CLAM-ABALONE SPAWNING AND REARING

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BY: Gerald Lukas
Fish Commission of Oregon

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CLAM-ABALONE SPAWNING AND REARING

INTRODUCTION

Laboratory studies during the 1971-72 project year consisted primarily of developing mass culture and planting techniques for Manila littleneck clams *(Venerupis semidecussata)*. Field studies continued utilizing laboratory-reared juvenile Manilas. These studies involved establishing experimental plots in several estuaries to evaluate the substrate potential as Manila habitat. Experimental clam plots, involving other clam species or the Manila clam, established in previous project years were monitored.

Adult red abalone *(Haliotis rufescens)* were obtained from California for spawning. Experiments were initiated for culturing diatoms in the laboratory as a primary source of food for juvenile abalone.

CLAM STUDIES

Methods

Clam Spawning, Larval and Juvenile Rearing

We were successful in mass rearing juvenile Manila littleneck clams using previously reported spawning and rearing techniques (Phibbs 1968, 1970). Heated sea water and potassium chloride (KCl) and/or Manila littleneck sex products were used to stimulate the adults to spawn after a one to two week conditioning period. A bactericide, sodium sulfamethazene (Sulmet) was added routinely at the start and with each water change during the larval phase of the Manilas' development. The Sulmet was used at a concentration of .05 gm/L.

A controlled temperature room in the laboratory was adapted to mass rear the juvenile clams during the first five months of their development. We used three types of containers which were found to be most suitable for mass culture. They were 16-liter glass carboys and 550 and 100-liter fiberglass tanks.

The glass carboys were used to hold fertilized Manila eggs during the first two days of their development. Normal Manila eggs held at temperatures of 20-22 C reached the swimming velliger stage in 48 hours. At the end of 48 hours, the Manila velligers were transferred to the 550-liter tanks and reared for two weeks. Feeding of the clam larvae began at this stage. An algae (*Isochrysis sp.*) was fed at a concentration of 20,000 to 30,000 cells/ml during the first week. The concentration was gradually increased as the clams grew. Water temperatures were maintained at 20-22 C and the salinity at 28 parts per thousand. Under these conditions, the clams completed metamorphosis at the end of two weeks and began to leave the water column to set on the bottom of the tank. These shallow tanks measured 130 x 68 x 14 cm deep. The water was renewed once a week.

The manila juveniles were held in these tanks until they reached a shell length of 1 mm, which took from three to five months. The clams were then transferred to a room where a supply of sea water pumped directly from Yaquina Bay was available. This water was heated to 15-16 C before being introduced into the tray of juveniles. The temperature of the raw sea water was gradually reduced during a two-week period to acclimate the clams to the temperature of the incoming sea water, which varies between 9.5 and 10.5 C. Water flow through the tanks ranged from 6.5 to 9.7 liters per minute. After the clams were transferred to the trays of running sea water, they were not fed laboratory-cultured algae and sustained themselves on phytoplankton occurring in the sea water.

<u>Clam Burrowing Study</u>

The clam burrowing study was initiated to determine conditions that would best permit juvenile Manila clams to burrow completely into a substrate. Five size groups of clams (2-5, 6-9, 10-13, 14-17, and 18-21 mm) were used to determine the ability to burrow into a coarse sand substrate in a one-half hour period. Groups of 50 clams were taken from sea water and were either transferred to the substrate and tested or they were held out of water for 1, 2, and 4 hours and then placed on the substrate.

In addition, the clams were tested on the substrate when it was covered with one-inch of water, when the water level was even with the substrate surface, and when the water was drained from the substrate.

Field Studies

Manila Littleneck Clams. Experimental plots were established in five different estuaries to test the suitability of the selected areas as sites for introduction of Manila littleneck juveniles. Sites selected in Tillamook, Netarts, Salmon River, Yaquina and Alsea bays consisted of a gravel-sand substrate which is most suitable as habitat for Manila clams.

At Salmon River, a 10 by 20-foot plot was planted with 10,000 Manila juveniles (50 clams/sq. ft.) in August 1971. In the four other bays, the plots were 10 by 40-feet. Twenty-thousand Manilas were planted in each of these plots at a density of 50 clams per square foot. These clams, when planted, ranged from 3.2 - 11.6 mm in length. The plots were established in September and October 1971.

An experimental plot was set up in upper Yaquina Bay on McCaffery's Island to determine optimum size and density of Manila juveniles at time of planting. Three different size groups of 10-month old clams (averaging 3.7, 7.0, and 11.4 mm) were planted at four different densities (50, 80, 125, and 200/ sq. ft.) in 4-foot square plots. This plot was established in October 1971.

A second size-density plot was established in April 1972, downstream approximately ¼-mile from the McCaffery's Island plot. Three different size groups of 16-month old clams (averaging 3.7, 6.5, and 11.0 mm) were planted at three different densities (80, 125, and 200/sq. ft.) in plots measuring 2 by 5-feet. In addition to the nine plots, a tenth plot was established with

Manila juveniles which were four months old. These clams averaged 3.6 mm and were planted at a density of 80/sq. ft. These Manilas grew exceptionally fast in the first four months of their life in the laboratory tanks. They were planted to compare their growth rate with the small clams in the other plots which had taken 16 months to attain the same length.

Results

Clam Spawning, Larval and Juvenile Rearing

Manila littlenecks were spawned during September, November, and December of 1971 and January 1972. These spawnings resulted in approximately two million juvenile Manilas which successfully reached the setting stage. To date, there are approximately 1.5 million juveniles being held in the laboratory. These juveniles will be planted during the summer of 1972 in selected areas which are judged suitable as Manila littleneck habitat.

Clam Burrowing Study

Preliminary results indicate that, except for the 18 - 21 mm clams, size is not a factor in ability of the clams to burrow. With the substrate covered by one-inch of water and the clams transferred immediately to the test substrate from the holding tray, 90 to 98 percent were buried in one-half hour. The largest size group were slower with only 70 percent buried in one-half hour.

When the water level was even with the substrate surface, 42 to 61 percent of the clams in the five size groups had dug in in one-half hour.

When the water was drained from the substrate, only three percent of the 18 - 21 mm clams had completely burrowed in. None of the clams in the other size groups burrowed in. At the conclusion of the drained sand test, enough water was added to cover the substrate to a depth of one-inch. When the water was introduced, the clam immediately began digging in. At the end of one-half hour, 88 to 95 percent of the four smaller groups were buried. Seventy-two percent of the largest group was buried.

Clams of one size group (6-9 mm) were held out of water for 1, 2, and 4 hours. This did not result in notable differences in percent of clams buried at the end of one-half hour when the substrate was covered with one-inch of water. The percent of 6-9 mm clams burrowed in ranged from 90 to 97 percent.

A similar holding test was conducted when the water level was even with the substrate. From 62 to 76 percent of the clams were buried after on-half hour with the beginning time of digging and the percent of completion possibly dependent on the amount of time held dry. An additional test was run in which the clams were refreshed in sea water for 15 minutes after being held dry. At the end of the test, 75 to 88 percent were buried.

Field Studies

Manila Littleneck Clams. The experimental plots established in five different estuaries during August-October 1971 were sampled in April-May 1972. Only one of the five plots was successful. This was a plot established on a gravel bar in lower Yaquina Bay. The ten by forty foot was established between 1.7 and 3.9-feet above mean lower low water (Figure 1).

The Manila clams in this plot had an average survival rate at the end of eight months of 16.5 percent (Table 1). Clam survival ranged from 1.2 percent at the lower tidal level to 34.8 percent at the higher tidal section of the plot. The average length of 10.0 mm was an increase of 4.3 mm since the clams were planted in September 1971. The average length of the clams in the upper portion of the plot was slightly smaller than those clams in the lower plot.

The experimental plots in the Salmon River estuary and Alsea Bay had been severely eroded during the winter due to severe flooding. No live clams or empty shells could be found in the plots. The planting in Tillamook Bay was also a failure. Seven percent of the plot was sampled and no live clams or empty shells were found. Because no trace of the clams was found, the area may also have been eroded during the winter.

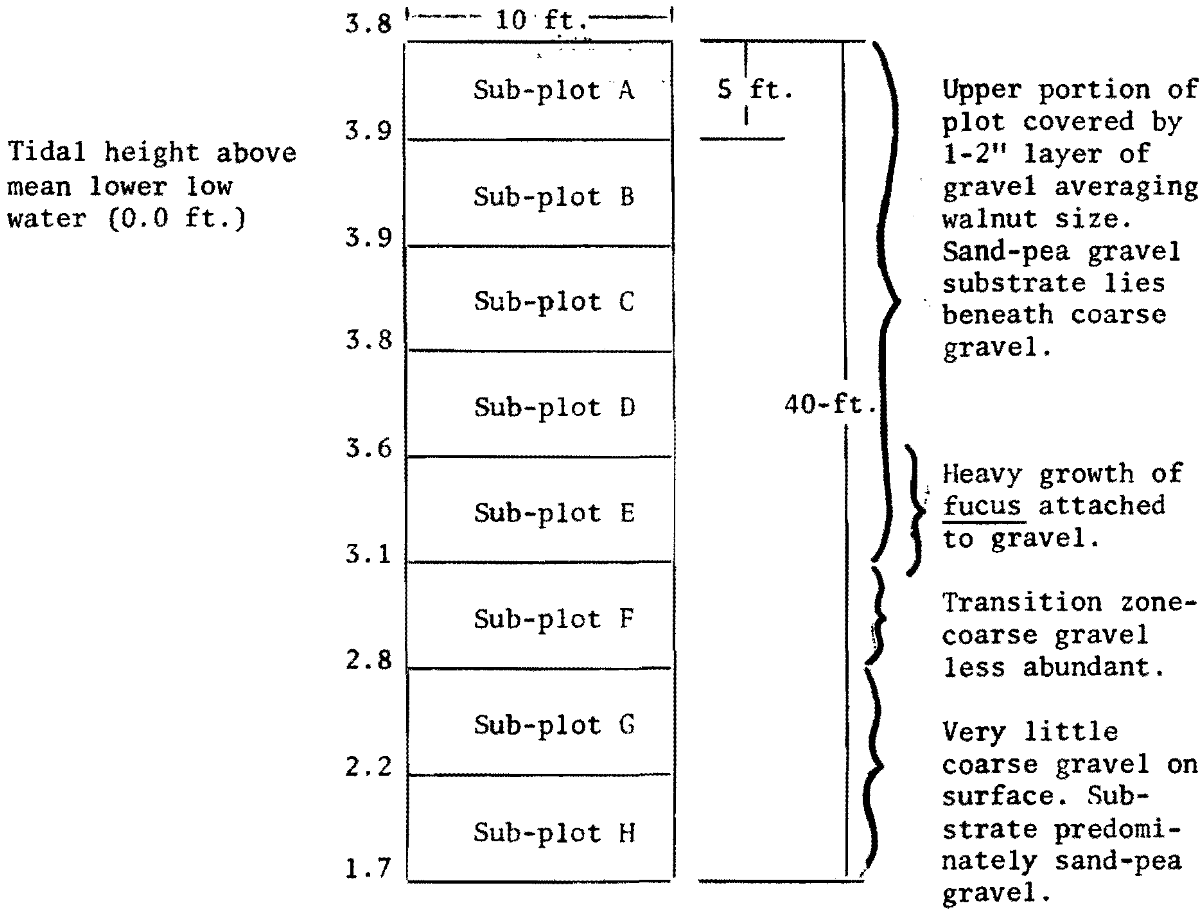

Figure 1. Diagram Depicting Tidal Height and Substrate Features of Sub-Plots of Experimental Manila Plot in Yaquina Bay.

Table 1. Mean Shell Length and Percentage Survival of Manila Littleneck Clams in Experimental Plot, Yaquina Bay, May 12, 1972.

Sub-Plot	Mean Shell Length (mm)	Percentage Survival
A	9.7	30.0
B	9.1	18.0
C	9.2	34.8
D	10.1	20.8
E	11.9	10.8
F	11.0	8.8
G	12.6	7.6
H	11.8	1.2
Total Plot Average	10.0	16.5

Less than one percent of the planted Manila clams survived in Netarts Bay. This bay lacks a major river system, eliminating the possibility that excessive runoff affected survival. The average length of the empty clam shells was 7.0 mm. At the time of planting, the clams averaged 5.5 mm. Some growth did take place before the clams died.

The size-density plot of Manila littlenecks established in Yaquina Bay in October 1971 also was a victim of the severe winter conditions. Samples of the plot revealed no survivors. A layer of silt had been deposited on the area during the winter and may have affected the survival of the clams.

The experimental Manila plot established on McCaffery's Island in upper Yaquina Bay in May 1970 (Phibbs 1970) was affected by winter flooding and silt deposition. No clams were found in the lower 15-feet of the 8 x 25-foot plot and only two live clams were found in the samples of the upper ten feet. These two clams (31 months old) were 32.6 and 35.1 mm long. The average length of the Manilas sampled in May 1971 was 21.4 mm.

Native Littleneck Clams. Native littleneck clams *(Venerupis staminea)* were planted in September 1970 in a plot containing various substrates (Table 2). This plot was sampled in May 1971 and again in May 1972. The average length of clams sampled in May 1972 was 26.4 mm, an increase of 13.4 mm in one year (Table 2). The artificial substrate in plot 2 (crushed rock, 3/4" - 1½") continued to demonstrate a relatively high survival rate for native littlenecks. In plots 3 - 6, the survival rates show no significant change. These figures may reflect uneven distribution of clams at time of planting. Siltation in this area was especially severe being ½ - 3/4" thick on the surface of the plot.

Butter Clams. The artificial substrate plots were also used for experimental plants of juvenile butter clams *(Saxidomus giganteus).* The plots containing butter clams were sampled in December 1970. At that time, the entire

Table 2. Mean Shell Length and Percentage Survival of Native Littleneck Clams, 7 and 19 Months After Planting.

Plot	Substrate Type1/	May 12, 1971		May 1, 1972	
		Mean Shell Length (mm)	Percentage Survival	Mean Shell Length (mm)	Percentage Survival
1	Control, silt overlying sand	12.5	2.0	-	0
2	Crushed rock 3/4" - 1½"	12.6	42.0	27.1	20.0
3	River rock 3/4" minus	14.0	4.0	26.0	6.0
4	Crushed rock 3/4" minus	13.0	6.0	25.0	12.0
5	River rock 3/4" - 1½"	12.0	2.0	29.02/	2.0
6	Crushed rock 1½" - 3"	13.7	8.0	26.8	8.0
Total Plot Average		13.0		26.4	

1/ Substrate described by Phibbs (1969).

2/ One clam.

area (4 by 4-feet) of each of the six plots was sampled. The clams were measured and replanted. When the plot was resampled in May 1972, it was noted that there was a mortality which we attributed to replanting. The average shell length of the dead clams was within 2 mm of the average shell length of live butter clams sampled in December 1970. Of 108 clams replanted in December 1970, 68.5 percent were recovered alive in May 1972, 16.7 percent were dead and the shells were recovered, and 14.8 percent were unaccounted for but were probably lost in replanting. Replanting the 48-month old butter clams resulted in a mortality of at least 16.7 percent, but probably approached 30 percent.

The average length of these 4-year old butter clams was 50.8 mm. The average length had increased 12.6 mm since December 1970. This growth rate may have been affected by digging and handling.

Discussion and Conclusions

Clam Spawning, Larval and Juvenile Rearing

Mass culture techniques have proven successful for Manila littleneck clams. The first series of spawnings have resulted in approximately 1.5 million Manila juveniles potentially available for planting. Further refinements of our culture techniques are needed to improve survival of the clams through the setting stage. The 1.5 million Manila juveniles will be used to continue field studies. However, not all of these clams will be planted during the late spring and early summer as originally planned. Only those juveniles from the September spawning are large enough to be planted at this time. It is our opinion that Manila clams should be at least 3 mm before they are planted to increase their chances for survival. Results from the size-density plots should provide information on the optimum survival of three different size groups of Manilas and may change the 3 mm figure.

The majority of the juveniles from the November through January spawnings are less than 3 mm and may not attain this length until mid-July. These clams did not grow as rapidly as had been anticipated. We plan to start spawning adult Manilas in August 1972 to provide larger size juveniles for the 1973 planting season. Our clam spawning efforts will be concluded by October. This should ensure that the juveniles would be ready for planting during the late spring or early summer. It is our opinion that a spring or early summer plant will increase clam survival by providing the juveniles ample time to become well established in the substrate before winter.

Clam Burrowing Study

The preliminary results of the clam burrowing tests have yielded information which will be used when Manila juvenile clams are planted in the field. The clams will still be planted on exposed clam flats rather than when the flats are covered with water. This will insure that the clams will be well distributed within the selected plot boundaries. It also will reduce the possibility of exposing the juveniles to predators in the water. Planting will be done in designated strips running parallel to the waters edge during the incoming tide. The water table should be near the surface in these strips. The clams will be refreshed before planting which should insure that at least 75 percent will be buried in one-half hour. As the tide level rises and covers the plot, it should stimulate most of the remaining exposed clams to begin burrowing.

Field Studies

Manila Littleneck Clams. The failure of four of five experimental Manila plots established in the fall of 1971 was attributed to the severe winter and excessive rainfall in western Oregon. The plots in Alsea Bay, Salmon River, and Tillamook bay were located in areas where erosion and scouring of the substrate occurred. These estuaries still have potential for Manila clam plants.

A spot check of the plot in Salmon River in October, three months after planting, showed that the clams were surviving and growing well. Plots will be re-established in these bays in areas less affected by winter flooding. The failure of the plot in Netarts is difficult to explain. The average length of the dead clams showed an increase of 1.5 mm over the average length at time of planting. The clams apparently survived and grew during the late fall, but were unable to live through the winter.

The success of the experimental plot in lower Yaquina Bay was encouraging. The average survival of 16.5 percent after eight months was exceptional considering that the Manila plot on McCaffery's Island showed a survival, in the gravel portion of the plot, of 12.5 percent after five spring and summer months. This plot provided new insight into the substrate requirements of Manilas which will be applied to future plantings. The plot had two distinct substrate differences. The lower portion of the plot consisted of a mixture of sand and pea-size gravel. The upper portion of the plot had a 1 to 2-inch layer of coarse gravel overlying the sand and pea-size gravel. Clams in the upper portion of the plot had notably higher survival rates than those in the lower plot. The layer of gravel probably acted as a buffer and protected the juveniles from the scouring effects of wave action. The clams in the lower portion did not have this protection and a large percentage were probably washed out of the plot. Future sites for Manila clam introduction should have a layer of coarse gravel on the surface if they are in areas which would be affected by wave action during winter storms.

The failure of the size-density plot on McCaffery's Island may have been the result of siltation and laboratory work needs to be done to determine the salinity tolerances of Manila juveniles of various ages. The high mortality of the other Manila plot on McCaffery's Island, established in 1970 and in existance in 1971, is an indication of the severe conditions that the Manilas encountered during the 1971-72 winter.

Native Littleneck Clams. The native littleneck clams in the sub-plot containing crushed rock (3/4 - 1½") continued to have the highest survival of the five different artificial substrate plots. The May 1972 sample was the last to compare the suitability of various artificial substrates. The entire area of each of the sub-plots has been sampled. Additional sampling will not show true survival due to mortality associated with replanting. The plot will be sampled in the future on a yearly basis to obtain data on clam growth.

Butter Clams. Butter clams occupy part of the artificial substrate plot. The sampling in May 1972 was done only to obtain data on growth. The results of the sampling showed that the four year old butter clams averaged about 51 mm. Another interesting aspect of the sampling was the occurrence of a planting mortality of at least 16.5 percent.

The artificial substrate plot has shown that both butter clams and native littleneck clams can survive in these plots. It is, therefore, conceivable that dredging spoils can be used to establish new and viable clam beds for these species. Location of these spoils could be critical. If the spoils are predominantly gravel, they should be deposited in an area where there is a constant current to prevent deposition of silt.

ABALONE STUDIES

Methods

Twenty-four adult red abalone were shipped by air freight from southern California to be used for spawning. No mortalities, immediate or delayed, occurred to these abalone. Ten days were spent inducing these abalone to spawn. The primary method used was thermal stimulation. Water temperatures were increased to 20-24 C for periods of up to one hour and then cooled to 14-19 C. Potassium chloride was also used at 1 gm/liter to induce the abalone to spawn.

Preliminary experiments were begun to culture diatoms in the laboratory for use as a food for the juvenile abalone. A fluorescent light fixture, with four 40-watt lamps, was suspended over a tank containing circulating sea water. Glass slides and pieces of plexiglass were suspended in the sea water to provide a substrate for the benthic diatoms to accumulate.

Results

About one-half of the abalone responded to the water temperature fluctuation and/or KCl and spawned. Several million eggs were released in clumps. When this occurs with bivalves, it indicates that the animals are forced to spawn and their eggs usually do not develop.

Preliminary results of the diatom experiment indicate that a large variety of diatoms will accumulate on the glass and plexiglass substrates. The majority were 50 μ or more in length. *Navicula sp.* and *Nitzeschia sp.* were the dominant forms.

Conclusions

The adult abalone used for spawning were obtained late in the spawning season and they may have already spawned prior to our receiving them. The fact that none of the eggs were viable, although the spermatazoa were, suggests that this is what did occur. The methods used to induce spawning were succesful although we were forcing the animals to emit non-viable sex products. These methods will be tried again in the next project year, but the abalone will be obtained much earlier in the spawning season to assure that at least some of the animals are gravid and will yield viable sex products.

The preliminary diatom culture experiments indicate that it is possible to start a community culture of benthic diatoms on selected suspended substrates. However, the lack of diatoms less than 50 μ is of some concern.

According to the Japanese abalone culturists (Ino 1968), newly set juvenile abalone require diatoms of 10 μ or less in length. A community culture of diatoms grown on suspended substrates may include a sufficient number of these diatoms, but they may be quickly crowded out by the larger diatoms. A uni-algal (single species) culture of a desirable diatom species would certainly be desired, but would be difficult to attain and maintain (Dr. McIntire, personal communication).

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