervals the water should be flushed from the tank and it would be replaced by clean water. Bacterial reductions for several holding times are given in Table 3. All frequencies for removing and renewing the water appear to work, so that biological factors should be used to choose the best frequency. For example, the oysters may not begin to pump until a half hour after being immersed, so that a longer interval would be better. Also, some

TABLE 3. *Reduction in Bacterial Levels With No Flow Through Tank*

Time	Hour	2 Hrs.	3 Hrs.	4 Hrs.	6 Hrs.
0	1000	1000	1000	1000	1000
12	142	145	152	162	186
24	20	21	23	26	35
36	3	3	3	4	7

means will be needed to maintain dissolved oxygen concentrations. Submerged air diffusers appear to be a very efficient and cost-effective method.

The model indicates that the oysters can never be packed too densely in the tank. Implicit in the model is the assumption that the water in the tank is mixed rapidly so that the concentration in the water does not vary significantly throughout the tank. Additionally, the removal of fecal matter is very important. Thus the need for a good circulation in the tank, rather than considerations of depuration rate, may dictate a limit to the loading rate. But from a mathematical point of view, increased loading rates will not hinder the depuration.

The specific flow rate is much more difficult to characterize. If a high flow rate is used, a great deal of energy will be expended unnecessarily. On the other hand, if a very low flow rate is used, not only will the depuration rate be slow but also the reduction in coliform counts will be insufficient. The best course of action is to run tests in the tank to document the rate of depuration for various flow rates. The model could then be used to determine a specific flow rate that insured the necessary reduction in bacterial levels without wasting energy.

In summary, the mathematical approach to depuration can be very fruitful. There is need for input from biologists as to the acceptability of the various assumptions and simplification, the correct values for the biological coefficients and the best means of improving the model. However, even at this stage, the combination of actual depuration runs and mathematical analysis can aid in the design and operation of depuration plants.

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THE ECONOMICS OF HATCHERY PRODUCTION OF PACIFIC OYSTER SEED: A RESEARCH PROGRESS REPORT

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ABSTRACT

An analysis of the economic viability of a Pacific oyster (Crassostrea gigas) *seed hatchery industry in the Pacific Northwest examines the demand for hatchery seed and the costs of producing hatchery seed. This is a progress report of the research, using limited market data and relying upon the production relationships prevailing at an experimental hatchery. The results suggest that hatchery production may be economically feasible, but a more accurate picture requires additional data from industry sources.*

INTRODUCTION

The Pacific oyster, *Crassostrea gigas*, has been cultured commercially in this country for about 50 years (Steele, 1964). Because there are so few areas in the Pacific Northwest where natural reproduction occurs, the industry has had to rely on Japan for a large proportion of its supply of oyster seed (oysters up to one year old). These seed are planted on growing grounds in Washington, Oregon, California, and British Columbia, where they mature in two or three years. Table 1 provides data on Pacific oyster production in the U.S., on imports of oyster seed from Japan, and on the production of oyster seed in what has become the principal domestic source — Washington's Hood Canal. As the table reveals, there has been quite a bit of fluctuation in domestic seed production and in seed imports from Japan.

In the market place, domestic Pacific oysters compete with imported oysters, whose volumes have recently increased, and with other oyster species, especially the Eastern, *Crassostrea virginica*, and European, *Ostrea edulis*, species. Data on these quantities also appear in Table 1. No analysis has yet been conducted of the final market for Pacific oysters (although this is currently under way at Oregon State University), but some results are available on the demand for oysters in the U.S. The Bureau of Commercial Fisheries (1970), Dunham and Bray (1974), and Suttor (Nash, 1969) all report price elasticity figures of less than unity. A recent study by Charbonneau and Marasco (1975) suggests that this varies substantially across regions and over time for fresh and frozen oysters. One would expect the demand for a specific species of oyster, such as the Pacific oyster, to be more price elastic than the demand for all oysters taken together.

Prices of both adult oysters and oyster seed have been rising over time (Table 2), the result of a variety of factors including rising consumer incomes and prices of substitute goods. Adult oyster prices have risen less rapidly than have seed oyster prices.

On the supply side, fluctuations in spawning and growing conditions along with increases in labor costs, i.e., those costs associated with tending the oyster grounds (including planting and harvesting) and those associated with oyster processing (including shucking), are important price-determining factors. While data on the labor costs appropriate to the oyster industry are not yet

available, some indication of the trend is suggested by the U.S. Bureau of Labor Statistics figures on average hourly earnings of workers in industries producing canned, cured, and frozen foods (Table 2). Hourly wage rates, as measured in current dollars, have increased over time.

Thus, the Pacific oyster industry in the U.S. has been experiencing uncertainties with respect to seed supply and production conditions, competition from imported oysters, and rising labor costs. These conditions, together with the competition for oyster lands which has come from recreationists and industrial users, have led to recent attempts to harvest oysters through less labor-

TABLE 1. *U.S. Oyster Production and Imports; U.S. Seed Production and Imports; 1947-1975*

Year	Pacific oyster production, U.S.[a]	Total oyster production, U.S.[a]	Pacific Coast seed oyster imports from Japan[b]	Pacific oyster seed production, Hood Canal[c]	Total oyster imports[d]
	. . . . . thousand pounds		*standard cases*	*std. case equiv.*	*thou. pounds*
1947	11,320	63,085	56,619	*	111
1948	9,564	61,610	32,869	*	160
1949	8,164	75,773	46,036	*	342
1950	8,080	76,415	46,726	*	446
1951	8,597	72,990	51,901	*	962
1952	9,957	82,242	83,290	*	595
1953	10,283	79,719	70,113	*	637
1954	10,855	81,922	65,528	*	1,056
1955	11,602	77,515	54,216	0	1,391
1956	11,881	75,133	100,634	1,000	1,928
1957	11,614	71,658	60,063	0	2,575
1958	11,197	66,395	61,119	2,000	5,015
1959	12,328	64,710	61,444	2,500	5,545
1960	10,983	60,010	44,291	3,500	6,597
1961	10,154	62,305	37,128.5	3,700	7,261
1962	10,714	56,037	41,499	5,200	7,387
1963	9,746	58,444	53,416	2,700	8,906
1964	9,934	60,534	41,160	0	8,154
1965	9,117	54,688	34,909.5	6,900	9,001
1966	*	51,223	16,102	9,200	12,028
1967	7,682	50,957	43,557.5	15,900	17,672
1968	*	55,600	38,415	6,200	15,550
1969	*	*	44,707	3,000	16,622
1970	7,915	53,602	26,079	5,000	15,484
1971	*	*	30,337	32,900	9,695
1972	8,362	56,058	7,321	33,400	22,309
1973	*	*	8,346	34,200	19,850
1974	*	*	12,406	46,700	16,010
1975	*	*	10,856	0	*

* Data not available.

[a] NMFS, NOAA, *Fishery Statistics of the United States*, various issues.

[b] State of Washington Dept. of Fisheries, *Washington State Seed Oyster Imports from Japan, 1975*.

[c] Personal correspondence, Ronald E. Westley, Washington State Dept. of Fisheries.

[d] BCF, *Basic Economic Indicators: Oysters*, May 1970; NMFS, *Current Fishery Statistics*, and NMFS, *Fishery Market News Reports* (Seattle), various issues.

intensive means and to culture oysters through methods which would permit more utilization of the water column on the oyster-rearing grounds (examples include raft culture, tray culture, stick culture).

During the last three years another development has taken place which could have profound effects on the industry. That development is the commercial raising of oyster seed under environmentally-controlled (hatchery) conditions. Drawing upon research results available from sources such as the experimental hatchery at the Oregon State University Marine Science Center, about six hatcheries in Washington and California are currently raising oyster seed for commercial purposes. This development is simply too recent to be able to analyze its impact on the industry. Nonetheless, it is the objective of the present study to examine the

TABLE 2. *Prices of Imported Oyster Seed and Shucked Pacific Oysters; Average Hourly Labor Earnings, 1947-1974*

Year	Price of Pacific oyster seed imported from Japan[a]	Wholesale price of shucked Pacific oysters (FOB Seattle, WA)[b]	Average hourly earnings: canned, cured, and frozen foods[c]
	dollars per case	*dollars per pound*	*dollars*
1947	5.86	0.37	*
1948	*	0.43	*
1949	*	0.47	*
1950	*	0.46	1.17
1951	6.92	0.50	1.25
1952	6.98	0.49	1.30
1953	7.27	0.46	1.35
1954	*	0.46	1.39
1955	6.19	0.46	1.43
1956	8.05	0.46	1.54
1957	8.67	0.47	1.60
1958	10.28	0.47	1.64
1959	*	0.46	1.70
1960	9.95	0.47	1.78
1961	9.88	0.55	1.85
1962	10.83	0.52	1.90
1963	11.00	0.52	1.92
1964	*	0.52	1.95
1965	*	0.52	2.01
1966	17.00	0.65	2.11
1967	*	0.77	2.22
1968	17.00	0.81	2.37
1969	16.50	0.81	2.51
1970	19.40	0.80	2.65
1971	20.50	0.80	2.83
1972	25.50	0.83	3.01
1973	29.00	1.12	3.25
1974	29.28	1.25	3.55

* Data not available.

[a] FOB Aberdeen, WA.; personal communication, Ronald E. Westley, Washington State Department of Fisheries; and (Steele, 1964.)

[b] NMFS, NOAA, USDC (Seattle), *Fishery Market News Reports*, various issues. The figures are receipts by Seattle wholesale dealers divided by pounds of shucked oysters.

[c] U.S. Bureau of Labor Statistics, *Employment and Earnings*, various issues.

nature of the demand for oyster seed and the costs associated with hatchery production of oyster seed, in the hope of being able to determine the viability of an oyster seed hatchery industry in the Pacific Northwest.

The Demand for Pacific Oyster Seed

A diagrammatic model of the Pacific oyster seed market is presented in Figure 1. In Figure 1, DD is the annual North American demand for Pacific oyster seed. This demand is derived from the demand for adult oysters, and is postulated to be such that the quantity of seed oysters demanded by oyster growers depends upon their expectations of the price at which they will be able to sell mature oysters, the current price of oyster seed, the quantity of available oyster-producing land in the Pacific Northwest, and the expected time path of wage rates during the growing period.

S_D is the domestic supply of Pacific oyster seed. It is postulated to be perfectly inelastic beyond some reservation price level, on the assumption that quantities of seed available this year are determined primarily by the environmental conditions prevailing during the spawning period of the previous year.

S_J is the supply of Pacific oyster seed from Japan. It expresses the hypothesis that the quantity of oyster seed supplied to the U.S. is a postive function of the price of oyster seed (in Japanese yen), Japanese production of oyster seed, and seed prices in Japan and other Japanese markets. Until recently, the U.S. has been the principal market for Japanese exports of seed. During the past five years, however, Western Europe (especially EEC members) has become an important market for Japanese seed. While, in the early years of the industry, the Pacific Coast Oyster Growers' Association was the principal seed importer, during the past 20 years several independent U.S. oystermen have been importing seed directly, rather than through the Association. Nonetheless, it is probably reasonable to assume that the large number of oystermen in the Pacific Northwest treat the price of oyster seed as exogenous; i.e., as being beyond their control.

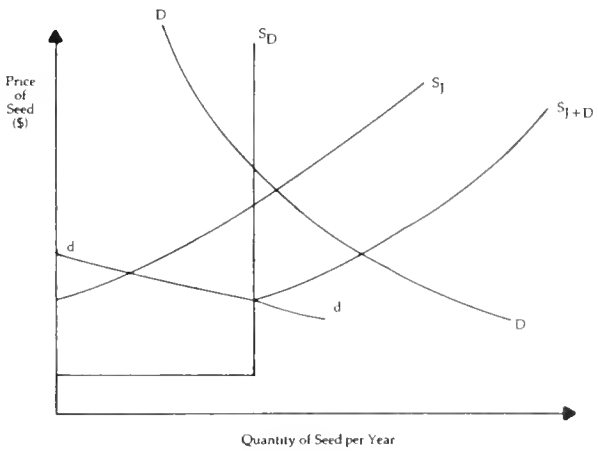

FIG. 1. *Supply and demand for Pacific oyster seed in the U.S.*

The curve dd is the curve on which the interest of this portion of the analysis focuses. It is the "net" derived demand for Pacific oyster seed. It can be interpreted as describing a functional relationship between alternative seed prices and the quantities of seed oystermen would purchase from sources other than Japan and domestic suppliers of wild (i.e., non-hatchery) seed. In other words, it is postulated that this is the demand for seed facing a hatchery industry.

Mathematically, the relationships may be described as follows:

$$(1) \qquad Q_t^{PS.\,D} = f(EP_t^{PO}, P_t^{PS}, L_t, EC_t)$$

$$(2) \qquad EP_t^{PO} = g(P_{t-1}^{PO}, \Delta Y_{t-1}, W_t^{os}, \Delta I_{t-1})$$

$$(3) \qquad EC_t = h(\Delta C_{t-1})$$

$$(4) \qquad Q_t^{PS.\,S.\,J} = j(P_t^{PS}/R_t, J_t^{OS}, S_t^{OS})$$

$$(5) \qquad D_t^{OS} = D_t^{OS*},$$

where

$Q_t^{PS.\,D}$ = Quantity (number of cases) of Pacific oyster seed demanded in year t;

$Q_t^{PS.\,S.\,J}$ = Quantity (number of cases) of Pacific oyster seed supplied by Japan to U.S. importers in year t;

EP_t^{PO} = Expected wholesale price of mature Pacific oysters in year t;

P_t^{PS} = Price per case of Pacific oyster seed in year t;

L_t = Acres of Pacific oyster-producing land in Washington, Oregon, and California in year t;

EC_t = Expected labor costs as between year t and year t+3;

P^{PO}_{t-1} = Actual wholesale price of mature Pacific oysters in year t-1;

ΔY_{t-1} = Change in per capita personal disposable income as between year t-1 and year t;

W^{OS}_t = World production of all oyster seed in year t;

ΔI_{t-1} = Change in U.S. imports of Pacific oysters between year t-1 and year t;

ΔC_{t-1} = Difference in wage rate for harvesting and shucking as between years t-1 and t;

R_t = Exchange rate between Japanese yen and U.S. dollar in time t;

J^{OS}_t = Japanese production of oyster seed in year t;

S^{OS}_t = An index of prices in Japan and foreign markets (excluding North America) for Pacific oyster seed in year t; and

D^{OS}_t = Quantity of domestic Pacific oyster seed supplied in year t. The * denotes that this quantity is determined by variables outside of the model.

Equation (1) originates in a Cobb-Douglas production function of the form:

$$\text{Oysters produced}_{t+3} = \alpha_0 \text{ Seed}^{\alpha_1} \text{ Labor}^{\alpha_2}_{t_0\, t+3}.$$

Combining Equation (1) with Equation (5) to yield the "net" demand curve yields

$$(6) \quad Q^{PS.D}_t - D^{OS}_t \equiv Q^{PS.\ D.\ J}_t = k(EP^{PO}_t, P^{PS}_t, L_t, EC_t) - D^{OS*}_t,$$

where $Q^{PS.\ D.\ J}_t$ is the quantity (number of cases) of Pacific oyster seed demanded from japan in year t. This transformation is necessary because data are not available on the total quantity of domestic wild seed (including British Columbia) available over the period of anaysis.

Equations (2) and (3) express postulated relationships among observable variables and expected (by oyster growers) future prices and labor costs. They are substituted into Equation (6) to express that equation in terms of observable variables. Thus, the two equations whose parameters are to be estimated are the "demand" equation (6′) and the "Japanese supply" equation (4):

$$(6') \quad Q^{PS.\ D.\ J}_t = k\left\{ g(P^{PO}_{t-1}, \Delta Y_{t-1}, W^{OS}_t, \Delta I_{t-1}), P^{PS}_t, L_t, h(\Delta C_{t-1}) \right\} - D^{OS*}_t$$

$$(4) \quad Q^{PS.\ S.\ J}_t = j(P^{PS}_t/R_t, J^{OS}_t, S^{OS}_t).$$

In this system, P^{PS}_t and $Q^{PS.\ D.\ J}_t$ ($= Q^{PS.\ S.\ J}_t$ in equilibrium) are treated as endogenous variables.

As indicated earlier, it is postulated the production of oysters can be approximated by a Cobb-Douglas function in seed and labor. A similar functional form is assumed for (2), (3), and (4). Thus, the functional form of (6′) becomes

$$(6'') \quad Q^{PS.\ D.\ J}_t = \alpha_o \left\{ (P^{PO}_{t-1})^{\beta_1} (\Delta Y_{t-1})^{\beta_2} (W^{OS}_t)^{\beta_3} (\Delta I_{t-1})^{\beta_4} \right\}^{\alpha_1} \cdot \left\{ (\Delta C_{t-1})^{\alpha_2} (P^{PS}_t)^{\alpha_3} \right\} + \alpha_4 L_t - D^{OS*}_t$$

and

$$(4') \quad Q^{PS.\ S.\ J}_t = \alpha_o \left(\frac{P^{PS}_t}{R_t} \right)^{\alpha_1} \left(J^{OS}_t \right)^{\alpha_2} \left(S^{OS}_t \right)^{\alpha_3}.$$

Time series data on the variables W^{OS}, L, and S^{OS} were not available at the time of analysis. Their omission results in biased estimates of the regression coefficients. This potentially major flow in the analysis should be corrected as data on the variables become available. In addition, while data on domestic production of seed from Hood Canal were available (Table 1), there are no data available on the quantities of Hood Canal seed actually planted. Furthermore, data on production of Willapa Bay seed and Pendrell Sound seed are based on estimates of the quality of seed set on National Marine Fisheries estimates of imports from Canada. Because of the difficulty of making the domestic production and the Japanese import data comparable, it was decided to include the D^{OS}_t variable in a multiplicative form in the "demand" equation, with its own exponent to be estimated.

Two-stage least squares procedures were used

to estimate the demand and supply parameters. The resulting equations are:

Demand for Pacific Oyster Seed:

$$\log Q_t^{PS,\,D,\,J} = \underset{(3.3920)}{18.1310} + \underset{(1.6663)}{3.0739}\log P_{t-1}^{PO} + \underset{(.5841)}{.3879}\log \Delta Y_{t-1}$$

$$+ \underset{(.0538)}{.0047}\log \Delta I_{t-1} - \underset{(.6020)}{.4644}\log \Delta C_{t-1} + \underset{(.0424)}{.0106}\log D_t^{OS*}$$

$$- \underset{(1.4202)}{3.4918}\widehat{\log P_t^{PS}}.$$

Supply of Pacific Oyster Seed by Japan:

$$\log Q_t^{PS,\,S,\,J} = \underset{(10.9828)}{36.7770} - \underset{(1.9220)}{4.1040}(\widehat{\log P_t^{PS}} - \log R_t)$$

$$- \underset{(.9474)}{1.2765}\log J_t^{OS}.$$

The numbers in parentheses are standard errors; the $\widehat{\ }$ sign indicates values predicted from the first stage.[1]

[1]The estimated stage I equation is:

$$\log P_t^{PS} = \underset{(.8772)}{3.3201} + \underset{(.3227)}{.8232}\log P_{t-1}^{PO} + \underset{(.1013)}{.2055}\log \Delta Y_{t-1} + \underset{(.0118)}{.0082}$$

$$\log AI_{t-1}$$

$$- \underset{(.1576)}{.0201}\log \Delta C_{t-1} + \underset{(.5099)}{1.5165}\log R_t + \underset{(.0084)}{.0223}\log D_t^{OS*}.$$

The J^{OS} variable was not included at this stage because of the limited number of observations. The R^2 statistics for the equation is .96.

The R^2 statistic for the demand equation is .75, while the Durbin-Watson (D-W) statistic for serial correlation is 2.64. The first statistic suggests that a fairly high degree of association exists between the quantity of Pacific oyster seed demanded and the explanatory variables of the demand equation. The size of the D-W statistic is in the "inconclusive" range. Only years for which data on all variables were available could be used— a total of 15 observations. A larger number of observations would help determine whether or not serial correlation is present.

Indeed, with so few observations, it is difficult to draw any "conclusions" from the analysis. However, some findings are worthy of note. First, the standard errors associated with the estimated coefficients for the two price variables are fairly low, relative to those coefficients. This suggests that oystermen may be sensitive to changes in both mature oyster prices and oyster seed prices in their seed-importing decisions. Indeed, the demand for imported seed appears to be price-elastic ($\varepsilon_{\hat{P}} = -3.4918$), suggesting that an increase (decrease) in Japanese seed prices would be associated with a more-than-proportional decline (rise) in purchases of Japanese seed, assuming no changes in the values of the other variables. The results also suggest that an increase (decrease) in the wholesale price of oysters would increase (decrease) the quantity of Japanese seed demanded at each of the values of the Japanese export price. A word of caution is in order here, however. These two price variables are highly intercorrelated, suggesting that it is difficult to sort out the separate influences of each on the quantities demanded. When the wholesale price variable was excluded from the equation, however, the price-elasticity figure changed to only -4.00, still in the "elastic" range.

Of some interest is the high standard error associated with the variable representing the domestic seed of Pacific oyster seed. One is tempted to conclude that this variable is not an important determinant of the imported quantities demanded. This may, indeed, be the case. An alternative explanation of the result, however, is that the data used, while the best available, do not accurately portray the actual quantities available, as perceived by the domestic growers when they make their import decisions. This may be particularly true of the Pendrell Sound seed. A third explanation is that the demand equation is simply mis-specified, a potential difficulty with all econometric models.

Specification error may have been a difficulty with the estimated supply equation. The R^2 statistic for that equation is .61 and the D-W statistic is 1.82. Because of data limitations, this analysis was conducted with only seven observations, and is included here only for purposes of illustrating the techniques used. Neither the R^2 nor the D-W statistic has much statistical meaning, given the small number of observations. However, it is of interest to note the negative coef-

ficients on both of the explanatory variables, where the economic model discussed earlier would lead one to hypothesize positive signs on both coefficients. A possible explanation is that the price variable is serving as a proxy for prices in Japan and other seed-importing countries (e.g., France). It so, one would expect that the greater the price, the smaller the quantity Japanese exporters would be willing to ship to U.S. buyers. In the discussion which follows, the supply of Japanese oyster seed is treated as being perfectly price-inelastic.

In Table 3 the estimated demand equation is employed to address the question: assuming that all values of the explanatory variables in that equation, except the price of oyster seed, were set at their 1974 levels, how much oyster seed would West Coast oystermen be willing to purchase at alternative prices? Subtracting from the Table 3 quantity figures an assumed amount of seed imported from Japan would yield estimates of the annual quantities of seed oystermen would be willing to purchase from hatcheries. Tables 1 and 2 reveal the import price to be $29.28, and the quantity imported to be 12,406 during 1974. Inserting the values into the estimated demand equation reveals that approximately 6,200 cases would have been demanded in 1974 at that price. In fact, industry sources maintain that approximately 7,500 cases were delivered in that year from West Coast hatcheries. Thus the model appears to do a reasonably good job of prediction. Perhaps the most significant result of this portion of the analysis is the finding that oyster seed buyers do, indeed, appear to be sensitive to changes in seed prices. It is reasonable to assume that this would also be true of hatchery-produced seed.

TABLE 3. *The Demand (Price-Quantity Relationship) for Imported Pacific Oyster Seed*[a]

Average market price	Estimated annual quantities of imported seed which oystermen would be willing to purchase
dollars per case	*number of cases*
20.00	68,398
25.00	31,379
30.00	16,602
35.00	9,691
40.00	6,080

[a] Assuming all other explanatory variables are set at their 1974 levels.

Again, the reader is reminded that these results must be interpreted with extreme caution. For one thing, this is no reason to expect 1974 conditions to prevail into the indefinite future. No account is taken of the differences in quality of the oyster seed over time and among sources. For example, if the yields of hatchery seed were greater than the yields of imported seed, and if oystermen knew this, the quantities demanded from hatcheries could be different than what is suggested in Table 3.

Furthermore, changes in the demand for oysters, themselves, would affect the demand for oyster seed. Indeed, there is currently some discussion in the industry of expanding export markets. The demand would also be affected by changes in the importation of oysters and oyster seed (for example, through lifting of the current embargo on Korean seed).

Given these cautionary remarks, perhaps the results reported here are helpful in suggesting what opportunities, under the postulated conditions, a commercial hatchery industry has with respect to seed sales. The next question is: what are the costs associated with such volumes? The discussion now turns to this question.

Seed Hatchery Production Costs

Among the characteristics that establish the competitive position of hatchery seed with other seed sources is the cost of production. The only public source of hatchery operating experience available in Oregon is through the Oregon State University pilot facility at the Marine Science Center (MSC) in Newport. Based on this source, costs have been estimated at a near-actual production of 15 bushels or 6 cases per week. Additional cost projections have been made for several other levels of output. In this preliminary report costs are summarized for what will be referred to as Plant I at 15 bushels per week capacity and Plant II at 800 bushels or 320 cases per week, which is nearer a realistic commercial production quantity.

The purpose of this portion of the study is to determine the costs of producing oyster seed in the Pacific Northwest. Other contributing objectives include:

1. identification of factors that influence production costs;
2. estimating costs in detail for each of six stages of operation employed in seed production; and
3. extension of these costs for two production capacities.

Fulfillment of these objectives will contribute to management capabilities.

1. Costs for comparing hatchery seed with imported and natural seed will be available.
2. Private hatchery operators will have detailed cost components with which to evaluate their production costs.
3. The impact of proposed changes in operating methods, facilities, or equipment upon specific cost items can be more readily estimated.

Determination of costs for the production of oyster spat by the MSC required identification of all the costs needed for the activities required to produce a usable product. In turn these various costs were assigned to six subgroups or stages of operation, illustrated in Figure 2.

By means of process charts, detailed diagrammatic descriptions were made correlating all activities for the production of oyster seed. Separate charts were made for algal food production and seed rearing. With the aid of process charts, it was possible to identify and assign the use of all resources as they were required by each activity. Fortunately, records of labor use were available. Cost figures were obtained from numerous sources including MSC records, equipment and material suppliers, utility companies, and building contractor estimates. A short excerpt of the detailed process chart for raising oyster seed is shown in Figure 3.

Classification and Determination of Costs

The costs associated with this study are the initial investment outlay, fixed costs, and variable costs. The initial investment outlay items consist mainly of buildings and equipment, and appear as production costs in the forms of depreciation, expenditure on interest on investment, etc. Acquisition cost of land is not included in this study. Fixed cost items associated with buildings and equipment include depreciation, interest on investment, insurance, taxes, repair, and maintenance. Supervision also is included as a fixed cost item. Variable cost items include expenses for labor, material, supplies, water, electric power, telephone, and other expenses directly related to oyster seed production.

1. Initial Investment Outlay

Costs for the oyster hatchery building were estimated on the basis of space requirements. Space requirements include rooms for (a) algal food production, (b) larval rearing and setting, (c) adult oyster conditioning and spawning, (d) algae lab, (e) analytical lab, (f) general office, (g) lunch and locker, (h) lavatory and storage, and (i) space allowance for aisles and an outdoor concrete pad for cultch preparation.

The building costs, including piping and wiring,

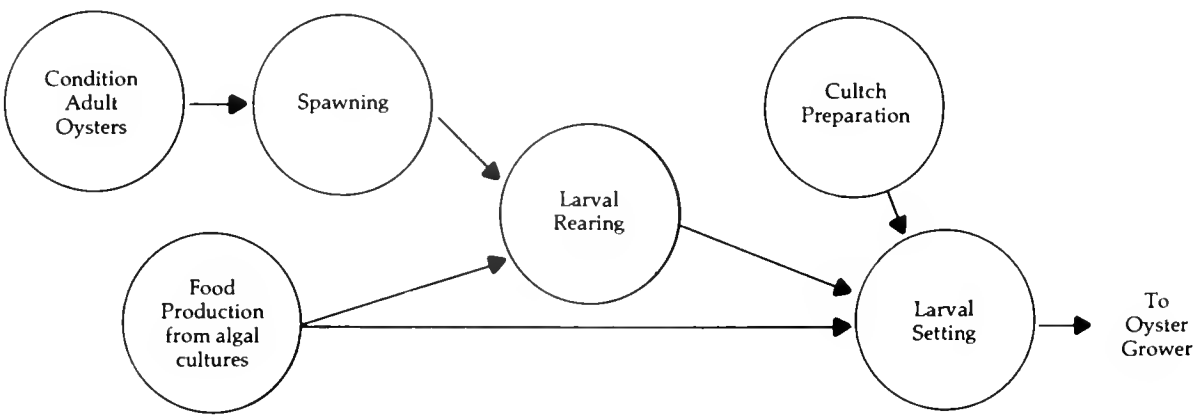

FIG. 2. *Relationship between six stages of oyster hatchery operation.*

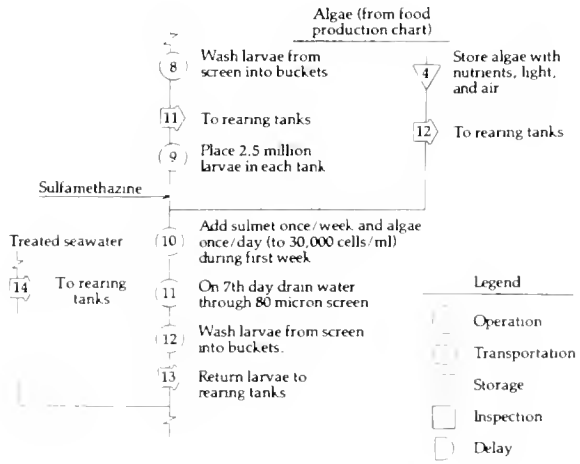

FIG. 3. *Excerpt of detailed process chart activities.*

were based on contractor estimates of $20 per square foot plus 8 percent for design fee, and $1 per square foot for a concrete pad in the cultch preparation area.

The number of various equipment units depends on the capacity output of the hatchery. The major expensive equipment units are Coulter Counter, autoclave, analytical balance, bay pump, dissecting scope, shaker, ultraviolet sterilizer, top-loading balance, and concrete mixer.

Table 4 shows the space requirements and the initial investment outlay for building and equipment, by stages of operation, for Plants I and II.

2. Fixed Costs

The following procedures and values were used in estimating fixed costs:

(a) Depreciation of building and equipment was calculated on the basis of 30- and 10- year lives, respectively (3.3 percent per year for building and 10 percent per year for equipment).

(b) Interest on investment was calculated at 5.2 percent on building and 5.5 percent on equipment, according to the following formula:

$$\text{Average interest} = \frac{i}{2}\left(\frac{n+1}{n}\right),$$

where i = interest rate, estimated as 10 percent for this study, and
n = number of useful years, calculated at 30 years for building and 10 years for equipment.

(c) Insurance and taxes were each estimated at 1 percent of *total initial investment* outlay.

(d) Repair and maintenance charges were computed at 1.5 percent of total initial investment outlay.

(e) Supervision costs were assumed to be 10 percent of direct labor costs.

TABLE 4. *Space Requirements and Initial Investment Outlay for Building and Equipment, by Stages of Operation, for Plant I (15 bu./wk.) and Plant II (800 bu./wk.)*

Stages of operation	Space requirements		Initial investment outlay for building		Initial investment outlay for equipment		Initial investment outlay for building and equipment	
	Plant I	Plant II	Plant I	Plant II	Plant I	Plant II	Plant I	Plant II
	— *square feet* —		— — — — — — — — *dollars* — — — — — — — —					
Conditioning	80	80	1,728	1,728	304	304	2,032	2,032
Spawning	80	80	1,728	1,728	63	66	1,791	1,794
Algae production . . .	520	5,520	11,232	119,232	26,365	115,050	37,597	234,282
Larval rearing	240	12,960	5,184	279,936	3,140	98,900	8,324	378,836
Larval setting	240	11,520	5,184	248,832	1,718	91,560	6,902	340,392
Cultch preparation .	400	2,000	400	2,000	312	2,496	712	4,496
Miscellaneous[a]	1,440	9,896	31,104	213,754	4,100	6,200	35,204	219,954
TOTAL	3,000	42,056	56,560	867,210	36,002	314,576	92,562	1,131,786

[a] Miscellaneous space includes general office, lunch, and locker rooms, lavatory, storage room, analytical lab, and aisles. Miscellaneous equipment includes bay pump, ultraviolet water sterilizer, and filters and valves, air compressor, and miscellaneous glass and plastic containers.

3. Variable Costs

The cost of direct labor at each stage of operation was determined by applying a wage rate of $4 per hour to the average man-hour expenditure required. These requirements were obtained from records maintained, for a 23-week period starting November, 1972, at the Oregon State University hatchery pilot operation. Production during this time was 15 bushels per week. The estimated average weekly man-hour expenditure, by stages of operation, is presented in Table 5.

TABLE 5. *Average Weekly Man-Hour Expenditure, by Stages of Operation, for Plants I and II*

Stages of operation	Plant I	Plant II
	hours/week	
Conditioning	0.2	0.5
Spawning	3.9	4.0
Algae production	13.1	410.8
Larval rearing	9.9	276.6
Larval setting	1.8	96.0
Cultch preparation	1.0	43.7
Miscellaneous[a]	3.7	41.0
TOTAL	33.6	872.6

[a] Preventive and corrective maintenance and other miscellaneous activities.

Costs of electricity are derived from light and power usage and estimated at 1¢/KWH. A one-horsepower motor consumes about .75 KW of electricity per hour of operation.

Both fresh and sea water are used in an oyster seed hatchery. Each larva consumes .00084 liters, or about .00022 gallons, of sea water per week. The major use of fresh water is in cultch preparation. At an output capacity of 15 bushels, or 6 cases, per week, fresh water is used at a rate of 2,800 gal./week for cultch preparation. This figure was computed by measuring the flow of water per second (2.6 gal./20 seconds) and the length of time required to clean each bushel of cultch (30 min./bushel).

Costs of waste and garbage disposal, chemical and bleach, telephone, office supplies, materials, and other miscellaneous costs were included in the total figures. Table 6 shows the estimated costs per week for utilities and supplies, and for stages of operation for Plants I and II.

4. Total Costs

Total costs and costs per bushel for Plants I and II, by stages of operation, are presented in Tables 7 and 8, respectively. These costs include both fixed costs and variable costs. Figure 4 shows the relative importance of cost functions of stage operations.

CONCLUSIONS

Table 9 shows the operational differences between Plants I and II. Direct labor cost is the single biggest item influencing production costs. Direct labor cost of Plants I and II is 27.3 percent and

TABLE 6. *Costs of Utilities and Supplies Per Week*

Item	Costs, by item Plant I	Plant II
	dollars/week	
Electricity[a]	32.04	781.97
Fresh water[b]	4.19	185.27
Waste and garbage disposal[c]	5.60	96.14
Chemicals and bleach	2.40	118.93
Material (uncleaned oyster shell)[d]	7.50	400.00
Telephone	10.00	25.00
Office supplies	3.00	20.00
Miscellaneous	7.00	15.00
TOTAL	71.73	1,642.31

Stages of operation	Costs by stages of operation Plant I	Plant II
	dollars/week	
Conditioning	7.26	7.26
Spawning	2.25	2.25
Algae production	8.11	361.07
Larval rearing	4.56	181.31
Larval setting	8.42	310.19
Cultch preparation	10.89	580.98
Miscellaneous	30.24	199.25
TOTAL	71.73	1,642.31

[a] 1¢/KWH for light and power.
[b] $1.20/1,000 gal.
[c] Sewer charge for ½ of water cost plus $3.50/week for garbage disposal.
[d] $0.50/bu. of uncleaned oyster shells.

TABLE 7. *Estimated Item Costs and Costs Per Bushel for Plant I, by Stages of Operation*

Item	Conditioning	Spawning	Algae production	Larval rearing	Larval setting	Cultch preparation	Misc.	Total
	dollars							
Initial investment outlays:								
1. Equipment costs	304	63	26,365	3,140	1,718	312	4,100	36,002
2. Building costs	1,728	1,728	11,232	5,184	5,184	400	31,104	56,560
TOTAL	2,032	1,791	37,597	8,324	6,902	712	35,204	92,562
Fixed costs:								
1. Depreciation	87	63	3,007	485	343	44	1,436	5,465
2. Interest on investment	107	93	2,034	442	364	38	1,843	4,921
3. Insurance	20	18	376	83	69	7	352	925
4. Repair & maintenance	30	27	564	125	104	11	528	1,389
5. Taxes	20	18	376	83	69	7	352	925
6. Supervision	4	81	272	206	37	21	77	698
TOTAL	268	300	6,629	1,424	986	128	4,588	14,323
Variable costs:								
1. Direct labor	42	811	2,725	2,059	374	208	770	6,989
2. Utilities, supplies, & materials	378	117	422	237	438	566	1,572	3,730
3. Other variable costs	21	46	157	115	41	39	117	536
TOTAL	441	974	3,304	2,411	853	813	2,459	11,255
TOTAL FIXED & VARIABLE COSTS	709	1,274	9,933	3,835	1,839	941	7,047	25,578
Costs per bushel:								
1. Fixed costs per bushel	.34	.39	8.50	1.83	1.26	.16	5.88	18.36
2. Variable costs per bushel	.56	1.25	4.24	3.09	1.09	1.04	3.15	14.42
TOTAL COSTS PER BUSHEL	.90	1.64	12.74	4.92	2.35	1.20	9.03	32.78
PROPORTION OF TOTAL COSTS (percent)	2.7	5.0	38.9	15.0	7.2	3.7	27.5	100.0

TABLE 8. *Estimated Item Costs and Costs Per Bushel for Plant II, by Stages of Operation*

Item	Conditioning	Spawning	Algae production	Larval rearing	Larval setting	Cultch preparation	Misc.	Total
	dollars							
Initial investment outlays:								
1. Equipment costs	304	66	115,050	98,900	91,560	2,496	6,200	314,576
2. Building costs	1,728	1,728	119,232	279,936	248,832	2,000	213,754	867,210
TOTAL	2,032	1,794	234,282	378,836	340,392	4,496	219,954	1,181,786
Fixed costs:								
1. Depreciation	87	64	15,440	19,128	17,367	316	7,674	60,076
2. Interest on investment	107	93	12,528	19,996	17,975	241	11,456	62,396
3. Insurance	20	18	2,343	3,788	3,404	45	2,200	11,818
4. Repair & maintenance	30	27	3,514	5,683	5,106	67	3,299	17,726
5. Taxes	20	18	2,343	3,783	3,404	45	2,200	11,818
6. Supervision	10	83	8,545	5,753	1,997	909	853	18,150
TOTAL	274	303	44,713	58,136	49,253	1,623	27,682	181,984
Variable costs:								
1. Direct labor	104	832	85,446	57,533	19,968	9,090	8,528	181,501
2. Utilities, supplies, & materials	378	117	18,776	9,428	16,130	30,211	10,361	85,401
3. Other variable costs	24	47	5,211	3,348	1,805	1,965	944	13,344
TOTAL	506	966	109,433	70,309	37,903	41,266	19,833	280,246
TOTAL FIXED & VARIABLE COSTS	780	1,299	154,146	128,445	87,156	42,889	47,515	462,230
Costs per bushel:								
1. Fixed costs per bushel	.01	.01	1.07	1.40	1.18	.04	.67	4.38
2. Variable costs per bushel	.01	.02	2.63	1.69	.91	.99	.48	6.73
TOTAL COSTS PER BUSHEL	.02	.03	3.70	3.09	2.09	1.03	1.15	11.11
PROPORTION OF TOTAL COSTS (percent)	.2	.3	33.3	27.8	18.8	9.3	10.3	100.0

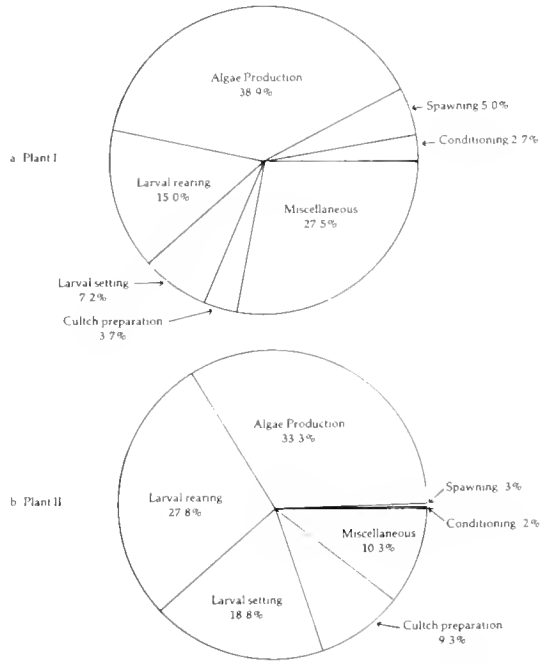

FIG. 4. *Costs of each stage as a percentage of total cost per bushel.*

39.3 percent, respectively, of the total costs per bushel. According to this study, Plant I operates at a total cost of $32.78 per bushel, and Plant II at $11.11 per bushel. The total costs of Plant I are about 195 percent higher than that of Plant II and, furthermore, the total costs of Plant II ($27.77/case or $11.11/bushel) are lower than the current market price of oyster seed (approximately $30/case or $12/bushel).

If Plant II were operating for 36 weeks each year, its annual output would be 28,800 bushels, or approximately 11,500 cases (1 case = 2.5 bushels). According to the Table 3 figures, where 16,602 cases could be sold at $30/case or $12/bushel, such a plant would be viable under the assumed conditions, plus the additional constraint that no seed be imported from Japan. Whether more than one or two plants could survive under these conditions is questionable, however. It must be remembered, furthermore, that the cost analysis was conducted with a hatchery which has a research rather than a cost efficiency objective. A commercial hatchery is concerned with maximizing profits, and would probably experience lower costs. For example, the most expensive item of equipment used at the OSU pilot hatchery is an $11,000 Coulter Counter. This equipment would not normally be used in a commercial hatchery. If, in this study, the Coulter Counter were eliminated, the total costs would drop by $2.70 per bushel or $6.75 per case. With this adjustment the total costs at Plant II would be $8.41 per bushel, or about $21 per case.

Another interesting finding of the study is that, in Plant II, cultch preparation cost, including $0.50 per bushel for uncleaned oyster shell, is $1.03 ($2.57 per case). This is about 9 percent of the total cost. Current market price of already-cleaned cultch is $4.50 per case. At least $2 per case is saved by cleaning oyster shell at the plant facility.

TABLE 9. *Operational Differences Between Plants I and II*

Item	Unit	Plant I	Plant II
Building size	square feet	3,000	42,056
Total initial investment outlay	dollars	92,562	1,181,786
Total fixed cost per year	dollars	14,323	181,984
Total variable cost per year	dollars	11,255	280,246
Total cost per bushel	dollars	32.78	11.11
Direct labor cost to the total cost	percent	27.32	39.30
Output capacity	bushels/week	15	800
Direct labor	persons	1	22
Shaker	numbers	2	57
Coulter counter	numbers	1	1
Concrete mixer	numbers	1	8
Minimum tank capacity	liters/tank	500	500

Additional data may yield different results. We are currently gathering production figures from commercial hatcheries and historical data on variables in the demand equations, for which data have not been available. We hope to be able to provide a more complete analysis, along the lines discussed here, in the near future.

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CAN THE OYSTER INDUSTRY LEARN FROM LIVESTOCK BREEDERS?

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ABSTRACT

Breeding organizations have united breeders and scientists in their endeavor to develop modern farm animal breeding methods. Now that shellfish hatcheries have made oyster domestication possible, a similar organization could be formed where oyster breeders and scientists could cooperate in oyster improvement. Such an organization would have three primary functions.

1. *To devise both economically and biologically feasible standards for oyster breeds. These standards would necessitate a numerical scoring system which would evaluate external characteristics of the animal and rate performance tests of the meats of siblings and progeny through heritability estimates. This would allow quantitative comparison of breeding oysters.*
2. *To initiate and maintain a national oyster registry for breeding oysters. Individual registry would require records of qualifying performance as a breeding oyster. Preferred registry could be granted to oysters with a consistently superior breeding performance.*
3. *To institute certification of seed and parent stock. Exchanges and sales of oysters would be enhanced and less hazardous economically if an oyster stock could be provided with a certificate that states breed, strain, origin, native environment, percentage of culls in the brood, health conditions such as disease resistance, freedom from associated organisms, and other pertinent information. Inspection before certification would be required.*

The proposed organization could be a vehicle for cooperation in oyster propagation improvement among government, breeders, growers, and consumers.

INTRODUCTION

The increased demand for food has recently led to the development of mariculture. In the early stage of fish farming, the emphasis has been on high production, but efforts of fish improvement seem of lesser importance. Fish improvement programs should not be sacrificed to the quest for high density cultures in optimum environments; they should be a part of every operation that propagates fish like other domesticated animals.

The American oyster (*Crassostrea virginica* Gmelin) is well-suited for artificial propagation (Loosanoff and Davis, 1963). The intricate methods for artificial shellfish propagation that have made oyster hatcheries and oyster domestication possible are a recent development.

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With controlled breeding established, shellfish improvement is attainable and necessary. Shellfish domestication is still in its infancy and most hatcheries can do little more than "farm a bunch of wild animals" (Menzel, 1971) because they cannot obtain improved broodstock. The science of genetics and modern animal breeding methods, developed and tested through centuries of farm animal improvement, can serve to achieve comparatively rapid progress in establishing desired characteristics in domesticated shellfish. Geneticists are already involved in such research (Cherfas, 1969; Longwell and Stiles, 1970; Shultz, 1970; Longwell, 1971; Menzel, 1971; Burton, 1972; FAO Fisheries Rept. No 119, 1972; Longwell, 1973).

At this time, the cooperation that exists between breeders and scientists for the improvement of terrestrial livestock has not yet been established for aquaculture. The experiences of cattle breeders as well as other culturists with breeders' organizations, agricultural extension programs, DHIA and Dairy Coops provide valuable examples of the accomplishments that can be affected by cooperation (Winters, 1939; Maliepaard, 1948). Similarly, the effort of all concerned with oyster improvement could be united. Such an organization would have three primary functions:

1. *Establishing standards for oyster breeds and point score.*

Though individual members of the oyster industry may have their own ideas about oyster improvement, there is no agreement on what the requirements for oysters for a certain purpose should be or what improvements are desired (FAO Report No. 119, 1972). There is not a well-defined goal for scientists to pursue. Improvement through line breeding, mass selection, hybridization of inbred lines or cross breeding of lines are possible methods (Longwell, 1976). Faster growth, uniformity of meats and shell, and improved meat quality are improvements likely to be achieved by these breeding methods. However, it has not been determined what improvements should be attempted aside from resistance to one specific disease (MSX) (Haskin, 1972).

Oyster domestication opens up a whole new realm of possibilities. There should be a certain amount of agreement among members of the industry and scientists to determine what kind of oyster is economically desirable and biologically feasible. It is not suggested that there should be agreement on *one* ideal oyster breed for the species *C. virginica*. There are different purposes for which oysters are used and, as in cattle, each could have its own breed. For example, *C. virginica* could be divided into three breeds: raw-bar oysters, shucking oysters, and soup oysters. Breeds would probably have to be subdivided into strains to fit local environmental conditions because part of the oyster's life cycle is spent in waters outside the hatchery. Different strains may also be desirable because consumers in specific localities are accustomed to a certain appearance and taste. Raw-bar oysters could be subdivided into Long Island, Chesapeake, Florida, and Louisiana strains. It may be practical to strive for uniformity in shucking oysters and soup oysters so they can be mechanically shucked.

The oyster industry, in its broadest concept, could consider all desirable and undesirable characteristics of oysters for a certain purpose and carefully weigh their importance relative to the harmony of the total animal. The industry could then conceive of a description or graphic representation of the desired oyster. This representation could serve as a guideline for the scientists working on oyster improvement. Geneticists would then be able to conduct experiments to determine if breeding for the desired characteristics is feasible. It would then be possible to create what is called a standard of perfection in livestock terminology. This would be the example of the ideal oyster that is economically desirable and eventually biologically feasible; the goal for all involved in oyster improvement to pursue. The standard of perfection would create the necessary unity of purpose in breeding direction. Whenever the standard of perfection is approached, new and higher standards could be established.

Certain shell characteristics have, through time, been associated with good oyster meats. Oystermen have always selected oysters by outward appearance. The actual description should be detailed and could contain requirements for specific breeds. A requirement for shucking oysters might be uniformity in shape for efficient mechanical shucking.

The quest for a standard of perfection for

oysters will have to be approached differently from those which apply to cattle, swine, or poultry because the oyster conceals most of its useful characteristics inside its shell. Evaluation of an oyster's exterior characteristics may tell something about the oyster itself and also allow some educated guessing about its meat qualities, but it does not reveal anything about its genetic capabilities as a breeding animal. Performance records of siblings and progeny are needed in case a line breeding approach is taken in place of mass selection (Acker, 1971; Byerly, 1964).

Scientific research can contribute information of what might be expected from oysters and to what extent the wishes of the oyster industry can be met. It can guide the organization when it wants to incorporate the latest scientific developments into revised standards. Scientists can design relevant standardized performance tests. In 1972, Lannan published a test for theoretical and realized predictions of selection progress (heritability estimates) which can be conducted by hatchery operators. Scientists can provide useful information on the effects of inbreeding (Bowman, 1959; Imai and Sakai, 1961; Battaglia, 1970; Castagna and Duggan, 1971; Longwell and Stiles, 1973) and any presumed or realized advantages of cross breeding (Longwell and Stiles, 1973).

Tests on siblings and progeny in the commercial hatchery should give information about a brood's progress, such as uniformity of the meats, average weight gain of the meats and percentage of culls. There may be other pertinent tests with which to monitor brood performance, but it is imperative to have uniform tests that do not burden hatchery operators. The final performance test should be how the oysters shuck out in pints of meats per bushel.

One spawning contains so many individuals that removal for selection and performance tests do not significantly deplete the brood. Oysters mature early and their value as breeding stock can be determined on the basis of progeny tests of their initial spawnings. Breeding stock determination can be made early enough in the oysters' life to allow its use for breeding stock for several years.

In order to make an agreed standard of perfection into a workable tool, it should be translated into a numerical score. The ideal exterior shell characteristics and the highest possible results of the sibling and progeny tests for a stock should receive numerical values according to their importance. The combined values would then be a perfect oyster's numerical rating. As genetic estimates of heritability for commercial characteristics becomes available and the results of studies for positive and negative correlations accumulate, industry and scientists could prepare selection indices that reflect priorities on the basis of both low or high heritability and commercial importance of the traits. The ideal specimen with its perfect score, would serve as a standard for comparison. Selection of breeding stock should be made from among oysters with the highest ratings in shell characteristics and performance. Breeding oysters should meet or exceed a minimum point score. Certain important characteristics might require a minimum score; oysters with a rating below the minimum for that characteristic would be disqualified from breeding.

2. *Registration*

Coordination in breeding direction could be achieved through the establishment of breeds and strains with their own standards of perfection. Scoring systems and maintenance of requirements for breeding stocks could determine the quality of animals used in oyster improvement while pedigree records could identify possible exceptional brood stocks.

Unless pedigree or mass selection records are registered with a breeders organization, the qualifications of such stocks would be known only to the owner. When the records are entered into a national registry, they become part of a combined effort in oyster improvement. Registered breeding stock might be exchanged or sold between breeders to the benefit of the breed.

Because individual oysters are hard to spawn, it may be advisable to enter entire qualifying oyster broods into a "provisional registry" and assign them a brood number. Entry into a "provisional registry" would depend on the results of progeny tests of more than one generation. When both parent and broods have qualified for registration, subsequent broods may also be registered. Individual oysters that show exceptional breeding characteristics might be entered into individual registration in addition to their brood registry.

The breeder would, in such cases, identify each spawner, keep the brood separate and keep a record of individual combinations. Individual oysters that consistently spawn exceptional broods might be entered into a "preferred registry."

3. *Certification*

In the future there may be three types of oyster producers: 1) breeders — hatcheries that actively pursue oyster improvement through scientific breeding programs; 2) augmenters — hatcheries that obtain the best possible brood stock and propagate large quantities of quality seed oysters; and 3) commercial oyster producers — planters and packers who buy quality seed and raise it to market-size and sell oysters to the public. Furthermore, if the shift to specialization in the cattle industry is any indication, it may be conceivable that future spat may be raised in one place, shipped to a good growing area, transferred to a fattening region, then to a region where the oysters would acquire the best possible taste and finally to the shucking house and consumer.

Specialization would require large amounts of high quality seed oysters to be bought, sold, and shipped. The seller could get a higher unit price if he could guarantee the performance that can be expected from seed offered for sale. A record of origin and environment could help a buyer judge whether or not the oysters are likely to perform for him. If oysters from a certain source do not perform, the buyer would then know that he does not want oysters from that source again.

Through registration of parent stock, brood and progeny performance tests, and final scrutiny by inspectors, a certificate of the merits of that brood could be issued. The inspection service could be a government subsidized branch of the organization (Anon., 1968). The certificate could contain information about breed, strain, origin, and environment as well as the percentage of culls, growth rate, disease resistance, health conditions, freedom from associated organisms, etc. Such certification would take the hazards out of seed buying. The superior performance of the seed would warrant a higher unit price which would include part of the cost of certification.

Certification could also help area protection, by allowing only certified disease-free oyster imports from compatible areas to be planted (Anon., 1968).

CONCLUSION

Responsibility for establishing breeds with their own standards, registration of breeding stock and certification may appear uncomplicated but a word of caution is appropriate. When descendants from wild populations are considered for the creation of a breed or entry into an established breed, we have to consider that the oyster's dependence on its environment may be difficult to overcome. Most wild oyster populations have adapted, through evolution, to their particular environment. When these populations become domesticated and their environmental conditions are changed, they may respond with erratic breeding and/or growth performance. An animal's highest performance is limited by its genetic capability to perform, but it will only perform at its highest capability when the environmental conditions are optimal. The genetic capabilities of oysters and requirements for optimal environment may vary between individuals and populations. Thus, it is necessary to discover what constitutes an optimum environment for individual oysters and populations of oysters before their genetic capability can be investigated.

Scientists could attempt to develop oysters that perform well in environments considered marginal; for instance, low salinity, high turbidity, etc. Selective breeding may create oysters that could be raised under optimal environmental "feed lot" conditions and others that perform satisfactorily under limited conditions.

The difficulty in setting standards for oysters that require such different environments to perform could be partly alleviated by including "recommended environmental conditions" on their certificates rather than confusing the common objective by too many sub-breeds and strains, each with their own standards. This would also prevent standards from deteriorating into stifling rigid rules that prevent the adoption of new developments, that once plagued cattle breeders (Bakker, 1948). Effectiveness of a breeders' organization would depend on the progressive efforts put forth by its members. It should be a forum and incentive for genetic experimentation and new developments should have its full support. It could be the vehicle through which government, scientists, growers, breeders and consumers cooperate in oyster improvement.

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ABSTRACTS OF THE TECHNICAL PAPERS PRESENTED AT THE 1975 NSA CONVENTION

A MARICULTURE SYSTEM FOR GROWING THE HARD CLAM, *MERCENARIA MERCENARIA*

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A relatively simple system of spawning and growing clams from egg to market size has been developed. This system utilizes the known technology for spawning clams and growing the larvae. The Glancy-Wells (centrifuged, incubated seawater) solarium method is used for growing algal food. The clams are spawned using thermal stimulant and stripped sperm. The larvae are grown in clarified incubated seawater until set. The post set are immediately transferred to flowing seawater tables where they are held until they reach approximately 2 mm (hinge to lip) size. They are then planted in a nursery area at 300 per sq. ft. on an intertidal flat. They are protected from crabs and other small predators by a crushed stone aggregate cover and prevented from being washed away by simple 2 foot high plastic screen current baffles.

In areas where larger predators such as rays are a problem, the area is further protected by a plastic screen fence. The system appears to be economically feasible. The cost of growing the clams is approximately $.015 each. The survival over winter in the present experiment is approximately 88%.

The system has the advantage of simplicity, has low energy demands, and is relatively inexpensive.

SYSTEMS ANALYSIS OF AN OYSTER COMMUNITY: AN EVOLVING MODEL

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Georgetown, South Carolina

A linear systems model is developed which describes energy flow through an intertidal oyster community. The model assumes that the intertidal oyster community is in a steady state. Computer simulation of the model leads to a stable system in a short period of time. The danger of utilizing data from other geographic areas is pointed out using the oyster as an example. In addition, it is shown that a systems model is a useful means of summarizing the known and emphasizing the unknown, thus helping to direct the research effort in a productive manner.

FRESHWATER AQUACULTURE OF THE TROPICAL PRAWN, *MACROBRACHIUM ROSENBERGII*, AND THE RAINBOW TROUT, *SALMO GAIRDNERII* USING THERMAL EFFLUENT DISCHARGES FROM AN ELECTRIC GENERATING STATION

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A cooperative research effort involving two companies, Public Service Electric and Gas. Co. and the Long Island Oyster Farms, as well as two institutions of higher learning, Trenton State College and Rutgers University, was mounted to investigate the possibilities of growing the freshwater prawn, *Macrobrachium rosenbergii*, as well as the rainbow trout, *Salmo gairdnerii*, to commercial sizes in aquaculture systems utilizing waste-heat water discharges of an electric generating station.

Two laboratories and several outdoor ponds were constructed at the Mercer Generating Station, Trenton, New Jersey, in the spring of 1974. Post larvae of the freshwater prawn were supplied by the Long Island Oyster Farms and stocked in laboratory tanks in May, 1974. Animals grew from 10 mm to 22 mm in one month at temperatures of 28°C; thermal effluent water from the generating station was passed through heat exchangers to boost temperatures to optimal levels. Prawns were stocked in outdoor ponds in mid-June at a density of 1/ft^2.

Pond temperatures averaged 25-30°C from June to mid-September; flow rates were adjusted to 100 gallons/minute. Netting and egg-crate shaped structures were suspended in the pond to increase surface area for prawn habitats. Animals were harvested biweekly for length-weight measurements; water quality was checked daily for dissolved oxygen, ammonia and pH and weekly for a wide range of dissolved and suspended solids.

Animals were harvested in early October, 1974; average growth rate was 0.6 inch/month although prawns at upper end of range grew 1 inch/month. Survival rate was 90.2% for 3 1/2 month pond experiment.

A program of brood-stock management and larval rearing was begun in late June, 1974. Adult animals were kindly donated by Florida Department of Natural Resources. Twenty thousand animals were raised through eleven larval stages to metamorphosis.

Seven-inch trout fingerlings were stocked in an outdoor pond starting in mid-November, 1974: final stocking occurred in late December, 1974. Most trout were removed in late April, 1974; animals averaged ten inches in length and weighed 0.4 pounds. This represented an average growth of 0.6 inches per month. No mortalities occurred after animals recovered from trauma of stocking; no diseases were observed during the 5-month sojourn in pond.

FABRICATED SUSTRATES - AN APPROACH TO THE INTENSIVE CULTURE OF *MACROBRACHIUM ROSENBERGII* (DE MAN)

Mark C. Evans

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Preliminary work at the Waste-Heat Aquaculture Facility, located at Public Service Electric and Gas Company's Mercer Generating Station, Trenton, New Jersey, has demonstrated the feasibility of intensive culture for the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). Three fabricated substrate designs, introduced to increase the surface area in a given volume of water, have all produced satisfactory growth rates and mortality levels in densely stocked tanks. As no significant difference between results is demonstratable at this time, it would appear that any increase in surface area per volume of water would produce a corresponding increase in the density to which prawns could be cultured. Work currently in progress should serve to confirm these results.

SURVEY OF SOUTH CAROLINA'S HARD CLAM *MERCENARIA MERCENARIA* RESOURCE

Robert C. Gracy

South Caroline Wildlife and
Marine Resources Department
Marine Resources Division

South Carolina clams have normally been harvested only incidental to oyster harvesting in the intertidal areas. Several reasons were responsible for the State's low clam production: shortage of hand labor, regulations prohibiting the use of most mechanical equipment, lack of knowledge of subtidal clam beds, and limited local markets.

In 1973 the Marine Resources Division initiated a clam survey of areas not under lease for oyster cultivation. The survey started March 1, 1973, and concluded June 30, 1975. To date, 18,000 square yard samples have been reconnoitred and four commercially valuable clam beds have been located. Systematic sampling was accomplished using a specially designed shallow draft boat 20 ft. long with an 8 ft. beam, equipped with a square yard set of hydraulic patent tongs. Information collected consisted of numbers of clams, species composition, grade size, location, bottom types and depth. This information was recorded on dai-

ly log sheets and transferred to computer cards for later retrieval. Experimental mechanical clam harvesting was permitted in 1974-75 and represents an increase of 1,402,476 lb. of clams over the previous season.

THE SUITABILITY OF MAINE WATERS FOR CULTURING OYSTERS *C. VIRGINICA* AND *O. EDULIS*

Herbert Hidu

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Studies have been underway since 1971 to evaluate Maine's diverse waters for commercial raft cultivation of American and European oysters. American oysters have been abundant in the past in Maine's upper estuaries and European oysters were introduced in 1949 in the hope that an outer coastal population would develop.

Intensive studies on the Damariscotta "Arm of the Sea" Estuary indicate that market oysters can be produced by raft culture in 2 to 3 years; American oysters in the protected warmed sites and European oysters in the colder sites. Cooperative experiments with private citizens have substantiated these results. Since Maine lacks a consistent natural seed source, hatchery production of northern stocks appears to be the viable option.

The Maine Aquaculture Law of 1973 has stimulated several entrepreneurs into pilot commercial oyster growout operations. Cooperative interaction has allowed us to make considerable advancement in system optimization. The most workable growout system for Maine waters appears to be a series of stacked floating trays tended by a shore-based service raft. The economics of Maine oyster production is discussed.

A STUDY OF SHELLFISH HATCHERY BACTERIAL DISEASES[1]

Louis Leibovitz, S. B. Hitchner, Paul Chanley, David Relyea, and Joseph Zatila

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Bacteriologic cultures were obtained from the water supply, algal cultures and shellfish larval cultures at 2 commercial shellfish hatcheries at approximate monthly intervals throughout a one year period. The dominant colony type of each bacterial culture sampled was selected and isolated as pure cultures, and subjected to identification procedures. Each of the identified selected isolates were tested for shellfish larval pathogenicity in a model system. The results of the pathogenicity tests were interpreted and possible application to further research needs and hatchery problems were discussed.

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[1] This work is a result of research sponsored by NOAA Office of Sea Grant, Department of Commerce, under Grant #04-3-158-39 to the New York State Sea Grant Program. The U. S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

OPERATING CHARACTERISTICS OF A HEATED, RAW SEAWATER, OYSTER FINISHING PILOT PLANT

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A research program to evaluate the growth response of oysters, *Crassostrea virginica*, to both environmental and fixed effects, has been in progress since December, 1973.

The growth in open flow of raw seawater has been tested for four levels of water temperature, up to ten levels of initial size of the experimental oysters, and a wide range of flow rates from 10 to 120 liters/oyster day.

Both growth data and weight of biodepositions

and sediments have been obtained over time at intervals of about sixty days.

Tentative mathematical models to describe the growth response are being developed through multiple regression analysis and correlation of the most significant variables.

A pilot plant was designed and built for this experiment. Its main components were: a heat exchanger for seawater, a degassing unit to prevent supersaturation of the heated water, a system of fouling free regulators to mix and keep constant flow rates of raw seawater, and a raceway system to hold the experimental oysters.

Attempts will be made to optimize the oyster growth process, and then to scale-up the pilot plant design.

CLAM MARICULTURE IN NORTHWEST FLORIDA: OBSERVATIONS ON SELECTION AND HYBRIDIZATION

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For the past three and a half years, attempts have been made to secure faster growing quahog clams by crossing the northern (*Mercenaria mercenaria*) and the southern quahog (*M. campechiensis*), and through selection for fast growth. Clams for parents have been obtained from areas extending from Maine to Texas. Thirteen successful rearings and plantings under natural conditions were made in 1972, 10 in 1973, 17 in 1974 and 48 in 1975. Replicates of each cross, upon reaching a size of 2 to 3 mm in the laboratory, selected for larger size and unselected controls, were planted and observed.

Some of the 1972 and 1973 crosses reached an average commercial size (over 50 mm long) in 14 to 15 months (some individuals over 60 mm) from time of spawning. The fastest growing clams were those "selected" groups of backcrosses, F_1 hybrid (from previous work) to the southern quahog. The emphasis in 1974 and 1975 has been to cross the larger pedigreed clams and also to backcross these with the northern quahog (which has the desirable commercial trait of remaining closed when removed from the water).

OYSTER DEVELOPMENT IN ATLANTIC CANADA — THE POTENTIAL AND THE PROBLEMS

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The oyster industry of Atlantic Canada has never retained its high production of earlier years, although considerable research and study has been carried on by government agencies and consultants.

A renewed interest in increasing the production to the estimated potential has developed in recent years. This has involved several Federal and Provincial government agencies and the establishment of new industry groups and firms.

The need for a co-ordinated approach to government funding and industry organization was recognized by the Federal Fisheries and Marine Service and a Maritime Oyster Development Committee was formed. This sixteen-member group is comprised of industry and government representatives.

An inventory of government funded programs has been completed and a plan for the maximization of benefits to the oyster industry is being drafted.

This paper briefly describes the background, the present situation, some of the problems and the potential of the oyster industry in Atlantic Canada.

INGESTED MATERIAL IN TWO SPECIES OF ESTUARINE BIVALVES: *RANGIA CUNEATA* GRAY AND *POLYMESODA CAROLINIANA* (BOSC)

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Stomach contents of two species of estuarine bivalves, *Rangia cuneata* Gray and *Polymesoda caroliniana* (Bosc), were collected from the

Ochlockonee River estuary (Franklin County), about 40 miles south of Tallahassee, Florida, and analyzed qualitatively and semi-quantitatively from May through November, 1972.

Items were qualitatively categorized as diatoms, algae (other than diatoms), unidentifiable detritus, and "other" (sand grains, spicules, etc.). At each monthly sampling period, five adult individuals of each species were randomly selected and preserved *in situ* with formalin. In the laboratory, the stomach contents were pipetted into formalin and then identified under a Zeiss phase-contrast microscope. Classification was down to genus if possible in the case of diatoms and algae.

A total of 48 species of phytoplankters were found in *R. cuneata* stomachs and 52 species were found in *P. caroliniana*. There were 34 species common to both clams and the most frequently occurring of these (occurring at least 50% of the time in both clams) were: *Asterionella sp.; Cyclotella sp.*, a colorless flagellate; *Diatoma sp.; Gymnodinium sp.;* two species of *Navicula;* two species of *Nitzschia; Stephanodiscus sp.*, a unicellular alga; and an unknown pennate diatom.

R. cuneata also was studied semi-quantitatively with regard to percentages of material ingested. In each of the months of July, September, and November, 20 to 25 adult individuals were collected at low tide and preserved *in situ*. Immediately following the November low tide collection, a high tide collection was made as a comparison. Stomach contents were suspended in formalin and 30 random fields were counted from well-shaken samples using the phase-contrast microscope at 800χ. Units of area were used to estimate relative amounts of material.

In all of the sampling months, detritus was found to be the most abundant material ingested. Mean levels of detritus ranged from 46% to 56% at the low tide collections, and to 81% at the November high tide collection. Corresponding detritus levels in water samples taken at the same times were from 60% to 66% at low tide, and 58% at high tide.

This study indicates that both *R. cuneata* and *P. caroliniana* are non-selective filter feeders. It was not determined which of the ingested material is actually utilized as food by the clams.

OYSTERS (*CRASSOSTREA VIRGINICA*) EXPOSED TO A COMPLEX INDUSTRIAL WASTE: SURVIVAL, GROWTH AND UPTAKE OF ANTIMONY COMPOUNDS

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Eastern oysters (*Crassostrea virginica*) were exposed continuously for 91 days in flowing sea water to a complex industrial waste which contained antimony compounds. Antimony was present in the waste as fine particles of antimony trioxide and as a soluble complex with ethylene glycol. Survival and growth (as determined by in-water weight) of oysters exposed to mean measured concentrations of 5.3 μg of antimony per liter of sea water (parts per billion, ppb) and <0.8 ppb of antimony did not differ significantly ($P<0.05$) from controls. Oysters did not accumulate antimony in tissues (whole-body) nor was antimony incorporated into the shells.

SEASONAL VARIATIONS IN TISSUE WEIGHT AND TOTAL SOLIDS OF THE CALICO SCALLOP, *ARGOPECTEN GIBBUS* (LINNE) AND THEIR RELATIONSHIP TO CHANGES IN GONAD CONDITION

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Seasonal tissue weight and total-solids data were collected in 1972 as part of an ecological study of the commercial calico scallop,

Argopecten gibbus (Linne), beds which existed that year south of Beaufort Inlet, North Carolina, at a depth of 20 to 25 m.

Seasonal tissue weights were examined by three methods: 1) wet weight, 2) weight-length ratio, and 3) growth-curve ratio. Because of significant correlations between wet-tissue weights and weight-length ratios to scallop lengths (gonad tissue data were exceptions), these methods were considered of questionable value as indicators of the life history of calico scallops. No correlation was found between calico scallop shell length and either growth-curve-ratio or total-solids data.

Total-solids and growth-curve-ratio data indicated that calico scallops off the North Carolina coast spawned between April and May and possibly the late fall in 1972. With spawning, all tissues exhibited a weight drop followed by gradual weight increase. Total-solids values for muscle and visceral tissue, subsequent to the spring spawning, did not increase or decrease, suggesting possibly that glycogen values or scallop condition remained relatively constant. During early and late summer gonad tissue, tissue-weight and total-solids data contained a number of peaks. It is theorized that one of these may have been an indication of an early September spawning by the calico scallops.

The supply of scallops diminished by October to an unprofitable level and commercial fishing was discontinued. Scallop tissue conditions, during this same period, did not suggest that demise of the fishery was caused by dying off of the scallops.

Seasonal chlorophyll-*a* values from the fishing area were not indicative of an upwelling condition and had no tracable relation to tissue values. A late fall bloom may have helped survival of larvae from a possible late fall spawning.

THE MARICULTURE POTENTIAL OF *TAPES SEMIDECUSSATA*(REEVE) IN AN ARTIFICIAL UPWELLING SYSTEM

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Three successive generations of *Tapes semidecussata*(Reeve) have been raised on mixed diets of three species of diatoms cultured in continuously flowing Antarctic Intermediate water. Parent stock which was introduced to the system at a length of 5 mm attained market size (38 mm) in 10 months. Population densities varied from 1600/ft² as juveniles to 160/ft² as marketable adults, and survival for the growth period was 64%. F_1 progeny, from time of fertilization, reached market size in 13 months. Larvae metamorphosed after 21 to 25 days and post-set survival was as high as 52%.

These preliminary studies show that *Tapes* can be spawned readily in a controlled system, grown rapidly in high densities with good survival, and consequently show a favorable potential for mariculture.

SCALLOP CULTURE IN MUTSU BAY, JAPAN

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In recent years, the production of sea scallops, *Patinopecten yessoensis*, in Japan has increased significantly. In 1973, 61,600 metric tons were harvested compared to approximately 4,000 metric tons in 1968. A major reason for this increase is the development of off-bottom techniques not only in the collecting of seed scallops but also in growing them to market size.

One of the centers for scallop culture in Japan is Mutsu Bay. Based on two trips to this area, one in 1970 and more recently in 1974, the author will describe the methods of culture now being practiced in the Bay. A brief description of harvesting and processing will also be presented.

RECIRCULATING SYSTEMS FOR EMBRYO INCUBATION AND LARVAL REARING OF THE FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DEMAN)

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Natural development of the freshwater prawn *Macrobrachium rosenbergii* will be compared to the more "traditional" culture methods being presently used by most culturists. The development of high-density recirculating systems at the aquaculture facility at the Mercer Generating Station, Trenton, New Jersey, will be discussed.

An early-larval rearing system incorporating an embryo incubator with automatic separation of larvae, and several late larval rearing systems will be described and evaluated with respect to economy of operation and larval survival. Several design criteria will be discussed.

PHYSICAL PARAMETERS OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* GMELIN, FROM THE WAREHAM RIVER

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Data from experiments conducted with the American oyster during the past several years at the Wareham River are analyzed to reveal relationships among length, weight, volume and density of whole oysters, oyster shell and meat.

The effects of variation due to genetics, growth environment and season are pooled to give general relationships which are useful in engineering design of aquaculture systems and in the design of experimental procedures and apparatus.

THE PRESENT STATUS AND FUTURE OUTLOOK OF SHELLFISH FARMING IN PUGET SOUND, WASHINGTON

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Shellfish farming in Puget Sound, Washington, involves principally culture and harvest of oysters, and harvest of natural crops of clams and geoducks. Present trends are decreasing production of oysters with some increase in clams and geoducks. Biologically, Puget Sound has enormous potential for increased production of oysters and geoducks due to the abundance of well protected, clean, nutrient rich water. Economic conditions have had a major impact on the oyster industry and economic conditions appear to be the major reason for the decline. Economic conditions appear favorable for the clam and geoduck fisheries. Recent enactment of legislation for control and management of shorelands has had some adverse effect of conduct of the shellfisheries and, depending upon further application of these laws, could be a major impediment in conduct of the shellfisheries in Washington State.

NSA PACIFIC COAST SECTION

OUT-BAY CULTURE

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A relatively new type of oyster farming is proposed called "Out-bay Culture". Its advantages and problems are discussed. Some data are presented which indicates this type of culture may be successful. In the future, oyster aquaculture will call for a ration which is yet to be formulated. The method also provides necessary control over the operation. Out-bay culture is speculative at the present and may or may not develop into a commercial reality.

RELATIVE ACUTE TOXICITY OF A PESTICIDE, HEAVY METAL AND ANIONIC SURFACTANTS TO MARINE ORGANISMS

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The relative acute toxicity of cadmium sulfate, methoxychlor, and two types of anionic surfactants (dodecyl sodium sulfate and linear alkylate sulfonates) was determined using various species of marine fish, crustacean, and bivalve mollusk larvae. Methoxychlor was most toxic to spot shrimp and Dungeness crab zoeae (48-hr median lethal concentrations or LC50's of 0.025 and 0.044 mg/1, respectively), of intermediate toxicity to chum salmon fry and larval Pacific herring (48-hr LC50's of 0.048 and 0.150 mg/1, respectively), and least toxic to three species of larval clams (native littleneck and the horse clams, *Tresus capax* and *T. nuttalli*) and larval Pacific oysters (range of 48-hr LC50's of 0.198 to 0.441 mg/1). No relationship was found between the toxicity of cadmium sulfate and sensitivity of a particular taxon. The 48-hr LC50 values ranged from less than 0.1 mg Cd^{++}/1 for larval horse clams (*T. capax*) to 14 and 115 mg Cd^{++}/1 for Pacific herring and brine shrimp nauplii, respectively. The anionic surfactant, dodecyl sodium sulfate, was most toxic to all four species of bivalve mollusk larvae (range of 48-hr LC50's from 0.58 to 0.89 mg/1) and least toxic to spot shrimp and Dungeness crab larvae (48-hr LC50's of 5.8 and 8.0 mg/1, respectively). Tests of three linear alkylate sulfonate formulations, composed of surface active agents having carbon chains of different lengths, were also conducted using larval Pacific oysters. The LAS formulation having a predominance of long carbon chains (e.g. 12, 13, and 14 carbons) was the most toxic (48-hr LC50 of 0.10 mg/1), and that with the shortest carbon chains (e.g. 10, 11, and 12 carbons) the least toxic (48-hr LC50 of 0.56 mg/l).

WINTER SPAWNING OF PACIFIC OYSTERS

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Vance Lipovsky

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Pacific oysters, *Crassostrea gigas*, from selected growing areas in Willapa Bay, were brought into the hatchery during January, February, and March of 1974 and 1975 for spawning. Eggs from

unconditioned oysters developed into larvae that were as viable as the eggs from oysters that had been conditioned for spawning. It appears that oysters which have not spawned during the summer will retain some viable gametes throughout the winter.

SYSTEM WORK DESIGN AND INTERIM REPORTS COVERING CURRENT AND PROPOSED INDUSTRIAL ENGINEERING STANDARDS FOR SHRIMP, CRAB, OYSTERS BOTTOM-FISH AND PRODUCT-MIX SPECIES

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Part One — Improvement of Direct-Labor Productivity

Workers will gain a deeper insight of skill levels, irregular acts and allowances (personal, fatigue and unavoidable delays) when they are exposed to **MAP** (Master Achievement Programming). Loop motion pictures and achievement tests illustrate desirable motion patterns and the most common errors and irregular acts. Each step is described by four basic acts: move, grasp, position and use. For self-appraisal and improvement, a Master's achievement test shows the time (in seconds) for high, average and low-skill performance.

Part Two — Improvement of Supervisory and Staff Productivity

Supervisors and supporting staff skills can be appraised by an investigation of **SAP** (Supervisor Achievement Programming). The work done by a crew being supervised can be used as a predominant measure of appraising supervisors and supporting staff. To get relative performance differences, two case studies with operating plants have been started. In both plants, the management agreed to furnish a complete record of past performance so a linear relationship can be calculated and assignable causes can be identified. Although this relationship is an important management tool, more important is the prompt analysis of large productivity differences between normal and standard times. When differences are high, immediate steps can be taken to avoid such repetitions in the future. Some possible assignable causes which will be investigated to discover productivity effects include personal time, yields, set-up time, fish condition, thefts, avoidable delays, safety, sanitation, skill levels, tool and equipment condition, unavoidable delays, consumer acceptance, fish size, errors in production data and other work on environmental factors yet to be determined.

Part Three — Improvement of Top-Level Productivity

Top Level Productivity — Owners, Plant Managers and Staff: **CAP** (Chiefs Achievement Programming) can be improved and evaluated when complete product-mix standards are included in a future plan and schedule chart. More effective resource planning, scheduling and utilization takes place when top level management can see the effects of their decision making. One cooperating processing manager stated that the processing of seafood (i.e. crab and shrimp) along with their traditional vegetables, showed a net profit (before taxes) of approximately $35,000. He mentioned that the seafood processing alone did not result in a direct profit but that by augmenting their entire production with the seafood processing, the savings resulted by providing a means to direct people and resources that would otherwise be idle. The $35,000 savings was validated by using actual inplant data in a Resource Planning and Management (RPM) chart. Also, RPM charts can be used to predict future results and to adjust rapidly with appropriate changes when the unexpected occurs. Other significant uses of the chart lie in the quick evaluation of the effects of mechanization, alternate marketing decisions, imperfect operations and the complexity of the input-output relationships among the various activities and resources.

AN APPROACH TO DEVELOPING A STOCK OF DISEASE RESISTANT OYSTERS

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A program currently underway at the University of Washington to develop strains of oysters

(*Crassostrea gigas*) that are resistant to the "summer disease" apparently caused by *Vibrio* sp. was presented. Briefly this study involves challenging adult oysters to a mortality-inducing situation in the laboratory, breeding the survivors together by single parent matings (one male x one female), and, after setting and growing these families, retesting them under the same conditions to check for increased disease resistance. Families which demonstrated increased resistance would be used to produce the next generation and for testing "on site" for increased resistance in a natural situation. To date, about 20 crosses have been made, which will be retested in the spring of 1976.

In addition, the families produced for the resistance studies will be monitored for single gene differences by electrophoresis of tissue enzymes and proteins. Data presented indicated that the frequencies of various genes have been changed in hatchery stocks, compared to naturally reproducing populations. This means that the genetic constitution of the oyster is being changed by artificial manipulation. In order to maintain the necessary genetic variability, avoid the problems of inbreeding, provide "markers" for further genetic manipulation, and distinguish specific stocks, gene differences as shown by electrophoretic means can be utilized as valuable tools in oyster culture.

SITE COMPARISON FOR THE CULTURE OF THE SPOT PRAWN *PANDALUS PLATYCEROS* BRANDT IN AND ADJACENT TO SALMON NET PENS

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University of Washington
Seattle, Washington

Clam Bay and Henderson Inlet in the central and southern basins of Puget Sound, respectively, were compared as potential prawn aquaculture sites. Seasonally warmer waters of the latter site were conjectured to accelerate growth.

Wild and laboratory reared prawns were held in nylon net pens with and without salmon and in benthic cages beneath commercial salmon net pens. Prawns were fed raw mussel (*Mytilus edulis*) and sea kelp (*Nereocystis leutkeana*), Oregon Moist Pellets (fish food) or geoduck clam (*Panope generosa*) processing wastes. Unsupplemented groups held in net pens and benthic cages could utilize fouling organisms or organic enrichment from the adjacent salmon pens.

Growth and survival of juvenile prawns were significantly higher at Clam Bay than at Henderson Inlet. One year old prawns averaged 7.13 grams which exceeded growth reported for wild populations off Vancouver Island, B.C. Rates of growth for Henderson Inlet benthic and Clam Bay surface yearlings were more rapid than the reported Vancouver Island populations with the exception of net pen, unsupplemented groups.

Henderson Inlet surface waters were unsuitable for prawn culture due to extreme temperatures (21.9° C in early June), protozoan fouling and dense plankton blooms. During the summer season surface reared juveniles and yearlings experienced 70% and 100% mortality, respectively. Conversely, benthic caged prawns at 10 meters had only 15% mortality during the same period.

Differences in growth and survival and possible applications to commercial culture are discussed.

RELATIONSHIP BETWEEN PACIFIC OYSTER SEED DENSITY AND FIRST YEAR GROWTH

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Growth of Pacific oyster seed was greater for densities of 8 or less per mother-shell within the density range tested, 1 to 40 per shell. Average size increased as the number per shell decreased within the 1 to 8 spat per shell densities. There was no statistical difference in average size among the 9 to 40 oyster per shell densities although average size declined slightly with increased density. At very low density, 10 or less per shell, there was no difference in average volume related to area of overwinter hardening. At densities from 11 to 40 per shell the average volume per oyster was greater for those over-wintered at North Bay compared with those at the Point Whitney Lagoon.