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Conference Paper

Culture of Mulinia lateralis and Crepidula fornicata embryos and larvae for studies of pollution effects

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Considerable emphasis has been placed on developing oulture systems for two species of molluscs which have no commercial importance, but are of considerable ecological significance. Several characteristics of the bivalve, Mulinia lateralis, and the gastropod, Crepidula fornicata, make them suitable as test organisms for studies of pollution effects. Mulinia lateralis has the following characteristics: a short generation time (approximately 60 days); a relatively high reproductive rate (3 to 4 million eggs at a single spawning); reasonable longevity (2 years); sex differentiation readily discernible through the shell of sexually ripe specimens; is relatively easy to culture; is small (adults 2. 7 to 20.0 mm long) and requires little space for rearing. Crepidula fornicata has the following characteristics: a short generation time (approximately 100 days); a satisfactory reproductive rate (about 6,000 larvae in a single brood release; females may produce 15 larval broods in a 6-month period); males and females can be easily identified because of their character-stacking behaviour (mature males can be externally identified); development of embryos can be observed within the egg capsule by growing mated pairs on transparent surfaces; larval releases can be predicted by noting color changes in the egg mass (pale yellow when first produced, while nearly black when release is imminent); earliest free-swimming larvae are large (about 400 $\mu)$ and can be grown to setting size (about 800 u) in 10 days in both static and flowing cultures; spat can be easily grown to maturity on flat, transparent surfaces and can be transferred from one surface to another; and although sexually mature specimens are consecutively protandric, they can quickly pass through the male phase without reproducing.

INTRODUCTION

Although studies of the effect of pollutants on marine invertebrates have been conducted for years, it was concluded at the Food and Agriculture Organization's (FAO) »Conference on Marine Pollution and its Effects on Living Resources and Fishing« held in 1970¹, that the range of organisms used for pollution toxicity tests should be considerably expanded. Moreover, it was concluded that research should be focused on certain species of organisms, which would satisfy the various basic objectives and requirements of experimental research, such as abundance of species, sedentarism, minimum life span, high sensitivity to pollutants and a size sufficiently large for making biochemical

and histological analyses. It was also noted that aquaculture projects could provide an excellent opportunity for the development of techniques for the culture of such organisms for pollution studies. Milford Laboratory at Milford, Connecticut, a part of the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration, has, over the past 40 years, developed the expertise for culturing marine molluses in the laboratory and has worked extensively with the embryonic and larval forms of two economically important species — the American oyster, Crassostrea virginica, and the hard-shell clam, Mercenaria mercenaria. Because of their long-standing commercial value, much is known of the spawning mechanisms of these estuarine molluses and the physiological requirements of their early developmental stages; consequently, studies have been conducted to determine the effects of pollutants on the young of these species.

More recently at Milford, however, considerable emphasis has been placed on developing culture systems for two species of molluscs which have no commercial importance, but are of considerable ecological significance. These organisms, Mulinia lateralis and Crepidula fornicata, have been cultured and found suitable as test organisms for studies of pollution effects because of their size, availability, short generation time, fecundity, and ease in culturing in the laboratory. Not only can the effects of pollution on these organisms be assessed by determining the percentage of larvae developing normally in contaminated test water, but studies can be made on the sublethal effects of contaminants on viability, growth, reproduction and mutagen-induced deviations in succeeding generations. In this presentation, certain aspects of culture techniques will be discussed for these two estuarine molluscs.

CULTURE OF MULINIA LATERALIS

Most commercial molluscs, such as the American oyster, Crassostrea virginica, hard-shell clam, Mercenaria mercenaria, surf clam, Spisula solidissima, and soft-shell clam, Mya arenaria, require a year or more to attain sexual maturity, have no externaly distinguishable sex differentiation, and, at least some, are presumably protandric. To maintain significant numbers of these molluscs under controlled conditions in the laboratory, as required for studies of pollution effects, considerable food, space and flowing sea water are needed.

Mulinia lateralis (Say) (Family: Mactridae), commonly known as the coot or little surf clam, has a number of characteristics that could make it useful for determining effects of pollution on shellfish. Factors which make it an efficient organism for such study include: (1) short generation time, (2) sex differentiation readily discernible through the shell in sexually ripe specimens, (3) relatively high reproductive rate, (4) ease of handling and culturing, (5) small space requirements, and (6) reasonable longevity².

Availability of Adults

M. lateralis is found in abundance in favorable environments from Malpeque Bay, Canada, to northeastern Mexico and in the West Indies³. In Long Island Sound, our study area. Sanders⁴ reported that M. lateralis was widely distributed but found that their abundance varied considerably during bimonthly sampling periods. Wass³ and Jackson⁶ studied M. lateralis populations and found

that they exhibited population explosions, followed by very high juvenile mortalities. In *M. lateralis*, population fluctuations have, on occasion, made it difficult to find sufficient numbers of adults for experimentation and it was found desirable to collect large stocks of adults when abundant and to maintain them in laboratory holding facilities. Collections can be made readily with an oyster dredge fitted with a small-mesh liner.

Maintenance of Juveniles and Adults

Juvenile and adult *M. lateralis* are best maintained in the laboratory in flowing sea water in trays or tanks with two inches of beach sand as substrate. The natural food supply in flowing sea water is normally sufficient to maintain these organisms in good physical condition, but supplemental feeding with algal cultures is recommended if such a source is available. Even though these clams grow rapidly in the laboratory, they achieve faster growth if placed outdoors in boxes of sand kept in tanks of running water during the warmer months of the year. Using this procedure, several different populations can be established within 3 to 4 months.

Conditioning and Spawning

Calabrese? studied the reproductive cycle of *M. lateralis* in Long Island Sound and found that they spawn naturally from July to September. No conditioning of the adults is necessary during this time to obtain viable gametes. Significant numbers of mature clams can be found in May and June and minimal conditioning is required for them to spawn. From October to December it is very difficult to produce mature animals in the laboratory, even with supplemental feeding and warmed sea water. During these months the clams are in an inactive stage following the spawning season or in early stages of gametogenesis. It may be possible to have *M. lateralis* available for spawning during this period by placing ripe animals in chilled sea water in late spring, which prevents them from spawning, and then briefly conditioning them before needed. These methods work successfully for other species of clams⁸.

M. lateralis respond well to the conditioning techniques developed by Loosanoff and Davis⁸ for ripening bivalves out of season. M. lateralis are taken from ambient temperatures during the colder part of the year and placed in running water trays. They are then acclimated by increasing the sea water temperature several degrees each day until the conditioning temperature is reached. It requires from 2 to 5 weeks at 18 to 20 °C to condition these animals for spawning.

One advantage of working with *M. lateralis* is the ease in separating males from females before the spawning attempt is made. The mature female gonad is pink-to-red-to-orange in color and the mature male gonad is white. The thin shells of *M. lateralis* make it possible to see the underlying gonad in the umbone region.

The spawning techniques described by Loosanoff and Davis⁸ are very successful for *M. lateralis*. Ripe adults are placed in finger bowls and the temperature is raised to about 28 °C in warm water bath. If thermal stimulation alone does not induce spawning in 30 minutes, a sperm suspension prepared

from a sacrificed male can be added as an additional stimulant. When the water in the spawning container has become cloudy with eggs or sperm the clams may stop spawning, but can be induced to start again if moved to clean water.

Fecundity

The fecundity of *M. lateralis* is high, although variable, as judged by the number of eggs discharged by individual females at a single spawning. The number of eggs released by a spawning female depends on the size of the animal, as well as the degree of development of the gonad³. Although the smallest number of eggs released by a single female is in the thousands and the greatest about 7 million, the average egg yield per female is 3 to 4 million.

Rearing Embryos and Larvae

Newly spawned eggs of *M. lateralis* are often irregular in shape but become spherical when suspended in sea water for a few minutes. The eggs should be fertilized with motile sperm as soon as possible after they are released, with care taken to avoid excess sperm introduction into the egg suspension since polyspermy and abnormal development may ensue.

Fertilized eggs, about 50 microns in diameter, may be separated from feces and other spawning debris by passing them through a sieve coarse enough to pass the eggs easily but fine enough to retain the debris. A sieve with mesh openings of 100 microns works well for *M. lateralis* eggs. Eggs can be conveniently cultured in one-liter or fifteen-liter containers using 15 μ filtered, ultraviolet-treated sea water at a density of 30 eggs/ml. At 20 to 25 °C the trochophore stage is attained in 9 hours and development proceeds to the straighthinge veliger stage in 15 hours³.

After 48 hours the straight-hinge veliger larvae, now 70 to 75 μ in length, are separated from the culture medium by pouring or siphoning the contents of the culture container through an appropriate sieve (openings about 40 microns). These 48-hour larvae are redistributed into clean culture media at a density of 15 larvae/ml. Larvae can be successfully reared to metamorphosis in 6 to 8 days at 25 ^{6}C on an algal diet. The larvae should be screened from the cultures and resuspended in fresh media every 48 hours to remove unconsumed food and metabolic waste products.

A combination of the chrysophytes, *Isochrysis galbana* and *Monochrysis lutheri*, is an excellent food for *M. lateralis* larvae. These algal species can be grown under carefully controlled conditions in semi-continuous unialgal cultures after the method of Ukeles⁹. These algae are added to the larval cultures at a density of 100 to 120 thousand cells/ml beginning at 48 hours and then every 24 hours.

Most M. lateralis larvae undergo metamorphosis and become benthic at a length of 200 to 220 μ . However, the size at metamorphosis is quite variable and individuals from 150 to 245 μ may possess both a foot and a velum and may alternately crawl and swim.

Optimum Culture Requirements

Calabrese^{10, 11} has studied the effects of temperature, salinity, and pH on embryos and larvae of M. lateralis and has determined their optimal requirements. Embryos developed satisfactorily (70% or more of maximum) into normal straight-hinge larvae at salinities from 22.5—30.0 ppt, temperatures from 15.0—25.0 °C and pH from 7.25—8.25. Larvae survived satisfactorily at salinities from 20.0—27.5 ppt, temperatures from 7.5—27.5 °C and pH from 6.50—8.75. Larvae grew satisfactorily, however, at salinities from 20.0—30.0 ppt, temperatures from 20.0—30.0 °C and pH from 7.00—8.50. Thus, optimum requirements for culturing M. lateralis from egg to metamorphosis are salinities from 22.5—27.5 ppt, temperatures from 20.0—25.0 °C and pH from 7.25—8.25 (Figs. 1, 2, 3).

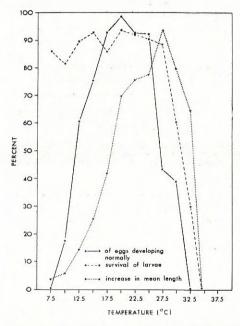


Fig. 1. The temperature tolerance of embryos and larvae of M. lateralis at 27 ± 0.5 ppt salinity, as indicated by the percentage of embryos that developed normally, percentage of larvae that survived and percentage increase in mean length of larvae. (after Calabrese¹⁴).

Growth of Juveniles

Recently metamorphosed *M. lateralis* survive and grow best and are most easily handled if they are kept in static cultures with frequent water changes until they are about 0.5 mm in length. At this size these clams grow more rapidly if placed in running water trays. Some nourishment is supplied by the foods naturally present in the sea water, but laboratory cultured algae are also routinely dripped into these trays to accelerate growth. Rapid growth of juveniles is attained at 22 to 24 °C. Even faster growth of juveniles can be achieved if placed outdoors in boxes of sand kept in tanks of running sea water during the warmer months of the year.

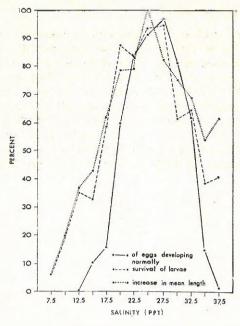


Fig. 2. The salinity tolerance of embryos and larvae of M, lateralis at 25 \pm 1 6 C, as indicated by percentage of embryos that developed normally, percentage of larvae that survived and percentage increase in mean length of larvae. (after Calabrese'').

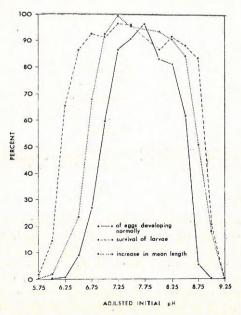


Fig. 3. The pH tolerance of embryos and larvae of M. lateralis, as indicated by percentage of embryos that developed normally, percentage of larvae that survived and percentage increase in mean length of larvae. (after Calabrese¹¹).

Generation Time

Using the culture methods for *M. lateralis* outlined above, the egg-to-egg cycle in the laboratory has ranged from 39 to 135 days with the average generation time about 60 days³. Although fully grown *M. lateralis* are 15 to 20 mm long, both male and female laboratory reared *M. lateralis* have spawned when only 2.7 mm in length.

CULTURE OF CREPIDULA FORNICATA

The prosobranch gastropod, Crepidula fornicata, is an important member of marine bottom communities along the Atlantic coast of North America from New Brunswick, Canada, to the Caribbean islands and Uruguay, and in northern Europe, including England, Wales, France, Belgium, Holland, Germany, Denmark, and Sweden¹². C. fornicata has also been reported from the Pacific coast of the United States¹³. C. fornicata is most often found associated with oyster beds where it is generally considered to be a competitor of the oyster for space and food. It may more directly affect oyster populations by removing oyster larvae from the water column by trapping them in food masses while feeding¹⁴ or by growing rapidly and smothering oyster spat¹⁵.

C. fornicata is a protandric, consecutive hermaphrodite¹⁶. Although adults are capable of some locomotion, this species forms sessile stacks composed of numerous individuals with very specific size, spatial, and sexual relationships. These stacks wind to the right, and the functional females are at the bottom and are the largest and oldest animals. The uppermost, smallest, and youngest animals are functional males, while some in the middle of the stack are in transition from the male to the female state¹⁶. The animals are oriented with their right anterior margins in contact, a position facilitating copulation. Females lay a gelatinous mass of egg capsules in front of the foot where they are covered by the anterior portion of the body. At each spawning numerous egg capsules containing about 250 eggs each are produced¹², and females are capable of spawning at least twice annually¹⁷.

Development of the embryos to an advanced veliger stage occurs in the capsules, and then the disintegrating capsules and larvae are actively expelled from the brood chamber by the female. The planktotrophic larvae are initially about 350 μ long and more than double in size while in the plankton. The larvae have been well described by Werner¹⁸. In the field, juvenile C. fornicata can reach the functional male phase in their first summer, and the youngest females become functional during their second summer¹⁹. In laboratory culture, C. fornicata has exhibited a number of characteristics which make it useful for determining the effects of pollution on gastropods. These characteristics include: (1) availability of adults on both sides of the Atlantic, (2) minimal space requirements, (3) ease in obtaining eggs and larvae for study, (4) sufficiently high reproductive rate, (5) ease in culturing young stages, (6) very rapid larval growth rate, and (7) a relatively short generation time.

Availability of Adults

Adult C. fornicata are found in abundance on most oyster growing grounds throughout their range. The ecological conditions necessary to support a C. fornicata population are so similar to the requirements of Ostrea edulis that

Walne¹² has indicated that an abundance of *C. fornicata* forms a very good indication as to the suitability of an area for oyster culture. Adult *C. fornicata*, attached to their natural substrate, can be left out of water for at least 12 hours without incurring any mortality if air temperatures are cool. Adult *C. fornicata* are best left to acclimate for a few days in a tank of flowing sea water in the laboratory before an attempt is made to separate them from their substrate for experimental purposes.

Maintenance of Adults

Stock populations of C. fornicata adults can be kept in tanks of flowing, unfiltered sea water. It is necessary to keep these tanks relatively free of silt to avoid suffocating the animals. For laboratory work, where it is necessary to observe the behavior of single individuals or of separate stacks of adults, the animals can be removed from their original substrate, placed on numbered panels, and suspended vertically in tanks of sea water. The animals may move over the surface of the panel, but they will not move off the panel unless under stress. If transparent plastic panels are used to contain reproductive stacks of adults, then the egg laying behavior of the bottom-most female can be observed through the panel. Coe20 found that most laboratory sea water systems do not supply adequate nutrition for the normal development of the reproductive systems of C. fornicata. At Milford, however, the food an oxygen requirements of adult C. fornicata are met by maintaining them in unfiltered sea water at a flow rate of 30 ml/animal/min. Adults have also been successfully maintained in static culture by renewing the water every day, aerating the culture, and supplementally feeding daily with cultured algae.

Conditioning and Spawning

Chipperfield¹⁷ has reported that the natural spawning period for a population of *C. fornicata* in Britain begins in the spring, when the water temperature rises to 10 °C, and continues until September. The Long Island Sound population used in our laboratory work appears to have the same natural season. Adults collected during this period would be expected to continue reproducing in the laboratory.

C. fornicata can be easily induced to breed out of season. Specimens collected from Long Island Sound off Norwalk, Connecticut, in November, when the ambient water temperature was 14 °C and egg laying had ceased, started producing eggs in a few weeks when held in the laboratory at both 18 and 25 °C. Another group, collected from New Haven Harbor, Connecticut, in March from water at 8 °C began producing eggs in as few as 5 days at elevated temperatures in the laboratory. We have used a temperature acclimation rate of no more than 3 °C/day in our studies and have observed no harmful effects caused by the temperature increases.

Reproduction of these animals under laboratory induced conditions can be easily studied by placing stacked pairs of animals on individual, numbered panels. In selecting pairs for this work, some care must be taken to insure that both a male and female are present. In these studies, therefore, only isolated pairs that had no scar areas on their shells, which would indicate positions previously occupied by small males, were selected or the top two animals from a stack were chosen. If both stacked animals are males initially, the lower animal undergoes transformation to the female phase.

C. fornicata will reproduce in tanks of flowing sea water at temperatures between 15 and 27.5 °C for at least six months. The most rapid development of the embryos occurs at 27.5 °C, the highest temperature studied. At this temperature larvae are liberated just 9 days after egg laying. At 15 °C the time to

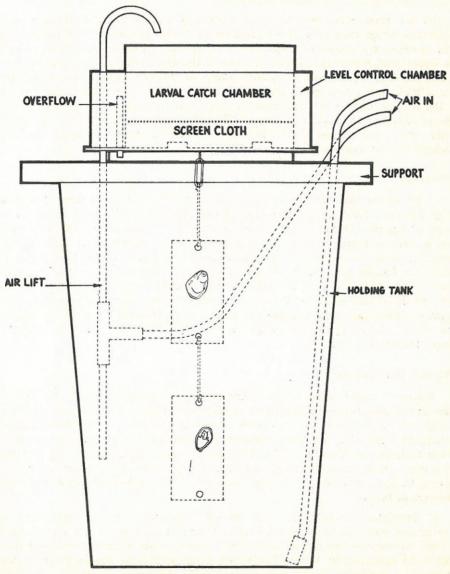


Fig. 4. Schematic of the apparatus used to collect C. fornicata larvae after they have emerged from the egg cases.

larval release is lengthened to 3 weeks. The number of larvae produced by laboratory-held females at high temperatures is significantly smaller than the number produced at lower temperatures and temperatures between 15.0 and 22.5 °C yield the greatest number of larvae per unit time.

As the embryos develop within the protected egg capsules, they change color and these changes can be observed if the female is kept on a transparent surface. Newly laid eggs are pale yellow and become dark brown or gray just before the advanced veligers are released.

In our procedure, females with dark egg masses are placed in a special apparatus which facilitates the collection of the larvae when they are released. This is necessary since the adults very efficiently filter their own larvae from the water, and numerous larvae are lost in the fecal mass. The larval collecting apparatus (Fig. 4) utilizes an airlift pump to deliver water containing larvae into a PVC and nylon mesh screen-cloth filter. The screen cloth has openings of 180 μ . The larvae are retained by the filter, and the water returns to the release chamber through a standpipe. The standpipe maintains a 2 cm layer of water above the screen cloth. This device collects about $70^{10}/_{0}$ of the larvae released at a recirculated flow rate of 60 ml/min.

Fecundity

Coe²¹ found that *Crepidula onyx* could spawn 10 times annually and produced between 5,000 and 20,000 eggs at each spawning. We have made no estimates of egg production for *C. fornicata*, but have made counts of larvae liberated at various temperatures. The number of larvae produced at each spawning decreases with increasing temperatures. At 15 °C about 10,000 larvae are produced per female per spawning, while at 27.5 °C the larval production is only about 1,500. Animals held at higher temperatures, however, produce consecutive broods of larvae faster than those held at lower temperatures and, consequently, about the same number of larvae are produced in a given length of time within the temperature range of 15.0 to 22.5 °C. Fecundity is reduced at temperatures above 22.5 °C.

Rearing the Larvae

Werner¹⁸ raised some *C. fornicata* larvae to metamorphosis by growing them in coarsely filtered sea water to which he did not have to add any additional nutrient. Pilkington and Fretter²² reared *C. fornicata* to metamorphosis on a cultured algal diet, but their method included using a pipette to transfer the larvae to fresh sea water periodically. This process is time-consuming and imposes a limit on the number of larvae used. The two methods described here for rearing *C. fornicata* larvae can be routinely used successfully to culture large numbers of larvae.

C. fornicata larvae were first reared successfully in our laboratory in a flowing sea water culture system similar to that diagrammed in Figure 5. In this system the larvae are placed in a PVC screen made with nylon mesh screen cloth with openings of 180 μ . The water level above the screen cloth is determined by the height of the surrounding PVC container which, in our system, is about 5 cm, and the volume of water in the larval chamber is 1 liter. Ultra-

violet-treated, 1 μ filtered sea water flows through the system at a rate of 10 liters/day, and cultured algae, kept chilled at 15 °C, are simultaneously introduced so that the food concentration is about 150,000 mixed algal cells/ml. At this food concentration, up to 3,000 larvae can be reared with no reduction in growth rate. C. fornicata larvae have been reared to metamorphosis in this system without ever cleaning the system or completely renewing the sea water in the growth chamber. Best results are obtained if the system is cleaned every second day. Under these conditions larvae begin to metamorphose in about 7 days at 25 °C and, on the average, more than $50^{\circ}/_{\circ}$ of the larvae complete metamorphosis.

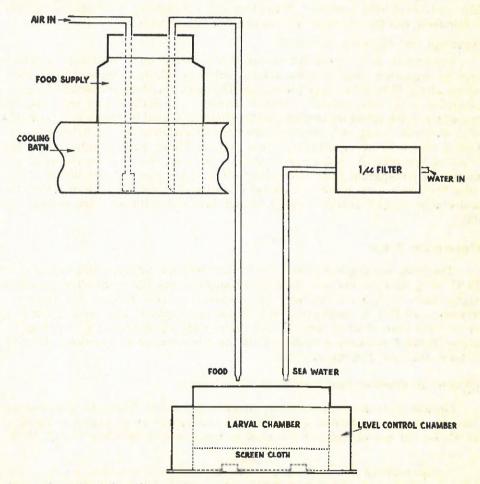


Fig. 5. Schematic of the culture system used to rear C. fornicata larvae in flowing sea water.

C. fornicata larvae can also be reared in static cultures, similar to those used traditionally in bivalve culture. Excellent results are achieved when 500 larvae are reared in 1-liter polypropylene beakers using 1 μ filtered, UV-treated sea water and an initial food concentration of 150,000 mixed algal cells/ml. The

larvae must be screened from the static cultures each day, resuspended in fresh sea water, and fed. When pouring these larvae onto a screen to remove them from the day-old culture media, it is important that a 2 cm layer of water be present above the screen surface. This buffer layer of water prevents physical damage on the screen and desiccation of the larvae. Some larvae in static cultures have metamorphosed in as few as 4 days at 25 °C, and between 50 and 90°/6 of the initial number normally survive the larval period.

Five algal species have been fed to C. fornicata larvae and their value in promoting larval growth in descending order of importance is as follows: Phaeodactylum tricornutum, Monochrysis lutheri, Isochrysis galbana, Chlorella 580, and Dunaliella euchlora. A mixture of all 5 species was superior to any single food, and this mixture was used in subsequent work.

Handling and Growing Juveniles

Settlement of *C. fornicata* larvae has occurred on the surface of every type of container used to grow them, including glass, polypropylene, PVC, nylon mesh, fiber glass, and plexiglass. The most satisfactory method to grow juveniles is to place recently attached animals in a shallow tray with flowing sea water. Fifty juveniles can be rapidly grown to sexual maturity at a flow rate of 1 liter/min using this method. The tray situation gives each juvenile potential access to every other juvenile and facilitates pairing behavior at the onset of maturity. As pairs are formed in the tray, they can be removed and placed on transparent panels for observation of the onset of egg laying. Juveniles have also been grown to sexual maturity in static cultures in which the water was aerated, renewed every second day, and cultured algae added each day.

Generation Time

The most rapid generation time for *C. fornicata*, 72 days, has occurred at 25 °C using flowing culture methods throughout the life cycle. Temperatures higher than 25 °C have not been investigated for each stage in the life cycle; however, at 27.5 °C embryonic and larval development and adult growth is more rapid than at 25 °C, and the life cycle might be shortened at this temperature. Table I presents a summary of the life cycle of *C. fornicata* at 25 °C in both static and flowing systems.

Effects of Various Environmental Parameters

Growth of C. fornicata at every stage is positively related to temperature. The growth rate of larvae increased with increasing temperatures from 10 to 30 $^{\circ}$ C. Larval metamorphosis occurred at temperatures between 15 and 30 $^{\circ}$ C,

TABLE I

Days required for various stages in the life cycle of C. fornicata at 25 °C.

	Egg Laying to Larval Release	Larval Period		First Pairing to Egg Laying	Total = Generation Time
Static	12	9	49	56	126
Flow	10	6	34	22	72

with maximum settlement occurring at 25 °C. Juvenile C. fornicata grow faster and reach sexual maturity sooner at 25 °C than at lower temperatures, Adult C. fornicata grow faster as temperatures increase from 15 to 27.5 °C.

C. fornicata larvae are not very tolerant of low salinities. Significant larval growth occurred only at salinities of 20 ppt and higher, while acceptable levels of metamorphosis occurred only at salinities above 22.5 ppt. Rapid larval growth and good survival through metamorphosis occurred at salinities up to 40 ppt, the highest salinity tested.

REFERENCES

- 1. Food and Agriculture Organization, Report of the FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing, FAO Fish. Rep., No. 99, FIRM/R 99 (EN) 1971.
- 2. A. Calabrese, Mulinia lateralis: Molluscan fruit fly? Proc. Natl. Shellfish. Assoc. 59 (1969) 65-66.
- 3. A. Calabrese, The early life history and larval ecology of the coot clam, Mulinia lateralis (Say) (Mactridae: Pelecypoda). Dissertation, Univ. of Connecticut, 1969, 101 pp.
- 4. H. S. Sanders, Oceanography of Long Island Sound, 1952-1954. The biology of marine bottom communities, Bull. Bingham Oceanogr. Coll. Yale Univ. 15 (1956) 345—414.
- 5. M. L. Wass, Study of a soft-bottom community in the lower York River, Virginia, Atlantic States Biologists Meeting, Charlottesville, Virginia (1956) Abstract.
- 6. J. B. C. Jackson, Bivalves: Spatial and size-frequency distribution of two intertidal species, Science 161 (1968) 479-480.
- 7. A. Calabrese, Reproductive cycle of the coot clam, Mulinia lateralis (Say), in Long Island Sound, The Veliger 12 (1970) 265—269, 2 text figs.
- 8. V. L. Loosanoff and H. C. Davis, Rearing of bivalve mollusks, in F. S. Russell (ed.), Adv. Mar. Biol. Academic Press, London 1, 1963, pp. 1-186.
- 9. R. Ukeles, Nutritional requirements in shellfish culture, in Proc. Conf. on Artificial Propagation of Commercially Valuable Shellfish, Univ. of Delaware (1971) 43-64.
- 10. A. Calabrese, Individual and combined effects of salinity and temperature on embryos and larvae of the coot clam, Mulinia lateralis (Say), Biol. Bull. (Woods Hole), 137 (1969) 417-428.
- A. Calabrese, The pH tolerance of embryos and larvae of the coot clam, Mulinia lateralis, The Veliger 13 (1970) 122-126.
- 12. P. R. Walne, The biology and distribution of the slipper limpet Crepidula fornicata in Essex rivers with notes on the distribution of the larger epibenthic invertebrates, Fish. Invest., London 20 (2) (1956) 1-50.
- 13. W. M. Chapman and A. H. Banner, Contributions to the life history of the Japanese oyster drill (Tritonalia japonica) with notes on other enemies of the Olympia oyster (Ostrea lurida), Biol. Rep., Dept. Fish., Wash. State, 49a (1949) 167-200.
- 14. P. Korringa, Crepidula fornicata as an oyster pest, Cons. Int. Explor. Mer., Rapp. Proc. Verb. 128 Part II (1951) 55-59.
- 15, V. L. Loosanoff and J. B. Engle, Little known enemies of young oysters, Science 93 (1941) 328.
- 16. J. H. Orton, On the occurrence of protandric hermaphroditism in the mollusc
- Crepidula fornicata, Proc. R. Soc. Lond. B. 81 (1909) 468—484. 17. P. N. J. Chipperfield, The breeding of Crepidula fornicata (L.) in the river Blackwater, Essex, J. Mar. Biol. Assoc. U. K. 30 (1951) 49-71.
- 18. B. Werner, Über die Anatomie, die Entwicklung und Biologie des Veligers und der Veliconcha von Crepidula fornicata L. (Gastropoda Prosobranchia), Helgol. wiss. Meeresunters. 5 (1955) 169-217.
- 19. W. R. Coe, Influence of natural and experimental conditions in determining shape of shell and rate of growth in gastropods of the genus Crepidula, J. Morphol. 71 (1942) 35-51.

20. W. R. Coe, Nutrition and sexuality in protandric gastropods of the genus Crepidula, Biol. Bull. (Woods Hole) 94 (1948) 158—160.

 W. R. Coe, The reproductive organs of the prosobranch mollusk Crepidula onyx and their transformation during the change from male to female phase, J. Morphol. 70 (1942) 501-512.

22. M. C. Pilkington and V. Fretter, Some factors affecting the growth of prosobranch veligers, Helgol, wiss. Mecresunters, 20 (1970) 576-593.

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Uzgoj embrija i licinki Mulinia lateralis i Crepidula fornicata u istrazivanjima utjecaja zagađivanja

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Naročita je pažnja posvećena usavršavanju uzgojnih metoda dviju vrsta moluska koji nemaju komercijalnu vrijednost, ali su zato u ekološkom pogledu vrlo značajni. Zbog nekih svojstava školjkaš Mulinia lateralis i puz Crepidula fornicata predstavljaju podesne organizme za istraživanja utjecaja zagađivanja. Pogodna svojstva M. lateralis su slijedeća: kratko vrijeme jedne generacije (oko 60 dana); relativno velika moć reprodukcije (3—4 miliona jaja u jednom mriješčenju); umjerena dužina života (2 godine); razlike u spolu vidljive kroz ljušture spolno zrelih primjeraka; relativno lak uzgoj; male dimenzije (odrasli od 2.7 do 20.0 mm dugi) te stoga i potreban minimalni prostor za uzgoj. Crepidula fornicata ima slijedeća povoljna svojstva: kratko vrijeme jedne generacije (oko 100 dana); dovoljna moć reprodukcije (oko 6 000 ličinki u jednom mriješčenju); zenke mogu proizvesti 15 ličinačkih stokova u 6 mjesečnom periodu); zbog karakterističnog vladanja — sakupljanja u hrpe, mužjaci se lako razlikuju od ženki (zreli mužjaci mogu se odrediti po vanjštini); razvojembrija umutar kapsule uočljiv je na prozirnim površinama; oslobađanje ličinki može se predvidjeti pračenjem promjena boja jajčanih masa (svijetlozuta — kada su jaja proizvedena, do skoro crna — pred samo izbacivanje); prve plivajuće ličinke velike su (oko 400 μ) i postižu veličinu za prihvat (oko 800μ) u roku od 10 dana; mladi prihvaćeni oblici mogu se lako uzgojiti do zrelosti na ravnim prozirnim površinama, i premjestiti se s jedne na drugu površinu; i iako su spolno zreli primjerci protandrični, mogu brzo proči fazu mužjaka, a da se ne razmnožavaju.