J. mar. biol. Ass. India, **46** (1) : 64 - 72, Jan. - June, 2004

Larval development and seed production in the 'whelk' *Babylonia spirata*

R. Sreejaya, Anjana Mohan, P. Laxmilatha and K.K. Appukuttan

Central Marine Fisheries Research Institute, Cochin – 682 018, India

Abstract

The larval development of the whelk, *Babylonia spirata* (Linnaeus, 1758) (Neogastropoda: Buccinidae) which forms a major component of the bycatches of shrimp trawlers of south-west coast of India was studied and its seed production techniques were developed. The broodstock, which were conditioned at low temperature of 26 - 28°C, spawned intermittently between January to April, and again during September to December, 2002. Each spawner laid an average 35 transparent egg capsules, which were firmly attached to the substratum by a slender stalk. Fertilized eggs of 260 to 280μm diameter started their development within the transparent egg capsule itself by spiral cleavage and progressively developed into morula, blastula, trochophore and veliger stages. The larvae hatched out as veliger between the 7th and 8th day after spawning and these were reared in the hatchery. Percentage of survival, feeding rate and settling percentage of the larvae were studied in detail. Optimum stocking density of the larvae was found to be 150/l which resulted in 65% settlement of the larvae. *Chaetoceros calcitrans* was given as feed till settling stage and after that the juveniles were fed with shrimp meat. Details of the spawning, morphology of capsule and growth of the larvae are presented in the paper.

Key words: Larval development in *Babylonia spirata*

Introduction

Babylonia spp. (Family: Buccinidae) commonly known as 'whelk,' 'Spiral Babylon' and 'Puramutta chank' (Dove egg shell) in local parlance and as 'Baigae' in trade are widely distributed in the Indo-Pacific region. In India, this species has been recorded from southeast and south-west coasts and in waters around Andaman and Nicobar Islands (Ayyakkannu, 1994). The whelks are important food species in Indo-pacific region (Ayyakkannu, 1994). India exported 300t of whelk meat during 1993-94 (Appukkuttan and Philip, 1994). The export statistics by MPEDA showed that India exported 921t and 704t of frozen whelk during 2000 and 2001 respectively. The high demand for export of whelk meat may lead to overexploitation and exploitation of undersized whelk resulting in the depletion of the wild stock. Appukkuttan and Ramdoss (2000) stressed the need for judicious exploitation and hatchery seed production for sea ranching to augment the production of the species.

Considerable work has been done on

the spotted babylon (*Babylonia areolata*) inhabiting the coastal areas of Thailand especially on the effect of stocking density on the growth and its substratum preference (Chaitanawisuti and Kritsanapuntu, 1997), juvenile rearing (Chaitanawisuti and Kritsanapuntu, 1997a) and nursery culture methods (Chaitanawisuti and Kritsanapuntu, 1998). This was further extended to development of grow out methods in flow through systems (Chaitanawisuti and Kritsanapuntu, 1999; 2000).

Studies related to reproduction and developments of gastropods in India are scanty. The pioneering attempts were made by Natarajan (1958) who described the egg masses and larval development of prosobranchs from the Gulf of Mannar. The perusal of the literature shows that considerable work has been done on various aspects of *Babylonia spirata* such as, spawning and larval development (Shanmugaraj *et al.*, 1994, Raghunathan *et al.*,1994), feeding behaviour and feed consumption (Patterson *et al.*, 1995b) and pen culture (Patterson *et al.*, 1995a). Though attempts were made in the east coast, the information on larval development and rearing from the southwest coast is apparently nil.

The authors express their sincere thanks to the Indian Council of Agricultural Research (ICAR), New Delhi for the financial assistance. We are grateful to Dr. Mohan Joseph Modayil, the Director, Central Marine Fisheries Research Institute, Cochin for providing the facilities for carrying out the research work. Thanks are also due to Dr. V. Kripa, Sr. Scientist, CMFRI for critically reviewing the manuscript.

Material and methods

The broodstock of *Babylonia spirata* were collected from trawl catches at Neendakara (Lat. 08° 56′ N and Long. 76°32′E) along the Kerala coast. The samples were then transported to molluscan hatchery at the Central Marine Fisheries Research Institute, Cochin in moist condition by covering them loosely with a jute bag or cotton soaked in sea water.

Live and healthy individuals were selected and maintained in the well aerated sea water in FRP tanks of 100 liter capacity. The animals were observed daily and the dead or unhealthy were removed. The tanks were cleaned and 70% of the water replaced daily with fresh seawater. The healthy whelks were allowed to acclimatize in the hatchery for two days after transportation. During the acclimatization period, the animals were allowed to starve and after that they were fed with shrimp meat and polychaetes. After acclimatization, they were transferred to 1 tonne capacity FRP tank provided with sand substratum and two bio-filters for better aeration and maintenance of water quality. Salinity, temperature and *p*H were regularly monitored and maintained within a range of 32 ± 2ppt, 28 ± 1°C and 8.1± 0.2 respectively.

The brood stock holding tanks were observed daily and egg capsules were carefully removed, cleaned in filtered seawater and transferred to the rearing tank.

The dimensions of the egg capsules such as length and width were measured using a digital caliper of 0.1mm accuracy. Number of eggs per capsule were counted by breaking the capsule and were measured under the microscope using the micrometer. The developmental stages were recorded under the stereo-zoom microscope.

Stock culture of micro algae viz. *Tetraselmis gracilis, Nannochloropsis salina, Isochrysis galbana* and *Chaetoceros calcitrans* were maintained in low temperature in 3 l Hafkin flasks following Gopinathan (1996). For feeding the larvae, the algal cultures were maintained in the hatchery in 4 l transparent pearl pet jars under artificial illumination. After estimating the concentration using a haemocytometer, the algae were harvested and fed to the veliger larvae of *B.spirata.*

Results

During the initial trials of transportation from the landing centre to the hatchery in seawater, mortality of the brood stock were observed for 2 to 3 days due to transportation stress. From subsequent trials, it was possible to minimize the mortality by transporting the whelk in a moist condition by covering them with a wet jute bag or wet cotton soaked in sea water.

The acclimatized brooders took average 15 days to spawn in the hatchery, though some took nearly two months to show the spawning activities. (Fig. 1A). The average size of the spawners was 36mm. Spawning occurred during night and continued up to the early morning hours. An errect 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.

Due to the transparent nature of the egg capsules, the eggs were visible and could be counted externally (Fig.1B). 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 errect position till the larvae hatched out. The average total length of the egg capsule was 27.8 ± 2.5mm and the capsular length excluding the stalk showed variation (Table 1). 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 them. There was positive linear correlation (r= 0.8764) between the average length of the egg capsule and average number of eggs (Table 1).

Larval development

First polar body was formed 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, (Fig.1C) which was followed by the second cleavage after one hour (Fig.1D). The divisions were clearly visible up to 16 cell stage (Fig.1E).

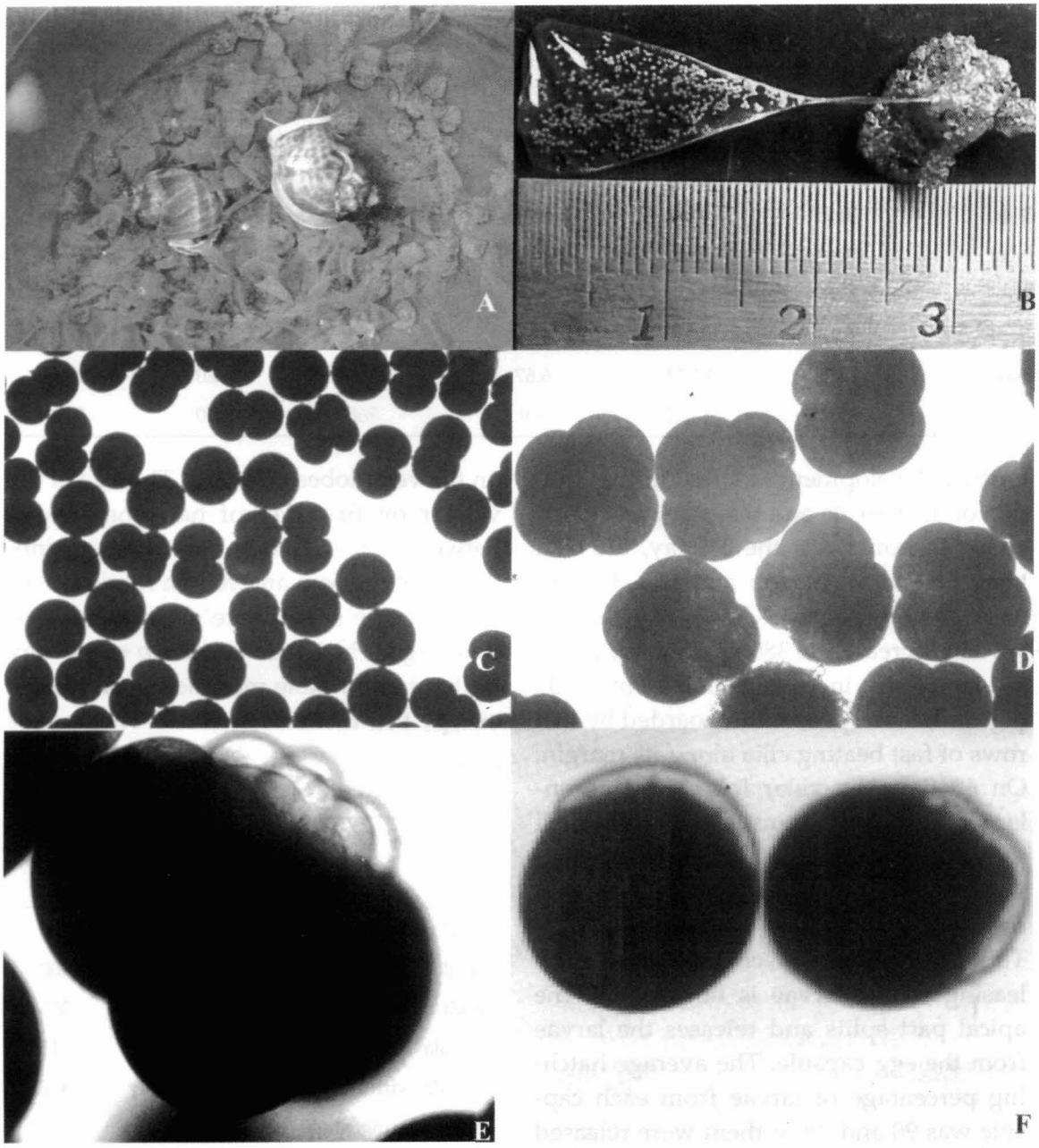

Fig. 1. *Developmental stages of* Babylonia spirata. *A. brooders with egg capsules, B. transparent egg capsule, C. 2 cell stage, D. 4 cell stage, E. 16 cell stage, F. morula*

Subsequently, it becomes an opaque mass due to large deposition of yolk in the egg. After 24 hours of spawning, the divisions were completed and the embryo got transformed into the morula stage with marginal cells at the anterior region (Fig. 1F).

Table 1. *Details of spawning* (B.spirata) *obtained in the hatchery during 2002*

Month	Average length of the egg capsule including stalk(mm)	Average capsule length (mm)	Average capsule width (mm)	Average number of eggs per capsule	Total no. of egg capsule obtained	No. of spawning days
Jan.	29.83	17.42	9.30	703	240	4
Feb.	29.8	17.5	9.78	652	60	1
Mar.	23.62	12.92	6.24	350	70	1
Apr.	29.0	16.2	9.3	700	130	3
Sep.	26.13	13.8	7.64	350	60	1
Nov.	26.2	14.22	6.67	358	120	1
Dec.	29.9	17.22	9.9	560	150	1

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 μm. Subsequently the larval size increased to 420 μm on 5th day and developed velum boarded by two rows of fast beating cilia along its margin. On 6th day, the velar lobes became 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. The measurements from egg to veliger are given in the Table 2.

The hatched out larvae swim to the surface of the water with fast moving cilia on the velar lobes (Fig. 2A). The size of the veliger on first day of hatching ranged between 450-470 μm. The larvae exhibited phototactism and fed on *I. galbana* or *C.salina*. Eye spots were clearly visible at this stage. This stage lasted up to 13th day without any visible morphological change except the increase in size. The foot is

Table 2 *Details regarding the development of fertilized egg of* B. spirata

Development	Day after spawning	Average Size (μm)
Fertilized egg	-	275
2-cell stage	2hrs	300
4-cell stage	3hrs	305
Morula	2rd day	315
Morula with marginal cilia	3rdday	322
Slow rotating stage	3rd day	340
Trochophore stage	4th day	380
Early veliger stage	5th day	420
Fully developed veliger	6th day	446
Veliger ready for hatching	7th day	450
Hatched veliger	7th-8th day	465

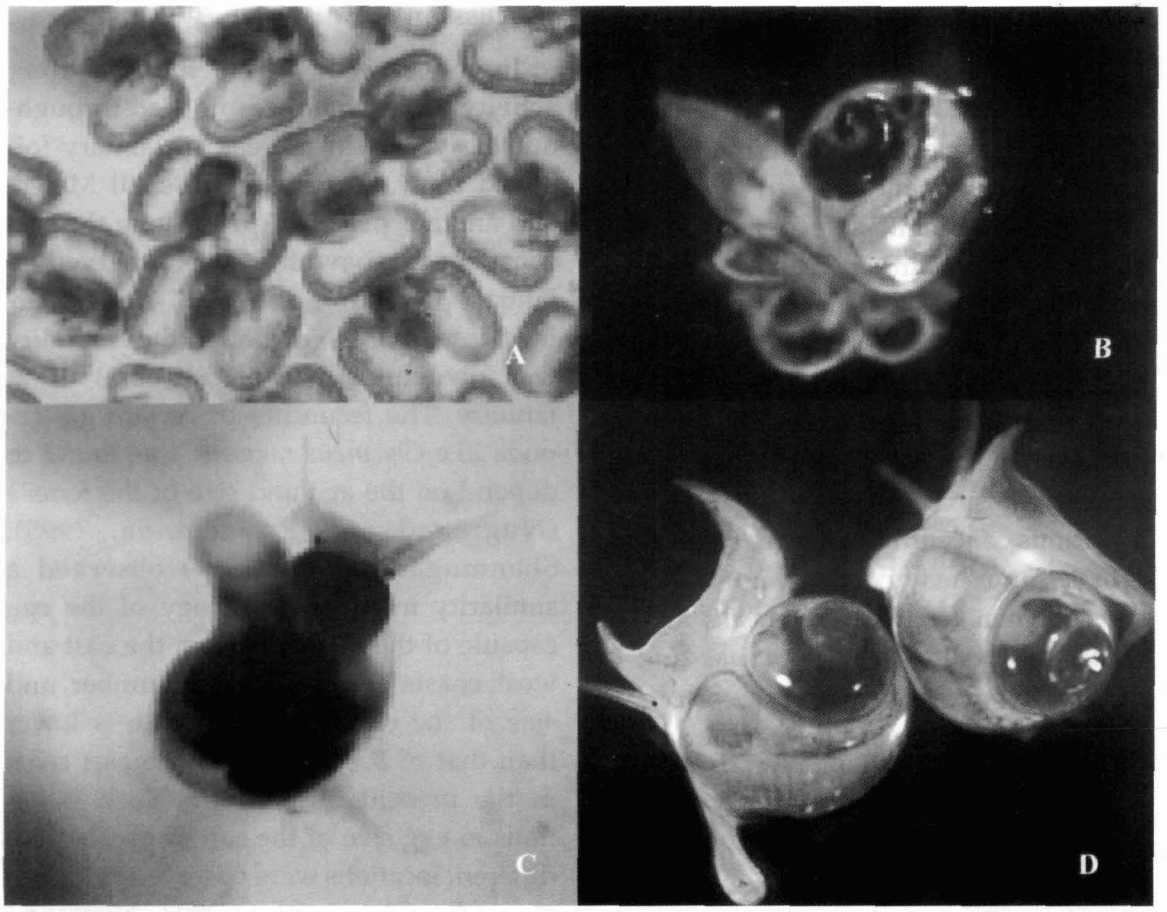

Fig. 2. *Developmental stages of* Babylonia spirata. *A. veliger larvae, B. larvae with fully developed foot, C. tentacles with eye, D. juvenile*

developed fully and protruded out on the 14th day of the fertilization (Fig. 2B). Subsequently, the velar lobes retrogressed and a pair of tentacles with eyes at the base were formed (Fig. 2C). Planktonic life of the veliger feeding on phytoplankton lasted up to 17th day. Degeneration of the velum and gradual development of radula and digestive tract indicate the transformation of the larval life to juvenile stage (Fig. 2D). The growth rate of veliger is depicted in the Figure 3.

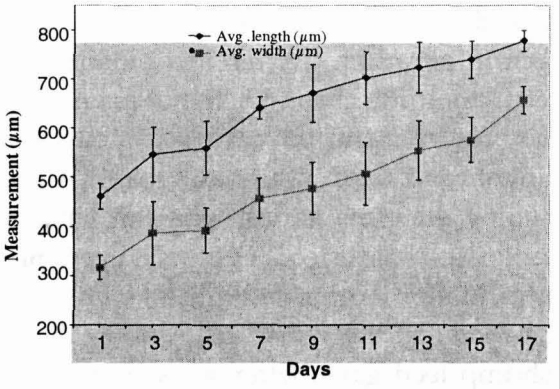

Fig.3. *Growth of the veliger from the day of hatching up to settlement*

Larval rearing

The larvae were transferred from the hatching tanks to the rearing tanks (Perspex/glass tanks) by filtering through a sieve of 400μm and stocked in seawater in the rearing tank at a density of 150 larvae/*l*. The salinity, *p*H and temperature were maintained at 32±1 ppt, 8±0.2 and 28±2°C respectively. Prior to stocking, the water for rearing was treated with hypochlorite and potassium permanganate solution to eliminate the unwanted microorganisms. Different algal feeding in various concentrations were tried. Poor growth and heavy larval mortality occurred when fed with *T. gracilis* and *N. salina*. Pure cultures of *I. galbana* and *C. calcitrans* were provided to the larvae up to the 17th day. The larvae were fed at the rate of 7000 cells $ml^{-1}hr^{-1}$.

Juvenile rearing

Metamorphosis of the larvae was completed in 17 to 19 days after the release from the capsule. The size of the juvenile at the settlement ranged from 800μm to 1.3mm. The settled juveniles were transferred to 5 liter beakers provided with gentle aeration. After settlement, the planktonic life changed. They became carnivores and started crawling along the bottom and sides of the rearing tank. Algae settled on glass slides, artificial shrimp feed, agar based feed, egg yolk, egg albumin, tubifex worms and rotifers were tried as food for the juveniles. Among these, shrimp feed gave better growth and survival. The settlement rate was 65%.

Discussion

In the present study, the spawning activity of *B. spirata* was noticed throughout the year with a peak in January followed by a gradual decrease till March, and another peak in April. Along the east coast, peak spawning was observed in January (Shanmugaraj *et al.*, 1994). During September – November, the fecundity was low, almost half of that observed in January. The fecundity of certain gastropods like *Chicoreus ramosus* was found to depend on the age and size of the female (Nugranad and Promchinda, 1995). Shanmugaraj *et al.* (1994) observed a similarity in the morphology of the egg capsule of the *B. spirata* from the east and west coasts. However, the number and size of the eggs in the capsule is lower than that of *B. spirata* from the east coast in the present observation. Such variations in egg size of the same species from different locations were observed in *Rapana venosa* by Chung *et al.*, 2002. Nugranad and Promchinda (1995) have reported variation in shape and size of the egg capsule laid by different female spawners of the same species from the same geographical area. Morphological differences in egg capsule such as shape, size and surface texture of species in the same genus of neogastropod have been observed and such differences have been associated with environmental factors such as physical stresses or geographical latitude (Chung *et al.*, 2002).

The development of the eggs within the egg capsule were fast and planktotrophic

veliger larvae hatched out within a week. The duration of development of the egg to hatching of veliger ranged from 7 to 8 days in the present study while it took 10 days along the east coast (Shanmugaraj *et al.*,1994). At present no reason could be attributed to the release of the larvae as it could change in the osmotic pressure. Nurse eggs were not observed in the present study which agreed with the observations of Chung *et al.* (2002). The larvae which hatched out as veliger (<0.5mm) was not found to consume nurse eggs during the development. The growth of the egg within the capsule was high, increasing from 275 μm egg to veliger of 465 μm while the increase in size of the egg (400 μm) to veliger (416 μm) was low in the development of the same species along the east coast (Shanmugaraj *et al.*, 1994). According to Han (1989) the larva is classified as veliger when the apical region becomes flat and the velum completely developed with long cilia. In the present study this stage was obtained on 5th day after spawning. Morton (1986) has reported that the residence time and the size at hatching were positively correlated to the nutritional resources of the egg capsule content. The low residence time and high hatching percentage substantiate the fact that the egg capsules were healthy. Development of bacterial or protozoan infection resulting in the retardation in the growth and survival was reported in the nudibranch, *Rostanga pulchra* by Chia and Koss (1978). Though such retardations were observed in the beginning of the trials, through proper water quality management it was possible to reduce the incidence of deterioration of capsules.

The larval development within the capsule was similar along the east and west coasts, while the hatching percentage and post settlement survival rates were higher in the present experiments.

References

Appukkuttan K.K. and M. Babu Philip. 1994. Gastropods- An emerging resource in the by- catch of shrimp trawlers at Sakthikulangara-Neendakara area. *Seafood Exp. J.*, **25** (21): 5-17

-------- and K. Ramdoss. 2000. Edible and ornamental gastropod resources. *In*: V. N. Pillai and N.G.Menon (Eds.) *Marine Fisheries Research and Management*. p 525-535. Central Marine Fisheries Research Institute, Cochin, India.

Ayyakannu, K. 1994. Fishery status of *Babylonia spirata* at Porto Novo, southeast coast of India. *Phuket Mar. Biol. Cent. Spec. Publ.*, **13**: 53-56.

Chaitanawisuti, N. and A. Krisanapuntu. 1997. Effect of stocking density and substrate preference on growth and survival of hatchery reared juvenile spotted *Babylonia areolata* Link 1807 (Neogastropoda: Buccinidae). *J. Shellfish Res.*, **16**:429- 433.

-------- and -------- 1997a. Laboratory spawning and juvenile rearing of the marine gastropod, spotted Babylon, *Babylonia areolata* Link 1807 (Neogastropoda:Buccinidae) in Thailand. *ibid.* **16**: 31-37

-------- and -------- 1998. Growth and survival of hatchery reared juvenile spotted babylon,

Babylonia areolata Link 1807 (Neogastropoda: Buccinidae). *ibid*, **17**: 85-88.

-------- and -------- 1999. Experimental culture of spotted Babylon, *Babylonia areolata* Link 1807 (Neogastropoda: Buccinidae) in Thailand. *Asian Fisheries Science*, **12**: 77-82

-------- and -------- 2000. Growth and production of hatchery reared juvenile spotted babylon, *Babylonia areolata* Link 1807 cultured to marketable size in intensive flow through and semi-closed re-circulating water systems. *Aquaculture Research*, **31**: 415-419.

Chia, F.S and R. Koss.1978. Development and metamorphosis of the planktonic larvae of *Rostanga pulchra* (Mollusca: Nudibranchia). *Marine Biology*, **46**: 109-119.

Chung, E.Y., S.Y. Kim, K.H. Park, and G.M. Park. 2002. Sexual maturation, spawning, and deposition of the egg capsules of the female purple shell, *Rapana venosa* (Gastropoda: Buccinidae). *Malacologia*, **44** (2): 241-257.

Gopinathan, C.P. 1996. Live feed culture-Micro algae. *Bull.Cent. Mar. Fish. Res. Inst.*, **48**: 110-116.

Han, K.O.1989. Handbook of culture of Abalone and other marine gastropods. CRC press, Inc-Boca, Rator, Florida. 348pp.

Morton, B. 1986. Reproduction, juvenile growth, consumption and effects of starvation upon the South China whelk, *Hemifusus tuba* (Gmelin) (Prosobranchia: Melonginidae). *Jour. Expt. Mar. Biol. Ecol.*, **102**: 257-280.

Natarajan, A.V., 1958. Studies on the egg masses and larval development of some prosobranchs from the Gulf of Mannar and Palk Bay. *Proc. Indian Acad. Sci.*, **46**: 170-228.

Nugranad, J.and T. Promchinda. 1995. Fecundity, size of egg capsules and hatched veligers of *Chicoreus ramosus* in captivity broodstocks. *Phuket Mar. Biol. Cent. Spec. publ.*, **15**: 69-73.

Patterson, J. K., A. Benny and K. Ayyakannu. 1995a. Pen culture of *Babylonia spirata* (Neogastropoda: Buccinidae) in Vellar estuary, Parangipettai, India. *ibid.* **15**: 59-60.

Patterson, J. K., C. Raghunathan and K. Ayyakkannu 1995b. Food preference, consumption and feeding behaviour of the scavenging gastropod *Babylonia spirata* (Neogastropoda: Buccinidae). *Indian J. Mar. Sci.*, **24**: 104-106.

Raghunathan, C., J. K. Patterson Edward and K. Ayyakannu. 1994. Long term study on food consumption and growth rate of *Babylonia spirata* (Neogasropoda: Buccinidae). *Phuket Mar. Biol. Cent. Spec. Publ.*, **13**: 207-210.

Shanmugaraj, T., A. Murugan and K. Ayyakannu.1994 Laboratory spawning and larval development of *Babylonia spirata* (L) (Neogastropoda: Buccinidae). *ibid.* **13**: 95-97.