CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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SEED PRODUCTION OF GASTROPODS

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Introduction

Marine gastropods are exploited all over the world for subsistence as well commercial reasons. Gastropods are used as food; they are a good source of protein and protein rich products. They are usually served as soup in many Japanese, Korean and Chinese restaurants. Commercially they in high demand in shell craft production such as lampshades, curios, fashion accessories like buttons, mother of pearl flakes from powdered scrap, formulation of lacquers and shampoos etc. The widespread use has often led to indiscriminate exploitation and it is important this resource be utilized in a sustainable manner. In this context, seed production of many commercially important gastopods has been successfully developed in hatcheries.

The green snail *Turbo marmoratus,, the* top shell, *Trochus niloticus, abalones Haliotis sp* have been successfully spawned in captivity and hatchery produced seed have been used for stock enhancement programmes. Broodstock maintenance, spawning, larval rearing and settlement methods are quite similar for most of the marine gastropods. The detailed seed production account of the commercially important whelk, *Babylonia spirata* is given below. *Babylonia* sp., commonly known as 'Whelk,' 'Spiral Babylon' and 'Puramuttai chank' (Dove egg shell) in local parlance and 'Baigai' in the trade sector, is a marine edible gastropod. It is widely distributed in the Indo Pacific region. In India, it is well represented in the Indian Peninsula at places such as Gulf of Mannar, Poompuhar, Nagapattinam, Madras and the waters around Andaman and Nicobar islands (Ayyakannu, 1994). *Babylonia* is a much sought after marine gastropod and it fetch a good foreign exchange. This edible gastropod is an important food species in the Indo-pacific region (Ayyakannu, 1994). The total quantity of whelk trade during 1993-94 was 300 tonnes and it increased to 500-600 tonnes during 1995-96.

Economic Importance of *Babylonia* spp

Baigai (*Babylonia)* have received considerable attention due to their economic importance and the increased demand for meat of these snails in the western countries. The boiled meat of the snail was Rs 40/kg (Ayyakannu, 1994), though it is not available in the market nowadays. Presently it is exported mainly to Japan. *Babylonia zeylanica* is sold at Rs. 30/kg shell on where as *Babylonia spirata* fetches Rs. 10/kg in the local. The shell has ornamental value and the operculum has medicinal importance and therefore no part of the whelk is wasted. The shells of *Babylonia* are used for interior decorations after cleaning, processing and polishing. A well polished whelk shell fetches Rs 3 / shell. Beautiful items such as curtains, pen stands, mementos, key chains and other novelties are made out of small shells. There is a good market for them not only in India but also in western countries. Handicraft items made from *Babylonia* shells are widely sold in almost all cities and tourists centres in India. The operculum popularly known as 'fish nail' is an important by- product for export and is valued at Rs.400 / kg. 100 kg gastropod shells yield 1 kg of operculum. The total export of operculum for 1992-93 is 2 t worth Rs. 4.14 lakhs (Statistics of Marine Products Export, 2001).

Two species of Babylonia occur in the Indian waters; *Babylonia spirata* and *Babylonia zeylanica.* These together form nearly 56% of the shrimp by catch. From the by-catch, *Babylonia spirata* and *Babylonia zeylanica* are segregated and separately auctioned due to their market preferences and considered as an emerging resource. In view of its high economic value and the increasing fishing pressure on the present stock, efforts were initiated for the seed production and farming of the whelks. The breeding, spawning and larval development of *Babylonia spirata* has been successfully carried out in the Central Marine Fisheries research Institute and is detailed below.

Breeding, spawnig and larval rearing of *Babylonia spirata*

i) Collection and transportation of the brood stock

The brood stock of *Babylonia spirata* were collected from landing centers of Neendakara and Sakthikulangara and transported to molluscan hatchery at CMFRI, Cochin for breeding and spawning studies. The best method to minimize the transportation stress and ensure complete survival the whelks were kept in cotton moistened with seawater or gunny sacs presoaked in seawater.

ii) Management of brood stock in the hatchery for spawning

70% of the fresh seawater was exchanged daily. The snails were allowed to acclimatize in the hatchery for two days after transportation. During this period no food was given to them. After acclimatization, they were fed with clam, squid, prawn, annelids, etc. From the observations, it was found that within five minutes they were able to locate the food and extended the proboscis to take the food. After two days of acclimatization, they were transferred to the tanks with sandy substratum for providing them a natural environment. The FRP tanks were provided with sand as the substrate bottom. The tanks were fitted with two biological filters to maintain water quality. The environmental parameters like salinity, temperature and pH were regularly monitored and kept with in the range of 32-35ppt, 26-29^{o}C and 8-8.3 respectively. *Babylonia spirata* had a distinct preference for clay (51.9%) as their substrate. The order of preference for other substrate was coarse silt (24.7%), coarse sand (17.3%), and gravel (6.1%).

iii) Spawning

The acclimatized brooders took average 15 days to spawn in the hatchery though some took nearly two months to show the spawning activities. The average size of the spawners was 36mm. Spawning occurred during night and continued up to the early morning hours. An erect position of spawners by pressing its foot in the substratum indicated spawning and any slight disturbance halted the spawning activity. The average number of capsules per spawner was 35-40 with 350-800 eggs per capsule.

Egg capsules: The eggs were laid in transparent vasiform capsules. Due to the transparent nature of the egg capsules, the eggs were visible and could be counted externally. The apical portion of the egg capsule was concave in appearance and the membrane in this region was thinner than the walls. The stalk of the egg capsule was firmly attached to the substratum to hold it in an erect position till the larvae hatch out. The average total length of the egg capsule was 27.8 ± 2.5mm and the capsular length excluding the stalk showed variation. The average width of the capsule at the apical region was 8.4 ± 1.5 mm. The average diameter of the fertilized egg was 275µm, irrespective of the size of the capsule and number of eggs in the egg capsule. There was positive linear correlation (r= 0.8764) between the average length of the egg capsule and average number of eggs in the capsule. The average size of the fertilized eggs in the egg capsule was 260-280 µm.

Hatching, larval rearing and larval development

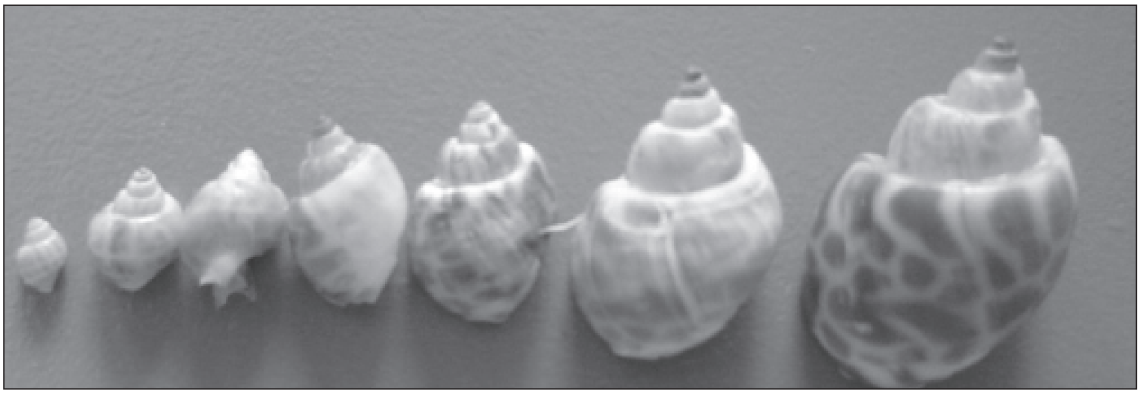
The capsules attached to the substratum with the help of the hold fast were transferred from the spawning tanks to the hatching tanks of 50 lit capacities with fresh filtered seawater and provided with gentle aeration. The salinity was maintained at 32 ppt, pH 8±0.2 and temperature 28±2^{o}C.

i) Fertilized eggs to planktonic larvae

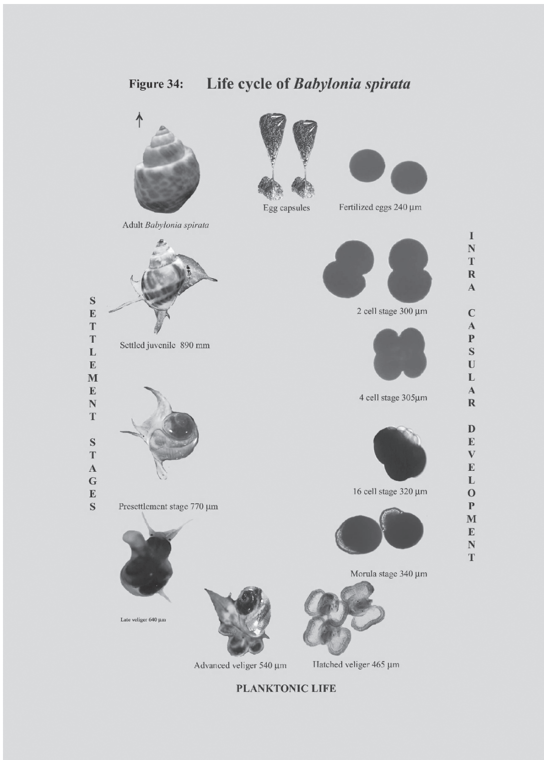
First polar body was released within 60 minutes after the release of fertilized egg capsule. The release of second polar body commenced at 90th minute. The first cleavage occurred 30 minutes after the release of the second polar body, which was followed by the second cleavage after one hour. The divisions were clearly visible up to 16-cell stage. Subsequently, it becomes an opaque mass due to large quantity of yolk in the egg. After 24 hours of spawning, the divisions completed and the embryo transformed into the morula stage with marginal cells at the anterior region. Further development resulted in the rotation of the morula and this stage lasted for about 48 hours.

On the 3rd day, the cilia were visible at the top and transformed to trochophore larva. On 4th day the larval size increased to 380 ìm. Subsequently the larval size increased to 420 ìm on 5th day and developed velum boarded by two rows of fast beating cilia along its margin. On 6th day, the velar lobes become enlarged and a thin, transparent larval shell was clearly visible. From this day onwards veliger larvae were fully developed and concentrated at the tip of the egg capsule. Though the exact mechanism of the releasing of the larvae is not known, the apical part splits and releases the larvae from the egg capsule. The average hatching percentage of larvae from each capsule was 90 and all of them were released by 7th and 8th day after spawning.

Juvenile *B. spirata*



Life cycle of *B. spirata*



Spawning of *B. spirata* in the hatchery

The larvae are plank tonic, swim towards the surface of the water and exhibit photo tactism. The larvae are transparent; possess bi-lobed velum fringed with cilia. The larval shell is fully developed. Eyespot is also developed.

i) Larval rearing

On the 7th day, the larvae were transferred from the hatching tanks to the rearing tanks (Perspex/glass tanks) by filtering through a sieve of 400µm and stocked seawater in the rearing tank at a density of 150 larvae/liter. The rearing conditions were salinity 32±1ppt, pH 8±2 and temp 28±2°C. Prior to stocking, the water for rearing was treated with hypochlorite and potassium permanganate solution to eliminate the unwanted microorganisms. Different algal feeding regimes were tried. Poor growth and heavy larval mortality occurred when fed with *Tetraselmis* sp. and *Nannochloropsis* sp. Pure cultures of *Isochrysis* and *Chaetoceros* were provided to the larvae up to the 17th day and larvae settled as juvenile. The larvae were fed at the rate of 7000 cells/ml/hr. Almost 95% of the larvae hatched out from the egg capsule and the survival rate was 60% from the hatching to settlement.

ii) Metamorphosis and settlement

The larvae feed and swim actively with the fast movement of cilia along the rim of the velum. Eyespot becomes clearer. This stage lasts up to the 13th day. The larval shell is fully developed and the foot protrudes out Operculum is seen as a scar; pair of tentacles develop at the base of the tentacles. Velum is 4-lobed, as a folding along the horizontal position. Active feeding of phytoplankton continues up to the 17th day. The plank tonic lifestyle begins to change and the larvae begin creeping and crawling along the bottom, actively searching for food and become carnivorous in nature, feeding on shrimp, clam squilla meat etc. Settlement begins when they attain the average size of 895 µm. At this stage the velum is shed, radula and digestive tract is developed and the juveniles secrete mucus along their path.

iv) Rearing of juveniles

Metamorphosis of the larvae completed 17-19 days after the release of the capsule. The settled juveniles were transferred to 5 liter beakers provided with filtered seawater and gentle aeration. After settlement, the feeding habit changes and they become carnivorous and the juveniles begin to creep and crawl along the bottom. Algae settled on glass slides, shrimp feed, agar based feed (composition agar 0.25gms, shrimp 1.5 gms, soyabean 0.25 gms and boiled egg albumin 0.25gms,in 100ml sea water) egg yolk; egg albumin, tubifex and rotifer were tried as food for the juveniles. However, only shrimp feed found as better for the growth and survival of the young ones. The survival rate after settlement was 70%. During the settlement stage, they attained 800-1000µ shell lengths. The juveniles had well developed radula and digestive tract suitable for carnivorous life and fully developed shell and operculum for protection.

The growth of Juveniles was recorded from day1 to 18 months of growth. The average total length on the 1st day was 1.5 mm. On the 15th day, the average total growth was 2.218 mm. After 1 month, an average total length of 2.3 mm was attained. After 45 days of growth, the average total length was 2.82 mm. At 2 months 3.84 mm of average total length was recorded. And after 75 days, 4.06 mm was attained. After 6 months, the average total length was 14.41mm, average width was 9.87mm and average weight gain was 0.92g. After 10 months the average total length was 23.33 mm average width 15.06 mm and average weight gain was 3.2 g. After 14 months, the average total length was 28.7mm average 29.15 mm average width 20.07 mm and average weight gain was 8.8 g. After 18 months, the average total length was 30.98 mm average width 21.29 mm and average weight gain was 9.99 g.

Preference of micro algal feed by larvae of *B. spirata*

Although the larvae showed the general acceptance of *Isochrysis galbana*, *Chaetoceros calcitrans* and *Tetraselmis gracilis* as feed, *Chaetoceros calcitrans* proved to the most preferred micro algal feed by the larvae followed by *Isochrysis galbana*. *Tetraselmis gracilis* found poor acceptance due to the fact that algae was not available to the larvae, since it remained at the surface of the water column. *Nannochloropsis salina* did not find acceptance at all, since there was no settlement and complete mortality occurred on the 2nd day it self.

Larval stocking density

Babylonia spirata larvae were maintained in 4 lit containers in pure, filtered sea water 0f 32 ppt, at 8 different stocking densities 75/lit, 100/lit, 125 /lit, 150/lit, 200/lit, 225/lit, 300/lit, 325/lit. They were provided with *Chaetoceros calcitrans* at the rate of 10,000 cells/ml/hr and gentle aeration. The growth and percentage of settlement were recorded till 17 days when complete settlement was observed. The optimum stocking density was found to be 150 nos/lit, giving very high settlement rate and good growth compared to other stocking densities. Lower stocking densities viz, 75/lit, 100/lit and 125/lit showed of better growth compared to that of 150 /lit, however the settlement was very poor in these stocking densities 49.5%, 44%and 52% respectively. Higher stocking densities 250 nos/lit, 225 nos/lit 300 nos /lit and 325 nos/lit resulted poor growth and very low settlement.

Effect of salinity on hatching of larvae

The egg capsules of Babylonia spirata were maintained in different 20, 30, 35 and 40‰ salinities in 2 lit pure filtered seawater in 3 lit capacity plastic containers and provided with gentle aeration. Complete water exchange was done on alternate days. Three egg capsules were introduced into each container of average size 26 ± 2mm total length; 16 ± 2mm capsule length and 10 ± 2 mm capsule width. From the experiment it was observed that no hatching occurred at lower salinities of 20 and 25 ppt. 50-70% hatching occurred on the 7^{th} day in the other salinity ranges, 30, 35 and 40 ppt and 25-30% hatching occurred on the 8^{th} day. Thus the ideal salinity range for the hatching of the eggs is 30-40 ppt.

The larvae were reared in different salinities ranging from 5 to 50‰ to study the effect of salinity on settlement % and growth of juvenile on the settlement stage. The larvae were stocked at the density of 150 larvae/ lit in different salinities in duplicates and growth was recorded till the day of settlement on 17^{th} day. In the 5, 10, 15 20 and 50‰ salinities, the larvae did not survive and there was total mortality. The settlement percentage was very low at 40‰ salinity (14%) and no settlement was observed in 45‰-till17th day. Good growth and maximum percentage of settlement obtained at 30‰ salinity (56%). So the ideal salinity required for the larval growth was confirmed as 30‰ with a pH ranging between 8.1-8.3 and the temperature 26-28^0 C.

Feed preference of *Babylonia spirata* juvenile in the hatchery

The survival of juveniles highest among those fed with shrimp feed (92.5%), followed by those fed with squilla (85%). The survival when fed with squid was 65% and those fed on clam was only 50%. Egg custard was found to be unsuitable as feed for juvenile as the total mortality observed after 10 days. Growth was highest among those fed with shrimp feed. However squid was more acceptable than squilla in terms of growth although there was better survival when fed with squilla.

The upgradation of the present larval rearing and hatchery technique will help to develop whelk farming on commercial basis in India, which will ultimately help in increasing production besides reducing the fishing pressure in the natural whelk stocks.

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