STUDIES ON SPAWNING AND LARVAL REARING OF THE WHELK, *BABYLONIA SPIRATA* (LINNAEUS, 1758) (NEOGASTROPODA: BUCCINIDAE)

Thesis submitted to Mangalore University
in partial fulfillment of the requirement for the award of
the degree of

Doctor of Philosophy
Under the Faculty of Biosciences.

By
Sreejaya.R. M.Sc,

Department of Post Graduate Studies and Research in Biosciences
Mangalore University, Mangalagangothri
Karnataka, India-574 199

December, 2008

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Certificate

*This is to certify that this thesis entitled "**Studies on spawning and larval rearing of the Whelk, Babylonia spirata (Linnaeus,1758) (Neogastropoda: Buccinidae)**" is an authentic record of research work carried out by Sreejaya.R under my guidance and supervision in Central Marine Fisheries Research Institute, Cochin, in partial fulfillment of the requirement for the award of Ph.D degree under the Faculty of Biosciences in Mangalore University. The thesis or part thereof has not previously been presented for the award of any Degree in any University.*

Cochin
Date:

Dr.K.Sunilkumar Mohamed,
Supervising Guide,
Principal Scientist & Head, Molluscan Fisheries Division,
Central Marine Fisheries Research Institute,
Cochin-682 018.

Declaration

*I do hereby declare that the thesis entitled ""**Studies on spawning and larval rearing of the Whelk, Babylonia spirata (Linnaeus, 1758) (Neogastropoda: Buccidae)**" is an authentic record of research work carried out by me under the guidance and supervision of Dr. K. Sunilkumar Mohamed, Principal Scientist & Head, Molluscan Fisheries Division, Central Marine Fisheries Research Institute, Kochi in partial fulfillment for the award of Ph.D degree under the Faculty of Biosciences of Mangalore University and no part thereof has been previously formed the basis for the award of any diploma or degree, in any University.*

Cochin
Date: *(Sreejaya.R)*

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(Sreejaya. R)

Dedicated to My Family

Contents

ACRONYMES

µm	Micrometer
AGR	Absolute growth rate
ANOVA	Analysis of Variance
avg	Average
Cc	*Chaetoceros calcitrans*
CCA	Crustose coralline algae
CITES	Convention on International Trade in Endangered Species
CL	Capsular length
CL	Capsular length
cm	Centimeter
CPUE	Catch per unit effort
CW	Capsular width
DO	Dissolved oxygen
DP	dry packing
Fig	Figures
g	gram
h	Hour
I g	*Isochrysis galbana*
IGR	Instantaneous growth rate
IR	Ingestion rate
l	liter
LC	Lethal concentration
ln Li	Natural Log of initial length
ln Lt	Natural Log of length at time t
Ns	*Nannochloropsis salina*
Ma	Accumulated mortality
min	minutes
ml	millilitre
mm	Millimeter
MP	moist packing
mt	metric tonne
Ni	Initial number of larvae
PL	Peduncle length
ppm	Parts per million
ppt	Parts per thousand
Sf	Final salinity
Si	Initial salinity
SL	Shell length
SW	Shell width
t	Tonne
Tg	*Tetraselmis gracilis*
TL	Total length
TRT	Treatment
TW	Total weight
UVR	Ultra violet radiation
V	Volume
Vf	Volume of freshwater
WP	Wet packing

PREFACE

The spiral Babylon *Babylonia spirata* (locally called 'Dove egg shell'- Pravumutta chank) is a gastropod resource which is being exploited rapidly for export to foreign countries mainly to Japan. The meat of this whelk is exported and also consumed by the fisher folks on the southeastern coast of India. The shells of these snails are used in the lime industry and for ornamental purposes. The operculum is exported to foreign countries for manufacturing medicine and perfumes (Thirumalavalavan, 1987). The export of these molluscs has led to the development of active fishery of this shellfish in different parts of India. As the demand for the whelk meat is increasing day by day and intensive exploitation is done by trawling in most centers, the chances of overexploitation and exploitation of undersized whelk leading to the depletion is quite possible. Measures are to be taken to avoid overexploitation and indiscriminate exploitation of the juvenile whelks and egg masses from the natural beds. Appukuttan and Ramdoss (2000) stressed the need for judicious exploitation and hatchery seed production for sea ranching to augment the production of the species. At present there is no technology available for large scale production of *B. spirata* in hatchery systems. Enhanced production through hatchery and sea ranching is an essential pre-requisite for the conservation of this resource. Knowledge on spawning, larval development and metamorphosis of a particular species will help in developing a successful hatchery technology to produce juveniles. Larval developmental studies are an essential pre- requisite for better understanding of the desired habitat, food preference, mortality and growth. Keeping this in view the present study on spawning and larval rearing of the whelk, *B. spirata* was taken up and is intended to provide the basic information on larval life cycle and to develop a hatchery protocol for rearing the species in controlled conditions.

The study was conducted on the whelk *Babylonia spirata* (Linnaeus, 1758) collected from the trawl landings at Neendakara and Sakthikulangara harbour in Kerala in the Southeast coast of India. *B. spirata* belongs to the Phylum Mollusca and Class Gastropoda of Order Neogastropoda in the family Buccinidae. The shell of *B. spirata* is smooth, ovoid with regular spiral rows of large rounded or squarish brown patches

on a white background. The spire is of medium height with rounded whorls while, the body whorl is inflated and the suture is enhanced with sharp edge. The columella is smooth and well developed. Aperture of the shell is large and ovate, constricted posteriorly by a thick ridge extending inwards on the columellar side. It grows to an average shell length of 5 to 6.5 cm. The species is discontinuously distributed in the Indian Ocean up to a depth of 150 m. The thesis is organized in the following manner.

The general introduction and review of literature of the work done are included in the chapter 1 of the thesis entitled 'INTRODUCTION AND REVIEW OF LITERATURE'.

The study was conducted during the period 2001-2006 in the hatchery of Central Marine Fisheries Research Institute (CMFRI), Cochin. For the development of protocol for broodstock maintenance in hatcheries five experiments on salinity, temperature tolerance, substrate and feed preference and on water quality were conducted and these are described in chapter 2 on 'BROODSTOCK MAINTENANCE AND REARING OF *BABYLONIA SPIRATA*'.

The broodstock maintained in the CMFRI hatchery spawned and observations were made on the copulation, spawning and brooding behaviour, feeding, variation in spawning periodicity, effect of the brooder size on fecundity and egg size etc. Experiments were also conducted to study the necessity of substratum for egg laying of whelk. These aspects are included in the third chapter titled 'SPAWNING OF *BABYLONIA SPIRATA*'.

B. spirata has an indirect development with an initial capsular phase followed by a planktonic phase. In the fourth chapter titled 'EGG CAPSULES OF *BABYLONIA SPIRATA* AND ITS REARING' the morphological and dimensional variation of the egg capsules have been studied. Moreover the experiments conducted to study the effect of quality of water, salinity, temperature, aeration and light on hatching rates and growth and development of larvae during the intra- capsular phase are described.

The embryonic development and changes during the intra- capsular phase and the post hatching period was studied and this is incorporated in the fifth section 'INTRACAPSULAR DEVELOPMENT AND GROWTH OF *BABYLONIA SPIRATA'*.

After studying the changes occurring in the larval metamorphosis, experiments were conducted to develop protocol for rearing the larvae in hatcheries. Experiments were conducted to study the effect of salinity, temperature, substratum, water exchange, stocking density, aeration, light and feeding on the larvae of *B. spirata*. These are described in the sixth chapter on 'LARVAL REARING OF *BABYLONIA SPIRATA*'.

After the settlement of the larvae, the juveniles were reared and their rearing was standardized by conducting a series of experiments and these are presented in seventh chapter entitled 'JUVENILE REARING OF *BABYLONIA SPIRATA'*.

Detailed description of the materials used and methods followed and the results obtained and discussions therein are presented in each chapter. The results of the all the experiments described in each chapter are summarized in the section 'SUMMARY'. The protocols for the development of a well established hatchery for broodstock rearing, spawning and rearing of the whelk, *B. spirata* are also documented in this section.

The references cited in the study are presented in the last section of the thesis 'REFERENCES'.

Chapter 1

Introduction and Review of Literature

1. INTRODUCTION AND REVIEW OF LITERATURE

1. 1. INTRODUCTION

Gastropods of the phylum Mollusca form an important resource with more than 80,000 species distributed in the aquatic and terrestrial biotopes. Several species of marine gastropods have been harvested from nature for their soft and succulent meat for many centuries. Trade of the beautiful gastropod shells with fascinating spiral whorls, dazzling colours, shapes and sculptures have contributed to the economic growth of several island nations and supported the growth of island based tourism (Hahn, 1989) and shell craft industries (Appukuttan and Ramdoss, 2000).

Among the farmed gastropods, the abalones, commonly known as ear shell molluscs, (‘awabi” in Japan) are the most important. An abalone fishery existed in Japan since early history (Hahn, 1989). The earliest reference to ama abalone divers is in a document from the region of Emperor Suinin around 30 A.D. The tradition of ama divers being exclusively female began in the sixth century when many men were taken to serve on war ships. Women who were left behind had to take care of themselves and they became self reliant. The government tribute was paid with awabi by the ama divers. From this beginning, it has become traditional for women to dive for abalones (Hahn, 1989).

One of the earliest records on gastropod trade is from Japan. Japan traded with foreign countries only through the port of Nagasaki prior to the beginning of the Meiji Era (1865 to 1912). Dried abalone was the most important export, constituting about 80% of marine exports (Hahn, 1989). During the Meiji Era, there was a strong desire in Japan to generate more money from exports by increasing the abalone harvest. Research on hatchery methods for abalone aquaculture began at the Tokai Regional Fisheries laboratory in 1959. The present abalone aquaculture techniques

are based on the pioneering work of Murayama (1935) on *Haliotis gigantean* and Ino on *H. discus* (1952). Modern methods of seed production and seaweed reforestation have greatly improved the harvests in Japan. Considering the global demand for abalone, several nations, initiated programs to transplant abalones and conduct research on abalone seed production and husbandry.

Aquaculture of economically important gastropods began in the 1970's and in 1979, Korea was the major nation farming the abalone (FAO, 2004). In 2002, there were more than nine countries farming abalone, with China producing the highest, 4500 tonnes (t), followed by Taiwan 3000 t. Other nations involved in abalone farming are South Africa (450 t), Japan (200 t), USA (169 t), Australia (162 t), Chile (150 t), Mexico (53 t), New Zealand (2 t) and other nations (10 t) (Gordon and Cook, 2004). World - wide more than 15 species are now being commercially cultivated with more than 1,000 individual farmers with individual production ranging from less than a tonne to over 200 mt (Gordon and Cook, 2004). Compared to the production from mariculture, harvests from natural beds have been considerably higher. The production from fishing gastropods increased tremendously from 30,499 t in 1950 to 1,21,657 t in 2003 (FAO, 2003).

FISHTECH (2007) has given a report of the current status of worldwide abalone farming. In California, USA, there are currently 13 abalone farms. The farmed size of abalone is normally 75 to 100 mm and they are marketed either as live or processed. The largest farm produces over 100 t and the smaller less than 10 t. Japan has many major seed farming operations, most of which are involved in ocean enhancement, totals of which are included under Fisheries; 30 to 40 million seeds are planted annually. Almost all Japanese farming consists of ocean bottom growing from farmed seeds. China, is the worlds largest producer with over 300 abalone farms with production expected to produce over 5,000 t in 2007, excluding lower value species. Most production is consumed within China. Taiwan currently has over 400 farms (many are small family run operations). Total production in 2007 is anticipated at over 2,800 t, including lower value species and most of the production is consumed in Taiwan. Farmed production from Australia/Tasmania, New Zealand is estimated at 600 t in 2007 and combined production in Chile and South Africa may

exceed 1,000 t in 2007. Production from Europe, Iceland and Pacific Rim countries should have a farm production of over 300 t.(http://www.fishtech.com/farming.html).

Whelks of the order Neogastropoda and family Buccinidae have been fished from several parts of Europe and Asia and they have formed nearly 30.9% of the global gastropod fishery production. *Buccinum undatum* is the major resource fished and other species of gastropod which have documented commercial fishery are *Strombus* spp., *Turbo cornutus, Haliotis* spp., *Busycon* spp. and *Cymbium* spp. (FAO, 2003). Muricids are important resource in several parts of the world. Attempts were made to develop the farming techniques of whelks in Asia since 1990's. The scavenging gastropod *Babylonia* is found only on sandy or muddy bottom of the Indo-Pacific coasts (Altema and Gittenberger, 1981). The spotted babylon, *Babylonia areolata* (Link, 1807) commonly known as the Hoy Wan in Thailand supports a commercial fishery (Chaitanawisuti and Kritsanapuntu, 1999b). The natural stock declined sharply in the 1990's and this was followed by an increase in both demand and price. This lead to an interest in aquaculture of this species for food production and replenishment of natural stock. After developing a sound technique for seed production of this species, methods were developed for monoculture and polyculture with compatible finfishes like sea bass. From an aquaculture point of view, the spotted babylon has many biological attributes and production and market characteristics necessary for a profitable aquaculture venture (Chaitanawisuti and Kritsanapuntu, 1999b; 1999c; 2006). The farming technology is also equally sound. Based on the study by Kritsanapuntu *et al.* (2006a; 2006b), the total yield of spotted babylon held in monoculture and polyculture with sea bass was 10.520 and 10.450 kg ha^{-1} respectively. The cost of production was USD 5.69 kg^{-1} and 6.95 kg^{-1} for monoculture and polyculture respectively. At a farm gate price (in 2003) of USD 9.00 kg^{-1} Kritsanapuntu *et al.* (2006a) have estimated a net return of USD 11,124 and 14,691 for monoculture and polyculture with sea bass. Based on these studies they have demonstrated that it is possible to culture *B. areolata* in earthen ponds such as abandoned/rested shrimp ponds where by unutilized shrimp ponds can be effectively used in several coastal areas in Thailand. Other species of Babylon like *Babylonia japonica* (Reeve 1842) in Japan, *B. formosae habei* (Altena and Gittenberger 1981) in China and *B. formosae formosae* (Sowerby 1866) in Taiwan

are exploited in their regions of distribution. Attempts have been made to study these resources to a limited extent (Zheng *et al.*, 2000; 2001; Chen *et al*., 2005).

The conchs, *Strombus* spp. are farmed in Turks and Caicos Islands and *Concholepas concholepas* in Peru with an estimated production of 25 t and 12 t respectively in the year 2003. Conch farm technology has been under development at the Caicos Conch Farm on the island of Providenciales in the Turks and Caicos islands since 1984. Conch world Provo, is owned by the Turks and Caicos shareholder company, Trade Wind Industries Ltd. (TWI) and is the only one of its kind. Twelve million dollars have been invested to develop conch farming to the commercial scale and it operates successfully today (http://www.turksandcaicos.tc/coralreefs/conch.htm). Archeologists have shown that it was an important source of quality protein for people long before Columbus came to the New World in 1942. Today conch is the preferred seafood in the Caribbean and Central America. Over 40 millions conchs are harvested annually.

The conch, *Strombus gigas* forms a very valuable fishery in the Caribbean and has been a chief source of protein for local people for at least 100 years (Brownell and Stevely, 1981). Attempts have been made to culture and conserve the gastropod which forms the source of livelihood of villagers of island nations (Hahn, 1989). A 2.5 year old queen conch yields upto 1kg of meat (75% protein by weight). There has been overfishing and the fishery proceeded from shallow to deeper habitats to maintain the level of catch (Hahn, 1989). He has inferred that the resource had reached declining levels incapable of sustaining continued harvest. Queen conch has already been placed in the list of "commercially threatened invertebrates" (Goodwin, 1983). Due of these facts, attempts were made to develop mariculture techniques, seed production methods and also to reseed the natural population. However these have not been as successful as the abalone mariculture and sea ranching programmes.

Sea ranching or reseeding of gastropod beds has also been promoted as a consequence of hatchery technology development. The seed produced in the hatchery of several economically important gastropods like abalone, trochids and conchs have

been used to enhance the natural stocks. When sea ranching programs were initiated several years back there were problems related to predation of released juveniles, lack of food etc. which led to low survival resulting in poor recovery rates. However through trial and error these techniques have been improved and now encouraging results are obtained. In an update on world abalone fisheries and aquaculture, Gordon and Cook (2004) have given a comparative account on the supply and market dynamics of the abalones during the 1970's and in the year 2002. The abalone world of the 1970's was one with minimal regulations of fisheries sector, little illegal catch and a cultured "market size" industry measuring production in kilograms, not tonnes (Gordon and Cook, 2004). In contrast, in the year 2002, two major abalone fishing countries (United States and South Africa) either closed the fishery entirely or threatened closure. Similar situations have occurred in several parts of the world.

Simizu and Uchino (2004) have described the success of abalone sea ranching. Abalone catch used to be 800 t a year in Chiba Prefecture on the Pacific coast of Central Japan and this was reduced to less than 100 t during the recent years (Simizu and Uchino, 2004). Surveys conducted revealed that the density of mature abalone was 0.019 individual m^{-2} which may be insufficient for successful reproduction (Shepherd and Brown, 1993; Shepherd and Partington, 1995; Badcock and Keesing, 1999). In 1998, 400,000 artificial abalone juveniles (3 cm in shell length) were released to the research field of 8,000 m^2 (Simizu and Uchino, 2004). The abalone seeds grew upto 10.2 cm shell length on an average in 3 years and 12.8 cm shell length in 4 years exceeding the legal catch size (12.0 cm), by the Chiba Prefectural Regulation of Fisheries Adjustment and commercial catches started in May, 2002. The catch per unit effort increased to a great extent from 3 kg day^{-1}-boat to 9 kg day^{-1} -boat. The inhabited area was considered to be expanded to 100,000 m^2 based on the catch logs by the local fisherman and as to May 2002, a production of 57,000 abalones was estimated to be attained by the excessive seeding.

Babylonia zeylanica (Bruguiere, 1789), known as Indian babylon is distributed mainly in Indian and Sri Lankan waters (Wye, 1991). In India, *Babylonia spirata* emerged as an important resource during the 1990's. This species is well represented

in the Indian Penisula in Gulf of Mannar, Poompuhar, Nagapattinam, Madras and in waters around Andaman and Nicobar islands (Ayyakkannu, 1994). The average whelk landing at Neendakara- Sakthikulangara composed of *B. spirata* and *B. zeylanica* for the period 2001-2003 was 487 t contributing 62.5% of the total gastropod landed during this period (Anjana, 2007). During the last 10 years there has been targeted fishery for this resource along the southwest and southeast coast especially off Central Kerala (Appukuttan and Philip, 1994; Philip and Appukuttan, 1997) and at Karnataka (Sasikumar *et al*., 2006). As the demand increased, modifications were made to gear for competent fishing at Kollam and Tuticorin. Whelk is fished using traps at Porto Novo (Ayyakkannu, 1994) and Karnataka (Sasikumar *et al*., 2006) and at Pondcherry using ring-nets (Chidambaram, 1997). The export of frozen *Babylonia* meat began in 1993 and since then it has been a regular item of the Indian marine products (Appukuttan and Philip, 1994). During the year 2000, 8.4 tonnes of *Babylonia* valued Rs 0.59 million were exported. Initially only frozen products were exported and since 2001, live whelks were also exported. In the year 2003, about 1115 t were exported as fresh/ processed to China, Japan, Southeast Asia, USA and European Union. Preliminary studies done at the Central Marine Research Institute and Centre of Advanced Study in Marine Biology, Parangipettai have indicated that the possibility of seed production of the *B. spirata* (CMFRI, 2002; Shanmugaraj *et al.,* 1994; Sreejaya *et al.,* 2004). Considering the growing significance of these resources, the topic for research was identified as "Studies on spawning and larval rearing of the whelk, *Babylonia spirata (*Linnaeus, 1758) (Neogastropoda: Buccinidae)" with a view to understand the spawning and larval development of *B. spirata* and develop a complete hatchery protocol for this resource.

As a prereqsite for the development of hatchery protocol the various factors affecting the growth and survival of early life history stages has to be studied. To develop this database, the objectives of the study have been formulated as given below.

1. Brood stock maintenance and spawning of *B. spirata.*
2. Capsular and larval development of *B. spirata* under controlled conditions.
3. Factors affecting growth, survival and settlement of *B. spirata* larvae.

4. Post settlement growth and survival of juvenile whelks.
5. Protocol development for whelk seed production.

The study is expected to provide information on the best management measures for the seed production of the whelk *B. spirata* which can support mariculture activities as well as natural stock enhancement programs. Scientific knowledge on the spawning and larval development of *B. spirata* will also be generated and understood.

1.2. REVIEW OF LITERATURE

1.2.1. Fishery of Gastropods

Many gastropods are harvested for their meat, at the same time the beautiful shape and colour of the shells have attracted and aroused the imagination of man to use them for commercial purposes (Ramdoss, 2003). However the search for food has constituted and still continues to form a major reason for shellfish exploitation. For many coastal populations of tropical areas, shellfish, as a substitute for fish, represents an important dietary component, especially for poor people. Other reasons for shellfish exploitation include the use of shells as a raw material for mother- of-pearl or for lime in pottery glazes, poultry food additives, or for personal adornment. Some marine species are collected to supply the interests of collectors for beautiful objects, or as a source of pharmaceuticals: a toxin recently extracted from the venomous Caribbean gastropod *Conus ermineus*, could be used to counteract the effects of some myasthenias and multiple sclerosis on muscle contractility. (http://www.ifremer.fr/doceiec). Abalone is harvested from several parts of the world and the production fluctuates between 10000 and 15000 t per year (Berthou *et al*., 2005). According to him, the abalone fishery in Tasmania reaching a total allowable commercial hatch of about 2500 mt. *Buccinum undatum* is another major resource fished and other commercially important gastropods which have a documented commercial fishery are *Strombus* spp., *Turbo* spp., *Haliotis* spp., *Busycon* spp., and *Cymbium* spp. (FAO, 2003). Muricids are important resources in several parts of the world.

Whelks are fished mainly from Europe. The European whelks (*Buccinum undatum*) fisheries began in the sixties and have increased in the recent years to fulfil the high demand of the Southeast Asian market. Basically harvested by traditional 8-16 m long potters with 500 to 1000 baited pots lifted per day and per boat, some areas are fully exploited. Traditionally in Canada, the whelk *B. undatum* has been harvested for several years using a variety of traps and boats especially by small, twine, conical pots known as Korean pots. Under a project, special stackable pots were fabricated and tested. The stability of the conical pot allowed fishers to extend the areas fished to deeper and more turbulent water while their stackability enabled vessels to carry more. The relatively light weight of poles as compared to buckets enabled the hauling and setting of the gear to be accomplished more quickly (FDP, 2002). The fishery of *Baccinanops globulosum*, a whelk along the Argentina coast began in the year 2000 and to prevent over exploitation, experimental licenses for fishing were issued via a public draw (Narvarte, 2006).

Ablone is harvested from several parts of the world and the production fluctuate between 10000 and 15000 t per year (Berthou *et al*., 2005). From mid eighties a new type of dragged gear locally called the 'rastell' and specially designs for catching *Murex* spp. began to be used on the Catallan coast. This beam trawl, modified without skates, is used by around 60 vessels, throughout the year, for 5 days a week during the recent years. The abalone fishery in Tasmania is one of the most important (Berthou *et al*., 2005), reaching a total allowable commercial catch of about 2500 mt (nearly half of Australia' total production). Two species are harvested manually by divers: *Haliotis ruba* and *H. laevigata*. This commercial fishery is under a quota management system. Access to the quota and the right to take the abalone is formally separated; there are about 350 holders of quota and only 125 divers licensed to harvest abalone. Approximately 4000 recreational abalone diving license per year and are restricted to a catch of 10 abalones per day.

Strombus gigas, the queen conch with a beautiful pink shell is a commercially important marine gastropod in the Caribbean Sea. The abundance of these resources has been affected by fishing pressure. This species was much abundant in common shallow intertidal waters and recently it began to be caught from deeper areas. The

guidelines on the requirements for responsible management of the fisheries exploiting Caribbean queen conch (*Strombus gigas*) with particular emphasis on the requirements to comply with relevant regulations of the Convention on International Trade in Endangered Species of wild Fauna and Flora (CITES) represented in the FAO (2008). The fishing method also progressed from simple hook and line to SCUBA diving up to 40 m depth. Similarly, crafts also changes from wooden canoes to far ranging fibreglass boats with powerful engines (Berg and Olson, 1989). The queen conch, which is extremely edible, was once part of a thriving fishery, and was utilized for food throughout its range (Randall 1964). In the 1960's the queen conch was second only to the spiny lobster as a fishery product (Randall, 1964). At that time 4 million were being exported from the Caicos Islands to Haiti (Berg, 1976). Another species which has been subjected to targeted fishing is *Bolinus brandaris*, a commercially important gastropod. This has been fished using artisanal fishing gear like trammel nets, basket traps and dragged gear, but later a modified gear 'rastell' began to be used for fishing this resource (Martin *et al.*, 1995).

The two main commercial mollusc species in Sudan are trochus (*Trochus dentatus*) and pearl oyster (*Pinctada margaritifera).* Although this fishery makes a low contribution to the overall Sudanese economy, it is of importance to coastal people as a source of income and an important source of animal protein found in the fresh fish markets in coastal areas (Review: Eltayeb 2004). According to him, the average annual Sudanese trochus (*T. dentatus* and *T. virgatus*) export between 1970 and 1998 was 521 t per year valued at USD 182,803. *T. dentatus* forms the backbone of the mollusc fishery (Eltayeb 1999).

The Indian marine gastropod production has been mainly contributed by three resources, the sacred chank *Xancus pyrum*, the turban shell *Turbo marmoratus* and top shell *Trochus niloticus*. The sacred chank has been harvested through a regular and organized by fishery mainly to meet demand from the bangle industry in West Bengal (Hornell, 1914; Ghazi, 1962; Jones, 1968). The fishery in Tamil nadu is controlled by the State Govt. which permits fishing by issuing license to fishermen. The annual chank landing varies from 1 to 1.5 million numbers in Gulf of Mannar and Palk Bay and an estimated 17, 000 to 20, 000 chanks are caught in trawl nets

along west coast in the southwest coast (Nayar and Mahadevan, 1974; Appukuttan *et al*., 1980; Alagarswami and Meiyappan, 1989; Narasimham, 2005).

Appukuttan *et al*. (1980) described the chank fishery of Kerala coast. The Kerala Government leases the right to collect chanks to co- operative societies. Chanks have been fished since early times along the Trivandrum coast by skin diving in the 10-20 m depth zone. Catamarans are used for transporting and each diver collects about 10 chanks per day. Chanks are also caught incidentally in bottom set gill nets and shrimp trawls. At Sakthikulangara, shrimp trawlers fishing at 40-50 fathom depth land considerable quantity of chanks (length 100-220 mm). Chanks are fished with 220-500 m long line, holding 500-1000 hooks. As hooks are dragged on the sea bed the foot of the chank gets hooked firmly.

Pota and Pattel (1986) have reported on the chank fishery of Gulf of Kutch. The Gujarat Fisheries Department controls chank fishing. Chanks are fished along the Gujarat coast in the Gulf of Kutch. The fishing area lies in 200 km coast line between Sachhana and Okha. The chanks are fished in the inter-tidal areas of patchy coral reefs and due to high tidal amplitude vast stretches of inter tidal areas are exposed at low tides. During the spring tides, the fishermen wade through the water, handpick the chanks and empty the catch basket known as 'Gumbha'. Thomas *et al*. (1998) reported on the high production of 20, 899 chanks in 1984-85. There was decline in chank catches from 1987-88 onwards. In 1996-97 only 798 chanks were caught. The fishermen take out the chank meat for their consumption. The Gujarat Fisheries Development Corporation undertakes marketing of the chanks.

The top shell, *Trochus niloticus* and turbo shell, *Turbo marmoratus* have been fished from Andaman and Nicobar islands (Amrithalingam, 1932; Setna, 1933; Rao, 1939; Appukuttan, 1979; Nayar and Appukuttan, 1983; Krishnamurthy and Soundararajan, 1999). Fishing methods for Trochus and turbo in the Andaman and Nicobar Islands is by diving and a power boat tows the smaller canoes (sampan) to the fishing area and each sampan with 3-4 divers is left in different fishing grounds. Imported or locally made goggles or glass masks are used by divers. Such sharing of resources has been observed along the Tamil Nadu coast in the chank beds known as

'sangunilam'. Fishermen reach the fishing ground in plank boat built boat with 10-15 fishermen in each boat. After which they skin dive (without any external respirator apparatus) and collect the chank from 16-24 m depth. In contrast to this, *Umbonium* sp. And *Oliva* sp. are collected from the sandy shore using scoop net from nearshore areas in the bay of Bengal (Ramdoss, 2003). *Chicoreus ramosus* and *Pleuroploca trapezium* are two important gastropods fished and utilized for shell craft industry along the Indian coast. The fishery information on landing centers in Palk Bay and Gulf of Mannar has been described by several workers (Ayyakkannu, 1992; Patterson and Ayyakkannu, 1992a, 1992b and Patterson *et al*., 1994).

Along Indian east coast at Porto Novo, the whelk, *B. spirata* have been fished using small traps with dried octopus and eel as bait (Ayyakkannu, 1994). Chidambaram (1997) has explained in detail the modified ring net used for fishing the whelk, the CPUE and seasonal variation in landing along Pondicherry coast. Along the west coast with emergence of shrimp trawlers, *B. spirata* and *B. zeylanica* began to be landed as a by-catch in Sakthikulangara and Neendakara (Appukuttan and Philip, 1994). When export market for whelks developed, targeted fishing of whelk by modification of trawlers also began (Philip and Appukuttan, 1997). The modified version of the gear has been described recently by Sabu *et al.* (2005).

1.2.2. Seed production and culture

Several gastropods including the haliotids, trochids, patellids, strombids, muricids and littorids are commercially important, but only a few have been domesticated. Among these, abalones and whelks are by far the most important.

Abalones

Abalones are large herbivorous gastropods with all species (nearly 100) in one genus, *Haliotis* (Hahn, 1989). Abalones are valued for their meat, shell and the lustrous rainbow coloured pearls which they produce. The technique for the hatchery production of abalone seed was developed in Japan in early 1960's and mass production began by 1974 (Kafuku and Ikenoue, 1983). The life history of *Haliotis discus* has been described by Kafuku and Ikenoue (1983). Hahn (1989) has given a

descriptive account of the biology of abalone with emphasis on gonad maturation, artificial induction of spawning, fertilization, larval development, settlement induction, growth and nutrition of abalones. He has detailed the aquaculture techniques and research and technology development on abalone seed production and aquaculture in Japan, California, Korea, France, New Zealand, Australia and Ireland. Based on the seed production techniques developed there are several hatcheries producing seed of commercially important gastropods (Hahn, 1989). Abalones form an important resource of China and Guo *et al*. (1999) have described the hatchery techniques and culture methods followed in China.

During the last two decades considerable work has been done on refinement of techniques of abalone seed production and farming for different locations and species. Much work was done on algal feed used for larval rearing of abalones (review: Jayabhand and Pahavasit, 1996; Shepherd and Steinberg, 1992) and at the same time attempts were made to develop artificial diets for abalone (review: Fleming *et al*., 1996). Stott *et al*. (2004) has also described an alternate culture system without using live feed in abalone culture.

The growth of post larvae and juvenile of abalones (Day and Fleming, 1992; Corazani and Illanes, 1998; review: Kawamura *et al*., 1998), and morphological developments in the larvae related to change in feeding habit from benthic algae to macro algae (Roberts *et al*., 1999; Kawamura *et al*., 2001) growth models and food conversion of cultured juvenile (Greenier and Takekawa, 1992), settlement of abalone larvae (Daume *et al*., 1999, 2000, 2001), nursery culture of abalone (Daume and Ryan, 2004) have been studied for different economically important species of abalone. Along with this the effects of environmental parameters such as temperature and salinity on early development were investigated (Chen and Chen, 1999; 2000; Lu *et al*., 2001, Zheng *et al*., 2000).

Abalone production systems related to water quality, production technology, type and size of cages and culture tanks, quality and quantity of feed and abalone size have been studied (Hooker and Morse, 1985; Fallu, 1991; Touche *et al*., 1993; Mercer *et al*., 1993; Mai *et al*., 1994). Morey (1998) reported on different hydraulic

systems used for the culture of green abalone suggesting that the water supply when entering from the upper part of the tank, was more advantageous than when entering from the bottom or end of the tank. Muller (1999) compared refuge system used on a pilot scale with red abalone. Abalone culture has gained importance in several new regions and accordingly attempts are also made to evaluate growth and survival of juveniles in different systems (Pereira and Rasse, 2007). There has been little published on the comparative culture technologies among different species of abalone probably because of the proprietary nature of abalone culture (Pereira and Rasse, 2007).

Sea ranching /reseeding for stock replenishment is an important part of abalone aquaculture. A large part of the seed produced by abalone hatcheries are sold to fisherman cooperatives at a subsidized rate for planting in open coastal waters. The fishermen cooperatives manage their reseeded areas and harvests the abalone when they reach marketable size (Hahn, 1989).

***Trochus niloticus* (Top shells)**

Top shells of the family *Trochidae* have been the subject of several studies. The tropical top shell *Trochus niloticus* is indigenous only to Indo-Malaysia and Yap and Palau in Micronesia but has been introduced in the tropical Pacific as far east as the Tuomotus of French Polynesia (Heslinga, 1981). *T. niloticus* is the most economically important gastropod in the tropical Pacific. The reproductive cycle of *T. niloticus* in King Solomon waters of Australia has been described by Gimin and Lee (1997). Attempts to produce viable *T. niloticus* larvae by fertilization of excised gametes (Rao, 1937), injection of KCl or exposure to H_2O_2 have been largely unsuccessful (Heslinga, 1981). Under appropriate laboratory conditions, however *T. niloticus* spawn on a predicable monthly schedule (Heslinga, 1981). The method for hatchery seed production of the top shell have been described by Heslinga and Hillman (1981) from Caroline islands and Bech (1997) from Thailand while Hahn (1989) gave a general account on the culture of top shell. Recently Lee and Amos (1997) reviewed status of top shell hatcheries in Australia, Indonesia and the Pacific. The growth of this gastropod in captivity and culture has been studied in Indonesia (Latama, 1999) and in Australia (Lee, 1997). In India, *T. niloticus* is found only in Andaman and Nicobar group of islands. Information on the gonad development,

spawning and sex ratio of this species in Andaman and Nicobar islands is available from the works of Amrithalingam (1932) and Rao (1936; 1937 and 1939). Nayar and Appukuttan (1983) and Krishnamurthy and Soundararajan (1999) have reported on successful spawning of *T. niloticus* collected from Indian waters.

Strombus gigas **(Queen conch)**

Queen conchs have been utilized throughout its range for aquaculture since the 1960's, and with the closure or heavy regulation of conch fisheries they now serve as the main source for enhancing stock populations (Shawl, 2004). Today much of the work produced on queen conchs is related to aquaculture, since the decline of conch populations has caused researchers to turn to hatcheries for individuals (Stoner, 1997). The queen conch has been farmed on a large scale in Turks and Caicos Islands since 1984 (Davis *et al.*, 1992). The culture of the queen conch, *Strombus gigas* has been tried in the Carribbean (Hahn, 1989). Queen conch is distinguished from other species by its large size, lack of pronounced spiral groove and pink coloured aperture. The spawning and development of this species has been reported (Randall, 1964; D'Asaro, 1966). The various aspects related to the larval development, metamorphosis and culture methods have been described by Hahn (1989); Sterrer (1992); Shawl (2004). Since the density of queen conch has decreased considerably in several regions due to over exploitation, reseeding has been tried and mariculture has been suggested as a method of increasing the population. (Hahn, 1989).

Turbo marmoratus **(Green snail)**

Green snail (*Turbo marmoratus* L.), locally known as Batulaga, Matabulan or Burgos is the biggest marine gastropod species of the genus *Turbo*, family Turbinidae (Gastropoda: Archaeogastropoda: Trochidea) (Eisenberg 1981; Abbott and Dance 1986; Wilson 1993). It is popularly known as green snail and found in several parts of Asia. Murakoshi *et al*. (1993) from Okinava and Dwiono *et al.* (2001) from Indonesia have reported on the broodstock maintenance, spawning and seed production. Attempts at reseeding or sea ranching using hatchery reared juvenile was tried in Palau, Vanuatu, New Caledonia and Pohnpei (Amos, 1997). However survival rates were discouraging. Nash (1993) and Yamaguchi (1988a;

1988b) have the opinion that much of the basic information required to judge effectiveness of reseeding is lacking.

***Chicoreus ramosus* (Muricid snail)**

Chicoreus ramosus, a muricid snail, which is widely distributed in Indo-Pacific was spawned and larvae reared successfully in Thailand (Bech, 1992.). Middlefart (1996) has described the egg capsules and early larval development of ten muricid gastropods from Thai waters. Morphology of the egg capsules collected from different natural beds has been described (Ruangchoy and Tantichodok, 1992). Nugranad *et al.* (1994) have given a detailed account of the techniques used for hatchery production of seed of *C. ramosus*. Within two years, through the hatchery produced seed, the F1 generation was raised (Nugranad and Promchinda, 1995). The second generation snails (F2) attained early sexual maturity and spawned when one year old (Traithong *et al.*, 1997). Sea ranching of the hatchery produced seed was tried by Bech (1994) and concluded that restocking of *C. ramosus* on artificial reefs is promising to establish self sustaining populations.

Whelks

The spotted babylon, *B. areolata* is an economically important gastropod in Thailand. Because of the depletion of the natural stocks concerted efforts were made to develop technology for its seed production and farming (Singhagraiwan,1996; Chaitanawisuthi and Kritsanapuntu, 1997a, 1997b; 1998, 1999a; 1999b; 1999c; Chaitanawisuthi *et al*., 2001a, 2001b).The spawning characteristics of the whelk have been studied (Poomtong and Nhongmeesub, 1996; Hua *et al*., 2001) and economic evaluation of monoculture and polyculture of *B. areolata* to marketable size using large-scale operation have been tried (Kritsanapuntu, *et al*., 2006b). Growth trails for polyculture of hatchery reared spotted babylon, *B. aerolata* and sea bass has been tried (Chaitanawisuti *et al.*, 2001a). In Japan, Kajikawa (1978) has reported on the artificial reproduction of the Japanese Ivory shell *Babylonia japonica* using irradiated sea water with ultraviolet ray. *B. formosae habei* is found in Taiwan and China and same aspects like reproductive behaviour (Ke and Li, 1991, 1992, 1993), copulation and egg laying (Chiu and Liu, 1994) and effects of three microalgae on survival, growth and metamorphosis of the larvae (Zheng *et al*., 2001)

and the effects of food availability on feeding and growth of juvenile (Chen *et al.*, 2005) have been studied.. Apart from the Babylon whelks few other gastropods have also been considered commercially important. The common whelk *Buccinum undatum* is the largest edible marine gastropod in North Atlantic waters reaching a maximum shell height of 150 mm (Hancock, 1967). This has been considered as a species suitable for aquaculture and laboratory trials have been conducted in the rearing of this species (Nasution and Roberts, 2004). The knobbed whelk *Busycon carica* is a prosobranch gastropod of family Melongenidae of economic importance and Power *et al.* (2002) have studied the development of this species in the egg capsule and reared the larvae to juveniles.

Concholepas concholepas is an economically important gastropod and it took several years and much effort until the first successful larval rearing, metamorphosis and settlement of larvae of this species could be reported (Di Salvo, 1988; Di Salvo and Carriker, 1994). However large scale farming attempts for this species are yet to be developed.

In India, the laboratory rearing and larval development of the spiral Babylon, *Babylonia spirata* has been described by Shanmugaraj *et al*. (1994) and the salinity tolerance of this species has been studied by Patterson *et al*. (1994). Sreejaya *et al*. (2004) have described the spawning, and larval development of *B. spirata.* The sacred chank, *Xancus pyrum* is an important gastropod of India and culture of this gastropod resource from egg capsule and growth of juvenile chank has been reported by Lipton and Selvakku (2000). The hatchery technique for seed production of this gastropod is yet to be developed. The spawning and larval development of *Rapana rapiformis* collected from the Indian east coast has been documented (Ramesh, 1999). *R. rapiformis* is the only available species of the family Rapanidae in Indian waters and its meat is consumed in certain parts of the country (Rajkumar, 1995; Ramesh, 1999). The spawning, larval development and settlement of this species have been described by Ramesh (1999). The larvae of *R. rapiformis* metamorphoses after 23-24 days.

For the development of a hatchery technique for a particular resource, it is pertinent that the optimal rearing environment which closely resembles its natural habitat is provided at all stages of rearing. The rearing systems should not induce stress in the brooders/ larval stages. A careful study of its behaviour and preferred requirements is essential for standardizing of the rearing protocols. Hence the important literature pertaining to the different sections of hatchery technology development for gastropods is given below.

1.2.3. Transportation of the molluscs

One of the primary steps in a hatchery development is transportation of broodstock. Mass transport of live broodstock and seed are considered a necessity for marine mollusc hatcheries since natural beds and culture areas are often located at distant sites far away from hatcheries (Poomtong *et al.,* 2000). Transportation experiments have been done in *Tridacna squamosa* and *Haliotis asinine* (Poomtong *et al*., 2000). Seed production techniques for bivalve molluscs are much more advanced and there are commercial hatcheries for oysters, clams, scallops and pearl oysters (Narasimham, 2005). Methods for treating and preserving bivalve molluscs in the live state have been patented (http/:www freepatentsonline.com). In the recent years live transportation of molluscs including whelks has become very popular and related to this several studies have been conducted (Baldwin *et al.,* 1992; Carefoot *et al.*, 1993). Chemical changes have been associated with stressed metabolic state of muscle tissue (Ivanovici, 1981). Ryder *et al.* (1994) have inferred that stress and probably survival time is related to animal size for *Haliotis iris* and *H. australis* such that larger animals are less stressed and should live longer out of water, although a recovery immersion period may be a useful management strategy. High value crustaceans especially lobster and crabs are transported live and to check the quality of the meat and survival of these resources studies have been conducted (McLeese, 1965; Winkler, 1987; Spicer *et al*., 1990; Whiteley and Taylor, 1992; Evans *et al.*, 1999). Marine finfishes are also packed live both for aquarium trade and also for other commercial markets. The trade of such live fishes is so developed that modified sealed containers are now available for transportation which preserve the

quality of the fish as well as gives high survival rates (http//:www freepatentsonline.com)

1.2.4. Food and feeding

Broodstock maintenance including their rearing medium, feed and feeding protocol have always received special attention in hatcheries (Hahn, 1989). In natural habitats gastropod molluscs, hunt for prey and are known to have an array of sensors and behavioural responses (Ferner and Weissburg, 2005). Gastropod chemoreception has been a productive area of research for more than half of a century (reviewed by Kohn, 1961; Mackie and Grant, 1974; Kats and Dill, 1998). This rich research lineage has broadened our understanding of the mechanisms and importance of chemosensation by gastropods, and the chemical identity of feeding stimuli has been a common focus of investigation (Sakata, 1989) leading to detailed studies of physiological responses (Elliot and Susswein, 2002). Many prosobranch gastropods are highly prey specific, others are less conservative and same may feed on animal carrion (Morton, 1990). Gastropods of the family Buccinidae are characteristic carnivores (Taylor *et al.,* 1980; Shimek, 1984; Himmelman and Hamel, 1993) and opportunistic scavengers attracted by chemical stimuli emanating from suitable food (Morton, 1990). Buccinids acquire their food by using an eversible proboscis at the end of which, the radula, a flexible chitinous ribbon bearing transverse rows of teeth is located (Hughes, 1986). The food preference, consumption and feeding behaviour of the scavenging gastropod *Babylonia spirata* has been studied by Raghunathan *et al.,* (1994) and Patterson *et al*. (1995). Feeding has also been related to spawning. A reduction in feeding with the onset of breeding has been observed for gastropod *B. undatum* (Martel *et al.*, 1986b; Stickle, 1973), *Nucella lapillus* (Feare, 1970).

1.2.5. Environmental factors for spawning process

Water quality is of prime importance in hatcheries. Among the abiotic factors, salinity, temperature, photoperiod, ammonia and dissolved oxygen are considered to be vital. The broodstock maintenance protocol of gastropod hatcheries is based on the preferred salinities of each species. For rearing the larvae and the juveniles the preferred salinity of the resource is provided to reduce mortality, increase growth and

production. Salinity is one of the most important environmental factors, which is relatively constant in the open sea, and varying considerably in the intertidal zones, estuaries and other biotopes. The living resources also adapt to survive such variations. The mechanism of salinity adaptation in marine molluscs has been reviewed by Berger and Kharazova (1997). The ability to exist at varying salinity, euryhalinity, depends on different adaptations. In addition to burrowing into bottom sediments, actively choosing the environment and escaping from unfavourable condition, marine molluscs may close their shells at abnormal salinity. Such reactions are due to the activity of peripheral detection located on head tentacles, mantle ridges and siphon surface (Freeman and Rigler, 1957; Vasilieva *et al.*, 1960; Davenport 1979, 1981).

In natural environment rapid salinity variation induces several molluscs to spawn. Gametogenetic activity of Haliotids is believed to be controlled by long-term endogenous rhythm with an annual lunar phase and an additional *zeitgeber* (time giver) from exogenous environment (Hahn 1989). It is believed that temperature, water quality, photoperiod, tides, exposure to air temperature, salinity, food supply and nutrition are factors that affect gonad development and breeding cycle of gastropod (Kinne, 1970; Webber, 1977; Tutschulte and Connell, 1981; Runham 1988; Hahn, 1989). Apart from acting as a stimulus for spawning, changes in temperature may also play an important role in the gonad maturation as well as regulating reproduction cycles (Uki and Kikuchi, 1975, Hahn, 1989; Well and Keesing, 1989; Kabir, 2001). Successful broodstock conditioning to produce mature animals on a preditable basis is essential for the success of hatcheries.

Studies have shown that photoperiod plays an important role in regulating the reproductive cycle of gastropods. For example, reproductive cycle of the pulmonate snail *Lymnea stagnalis* (Bohlken and Joosse, 1982; Dogterom *et al.*, 1983; Bohlken *et al*., 1987), *L. peregr*a (Lundelius and Freeman, 1986), the nudibranch *Aplysia california* (Wayne and Block, 1992) are mediated by photoperiod. Lundelius and Freeman (1986) confirmed that a photoperiod signal is received by a photoreceptor located in the central ganglia. The signal then activates the neurosecretory cells within the cerebral ganglia to release hormones that stimulate the development of the

reproductive organ. For the maturation of red abalone, Freeman *et al*. (2006) has found that temperature, vigorous water motion and adequate food supply are more important than photoperiodicity.

1.2.6. Spawning behaviour and egg capsules

An attempt to review reproduction in prosobranchs and opisthobranchs by describing their egg masses has been made by Soliman (1987). Soliman (1991) has made a comprehensive review of the spawning, development and metamorphosis of the prosobranch and opisthobranch gastropods from the north eastern Red Sea. For almost every species, data on the breeding season, size and number of eggs laid and period and types of development have been detailed. The early embryology, larval structure and behaviour are also summarized.

Several gastropods exhibit characteristic spawning behaviour (Castilla and Cancino, 1976). Behaviour plays a major role in the reproduction of marine gastropods, particularly for species which copulate and then deposit egg capsules. For the common whelk, *Buccinum undatum* and most neo and mesogastropods the production and storage of gametes, copulation, storage of sperm by the female, fertilization and production and deposition of the egg capsules are all integral steps for reproduction (Martel *et al*., 1986a). Within the family Muricidae spawning has been observed in several species, such as *Nucella lapillus* (Pelseener, 1935); *Urosalpinx cinerea* (Hancock 1957), *Ocenebra erinacea* (Lebour, 1936; Hancock, 1957); *N. calcar* (Gallardo, 1973); *Trophon spp*. (Lebour, 1937: Thorson, 1940) and *M. virgineus var.ponderosa* (Natarajan, 1957).

Gallardo (1973) have summarized the literature on the number of eggs and larvae in the family Muricidae. In neogastropods the female store the sperm in the pallial oviduct after copulation (Fretter, 1941). This complex organ has been the subject of many studies (Johansson, 1953, 1957; Fretter and Graham, 1962; Houston, 1971) and Fretter (1941) was the first to describe clearly its compartments and their function for the genera *Nucella, Ocenebra, Nassarius* and *Buccinum.* Attraction of males to female during copulation period has been reported for *B. undatum*. This has also been observed for other neogastropods (Magalhaes, 1948; Pearce and Thorson, 1967;

Edwards, 1968; D'Asaro, 1970a). This aggregatory behaviour may be provoked by pheromones released by gravid females. Multiple copulations have been observed for *B. undatum*. Martel *et al.* (1986a) and for other gastropods for example, *Kelletia kelletia* (Rosenthal, 1970); *Urosalpinx cinerea* (Hargis and MacKenzie, 1961); *Cepaea nemoralis* (Murray, 1964), *Neptunea antiqua* (Pearce and Thorson, 1967) and *Eupleura caudata* (Hargis and MacKenzie, 1961). *Concholepas concholepas* is a dioecious species whose mating behaviour has been described by Castilla (1974). The common spider conch *Lambis lambis* of the family strombidae is abundant in the shallow waters of the Indo-Pacific. The spawning and development of this species is described by Hamel and Mercier (2006b). They have also described the copulation and spawning of the common egg shell *Ovula ovum* in the Marshall islands. The spawning of the purple snail *Plicopurpura pansa* has been described by Naegel (2004).

Primitively, gastropod eggs were laid singly, uncovered and they were externally fertilized (Soliman, 1991). However it has been observed that many gastropod species package their fertilized eggs in jelly masses, sand collars or complex semi rigid egg capsules (reviewed by Thorson, 1950; Beeman, 1977; Berry, 1977; Webber, 1977; Pechenik, 1979; Hodgson, 1999; Klussmann-Kolb and Wagle, 2001). The functional properties and adaptive value of such encapsulating materials are generally uncertain particularly for species in which individuals escape as long- lived swimming veliger larvae (Pechenik, 1979, 1986). However it is believed that benthic egg masses provide protection to the developing embryos from environmental stresses and predation (Thorson, 1950; Pechenik, 1979; Strathmann 1985). Gastropod intracapsular embryonic development and mortality may be influenced by temperature, salinity, ultraviolet radiation (UVR), oxygen availability, water flow, fouling, embryonic position, predation and parental history (Prezeslawski, 2004). Prezeslawski (2004) has reviewed the effects of environmental stress on embryonic development within intertidal gastropod egg masses.

Neogastropod egg capsules are produced in an amazing variety of shapes and sizes, ranging from flat hemispherical disks to tall erect vases, and spanning a size range from millimetrs to many centimetres in length (Bandel, 1973; D'Asaro, 1991, 1993,

1997). Despite the varied form of these capsular cases, almost all neogastropod capsules contain a central fluid filled chamber that houses embryos for some portion of their development (Rawlings, 1990, 1999; Yaroslavtseva and Sergeyeva, 2002). The features of the neogastropod egg capsules have been studied in detail by several authors (Pechenik, 1975; Rivest, 1983; D'Asaro, 1997). It is believed that the distinctive shape of neogastropod capsules derives from an unusual moulding process that occurs within a ventral pedal gland in the base of the females foot. Details regarding the various processes helping the capsule formation, molding in the final form and hardening it (sclerotization) has also been studied (Price and Hunt, 1973, 1974, 1976). The capsule case has been found to be composed of proteins and carbohydrates (Bayne, 1968; Sullivan and Maugel, 1984; Colman and Tyler, 1988; Hawkins and Hutchinson, 1988; Miloslavich, 1996b). Evidence for lipid in capsules of same species has also been reported (Miloslavich, 1996a).

Based on the investigation carried out in the Gulf of Mannar and Palk Bay Natarajan (1957) reported on the egg masses and larval development of 32 species of prosobranchs. This study provides first time reports on the breeding biology of *Cerithium morus*, *Cerithium spp*, *Murex virgineus var ponderosa*, *Murex trapa*, *Thais tissoti*, *Thais spp*., *Pyrene zebra*, *P. versicolor*, *Pyrene spp*, *Nassa jacksoniana*, *N. costata*, *N. thersites*, *Ancilla spp* and genus *Araseosus*. He has briefly reported the earlier work done on the breeding biology of gastropods in other geographic locations.

The number of eggs laid by a gastropod is inversely proportional to egg size (Soliman, 1991). According to the available data, the maximal size attained in prosobranchs markedly exceeds that in opisthobranchs (Natarajan, 1957: Eisawy and Sorial, 1968: Gohar and Eisawy, 1967a: Thompson, 1967). In many prosobranchs (principally neogastropods) the majority of eggs laid act as nurse eggs subserving as food for the very small fraction of viable eggs which proceed to full development (Gohar and Eisawy, 1967). According to Thorson (1940), 50,000 to 100,000 nurse eggs may exist per embryo in *Volutopsius norwegicus*. Nurse eggs are not reported to exist in opisthobranchs.

In *B. undatum* capsules size was strongly correlated with female size (Valentinsson, 2002). For prosobranch gastropods the general trend is that the number of eggs increases with female size and that egg size is dependent of female size (Spight *et al.*, 1974; Spight and Emlen, 1976; Miloslavich and Defresne, 1994). However the production of more eggs in large females does not necessarily result in greater number of hatchlings in instances where there are nurse eggs (Valentinsson, 2002). The egg size and number variations related to maternal size and age in was studied in Opishobranchiata, *Haloa japonica* by Ito (1997). Numerous studies of gastropods also report that larger females produce larger clutches; *Buccinum cyaneum* (Miloslavich and Dufresne, 1994). *Thais lamellose* and *T.emarginata* (Spight and Emlen, 1976), *Fissurella barbadiensis* (Hughes, 1971) and *Crepidula dilatata* (Chaparro *et al*., 1999).

1.2.7. Metamorphosis and development

The mechanism through which such stimuli mediate metamorphosis is incompletely understood. The process has been studied by testing the effects of elevated ion concentrations (Yool *et al.*, 1986; Pechenik and Gee, 1993; Pechenik *et al.*, 1995, Wendt and Woollacott, 1995; Woollacott and Hadfield, 1996; Bryan and Qian, 1998), neuroactive compounds (Coon *et al*., 1985; Carpizo-Ituarte and Hadfield, 1998; Couper and Leise, 1996; Froggett and Leise, 1999) and pharmacological agents (Baxter and Morse,1987; Clare *et al.*, 1995; Pechenik and Qian, 1998; Holm *et al.*, 1998; Yamamoto *et al.*, 1998; Biggers and Laufer, 1999; Pires *et al.*, 2000a, 2000b) and by documenting the development of specific nervous pathways (Kempf *et al*., 1997). The signal transduction pathways involved in metamorphosis have been difficult to dissect due to the small size of the larvae and uncertainties associated with interpreting the results of the whole-organisms experiments (Pawlik, 1990). The normal metamorphosis of the gastropod larvae may involve one or more inhibitory pathways as suggested by Pechenik and Qian (1998). Experimental evidence suggests that the larvae of the gastropod *Ilyanassa obsolete* metamorphosize when they stop producing an inhibitor, nitric oxide (NO) and when existing NO is sufficiently removed from circulation or degraded (Froggett and Leise, 1999).

Based on the studies of Thorson (1946, 1950), Mileikovsky (1971) and Todd (1981) and Soliman (1991) the main types of developmental patterns among gastropods are (i) planktotrophic development with typical veliger larvae feeding during their short or long pelagic existence; (ii) lecithotropic development which may be pelagic or non- pelagic; and (iii) direct or capsular development . Each development type is correlated with a specific egg size range (Soliman, 1991). Soliman (1991) inferred that the egg diameter range for the three types was 60-80 µm, 140- 440 µm and 300-330 µm respectively. The same author has inferred in the review that the major factors affecting metamorphosis are, in chronological sequences: food condition, acquiring competence for metamorphosis and suitable substrata for settlement and metamorphosis.

Developmental rate is influenced by temperature and salinity in marine organisms (Fretter and Graham, 1962; MacInnes and Calabrese, 1979; Lucas and Costlow, 1979; Johns, 1981a, 1981b, 1982; Tettelbach and Rohdes, 1981; Pechenik, 1982, 1983; Laughlin, 1983; Roller and Stickle, 1985, 1989; Thiyagarajan,2003; Desai, 2006). It has been observed that larvae raised in low salinity exhibit slow growth rates, increased mortality, and a possible delay of metamorphosis (Bivalves: Gunter, 1955; Gastropods: Scheltema, 1965, Rosenberg and Rosenberg, 1973; Zimmerman and Pechenik, 1991; Crustaceans: Costlow *et al.*, 1971). Larvae raised under condition of low temperature or insufficient food show similar effects; the larvae take longer time to develop and frequently delay metamorphosis in stressful physical conditions (Bivalves: Lucas and Costlow, 1979; Lima and Pechenik, 1985; gastropods: Scheltema, 1962, 1967, Polychaete: Qian *et al.*, 1990; multiple taxa: Pechenik, 1987,1990).

It has been observed that in gastropods with direct development, embryos use the yolk to fuel development (Miloslavich, 1996b) and additional nutrition may be obtained from albumin fluid within the egg capsule (Stockmann-Bosbach and Althoff, 1999; Penchaszadeh and Rincon, 1996). Embryonic development of buccinids have been studied in the red whelk *Neptunea antiqua* (Pearce and Thorson, 1967), *Searlesia dira* (Rivest, 1983), *Colus stimpsoni* (West, 1973), *B. cyaneum* (Miloslavich and Dufresne, 1994) and *B. undatum* (Naustion, 2003).

Developmental biology of opisthobranchs, especially cell–lineage and cleavage, organization of larvae during development, timing of development and biological data etc., have been studied by several workers and reviewed by Dohmen (1992). Embryogenesis and morphogenesis of the larva have been studied by Thompson (1958) and older larval stages and hatchlings are described by Chia and Koss (1978); Bickell and Chia (1979) and Bickell and Kempf (1983). Little information is available on development of larval organs of shelled opisthobranch such as Bullomorpha (Smith, 1967). Detailed review of several aspects of embryonic and larval development of the genus *Haminaea* and other cephalaspideans has been given by Schaefer (1997).

Torsion is an important part of gastropod development. Yonge (1947) brought together for the first time the numerous theories concerning torsion. This subject has since been reviewed extensively (Eales, 1949: Crofts, 1955: Morton, 1958: Ghiselin, 1966). The majority of theories on torsion fall into two categories, those which attempt to explain the evolution of the process of torsion as an adaptation to the advantage of the larvae, and those which consider it to be a necessary adaptation for the adult (Underwood, 1972). Underwood (1972) has made a reappraisal of torsion in gastropods. He has proposed that the two components of torsion may have evolved independently. The first relatively rapid component of torsion, resulting in 90^0 of displacement of the mantle cavity in relation to visceral mass, may be of advantage to the swimming larvae. The final slower component of the newly metamorphosed benthic snail, and is the only component of torsion found in those gastropods which have no free swimming larvae.

1.2.8. Substratum preferences

The substratum is a significant ecological factor which influences the distribution of organisms (Clampitt, 1970, 1973; Harman, 1972). The substratum provides attachment, shelter and nourishment that are the fundamental needs of the organism (Nair and Thampy, 1980). Being benthic, several bivalves and gastropods are known to have a preference for particular type of substratum. Spawning areas are also specific for many neogastropods. Many species exhibit remarkable specificity for

certain spawning substrate or locations within intertidal or shallow subtidal marine environments (Spight, 1974; Brenchley, 1981). Individuals of the muricid gastropod, *Nucella lamellosae*, for example, continue to spawn in the same spawning location year after year (Spight, 1974). Behavioural responses are also known to vary based on the types of the substratum. This ranges from firm attachment to burrowing. Substratum preference of marine prosobranch gastropods, *Chicoreus ramosus, Fasciolaria trapezium, Murex tribulis* and *C. virgineus* has been studied by Patterson and Ayyakkannu (1992). Rao and Sukumar (1981) studied the response of a tropical gastropod *Cerithidea cingulata* to different types of substrata. One of the most important aspects of substratum preference by gastropod is for laying egg capsules (Barnett *et al.*, 1980). Substratum as a factor in the distribution of two prosobranch freshwater snails was studied by Vaidya (1979). Substrate is important in abalone hatcheries and for the settlement of abalone larvae, specialized substrates are used. The substrata used are translucent corrugated, hard plastic sheets and these are called “wavy plates” (Hahn, 1989). The plates develop algae and this act as feed for the settling larvae.

Many benthic invertebrate species, have planktonic larvae in their life histories (Thorson, 1950). Eventually, the larvae reach a point in development at which metamorphosis to the benthos becomes possible, and then they begin assessing the suitability of substrata for settlement and metamorphosis (Wilson, 1958; Crisp, 1974). Termination of larval life involves two steps, settlement, which is reversible, and metamorphosis, which is an irreversible morphological alteration to adult form (Crisp, 1974). For gastropod veligers, metamorphosis is defined as the loss of the larval swimming organ, the velum. Loss of the velar lobes is the first gross morphological indication that the transition from larval to adult form is taking place (Bonar and Hadfield, 1974). If the gastropod larvae fail to encounter a suitable substratum, they may enter a “delay period” in which metamorphosis is postponed and additional substrata may be examined (Scheltema, 1961; Kriegstein *et al.*, 1974; Hadfield, 1978; Switzer-Dunlap and Hadfield, 1977). The ability of the larvae to delay metamorphosis is not restricted to gastropods but has been reported in many other groups of marine invertebrates (Wilson, 1948; Ryland, 1959; Hinegardner, 1969; Chia and Spaulding, 1972; Lewis, 1978). Pechenik (1987) has stated that

duration of the plankton period of benthic marine invertebrate is a function of the developmental rate which is influenced by such environmental factors as temperature, salinity, dissolved oxygen concentration, pH, pollutants, availability of food and suitable substrates.

Discrimination among substrate is well documented in marine invertebrate larvae (reviewed by Crisp, 1974; Pawlik, 1992; Rodriguez *et al.,* 1993). Some studies have documented settlement preference in multiple choice experiments (Kirchman *et al.*, 1982; Miron *et al.,* 1996; Stoner *et al.*, 1996, Wieczorek and Todd, 1997; Daume *et al.,* 1999). Substrate choices made by marine larvae settling in still water and in flume flow was studied by Butman *et al*. (1988). Considerable work has been done on the settlement of the abalone. Coralline algae are among the most effective larval settlement cues for all species of abalone tested to date (Roberts, 2001a). Other natural surface such as biofilms can induce larval metamorphosis but few approach the speed and consistency of crustose coralline algae (CCA) (Roberts, 2001a, 2000b).

1.2.9. Larval feeding

Gastropod larvae generally spend two to six weeks during development as in the plankton (Thorson, 1946) during which time the veligers utilize phytoplankton as food. The mechanism of food collection has been summarized for several species of gastropods (Fretter, 1967, Fretter and Montgomery, 1968;). Larval feeding can affect the development, condition, dispersal and survival of successive stages in marine invertebrate life cycles (Basch, 1996). Descriptive studies of larval feeding and development in several invertebrates date back over a century (Metschnikoff, 1870; reviews: Thorson, 1946; Young, 1990). Attempts have also been made to experimentally quantify feeding requirements for larval de crustose coralline algae velopment through metamorphosis (reviews: Loosanoff and Davis, 1963; Kinne, 1977; Strathmann, 1987; Olson and Olson, 1989; Young, 1990).

In the hatchery technology development it is essential that the larvae receive optimum feed. Low or high feeding protocol may affect the larval development. Larval responses to food limitation or starvation have been studied for many years,

particularly for crustaceans, molluscs, polychaetes and echinoderms (Perron and Turner,1977; Dawirs, 1984; Anger, 1986; Staton and Sulkin, 1991; Wehrtmann, 1991; Hansen,1993; Qian and Chia, 1993; Allison, 1994; Anger, 1995a, b; Schuh and Diesel, 1995; McEdward and Qian, 2001; Giménez,, 2002; reviewed by Thorson, 1950; Olson and Olson, 1989; Morgan, 1995). In response to low food supply larvae of at least some species show reduced rates of growth and development (Perron and Turner, 1977; Qian and Chia, 1991, 1993; Wehrtmann, 1991; His and Seaman, 1992; Hansen, 1993; Allison, 1994; Anger, 1995a,b; Eckert, 1995; Basch, 1996; Pechenik *et al*., 1996a; Qiu and Qian, 1997a, b; Giménez, 2002), decreased metamorphic success (Qian and Chia, 1993; Eckert, 1995; McEdward and Qian, 2001) and smaller size or low energy content at metamorphosis (Qian and Chia,1993; Hart and Strathmann, 1994; Eckert,1995; Meidel *et al*., 1999; Miller and Emlet, 1999; McEdward and Qian, 2001; Vaitilingon *et al.*, 2001).

Once the larvae settle as juveniles, most often their feed preferences changes. According to these changes the culture protocols and the production systems are modified. Several studies have been conducted to improve the growth rates and production per unit area of commercially important gastropods like the abalone (Hahn, 1989; Pereira and Rasse, 2007), whelks (Chaitanawisuti and Kritsanapuntu, 1999b, 2006; Harding and Mann, 2001); trochids (Heslinga, 1981; Heslinga and Hillman, 1981) and queen conches. Based on the research and development in hatchery for gastropod production system, farming and reseeding attempts have also intensified and this is evident in the increased global gastropod production (Gosselin and Chia, 2004).

1.2.10. Conclusion

Considerable work has been done on the spotted babylon (*Babylonia areolata*) inhabiting the coastal waters of Thailand, especially on the effect of stocking density, growth and substratum preferences. (Chaithanawisuti and Kritsanapuntu, 1997a), juvenile rearing (Chaithanawisuti and Kritsanapuntu, 1997b) and nursery culture methods (Chaithanawisuti and Kritsanapuntu, 1998). This was further extended to development of grow out methods in flow through systems (Chaithanawisuti and

Kritsanapuntu, 1999a; 1999b, 2001a; Chaithanawisuti *et al*., 2001b, 2002). Studies related to reproduction and development of gastropods in India are scanty. The pioneering attempts were made by Natarajan (1958) who described the egg masses and development of prososbranchs from the Gulf of Mannar.

In India possibilities of destruction of *Babylonia* egg masses and juvenile whelk due to intensive fishing has been indicated by Appukuttan (1996). Hatchery production and sea ranching of the seed has been identified as a conservative measure to such population damages by Shanmugaraj *et al.,* (1994), which justifies the need to develop a hatchery technology for the production of the whelk. Though studies on the larval development of *B. spirata* were made from the east coast, there is no information on larval development and rearing from southwest coast. So attempts were made to understand the spawning and larval development for the seed production of *Babylonia spirata* to provide a baseline data to standardise the culture of the species. For the optimization of the conditions for healthy larval growth, it is necessary to know the various factors which influence larval life stages in controlled hatchery conditions thus helping to influence the growth, survival and yield of the larval stock. The present study entitled "Studies on spawning and larval development of the whelk, *Babylonia spirata* (Linnaeus, 1758) (Neogastropoda:Buccinidae)" was designed to investigate in detail the various aspects of biology, such as broodstock maintenance, spawning, larval development , rearing and settlement of the juvenile which would form a basic platform to develop the hatchery technology of the whelk for a commercial level seed production.

Chapter 2

Broodstock maintenance and rearing

2. BROODSTOCK MAINTENANCE AND REARING

2.1 MATERIALS AND METHODS

The broodstock of *B. spirata* collected from trawl catches of Neendakara fishing harbour (Lat 08^0 56'N and Long 76^0 32'E) along the Kerala coast were transported to the molluscan hatchery of Central Marine Fisheries Research Institute. The measurements of the whelks were taken using the digital vernier calipers of 0.1 mm accuracy. The measurements used were total shell length (SL) from apex to the tip of the columella, shell width (SW) the width of the body whorl. The total weight (TW) was weighed as the total whole animal wet weight with an electronic balance of 0.01 g accuracy.

2.1.1. Standardization of transportation method of broodstock of *B. spirata*

Brooders of *B. spirata* with average shell length (SL) 41.3 ± 2.1 mm, shell width (SW) 28.5 ± 0.7 mm and total weight (TW) 18 ± 2.5 g were used for the experiment and these were exposed to different packing conditions. The treatments were *viz*, (i) wet packing (WP), (ii) moist packing (MP) and (iii) dry packing (DP). The different types of packing methods tried during transportation experiment are given in the Table 2.1.

For each treatment 75 whelks (n= 15 nos x 5 sampling) were used. At five hour interval such as 5, 10, 15, 20 and 25 h one group from each treatment was released into separate plastic troughs of 30 l well aerated water of ambient salinity 32 ppt. The activity and mortality of whelks were recorded for each treatment at 5 h interval during the following 50 h.

TREATMENT 1. Transportation in water (TRT- WP)

In this experiment one treatment was transportation in water with aeration (TRT-WP- 1) and the other without aeration (TRT- WP-2). Each experiment was done in 5

l of water with 15 animals. The salinity of the water used was 32 ppt, temp 28^0C and pH 8.1 ± 0.2. The amount of dissolved oxygen (DO) in these treatments was analyzed in the beginning and at the conclusion of the experimental period. The ammonia in the water was estimated based on indophenol blue reaction of phenol hypochlorite method (Solorzano, 1969).

Table- 2.1. Different packing conditions of brooder *B. spirata* during transportation.

Sl. No	Treatment Packing condition	Treatment code	Mode of packing
1	Wet packing (WP)	TRT- WP-1	Completely immersed in water, stocking density 3 whelks l^{-1}, with aeration provided.
	Wet packing (WP)	TRT- WP-2	Completely immersed in water, stocking density 3 whelk l^{-1}, no aeration.
2	Moist packing (MP)	TRT- MP-1	No water, covered with moist jute bag, exposed to ambient temperature. Stocking density 1 whelk/10cm^2.
	Moist packing (MP)	TRT- MP-2	No water, covered with moist jute bag, exposed to low temperature (controlled) Stocking density 1 whelk /10 cm^2.
3	Dry packing (DP)	TRT- DP-1	No water, whelks placed in a plastic trough and exposed to ambient temperature without moist covering.

TREATMENT 2. Transportation under moist packing in jute bag (TRT- MP)

Moist packing and transportation of the whelks was done in two different conditions, one in ambient atmospheric temperature (TRT- MP-1) and the other in reduced temperature of 24^0C (TRT- MP-2). Low temperature condition was created by packing ice in insulated ice boxes. Temperature was recorded with a thermometer having the range of 0 -50^0C. Jute bags used for keeping the whelks moist were thoroughly washed with seawater to remove dirt and odour and soaked in seawater of 32 ppt for 1h prior to the initiation of the experiment. Each experimental group contained 15 whelks and those used for (TRT- MP-2) were kept in an ice box at

24^0C. These were wrapped in the pre- treated jute bag. Releasing of the whelks in the water at 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} hourly interval was same as described in 2.1.1. and after every 5 h interval activity and mortality were recorded.

TREATMENT 3. Transportation in dry method (TRT- DP)

Dry packing and transportation was carried out by keeping the whelks in a plastic container without water and moist conditions. Each experiment was conducted in triplicate and contained 15 whelks. The troughs were covered with wide- meshed net to prevent the escape of the animals. These experimental containers were kept in shaded places to avoid desiccation and drying of whelks. The treatment period and observations made were same as described earlier.

2.1.2. Determination of substratum preference of adult *B. spirata*

B. spirata of average SL 42.0 ± 2.0 mm, SW 27.7 ± 1.4 mm and TW 18.23 ± 1.6 g were acclimatized to the laboratory conditions for three days prior to the initiation of the experiments. The response of the whelks to four types of substrata *viz*, gravel (1 – 2 mm), coarse sand (1.00 – 0.50 mm), fine sand (0.25 – 0.10 mm) and silt (0.05 - 0.002 mm) was studied. The different substrates were collected from specific ecosystems around Cochin. Sub samples of each lot were taken and grain size analyzed by sieving through sand sieves and by textural analysis test. Substrates other than silt were washed repeatedly to remove dirt and other unwanted matter. After cleaning the test substrates, they were dried and used.

2.1.2.1.Response of *B.spirata* to varied substrates during short term exposure

An experiment was conducted to study the response to varied substrates during short term exposure was carried out for a period of seven days. Three circular tanks each of 1t capacity were used and in each, the tank bottom was divided into four sections without any physical partitions into equal quadrants area of 800 cm^2 (Fig. 2.1) and the substrate samples were spread separately in each section upto 10 cm height.

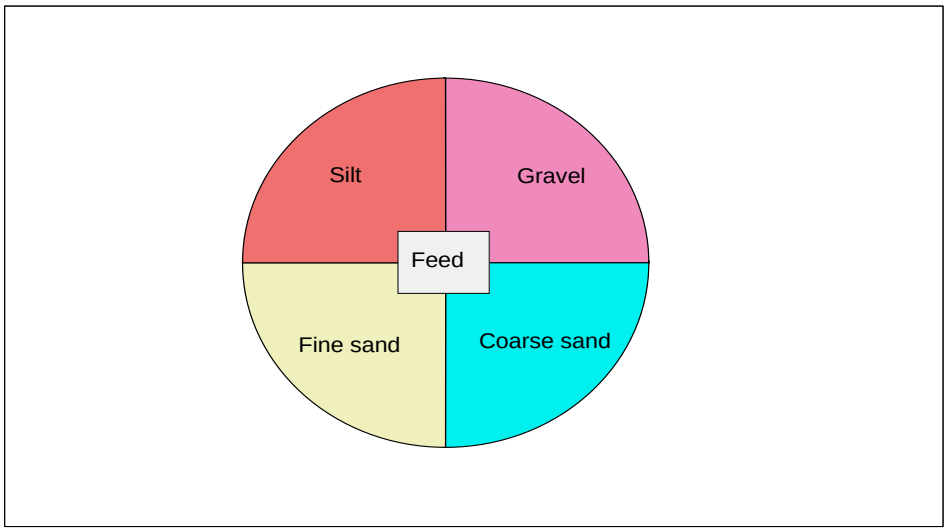


Fig. 2.1. Diagrammatic representation of the experimental setup in the tank bottom

Twenty animals were used and they were released into the centre of each experimental tank where the area was cleared. Sea water of 32 ± 1 ppt was added up to the height of 50 cm over the substrata and was changed on alternate days. The water temperature during the experimental period was 28 ± 2^0C and was aerated. A glass plate was placed in the centre of the tank to release the snails and for feeding. The response of the animals to different types of substrata was studied by counting their numbers at intervals of 2, 5, 10, 15 and 20 h after feeding till the next feeding schedule and the average occurrence was estimated. Whelk behaviour in each substrate was also observed.

Feed acceptance: The whelks were fed with 50 g of shrimp or squilla meat every day at 1000 h. The meat was cut into small pieces (1 to 2 mm) and placed in the central region in the glass petri-dish. The left over feed was removed after 1 h.

2.1.2.2. Response of *B. spirata* to varied substrates during long term exposure

The procedure of this experiment was the same as that of Expt. 2.1.2.1 as mentioned above but was continued to 25 days to study the response when exposure duration is prolonged. The number of whelks occurring in each section of the tank was counted

daily pooled and percentage of occurrence on 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day was estimated.

2.1.3. Food and feeding of *B. spirata.*

2.1.3.1. The feeding behaviour and feed preference of *B. spirata*

Twenty acclimatized whelks which were healthy and active in the hatchery condition were selected to study the feed preference. Prior to the experiment, the whelks were starved for two days. Then they were stocked in a rectangular tank of 1 t capacity having the bottom area of 2 m^2. Eight different kinds of feed *viz* shrimp, squilla, clam, mussel, squid, fish, polychaete worms and pellet feed were provided as feed in different parts of the tank in glass plates. Whelks were introduced in the centre of the tank and observed for their feeding behaviour and food item which they prefer most. The distance over which the snail could detect the feed and the time taken for it to reach near to the food were studied. The whelks were allowed to feed for one hour after which the feed was removed. Before and after feeding the quantity of feed in the tank was weighed.

2.1.3.2. Estimation of the consumption rate of different feed.

B. spirata (n=60) having average SL of 42.3 ± 3.0 mm, SW 28.4 ± 1.6 mm and TW 18.9 ± 2.7 g were selected for the experiment. They were acclimatized to laboratory conditions of salinity 32 ± 2 ppt, temperature 28 ± 2^oC and pH 8.1 ± 0.2. Meat of two species of bivalve, *Perna viridis* and *Mertrix meritrix*, two species of crustaceans, *Penaeus indicus* and *Oratosquilla nepa* and cephalopod *Loligo duvauceli* and fish *Ambassis* were used during the experiment. Since the snails did not consume pellet feed and polychaete worms (section 2.1.3.1) these were not treated in the present experiment. Ten snails were kept separately for each treatment. Each snail was kept in separate plastic troughs of 1 l capacity and 2.5 g feed provided. The quantity of leftover feed was weighed after 1h. From this, the percentage of feed consumed relative to the total wet weight of the animal was calculated by using the formula,

$$\% \text{ of feed consumed} = \frac{\text{Total feed provided (g) - Weight of unused feed (g)} \times 100}{\text{Wet weight of whelk (g)}}$$

The food was provided everyday at 1000 h and the procedure was repeated in the afternoon at 1500 h. The experiment was repeated for 7 days.

2.1.4. Salinity tolerance of adult *B. spirata*

2.1.4.1. Experiment to determine the salinity tolerance of *B. spirata*

Salinity tolerance of adult *B. spirata* of SL 40.86 ± 4.3 mm, SW 28.13 ± 1.64 mm and TW 18.25 ± 3.16 g was studied by exposing the whelks to varied salinities *viz*, 15, 20, 25, 30,35, and 40 ppt. The low salinity seawater for the experimental trials were prepared by adding fresh water to the 35 ppt natural sea water. The vol. of fresh water (*l*) required for making desired salinity (ppt) from the natural sea water of known salinity 35 ppt based on the following formula (Upadhyay, 1994).

$$Vf = \frac{V}{Sf} \times (Si - Sf)$$

Vf = Vol.of freshwater (to be added.)

V = Vol.of seawater (to be diluted)

Si = Initial salinity (of seawater to be diluted)

Sf = Final salinity (of seawater after dilution) (This is the required salinity)

The salinity was measured using a refractometer (ATAGO 0-100 ppt, Japan). The experiments were conducted in 20 liter plastic troughs at a stocking density of one whelk in 2 l of seawater. All the experiments were conducted in triplicates. Salinity of 32 ppt was used as the control. The experimental containers were provided with silt substratum. Daily 25 g of food was placed in the tanks and the snails were allowed to feed for 1 h following which the feed was removed and water was changed. The test medium was aerated through out the experiment. Observations were made on the activities of whelks in all tanks on their behaviour and feed acceptance and mortality were recorded everyday for 10 days. Based on the number of whelks dead, the percentage of mortality was estimated.

From the results of the Expt 2.1.4.1, a second experiment, Expt 2.1.4.2 was conducted to find out the LC_{50}

2.1.4.2. Experiment to determine LC_{50}

To find out the LC_{50}, the whelks were exposed to 25, 26, 27, 28, 29 and 30 ppt since the mortality in 25 ppt was less than 50% and in 30 ppt the survival was 100%. The required salinity was prepared as described in the section 2.1.4.1 and the same number of animals was used. The experimental procedure was same as that for 2.1.4.1.

2.1.5. Temperature tolerance and behavioural changes of *B. spirata* to different temperatures.

B. spirata (n=160) having an average SL of 40.86 ± 4.3 mm, SW 28.13 ± 1.64 mm and TW 18.25 ± 3.16 g were selected for the experiment. They were exposed to six different temperatures *viz,* 20, 22, 24, 26, 28, 30, 32 and 34^{o}C. Temperatures of 20 to 24^{o}C were regulated in the air conditioned broodstock room. Water temperature of 26 and 28^{o}C were obtained in the ambient temperature, while 30 to 34^{o}C were maintained with the help of an aquarium heater. All treatments were carried out in duplicate and tanks were well aerated throughout the experiment. In all treatments observations were made on the behaviour of the whelks and their feed acceptance. Experiments were terminated on 10th day and through out the experimental period in all treatments the hydrological parameters like salinity 31 ± 2 ppt and pH 8.1 ± 0.3 were maintained.

2.2. RESULTS

2.2.1. Standardization of transportation method of broodstock of *B. spirata*

TREATMENT 1. Transportation in water (TRT- WP)

Survival was 100% in both treatments TRT- WP-1 and TRT-WP- 2 after 5 h of transportation. The percentage of survival decreased with increased duration of treatment period *viz,* 10, 15, 20, and 25 h to 93%, 87%, 73% and 47% respectively. The same decreasing trend was also observed in the experiment without aeration, the percentage survival was 73, 60, 33 and 0 % respectively. The results of the treatments TRT-WP-1 and TRT-WP-2 are shown in the Fig. 2.2 & 2.3 respectively.

The dissolved oxygen (DO) in the both treatments decreased with increased duration, while the concentration of ammonia in the experimental container increased. After 25 h of treatment the amount of DO and ammonia in the TRT-WP-1 was 3.14 ± 0.1 and 9.77 ± 0.2 while in the TRT-WP-2 it was 1.2 ± 0.03 and 12.6 ± 0.34 respectively. The amount of DO and ammonia in the water during the observation time are shown in the Table 2.2.

Table- 2.2. Level of dissolved oxygen and ammonia content in the wet packing methods

Treatment period (h)	TRT-WP-1		TRT-WP-2	
	DO (mg l^{-1})	NH_3 (µmol l^{-1})	DO (mg l^{-1})	NH_3 (µmol l^{-1})
0	6.00 ± 0.30	0.39 ± 0.60	4.20 ± 0.20	0.37 ± 0.42
5	5.30 ± 0.20	3.18 ± 0.50	3.50 ± 0.10	1.71 ± 0.50
10	4.90 ± 0.10	3.30 ± 0.30	2.50 ± 0.10	6.00 ± 0.23
15	4.70 ± 0.03	3.90± 0.20	2.20 ± 0.02	7.50 ± 0.30
20	4.50 ± 0.05	4.60 ± 0.30	1.90 ± 0.10	9.64 ± 0.40
25	3.14 ± 0.10	9.77 ± 0.20	1.20 ± 0.03	12.6 ± 0.34

TREATMENT 2. Transportation under moist packing in jute bag (TRT- MP)

Survival was 100% under moist condition both in atmospheric temperature (TRT-MP-1) and in low temperature (TRT-MP-2) at the end of 5 h. After 10 h of treatment in TRT- MP-1 the percentage of survival decreased to 47%, while in the lower temperature the survival was 93%. Within 15 h in TRT-MP-2 the percentage of survival was 67 while in TRT- MP-1 it was very low (20%). Mortality was 100% at 20 h and 25 h transportation in atmospheric temperature, while in TRT-MP-2, 53% and 27% whelks survived. The results of the treatments were shown in the Fig. 2.4 & 2.5.

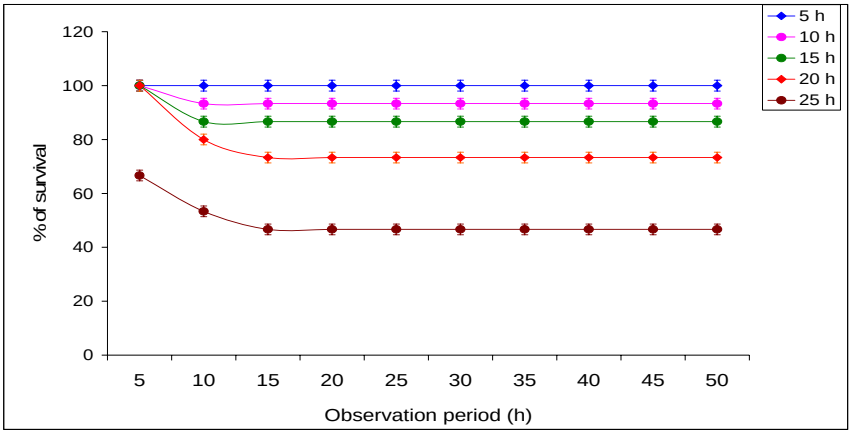


Fig. 2.2. Survival of *B. spirata* during the post- transit period in the treatment TRT-WP-1

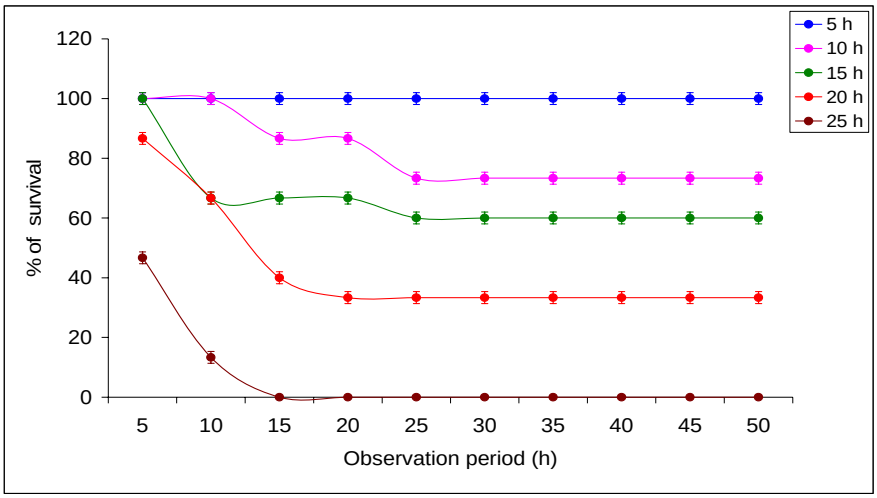


Fig. 2.3. Survival of *B. spirata* during the post- transit period in the treatment TRT-WP-2

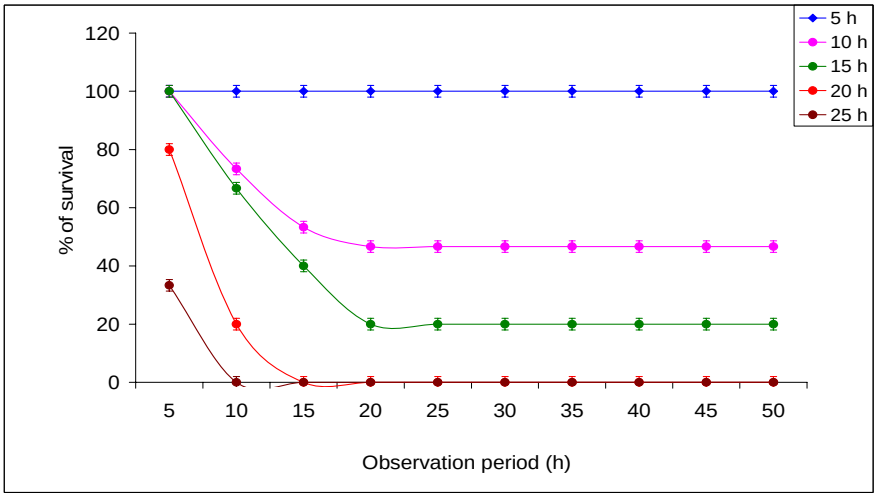


Fig. 2.4. Survival of *B. spirata* during the post- transit period in the treatment TRT-MP-1

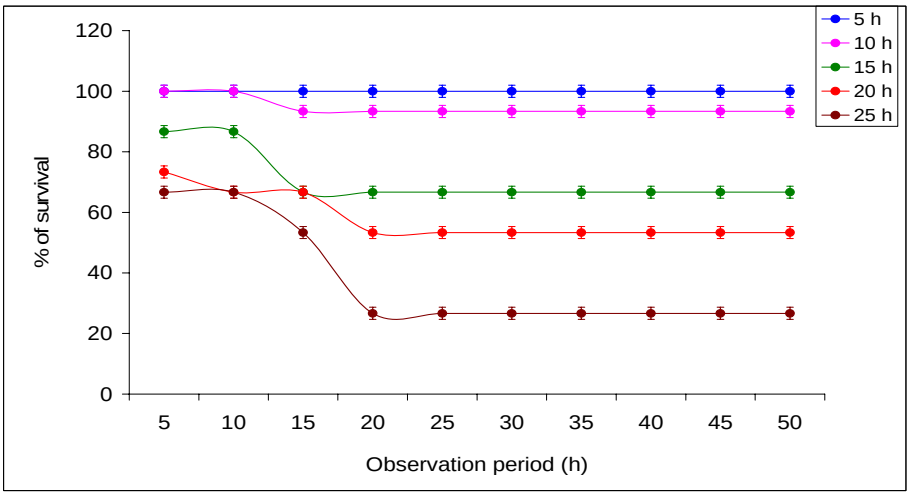


Fig. 2.5. Survival of *B. spirata* during the post- transit period in the treatment TRT-MP-2

TREATMENT 3. Transportation in dry method (TRT- DP)

Percentage of survival was very low in the dry packing method. Only 40% whelks survived after 5 h of transportation. The whelks were live even after 20 h of treatment but they did not survive during the post transit period when they were stocked in sea water. Mucous production was very high during exposure to atmosphere and their muscles relaxed and could not be fully retracted in to the shell. The result of the treatment is shown in the Fig. 2.6.

After 15 h of post transit period further mortality was nil in all the above experiments. The final survival of *B. spirata* after 15 h of post transit period all the treatments are shown in the Fig. 2.7.

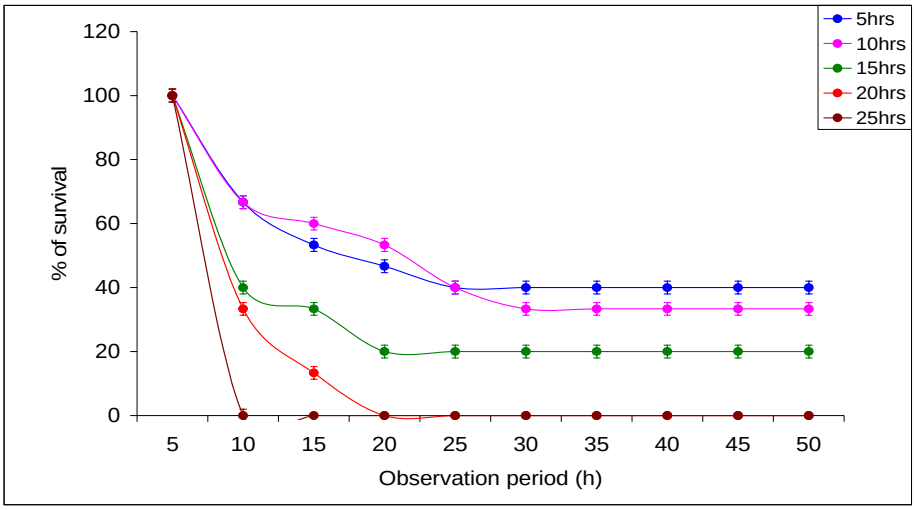


Fig. 2.6. Survival of *B. spirata* during the post- transit period in the treatment

TRT-DP.

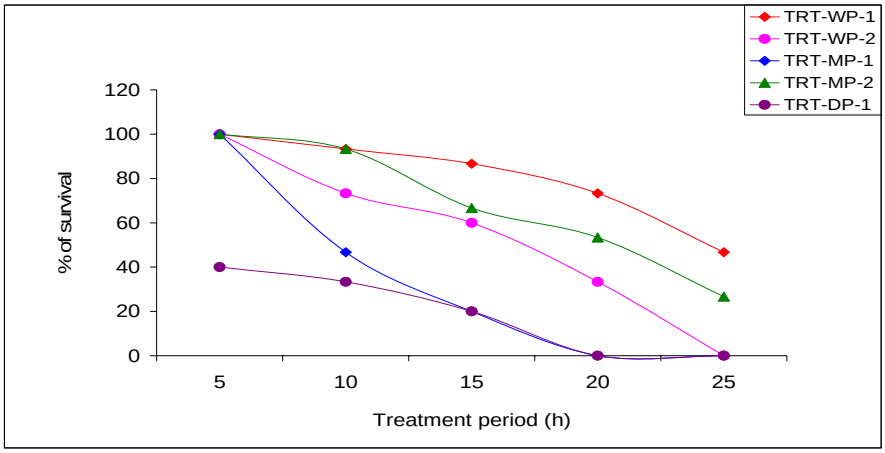


Fig. 2.7. Final survival of *B. spirata* in different packing conditions

2.2.2. Determination of substratum preference of adult *B. spirata*

2.2.2.1. Response of *B. spirata* to varied substrates during short term exposure

The response of whelks to different substratum varied during the experimental period. The average percentage of occurrence of *B. spirata* in varied substratum is shown in the Fig. 2.8. The highest percentage of occurrence was in the silt substratum with an average of 47.6 ± 0.6 during the one week experimental period. The average percentage of occurrence of the whelk in the fine sand substratum was 26.2 ± 1 while in the coarse sand and gravel it was low 17.9 ± 0.9 and 8.3 ± 1.2 respectively. The substratum preference study clearly indicated that *B. spirata* preferred silt substratum and the variation in the occurrence of whelks in each substratum was significant ($P<0.05$).

Feed acceptance: All the experimental animals consumed feed within 5-10 minutes when feed was provided and were found to move to the preferred substratum after feeding.

2.2.2.2. Response of *B. spirata* to varied substrates during long term exposure

The percent occurrence of *B. spirata* in different substratum during the long term exposure is shown in the Fig. 2.9. In the silt substratum the percentage of occurrence showed much variation during long term exposure. The average percentage during

this period increased from 36% on day 5 to 73% during 25th day. Initially the percentage occurrence in the fine sand substratum was lower (23%) than in the coarse sand (32%). However in the subsequent days the average percentage of whelks in fine sand substratum was higher, ranging between 23 and 31%. The average percentage of occurrence in coarse sand substratum was higher (32%) on 5th day which gradually decreased to 2% by 25th day. In the gravel substrate also the occurrence was low (9%) on 5th day and by 25th day there were no whelks in gravel substrate.

Whelk behaviour: When the whelks were released in to the centre of the tank, they moved and dispersed to different substrates in experiment 2.2.2.1 and 2.2.2.2. The whelks which entered the gravel and coarse sand substrates remained on the surface of the substrate. In the sand substratum they were partially buried with their siphon exposed while in the silt substratum the whelks were completely buried with only their siphons exposed above the substratum.

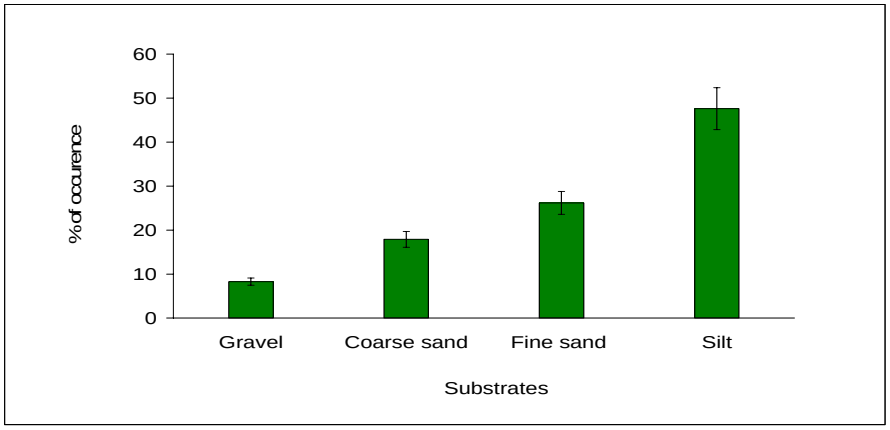


Fig. 2.8. Average % of occurrence of *B. spirata* in the four different substrata studied during the short- term exposure of 7 day

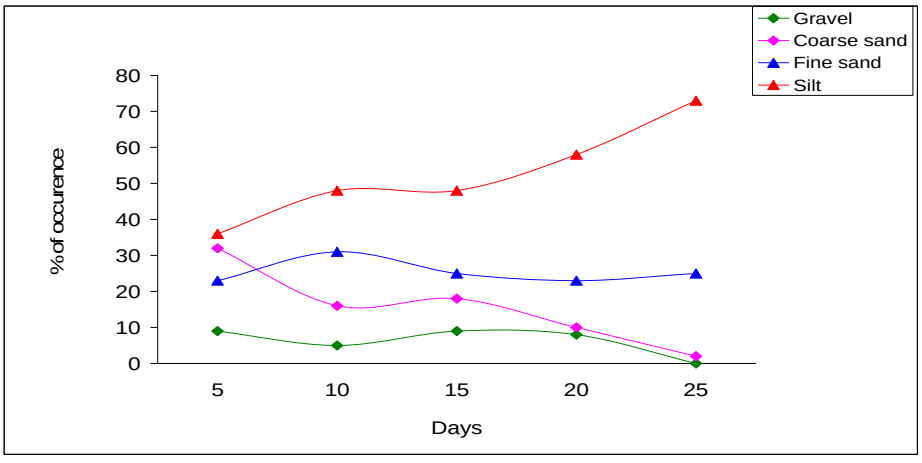


Fig. 2.9. Percent occurrence of *B. spirata* in different substratum during long term exposure

2.2.3. Food and feeding of *B. spirata.*

2.2.3.1. The feeding behaviour and feed preference of *B. spirata*

As soon as they were introduced in the tank, all whelks dispersed to different parts of the tank in search of feed. While moving they extended their proboscis. Greater affinity was observed for the squilla and shrimp meat. Thirty percent of the whelk accepted the squilla within 10 min of sighting the feed, which was kept in a corner of the rearing tank. At the same time 35% whelks showed affinity towards the shrimp meat which was kept in other corner of the tank. No response were observed to the pellets feed even though 15% of them passed over it. Fifteen percent of the whelks fed on the mussel meat while 10% on clam meat. Ten percent whelk extended the proboscis over the fish feed but did not take it and 5% moved near to the squilla and fed on it. Only 5% fed on squid meat and no whelk responded to the polychaete worms. Out of 15 g of each type of feed provided only shrimp and squilla were completely consumed. Only 40% of mussel and 23% of clam meat was consumed by the whelks.

Feeding behaviour: When the snails got the sense of the feed, they stopped their movement for few minutes and extended their proboscis (Fig. 2.10) When the feed was sensed by the proboscis, they went near the feed and contracted the proboscis and sucked it fully.

Fig. 2.10. Adult *B. spirata* extending the proboscis for feeding

Soft parts were sucked more easily and more time was spent on hard portions like foot and adductor muscles of the bivalve. While feeding on shrimp and squilla, the calcareous exoskeleton was left out and they sucked the meat fully. The observations on feeding behaviour and percentage of animals which consumed the feed in the experiment are shown in the Table 2.3.

Table- 2.3. Feed preference and feeding response to different types of feed by *B. spirata*

Type of feed provided	**Time or sensing the feed (min)**	**% of preference**	**Feeding behaviour**
Penaeus indicus	10	35%	Within 10 min responded to the feed, soft part sucked in and exoskeleton left out.
Oratosquilla nepa	10	35%	Within 10 min responded to the feed. soft part sucked in and exoskeleton left out.
Perna viridis	12	15%	Only soft part of the meat consumed, adductor muscle and foot not consumed
Meritrix meritrix	15	10%	Consumed the soft part of the meat, more time spent for ingesting the foot region.
Loligo duvauceli	20	5%	After 20 min of wandering for food, consumed squid meat.
Ambassis	25	Nil	Passed over the feed, extended the proboscis, food not consumed.
Polychaete worms	Nil	Nil	Passed over the feed without consuming it.
Pellet feed	Nil	Nil	whelks passed over the feed, without consuming the feed.

2.2.3.2. Estimation of the consumption rate of different feed.

The percentage of food consumed by wet body weight of the animal varied with the type of feed. Whelks consumed the feed only in the morning hours and they did not

respond to the feed in the afternoon. The whelks actively fed only on alternate days of the experiment. The weight of feed consumed by the whelk during the experimental period is shown in the Fig. 2.11.

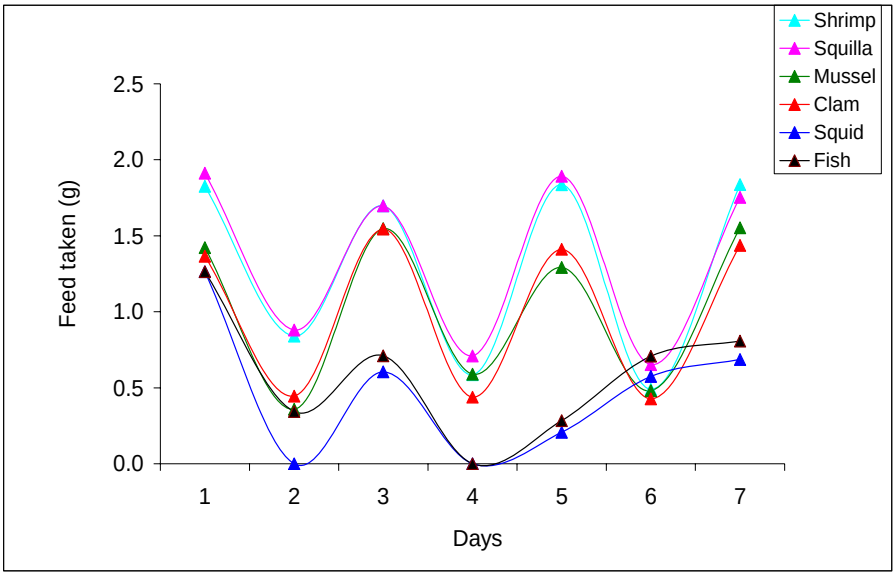


Fig. 2.11. Feed (g) consumed by *B. spirata* during the experimental period

The consumption rate in percentage of body weight of shrimp and squilla meat were almost same, 9.5 and 9.2% respectively on active feeding days. While for the bivalve feed the value was 7.9% for both mussel and clam. Fish and squid feed had little acceptance and the consumption rate was 3.9 and 3.8% respectively. During the low feeding days rate of consumption decreased in the order of squilla, shrimp, mussel, clam, fish and squid. The percentage of feed consumed by body wet weight of the whelk on active and a low feeding days is shown in the Fig. 2.12.

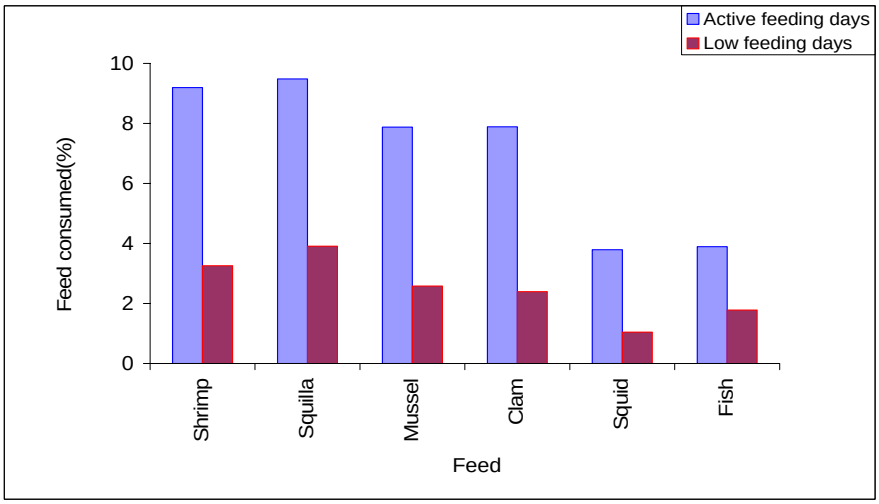


Fig. 2. 12. Percent of feed consumed by body weight of whelk

2.2.4. Salinity tolerance of adult *B. spirata*

2.2.4.1. Experiment to determine the salinity tolerance of *B. spirata*

Survival was 100% in 28- 40 ppt salinities. In 10 and 15 ppt 47% mortality was noted within 12 h of exposure. In 20 ppt also high mortality was noted, 50% mortality occurred in 16 h of exposure to salinity. Within 24 h complete mortality were recorded. In all the low salinities the animals firmly closed the operculum and did not bury into the substratum. Only 20% of the whelks survived in 25 ppt salinity and during the experimental period they were inactive. Whelks which were live showed slight movement over the substratum and also along the walls of the container. They did not respond to the feed on 2nd day. The quantity of food consumed during subsequent day was also low, 1.5% by body weight of the whelk.

2.2.4.2. Experiment to determine of LC_{50}.

Fifty percent survival was recorded in 26 ppt salinity at the end of the experiment. Within 24 h, 20% mortality was recorded which increased to 50% on 8th day, following which there was no mortality. Those which survived remained partially buried in the substratum. Feed was consumed but the quantity consumed was low. In 27 ppt salinity 10% mortality occurred in 24 h and thereafter no mortality was recorded. The live whelks accepted feed and remained completely buried in the substratum exposing their siphon. Complete survival was recorded in all salinities above 28 ppt. In these treatments, the whelks were completely buried through out the experimental period and responded to the feed within 5 min. The percentage survival of *B. spirata* in different salinity is shown in Table 2.4**.**

Table- 2.4. Percentage survival of *B. spirata* in different salinities

Days	Salinity ($^0/_{00}$)											
	10	**15**	**20**	**25**	**26**	**27**	**28**	**29**	**30**	**35**	**40**	**32 (Control)**
1	0	0	0	100	100	100	100	100	100	100	100	100
2	0	0	0	50	80	90	100	100	100	100	100	100
3	0	0	0	33	80	90	100	100	100	100	100	100
4	0	0	0	30	80	90	100	100	100	100	100	100
5	0	0	0	30	80	90	100	100	100	100	100	100
6	0	0	0	30	63	90	100	100	100	100	100	100
7	0	0	0	30	63	90	100	100	100	100	100	100
8	0	0	0	30	50	90	100	100	100	100	100	100
9	0	0	0	20	50	90	100	100	100	100	100	100
10	0	0	0	20	50	90	100	100	100	100	100	100
Final survival %	**0**	**0**	**0**	**20**	**50**	**90**	**100**	**100**	**100**	**100**	**100**	**100**

2.2.5. Temperature tolerance and behavioural responses of *B. spirata* to different temperatures.

In temperature below 24^{o}C, all the whelks were inactive and lay above the substratum in a placid condition. At 20^{o}C they closed the operculum tightly. In 22 and 24^{o}C the whelk remained in an inverted position exposing their foot upwards. Intake of food was negligible. At 26^{o}C the whelks buried in the substratum, a response to the feed was slow and was active. At control temperature of 28^{o}C, 100% whelks lead a normal life of movement, feeding and burrowing. In 30^{o}C the activities were same as in 28^{o}C. In temperature of 32^{0}C, the whelks widely dispersed and buried in the substratum. Response to the feed was low and only 80% of whelks consumed the feed. But in 34^{o}C most of the whelks were inactive; their muscles did

not retract into the shell completely. This condition was worse than in low temperature. On 10th day 30% mortality was recorded. When the whelks exposed to lower temperature of 20 to 26°C were brought to the ambient temperature (28°C) all become active. However when whelks in high temperature (34°C) were brought to the ambient temperature of 28°C, again 30% mortality occurred. Summary of the observation were shown in the Table 2.5.

Table- 2.5. Percentage of survival and behavioural difference of the whelks in different temperature conditions.

Temp (°C)	**% of active whelk**	**% of whelk which consumed feed**	**Behavioural responses**
20°C	Nil	Nil	Tightly closed the operculum and retrieved into the shell, lay above the substratum, no response to feed, no mortality.
22°C	Nil	Nil	Inactive, no response to feed, lay in an inverted position exposing foot upward above the substratum, no mortality
24°C	10	Nil	90% inactive lay above the substratum in an inverted position exposing the foot upward. 10% buried their foot in the substratum, no response to feed, no mortality
26°C	100	100	100% whelks active, partially buried in the substratum responded to the feed as soon as they were provided with feed. 100% survival.
28°C	100	100	More active than above, completely buried in the substratum exposing only their siphon, responded immediately when feed was provided, occasionally came out above the substratum and aggregation of whelks was observed.
30°C	100	100	Completely buried in the substratum exposing only their siphon, immediately when feed was provided, occasionally came out above the substratum and buried, aggregation of whelks observed.
32°C	100	80	All active, responded to feed, widely dispersed and buried in the substratum, excess mucous production but no mortality.
34°C	20	Nil	Fifty percent inactive during the initial stage of experiment and 30% mortality on 10th day of the experiment, no food intake,

			muscles stiff and rigid foot, not retracted into the shell, body whitish and in dry condition.

2.3. DISCUSSION

Among the three methods of packing, moist packing in low temperature was found to the best. In wet packing method the survival was low since there was hypoxic condition and successive ammonia production. A similar observation on ammonia excretion has been reported by Patterson *et al.* (1996) on the same species. They found that the rate of ammonia excretion increased with time. Ammonia forms the bulk of excreted nitrogen (Hughes, 1986) and factors such as body size (Little, 1981), temperature (Emmerson, 1969) and salinity (Stickle and Bayne, 1982) are known to influence ammonia excretion. Another important factor observed by Patterson *et al.* (1996) is that the rate of ammonia excretion increased when the animals were starved. In the present experiment the whelks which were transported were in the starved condition. This also would have increased the ammonia excretion rate resulting in unfavorable condition which leads to mortality of whelks. In the moist packing methods the survival rates were high especially in low temperature condition. It is well known that under low temperature the metabolic rate decreases and since there was no solute, the product of metabolism like ammonia was not released to the external environment. Hence an unpleasant surrounding as what happened in wet packing did not occur which reduced the mortality. In the dry packing method the broodstock experienced dessication. Though there was no solute, the temperature was higher and they were exposed. This led to dessication. *Littorinid* and *Acmacid* gastropods living in the intertidal zone have been known to withstand dessication up to several months, during which their haemolymph may attain solute concentration equivalent to 300% seawater (Wolcott, 1973; Emson *et al.,* 2002). Such incredible ability to tolerate very high internal solute concentration is apparent in another gastropod the *B. naticoidea* which inhabits an environment with extreme salinities of above 65 ppt (Van Gaest *et al.,* 2007). However *B. spirata* when transported by the dry packing method was found to be alive when the duration was low (less than 5 h) but did not survive the recovery period. The animals were found

to be highly stressed as evidenced by the high mucous production. From the results of the transportation experiment it is recommended that the best method to transport broodstock is by moist packing in low temperature for a maximum period of 10 h.

In the short and long term study on substrate preference, it was observed that *B. spirata* preferred silt substratum followed by fine sand and did not prefer coarse sand and gravel. The fact that the percentage of whelks distributed in the silt substratum increased and almost doubled (73%) by 25th day indicates that whelks had clear preference for silt substrate. Similarly increased occurrence of whelks in fine sand with increase in duration and their complete avoidance of gravel substrates also confirm their specific needs. Moreover their behaviour after feeding by returning back to the preferred substrate also reconfirms their habitat preference. Studies made by Mohan (2007) done concurrently with the present study on the soil texture in the Kollam zone, from where the brooders was collected support the inference that *B. spirata* prefers silt while *B. zeylanica* was observed mainly in the sandy beds. The soft bottom nature supporting the burrowing nature of *B. spirata* reported by Mohan (2007) also support the burrowing nature of the same species in the laboratory. She also reported the variation in the density or abundance of the whelk *B. spirata* and *B. zeylanica* within the same beds apart from the specific substratum.

Patterson and Ayyakkannu (1992) have also observed such preference to soft sediment by the muricid gastropod *Murex tribulus*. Though this gastropod showed major preference to mud, it was also found in the sand. *Chicoreus ramosus* is a species which basically prefers hard substratum but Patterson and Ayyakkannu (1992) have collected these in small numbers from coral beds, mud and sandy sea bottom region in the Bay of Bengal indicating their capability in withstanding soft sediment substratum also. Similar behaviour of other gastropods like *Chicoreus ramosus* to coral substrate, *Fasciolaria trapezium* to sand substratum, *Murex tribulus* to muddy substrate and *Chicoreus virgineus* to stone substratum has been observed (Patterson and Ayyakkannu, 1992). *Chicoreus ramosus* returned to its preferred substrate after feeding. In the present study it was observed that the whelks lay buried in the sand substrate with their siphons exposed. Similarly *M. tribulus* also was found to bury in mud or sand substrate and expose the siphons. Most of time *F.*

trapezium was found with its whole shell burried in sand with only the apical part of the shell exposed. Contrary to this *C. ramosus* and *C. virgineus* firmly attached to the hard substrate. Burrowing in the sand has been considered as a protective action of snails against heavy wave action (Vaidya, 1979). Driscoll and Brandon (1973) have found that the factors most clearly correlated with the distribution of the species were the clay content, abundance of dead shell material and substratum stability.

Another snail which is able to discriminate between different types of substrates is the tropical estuarine gastropod *Cerithidea cingulata* (Rao and Sukumar 1981). Texture of the substratum seems to be an important factor in determining the distribution pattern of *C. cingulata* in the field. They observed that these snails were absent near the mouth of the estuary and in other places where the substratum was sandy. Substrate preference experiments have shown that unlike *B. spirata* these snails avoided fine mud and sand and favoured mixed substrata. Such preference to a specific substratum has been attributed by the authors to the feeding habits of and to physical factors such as increased difficulty in movement about in fine mud and coarse sand. Similarly Wells (1978) observed that population density of the mud snail *Hydrobia ulvae* was lowest in finest sediments and coarsest sand and found that these snails favor intermediate grain size.

Preference to particular substrates has been observed in freshwater prosobranch gastropods also. *Viviparus bengalensis* and *Melania scabra* have shown preference to sand over stone even in the absence of feed. Vaidya (1979) has suggested that such behaviour may impose restrictions on their distribution alone. Other snails like *Physa parkeri* and *Heliosoma antrosa* also have shown preference to sand in contrast to *P. integra* (Clampitt, 1973). Observations made by Hanumante *et al.* (1977) show that the pulmonates snails *Indoplanorbis exustus* preferred hard substratum like stones over sand, while *Lymnea auricularia* did not favour any of the substrata.

In the present study it was observed that *B. spirata* has a long extendable proboscis and it can sense the feed. Moreover it had significant preference to squilla and shrimp meat. In a study on *Buccinum isaotakii*, Ilano *et al.* (2005) observed similar

behaviour, as *B. spirata* such as extending the proboscis when feed is sighted. The response of *B. isaotakii* to the presence of feed started by extending its proboscis when approaching the feed, attacking it and finally ingesting the food similar to the response of *Neptunea antique* to its polychaetes prey (Pearce and Thorson, 1967).

The food when probed with proboscis was cut or punctured with the radula by *B. spirata* and also in the case of *B. isaotakii* (Ilano *et al.*, 2005). An entirely different feeding behaviour is seen in intertidal muricids where the snails penetrate the shell of their prey by chemical activities of carbonic anhydrase, chelating agents and enzymes secreted by the accessory boring organ situated in the foot, and together by mechanical scraping action of the radula (Carriker, 1981). Some studies have also shown that certain muricids can produce various chemicals such as serotonin from the accessory salivary glands (Andrews *et al.*, 1991; West *et al.*, 1994) as well as choline esters from the hypobranchial gland (Roseghini *et al.*, 1996) to paralyze and capture the prey. Similar to the present observation on the feeding behaviour of *B. spirata*, Patterson and Ayyakannu (1992) have observed the same whelks suck in the soft part of the bivalve meat and also to spend more time for sucking the hard part like the adductor muscle.

The feed preference experiments conducted in the laboratory for adult *B. spirata* shown that they are not active feeders but they can sense the presence of feed 1 m apart from them. Another important observation was that the snails refused to accept pellet feed and fish meat even when they moved over it while trying to reach the squilla or shrimp meat. This confirms their strong chemoreception. Scavenging snails have been reported to be actively sensitive to such chemical messages emanating from food material (Shimek, 1984) and in surface film. Such chemical stimuli were observed to extend over longer distances (Morton, 1990). While subtidal organisms like *B. undatum* (Himmelman, 1988) took more time to locate bait 2 m upstream, scavenging snails reached them quickly (Morton and Briton, 1991). Studies on the slow moving gastropod *Busycon carica* by Ferner and Weissburg, (2005) revealed that the animal can locate the odour source from 1.5 m downstream with no significant effect of flow treatment. *B. isaotakii* also displayed distance chemoreception rather than contact chemoreception (Ilano *et al.*, 2005)

Considerable variation has been observed in the preferred feed of *B. spirata* in the present study and that observed by Patterson and Ayyakkannu (1992). Shrimp and squilla meat were the preferred food with 35% of whelks feeding on each of the feed. In the study conducted by Patterson and Ayyakkannu (1992) *B. spirata* preferred oyster meat to shrimp meat. This discrepancy may be due to the fact that in the present study oyster meat was not one of the treatments instead meat of green mussel and clams were provided. However in both the studies meat of bivalves were consumed. Morton and Briton (1991) have also observed that scavengers like nassarid *Nassarius pyrrhus* and *B. undatum* feed on a wide variety of invertebrate prey and carrion including bivalves (Neilsen, 1975) and polychaetes (Taylor, 1978).

Four species of *Neptunea* were reported to feed on bivalves and polychaetes and secondarily upon carrion, although other prey taxa also were represented in their diet (Shimek, 1984). Their preference for squid was also low which is similar to that of *B. isaotakkii* (*Ilano et al*., 2005). However, in the present study the whelks also did not consume pellet feed and showed preference to fresh feeds. Similar observations have been made on *B. isaotakkii* (Ilano *et al.,* 2005). They observed that this species hardly detected boiled feed but responded very well to fresh feed. These observations support the view that perception of food depends on the nature of the stimulatory substance in it as well as its dispersion in water (Patterson *et al.,* 1995). The feed consumption rate was considerably higher (9.2 to 9.5%) for the preferred feed than observed (3.05%) by Patterson and Ayyakkannu (1992). This may be due to the difference in size of the experimental animals.

Another important observation was that the whelks exhibited active feeding days alternating with passive feeding. Such passive feeding days has also been observed in *B. isaotakii* (Ilano *et al.,* 2005) when the snails fed less on their preferred feed items which was sardine and scallop meat. Based on this experiment the rearing protocol for broodstock can be fixed as feeding on alternate days with preferred feed such as shrimp or squilla. Ilano *et al.* (2005) has observed size related variation in *B. isaotakii* and several gastropods like *Bullia digitalis* (Stenton- Dozey and Brown,

1988), *Hemifusus tuba* (Morton, 1986), *Babylonia lutosa* (Morton, 1990) and *Neptunea arthritica* (Fujinaga and Nakao, 1999).

Salinity is an important abiotic factor that has a profound effect on marine organisms (Newell, 1976; Vernberg and Vernberg, 1972). In the present study the adult *B. spirata* were found to be stressed in salinities from 5 to 20 ppt and salinities lower than 20 ppt were lethal. Few whelks tolerated short periods of experimental exposures in salinities 20 to 25 ppt. Their behavioural response during this period of exposure to low salinity was that of avoidance. They completely closed the operculum and also abstained from burrowing and were lying with a closed operculum on the surface of the substrate. Such instances where the gastropod lay inactive above the substrate have been reported in *Bathynerita naticoidea* (Van Gaest *et al.*, 2007). It is well established that aquatic animals overcome problem caused by salinity fluctuations by their behavioural responses. Burrowing has been considered as an escape strategy for unfavorable abiotic conditions like low or high salinities for marine molluscs (Berger and Kharazoa, 1997). Patterson *et al.* (1994) have also observed behavioural responses of *B. spirata* exposed to lower salinities. The tolerance levels obtained in the present experiment were similar to that obtained by Patterson *et al.* (1994) with only marginal differences. In the present study 100% survival was obtained in salinities above 28 ppt while *B. spirata* in the experiments conducted by Patterson *et al*. (1994) had complete survival above 29 ppt salinity. However all the behavioural responses were similar. In the present experiment it was also observed that few whelks tried to climb the wall of the tank in salinities 20 to 28 ppt which also is a means to escape to a better environment. The “shell closing mechanism” has been suggested as the principal response of intertidal molluscs to survive short periods in lower salinities (Hoyaux *et al*., 1976). Similar closing mechanism has also been observed in bivalves, *Katelysia opima, Crassostrea virginica* and *Mytilus edulis* (Mane, 1974; Hand and Stickle, 1977 and Charles and Austin, 1978) and gastropods *Murex pomum* and *Murex tribulus* (Sander and Moore, 1979; Raghunathan and Ayyakkannu, 1992). Davenport (1979) found that the mussel *Mytilus edulis* survived by retaining water within the mantle cavity after the shell valves were closed in response to falling environmental salinities.

In the present study tolerance to low salinities were observed for short periods and the whelks succumbed to death with increased duration. For marine organisms additional energy is required for osmotic adjustment in hyposaline environments, with the result that animals are unable to maintain a positive energy budget below threshold salinity. Such instances, where the gastropods can withstand normal salinities has been reported in the neritid *Bathynerita naticoidea* where the neritid survived for a few hours in salinities above 65 ppt (Van Gaest *et al*., 2007). The authors indicated this as the lack of osmoregulatory ability of the gastropod in high salinities. At low salinities however same gastropods have same osmoregulatory ability (Andrews, 1988). Marine neritid species such as *Nerita fulgurans*, however often have blood that is isosmotic with urine suggesting that they osmoconform (Fretter and Graham, 1962; Andrews, 1988). In the present study 28 ppt salinity was found to be threshold salinity of *B. spirata*. Based on salinity tolerance range of several gastropods, Thivakaran and Kasinathan (1990) have inferred that salinity tolerance of a species is influenced by the salinity regime of the habitat. Burrowing has been considered as an escape strategy for unfavorable abiotic conditions like low or high salinities for marine molluscs (Berger and Kharazoa, 1997).

In the present study, *B. spirata* was found to tolerate salinities greater than 26 ppt. This supports the inference made by Thivakaran and Kasinathan (1990), since the *B. spirata* collected were from the whelk beds in the coastal regions off Kollam where the salinity usually is above 26 ppt (Mohan, 2007). Even if the salinity drops, it is for a short period after which the whelk beds are replenished with high saline water. Based on the results of the salinity tolerance experiment, the broodstock rearing protocol has been elucidated by proposing that the broodstock must be maintained in salinities greater than 28 ppt.

Temperature tolerance studies indicated the behavioural response of *B. spirata* to low and high temperature. Their response to low temperature and high temperature varied, in the former, they either closed the operculum and retreated into the shell or lay in an inverted position exposing the foot. In the latter they buried in the substratum. The specific behaviour of closing the operculum in low temperature may be to avoid losing the body heat to the cold environment and the inactive stage can

be due to low metabolic activity. Contrary to this, the whelks buried into the substratum when temperature was raised from ambient water temperature. This particular behaviour may be an escaping mechanism to avoid the unpleasant higher temperature conditions of the surrounding water since substrate provides a protection. The temperature range 26 to 30^{o}C was found to be optimum based on their behavioural responses and ready acceptance of feed and feeding rate. Temperature above 34^{o}C was found to be lethal with stiffening of muscles and abstainement from feeding. Based on these observations, the temperature regime for broodstock of *B. spirata* is suggested as 26 to 30^{o}*C.* The population changes of *Nassarius reticulates* in Sweden has been found to be highly influenced by variation in temperature (Tallmark, 1980). In the field he had observed that the different activities of snails like, locomotory activity, inshore migration , spawning, growth, offshore migration and quiescence in different years were commenced at the same water temperature but in different dates.

Chapter 3

Spawning of Babylonia spirata

3. SPAWNING OF *BABYLONIA SPIRATA*

3.1. MATERIALS AND METHODS

3.1.1. Broodstock acclimatization and spawning

The broodstock of *B. spirata* collected from trawl catches of Neendakara fishing harbour (Lat 08^0 56'N and Long 76^0 32'E) along the Kerala coast were transported to the molluscan hatchery of Central Marine Fisheries Research Institute. Moist packing as described in section 2.1.1 was adopted for the transportation of brooders and they were stocked in the 1 t tank without any substratum during the acclimatization period of two days. No feed was provided to the broodstock during the period of acclimatization. The water quality in the spawning tank was monitored regularly and they provided the basic conditions as per the inference made from the experiments described in section 2.1.4 and 2.1.5.

Every month 50 whelks (M:F = 1:1) were stocked in a 500 l tank having the bottom area 3846 cm^2 with 1925 cm^2 area having silt substratum. Fresh brooders were not available during the month of June and July, when there was no landing of the whelk. The old stocks were replaced by fresh ones every month. Spawning details were recorded every month from January 2001 to December 2002. The shell length (SL) and shell width (SW) of the brooders were taken using the digital calipers (Mitutoyo, Japan) of 0.01 mm accuracy and the total weight (TW) was weighed before they were stocked in the spawning tank. The fecundity, spawning period, spawning frequency and activities specific to the spawning season such as spawning behaviour and feeding behaviour were observed and studied.

3.1.2. Effect of size of brooder on fecundity and size of egg

Fecundity of *B. spirata* was estimated from the mean number of eggs in the egg capsule obtained in a particular spawning. Three size groups of whelks were tested which were included under three size classes, (a) 45-50 mm, (b) 40-45 mm and (c) 35-40 mm. First group contained the whelks having an average the SL 46.8 ± 2.1

mm, SW 30.7 ± 1.6 mm and total weight 20.3 ± 1.5 g. Second with medium sized brooders having SL 42.8 ± 2.1 mm, SW 28.7 ± 1.1 mm and TW 18.0 ± 2.0 g and third were small size whelks having an average the SL 37.6 ± 1.1 mm, SW 25.8 ± 0.6 mm and TW 14.0 ± 2.6 g. Each group of experiment was in triplicate by rearing three pairs of male and female separately. The capsules obtained were carefully collected. Ten egg capsules from each group were taken and the number of eggs counted. The averages were calculated and fecundity was estimated by multiplying the number of eggs with number of egg capsules.

3.1.3. Requirement of substratum for spawning

An experiment was set up to study the effect of substratum on spawning activity. Experiments were conducted in the month of January 2001 when there was frequent spawning. Rectangular fiber glass tank of 50 l capacity was used for the experiment. Ten whelks (sex ratio 1:1) were stocked in 30 l seawater. Brooders which were acclimatized two days in the hatchery were used for the experiment. The spawner having an average SL 38.84 ± 2.4 mm and TW 14.2 ± 2.4 g were fed with shrimp before the initiation of the experiment. Experiments were in two sets (1) with fine sand substratum and (2) without any substratum. For the first set of experiment out of 1632 cm^2 bottom area of the tank, 400 cm^2 were provided with substratum. Water quality and feed was maintained as per the result of the experiments described in chapter 2. Spawning tank was under observation through out the experimental period of 15 days. The experiments were in triplicates.

3.2. RESULTS

3.2.1. Brood stock acclimatization and spawning

After two days acclimatization in the hatchery the whelks actively consumed the feed and positively responed to the substratum. The acclimatized brooders took 2-10 days to spawn in the hatchery if the conditions for spawning were favourable to them. Spawning was obtained from the 2^{nd} day after acclimatization and could be prolonged up to 22 days.

Copulation: During copulation, the female positioned itself on the right side of the male, and mounted slightly over the male (Fig. 3.1). Both remained immobile throughout the process of copulation for 2-3 h. During this copulation process, the transfer of gametes happened and it is stored in the seminal receptacle of the female. After copulation the male moved away first and the female remained there itself in an inactive stage exposing the foot out of the shell. Copulation was observed both in the day time and night hours.

Spawning behaviour: It was observed that the whelks usually remain buried in the sand substratum exposing only the siphon above the surface when there was no tendency for spawning (Fig. 3.2). However during the spawning period 90% of the brooders moved more towards the surface of the substratum and were only partially buried (Fig. 3.3) However prior to spawning process, aggregation of the spawners was observed, wherein all the adult females moved and congregated in the same area. The males at this time buried in the sand away from the spawning site of the females.

During spawning the female *B. spirata* came above the substratum and remained in an erect position by pressing the foot down and lifting the shell and body whorl up exposing the neck region (Fig. 3.4). This particular posture is an indication of the spawning activity and any slight disturbance halted the spawning activity. Otherwise the female spawner remained in the same posture for nearly 24 h. Even though spawning process started in the early night, 90 % of the spawning occurred in the early morning hours.

Single egg capsule was released at a time during the process of spawning. *B. spirata* took 5-10 min to release a single capsule, the interval between each laying varied slightly. It took an average of 60-90 min for the deposition of 30-35 egg capsules. During spawning the female covered the laying egg capsule with its foot. On completion of the formation of the egg capsule and its attachment to the substratum the whelk leaves the capsule. The egg capsules become hardened on contact with the sea water, otherwise it has a jelly like appearance. In most of the spawnings, the first

laid capsule was a jelly mass without stalk and substratum for attachment and contained only a few numbers of eggs in it.

Brooding: Brooding behaviour was not observed in *B. spirata*. After laying the eggs, the females moved away from the egg mass and subsequently the females scattered to different region of the tank and resumed normal behaviour by burrowing themselves in the sand substratum and accepting the feed. The spawner after the process of spawning, along with the egg capsule is shown in the Fig. 3.5.

Feeding behaviour: Behavioural changes were observed in the feeding activity, during breeding period. Whelks usually sense the feed and come out of the substratum to take the feed within 5 min when the feed was placed anywhere in the tank. However during the spawning period, the females did not accept feed even if the feed was placed near the spawner. Complete starvation was observed till spawning and the normal feeding behaviour was resumed after spawning.

Fig. 3.1. Copulation of *B. spirata*

Fig.3.2. *B. spirata* exposing only the siphon above the substratum

Fig.3.3. *B. spirata* partially buried in the substratum

Fig. 3.4. Spawner position before spawning process

Fig.3.5. Spawner with egg capsule

Spawning period: During the year 2001 spawning was observed from January to April, September to October and December indicating spawning during seven months out of 10 months when the brooders were kept for spawning in the hatchery. In the following year also the same frequency of spawning was observed. There was no spawning during October 2002, instead spawning occurred during November. The spawning calendar of *B. spirata* during 2001-2002 are shown in the Fig. 3.6

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2001					X	X	X	X			X	
2002					X	X	X	X		X		

Fig 3.6. Spawning calendar of *B. spirata* during 2001 - 2002.
(Coloured columns indicating the spawning activity)

Frequency of spawning: The spawning periodicity was low, restricted to a single day in March 01, October 01, March 02, September 02, November 02 and December 02. The total number of egg capsules obtained during the spawning and the frequency of spawning in each month is shown in the Fig. 3.7. However longer spawning duration of 16 days was also obtained during the study period in the month of December 2001 followed by the 15 day duration in September 2001. During April 2002 three spawning were obtained and the duration between these was 13 days. The total number of egg capsules obtained during the spawning and the average number of spawners in each month are shown in the Fig 3.8**.** Monthly spawning details and spawning frequency of *B.spirata* during the year 2001-2002 are shown in the Table 3.1.

Table- 3.1. Spawning frequency, no. of egg capsules and duration between spawning during the period 2001 and 2002.

Month	Spawning date	No. of egg capsules	Spawning frequency	Duration between spawning (days)
Jan- 01	24.01.01	120	4	6
	28.01.01	80		
	29.01.01	62		
	30.01.01	120		
Feb- 01	16.02.01	230	2	4
	20.02.01	183		
Mar-01	19.03.01	92	1	0
Apr-01	19.04.01	80	3	4
	20.04.01	86		
	23.04.01	80		
Sept- 01	15.09.01	40	4	15
	18.09.01	100		
	21.09.01	43		
	30.09.01	220		
Oct- 01	02.10.01	180	1	0
Dec- 01	11.12.01	200	3	16
	24.12.01	200		
	27.12.01	250		
Jan -02	10.01.02	120	4	6
	11.01.02	40		
	14.01.02	80		
	16.01.02	65		
Feb -02	02.02.02	80	3	8
	05.02.02	150		
	10.2.02	130		
Mar -02	31.03.02	82	1	0
Apr -02	01.04.02	80	3	13
	06.04.02	80		
	14.04.02	40		
Sept -02	26.09.02	300	1	0
Nov -02	30.11.02	160	1	0
Dec -02	28.12.02	450	1	0

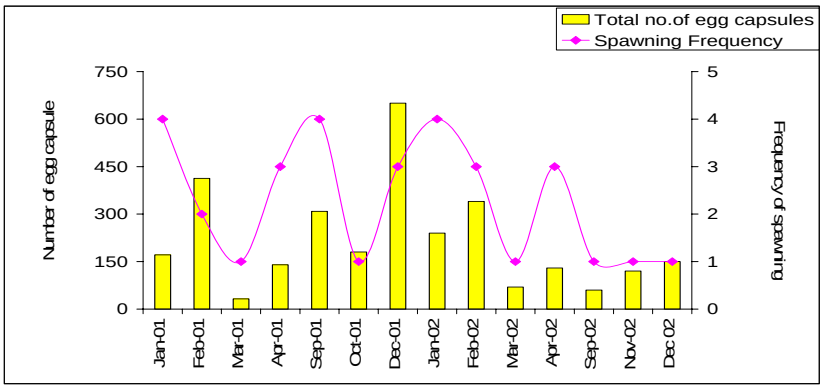


Fig. 3.7. Total number of egg capsules obtained and frequency of spawning during 2001-2002.

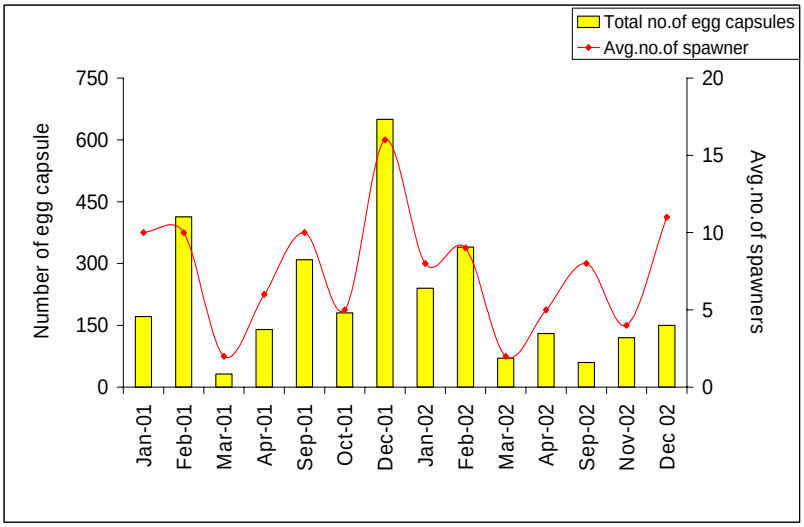


Fig. 3.8. Total number of egg capsules obtained and average number of spawners during 2001-2002.

Seasonal variation in the fecundity of *B. spirata*

All eggs in the egg capsules were in fertilized condition. The average size of the fertilized egg inside the capsule was 240 µm in diameter irrespective of the size of the spawner, season and size of the egg capsule. Immediately after spawning the developmental processes were initiated. Progress of development of all eggs inside the capsule showed that each egg was fertilized and had the capacity to develop into full grown veligers.

The size of the brooder varied in different months of the year 2001 and 2002 and ranged between 35- 45 mm. Out of 14 months of the study period, brooders with the shell height 35-40 mm were obtained in 12 months. The average number of egg

capsules produced per female was always 40 ± 5 mm, but variations were significantly different (P< 0.5) in the capsular size and number of eggs present in it. So the fecundity of the whelks varied in different months.

Fecundity of whelk was found to be low in March of both the years which was 13260 ± 348 and 13860 ± 583 in 2001 and 2002 respectively. In September 2002 also fecundity was low 13869 ± 366. Fecundity was higher in January and February of both years. Higher fecundity was recorded as 26930 ± 4178 in January 2002 followed by 26040 ± 700 in February of the same year. In 2001, higher fecundity recorded was 25640 ± 1339 in December followed by 25560 ± 907 in February and 24520 ± 2441 in January. The average size of the spawner was high in the month of April 2001 (44.24 ± 1.67 mm) and the fecundity recorded was 22600 ± 3568. The monthly variation in average size of the spawner, total length (TL) of the egg capsule and capsular length (CL) of the egg capsule and fecundity in different month of the study period are given in the Table 3.2

Table- 3.2. Monthly variation in the average spawner size fecundity and during the period 2001 and 2002

Month	Average SL of the spawner (mm)	Total length of egg capsule (mm)	Capsular length (mm)	Fecundity (no.of eggs/animal)
Jan-01	39.22	31.92	12.3	24520
Feb-01	37.4	29.92	16.66	25560
Mar-01	39.41	24.63	8.88	13260
Apr-01	44.24	29.97	10.68	22600
Sep-01	35	29.20	16.87	22737
Oct-01	36.1	27.79	16.34	23700
Dec-01	38.5	31.22	12.21	25640
Jan-02	37.6	28.91	12.42	26930
Feb-02	41.4	29.78	12.18	26040
Mar-02	37.2	23.35	11.27	13860
Apr-02	39.7	28.41	12.67	22800
Sep-02	38.8	26.13	11.81	13869
Nov-02	39.9	27.32	12.07	20700
Dec-02	39.2	29.87	15.73	22432

3.2.2. Effect of size of brooder on fecundity and size on egg

The fecundity of *B. spirata* varied with size. The egg capsule produced by the larger female was bigger. The average total length of the egg capsule produced by the three size groups were 33.67 ± 0.58 mm for larger female, 29.07 ± 0.5 mm for medium size group and 25.93 ± 0.90 mm for smaller group. The number of eggs in the egg capsule also varied according to the capsular size of the egg capsule. As the capsular size increased, the number of eggs in the capsule also increased. So the fecundity was higher for larger females and larger capsules. The fecundity obtained for the three size groups are 27594 ± 51, 21801 ± 77 and 18903 ± 70 for larger, medium and smaller whelks respectively. The size class and average fecundity is shown in the Fig 3.9.

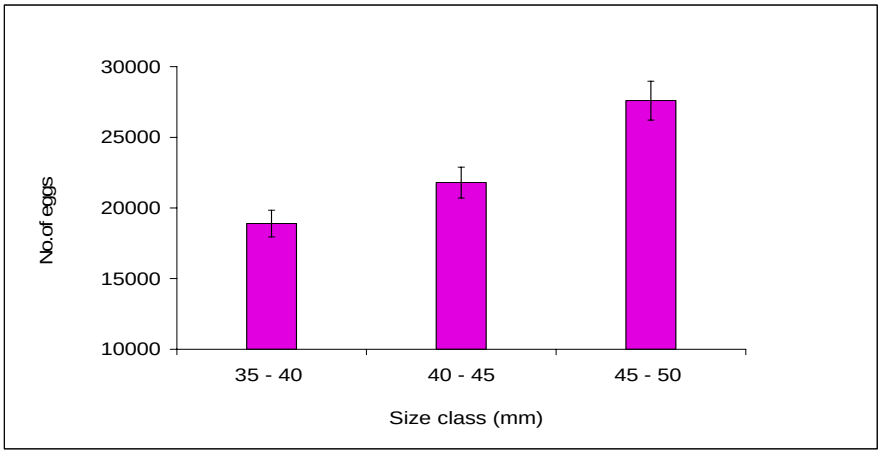


Fig. 3.9. Fecundity of *B. spirata* for different size class

3.2.3. Requirement of substratum for spawning

In the first set of experiment when the whelk was released in the region without substrata they moved towards the substrate and buried within 30 min. Presence of substratum was preferred by the spawning females for laying the eggs. In the tank provided with sand substratum, it was observed that the females moved to a common area and egg capsules were deposited by all females in the same region. No egg capsules were obtained from the exposed region. The spawning occurred in this set on 2nd day of the experiment and continued on the following day. On the first day of

spawning, 75 egg capsules were obtained from an area of 225 cm^2 and on the next day 80 egg capsules were obtained from the same area.

In the second set of the experiment without substratum, the spawning was delayed. The egg capsules were obtained only on 10^{th} day of the experiment and the number was also low (n= 20). The egg capsules were scattered in the different region of the tank, 25% cemented to the tank bottom which were standing erect, while 75% fell on the bottom of the tank. On 13^{th} day also 10 egg capsules were obtained out of which three were cemented to the tank bottom. No further spawning was recorded till the day of termination of the experiment on 15^{th} day.

3.3. DISCUSSION

B. spirata exhibited specialized spawning behaviour especially activities like half buried nature instead of complete burying and they showed spawning aggregation and pair formation. Several gastropods showed specific spawning behavior. Aggregation is a common feature of buccinids also. The attraction of males to females during copulation period as observed for *B. undatum* (Martel *et al*., 1986a) and in *B. spirata* in the present study has also been reported for other neogastropods (Magalhaes, 1948; Pearce and Thorson, 1967; Edwards, 1968; D'Asaro, 1970a). This aggregation behaviour may be provoked by pheromones released by gravid females (Martel *et al*., 1986a). *B. undatum* also shows spawning migration, often moving towards the shore in Atlantic Nova Scotia (Kenchington and Glass, 1998). They have observed females mating with more than one male and have observed the whelks to store the sperm for nearly 8 weeks. Multiple copulation have been reported for a number of gastropods for example, *Urosalpinix cineria* (Hargis and MacKenzie, 1961), *Cepaea nemoralis* (Murray, 1964), *Neptunea antique* (Pearce and Thorson, 1967), and *Eupleura caudate* (Hargis and MacKenzie, 1961). For the last two species, two males copulating simultaneously with a female is also described. The genetic importance of polygamy for neogastropods with internal fertilization is discussed by Hargis and MacKenzie (1961), and Houston (1971). As a result of multiple copulation the juveniles hatching from a single egg mass have greater genetic variability. This is probably particularly important for the species which have

a slower rate of gene flow due to their completely benthic development. Contrary to these instances of polygamy, a specific instance of pairing between the same male and female has been described in the common egg shell, *Ovula ovum* in Majuro, Marshall islands (Hamel and Mercier, 2006a). They tagged the snails and found that no multiple copulation involving different individuals was ever recorded.

Hamel and Mercier, (2006a) observed that the snails *O. ovum* formed pairs as the breeding period approached, often 2-6 days before copulation. The female was observed to carry the generally smaller males on its back without any signs of copulation for 3 to 4 days. In those instances the same male was found on the back of the same female every day, yet they moved separately during night and remained close to each other. Most copulations occurred in the morning between 0400 and 1000 h, although a few were recorded at various times of the day. Copulation typically lasted between 34-72 min. In the present study pairing and some sort of intimacy between male and female whelks was observed, 4-10 days before spawning, but polygamy was not evident. Studies on spawning of *Lambis lambis* by Hamel and Mercier (2006b) observed that pairing and copulation occurs in the middle of the day. Observation on the spawning behaviour of *B. areolata* by Hua *et al*. (2001) reported that the adults often mate in the evening and at night 2-3 days before spawning. In the case of *B. undatum* copulation takes place 3 weeks to two months after copulation (Martel *et* al., 1986a). Contrary to this the copulation was often observed while the female was in the process of depositing egg capsules in the case of *Busycon carica* (Power *et al*., 2002). Studies on the spawning behaviour of the intertidal snail *Cerithidea cingul*ata by Sreenivasan (1997) during the nuptial act, the male clings to the female close to the right pallial side and hold is stronger to the extent of both of them could be lifted off the ground. He reported that mating occurred during day time as well as in the early part of the night and extends for more than one hour. Position of the male and female varied during copulation but usually male is on the right side of the female. In the present study the copulation process were observed both in the day and night hours, and it lasted for 2-3 h, during that time both animals were in an immobile posture. During copulation the female

positioned on the right side of the male and mounted slightly over the male in the present study. Any slight disturbances in the water separate them apart.

Egg laying can start immediately or can be extended up to 2 months after copulation. Ramorino (1975; 1979) reported that females of the Chilean muricids *C. concholepas* can store viable sperms in the seminal receptacle for several months after copulation. During the present study when female whelks collected during March were stocked separately without male whelks the females spawned. The same brood spawned in April and May indicating that sperms stored were viable and egg laying can be prolonged up to 8 to 10 weeks. Similar observations have been made in *B. undatum* also (Kenchington and Glass, 1998). Martel *et al*. (1986a) have reported in the same species that egg laying lasted several months after copulation. The storage of sperm by females is probably advantageous as females are independent of males when selecting egg laying sites. In fact, Martel *et al*. (1986a) have found that females leave the deeper sediment bottom to shallow and where they deposit their eggs. If a female is interrupted during egg laying she could probably resume this activity. The storage of sperm thus permits the selection of favourable time and place for egg laying.

Egg laying was found to be in groups, often occurring in the same area. In *B. undatum* upto 15,000 egg capsules in a collective egg mass has been reported (Dons, 1913). Egg laying in groups has been reported for many gastropods, including *Urosalpinx cinerea, Dicathais aegrota, Nucella (Thais) lapillus, Thais emarginata, Thais lamellos*e and *Ceratostoma foliatum* (Hancock, 1960; Phillips, 1969; Feare, 1970; Houston, 1971; Spight, 1974; Spight *et al*., 1974). It may be a mechanism to make optimum use of favourable egg laying sites. Other gastropods also migrate during the egg-laying period (Thorson, 1950; Carriker, 1955). Such migrations probably serve in finding sites favourable for development of embryos and larvae or where predation on egg capsule is limited. At Cap du Corbeau (in Canada) the migration of *Buccinum* consisted of a shoreward movement of adults over a short distance to the region of large boulders in shallower water, whereas at Anse des Noyes (Northern Gulf of St. Lawrence, Canada). There is probably a random movement in search of scattered boulders. Thus, the type of movement during the egg-laying period probably depends on the relationship between feeding areas and

favourable egg-laying sites. Such congregation also helps fishers. An interesting example is given by Castilla and Cancino (1976) whereas productive divers look for " *Concholepas spot*" or congregation of the snails in order to collect simultaneously several specimens. In these spots the snails were usually one on the top of the other and when dislodged large clutch of egg capsules were deposited.

The process of egg laying in *B. spirata* occurred mostly during early morning hours and during night. Few instances were recorded where the egg laying activity took up to early evening hours in the present study. Deposition of egg capsules by *B. areolata* (Hua *et al.*, 2001) reported that the snails deposit egg capsules in the evenings and at nights especially in the spring tide nights. However in *O. ovum* egg laying took as long as 74 h although average was between 32 and 48 h. (Hamel and Mercier, 2006a). In *Concholepas concholepas* spawning activities are known to last from 8 to 77 h (Castilla and Cancino, 1976). They have observed two kinds of spawning activity *viz,* discontinuous spawning, where capsules were laid for periods of 8 to 12 h with resting periods of 21 h and one long and continuous spawning activity which has been observed to last as long as 77 h. Gathering of muricids during the spawning season are frequently reported for the species *Urosalpinx cinerea* (Federighi, 1931; Hancock,1959) and for *Ocenebra poulsoni* and *Shaskyus festivus* (Fotheringham, 1971). For the gastropod *B. areolata* (Hua *et al.*, 2001) the spawning has been recorded during night, while for *B. undatum* (Martel *et al.,* 1986a) and *Chicoreus ramosus* (Ruangchoy and Tantichodok, 1992) the egg laying occurred both during the day and night.

In *B. spirata* brooding by females were not observed as females move away from the egg capsule as soon as the egg was deposited similar to that in *Ovula ovum* (Hamel and Mercier, 2006a) and *Chicoreus ramosus* (Nugranad and Promchinda, 1995). In contrast to this, in the spider conch *Lambis lambis* Hamel and Mercier (2006b) have observed the females remain to close to the spawn covering it entirely or in part with their shell from egg laying till early veliger stage. This protective behaviour ceased at the beginning of the third day of development. The females moved away a few hours before veligers hatched from the filament on day 3. The prosobranch *Bullia*

melanoides produce egg capsules which remain attached to the female who guards them until they are released (Ansell and Trevallion, 1970).

Starvation or reduced intake of feed was observed as a prelude to copulation and spawning in the present study. Similar behaviour has been reported for *B. undatum* (Martel *et al*., 1986a; 1986b), for the dog whelk *Nucella lapillus* (Feare, 1970) and for the intertidal prosobranch *Thais lamellose* (Stickle, 1973) and for the muricids snail *Concholepas concholepas* (Castilla and Cancino, 1976).

In the present study it was observed that *B. spirata* deposited the egg capsules on a small disc like flaccid structure on the sand grains. Lesser number of egg capsules was obtained from the spawning tank which was not provided with substratum. When egg capsules were laid on the tank without substrate, the capsule stakes bent and often resulted in fungal infection. The necessity of substratum for depositing the egg capsules were also recorded for many gastropods. The egg capsules of *Ovula ovum* found firmly attached to the substratum as reported by Hamel and Mercier (2006a). The egg capsules of the muricid snail *Rapana venosa* found attached to the sand or small gravels as reported by Chung *et al*. (2002), while it was in clusters in the melonginid gastropods *Hemifusus pugilinus* (Patterson and Ayyakkannu, 1997), *Pleuroploca trapezium* (Raghunathan and Ayyakkannu, 1994). Similar to the observations of egg capsules of *Chicoreus ramosus* by Nugranad and Promchinda (1995) and Ruangchoy and Tantichodok (1992), no egg capsules were found attached to the shell of the whelk in the present study. Prosobranchs like various species of *Natica* deposited their egg capsules in the interstices of sand grains (Fretter and Graham, 1962), *Bullia melanoides* deposit egg capsules on the female shell itself (Ansell and Trevallion, 1970). Barnett *et al*. (1980) reported that *Nassarius reticulatus* showed strong tendency to lay the eggs capsules on the available red algae, although when no weed was available they deposited the egg capsules on the wall of the aquarium tank. The substratum on which egg capsules deposited is known to vary. *B. undatum* lays the egg capsules on irregular surface and faces of boulders and strips of kelp (Kenchington and Glass, 1998). Martel *et al*. (1986b) are of the view that depositing egg mass on such areas makes it less accessible to the predators like sea urchins.

Almost year - round spawning has been observed for *B. spirata* and studies on other whelks like *B. areolata* have also suggested similar prolonged spawning. However the intensity of spawning and the number of egg capsules laid also showed variation. Peak was observed during the month of December with maximum number of egg capsules. Such prolonged spawning with peak has been observed in *B. areolata* also (Hua *et al.*, 2001). Related to this was the size of capsule in relation to the size of the brooder. It was observed that larger egg capsules were laid by larger female whelks. In the present study the average number of egg capsules produced per female was 40 ± 5 but significant variation in capsular size and number of egg present resulted in varied fecundity during different months. Hua *et al*. (2001) have stated that fecundity of *B. areolata* increased with size of brooders and depended on their health and feeding. However the average capsules per individual was almost similar (38 nos) to that observed in the present study.

In *B. undatum* capsule size was strongly correlated with female size (Valentinsson, 2002). For prosobranch gastropods the general trend is that the number of eggs increases with female size and that egg size is dependent of female size (Spight *et al.*, 1974; Spight and Emlen, 1976; Miloslavich and Dufresne, 1994; Ilano *et al.,* 2004). However the production of more eggs in large females does not necessarily result in greater number of hatchlings in instances where there are nurse eggs (Valentinsson, 2002). Numerous studies on gastropods also report that larger females produce larger clutches; *Buccinum cyaneum* (Miloslavich and Dufresne, 1994); *Thais lamellose* and *T.emarginata* (Spight and Emlen, 1976) and *Fissurella barbadiensis* (Hughes, 1971).

The size of the egg was almost similar (242 µm) for *B. areolata* (Hua *et al.*, 2001) and in the present study (240 µm), but was much smaller than that observed in *B. spirata* (400 µm) by Shanmugaraj *et al.* (1994). In *C. concholepas* it has been observed that the large capsules are laid by large females (Castilla and Cancino, 1976).

Chapter 4

Egg capsules of Babylonia spirata and its rearing

4. EGG CAPSULES OF *BABYLONIA SPIRATA* AND ITS REARING

4.1. MATERIALS AND METHODS

Immediately after the termination of the spawning process the egg capsules along with its base were carefully removed from the spawning tank. These egg capsules were cleaned and washed in filtered sea water and transferred to the rearing tank provided with aeration (Fig. 4.1). The developmental stages were observed under a light microscope. Without breaking the capsule, the diameter of eggs and embryos inside each capsule were measured using the ocular micrometer under 10x magnifications. Ocular micrometer was calibrated by comparing the ocular micrometer with a calibrated stage micrometer.

The number of eggs per capsule was counted by breaking the egg capsule at the apical region and squeezing out the eggs from the bottom of the capsule into a petridish. Counting was done under the microscope. The morphology of the egg capsule, seasonal variation on the fecundity, allometric relation and growth rate were tested by well planned experiments and results were analyzed by one way ANOVA using SPSS 7.5 software.

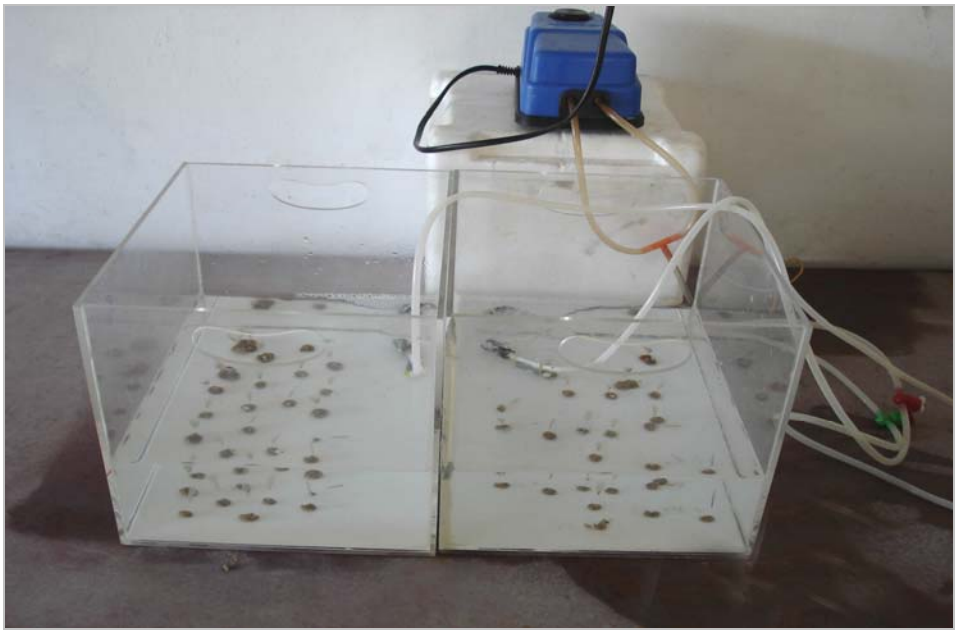


Fig. 4.1. Egg capsule rearing tank.

4.1.1. Morphology of the egg capsule

The morphology of the egg capsules of *B. spirata* was studied under the microscope and the thickening of the side walls measured. Photographs during development phases were taken under a stereozoom microscope using a camera attachment and also with the help of a manually operated camera using an eyepiece adaptor. The variation in the development of larvae in the egg capsule with and without cementing of peduncle was studied. Three sets with 10 cemented capsules in each were taken carefully and replanted in the experimental tank with 3 l of seawater. Three different sets (10 capsules each) which were uncemented and free were taken and kept in an identical 3 l container. Both the treatments were aerated and the salinity in the experimental containers was maintained 32 ppt and temperature at 27± 1^0C. Observations were made on the growth and development of the egg till the day of hatching of the veliger.

4.1.2. Variation in the dimensions of the egg capsule

The dimensions of the egg capsules were measured using a digital vernier of 0.01 mm accuracy. From each spawning, 30 egg capsules were taken and their total length (TL), capsular length (CL), peduncle length (PL) and capsular width (CW) at the broader apical portion were measured. Diagrammatic representation of the egg capsule is shown in Fig. 4.2.

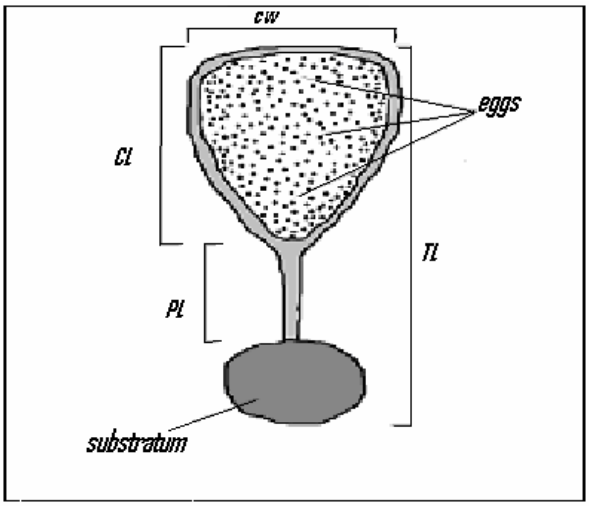


Fig. 4.2. Diagrammatic representation of the egg capsule

4.1.3. Percentage of hatching of the veliger

Observations were made to study the percentage of larvae hatched out from the egg capsules in different spawning period. Three egg capsules were selected from six spawning in different months (Jan 01, Mar 01, Dec 02, Jan 02, Mar 02 and Dec 02) to study the percentage of larvae released out from a capsule during the process of hatching. Individual egg capsules were taken and the numbers of eggs were counted under the light microscope. Then each of capsule was kept for hatching in separate beakers of 1 l capacity. The salinity of the rearing water was maintained 32 ± 2 ppt , pH 8.1 ± 0.2 and temperature 27 ± 1^{o}C. Water exchange was done during alternate days and the mild aeration was provided throughout. No disturbances were made when the hatching process was progressing. Number of larvae hatched out were counted and stocked in the rearing tank for further development. The egg capsules were left undisturbed to complete the hatching and the larvae which hatched out on the following day were counted and stocked in separate rearing tank. Experiments were conducted in triplicate and repeated for different spawning periods.

4.1.4. Effect of water treatment during incubation of egg capsule

Experiments were conducted to determine the need for the treatment of water with hypochlorite solution. The water used for the experiment was treated with hypochlorite solution at a concentration of 30 ppm and aerated well for 24 h. After 24 h it was dechlorinated with sodium thiosulphate and presence of chlorine was tested with toludine solution. Chlorination status of water was confirmed by carrying out ortho toluidine test where in 1 ml declorinated water a drop of ortho toluidine reagent was added. The samples not showing yellowish coloration were labelled as negative (Jacob, 1967). The effect on this treatment was compared with non treated water by rearing the egg capsules in both treatments. The salinity of the rearing water was maintained 32 ± 2 ppt, pH 8.1 ± 0.2 and temperature 27 ± 1^{o}C. Water exchange with stored treated water was done during alternate days and the mild aeration was provided throughout the experimental period.

4.1.5. Water exchange during incubation

Experiments were conducted to determine the need of water exchange on capsular development during the period of incubation. Ninety egg capsules were selected for the experiment and separated in to nine groups, each group with ten egg capsules. Three sets of experiments were designed (1) without water exchange till the day of hatching (2) with water exchange on alternate day and (3) with complete water exchange on every day. Aeration was provided in the entire experimental tank and the salinity was maintained at 32 ± 2 ppt and pH 8.1 ± 0.2. Observations were made daily on the development of the larvae inside the egg capsule under the light microscope. The water used for all the experiment was treated with 30 ppm hypochlorite solution prior to the initiation of the experiment.

4.1.6. Effect of salinity on egg capsule

The egg capsules with embryos in the morula stage having a slight rotation of the embryo were exposed to different salinities to determine whether there is influx of water into the capsule. Individual egg capsules were immersed in 15, 20, 30, 32 and 35 ppt salinities and weighed at intervals of 5, 10, 15, 20, 25, 30 and 60 minutes. At each weighing, the egg capsules were blotted carefully and weighed. The egg capsule was also observed under the microscope through out the experimental period. After 1 h and 2 h exposure to these salinities a small incision was made on the egg capsule and the albuminous fluid was squeezed out and the salinity of this fluid was measured using a refractometer.

4.1.7. Effect of salinity on hatching

Healthy egg capsules (n= 18) which were erect and of average TL 32.2 ± 2.3 mm, CL 19.8 ± 1.3 mm and CW 10.7 ± 1.0 mm which contained 620 ± 50 numbers of eggs were selected for the experiment immediately after spawning. The fertilized eggs which were in a stage to release the polar body were chosen for the experiment. The selected egg capsules were maintained individually in the glass beaker of 1 l capacity. They were exposed to the salinities 20, 25, 30, 35 and 40 ppt and the experiments were conducted in triplicate. All the capsules were observed for their developmental stages under a light microscope. Aeration was provided to each

beaker and the developmental stages were observed daily. The days required for completion of the metamorphosis, hatching and the number of veligers which were released from each capsule was recorded.

4.1.8. Effect of salinity on growth and development during incubation

Three egg capsules of average TL 32.45 ± 2.69 mm, CL 21.02 ± 1.67 mm, CW 11.08 ± 2 mm were used to study the effect salinity on growth and development of the fertilized eggs. Experimental set up was same as described under section 4.1.7. The developments of the larvae inside egg capsule were monitored regularly under the light microscope. The morphological features in different salinities were recorded and the movement of larvae inside the capsule was observed. Measurements of the larvae were taken on 1^{st}, 3^{rd}, 5^{th} and 7^{th} day of experiment using the stage and ocular micrometer.

4.1.9. Effect of temperature on growth and development

Experiments were conducted to study the effect of temperature on the growth and development of the egg to veliger inside the egg capsule. Healthy egg capsules (n= 75) which were erect and cemented to sand grain having an average TL 28.91 ± 2.7mm , CL 16.50 ± 3mm, and CW 12.42 ± 1.2 mm were selected for the study. The capsules were reared at the temperatures 22, 24, 26, 28 and 30^0C. The seawater used for the experiment was dechlorinated and filtered. Developments inside the egg capsules were monitored daily and measurements taken. The aeration was provided to the tank so as to maintain the temperature uniform in the surface and bottom of the water column. Lower temperatures were maintained in an air conditioned room while temperature of 30^0C was maintained using aquarium heaters. Salinity of the water was kept constant as 32 ppt and water was exchanged on alternate days. The experiments were conducted in triplicate.

4.1.10. Effect of aeration on growth and development

An experiment was conducted to study the effect of aeration on the development and release of the veliger during the period of incubation. Water parameters were maintained as described under section 4.1.4. Sixty egg capsules were selected for the study and grouped into two sets. One set was provided with aeration while the other

without aeration. Experiments were conducted in triplicate where in treatments were stocked with 10 egg capsules in 3 l of dechlorinated filtered sea water.

4.1.11. Effect of light on growth and development of the veliger

Experiments were conducted to study the effect of light on the growth and development of the egg to veliger inside the egg capsule. Healthy egg capsules (n= 75) which were erect and cemented to sand grain having an average TL 27.81 ± 2.3 mm , CL 15.50 ± 30 mm, and CW 12.45 ± 1.3 mm were selected for the study. To study the effect of light on growth and development of the veliger one set of the experiment was covered with black cloth to provide darkness to the experimental unit and the other was kept open. Aeration was provided to both set of experiment through out the study period. The development of the larvae inside the egg capsule and the number of days required for hatching of the veliger from the egg capsule were monitored daily.

4.1.12. Changes in the weight of the egg capsule during the period of development of the embryo

Fifteen egg capsules of average TL 28.91 ± 2.7 mm , CL 16.50 ± 3 mm, and CW 12.42 ± 1.2 mm were selected for the experiment. Measurements of each capsule were made using the digital caliper (Mitutoyo, Japan of 0.01mm accuracy). The weight of the egg capsules were taken daily from the day of spawning till the day of hatching of the veliger. Each capsule were numbered and kept separately in individual beakers for the observing the development. All the optimum conditions required for the development of the embryo were satisfied based on the result of the above experiments.

4.2. RESULTS

4.2.1. Morphology of the egg capsule

The female whelks deposited the egg capsules which were produced by the pallial duct. The egg capsules were attached firmly to the substratum by a slender peduncle in an erect position. (Fig. 4.3). The egg capsules were cemented to the sand substratum in such a way that the peduncle is surrounded by the sand particles which

adhered to each other. The circular or oblong cemented sand base ranged between 10 to 12 mm diameter. The egg capsules are inverted triangular in shape and transparent. The eggs were visible within the capsule and could be counted without breaking the capsule under the microscope.

Fig. 4.3. Egg capsule with substratum

The peduncle of the capsule rested on a flat base of an average diameter of 6.26 mm which helps in attaching to the sand particles. A rigid base was seen only when there was a sand substratum and this was helpful in stocking or replanting the capsules to other rearing tanks. These capsules with sand base were erect and swayed in the water and were healthy. Development in all these capsules was normal and disease outbreak was nil. In such erect egg capsules the larvae were released in to the water column and these moved away for further growth.

The walls of these capsules were double layered, varying in thickness. The apical portion of capsular wall was thinner than the rest of the region. The sidewalls were

139 ± 12 μm in thickness while the apical region through which the larvae released were 81.33 ± 15 μm. The average thickness of the peduncle was of 340 ± 55 μm.

When no sand substratum was present, the flaccid membrane base was seen but the peduncle could not support the capsule to maintain the erect position. Hence all capsules were bent and often fell on to the substratum. Fungal and ciliate attacks were seen in bent capsules and the developments were not normal. Decaying of the eggs in the egg capsules without sandy base resulted in the thickening of the capsular membrane and the thickening spread to the lower part of the capsule resulting in complete mortality of the embryo within the capsule (Fig. 4.4 & 4.5)

Only in 5% of the total number of bent egg capsule the developments were normal and the larvae released were almost on the bottom of the rearing tank and they could not move or develop to planktonic stage in development.

4.2.2. Variation in the dimensions of the egg capsule

Size of the capsule

The size of the capsules laid by *B. spirata* showed wide variation. The maximum length of the egg capsule obtained during 2001 was 39.08 mm in September and minimum 20.01 mm in October. During 2002, a largest size egg capsule of length 32.74 mm was obtained in the month of January. In the same year the smallest egg capsule obtained was of 22.1 mm in March. The average length of the egg capsule in each month ranged between 24.63 mm and 31.92 mm during the study period.

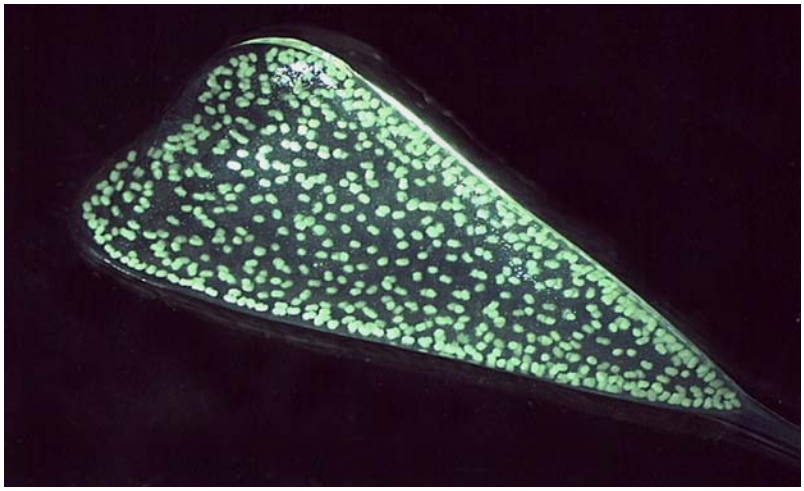


Fig. 4.4. Healthy egg capsule with eggs

Fig.4.5. Infected Egg capsule

Capsular length

Length of the egg capsule also showed variation throughout the study period. During 2001 minimum length of the egg capsule observed was in September and March, the length being 8.59 mm and 8.6 mm respectively. Maximum CL obtained was during the month of September, 23.65 mm, while in 2002, the maximum CL was 17.6 mm in December and minimum, 9.1 mm in April. Monthly variation in the total length and capsular length of the egg capsule are shown in the Fig 4.6.

Capsular width

Width of the egg capsule also showed variation in the maximum and minimum measurements which were 15.00 mm, and 5.00 mm respectively in October 2001 and September 2001. In April 2002 maximum width was 12.50 mm and minimum was of 5.60 mm. However the average size of the capsular width ranged between 7.64 and 10.96 mm during the study period. Monthly variation in the capsular length and capsular width of the egg capsule are shown in the Fig 4.7.

Peduncle length

Peduncle length of the capsule varied during the study period. The shortest peduncle length observed was 5.70 mm in Sept 2001 and the longest 23.62 mm was also in the same month. During 2002 also longest peduncle of length 19.57 mm was obtained in January, while shortest 10.8 mm was during January and March.

Number of eggs inside the egg capsule

The number of eggs showed variation in each month. The maximum number of eggs obtained during the study period was in the month of January 2002. During the month of March in both years the number of eggs recorded was low, 320 in 2001 and 337 in 2002. The average number of eggs recorded during the study period was maximum in the month of January 2002 was 673 ± 104 followed by the February 2002 and December 2001. The minimum and maximum values of TL, CL and number of eggs are shown in the Table 4.1. Monthly variation in the average number of eggs during the year 2001 to 2002 is shown in the Fig. 4.8.

Table 4.1. Minimum and maximum measurements of the egg capsules during the period 2001 and 2002

Month	Minimum total length (TL)	Maximum total length	Minimum capsule length	Maximum capsule length (CL)	Minimum no. of eggs	Maximum no. of eggs
Jan-01	29.00	34.00	10.60	13.60	542	668
Feb-01	22.00	36.20	10.80	19.60	595	660
Mar-01	23.10	25.40	8.60	10.00	320	340
Apr-01	27.00	34.44	9.00	14.44	450	690
Sep-01	21.38	39.08	8.59	23.65	500	640
Oct-01	20.10	33.98	9.93	21.90	542	618
Dec-01	28.60	33.40	9.80	13.60	583	682
Jan-02	23.40	32.74	10.50	14.20	500	810
Feb-02	28.89	30.13	10.99	13.43	627	673
Mar-02	22.10	25.20	9.50	12.60	337	368
Apr-02	24.10	32.40	9.10	16.10	520	650
Sep-02	25.15	27.19	10.19	13.69	335	360
Nov-02	23.50	28.80	10.87	13.30	333	368
Dec-02	26.80	32.40	12.00	17.60	520	600

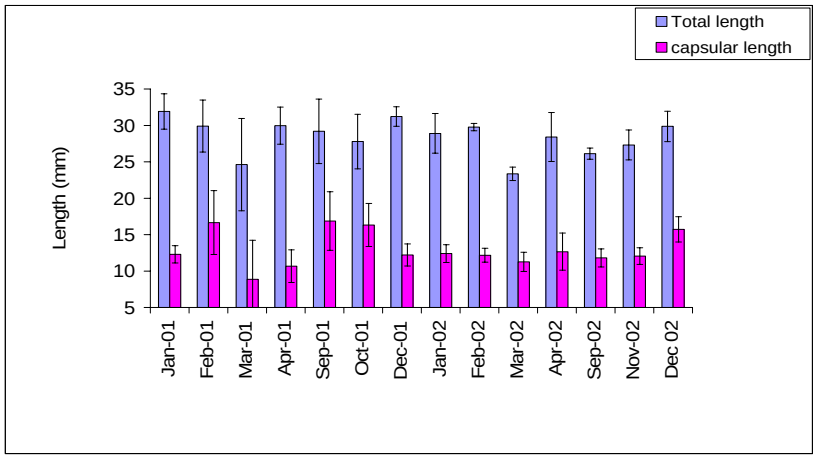


Fig. 4.6. Monthly variation in the average total length and capsular length of the egg capsule during 2001-2002

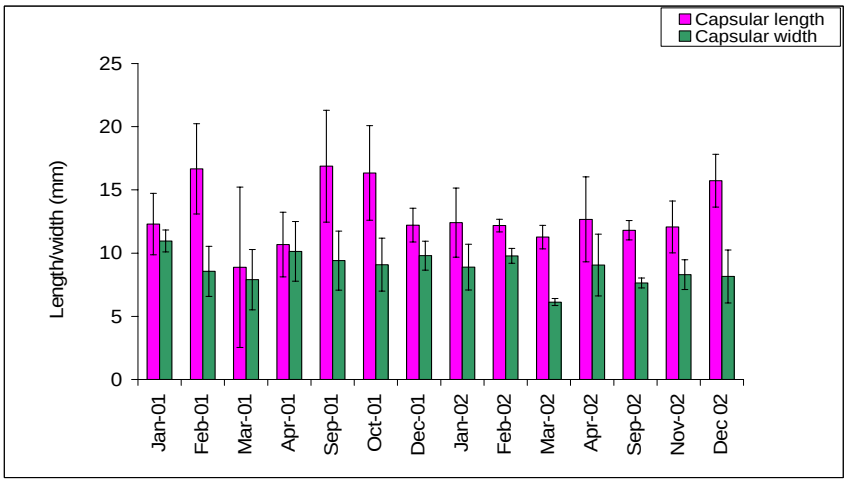


Fig. 4.7. Monthly variation in the capsular length and capsular width of the egg capsule during 2001-2002

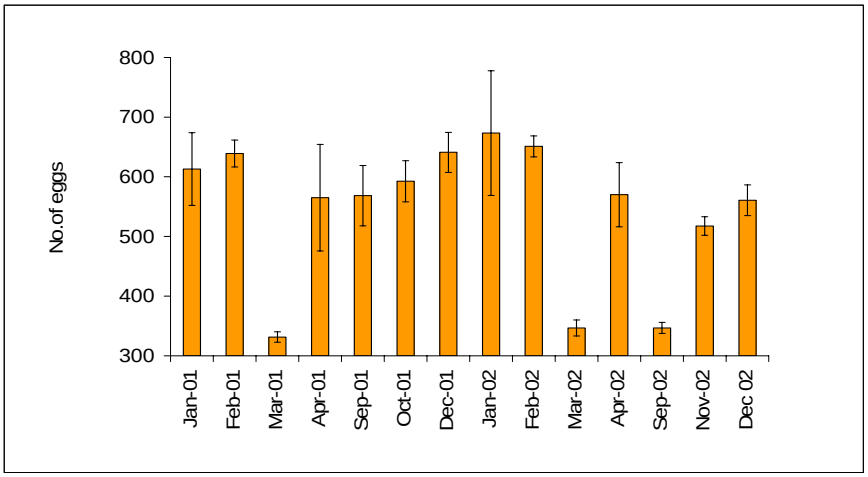


Fig. 4.8. Monthly variation in the average no. of eggs during the year 2001-2002

4.2.3. Percentage of hatching of the veliger

Hatching process of the larvae from all the capsules (n=16) started on 7th day after spawning and the process was completed on 8th day. Immediately after hatching the healthy larvae swims to the upper layer of water column and weak ones remain at the bottom of the container. Though the average number of eggs in different months varied (avg = 631, 344, 620, 767, 321 & 569), the percentage of hatching did not show much variation. The average % of hatching on 7th day was 66.6 ± 3 and 28.2 ± 3 in the following day. The average percentage of larvae released was 94.7 % in all experiments irrespective of the number of eggs in the capsule. There was high positive correlation between the number of eggs and the number of veliger released

(r = 0.999). The percentage of hatching and the average number of larvae released from the egg capsule are shown in the Fig 4.9.

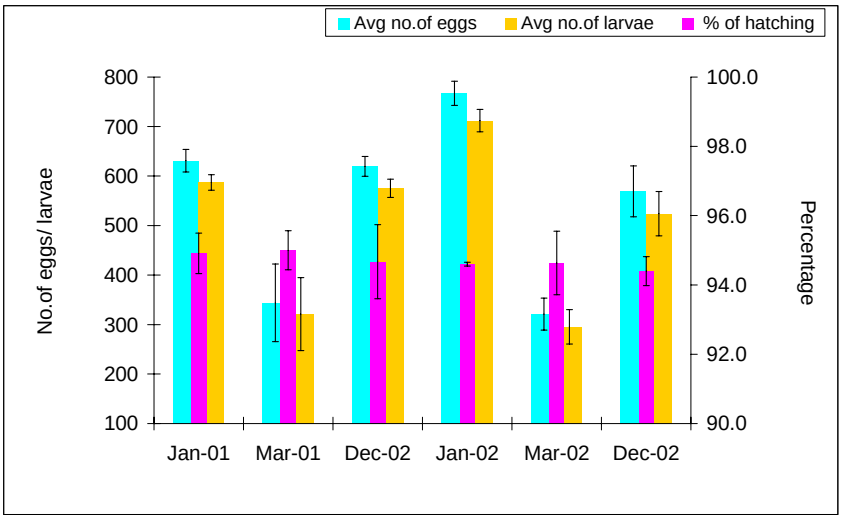


Fig. 4.9. Percentage of hatching of the veliger in different spawning

4.2.4. Effect of water treatment during incubation

Fungal infection was observed in the egg capsules which were placed in the seawater without any prior treatment. None of the egg capsules survived and reached the stage of releasing the larvae. The infection was recorded from the 3rd day of the experiment and 50% got infected by the 5th day. Complete mortality was recorded on 7th day of the experiment. There was no mortality and infection to the capsules in pretreated water. The number of days required for the release of the larvae was same in both treatments.

4.2.5. Water exchange during incubation

In the treatment without water exchange fungal attack of the egg capsules were recorded. During the fifth day of the experiment 10% of the capsules were removed from the experimental tank due to the fungal infection. The infection gives a pink colour to the capsular membrane. Developmental stages were retarded in this condition and all the embryos clumped together as a single mass. During the seventh day only 70% of the egg capsules were free from the infection and the process of hatching started on the following day. Only 30% hatching occurred on the eighth day

and the larvae released remained in the bottom of the container and were mostly inactive and unhealthy. Hundred percentage survival of the egg capsules were recorded for the other two treatments. The release of larvae from the capsule started on the 7th day and 62% were released on this day. The larvae were healthy and active in the water column. In these treatments 95% of the eggs hatched out as veliger and the hatching process was completed on 8th day after spawning. Comparison of the three treatments are given in Table 4.2.

Table- 4.2. Comparison of the effect of water change on incubation of the egg capsule.

Details	TREATMENTS		
	No water exchange	Water exchange on the alternate day	Complete water exchange on every day
Survival of the egg capsule	70%	100%	100%
Days taken for release of the larvae	8-9	7-8	7-8
% of releases of the veliger	40%	95%	95%
Survival % of the veliger	10%	85%	85%

4.2.6. Effect of salinity on egg capsule

During the beginning of the experiment, the internal salinity of the egg capsule was 42 ppt. After 1h exposure to the lower salinities the salinity of the albuminous fluid inside the egg capsule was found to be lower. The salinity after 1h in 15, 20, 25, 30 and 32 ppt were 20, 25, 30, 35 and 37 ppt respectively. After 2 h of exposure, the salinity of the external and internal medium of the egg capsule was found to be the same. The thickness of the albuminous fluid was lost due to the invasion of water into the egg capsule.

The loss in average weight of the egg capsules in 15, 20, and 25 ppt salinities were 0.0215, 0.0196 and 0.0143 g respectively while in 30 ppt the decrease in weight was only 0.0062 g and in 32 ppt it was 0.0025 g. In 35 ppt 0.0017 g of decrease was observed after 1 h exposure.

Immediately after 3 minutes exposure to the lower salinities the embryo in morula stage stopped their rotation and the cells were broken. No further development occurred in these egg capsules and 100% mortality of embryos was observed. The morphological changes in the embryo in control salinity and lower salinity are shown in the Fig. 4.10 & 4.11.

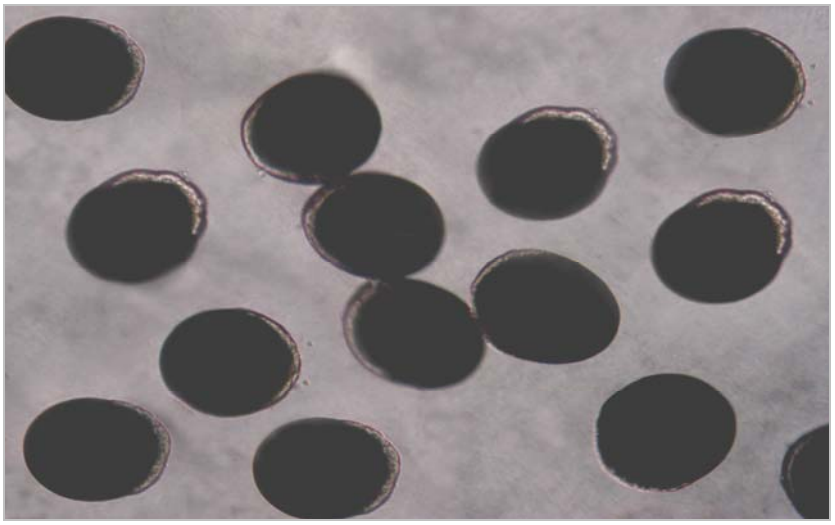


Plate 4.10. The embryo in the control salinity

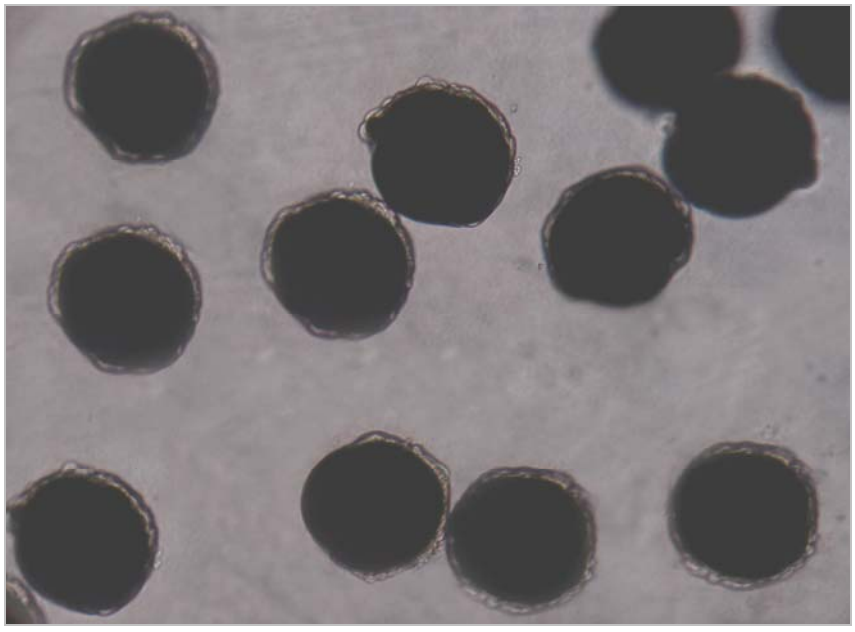


Plate. 4.11. The morphological changes in the embryo in lower salinity

4.2.7. Effect of salinity on hatching

In 20 and 25 ppt salinity no hatching occurred due to the death of the embryo inside the capsule. Only 10% could attain the divisional stages and 100% mortality was recorded on the third day. In 30, 32, 35 and 40 ppt the hatching was recorded on the 7th day and the hatching was completed on the following day.

The percentage of veliger released was found to be highest (95%) in the control salinity while it was 92% in both 30 and 35 ppt salinities and in higher salinities the hatching percentage decreased (90 %). The result of the experiment is shown in the Fig 4.12**.** More than 60% of larvae were released on the 1st day of hatching (7th day of egg release) in all treatments. Percent of hatching varied in different salinities on the 7th day. In the control 68% of larvae were released on the 7th day while it was 60% in 30 ppt, 65% in 35 ppt and 60% in 40 ppt salinities. On the 8th day the percentage of larvae released ranged between 26-32%.

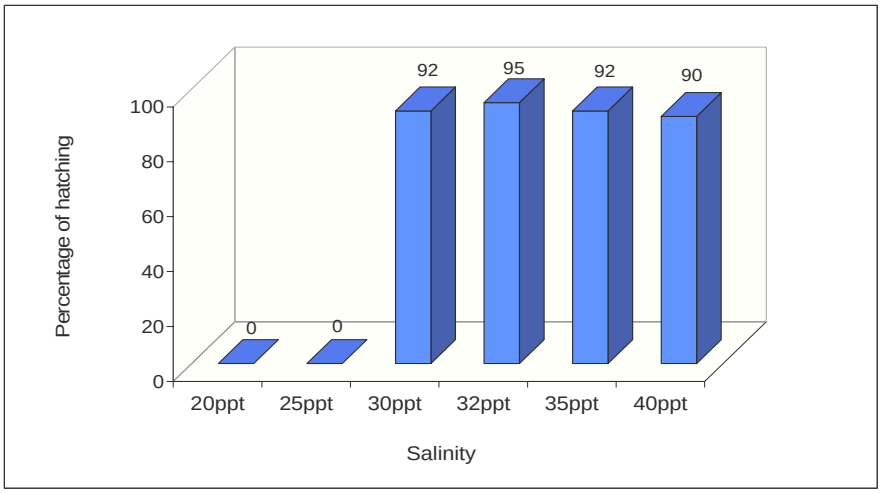


Fig. 4.12. Percentage of hatching of the egg capsules at different salinities

4.2.8. Effect of salinity on growth and development during incubation

The embryo could not complete the divisional stages of normal development in salinities 20 and 25 ppt. The division of the fertilized eggs was found to be very slow in 25 ppt compared to other salinities on the 1st day. Only 50% of the eggs released polar body after 24 h. From this only 20% attained the two cell stage and remaining did not developed further. Four- cell and morula stage was observed for a few eggs

(1%) after 24 h. After this there was no further development and resulted in complete mortality. On the 1st day of experiment the size of the fertilized egg was 240 µm. Few eggs had reached morula stage and these had a diameter of 282 µm.

Mortality of the eggs in the albuminous fluid of the egg capsule led to decay and fungal infection. The transparent nature of the egg capsule was lost and they became an opaque mass with yellowish colour.

In the control salinity normal development was observed. Release of the polar body was observed 30 minutes after spawning and 95% of the eggs released the polar body in 45 min. All the eggs attained the two-cell stage within 1h after the release of the second polar body and reached the four- cell stage in the 2nd h.

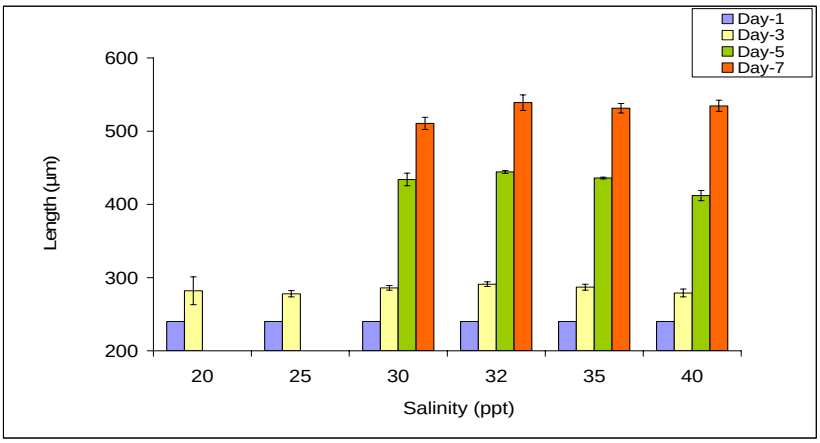


Fig. 4.13. Average length of the growing embryo and veliger larvae within the egg capsule at different salinities

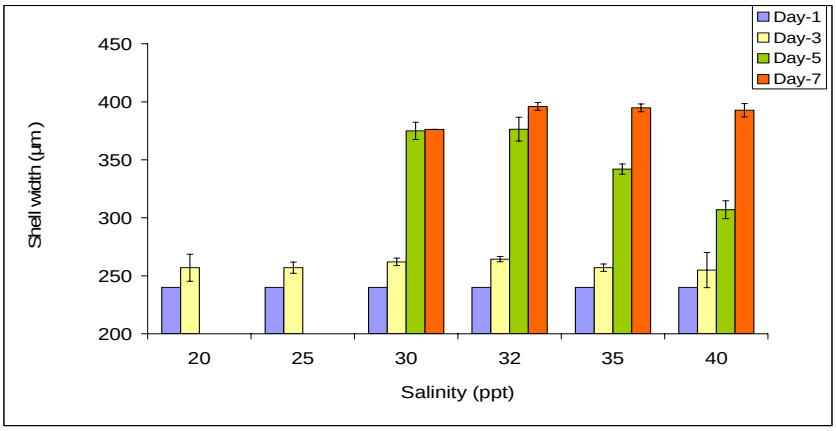


Fig. 4.14. Average width of the growing embryo and veliger larvae within the egg capsule at different salinities.

Embryo developed to morula after 24 h. On the 3rd day slight rotation of the embryo began inside the egg capsule. The veligers attained an average shell length of 460 µm and shell width of 396 µm on the day of hatching. The variation in the average shell length and width of the larvae in different salinities and the size of the veliger larvae on the day of hatching was not significant (P> 0.05) for the salinities 30, 35 and 40 ppt. The shell length and shell width of the veliger on alternate days of experiment shown in Fig. 4.13 & 4.14.

4.2.9. Effect of temperature on growth and development

The growth and development was delayed in lower temperatures. Divisional stages took more time than in the control temperature of 28^0C. In control temperature all the eggs inside the capsule started to release the polar body in 30 min after spawning and release of the second polar body commenced at 90 min. But at lower temperatures from 20-24^0C the release of the first polar body began after 90 min and second polar body at 2 h from the time of spawning. All the eggs in the control temperature attained the two-cell stage 30 min after the release of the second polar body while in lower temperatures it took 1.5 h. All the eggs attained four-cell stage in control temperature after 1h of the first division while in lower temperature it was after 2 h. The morula retained the marginal cilia stage for 48 h in control salinity and started rotation inside the capsule on 3rd day. Lower temperatures extended the duration of morula stage up to 72 h and rotation began only after 4th day. Development of velum was observed in the control temperature on 4th day while in lower temperatures it was on the 5th day. At the temperature of 26^0C the development was same as in the control temperature. In 32^0C, the division was faster than lower and the control temperatures. All the eggs started to release the polar body in 30 min after spawning and all the eggs completed the release of the polar body after 45 min. The two- cell stage was attained by all the eggs at 60 min and four-cell stage in 2 h. The 4 cell stage was observed only for 2 h and morula stage was reached in 12 h. The morula started rotation at 24 h and developed velum on the 2nd day.

The larvae stated to release the veliger at control temperature on 7th day and completed the process on next day. In 26^0C also the same result was observed. But in

lowered temperatures, from 20-24 ^{0}C, the larvae were released only on 11th and 12th day after spawning. With the increase of temperature the development was faster and the capsule started to break open on 5th day and released 60 % of the larvae on 6th day. The percentage of larval release was 80, 82, 86, 94, 95 and 85 in 20, 22, 24, 26, 28 and 30^{0}C respectively. Mass mortality was observed for the larvae released in 30^{0}C treatment on the following day. At lower temperatures the larvae remained in the bottom of the container and retracted the velum into the shell. Planktonic phase was normal only in 26 and 28^{0}C and movements in the column of water was possible with fast movement of cilia on the velum.

4.2.10. Effect of aeration on growth and development of the veliger.

Aeration had influence on the hatching process of the larvae. In the experimental tank provided with aeration, the larvae which were released actively swam in the water column while in the treatment without aeration they usually remained in the bottom of the tank. Percentage of larval release on the first day of hatching was also low (30%) while it was 65% in the treatment with aeration. The growth and developmental stages during the period of incubation was observed to be the same in both treatments. The larvae released from the egg capsules had the size of 460 µm in both cases.

4.2.11. Effect of light on growth and development

Light had no influence on the hatching process of the egg capsule. Release of larvae in both the treatments such as with light and without light occurred on the 7th day after spawning. In both treatments the larvae were active in the water column. The size of the larvae was same.

4.2.12. Changes in the weight of the egg capsule during the period of development of the embryo

The albuminous fluid inside the egg capsule was found to become thin as the development progressed. Gradual decrease in the weight of the egg capsule was observed till the 4th day. Developments of the velar structures were observed from

the 5th day and thereafter the capsular weights were found to increase. The observation on the average weight of the egg capsule recorded is shown in Fig. 4.15.

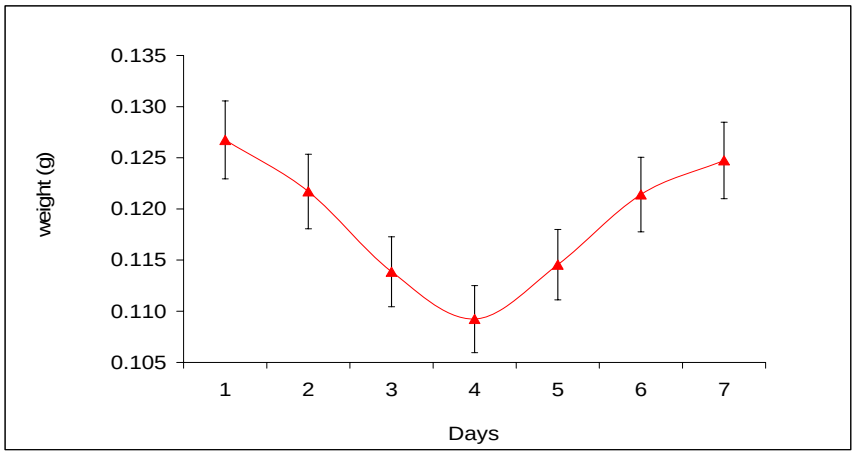


Fig. 4.15. Changes in the weight of the egg capsule during development.

4.3. DISCUSSION

The number of eggs in capsule observed in the present study were almost similar to that observed for *B. areolata* by Hua *et al*. (2001) but slightly lower than that observed in *B. spirata* (900 nos) along the east coast by Shanmugaraj *et al.* (1994). Such instance, where difference in number of eggs for the same species in different region/studies has been observed, a typical example is that of *B. undatum* (Valentinsson, 2002). The number of eggs deposited per capsule observed by Valentinsson (2002) was (700-2,300) were similar to values reported by Portman (1925; 500-3,200), Hancock (1967; 3000), Fretter and Graham (1964; 500-3,000) and Martel *et al.* (1986a; 2,700). However, the number of eggs per capsule was strongly dependent on female size, some variability was to be expected in the number of eggs per capsule. A common feature of *Buccinum* species is that most eggs are ingested by developing embryos as nurse eggs. In the study by Valentinsson (2002) only 0.2-1.2% of the eggs completed development, which compared well with 1% reported by Staiger (1951), and 1.1% reported by Martel *et al.* (1986a). But in *B. undatum* the number of eggs in the capsule were much higher (~ 2700) than in the whelks of *Babylonia spp*. Similar variation has been observed in different species of *Haliotis*. For example in *H. varia*, Minch (2000) observed lower fecundity at Cave Ranh Bay than at Nhatrang Bay.

Natarajan *et al*. (1957) has reported that the egg masses of marine prosobranchs are differ in characteristic their shape and appearance such as gelatinous ribbons (*Cerithidea fluviatilis, Cerithium morus*), peculiar sand encrusted collar-like structures (*Natica marochiensis*), large slimy wavy bands (*Tonna dolium*) and capsules of various shapes and sizes (*Murex virgineus, Thais bufo, Nassa jacksoniana, Ancilla spp., Conus araneosus,* etc.). In the holo-pelagic *Janthina prolongata* the egg capsules were observed to be suspended underneath the float. In *Cellana sp* and the capsules are usually parchment-like., the eggs are extruded singly in hundreds and fertilization is external. The various structure, shape and colour of the egg capsules described for other gastropods were, thick, yellowish, ampulliform egg capsule for *Rapana rapiformis* (Thorson, 1946), vasiform, semi transparent and creamy white egg mass for *Chicoreus ramosus* (Ramesh *et al.*, 1992), vasiform, opaque, pale violet egg capsule for *Pleuroplaca trapezium* (Raghunathan and Ayyakannu, 1994), vasiform, yellowish, white opaque egg mass for *Hemifusus pugilinus* (Patterson and Ayyakkannu, 1997) and inverted triangular egg capsule for *Babylonia areolata* (Hua *et al.*, 2001). In the present study also the egg capsule obtained were transparent, inverted triangular in appearance with a double layered wall. The eggs are visible with the naked eye. Development within the egg capsule started immediately after egg laying in *B. spirata* and similar progress has been observed in *B. areolata* (Hua *et al*., 2001). Moreover the shape of the egg capsules of these two whelks, viz *B. spirata* and *B. areolata* were similar. Variation in shape of egg capsules has been reported by several workers (Thorson, 1940; Amio, 1955; Natarajan, 1957; Fretter and Graham, 1962; Gohar and Eisawy, 1967a; 1967b; D'Asaro, 1970a; Soliman, 1987).

The capsular wall was found to be double layered in *B.spirata* in the present study and is in accordance with observation on *B. spirata* along east coast (Shanmugaraj *et al*., 1994) and *B. areolata* (Hua *et al.,* 2001). In contrast to this simple structure, the capsule of the muricids snail *Plicopurpura pansa* is multilaminated with tough fibers (Naegel, 2004) and this is as a protection against the biological and physical stresses (Strathmann, 1987; Rawlings, 1990; 1999). The simple structure of capsular wall of *B. spirata* may be due to the fact that the animal is inhibiting subtidal regions which are not exposed to severe wave action.

The capsule was found to a thin at the apical end and similar observations have been made on other whelks. This is the region through which the larvae are released. Ramesh *et al.* (1992) has termed this as the "escape aperture" in *Chicoreus ramosus*. Soliman (1987) reported that at hatching the whole upper wall of the neritid capsule detaches, thus liberating the larvae. In neogastropods, in particular (and in certain mesogastropods, eg. *Tonna olearium*) the egg capsule has an exit hole with fixed shape and position. The exit hole remains close throughout development but opens at hatching to permit the release of larvae.

The eggs of *B. spirata* were transparent and even in *B. areolata* the same nature was observed by Hua *et al.* (2001). In the present study it was observed that the egg capsules turn pink and opaque when the rearing condition deteriorated, for example when water was not changed properly and also when the capsule were infected by bacteria or by fungus due to low saline condition or by bending of the capsule when substratum was not provided. Similar situation when the eggs became non-viable has been observed in *P. pansa* (Naegel, 2004). Gallardo (1973) as well as Riquelme and Chavez (1991) noted that the development of purple colour in non viable capsules was due to bacterial infection.

The hatching rate has been found to be high in the present study with more than 94.7% of the embryos developing to veliger. Similar ranges were observed for *B. areolata* (Hua *et al.*, 2001) and *B. spirata* (Shanmugaraj *et al*., 1994), but the percentage of hatching was low in *B. undatum*, in this species there was no planktonic larval stage and the young ones emerge as fully developed miniature adults. Hatching rate of green snail *Trochus marmoratus* in Okinava varied from one spawning to another and ranged from 14.6 to 100% (Murakoshi *et al*., 1993).

B. spirata eggs did not survive in low salinities and hatching rate were high only in salinities above 30 ppt, which was indicating that the early stages in the life history of *B. spirata* were less tolerant to lower salinities than the adult. This is clear evidence to the fact that salinity extremes can affect embryonic mortality in egg masses (Prezeslawski, 2004) but exceptionally Scheltema (1965) did not find any

significant difference between levels of salinity that proved lethal to adult and hatched veligers of *I. obsoleta*. In the present set of experiments on salinity it was observed that within minutes of placing the egg capsules in low salinity, the capsule weight reduced indicating the efflux of salts. The weight loss was lower in 30 ppt than in 15 to 25 ppt. Woods and DeSilets (1997) conducted similar experiments on the gelatinous matrix of *Melanochlamys diomedea* egg capsules and found that the rate of efflux was slow and the embryos themselves were tolerant. However the contrasting difference between the response of *M. diomedea* egg capsule and that of *B. spirata* may be due to the variation and stability found in the habitats of these two species. The habitat of the former species is prone to freshwater influx hence the gelatinous egg mass do seem to be protected to some degree against the changes in the gelatinous matrix. *B. spirata* however is found in an environment which has more stable salinity profile and the low tolerance to lower salinities can be attributed to this. Studies have shown that as salinity deviates from within a species normal habitat, the mortality of gastropod embryos increases (Struhsaker and Costlow, 1969; Pechenik, 1982; Woods and DeSilets, 1997). However, as embryos develop they seem to become more tolerant to a wider range of salinities (Struhsaker and Costlow, 1969; Pechenik, 1983; Richmond and Woodin, 1996).

The low salinity (20 and 25 ppt) was found to affect the cell division and growth of the embryo. Only 1% eggs developed to morula stages and thereafter the eggs became inactive and the capsule became an opaque mass. This shows that the osmoregulation capacity of B. *spirata* is low. Delayed development has been observed in several gastropods like *Ilyanassa obsolete*, *Nucella lamellose* and *N. lima* (Pechenik, 1982). Based on these experiments it is suggested that the rearing of the capsules in the hatchery should be done only in salinities greater than 30 ppt even though the adult can tolerate the salinities slightly lower than 30 ppt. The fact that the shell width and length of veligers hatched from 30, 35 and 40 ppt salinities was not significantly different ($P > 0.05$) indicates that *B. spirata* egg capsule can tolerate salinity variations in higher range than in the lower range.

Temperature also affected the embryonic development, hatching percentage and time of hatching in *B. spirata*. Temperature of 26 to 28^{o}C did not hinder the

developmental process while in 20 to 24^{o}C the process was delayed and in 30^{o}C the embryonic development was faster but the larvae did not survive. For gastropod embryos within capsular egg masses it has been suggested that hatching time can be estimated knowing only the taxon and the temperature (Spight, 1975; Palmer, 1994). In the present study it was observed that the embryo cannot tolerate those lower temperatures which were tolerated by the adults. Prezeslawski *et al*, (2004) reported that embryos become stressed and often die if exposed to extreme temperatures relative to their natural environment and seem more vulnerable to temperature extremes than adults. Thompson (1958) found that adult nudibranch *Adalaria proxima* spawned and remained healthy at a relatively high 13^{o}C, but this temperature was lethal to its eggs. Despite vulnerability to both high and low temperature extremes, gastropod embryos may be more tolerant of lower temperatures within their range than higher temperatures. Struhsaker and Costlow (1969) found that planktotrophic larvae of *Littorina picta* had a high survival rate at temperatures lower than their established optimal developmental temperatures, but the larvae had lower survival rates at higher than optimal conditions. Similar observations have been made on encapsulated gastropod embryos.

One interesting observation was the higher developmental rate at 30^{o}C and early hatching (*viz*, 5th day instead of 6th or 7th day) but the larvae did not survive. Dehnel and Kong (1979) have also observed a similar development in *Cadlinna luteomarginata* along the coast of British Columbia where the hatching time was fourfold at 15^{o}C than at 5^{o}C. The embryo also degenerated by 4th cleavage stage at 20^{o}C, the average summer temperature. Thus the embryos of this species seemed much more tolerant of lower temperature than higher temperature within their natural thermal range. Although there is a lethal low and high temperature for gastropod embryos, some embryos are able to protect against high temperatures to a certain extent. Recent research has revealed the presence of heat shock proteins inside the gelatinous egg masses of the cephalaspid *Melanochlamys diomedea* (Podolsky and Hoffmann, 1998; Podolsky, 2000). These proteins allow embryos to withstand high temperatures, such as those reached during low tide on a summer day, by preventing the degradation of proteins during heat stress and facilitating the refolding of proteins. These thermally protective proteins develop as the embryos mature

(Podolsky and Hoffmann, 1998). Thus undeveloped embryos are especially vulnerable to high temperatures and they become less vulnerable to temperature extremes as they develop (Thorson 1950). It is presently unknown whether egg masses of other species contain thermally protective proteins. Low temperatures can affect embryonic development by prolonging or halting it. Scheltema (1967) found that the embryonic development of the neogastropod *I. obsolete* slowed significantly as the temperature dropped. The embryos ceased development at lower threshold of the species temperature range. Strathmann and Chaffee (1984) point out that lower temperatures decrease embryonic metabolic rates, thus resulting in slower development as it happened in *B. spirata*. In addition, they suggested that lower temperatures could decrease intracapsular fluid viscosity and diffusion rates, thereby decreasing oxygen availability to the embryos.

Aeration was found to be essential for development of embryos while light did not affect the development in *B. spirata*. Prezeslawski *et al.* (2004) has found that oxygen availability can dramatically influence the development of embryos with egg masses. In a series of experiments, Strathmann and Strathmann (1995) demonstrated that oxygen limited the embryonic development within gelatinous opisthobranch egg masses. They found that embryos of these species showed arrested development during hypoxia until they were returned to a normal oxygen level. Furthermore, lower oxygen availability throughout the developmental period reduced shell length at hatching. These observations have recently been supported in the capsular egg masses of *Chorus giganteus* (Cancino *et al*., 2003). Oxygen availability within egg mass is inextricably linked to water flow, embryonic position within the egg mass and fouling. Among gelatinous egg masses, flowing water accelerates the rate of embryonic development by decreasing hatching time and increasing embryo activity (Eyster, 1986) because it most likely increases the overall oxygen supply to the egg mass through diffusion (Strathmann and Hess, 1999). Strathmann and Chaffee (1984) found that flowing water decreases asynchronous development within a spherical gelatinous mass, where still water promotes relatively high developmental variation. The same study found no effect of still water on development among elongated gelatinous ribbons. Biermann *et al*. (1992) also found a significant interaction between water flow and egg mass thickness. There was no difference in

embryonic development rates between egg ribbons maintained in still water and those exposed to a strong current; but when the thin ribbons of egg masses were laid forming a thicker structure, there was a noticeable developmental retardation in still water. The egg capsules of *B. spirata* are closely laid but there is enough space for water flow in between. Hence mild aeration provided water circulation resulting in healthy larvae.

Chapter 5

Intra capsular development and growth of Babylonia spirata

5. INTRA CAPSULAR DEVELOPMENT AND GROWTH OF *BABYLONIA SPIRATA*

5.1. MATERIALS AND METHODS

5.1.1. Development inside the egg capsule

Immediately after spawning, the egg capsules were transferred to the rearing tank. The egg capsules were placed in the acrylic tank and were supplied with de-chlorinated, filtered, aerated sea water of 32 ppt salinity and temperature 26^0C to 28^0C. Egg capsules were not disturbed during the incubation period. Complete exchange of water was done on alternate days. On each day five egg capsules were placed in 2 ml of water were collected and observed under the light microscope fitted with photographic attachment. The metamorphic changes were recorded and behavioural pattern of the larvae observed. On each sampling day fresh egg capsules were taken for observation and these were kept in another rearing tank for further development. Measurements of the egg and larvae were recorded using the ocular micrometer as described under the section 4.1.

5.1.2. Post hatching

After hatching or release of swimming veliger larvae from an egg mass, the veligers were filtered gently from egg mass culture water using 300 µm sieve and cultured in aerated filtered sea water (30-32 ppt) at temperatures of 26^0C to 29^0C at a stocking density of 150 larvae l^{-1} . Immediately after transferring to the culture tank, the larvae were fed with the microalgal feed of *Isochrysis galbana (Ig) or Chaetoceros calcitrans (Cc).* Healthy, active larvae (n=5) at the surface of the water column were placed 2 ml of water in an embryo cup and observed under the 10x of the light microscope. When the veligers stopped swimming and rested on the bottom of the dish with extended vela, they were measured using the micrometer and photographs were taken with the stereozoom camera attached to the microscope .

5.1.3. Post settlement

The larvae which settled as juvenile were collected from the rearing tank and stocked in juvenile rearing tank and were fed with the shrimp paste. After feeding, the juveniles were washed with sea water and stocked in filtered sea water with 32 ppt salinity and provided with aeration. No substrate was provided during earlier juvenile stage. Feeding was done once daily. The shell length (SL) and shell width (SW) of the juvenile were measured using the digital calipers of 0.01 mm accuracy.

5.2. RESULTS

5.2.1. Development inside the egg capsule

All the eggs inside an egg capsule were spherical in appearance and had an average diameter of 240 μm irrespective of the size of the egg capsule, spawner size and season of spawning. The fertilized egg had no noticeable morphological changes immediately after spawning. The first recognizable change that happened under the light microscope was the release of the polar bodies. The first polar body was released at the 60^{th} minute after spawning (Fig. 5.1). Release of the second polar body commenced at 90^{th} minute.

The cleavages began after the discharge of the 1^{st} and 2^{nd} polar bodies. The first cleavage occurred at 30^{th} minute (2 h after spawning) after the release of the second polar body which resulted in the two-cell stage (Fig. 5.2). The average size of the two-cell stage was 299 ± 23 μm which had two equal rounded portions cleaved by a furrow (cleavage furrow) in between. The average diameter was 140 ±10 μm. All the divisional stages inside the egg capsule were almost synchronized; all eggs attained the same divisional stages within 30 minutes from the appearance of first cleaved cell.

After 1 h interval (3^{rd} h after spawning) second cleavage of the egg resulted in the 4-celled stage (Fig. 5.3). The average size of the 4-cell stage was 305 ± 16 μm. The divisions were clearly visible up to 4 cell stage. Before the 3^{rd} cleavage the eggs become an opaque mass in the posterior region and a transparent anterior region (Fig. 5.4).

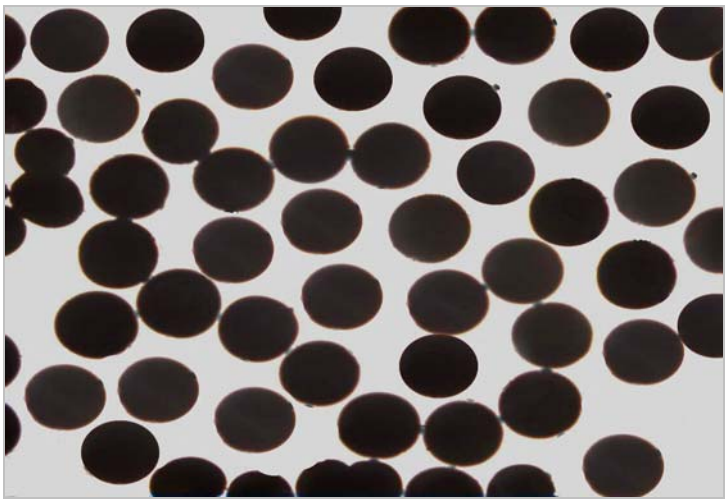


Fig. 5.1. Fertilized eggs with polar body (5x)

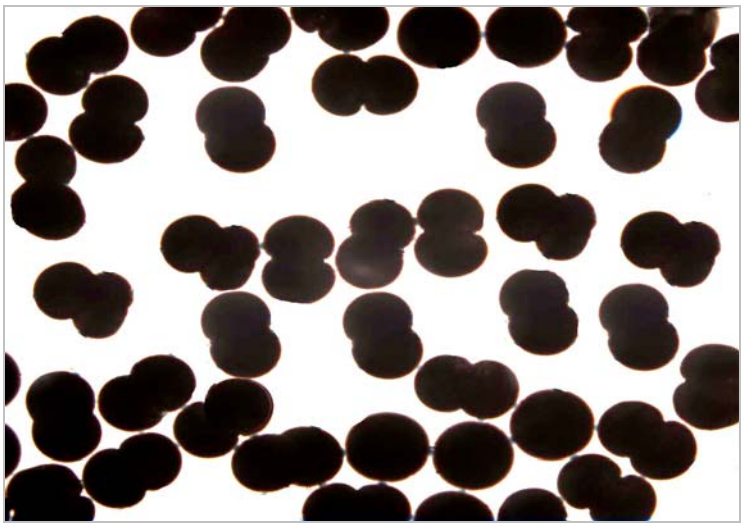


Fig. 5.2. 2-cell stage (5x)

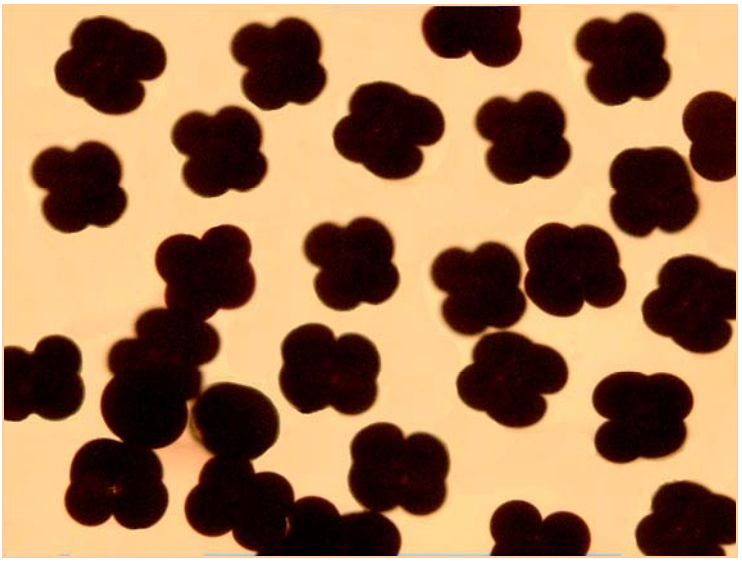


Fig. 5.3. 4-cell stage (5x)

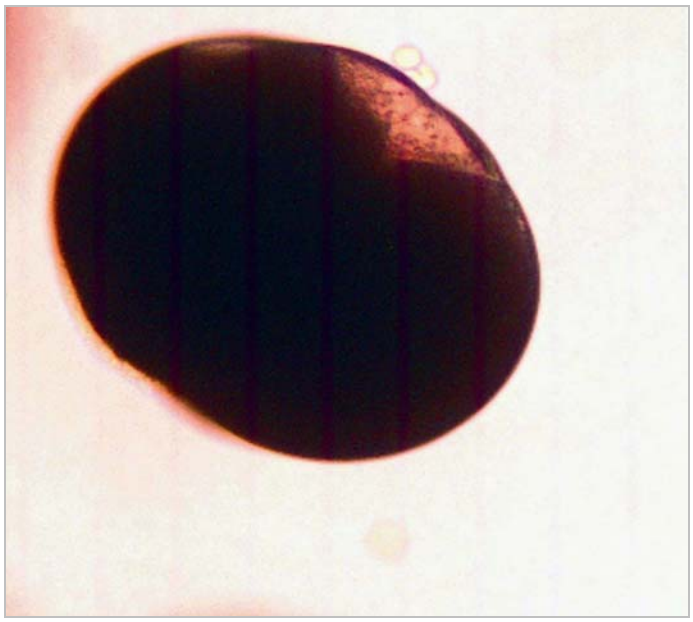


Fig. 5.4. Micromeres above the macromeres (40x)

The transparent portion was composed of the micromeres which are seen clearly above the opaque macromere. The number of cells in the micromere portion could be counted as it was transparent and visible under the microscope. The eggs attained the 8-cell stage after 04:30 h after spawning (Fig. 5.5). After 1 h all the eggs reached the stage of 16-cell stage (Fig. 5.6). The 32-cell stage was recorded at the 6^{th} hour after spawning (Fig. 5.7). Further it become an opaque black structure without any movement and measured 310 ± 10 µm in diameter.

After 24 h of spawning, the divisions were completed and the embryo transformed into the morula with marginal cells at the apical region (Fig. 5.8). The marginal cilia had a width of 20 µm and extended $1/3^{rd}$ area of the anterior marginal region. At the initial stages of morula stage there was no movement and it remained in this condition till 48 h. The size at this stage was 315 ± 24 µm.

Following these developments slow rotation of the embryo inside the membrane began with the help of the anterior marginal cilia and the embryo became elongated measuring 322 ± 20 µm in length. The embryo rotated inside the egg membrane. It took 6 seconds to complete a full rotation. The rotation was 180^{o} clockwise followed by a reverse rotation which completed one full rotation. This process continued for

till 3rd day. Thereafter the rotation became faster and the embryo turned a full rotation of 360^0 in the egg membrane which was completed in 15 sec (Fig. 5.9).

More frequent movement was observed inside the egg membrane on the 3rd day of development (Fig. 5.10). The egg membrane became thin and finally the larvae were released out. The released trochophore larvae had a shell length of 360 ± 12 µm.

On the 4th day of development the trochophore measured 380 ± 8 µm. The velum gradually began to come out from the embryonic shell the protoconch, with a few cilia on the corners of the velum. Slight movements of the velum with cilia were observed. The larvae rotated freely within the egg capsule.

Fig. 5.5. 8-cell stage (40x)

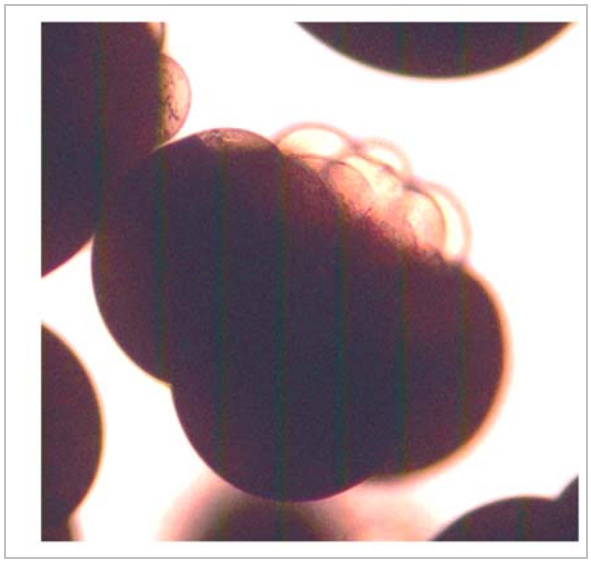


Fig. 5 .6. 16-cell stage (40x)

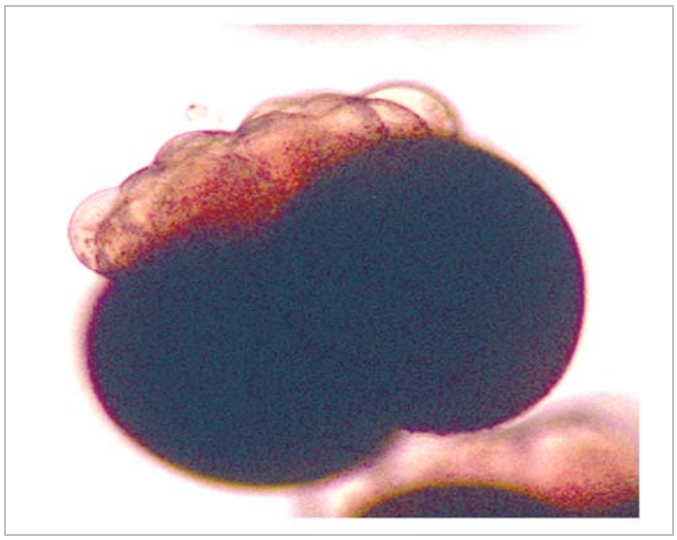


Fig. 5.7. 32-cell stage (40x)

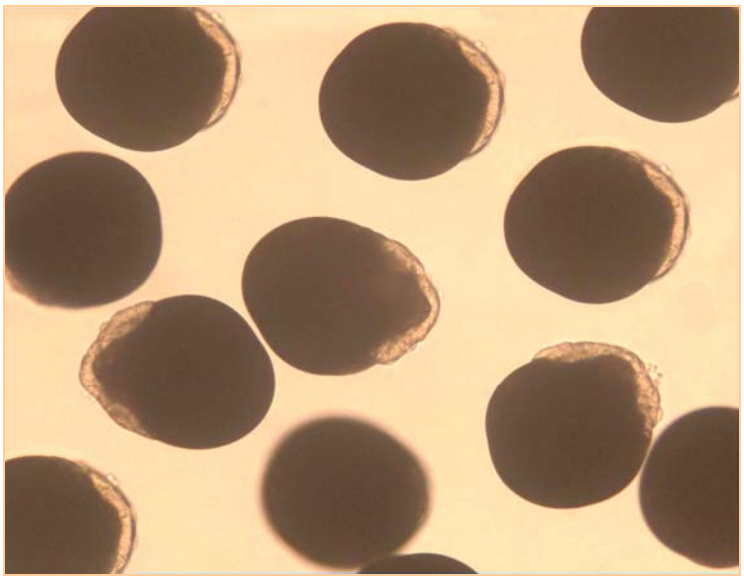


Fig. 5.8. Morula stage (10x)

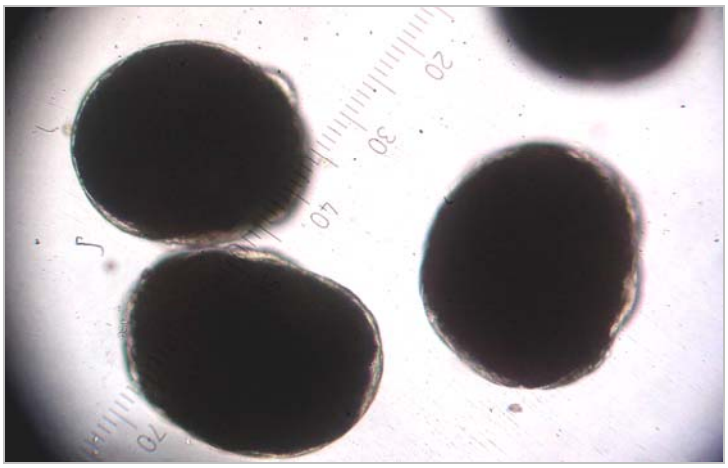


Fig. 5.9. Rotating Morula stage (10x)

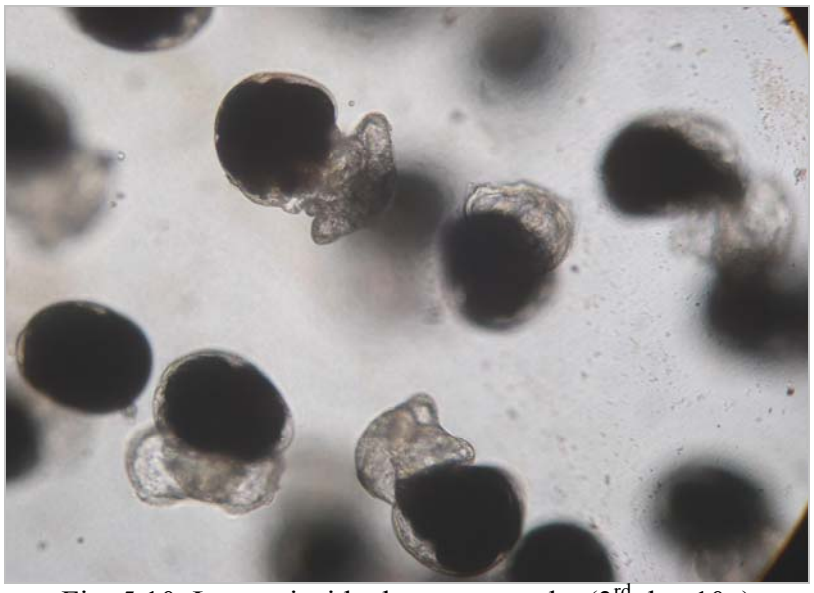


Fig. 5.10. Larvae inside the egg capsule (3^{rd} day 10x)

The protoconch was transparent and a knob like opaque portion was visible through the shell. Simultaneously the thickness of the fluid inside the egg capsule was found to be reduced (Fig. 5.11).

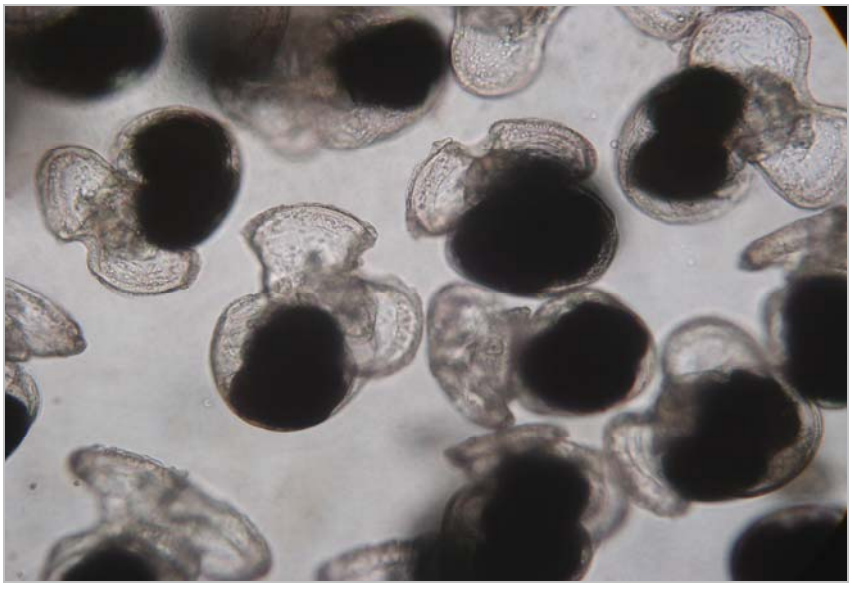


Fig. 5.11. Larvae inside the egg capsule (4^{th} day, 10x)

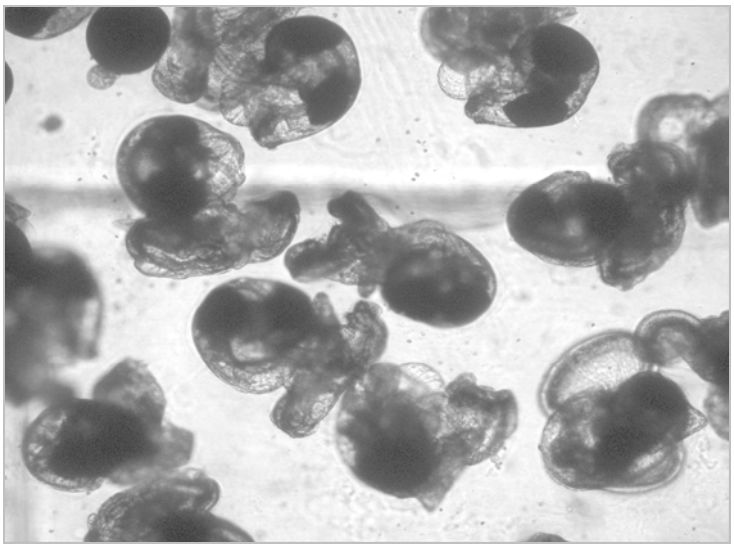


Fig. 5.12. Larvae inside the egg capsule (5th day, 10x)

On the 5th day the larvae developed as veliger with the apical region becoming flat and with long cilia on the velum. Though the velum was fully formed, it did not spread completely around the larval shell (Fig. 5.12). It was restricted to the anterior part of the larval shell. The larval shell grew dorsally and ventrally until it covered the body of the larvae just below the velum. Cilia along the margin of the velum increased in length and started beating resulting in the movement of the veliger inside the egg capsule instead of slow rotation observed the previous day. The larval heart became visible through the transparent shell and the beating could be counted (65 beats/minutes).

On 6th day the velar lobes became enlarged and almost covered half of the larval shell. Through the thin transparent larval shell internal structures were visible clearly. The single mass of opaque portion found on the previous day reduced in size and became bi-lobed. Embryonic kidney was also visible below the velum. Gills were also present. The jelly inside the egg capsule became thinner. No retraction of the larvae into the shell was observed on this day and all were in a state of continuous movement. The well developed veliger ready for hatching measured 445± 31 μm in shell length with a width of 313.6 ± 15 μm. All larvae moved and concentrated towards the tip of the egg capsule near the aperture and along the margins of the egg capsules ready for hatching out of the capsule. The albuminous fluid inside the egg capsule now became decreased on the progress of development

and completely disappeared on the day of hatching. Though the exact mechanism for breaking of the aperture is not known yet, the apical portion split open and the veligers were released from the capsule on the following day. (Fig. 5.13). On the day 7^{th} day the apical aperture of the egg capsule opened and the larvae were released. The releasing of the veliger out of the capsule continued during the next day also.

Hatching of the veliger out of the capsule was not synchronized. Only 60 % were released on the first day and the remaining larvae concentrated towards the base of the capsule and were released on the following day. Only the undeveloped eggs remained in the egg capsule after the release of the developed veliger. Newly hatched veligers had a fully developed well spreaded velum and a pair of eye spot. The velum of the larvae fully covered the larval shell. The newly hatched veliger had a thin, fragile, transparent, globose shell through which the internal organs were clearly visible. The margin of the velum increased in thickness and cilia in two rows were observed to beat very fast continuously. The velum of the larvae was almost double the size of shell and margins were pigmented, light yellow in colour. The inner portion of the velum was transparent. The hatched larvae swam to the surface of the water with fast moving cilia on the velar lobes. The average shell length of the veliger on the day of hatching was 460± 27 µm had a shell width of 315 ± 28 µm (Fig. 5.14).

5.2.2. Post hatching

Eye spot of the larvae was clearly visible from the first day of hatching. The healthy larvae were found on the surface of the water with fast ciliary movements. They moved as a single mass, so that the velum of one was close with the other without any gap in between. The average size of the larvae on 8^{th} day was 476 ± 24 µm in shell length and 330 ± 24 µm shell width. (Fig. 5.15). This stage lasted upto 13^{th} day with no marked changes in morphology except the increase in the shell length and pigmentation of the velum. The deep yellow coloured pigmented velar lobes extended to the maximum on 10^{th} day. (Fig. 5.16). The shell length of the larvae on this day was 559 ± 27 µm with a width of 436 ± 20 µm. From the 13^{th} day the transparent colour of the velum changed gradually to a light brown as a result of pigmentation. The margins became thickened and coloured as well. The larval size

increased to 670 ± 59 µm in shell length and 480 ± 53 µm shell width. (Fig. 5.17). Well differentiated morphological changes were observed from the day-14th onwards. On 14th day the larval foot was noticed. A transverse groove began to develop through the centre of the velum. (Fig. 5.18).

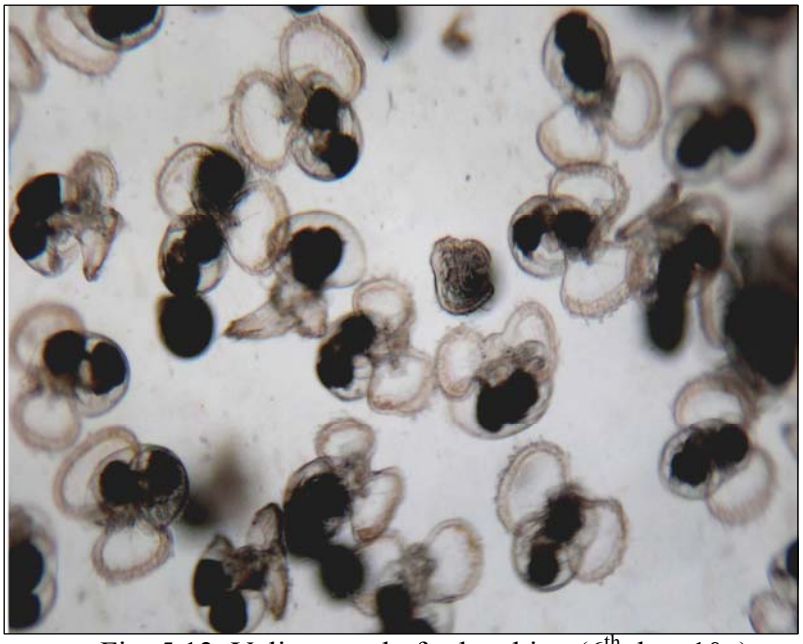


Fig. 5.13. Veliger ready for hatching (6th day, 10x)

Fig. 5.14. Hatched veliger (7th day, 40x)

Fig. 5.15. Veliger on 8th day of pelagic life (40x)

Fig. 5.16.Veliger on 10th day of pelagic life (40x)

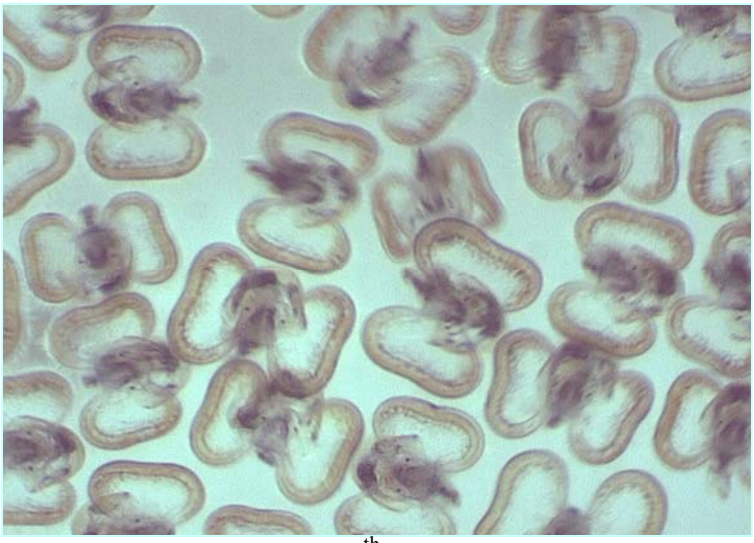


Fig. 5.17. Veliger on 13th day of pelagic life (10x)

Fig.5.18. Veliger on 14th day of pelagic life (10x)

The operculum also became visible as a scar on the metapodial region on 15th day. As the velar groove bifurcated, the bi-lobed velum had four lobes. The protoconch 1 was clearly visible on this day (Fig. 5.19). The ciliary movements on the velum tended to decrease and from the following day and the velum diminished in size and started to disintegrate. On subsequent days the number of cilia and ciliary beats diminished and the larvae showed a common tendency of retracting in to the shell frequently.

On 16th day there was the retrogression of the velar lobes and formation of various organs of the larvae. The larvae measured 745 ± 20 µm in shell length and 560 ± 26 µm shell width. The pelagic life of the larvae ceased and they gradually entered a creeping stage. At this time the larvae possessed rudimentary velar parts (Fig. 5.20). The velum at this stage was visible through the shell when the body of the larvae was withdrawn. It was observed fully extended during swimming or was seen protruding at the anterior of the shell during crawling. They spent most of the time crawling on the bottom of the rearing container or retained the ability of swimming by a feeble movement in the column of water. They remained in this stage for a period of 30-40 h (Fig. 5.21). Complete resorption of the velar lobes occurred during the final stage of metamorphosis (Fig. 5.22). Then they possessed a well defined metapodium with the operculum attached to the posterior end. Larvae had paired tentacle with prominent eyes at its base in the final stage of the veliger. On 19th day the larvae

completely metamorphosed to an young juvenile. The presence of substratum was not necessary for the settlement of the juvenile. At this stage they were found to be actively crawling on the bottom of the tank or along the wall of the container. During settlement the juveniles possessed a well defined siphon, tentacles, foot, operculum and a larval shell (protoconch II) and a transparent body whorl. (Fig. 5.23). The juvenile at the settlement had an average shell length of 920 ± 80 µm and width of 803 ± 70 µm. At this stage the shell was smooth and thin without sculptures characteristic of adult *B. spirata*. The various developmental stages during the process of metamorphosis are summarized in the Table 5.1 and 5.2.

Fig. 5.19. Veliger on 15th day of pelagic life

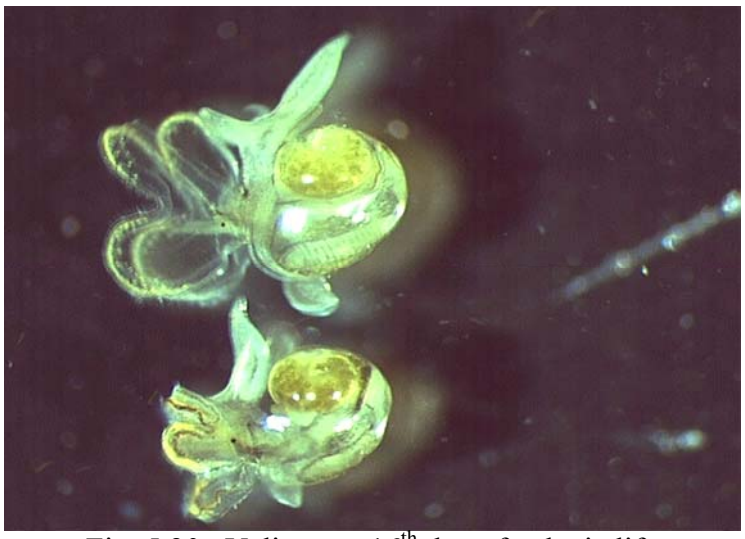


Fig. 5.20. Veliger on 16th day of pelagic life

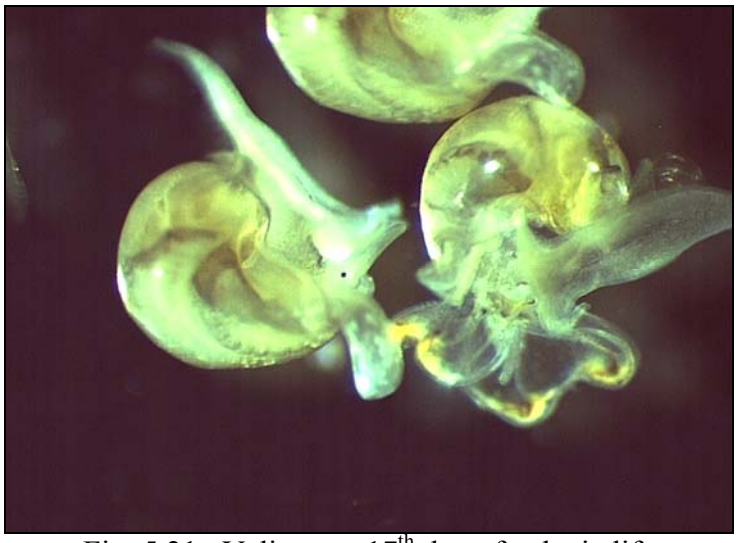


Fig. 5.21. Veliger on 17th day of pelagic life

Fig. 5.22. Veliger on 18th day of pelagic life

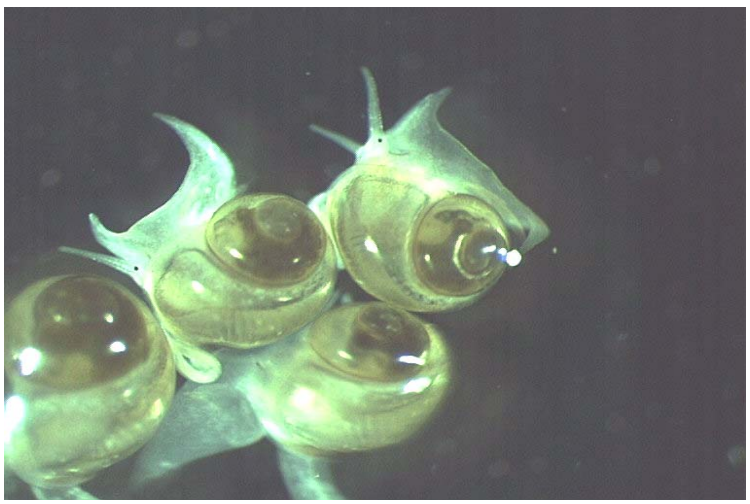


Fig. 5.23. Settled juvenile

Fig. 5.24. One week old juvenile

Table-5.1 Developmental stages of the fertilized eggs in side the egg Capsule

Day	Measurement	Morphological features
0 h (Immediately after spawning)	240 µm	Spherical appearance, not transparent, uniform in size.
01:00 h		Release of the first polar body.
01:30 h		Release of the second polar body.
02:00 h	299 ± 23 µm	First cleavage happened which cleaved the spherical egg into two equal rounded portions by a cleavage furrow. (2-cell stage).
03:00 h	305 ± 16 µm.	second cleavage of the egg resulted in to 4-celled stage.
04:30 h		8-cell stage
05:30 h		16-cell stage
06:00 h		32-cell stage
08:00 h	310 ± 10µm	Become an opaque black structure without any movement.
24:00 h	315 ± 24 µm	The divisions were completed and the embryo transformed into the morula with marginal cells at the apical region. This stage lasted upto 48 h.
48:00 h	322 ± 20 µm	Slow rotation of the embryo inside the membrane began with the help of the anterior marginal cilia and the embryo became elongated. The embryo rotated inside the egg membrane, 180^{o} clockwise followed by a reverse rotation which completed one full rotation.
48:00-72:00 h	340 ± 10 µm	Rotation became faster and the embryo turned a full rotation of 360^{0} in the egg membrane which was completed in 15sec.

72:00 h	360 ± 12 µm.	More frequent movement was observed inside the egg membrane. The egg membrane became thin and finally the trochophore larvae were released out.
4th day	380 ± 8µm	The velum gradually began to come out from the embryonic shell the protoconch, with a few cilia on the corners of the velum. Slight movements of the velum and the larvae rotated freely within the egg capsule.
5th day	SL- 420± 12 µm SW-300 ± 25 µm	The larvae developed as veliger with the apical region becoming flat and with long cilia on the velum. The velum was fully formed, but it did not spread completely around the larval shell.
6th day	SL- 445± 31 µm SW-313±15 µm	The velar lobes became enlarged and almost covered half of the larval shell. The embryonic kidney and gills visible through the transparent shell. The well developed veliger had no retraction into the shell and all were in a state of continuous movement.
7th day	SL- 460± 27 µm SW-315 ± 28 µm	The apical aperture of the egg capsule opened and the larvae were released. 60% were released on the first day and the remaining larvae on the next day.

Table- 5.2. Metamorphosis of the veliger larvae of *B. spirata* after hatching

7-8th day	SL- 476± 24 µm SW-330 ± 24 µm	Eye spot of the larvae was clearly visible from the first day of hatching. The healthy larvae were found on the surface of the water with fast ciliary movements. They moved as a single mass.
9-12th day	SL- 559± 27 µm SW-436 ± 20 µm	The pigmentation over the velar lobes became deep yellow coloured.
13th day	SL- 670± 59 µm SW-480 ± 53 µm	The transparent colour of the velum changed gradually to a light brown as a result of pigmentation. The margins became thickened and coloured well.
14th day	SL- 690± 20 µm SW-492 ± 12 µm	Well differentiated morphological changes observed, developed the larval foot and a transverse groove which began to develop through the centre of the velum.
15th day	SL- 720± 20 µm SW-520 ± 30 µm	The operculum also became visible as a scar on the metapodial region, Velum bifurcated and had four lobes. The ciliary movements on the velum decreased.
16th day	SL- 745± 20 µm SW-560 ± 26 µm	The pelagic life of the larvae ceased and they gradually entered a creeping stage. The larvae possessed rudimentary velar parts. They remained in this stage for a period of 30-40 h.

18th day	SL-820± 420 µm SW-740 ± 30 µm	Complete resorption of the velar lobes occurred during the final stage of metamorphosis. The larvae possessed a well defined metapodium with the operculum attached to the posterior end.
19th day	SL- 920± 80 µm SW-803 ± 70 µm	The larvae completely metamorphosed to an young juvenile. The juveniles possessed a well defined siphon, tentacles, foot, operculum and a larval shell (protoconch II) and a transparent body whorl. The juvenile shell was smooth and thin without sculptures.

5.2.3. Post settlement

The first teloconch of the juvenile became well distinguished on the first day of the settlement. The planktonic filter feeding mode suddenly changed into that of a carnivore. From the first day of settlement they fed on the shrimp paste which was provided to them. The internal gills were visible through the transparent shell. The larval shell got sculptured one week after settlement. (Fig. 5.24). Increase in shell length become faster and the juvenile attained the size of 2.32 ± 1 mm shell height and 1.81 ± 0.6 mm shell girth after 30 days of development. The second whorl of the shell developed two week after settlement (Fig. 5.25). After 45 days the shell thickness increased and the internal organs were not visible. The third body whorl became developed at 2.5 month old settled juvenile. At this time patterns on the body whorl became deeply coloured (Fig. 5.26). The larvae started to secrete the mucous and were observed to fall while climbing the walls of the tanks. Till then they were unable to return below the water level in case they moved above the water level. This used to result in complete desiccation followed by mortality.

The juvenile attained the full feature of the adult by 50 days of development from the date of spawning and they buried under the fine sand substratum similar to that of the adult. Only the siphon projected out of the substratum and they sensed the presence of food within minutes as soon as they were fed and responded by coming out of the substratum and extending the proboscis in search of feed (Fig. 5.27). After feeding they again returned to the substratum and lay buried. The size of the 50 days old juvenile was 5.8 ± 1.4 mm shell length and 4.5 ± 0.6 mm shell width (Fig. 5.28). Life cycle of *B. spirata* is shown in plate 1.

Fig. 5.25. Two week old juvenile

Fig. 5.26. 2.5 month old juvenile

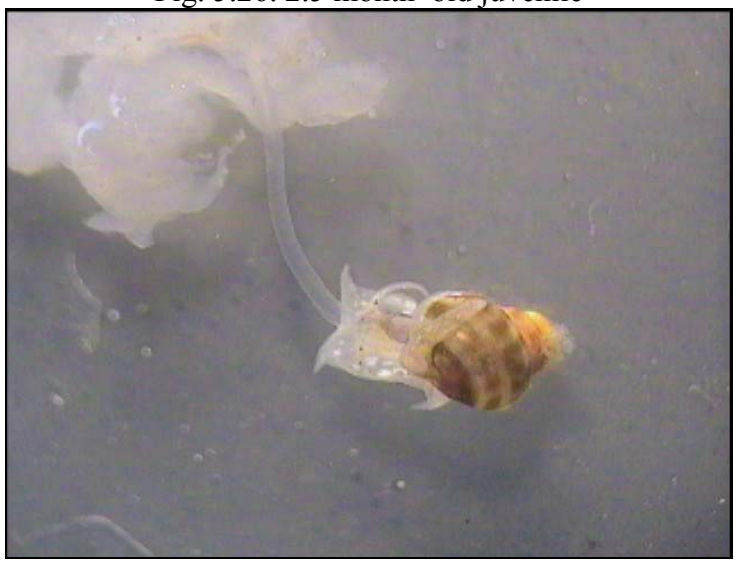


Figure 34: **Life cycle of *Babylonia spirata***

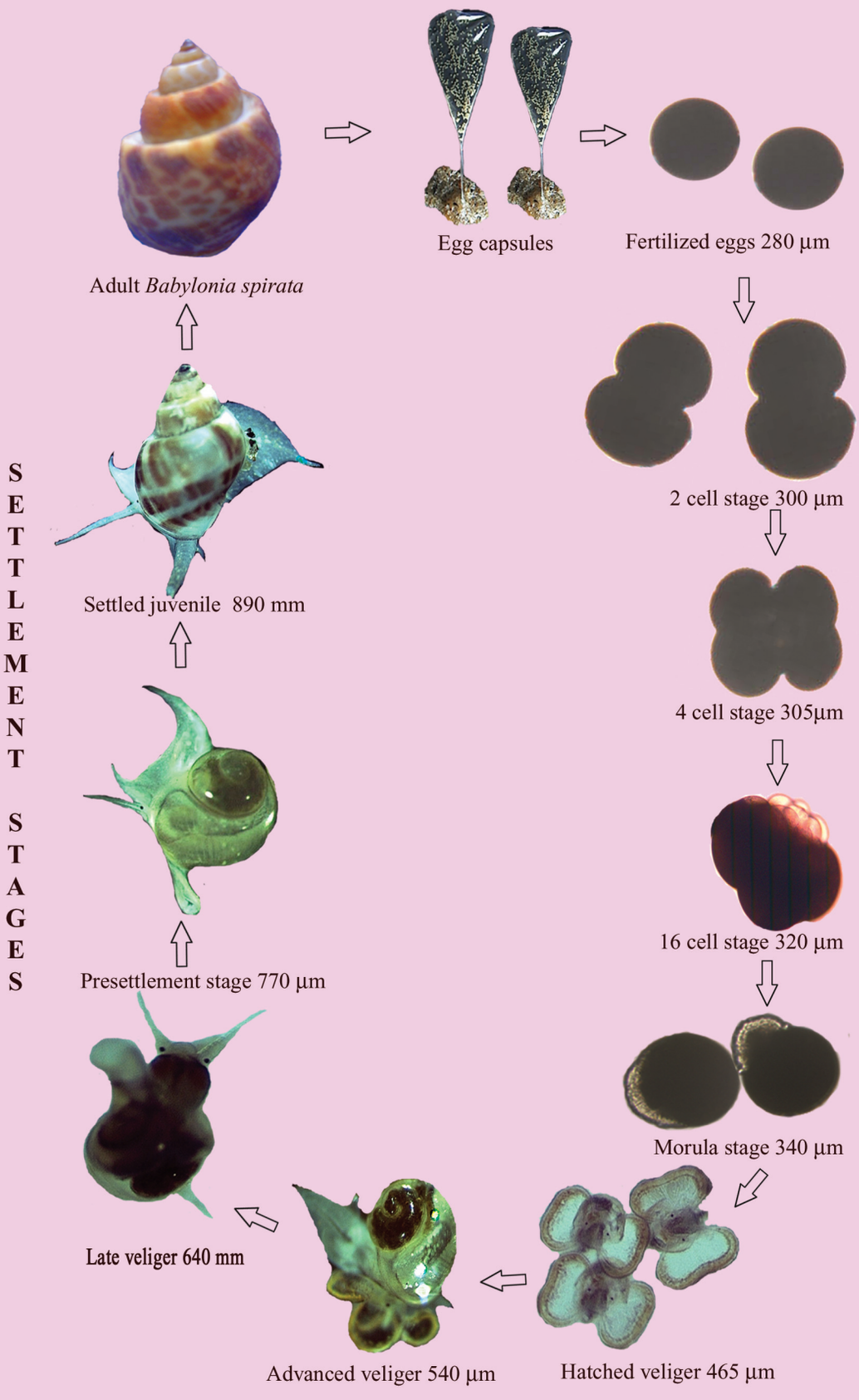


Fig. 5.27. Juvenile extending the proboscis for feeding

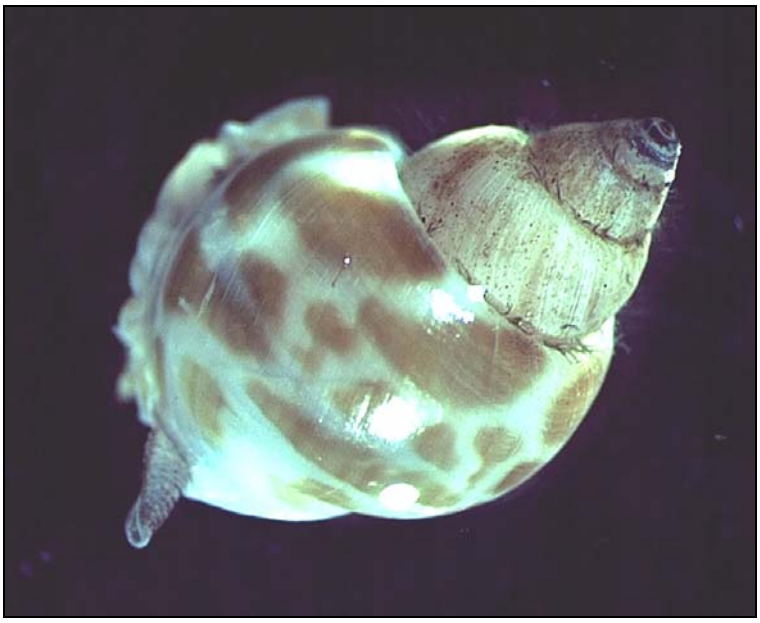


Fig. 5.28. 50 day old juvenile

5.3. DISCUSSION

Within the egg capsule the most significant development was the formation of protoconch by 3rd day. The formation of ciliated velum which gradually increased in pigmentation and enlargement of the cilia was another major development. The larvae, the protoconch and developed velum showed movement within the capsule. Similar movements of the veliger larvae in the encapsulated stage have been reported for *B. spirata* (Shanmugaraj *et al.,* 1994) and in *B. areolata* (Hua *et al*., 2001). The larvae of *B. spirata* within the capsule are similar to that of *B. areolata* with ciliated velum which was like the wings of a butterfly.

The intracapsular development has been found to be slightly different in *B. areolata* compared to that of *B. spirata* in the present study. The first and second polar body were released within 1h and 2 h respectively while in *B. areolata* the embryonic development were slightly delayed (Hua *et al*., 2001). Similarly larvae of *B. spirata* reached the four celled stage in 3 h while in *B. areolata* (Hua *et al*., 2001) and *Doridella steinbergae* (Bickell and Chia, 1979) it took more than 10 h to reach this stage. In other gastropods like *G. cineraria* (Underwood, 1972) it completed the

four-cell stage comparatively early (1.4h). Similar variations were observed in gastrula and development of morula.

It is well established that (reviewed by Mileikovsky, 1997) there are three different developmental stages of larval development, i) pelagic development ii) direct development and iii) viviparous development. *B. spirata* had pelagic development and in all the months only this type of development was observed. Though almost all species have one of these types of development, mentioned above, in some species for example, in prosobranch gastropod *Polinices triseriata (*Naticidae) two types of development were observed. In the Canadian coastal waters this species possess direct development during years with wet cool summers, but semi-planktonic development during years with hot dry summers. In latter case, its larvae, after hatching, spend a few hours in the plankton; they are unable to determine their position in the water column and passively drift about with the prevailing water currents (Giglioli, 1955). He further proposed that both these types of development are present simultaneously each year, but that one type predominates according to weather conditions.

After the incubation period the larvae which hatched out were 460 ± 27 μm and the larvae of *B. areolata* were also almost similar. The larvae were phototactic and planktotrophic. Smith *et al.* (2005) found the larvae of *Polinices pulchellus* to be negatively phototactic. Digestive organs were developed and they were capable of feeding on micro - algae. Very interesting observation was the continuous beating of the long cilia on the vela and the movement of the larvae. The movement of the cilia on the velar lobes not only helps the larvae to swim but also create water currents which bring food to their mouth (Hua *et al.,* 2001). The movements of *P. pulchellus* as described by Smith *et al*. (2005) is that the larvae swam with the veliger shell directed downwards with the aperture and expanded velum oriented upwards.

When the larvae were hatched they had a well spread velum, a pair of eye spot and a transparent larval shell. Similarly the larvae of *Chicoreus ramosus* were also transparent but the shell was pale yellow in colour with minute purple colour (Ramesh *et al*., 1992). During the period, immediately after hatching (day 8-13) the

larvae mainly fed on micro - algae. The pigmentation increased as the margins became thick and rudiments of internal organs developed. Similar observations have been made for other neogastropods (Hua *et al.*, 2001) and in *C. ramosus* (Ramesh *et al*., 1992). In Naticids also the development after hatching was found to be similar (Smith *et al*., 2005). Within the transparent protoconch the beating of the heart could be observed in the present study. In the study of *B. spirata*, similar observations have been made by Shanmugaraj *et al*. (1994) and in *C. ramosus* (Ramesh *et al*., 1992). Although a larval heart is a structure typical of prosobranch veligers, it is present only occasionally in opisthobranch veligers. The presence of a larval heart has been reported for all aplysiids, for cephalaspid *Philine* and *Acteocina* and for a single nudibranch, *Adalaria*. The small, vesicular, regularly pulsing larval heart is usually situated beneath the floor of the mantle cavity and although its function is not known, Thompson (1972) has suggested that its regular pulsations accelerate the flow of water through the mantle cavity. In several species, an undifferentiated mass of cells can be seen prior to metamorphosis in the body some distance behind the larval heart. These cells are the rudiment of the adult heart, which develops independently of the larval heart during or following metamorphosis.

In *B. spirata* as the larvae developed, the bilobed velum developed a small bend in the central part making it partly four lobed. Simultaneously the protoconch-2 increased in size. However the notch did not deepen and there was no complete bifurcation of the velum into four lobes as observed for Naticids like *Polynicia* (Smith *et al*., 2005) and for *C. ramosus* (Ramesh *et al*., 1992). In the case of *C. ramosus* different observations have been reported regarding the nature of the velum. Bussarawit and Ruangchua (1991) observed that the veligers of *C. ramosus* have a four lobed velum which gradually degenerates, when the larvae become benthic after about 10 days of development. Contrary to this Ramesh *et al.* (1992) observed the larvae of the same species to have a distinct bilobed velum immediately after hatching which then divided into four lobes. Ten days after hatching the larvae were found actively swimming in contrast to the findings in Thailand. On 25th day the vela of the larvae of *Polinices pulchellus* had bifurcated into four velar arms while in *B. spirata* it was observed that the cilia of the vela were partially lost and the vela showed retrogression. The lobes became shorter and thicker, were lost, and the

movements slowed down. On the 12th day torsion was seen with the formation of single whorl shell. Before settling the larvae of *B. spirata* and *P. puchellus* (Smith *et al*., 2005) observed discontinuous movements, the velar cilia stopped beating and the larvae sank short distances before swimming was resumed. During 17-18th day the larvae swam as well as creeped short distances consequent to the resorption of the velum. In the present study it is firmly observed that the vela are resorbed.

The exact mechanism by which the vela are lost has been studied in few prosobranch by Fretter (1972) who stated that in *Lacuna vincta*, a primitive neogastropod and *in Nassarius incrassatus* the velum is retracted into the shell by two dorsal and two ventral groups of muscles. The velum is bilobed in the former species while it is four lobed in the latter. He further states that sometimes at metamorphosis the velum is eaten or cast off in total or in part. This is preceded by spasmodial contractions of its retractor muscle which will be broken from the head. In *N. obsoletus* Scheltema (1956) observed that the velar lobes were cast off. Similarly in the present study it was observed that the cilia along the margin of the velar lobes were cast off. But the velar lobes reduced in size and were not cast off.

Torsion in *T. niloticus* has been described by Heslinga (1981). In this species the first phase of torsion occurred between 14 and 18th hour, rotating the shell and visceral hump through 90^0 with respect to head and foot. In the present study such torsion was seen only towards the end of development. This must be because the entire developmental period of *T. niloticus* larvae was short (3-10 days) where as for *B. spirata* the development was much more prolonged and tentacles developed only by 16th day before settlement. The torsion observed by Underwood (1972) during the development of *G. cineraria* is almost similar to that observed in the present study but with delayed duration. In *Gibbula cineraria* the first torsion was observed in 8 h. The remaining part of the torsion was completed within four days when the larvae were, at least partially able to retract in to the shell. By 96 h though the process of torsion was complete, the larvae were never observed to retract in to the shells. In the present study also the most effective retraction even after disturbance, appeared to be ineffective as a defense mechanism. This was particularly evident when they had ciliate infection as the operculum was left somewhat open and the velum and foot

were always out of the shell. During the final torsion the velum became reduced and the larvae spent long period of time motionless on the bottom and could only swim in jerky manner. It has been suggested that torsion may have been an evolutionary adaptation which arose in the larvae of limpet like ancestral molluscs and was advantageous to the newly settled larva helping it to balance the shell during settlement (Ghiselin, 1966). Third and last phase of torsion was observed before settling when a two whorl shell resembling the adult shell except for the sculptures was developed. In *B. areolata* also the developments were similar including the disintegration of the velar lobes and development of siphonal canals (Hua *et al*., 2001). With the development of proboscis and siphonal canals on 17/18th day the feeding habit changed and the larvae started accepting shrimp paste. Associated changes in feeding and movement were also noted. Even though the larvae moved very close to each other on several occasions, they never attacked each other. Contrary to this, cannibalism was observed in *P. pulchellus* (Smith *et al.*, 2005) and is widely reported among naticidae (Dietl and Alexander, 1995).

The duration for the metamorphosis of the larvae from pelagic to creeping stage was found to be different. However in certain gastropods like the *P. pulchellus*, the larvae which were able to crawl (pediveliger) also had the ability to swim (Smith *et al.,* 2005). The pediveliger foot also had an operculum. Competent pediveliger lost their vela and metamorphosed within 12 to 24 h of exposure to sediment collected from the adult habitat. Metamorphosis was never observed in the absence of sediment. Under these conditions *P. pulchellus* pediveliger changed little in appearance and survived for approximately six months. In herbivorous gastropods like *T. niloticus* (Heslinga, 1981) and species of the family Haliotidae (Hahn, 2000) settlement has been found to be induced by the presence of benthic algal film. In contrast to this, in the present study as well as in other commercially important gastropods like *C. ramosus* (Ramesh *et al.*, 1992) the larvae metamorphosed without any substratum.

In the present study complete settlement was observed by 18-19th day and Shanmugaraj *et al.* (1994) observed that the larvae of *B. spirata* along the east coast of India also completely metamorphosed and settled on 19th day. In some species different periods of metamorphosis has been observed. The possibility for this

change is that the environmental condition like temperature must have been different. Instances where variation in abiotic conditions have delayed metamorphosis has been reported (Pedersen and Page, 2000; Smith *et al.*, 2005). In the present study *B. spirata* was found to have a planktonic life of (19 days). This mode of planktonic life enhances the opportunities for dispersal, colonization of new habitats and genetic exchange (Scheltema 1971; Strathmann, 1974). Roller and Stickle (1989) observed telephaic veligers in the muricid gastropod *Thais heomostoma.* In the muricid family, however the development pattern of the larvae is quite different from species to species. For instances, in *Murex florifer* and *M. pomum* (D' Asaro, 1970a); *Nucella lapillus* (Lebour, 1937) and *Urosalpinx cinerea* (Hancock, 1959) the larvae hatched out as juveniles (direct development). Webber (1977) suggested that the occurrence of direct development is related to latitude, and he also observed that high level percentage of direct development is restricted towards the arctic environment.

The size at metamorphosis was 880-1000 μm for *B. spirata* in the present study while for *B. spirata* along the Indian east coast the settled juvenile measured 1436 μm. In many species of the family buccinidae indirect larval development is the dominant pattern (Patterson *et al.*, 1994). It is characterized by large number of small eggs, small sized planktotrophic pelagic larvae and short period before metamorphosis into the juvenile stage. According to Middlefart (1996) the muricid species *Thais tissoti* and *Morula granulata* exhibit the same developmental mode.

In *B. spirata* it was observed that embryonic development and early larval stages took place within the egg capsule and the larvae hatched out on 7^{th} or 8^{th} day after spawning. In *B. spirata* which were reared along the east coast of India hatching was little delayed (10^{th} day) (Shanmugaraj *et al*., 1994) and in *B. areolata* it was slightly earlier, 6^{th} day (Hua *et al*. 2001). The difference may be due to the environmental variations. The variation could be due to air bubbling or water circulation in tanks (Castilla and Cancino, 1976). Compared to the short gestation period, the incubation period of *B.undatum* is very prolonged 5 to 8 months (Kenchington and Glass, 1998) while that of *Lambis lambis* is very short (3 days) (Hamel and Mercier, 2006b). In the case of *B. undatum* the developing embryos feed on the nurse eggs and Martel *et al*. (1986b) have estimated that of 3,40,000 eggs in a single mass (140 capsules) ~

3700 hatched as juveniles. They have no planktonic larval phase implying that dispersal is limited (Gendron, 1992; Lanteigne and Davidson, 1992). In the knobbed whelk *Busycon carica* also there is no planktonic stage and the young ones emerge as fully developed miniature of adult and for C. *concholepas* also long duration (69 to 128 days) has been observed for the hatching to be initiated.

Chapter 6

Larval rearing of Babylonia spirata

6. LARVAL REARING OF *BABYLONIA SPIRATA*

6.1. MATERIALS AND METHOD

6.1.1. Salinity tolerance and effect of salinity on growth and settlement of the hatched veliger

Experiment was conducted to study the salinity tolerance of veliger larvae of *B. spirata*. The stocking density of the larvae in the experimental containers was 150 larvae l^{-1}. Tolerance was studied by exposing veliger larvae to varied salinities such as 25, 30, 32, 35, 40 and 45 ppt. The lower salinities for the experimental trails were prepared by the formula described in the section 2.1.4.1. Higher salinities were prepared by naturally evaporating the sea water. The experiment was terminated on complete settlement of the veliger in all salinities. The measurements of the veliger were taken every third day to determine the difference in growth in varied salinities. The settlement percentage and duration of time required for settling in different salinities were monitored and recorded. The mean temperature and pH during the experiment was maintained constant, 27 ± 1^0C and 8.2 ± 0.2 respectively. Gentle aeration was provided in all vessels throughout the experiment. All the experiments were conducted in triplicate. The instantaneous growth rate (IGR) of the veliger was calculated from the following formula (Corazani ,1997).

$$\text{Instantaneous growth rate (IGR)} = \frac{\ln Lt - \ln Li}{t}$$

Where, ln Lt = Natural Log of length/ width measurements at time t

ln Li = Natural Log of initial measurements of length/ width

t = Time in days

The survival was determined by at each day of the culture period expressed as a percentage (%S) using the formula,

$$\%S = \frac{Ma}{Ni} \times 100$$

Where, Ma = Accumulated mortality and Ni = Initial number of larvae

6.1.2. FEEDING THE VELIGER

Culture of algal feed

Natural phytoplankton content in the chlorine treated sea water is insufficient to support the optimum growth of high densities of larvae. Stock culture of the microalgae such as *Isochrysis galbana*, *Chaetoceros calcitrans, Tetraselmis gracilis* and *Nannochloropsis salina* were maintained an air-conditioned room in the hatchery. Stock cultures were kept in 3 l Haffkeine flask. The seawater was dechlorinated, boiled and cooled and then enriched with Walne's medium and vitamins (Gopinathan, 1996). Ten ml of the inoculum in the growing phase was transferred to the culture flask and placed in an illuminated rack (1000 lux) (Fig. 6.1). After four days of culture when the cell density reached 300 x 10^4 cells ml^{-1}, these were used for feeding the veliger.

Fig. 6.1. Culture of microalgae as feed for the veliger in the laboratory

Estimation of stock density of algae : Accurate estimates of cell density were made using a haemocytometer. Regular counting of the algal cells was done before feeding to estimate the quantity of the feed required for the larvae. The calculations were made as follows,

Average number of cells ml^{-1} = Average number of cells in 0.004m^3 x 250 x 10^3

Calculating food rations to larvae: The volume of harvested culture required to feed the larvae in the rearing tank to achieve the particular cell density were calculated from the following equation,

$$\text{Volume of algae to feed (l)} = \frac{\text{Required cell density (cells per µl)} \times V}{\text{Cell density of harvested algae (cells per µl)}}$$

Where, V = Volume of water in the larval culture tank in liters.

6.1.2.1. Necessity of supplementary feed for the survival of newly hatched veliger.

Experiment was conducted to study whether the algal feed is a necessary for the hatched veliger of *B. spirata* from the day of hatching. The veligers were stocked at density of 150 larvae l^{-1} . The salinity and temperature of the rearing medium was 32 ± 1 ppt and 27 ± 1^{o}C respectively. One set of treatment in the experiment was rearing the larvae without algal supplement while the other treatment was provided with 1 x 10^5 cells ml^{-1}. The experiment was conducted in triplicate and the percentage survival on the following day was recorded.

6.1.2.2. Ingestion rates (IR) by the veliger

Experiment was conducted to study the number of algal cells required for the veliger of *B. spirata* during the pelagic phase of the life cycle up to settlement. The ingestion rate (IR) or feeding rate is defined as the number of algal cells an organism consumes per unit time (Malcouf and Bricely, 1989). The stocking density of the larvae was maintained at 150 larvae l^{-1} and fed with *Isochrysis galbana*. In one tank the algal cells without larvae were kept to test the possible multiplication of algal cells during the experiment. The mean temperature, pH and salinity during the experiment were 27 ± 1^{0}C, 8.2 ± 0.2 and 32 ppt respectively. The samples were taken and fixed with 4% diluted formalin and the number of algal cells was counted in each ml using the haemocytometer. The initial concentration immediately after feeding was recorded. Subsequent samples were taken at 10 h and 24 h after feeding and water was changed daily. The ingestion rate (IR) of the algal cell was calculated using the following formula. (Walne, 1963)

$$\text{Ingestion rate (IR)} = \frac{C_1 - C_2}{T} \times \frac{V}{N}$$

C_1 = Initial cell concentration (cells ml^{-1})

C_2 = Final cell concentration (cells ml^{-1})

T = Duration (h)

V = Volume of water (l)

N = Number of larvae

6.1.2.3. Acceptance of different types of algal cells and its effect on growth and settlement of veliger of *B. spirata*

Experiment was conducted to study the acceptance of different types of algal cells by the veliger larvae of *B. spirata* and the effect of feed on larval growth and settlement. The different types of algal feed used in the experiment were *Chaetoceros calcitrans* (TRT-1-*Cc*) *Tetraselmis gracilis* (TRT-2-*Tg*) and *Nannochloropsis salina* (TRT-3-*Ns*). The *Isochrysis galbana* (TRT-4-*Ig*) was treated as control of the experiment and all the treatments were in triplicate. Culture was maintained as described earlier and harvested on the fourth day when the cell density became 3 x 10^6 cells ml^{-1}. Quantity for feeding during the metamorphosis was based on the result of the experiment mentioned in the section 6.1.2.2. The growth of the veliger was measured on alternate days of the experiment and the survival percentage and number of days required for the larvae to settle down was recorded. For testing the significance of the variation, ANOVA was performed using SPSS software.

6.1.2.4. Effect of combination of the algal feed on the growth and settlement of veliger of *B. spirata*

The effect of monoalgal diet (TRT-1) and combination of the algal feed (TRT-2) for rearing the veliger of *B. spirata* were tried for rearing the larvae in the hatchery. The control of the experiment was a monoalgal (*Chaetoceros calcitrans)* treatment. The combination of algal feed contained equal number of cells of *Isochrysis galbana* and *Chaetoceros calcitrans* ml^{-1}. The stocking density was 150 veliger l^{-1}. The quantity of feed was increased as the growth progressed based on the result of the experiment 6.1.2.2. The growth of the larvae was measured periodically from the beginning of the experiment to the settlement day. The experiment was conducted in triplicate and the growth of the veliger was measured in terms of increment of shell length and

shell width. The temperature, pH and salinity during the experiment were 27 ± 1^0C, 8.2 ± 0.2 and 32 ppt respectively and aeration was provided.

6.1.2.5. Effect of low and high concentration of the algal feed on growth and settlement of veliger of *B. spirata*

Based on the earlier feed trails, an experiment was conducted to study the effect of low and higher concentration of algal feed on growth and survival of the veliger. The larvae were fed with *Chaetoceros calcitrans*. The average number of cells during the starvation and excess feeding experiment is shown in the Table 6.1. The first treatment was with lower concentration of algal cells (TRT-1), while the other was with two higher concentrations than the control of the experiment (TRT-2 and TRT-3). The control of the experiment was in the concentration based on the result of the experiment 6.1.2.3 (TRT-4). The IR was calculated every day and the growth measurements were taken on alternate day up to settlement. All treatments were in triplicate.

Table 6.1. The concentration of algal feed supplied in treatments.

Treatment	Days after hatching		
	1-2	3-15	16-19
TRT-1	5×10^3	10×10^4	5×10^3
TRT-2	4×10^4	7×10^4	4×10^4
TRT-3	5×10^4	8×10^4	5×10^4
TRT-4 (control)	3×10^4	6×10^4	3×10^4

6.1.2.6. Effect of temperature on IR of algal feed, growth and settlement of veliger of *B. spirata*

Experiment was set up to study the effect of temperature on IR of algal feed, growth and settlement. Different temperature ranges selected for the study were 22-24, 24-26, 28-30, 30-32^0 C. The ambient water temperature in the range of 26-28 ^{0}C was treated as the control of the experiment. The experimental set up was same as described earlier. The IR of algae was counted every day and measurements of the veliger were taken periodically. The veligers were first acclimatized to the lower and higher temperatures by gradual acclimatization at the rate of 1^0 C for 30 min. The

data obtained for shell length (SL), shell width (SW), percentage of settlement, number of days required for settlement and ingestion rate (IR) were analysed for optimum temperature for rearing the larvae.

6.1.3. Effect of stocking density on growth and settlement of veliger of *B. spirata.*

Experiment was conducted to study the effect of stocking density on growth and settlement of the veliger. The veligers were stocked at densities 75, 100, 125, 150, 175, 200, 300, and 500 larvae l^{-1}. They were fed daily with concentrations of *Chaetoceros calcitrans* based on the result of the experiment 6.1.2.3. The water parameters like salinity, temperature and pH were maintained at 32 ppt, 27 ± 1ºC and 8.1 ± 0.1 respectively for all the treatments. The exchange of water was changed on alternate day of the experiment and the larvae counted in each container. All tanks were aerated gently and treatments were in triplicates. The growth of the larvae monitored on alternate day of the experiment. The absolute growth rate (AGR) of the veliger was calculated to find out the growth rate in different stocking densities based on the formula,

$$\text{Absolute growth rate (AGR)} = \frac{L_1 - L_0}{t_1 - t_0}$$

Where, L_1 and L_0 are shell length at time t_1 and t_0 respectively (Wolf and Garrido 1991).

6.1.4 Effect of substratum on growth and settlement of veliger of *B. spirata.*

Experiment was conducted to study the effect of substratum on settlement of the veliger. The treatments were maintained at 27ºC having salinity 32 ppt and pH 8.1. The tanks were provided with aeration and supplied with *Chaetoceros calcitrans* as feed. The control of the experiment was without substrates (TRT-1) for the settlement. Three types of substrates were used (1) nylon net (TRT-2), (2) clean glass slides (TRT-3) and (3) glass slide coated with mat forming algal cells (TRT-4). The number of days required for the larvae to settle and the percentage of settlement was monitored. All the treatments were in duplicates.

6.1.5. Effect of water exchange during the rearing period on growth and settlement of veliger of *B. spirata*.

Experiment was conducted to study the need for the exchange of water during the period of metamorphosis of the veliger. Five different water exchange regimes were tested during the experimental period those were (1) no water exchange throughout the experiment (TRT-1), (2) Complete water exchange daily (TRT-2), (3) 50% water exchange daily (TRT-3), (4) 50% water exchange on alternate days (TRT-4), (5) Daily addition of $1/4^{th}$ of the water to the rearing tank (TRT-5). The control of the experiment was complete water exchange on alternate day of the experiment (TRT-6). The concentration of ammonia in all the treatments was measured daily. Each treatment was duplicated and the stocking density of veliger was maintained at 150 l^{-1}. The water temperature was 27 ± 1ºC and salinity 32 ppt. Growth and survival were monitored and recorded on alternate days during the experiment.

6.1.6. Effect of aeration on growth and settlement of veliger larvae of *B. spirata*

Experiment was conducted to study the effect of aeration on growth and settlement of the veliger during metamorphosis. Two sets of treatments were set up (1) with gentle aeration (TRT-1) and (2) without aeration (TRT-2). All other rearing procedures were based on the results of experiments conducted earlier. The dissolved oxygen level and concentration of ammonia in the rearing medium were monitored on alternate days before exchange of water. The growth, number of days required for the larvae to settle and percentage of survival was recorded.

6.1.7. Effect of light on growth and settlement of veliger of *B. spirata*

Experiments were also conducted to study the effect of light on growth and settlement of the veliger during metamorphosis. Three treatments were set up such as (1) reared in 24 h of light (TRT-1), (2) reared in 24 h darkness (TRT-2) and (3) the control of the treatment was the rearing of the larvae in 12 h of light and 12 h of darkness (TRT-3). Water was changed on alternate day of the experiment and all the treatments were provided with aeration. Larvae were fed with *Chaetoceros salina* as feed at required quantity based on the progress of metamorphosis. The number of days required for the larvae to settle and percentage of survival was recorded.

6.2. RESULTS

6.2.1. Salinity tolerance and effect of salinity on growth and settlement of the hatched veliger

The duration of pelagic life of *B. spirata* veliger in different salinities was different. In 32 ppt salinity (control) the larvae showed the tendency to settle down on 18th day after hatching out from the egg capsule. The percentage of survival on settlement day of the juvenile from the day of hatching to settlement is shown in the Fig. 6.2. The percentage settlement was also higher in control salinity when compared with other salinities. Out of 76% of the larvae which survived on 10th day of development, 58% survived and settled in the control salinity. In 25 ppt salinity the settlement day was prolonged and it took eight more days (19th -26th day) for the complete settlement of the juvenile. The percentage of settlement was also low, only 28% settled as juvenile. In 30 ppt salinity the first settlement was observed on day 19th and duration for settlement of all the larvae was same as that of control salinity (4 days). In 35 ppt settlement of the metamorphosed juvenile was observed on 20th day. The duration of settlement in 35 ppt was seven days and settlement was 57%. Much prolonged pelagic life was observed in higher salinities. In 40 ppt settlement was found to begin only on 22nd day and only 15% of settlement was attained on complete settlement of the larvae. Only 3% survival and settlement was recorded in 45 ppt salinity on the final day.

The growth of the larvae of *B. spirata* also showed significant variation ($P< 0.05$) in all salinities. The average shell height and shell width of the veliger on the initial day of the experiment was 477 ± 7 µm and 330 ± 4 µm respectively. On the final day of settlement the average measurements in different salinities showed variation in shell height and shell width. Maximum shell length and shell width was attained in control salinity (32 ppt) which were 919 ± 36 µm and 800 ± 44 µm respectively. In 25 ppt salinity the growth decreased and the settled juvenile had 783 ± 49 µm shell length and 693 ± 68 µm hell width. Though the survival and settlement was low in 45 ppt salinity, those which survived had an average shell length of 793 ± 14 µm and shell width 693 ± 28 µm. The average shell length of the settled juvenile in 30 ppt salinity

was 903 ± 14 µm having the shell width 783 ± 7 µm. The average shell length and shell width of the veliger during the metamorphosis in different salinities and result of the ANOVA is summarized in the Table 6.2. The percentage of settlement rate during metamorphosis of *B. spirata* veliger in different salinities is shown in the Fig. 6.3. The instantaneous growth rate (IGR) of the veliger during metamorphosis was computed and is represented in the Fig. 6.4 & 6.5.

The average shell length, shell width and settlement percentage showed an increase in trend up to 25-32 ppt thereafter it decreased. So the salinity ranges were grouped into two categories (i) 25-32 ppt and (ii) 35-45 ppt. The average shell length, shell width and percentage of settlement showed strong positive correlation with the salinity range 25-35 ppt which was r= 0.9867, 0.9881, 0.9756 respectively. While in the salinity range 35-45 it was negative correlation, for the shell length (r= -0.834), shell width (r= -0.834) and settlement percentage(r=-0.964) respectively. The days required for the veliger larvae to settle showed positive correlation with salinity (r=0.888).

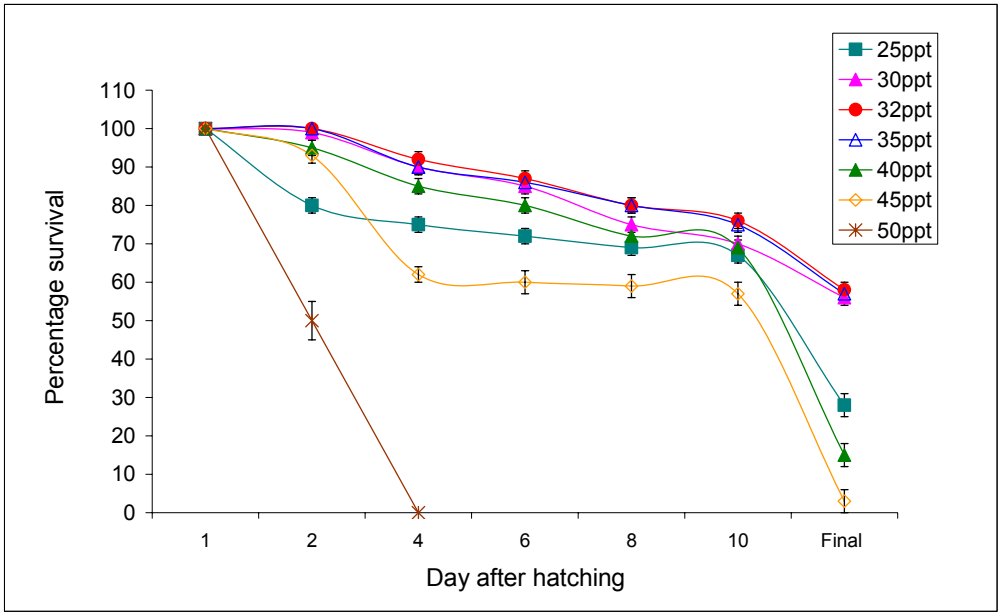


Fig. 6.2. Percentage of survival of *B. spirata* veliger in different salinities

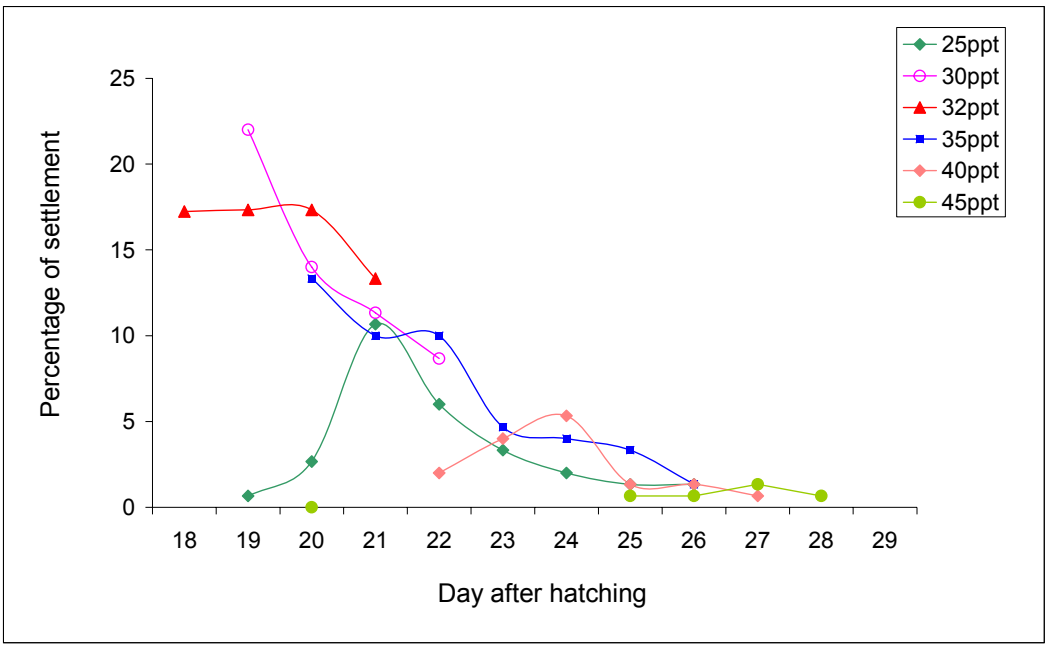


Fig. 6.3. Percentage of settlement rate during metamorphosis of *B. spirata* veliger in different salinities

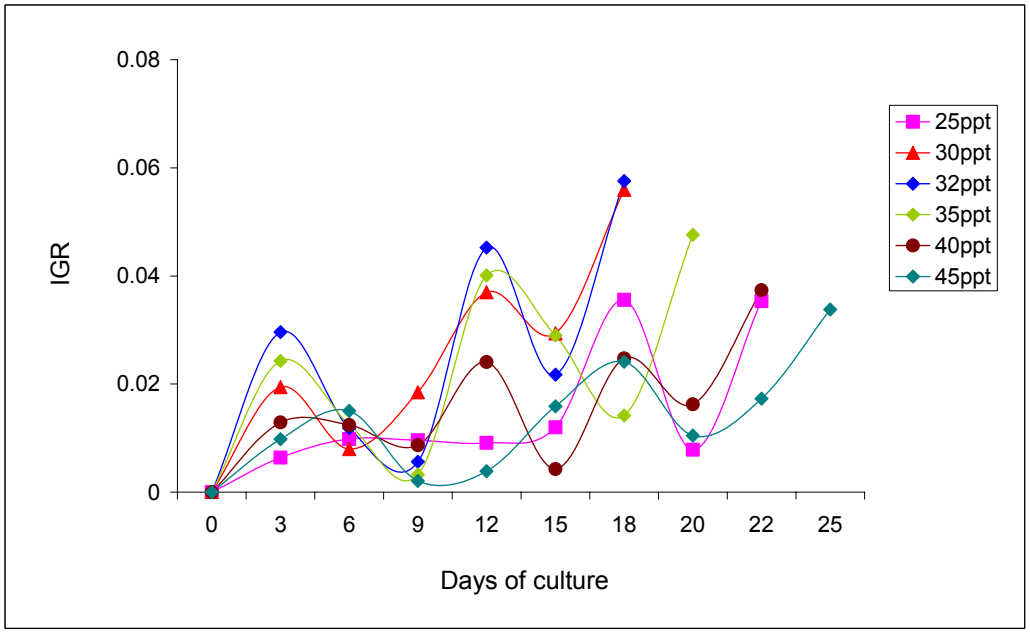


Fig. 6.4. The instantaneous growth rate (IGR) in shell length of the veliger till settlement

Table- 6.2. Average shell length and shell width (μm) of the veliger during development in different salinities and results of ANOVA. Result of Duncan Post-Hoc Multiple Test are indicated in superscript. Non-identical superscripts row wise indicates significant difference ($P< 0.05$).

Day *		**F value**	**Sig**	**25ppt**	**30ppt**	**32ppt**	**35ppt**	**40ppt**	**45ppt**
1	SL*	0.523	0.755	477 ± 7^{a}	470 ± 5^{a}	477 ± 7^{a}	477 ± 7^{a}	477 ± 7^{a}	477 ± 7^{a}
	SW*	0.036	0.999	329 ± 5^{a}	329 ± 5^{a}	329 ± 5^{a}	329 ± 5^{a}	329 ± 5^{a}	329 ± 5^{a}
3	SL	5.523	0.007	483 ± 3^{a}	498 ± 15^{ab}	521 ± 14^{c}	512 ± 10^{bc}	$495 \pm 7^{a\,b}$	486 ± 5^{a}
	SW	6.666	0.003	339 ± 6^{a}	356 ± 14^{ab}	377 ± 12^{c}	370 ± 10^{c}	$353 \pm 8^{a\,b}$	342 ± 9^{a}
6	SL	9.873	0.001	506 ± 19^{a}	514 ± 8^{a}	546 ± 4^{b}	539 ± 1^{b}	520 ± 3^{a}	514 ± 9^{a}
	SW	5.181	0.009	378 ± 16^{ab}	385 ± 5^{abc}	403 ± 4^{c}	395 ± 2^{bc}	$373 \pm 3^{a\,b}$	361 ± 17^{a}
9	SL	0.004	0.005	526 ± 6^{a}	553 ± 12^{bc}	559 ± 18^{c}	546 ± 5^{b}	$534 \pm 3^{a\,b}$	514 ± 9^{a}
	SW	127.9	0.000	404 ± 2^{b}	433 ± 6^{c}	436 ± 3^{c}	430 ± 3^{c}	384 ± 11^{a}	379 ± 7^{a}
12	SL	136.5	0.000	545 ± 3^{a}	641 ± 12^{c}	669 ± 11^{d}	641 ± 12^{c}	598 ± 7^{b}	537 ± 5^{a}
	SW	20.296	0.000	420 ± 11^{a}	459 ± 2^{bc}	478 ± 26^{cd}	454 ± 7^{c}	498 ± 21^{d}	410 ± 2^{a}
15	SL	342.02	0.000	572 ± 3^{a}	721 ± 13^{c}	730 ± 6^{c}	720 ± 10^{c}	604 ± 5^{b}	554 ± 25^{a}
	SW	58.664	0.000	436 ± 5^{a}	544 ± 14^{bc}	553 ± 6^{c}	544 ± 7^{bc}	491 ± 38^{b}	427 ± 26^{a}
18	SL	101.13	0.000	783 ± 49^{a}	902 ± 14^{c}	919 ± 35^{c}	879 ± 28^{b}	813 ± 45^{a}	793 ± 12^{a}
	SW	133.99	0.000	693 ± 68^{a}	783 ± 7^{d}	800 ± 44^{d}	775 ± 15^{c}	692 ± 13^{c}	707 ± 56^{b}

* Day after hatching of the veliger from the egg capsule

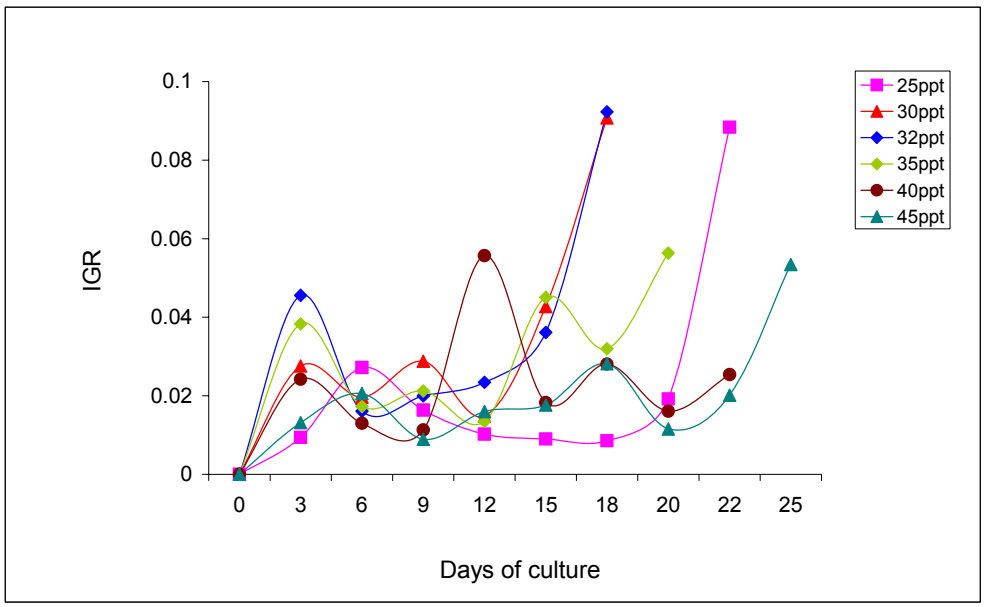


Fig. 6.5. The instantaneous growth rate (IGR) in shell width of the veliger till Settlement

6.2.2. FEEDING THE VELIGER

6.2.2.1. Necessity of supplementary feed for the survival of newly hatched veliger

The water treatment during the larval rearing removed all the natural phytoplankton in the sea water. From the experiment it was confirmed that the supplement of additional algal feed was necessary for the survival of the veliger from the first day of hatching. The veligers supplied with algal feed showed 90% survival on the following day, while those in treatment without feed, the survival was only 30% on the second day. Out of the 30% which survived on the second day complete mortality was recorded on the following day.

6.2.2.2. Ingestion rate (IR) by the veliger

There was variation in the consumption of algal cells during the initial stage of pelagic life cycle and further progressive growth. On the first day of hatching the ingestion rate per larvae was 7755 ± 213 cells ml^{-1} h^{-1}. From the third day onwards the ingestion rate doubled. A rapid decrease in the rate of consumption was noticed on 15^{th} day and the pelagic, planktonic veliger settled as juvenile and become carnivorous on 18^{th} day. The pelagic phase of the life cycle could be grouped into three stages based on the feeding rate. From the first day to second day the average number of cells ingested per larvae was 8195 ± 124 cells ml^{-1} h^{-1} (Phase I). From the

third day to the 13th day the ingestion rate increased to 18858 ± 631 cells ml^{-1} h^{-1}(Phase II), while during 15 to 18th day the average cells required was 9635 ± 651 cells ml^{-1} h^{-1} (Phase III). The average ingestion rate per veliger during the 14th days of pelagic life was 16392 ± 756 cells ml^{-1} h^{-1}. The average ingestion rate (IR) of the veliger during larval period is shown in the Fig. 6.6.

Diurnal variation in feeding was noticed. Difference was also observed in the first 10 h (day time) of feeding and next 14 h (night hours). Higher rate of feeding was noticed immediately after the water exchange during day time and it decreased during night hours. The average rate of consumption during the pelagic phase of the life cycle per larvae doubled during the day time (10h) which was 20977 ± 1198 cells ml^{-1} h^{-1}, while during the next 14 h (night time) it was 11731 ± 821 cells ml^{-1} h^{-1}. The difference in the ingestion rate (IR) during day time and night hours during the metamorphosis of the veliger are represented in the Fig.6.7.

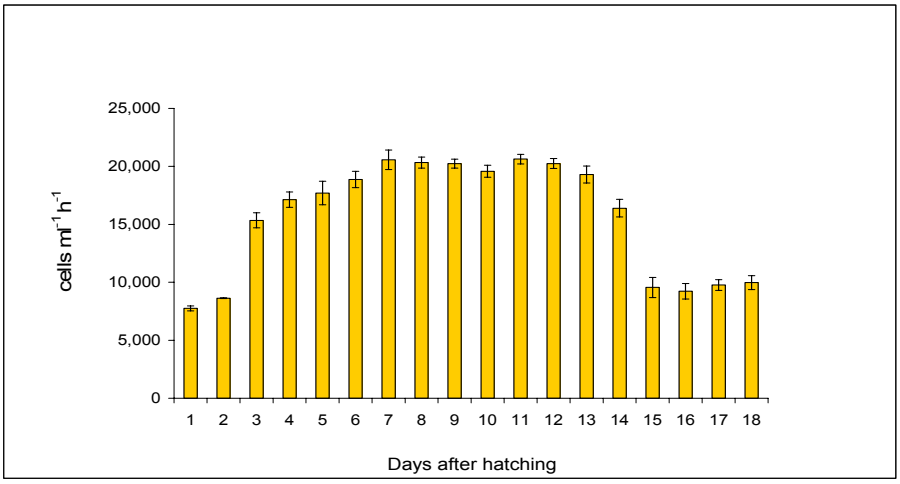


Fig. 6.6. Ingestion rate during planktonic larval period of *B. spirata* veliger

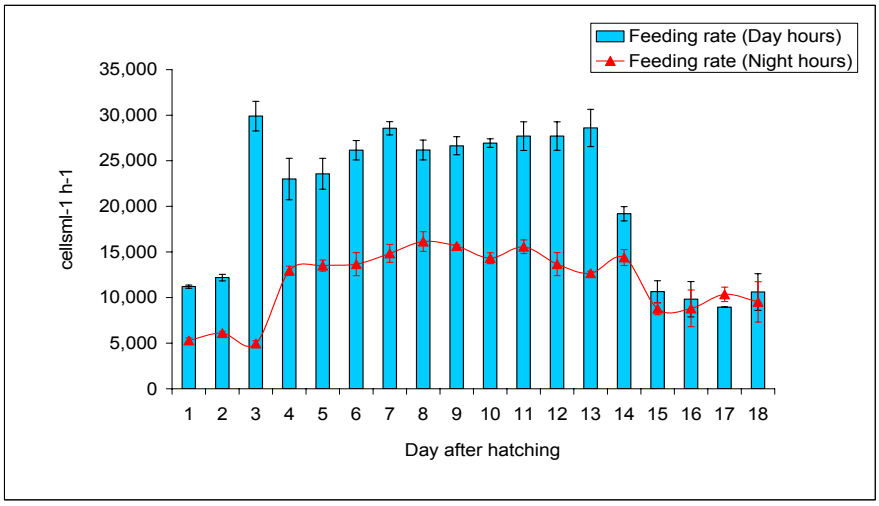


Fig. 6.7. Difference in Ingestion rate during day time and night hours of *B. spirata* veliger

6.2.2.3. Acceptance of different types of algal cells and its effect on growth and settlement of veliger of *B. spirata*

The average IR from the 1st day up to settlement is shown in the Table 6.3. None of the veliger larvae could survive on smaller nano plankton feed. Complete mortality was recorded on the fourth day in the rearing tank fed with *N. salina* (TRT-3-*N*s) The IR observed on the first day was also negligible (2075 ± 307 cells ml^{-1} h^{-1}) when compared with other algal feed. The veligers were weak and could not consume the algae which resulted in mortality. The *T. gracilis* used for the rearing (TRT-2-*Tg*) also could not support the life till the end of metamorphosis. The average IR of the on first day of pelagic life was 4619 ± 500 cells ml^{-1} h^{-1}. The larvae survived up to seventh day after hatching and complete mortality occurred on 9th day. The veliger could survive up to settlement only on *I. galbana* and *C. calcitrans* as feed (TRT-1-*Cc* and TRT-4-*Ig*). In both treatments the IR increased as the development progress with time. The average IR for the first two days on *I. galbana* and *C. calcitrans* were 8060 ± 156 and 8360 ± 110 cells ml^{-1} h^{-1} respectively. Rapid increase in the IR was observed from 3rd to 12th day in TRT-1-*Cc* and TRT-4-*Ig*. The IR during this period for *I. galbana was* 19064 ± 551 cells ml^{-1} h^{-1}. while for *C. calcitrans* the IR was increased from 3rd day to 14th day and the average IR during this period was 19329 ± 643 cells ml^{-1} h^{-1}. On 13th day slight decrease in the IR was observed TRT-4-*Ig* while in TRT-1-*Cc* decrease was observed on 15th day. Ingestion rate decreased from the day 14 to settlement. The average IR during this period for *I. galbana* and *C. calcitrans* were 11112 ± 1223 and 13277 ± 436 cells ml^{-1} h^{-1} respectively. The average ingestion rate of the veliger from the day of hatching to settlement in different algae is shown in Table 6.3. The comparison of the IR in *I. galbana* and *C. calcitrans* is represented in the Fig. 6.8.

The percentage of survival on settlement day of the juvenile which were fed on both species was 59%. The number of days required for the larvae to settle as juvenile was also same, 19th day after hatching in both treatments. The average shell length and shell width of the juvenile on the day of settlement in treatment where *C. calcitrans* (TRT-1-*Cc*) provided as feed was 934 ± 66 μm and 820 ± 83 μm respectively, while in the treatment *I. galbana* (TRT-4-*Ig*) as feed was 900 ± 72 μm shell length and 788

± 57 µm shell width. The comparison of the growth of veliger feeding in both species of micro algae was shown in the Fig.6.9.

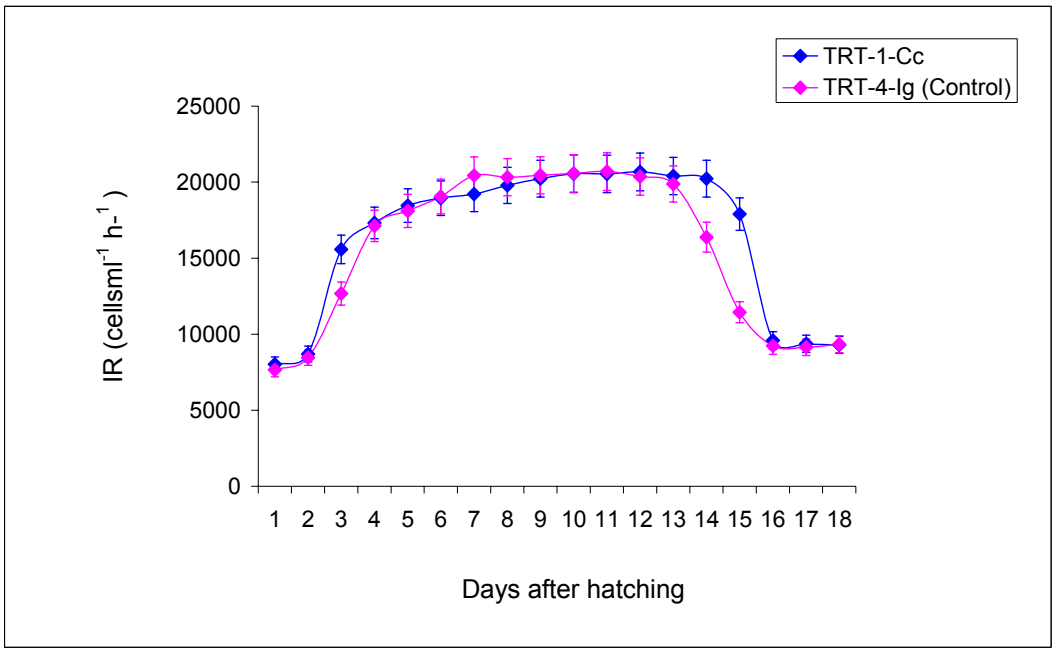


Fig.6.8. Ingestion rate(IR) of *Isochrysis galbana* and *Chaetoceros calcitrans* provided as feed to the veliger of *B. spirata*

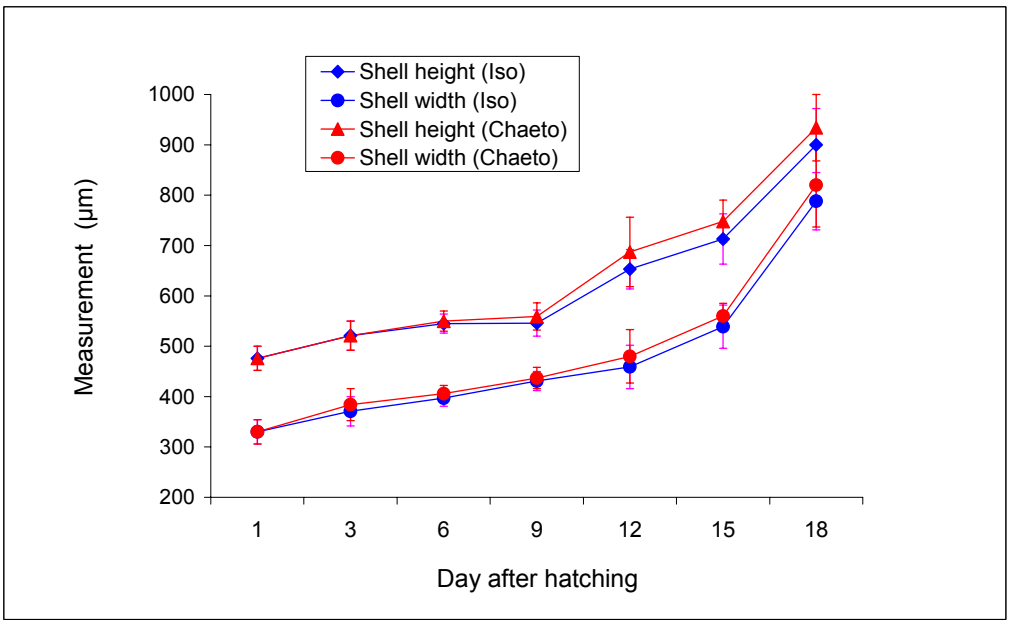


Fig. 6.9. Comparison of the growth of the veliger fed on *Isochrysis galbana* and *Chaetoceros calcitrans*

Table.6.3. The average IR (cells ml^{-1} h^{-1}) of the veliger on different kind of microalgae up to settlement.

Day	TRT-1-Cc	TRT-2-Tg	TRT-3-Ns	TRT-4-Ig (Control)
1	8022 ± 157	4619 ± 500	2074 ± 307	7661 ± 140
2	8699 ± 62	4977 ± 759	2117 ± 581	8459 ± 173
3	15581 ± 487	5416 ± 406	262 ± 1046	12676 ± 597
4	17321 ± 389	5326 ± 419		17128 ± 323
5	18462 ± 614	6785 ± 1384		18104 ± 609
6	18948 ± 1004	9785 ± 2043		19053 ± 436
7	19213 ± 1201	14551± 4759		20435 ± 645
8	19788 ± 847	1149 ± 2672		20325 ± 474
9	20231 ± 467			20451 ± 576
10	20550 ± 616			20578 ± 521
11	20547 ± 458			20697 ± 477
12	20675 ± 580			20375 ± 505
13	20405 ± 587			19878 ± 902
14	20231 ± 467			16382 ± 755
15	17896 ± 651			11448 ± 450
16	9588 ± 242			9235 ± 672
17	9373 ± 348			9150 ± 668
18	9298 ± 472			9344 ± 3572

6.2.2.4. Effect of combination of the algal feed on growth and settlement of veliger of *B. spirata*

When mixed feed of *I. galbana* and *C. calcitrans* was provided the ingestion rate (IR) followed the same pattern as that for mono algal feed. The IR increased from the first day of pelagic life up to 12th day and reduced from the 13th day up to settlement. The average IR when mixed feed was provided was lower than in the control The average IR during the pelagic phase was 15868 ± 531 cells ml^{-1} h^{-1} while in the control was 16162 ± 374 cells ml^{-1} h^{-1}. The percentage of settlement found to be same in the treatments (60%) as well as in the control. The settlement was on 18th

day after hatching. There was also no difference (P>0.05) observed in the growth of the veliger. The mean size of the juvenile on the day of settlement in TRT-2 was 936 ± 62µm shell length and 841 ± 80µm shell width while in the control (TRT-1) it was 920 ± 80 and 829 ± 80 µm respectively. The result of the experiment is shown in the Table 6.4.

Table 6.4. Ingestion rate of the larvae when mixed algae (*Cc* + *Ig*) and monoalgal feed were supplied during the pelagic phase**.**

Days after hatching	Ingestion Rate (IR) (cells ml^{-1} h^{-1})		Average measurements (µm) ± SD			
			Mixed algal diet (Cc+Ig)		Control (*Chetoceros calcitrans*)	
	Mixed feed	Monoalgal feed (Control)	Shell length	Shell width	Shell length	Shell width
1	7864± 81	8087 ± 8	476 ± 24	330 ± 24	476 ± 24	330 ± 24
2	8494 ± 65	8699 ± 62				
3	15212 ± 475	15583 ± 489	521 ± 29	371 ± 29	521 ± 29	378 ± 31
4	16911 ± 378	17321 ± 389				
5	18024 ± 600	18462 ± 614				
6	18504 ± 801	18960 ± 299	545 ± 19	397 ± 16	546 ± 27	403 ± 15
7	19339 ± 930	20435 ± 330				
8	19796 ± 563	20325 ± 330				
9	19796 ± 563	20325 ± 330	588 ± 39	454 ± 17	559 ± 27	436 ± 20
10	20284 ± 651	21017 ± 342				
11	20101 ± 566	20634 ± 336				
12	20077 ± 620	20451 ± 330	654 ± 39	469 ± 41	670 ± 59	480 ±53
13	19943 ± 624	20451 ± 330				
14	18458 ± 808	18239 ± 873				
15	15822 ± 487	14432 ± 344	724 ± 29	567 ± 35	730 ± 24	554 ± 26
16	9237 ± 434	9328 ± 662				
17	8976 ± 389	9008 ± 451				
18	9018 ± 339	9167 ± 208	936 ± 62	841 ± 80	920 ± 80	829 ± 80

6.2.2.5. Effect of low and high concentration of the algal feed on growth and settlement of veliger of *B. spirata*

The average number of algal cells consumed by the larvae in the control of the experiment (TRT-4) was 17886 ± 5527 cells ml^{-1} h^{-1}. In the treatment where the larvae were provided with low supply of feed (TRT-1) the ingestion rate (IR) was only 6090 ± 2037 cells ml^{-1} h^{-1}. In the treatments where excess feed was given

(TRT-2 and TRT-3) the IR was found to be high, 18017 ± 5923 and 18548 ± 5647 cells ml^{-1} h^{-1}respectively. The average IR of the veliger in different treatments is shown in the Fig. 6.10.

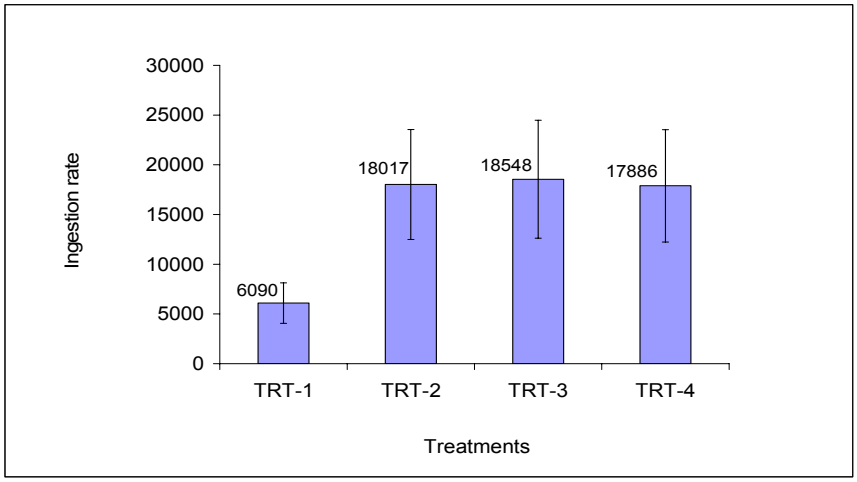


Fig 6.10. Ingestion rate (IR) of veliger of *B. spirata* in different concentration of algal cells

The number of days required for completion of the metamorphosis and settlement of the veliger varied in treatments. In the control treatment (TRT-4) the veliger settled as juvenile on 19th day of the experiment. In TRT-1 the growth rate of veliger was slow and required 29 days for the settlement. In TRT-2 and TRT-3 the veliger metamorphosed earlier and settled as juvenile on 17th and 15th day after hatching.

The percentage of survival was very low (30%) in TRT-1. In TRT-2 though the settlement was early, the percentage of settlement was low (55%) than the control (60%). In higher concentration of feed the percentage of settlement was found to decrease (51%). The average percentage of settlement in different feed concentration is shown in the Fig. 6.11.

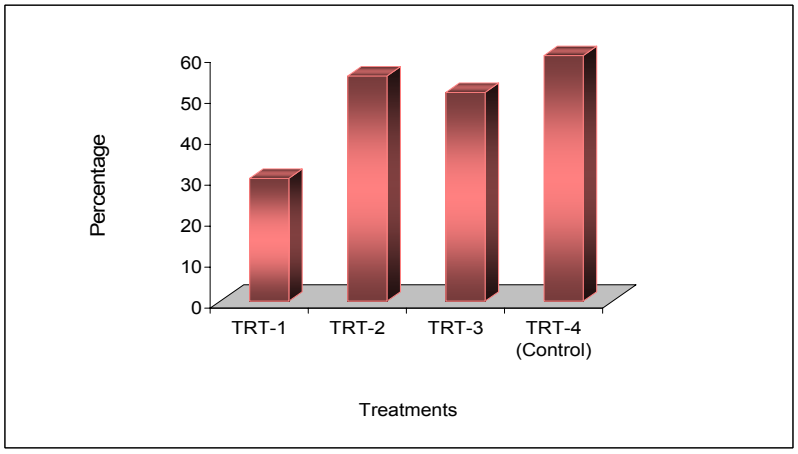


Fig. 6.11. The percentage of settlement on the juvenile *B. spirata* in different treatment

The average growth in shell length and shell width on the day of settlement in different treatments did not show significant variation (P> 0.05) The shell length of the juvenile on the day of settlement were 920 ± 20, 960 ± 20, 960 ± 20, 960 ± 20 μm in treatments TRT-1, TRT-2, TRT-3, TRT-4 respectively. The average shell width was 820 ± 20, 840 ± 20, 840 ± 20 and 860 ± 20 μm in respective treatments.

6.2.2.6. Effect of temperature on IR of algal feed, growth and settlement of veliger of *B. spirata*

Temperature had significant effect on growth and settlement of the veliger. In lower temperature (22-24ºC), time taken to settle was much prolonged and it took 29 days for settlement. In 24- 26 ºC, 23 days was required for the veliger to settle. In the control treatment (26-28^{o}C) the veliger settled as juvenile on 19th day after hatching. In higher temperatures the metamorphosis was earlier, it took 13-15 days for settlement. The settlement was much earlier (13th day) in 30-32ºC, while in 28-30ºC it was on 15th day.

The average size of the larvae also showed significant variation based on the day of settlement. Maximum growth was obtained in 26-28ºC, where the juvenile was 1020 μm in shell length and 950 μm in shell width. The average shell length of the juvenile on the first day of settlement in the temperature ranges 22-24, 24-26, 26-28, 28-30 and 30-32ºC were 920 ± 20, 982 ± 25, 983 ± 26, 946 ± 54 and 911 ± 9 μm respectively. The average shell length and width of the veliger during metamorphosis at different temperatures is shown in the Fig. 6.12 and 6.13. Similarly the average shell width was 859 ± 68, 917 ± 31, 917 ± 31, 859 ± 69 and 814 ± 7 μm in the above respective temperatures. Maximum growth in shell length (983 ± 26 μm) of the veliger during rearing period was obtained in control of the experiment (26-28^{0}C).

The percentage of settlement varied in different temperatures. Maximum percentage of settlement (61%) was obtained in temperature of 26-28ºC. At higher temperatures (28-30º and 30-32ºC) 56% veliger settled as juveniles while it was 40% and 58% in 22-24 and 24-26ºC respectively. In higher temperatures the survival after settlement was very low. None of the larvae survived after the settlement in 30-32 ºC while it was 50% in 28-30ºC. The survival of the juvenile after settlement was 70, 80 and

82% in 22-24, 24-26 and 26-28ºC respectively. The percentage of settlement and survival percentage after settlement are shown in the Fig. 6.14.

Feeding rate of the veliger at different temperatures also varied. An increase in the ingestion rate (IR) was observed at higher temperature. The average IR per larvae ml^{-1} h^{-1} is represented in the Fig. 6.15. The average IR in different temperature regimes of 22-24,24-26, 26-28, 28-30 and 30-32^{0}C were 14928 ± 4183, 15871 ± 4756, 16350 ± 5367, 19053 ±5515 and 21285 ± 6890 respectively.

In the control of the experiment (26-28^{0}C), the veliger settled on 19th day and the growth was maximum. The settlement % and survival after settlement was also higher at this temperature. So the optimum temperature for rearing the larvae was found to be 27^{0}C.

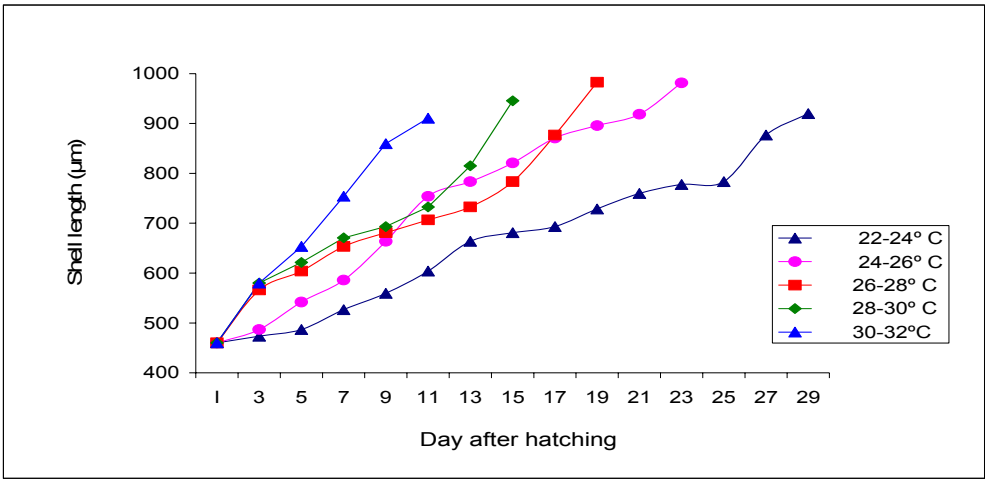


Fig. 6.12. Average shell length (µm) of the veliger of *B. spirata* in different temperatures

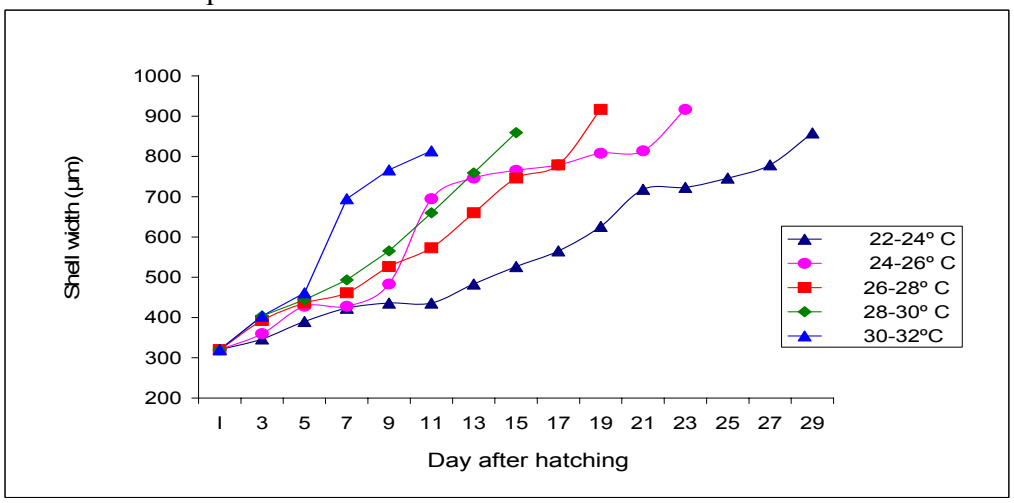
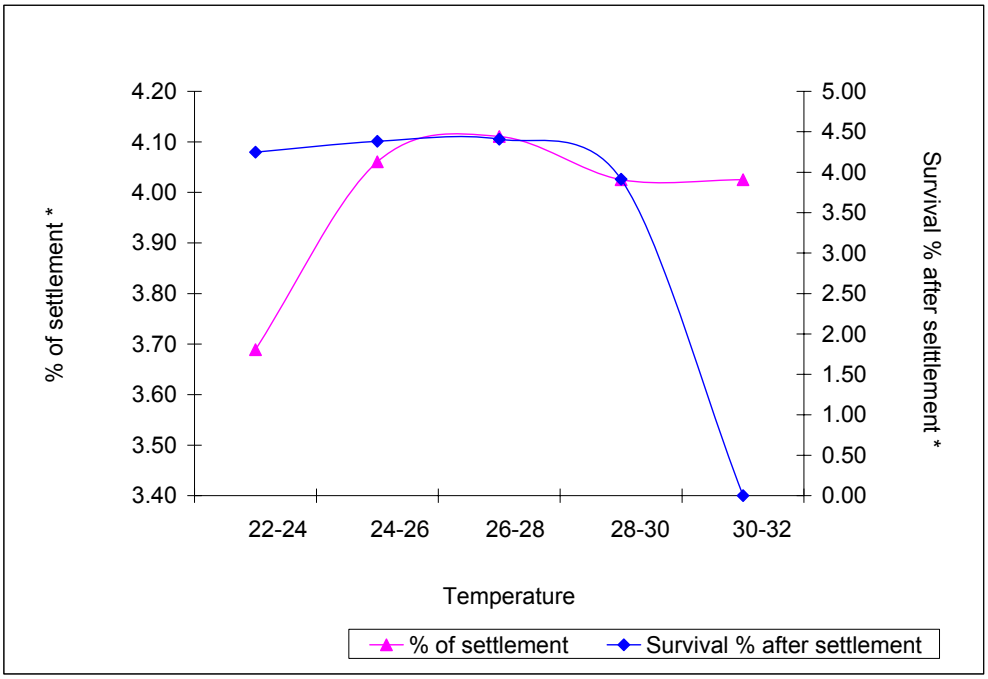


Fig. 6.13. Average shell width (µm)of the veliger of *B. spirata* in different temperatures



* Natural log of values are given

Fig. 6.14. Percentage of settlement % and survival % after settlement of the juvenile *B. spirata* at different temperatures

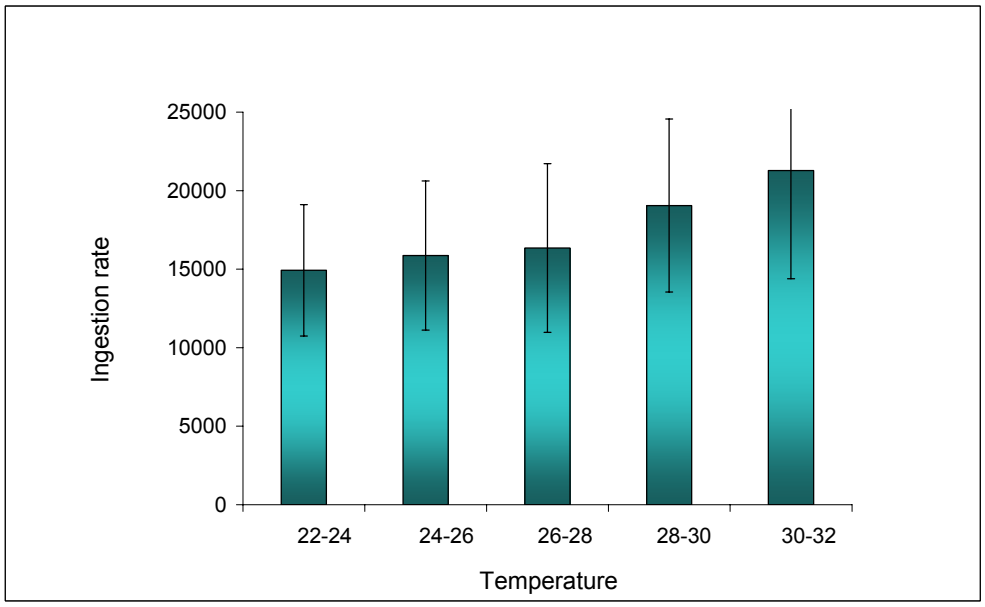


Fig. 6.15. Ingestion rate (IR) of the veliger of *B. spirata* at different temperature regimes

6.2.3. Effect of stocking density on growth and settlement of veliger of *B. spirata.*

The percentage of settlement varied in different stocking densities. Maximum percentage of settlement (62%) was obtained in the stocking density of 150 l^{-1}. More than 50% settlement was recorded in the stocking density up to 150 l^{-1} and above this density the settlement percentage was below 50. At the stocking density of 75, 100 and 125 l^{-1}, the average settlement percentage was 52. The average settlement percentage was 46, 18, 5, 1 and 1% in the stocking densities 175, 200, 300, 400, and 500 larvae l^{-1} respectively. The average percentage of settlement in different stocking density is shown in the Fig.6.16.

The duration of pelagic life also varied in different stocking densities, and it was much prolonged in higher stocking densities. The settlement process began on 17th day in control stocking density and was completed on 23rd day, while in lower concentrations it was from 19th to 23rd day. In the stocking density 175 larvae l^{-1} the settlement process started on 23rd day and was completed on 27th day. In all the higher stocking densities (300, 400, and 500) the settlement was much delayed and only a few larvae settled on 23rd day. The average measurements of the settled juvenile in different stocking densities are shown in the Table 6.5. Absolute growth rates of shell length and shell width of the veligers in different stocking densities are shown in the Fig. 6.17 & 6.18.

Table 6.5. Average size of the settled juvenile in different stocking densities.

Treatment	Density larvae/l	Average shell length (μm)	Average shell width (μm)
TRT-1	75	966 ±8	870 ± 14
TRT-2	100	966 ± 8	900 ± 0
TRT-3	125	976 ± 6	880± 0
TRT-4	150	970 ± 14	870 ± 14
TRT-5	175	920 ± 28	850 ± 14
TRT-6	200	920 ± 2	810 ± 14
TRT-7	300	903 ± 4	803 ±4
TRT-8	400	903 ±4	803 ±4
TRT-9	500	900 ± 0	800 ± 0

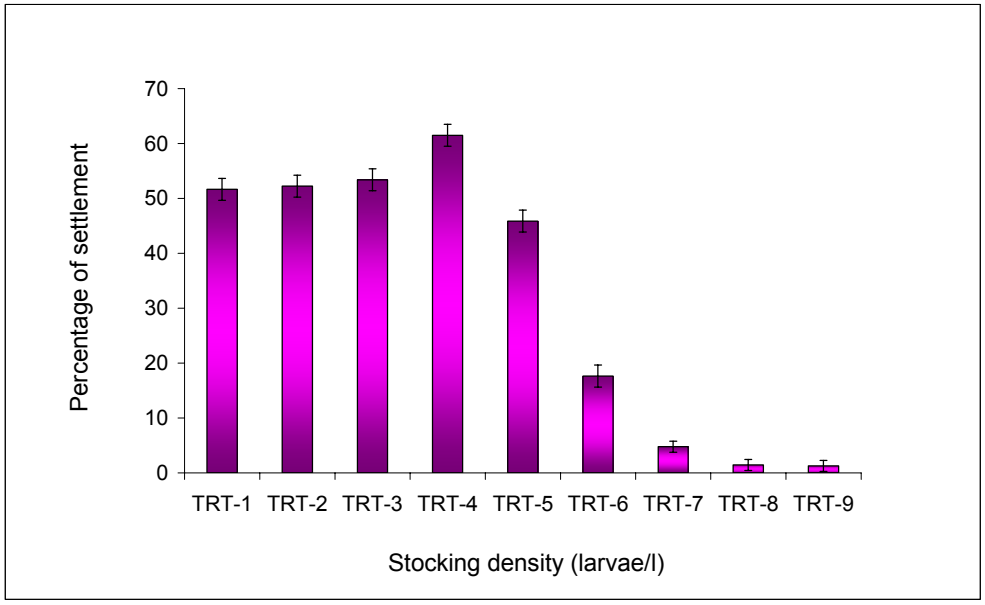


Fig. 6.16. Percentage of settlement of the veliger at different stocking densities

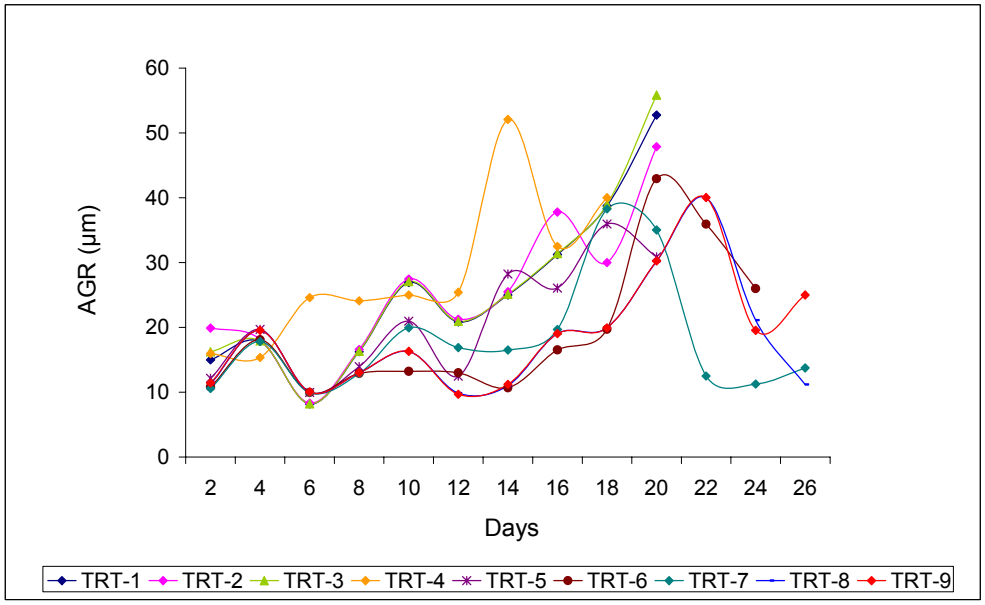


Fig. 6.17. Absolute growth rate (AGR) in shell length of the veliger in different stocking densities

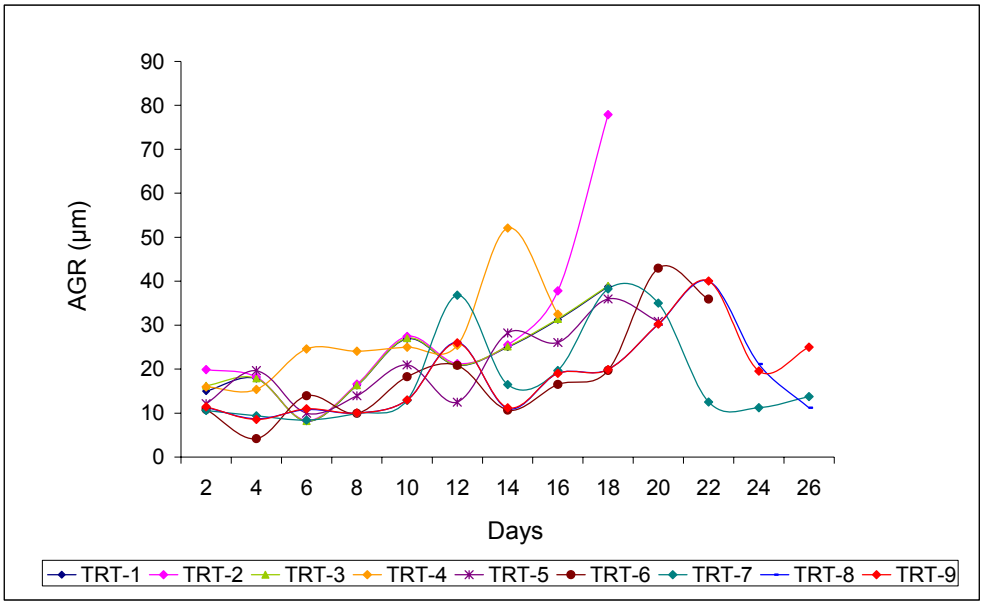


Fig. 6.18. Absolute growth rate (AGR) in shell width of the veliger in different stocking densities

6.2.4. Effect of substratum on growth and settlement of veliger of *B. spirata*.

There was no difference in the percentage of settlement and number of days required for the veliger to settle as juvenile in the tanks provided with substratum and without any substratum. The larvae started to settle on 18th day of pelagic phase and completed the settlement on 22nd day. The settled larvae were found to crawl along the bottom and walls of the container. While crawling, some were found on the substrates, but did not remain there for a long time. The average shell length 980 ± 30 µm and shell girth 893 ± 59 µm and did not show variation between treatments.

6.2.5. Effect of water exchange during the rearing period on growth and settlement of veliger of *B. spirata*.

Maximum settlement (62%) was recorded in TRT-6, where complete water was changed on alternate days of the experiment The percentage of settled juveniles in the treatment TRT-1, TRT-2, TRT-3, TRT-4, TRT-5 and TRT-6 were 27, 45, 58, 57, 50 and 62 % respectively. The percentage of survival upto settlement of the veliger on every 3rd day of culture is represented in the Fig. 6.19.

In treatment TRT-1 the concentration of ammonia was high (78.77 µmol l^{-1}). The concentration of ammonia in the rearing tanks is presented in the Fig. 6.20. The percentage of settlement of the juvenile showed a negative correlation with concentration of ammonia in the rearing tank (r= -0.703).

Difference was also observed in the average shell length and shell width of the veliger during the period of metamorphosis. Maximum shell length of 966 ± 12 µm and shell width of 940 ± 0 µm were attained in the treatment TRT-6. The shell length (860 ± 20 µm) and shell width (773 ± 12µm) measurements were low in the treatment TRT-1, where no water was changed throughout the experiment. The average value of ammonia, % of survival, shell length, width and result of ANOVA during different treatment regimes are shown in the Table 6.6. The average values of the different treatments and ANOVA results are shown in the Table 6.7.

Table 6.6 Average value of ammonia, % of survival, shell length and width and result of ANOVA during different treatment regimes.

Treatment	**NH_3 (µmol/l)**	**Survival %**	**Shell length (µm)**	**Shell width (µm)**
TRT-1	26.80 ± 29	52.29 ± 33	624.0 ± 131.7	490.71 ± 147.8
TRT-2	0.601 ± 0.2	83.86 ± 16.75	629.57 ± 140.31	509.7 ± 164.93
TRT-3	4.35 ± 4.0	79.0 ± 18.72	626.57 ± 149.33	502.86 ± 175.95
TRT-4	8.47 ± 8.0	73.42 ± 21.80	641.0 ± 146.5	501.86 ± 176.65
TRT-5	20.45 ± 25	80.86 ± 17.7	648.86 ± 145.25	530.43 ± 192.68
TRT-6	9.42 ± 7.1	82.71 ± 14.56	683.29 ± 169.53	573.43 ± 234.22
F-value	**2.621**	**2.163**	**0.159**	**0.187**
Sig- P value	**0.040**	**0.080**	**0.976**	**0.966**

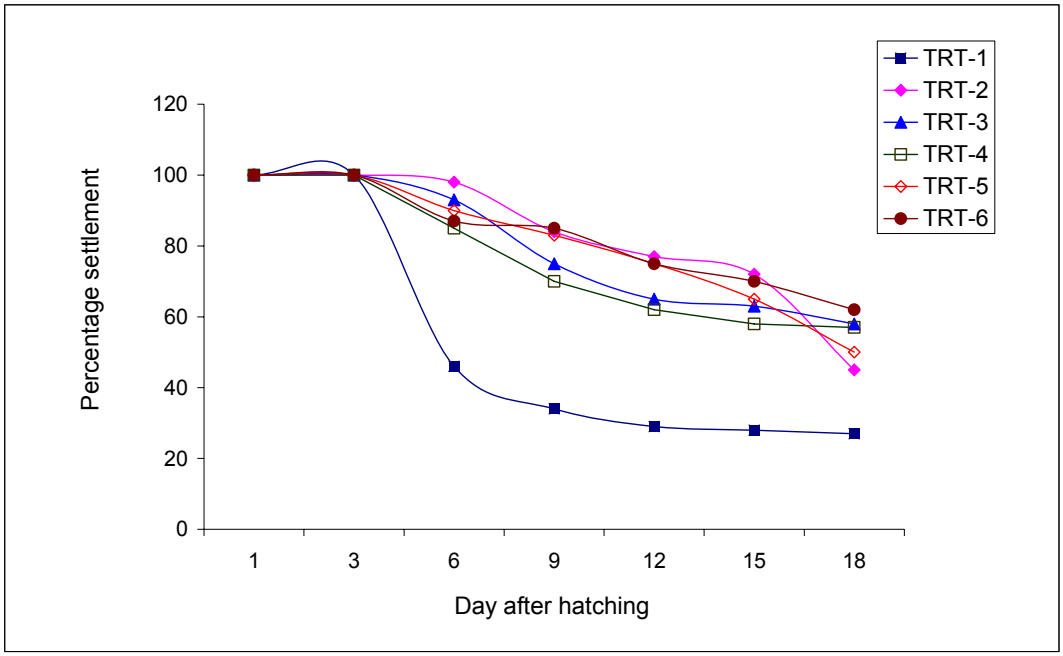


Fig. 6.19. Percent settlement of veliger in different water exchange treatment

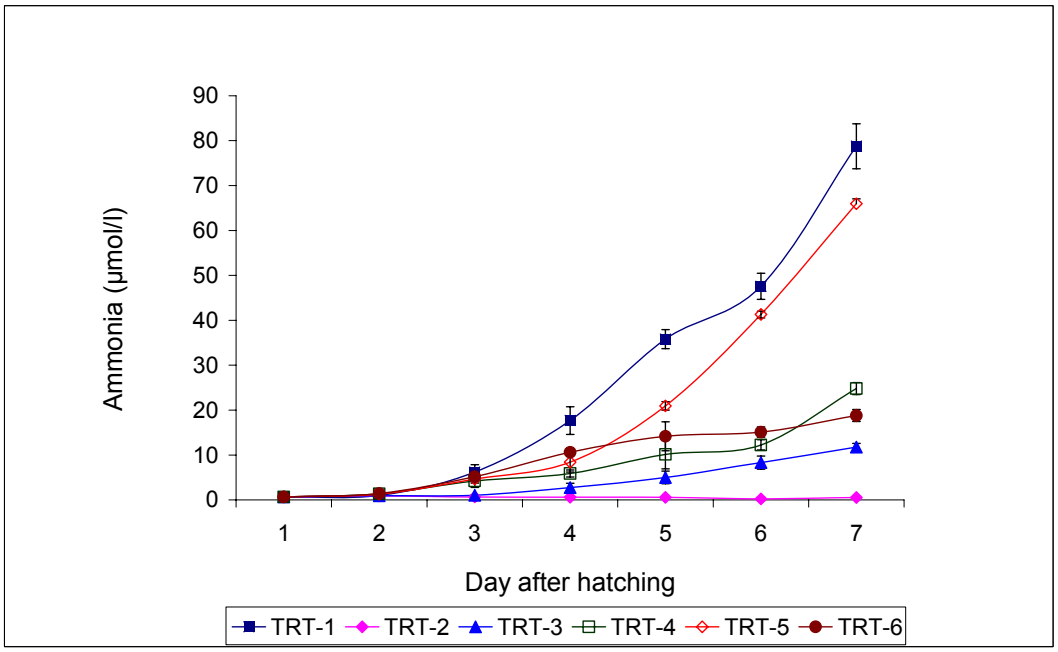


Fig. 6.20. The concentration of ammonia (µmol/l) in the different water exchange treatment

6.2.6. Effect of aeration on growth and settlement of veliger of *B. spirata* during the rearing period.

Survival and settlement was found to be low in the larval rearing tank which was not provided with aeration. The percentage of settlement in the aerated treatment was 60%, while in the non -aerated treatment it was 36%. Similarly growth was also retarded in the non-aerated culture tank. The average shell length and shell width of

the juvenile on the day of settlement in the aerated water was 956 ± 14.4 µm and 903 ± 13.6 µm , where in non- aerated tank it was 909 ± 9µm and 828 ± 8.2µm respectively. The average growth in shell length and shell width of the larvae during the period of rearing in both treatments is shown in the Fig. 6.21. The average dissolved oxygen (DO) level in the non aerated tank was much low, 3mg l^{-1} while in the aerated tank it was 13 ±0.2 mg l^{-1}. Though water was changed every alternate day of the experiment, increased production of ammonia was noticed in the non aerated treatment. The average ammonia content in the tank provided with aeration was 7 ± 0.1 µmol l^{-1} , while in the other treatment it was 16 ± 0.2 µmol l^{-1}. The amount of ammonia in the rearing tank in both treatments was found to increase with the progression of metamorphosis. The amount of ammonia in the culture tank on every 3rd day is shown in the Fig. 6.22. The number of days required for the completion of metamorphosis of the veliger to juvenile also varied. In the aerated it took 18 days while in the non-aerated tank it was prolonged to 22 days.

The amount of ammonia in the rearing tank showed a strong positive correlation (r= 0.94) with the progress of metamorphosis, while it was related negatively (r= -0.550) with dissolved oxygen in the rearing tank. Shell length and shell width was positively correlated (r= 0.87 and r= 0.89) with the production of ammonia in the rearing tank. The percentage of survival was positively correlated (r= 0.58) with the amount of dissolved oxygen in the rearing tank. The significant values of the Pearson correlation analysis is presented in the Table 6.9.

	Ammonia	Dissolved Oxygen	Shell length	Shell width	Survival %	Day required for complete metamorphosis
Ammonia	1.00	-0.55**	0.87**	0.89**	-0.93**	0.94**
Dissolved Oxygen	-0.55**	1.00	-0.17	-0.25	0.58**	-0.35*
Shell length	0.87**	-0.17	1.00	0.98**	-0.86**	0.97**
Shell width	0.89**	-0.25	0.98**	1.00	-0.86**	0.95**
Survival %	-0.93**	0.58**	-0.86**	-0.86**	1.00	0-.94**
Day required for complete metamorphosis	0.94**	-0.35*	0.97**	0.19	-0.94**	1.00

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table.6.9 . Significant correlation values in Pearson correlation analysis

Table. 6.7. Average shell length (SL) and shell width (SW) (μm) of the veliger during development in different water exchange regimes and results of ANOVA. Results of Duncan Post-Hoc Multiple Range Test are indicated in superscript. Row-wise values with identical superscripts are similar.

Treatment		**TRT-1**	**TRT-2**	**TRT-3**	**TRT-4**	**TRT-5**	**TRT-6**	**F-Value**	**Significance**
Day 1	SL	476 ± 0^{a}	476 ± 0^{a}	476 ± 0^{a}	476 ± 0^{a}	476 ± 0^{a}	476 ± 0^{a}		
	SW	330 ± 0^{a}	330 ± 0^{a}	330 ± 0^{a}	330 ± 0^{a}	330 ± 0^{a}	330 ± 0^{a}		
Day3	SL	520 ± 0^{a}	527 ± 2^{a}	527 ± 2^{a}	527 ± 2^{a}	520 ± 0^{a}	553 ± 12^{b}	**5.200**	**0.009**
	SW	371 ± 0^{a}	371 ± 0^{a}	371 ± 0^{a}	371 ± 0^{a}	371 ± 0^{a}	371 ± 0^{a}		
Day6	SL	560 ± 0^{ab}	540 ± 0^{b}	537 ± 12^{a}	566 ± 41^{b}	620 ± 20^{c}	627 ± 12^{c}	**12.800**	**0.000**
	SW	400 ± 0^{a}	401 ± 8^{a}	400 ± 0^{a}	393 ± 12^{a}	400 ± 0^{a}	447 ± 12^{b}	**21.614**	**0.000**
Day9	SL	588 ± 6^{b}	592 ± 7^{b}	559 ± 1^{a}	600 ± 0^{b}	596 ± 7^{b}	620 ± 20^{c}	**14.109**	**0.000**
	SW	454 ± 0^{b}	451 ± 10^{b}	420 ± 0^{a}	420 ± 0^{a}	453 ± 23^{b}	506 ± 12^{c}	**23.480**	**0.000**
Day12	SL	640 ± 0^{a}	657 ± 12^{ab}	670 ± 0^{b}	670 ± 17^{b}	680 ± 17^{bc}	700 ± 17^{c}	**7.232**	**0.002**
	SW	507 ± 12^{a}	587 ± 12^{c}	540 ± 0^{b}	540 ± 0^{b}	613 ± 12^{d}	640 ± 0^{e}	**115.73**	**0.000**
Day15	SL	724 ± 0^{a}	735 ± 9^{ab}	727 ± 12^{a}	740 ± 0^{b}	743 ± 6^{b}	840 ± 17^{c}	**138.39**	**0.000**
	SW	640 ± 0^{a}	655 ± 22^{a}	640 ± 0^{a}	640 ± 20^{a}	706 ± 23^{b}	820 ± 0^{c}	**65.158**	**0.000**
Day18	SL	860 ± 20^{a}	880 ± 20^{ab}	900 ± 20^{b}	906 ± 12^{b}	906 ± 12^{b}	966 ± 12^{c}	**14.550**	**0.000**
	SW	733 ± 12^{a}	773 ± 12^{b}	820 ± 0^{c}	820 ± 0^{c}	840 ± 0^{d}	940 ± 0^{e}	**330.5**	**0.000**

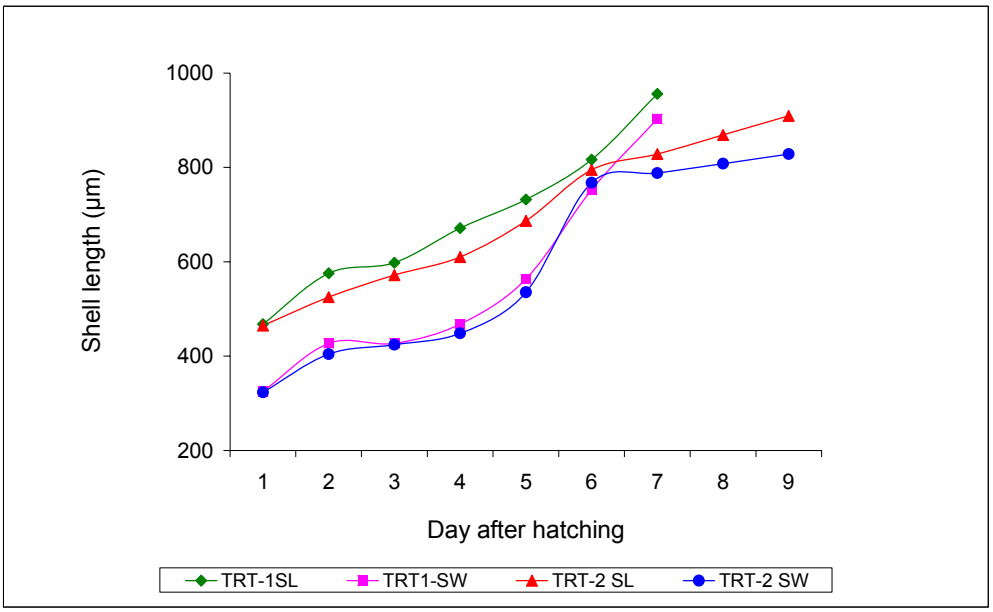


Fig. 6.21. Average growth in shell length (SL) and shell width (SW) of the veliger in aerated and non-aerated treatment

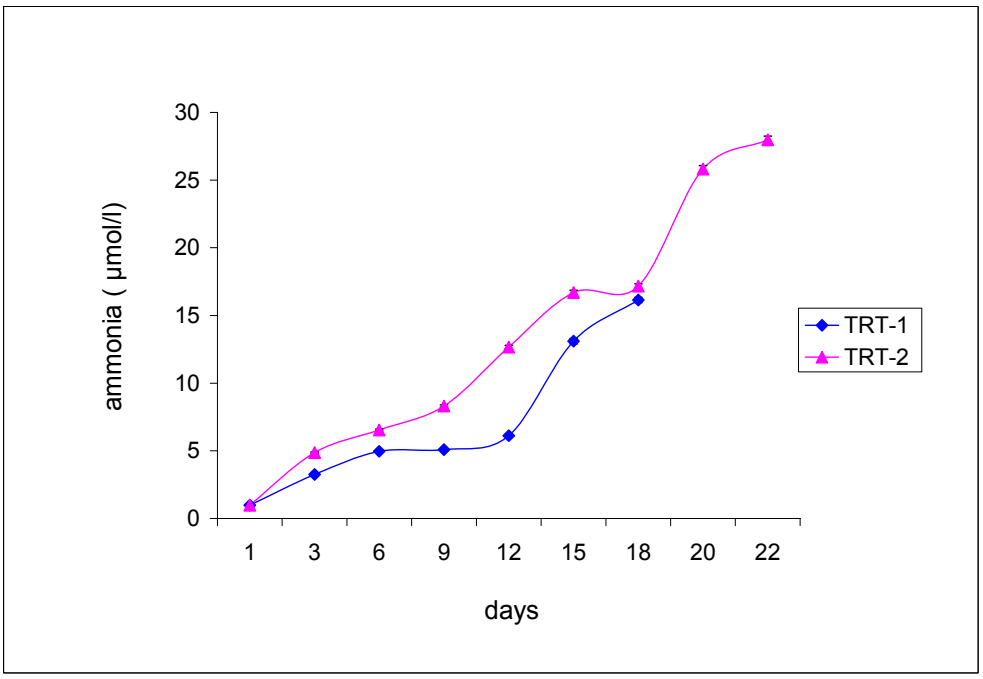


Fig. 6.22. The amount of ammonia in the rearing tanks in aerated and non-aerated treatment

6.2.7. Effect of light on growth and settlement of veliger of *B. spirata* during the rearing period.

In all treatments the veliger settled as juvenile on 18th day after metamorphosis. The settlement was 61% in TRT-1 and TRT-3, while it was 60% in TRT-2. The average

shell length of the settled juvenile in TRT-1 and TRT-2 was same, 949 ± 9.4 µm, while in TRT-3 it was 954.75 ± 5.68 µm. The shell width in TRT-1 was 896.88 ± 8.9 µm and in TRT-2 it was 848.4 ± 8.4µm. In TRT-3 the shell width measured was 901.94 ± 5.37 µm. The average amount of ammonia produced in the rearing tank showed variation during the culture period, in TRT-1 it was 7.6 ± 5 µmol l^{-1} while in TRT-2 and TRT-3 it was 9.38 ± 5.5 µmol l^{-1}. The average amount of dissolved oxygen present in the rearing tank through out the experimental period was 12.79 ±1.2 mgl^{-1}, 12.71 ± 1.1 mg l^{-1}, 12.79 ± 1.2 mg l^{-1} respectively in TRT-1, TRT-2 and TRT-3 respectively. The average values of dissolved oxygen and ammonia present in the culture tank is shown in the Table 6.10. The average growth on every 3rd day in shell length and shell width of the veliger during metamorphosis in the treatments up to juvenile is shown in the Table 6.11. No significant variation obtained in the treatments and hence light had no much influence in the growth of the veliger during the development.

The values of Pearson correlation with ammonia in each treatment is shown in the Table 6.12. The Pearson correlation showed negative correlation (r= -0.881 and r= -0.885) with the ammonia and percentage of settlement in TRT-1 and TRT-3 respectively, while it had strong negative correlation (r= -0.969) in TRT-2. The correlation value of shell length and shell width with ammonia in TRT -1 and TRT-3 was same (r= 0.988 and 0.986 respectively) while in TRT-2 it was r= 0.957 and 0.887 respectively.

Table 6.10. Average amount of dissolved oxygen and ammonia in the larval rearing tank.

	Dissolved oxygen (mg/l)			**Ammonia (µmol/l)**		
Day	**TRT-1**	**TRT-2**	**TRT-3**	**TRT-1**	**TRT-2**	**TRT-3**
1	10.67±0.11	12.42±0.12	10.73±0.06	0.86 ± 0.01	0.95 ± 0.01	0.86 ± 0.01
3	11.82±0.12	12.32±0.12	11.88±0.07	3.69 ± 0.04	4.4 ± 0.04	3.71 ± 0.02
6	13.64±0.13	13.68±0.14	13.71±0.08	5.29 ± 0.05	6.85 ± 0.07	5.32 ± 0.03
9	12.78±0.13	10.55±0.10	12.85±0.08	6.26 ± 0.06	9.84 ± 0.10	6.30 ± 0.04
12	12.73±0.13	13.74±0.14	12.80±0.08	6.67 ± 0.07	13.70±0.14	6.70 ±0.04
15	13.74±0.14	13.64±0.13	13.81±0.08	11.68±0.12	14.14±0.14	11.74±0.07
18	14.14±0.14	12.63±0.13	14.22±0.08	15.64±0.15	15.76±0.16	15.73±0.09
AVG	**12.79 ± 1.2**	**12.71 ± 1.1**	**12.71 ± 1.2**	**7.6 ± 5**	**9.38 ± 5.5**	**9.38 ± 5.6**

Table 6.11 Average growth of the veliger of *B. spirata.*

Day	Avg. shell length (µm)			Avg. shell width (µm)		
	TRT-1	**TRT-2**	**TRT-3**	**TRT-1**	**TRT-2**	**TRT-3**
1	464.6±4.6	464.6±4.6	464.6 ±4.6	343.4 ±3.4	343.4±3.4	343.4±3.4
3	585.8±5.8	525.2±5.2	589.10 ±3.51	424.2 ±4.2	404 ±4.0	426.59±2.54
6	626.2±6.2	606±6	629.73 ±3.75	444.4 ± 4.4	444.4±4.4	446.91±2.66
9	666.6±6.6	626.2±6.2	670.36 ±3.99	484.8 ± 4.8	484.8±4.8	487.53±2.90
12	727.2±7.2	747.4 ±7.4	731.30 ±4.35	559.54 ±5.54	535.3 ±5.3	562.70±3.35
15	828.2±8.2	828.2±8.2	832.87 ±4.96	787.8 ±7.8	787.8±7.8	792.24±4.72
18	949.4±9.4	949.4±9.4	954.75 ±5.68	896.88 ±8.9	848.4±8.4	901.94±5.37

Table. 6.12. Significant Pearson correlation values of ammonia in each treatment

Treatment	% of settlement	Shell length	Shell width
TRT-1	-0.881	0.988	0.986
TRT-2	-0.969	0.957	0.887
TRT-3	-0.885	0.988	0.986

6.3. DISCUSSION

In the present study salinity was found to influence settlement, survival and growth of the larvae. Hatching was found to be by 18th day in 32 ppt salinity and lasted for four days resulting in cumulative survival of 58%. With increase salinity there was a gradual delay in settlement and reduction in survival rate. The survival rate was very low, reaching nearly 3% in 45 ppt. Salinity also affected the growth of the settled larvae with larger shell length and width in 32 ppt than in higher salinities. Chiu *et al*. (2004) and Pechenik (1987) has reported that duration of the planktonic larval stage before settling and metamorphosis into juveniles is a function of developmental rate, which is influenced by environmental factors such as temperature, salinity, dissolved oxygen, concentration, pH, pollutants, availability of food. In the present study there was a short delay of 6 days to initiate settlement in higher salinities and there was considerable difference in shell length indicating that growth rates were also affected. The low survival rates also indicate that metamorphosis was affected in higher salinities. It is well known that ability to delay metamorphosis vary widely among species, both in the length of time that metamorphosis can be delayed, and in

the eventual fate of the larvae in the continued absence of a triggering cue (Pechenik, 1984; Pechenik *et al*., 1998). At 32 ppt growth and differentiation of the larvae of *B. spirata* were optimal as indicated by the large shell and development into healthy juvenile. This supports the fact that growth, which is a measure of increased body size or weight or cell number and differentiation (morphological or physiological changes that reflects temporal shifts in gene expression) are correlated as indicated by Pechenik (1984).

The experiment conducted to test the necessity of feed for veligers immediately after hatching indicated that it is essential to feed the larvae without which 30% mortality was recorded on the 2nd day and complete mortality on the 3rd day. Earlier studies have shown that low food supply may kill planktotrophic meroplanktons either directly through starvation or indirectly by forcing the larval period to elongate thereby increasing the time to be preyed upon as plankton (Olson and Olson 1989, Pechenik and Gee, 1993). Unlike *B. spirata* the newly hatched larvae from the benthic egg capsules of *Lacuna vincta*, *Rissoa parva, Crepidula fornicata, Nassarius incrassatus* and *N. reticulates* do not feed for the first 24-72 h, that is until the yolk lasts.

The ingestion rate of the plankton larvae showed an increasing trend in the initial phase of pelagic life and gradually reduced when it was time for it to settle as juvenile. Moreover there was considerable variation in the feeding rate between day and night hours. Similarly Fretter and Montgomery (1968) have observed that gastropod veligers show differential feeding rates. They have stated that veligers which are starved for a day and then given food swim near the surface of the water and the majority undergo a period of intensive feeding activity as indicated by the vigour of the velar cilia. In a medium with high concentration of a good food which the veliger can digest, most individuals will fill the stomach within a few minutes and then stop feeding, sink to a lower level and remain there for a while. Others take in an occasional cell. In low concentration of the same food, single cells are collected intermittently and feeding may be more or less continuous.

In the present study complete mortality was recorded in treatments with *Nannochloropsis salina*. The larvae could survive only on a diet of *Isochrysis galbana (Ig)* or *Chetoceros calcitrans (Cc)* or mixed diet of *Ig* or *Cc*. Fretter and Montgomeny (1968) have reported that cells of some algal species such as *Isochrysis galbana* break easily as they are battered against the gastric shield so that their contents are readily available for digestion. Few of the flagellates and diatoms which they tested had resistant walls, with areas of weakness which the digestive juices penetrate, but often only after some mechanical injury has been inflicted by the gyrating action of the stomach. Some cells like the *Asterionella japonica* forming chains about 50µ long, tended to clog the large preoral cilia and were not ingested unless a chain had been broken into individual cells. In the present study the high mortality in the larvae fed with *Tetraselmis gracilis* could have been due to the inability to digest the cell. In combination feeds the larvae was able to utilize both *Ig* and *Cc* as indicated by the similar shell length and shell width. Almost similar growth rates were observed when *Ig* and *Cc* were fed. *Ig* has been found to be good food for other gastropod also. Latama (1999) found highest growth and survival of the top shell *Trochus niloticus* larvae on feeding *Ig*. Very poor survival was found in the treatment with *Tetraselmis gracilis* and *Nannochloropsis salina* in the present study. For the asteroid larvae of *Asterina miniata,* linear growth, differentiation and survival rate were similar with mixed and unialgal diets. For the same larvae, at high food levels (5 $x10^4$ cells ml^{-1}) developmental time was little affected by diet composition. In contrast, at one order of magnitude lower food level, developmental time was much faster in the mixed, relative to the unialgal diets. In the present study low feed resulted in low survival and it took nearly 10 days more to settle than in optimum feeding. Pechenik *et al.* (2002) observed that starving the larvae of the prosobranch gastropod *Crepidula fornicata* reduced the initial growth rate. They suggest that starvation early in larval life could impact proper gill formation later if specific energy stores or materials to be used in gill formation are normally sequestered early in larval life. Klinzing and Pechenik (2000) observed one interesting factor in the development of the larvae of *Crepidula fornicata.* It was noted that the dramatically reduced food concentration induced disproportionate growth in the velar lobes. Similar effects have been reported for some larval

echinoids (Boidron- Mittairon, 1988; Hart and Scheibling 1988; Fenaux *et al.,* 1994). In the laboratory low food concentration and nutritionally inadequate diet have been shown to decrease growth rate and prolong development for many invertebrate larvae including those of crustaceans (West and Costlow, 1987), polychaetes (Hansen, 1993); sea stars (Allison, 1994; Basch, 1996), gastropods (Pillsbury, 1985) and oysters (His and Seaman, 1992). In high concentration though the settlement was earlier by 2 days the survival was 9% less than the optimum. In higher algal biomass, debris must have formed and during the rearing of larvae of three prosobranch gastropods viz, *Hyanassa obsoleta, Crepidula fornicata* and *B. formosae* the shells became badly fouled with the debris if higher cell densities were used.

Temperature was found to have significant effect on the growth and settlement of the veliger. The settlement was found to be delayed by 10 days in low temperature. Higher temperature accelerated the settlement but survival was low. The ingestion rates at higher temperature were comparatively more than at low and ambient temperature. Temperature has been found to influence metamorphosis and settlement in other invertebrates also. Acute exposure to elevated temperature for at least 1 h promoted metamorphosis in competent larvae of *Crepidula fornicata* (Gaudette *et al*., 2001). Exposure of the larvae to lower temperature regimes (eg: a shift from 20^0C to 25^0C through 30^0C or from 25^0C to 28^0C or 30^0C) was not effective in inducing metamorphosis. By contrast, a 2h exposure to 32^0C induced metamorphosis significantly above the control levels regardless of whether larvae were reared at 20^0C or 25^0C. Gaudelte *et al.* (2001) concluded that the absolute temperature was more important than magnitude of the temperature increase for inducing metamorphosis. Contrary to this, in some instances temperature increases have resulted in negative effects. *Hydracina echina* larvae reared at 18^0C disintegrated when tested at 32^0C (Kroiher *et al.,* 1992). Substantial number of *H.echinata* larvae reared at 18^0C metamorphosed upon exposure to temperature elevated by 9 or 10^0C (to 27 or 28^0C) metamorphosed when temperature was raised to 29^0C. Similarly larvae of ascidian, *Ciona intestinalis* showed statistically significant increase in metamorphosis when exposed to temperature elevated by either 7 or 10^0C (from 18^0C to 25 or 28^0C) (Kroiher *et al*., 1992). Small temperature increases also stimulated metamorphosis of oyster (Lutz *et al.,* 1970) and Chiton larvae (Pechenik

1984). But in the case of Chiton larvae, the effects were seen only after a week or more had elapsed since the temperature increase. The reason for stimulation of metamorphosis may be the chemical change. Kroiher *et al*. (1992) noted that in Hydractinia, the presence of compounds that serve as methyl group donors suppresses metamorphosis. Accordingly, they proposed that thermal stress and heat proteins may elevate the concentration of methyl group acceptors (possibly the heat shock proteins, themselves) and thereby trigger metamorphosis. Alternately, the normal metamorphosis may involve one or more inhibitory pathways as suggested by Pechenik and Qian (1988). Experimental evidence by Froggett and Leise (1999) suggested that the larvae of the gastropod *Ilyanassa obsoleta* metamorphose when they stop producing an inhibitor, nitric oxide (NO), and when the existing NO is sufficiently removed from circulation or degraded. Gaudette *et al*., (2001) have implied that the competent larvae of *C. fornicata*, although capable of metamorphosis, may also be prevented by doing so by the presence of an inhibitor possibly a protein. Thermal stress might act to induce metamorphosis indirectly by denaturing and inactivating this protein (Pechenik and Qian, 1998). In the present study also the early settlement of the larvae might be due to such chemical changes.

In *B. spirata* stocking density affected the settlement period of the larvae and optimum was found to be 150 larvae l^{-1}, above which the metamorphosis and settlement was found to be delayed. Development and growth was also inversely related to concentration in clams such as *Spisula solidissima smiilis* (10, 20, 30 and 50 larvae ml^{-1}) (Hurley and walker, 1997), *Mercenaria mercinaria* (250, 500, 750, 1000 and 3000 eggs ml^{-1}) (Loosanoff and Davis, 1963) and *Scapharca broughtonii* (1,8,14 and 24 larvae ml^{-1}) (Wang *et al.,* 1993). In marked contrast Shieh and Liu (1999) found that the larvae of *B. formosae* responded to high larval concentration by metamorphosing precociously and its mortality decreased. However the authors have indicated apprehensions on the acceleration of development if the concentration was increased above 8 larvae ml^{-1}.

In laboratory studies, larval crowding has been found to be an additional factor contributing to developmental plasticity (Loosanoff and Davis, 1963). Hinegardner (1969) suggested that one mature larvae ml^{-1} is optimum for laboratory culture of sea

urchins. Studies in invertebrate larval development have usually maintained their rearing ranges of 0.2- 2 larvae ml^{-1} for example with bryozoan (Wendt, 1996), asteroid (Basch,1996) and gastropods (Pechenik *et al.,* 1996). Higher concentration such as 10-50 larvae ml^{-1} or higher have been used for barnacle (Pechenik *et al.,* 1993), clams (Loosanoff *et al.,* 1951; Hurley and Walker, 1997) and gastropod (Chaitanawisuti and Kritsanapuntu, 1997). Chaitanawisuti and Kritsanapuntu (1997a) have cultured the larvae of *B. areolata* at a density of 10 larvae ml^{-1}. The negative effect is generally because of the reduced larval feeding efficiency, physical interactions among the larvae and or accumulation of soluble wastes which lowers feeding times and rates (Basch,1996).

In the present study there was no difference in the percentage of settlement and growth of the larvae in the treatment with substratum and without substratum. However in gastropods like *Chicoreus ramosus*, settlement was related to the nature of the substratum (Bech, 1992). Veligers of this gastropod preferred coarse to fine sediment. On an average 72.8% of the veligers settled on pebbles, 22.3% on granule and 4.9% on sand. Different results have been obtained for bivalves Zhang and Yang (2006) have observed that the pediveliger larvae of *Ruditapes philippinarum* reared without substrate had a greater metamorphosis rate those reared with various types of sand as substrate.

Experiments on water quality maintenance in the larval rearing protocol indicated that it is optimal to change the water on alternate days and it is essential to aerate the tanks. The reason for high mortality in treatment without water exchange can attributed to the high ammonia content. Zhang and Yang (2006) have noted that a water exchange rate of 50% twice daily provided optimal larval growth for the clams *R. philippinarum* The treatments without aeration and water exchange were found to be detrimental to the larvae. Similarly complete darkness also showed poor performance. Similar observations were made in the rearing of the clams *R. philippinarum* larvae. High light intensity was found to be critical for the normal growth and survival for the juvenile giant clam *Tridacna gigas* (Lucas *et al.*, 2001). The growth pattern of juvenile hard clam was found to be affected by light and dark condition (Cenni *et al.,* 1999). There preference might be because of the

resemblances to natural habitat, where both light and darkness prevail since it is planktonic. The study indicated that a strong negative correlation of ammonia with settlement.

Chapter 7

Juvenile rearing of Babylonia spirata

7. JUVENILE REARING OF *BABYLONIA SPIRATA*

7.1. MATERIALS AND METHODS

7.1.1. Growth and survival of *B. spirata* in different types of feeds.

An experiment was conducted to study the growth and survival of the juvenile *B. spirata* fed with six different types of feed. Meat of the fish *Ambassis*, bivalve *Meritrix meritrix*, two species of crustaceans, *Penaeus indicus* and *Squilla serrata*, cephalopod *Loligo duvaucelli* and boiled egg albumin were used during the experiment. The control of the experiment were juveniles which were not fed during the experimental period. Actively feeding *B. spirata* juvenile (n=280) of 30 days old having average shell length of 2.23 ± 0.46 mm, shell width 1.63 ± 0.43 mm were selected for the experiment. Twenty snails were used for each treatment and these were kept in duplicate. Each group was maintained in 5 l of filtered seawater and the rearing tank was provided with aeration. Feed was macerated into paste form and fed to the juvenile in a Petri plate. After feeding, the water was changed and the juveniles were stocked in fresh filtered sea water provided with aeration. Growth was measured in terms of individual shell length (SL) and shell width (SW) using a digital caliper at every 5 day interval. The total weight of the group was weighed to determine the increment in the wet weight. The absolute growth rate (AGR) was calculated by the formula given in the section 6.1.3. The survival percentage was recorded at every 5^{th} day when the measurement was taken and the experiment was terminated on the 15^{th} day. Survival percentage was calculated from the difference between the number of juveniles stocked and number live at the termination of the experiment, expressed as the percentage of initial stocking. The juveniles were fed once in a day at 1000 h. The hydrological parameters like salinity 31 ± 2 ppt, pH 8.1 ±0.3 and temperature 26- 28^{o}C were maintained throughout the experimental period.

Statistical analysis was performed with the SPSS statistical package. Differences in growth and survival of the juvenile *B. spirata* between treatments were determined by one- way analysis of variance (ANOVA).

7.1.2. Effect of size variation on the consumption rate of juvenile *B. spirata.*

B. spirata juvenile of different shell lengths were selected for the experiment to study the consumption rate at different growing stage. The juveniles (n=75) belonging to five different size classes 1-5, 5-10, 10-15, 15-20, and 20-25 mm were selected for the study. Meat of *Penaeus indicus* was used as feed. The quantity of feed was weighed before feeding and weigh of the left over feed was also taken. The experiment was conducted in triplicate. Excess feed was removed. The juveniles were allowed to feed once in a day for 1 h and the water was changed after feeding. At the time of feeding, no aeration was provided. Temperature and salinity were maintained at 32 ± 2 ppt and 28^0C respectively. As the individuals were small, the feed consumed relative to the total weight of the group was calculated. The mean food consumption per individual per meal was calculated as the total food consumption of the group divided by the number of individuals in each group. The percentage of feed consumed relative to the wet weight of the individual was calculated based on the formula described under section 2.1.3.2. To estimate the growth performance, all the juveniles from each treatment group were measured individually for shell length, shell width and total weight at 15 days intervals. The experiment was terminated after 30 days.

7.1.3. Preference of substratum by juvenile *B. spirata*

Juveniles *of B. spirata* of average shell length 10± 2 mm, shell width 8.82 ± 1.2 mm and total weight 0.92 ± 1.5 g were selected for the study. The response of these juvenile whelks to four types of substrata *viz,* gravel (mean size 1 – 2 mm), coarse sand (mean size 1.00 – 0.50 mm), fine sand (mean size 0.25 – 0.10 mm) and silt (mean size 0.05 - 0.002 mm) was studied. Substrates other than silt were washed repeatedly to remove dirt and other unwanted matter. After cleaning the test substrates, they were dried and used. Three rectangular tanks each of 30 l capacities were used and in each, the tank bottom was divided into four sections without any physical partitions and the substrate samples were spread separately in each section upto 2 cm height. Twenty animals were used and they were released into the centre of each experimental tank where the area was cleared every day after feeding. The

occurrences of juvenile whelks in different substrates were counted every day before feeding and all were picked out of the tank for feeding. After feeding again they were released in to the centre of the tank. Sea water of 32 ppt was added up to the height of 20 cm over the substrata and was changed on alternate days. The water temperature during the experimental period was 28 ± 2^0C and it was aerated well. Behaviour of the juvenile whelks in each substrate was also observed. The percentage of occurrence was estimated from the result of the number of occurrence of the juveniles daily in each substrate.

7.1.4. The effect of substratum on the growth of the juvenile *B. spirata.*

An experiment was conducted to study the effect of substratum on growth and survival of the small juveniles of *B. spirata*. Three month old *B. spirata* juveniles of (n= 40) having average shell length 9.60 ± 0.39 mm, shell width 6.29 ± 0.32 mm and weight 0.16 ± 0.03 g were selected for the experiment. One set of the treatment was provided with fine beach sand (TRT-1) and other without any substratum (TRT-2). The experiment was conducted in duplicate for the period of 60 days and the absolute growth rate (AGR) for shell length, shell width and weight was estimated as described in section 6.1.3. The behavioral responses were noted during the experimental period. The percentage of survival was noted at the end of the experiment.

7.1.5. Salinity tolerance of juvenile B. *spirata*

Salinity tolerance of adult *B. spirata* (n=210) of shell length12.22 ± 1.10 mm, shell width 8.54 ± 1.08 mm and total weight 0.28 ± 0.06 g was studied by exposing the whelks to varied salinities *viz*, 15, 20, 25, 30, 32 (Control), 35, and 40 ppt. The different salinities for the experimental trails were prepared by the formula described in section 2.1.4.1. The experiments were conducted in 10 l FRP tank with 5 l of seawater. Each set of treatment had 10 numbers of young juveniles and these were kept in triplicate. The juveniles were gradually acclimatized to the lower salinities. The test medium was aerated well throughout the experiment. Observations were made on the activities of whelks in all tanks on their behaviour and their feed acceptance and mortality were recorded. Based on the number of dead whelks, the

percentage of mortality was estimated. The experiment was terminated after 60 days or on complete mortality in lower salinities.

7.1.6. Temperature tolerance of juvenile *B. spirata*

B. spirata (n=80) having an average shell length of 12.10 ± 1.08 mm, shell width 8.46 ± 1.05 mm and wet weight 0.27 ± 0.58 g were selected for the experiment. They were exposed to six different temperatures *viz* 20, 22, 24, 26, 28, 30, 32 and 34^{o}C. Temperatures of 20 to 24^{o}C were regulated in an air conditioned broodstock room. Water temperature of 26 and 28^{o}C were obtained in the ambient temperature, while 30 to 34^{o}C were maintained with the help of an aquarium heater. The juveniles were gradually acclimatized to the lower or higher temperatures. All treatments were carried out in duplicate and tanks were well aerated throughout the experiment. In all treatments observations were made on the behaviour of the whelks and their feed acceptance. Experiments was terminated on 60th day and through out the experimental period in all treatments the hydrological parameters such as salinity 31 ± 2 ppt and pH 8.1 ±0.3 were maintained uniformly. The growth of the young ones was measured at the end of the experiment.

7.1.7. Growth rate of juvenile *B. spirata* in the hatchery

Juveniles of *B. spirata* were reared in the hatchery till they attained the shell length and shell width similar to the adult. For rearing the juveniles of 1.5 ± 0.56 mm were selected. They were maintained in the hatchery in seawater having the salinity 32 ± 2 ppt and pH 8.1 ± 0.2. The temperature was ambient ranging from 26- 28ºC. The whelks were provided with fine silt as substratum and shrimp as feed. A sand filter was provided at the centre of the rearing tank with a filtration capacity of 3 l min^{-1}. The feed was provided in the rearing tank it self and the aeration was turned off at the time of feeding. The juveniles were allowed to feed once in a day with adequate feed and the residual feed was removed 1hr after feeding. The sand was replaced with fresh dried sand once in a month. Fifty percentage water exchanged daily and replenished with filtered sea water. The shell length, shell width and total weight were measured using digital recorders every month. From the increase in shell

dimensions the average growth rate of the juveniles under hatchery conditions was calculated.

7.2. RESULTS

7.2.1. Growth and survival of *B. spirata* in different types of feeds.

Mortality percentage was higher in the control treatment where the juvenile were not supplied with feed. Only 45% juvenile survived by day 5 in control treatment. Complete mortality was recorded on 10^{th} day of the experiment due to starvation of the juvenile. The survival percentage was found to be higher in the treatment with shrimp as feed (93%). The percentage of survival was found to be decreasing in the order squilla (85%), squid (65%), clam (55%), fish (45%). In the treatment using egg albumin none survived and complete mortality was recorded by 10^{th} day of the experiment. The result of the survival percentage in different types of feed is shown in the Fig 7.1.

The maximum SL of juveniles on 15^{th} day of the experiment was obtained with shrimp meat as feed (3.6 mm) followed by squilla meat as feed (3.32 mm) and fish meat as feed (3.24 mm). The SL obtained after 15 days of growth in clam and squid meat as feed was 2.93 and 2.86 mm respectively. The increment in the SL during the experimental period is shown in the Fig 7.2.

The maximum SW (3 .25mm) was also obtained in the shrimp meat as feed after 15 days of the experiment. The average shell width obtained with fish, squilla, squid and clam meat as feed were 2.74, 2.68, 2.28 and 2.32 mm respectively at the termination of the experiment. The increment in the SW during the experimental period is shown in the Fig 7.3.

The maximum weight of the *B. spirata* juvenile after 15 days of growth was in the treatment with shrimp meat as feed (0.56 g) followed by squilla meat as feed (0.51 g). The average weight attained in fish, squid and clam meat as feed were 0.32, 0.26 and 0.24 g respectively. The average weight in different treatments during the experimental period is shown in the Fig 7.4.

The absolute growth rate (AGR) for length, width and total weight was maximum in the case of shrimp meat as feed, 0.89, 0.11 and 0.03 respectively. The average AGR during 5 day intervals are shown in the Table 7.1 and represented in Fig 7.5.

Table-7.1. The average AGR for length, width (mm day^{-1}) and weight (g day^{-1}) of *B. spirata* juvenile on different types of feed for 15 days

Growth measurements	Fish	Prawn	Squilla	Squid	Clam	Egg albumin	Control (No feed)
Length	0.06	0.09	0.07	0.05	0.04	0.02	0.00
Width	0.08	0.11	0.07	0.05	0.05	0.00	0.00
T. Weight	0.01	0.03	0.02	0.01	0.01	0.00	0.00

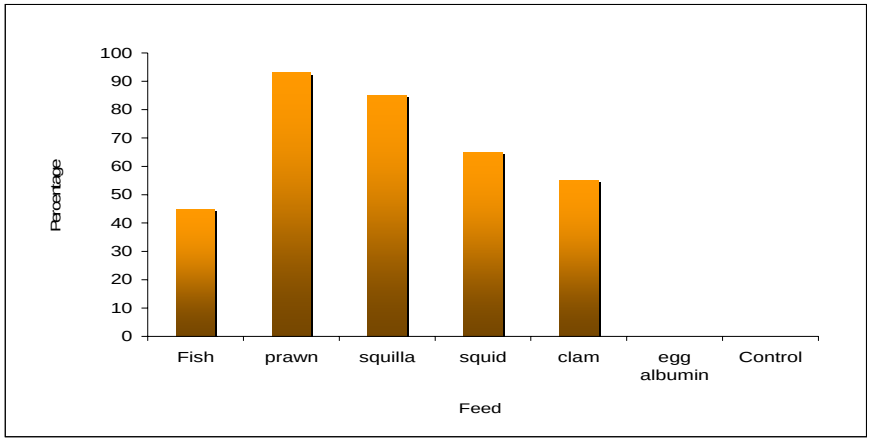


Fig 7.1. Final survival percentage of *B. spirata* juveniles fed with different types of feed.

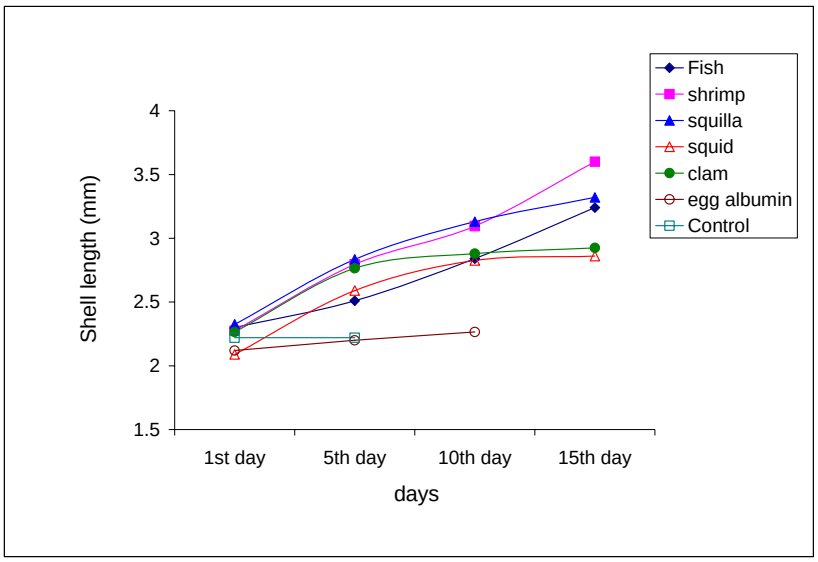


Fig 7.2. The average shell length (mm) of the juvenile *B .spirata* fed with different feeds

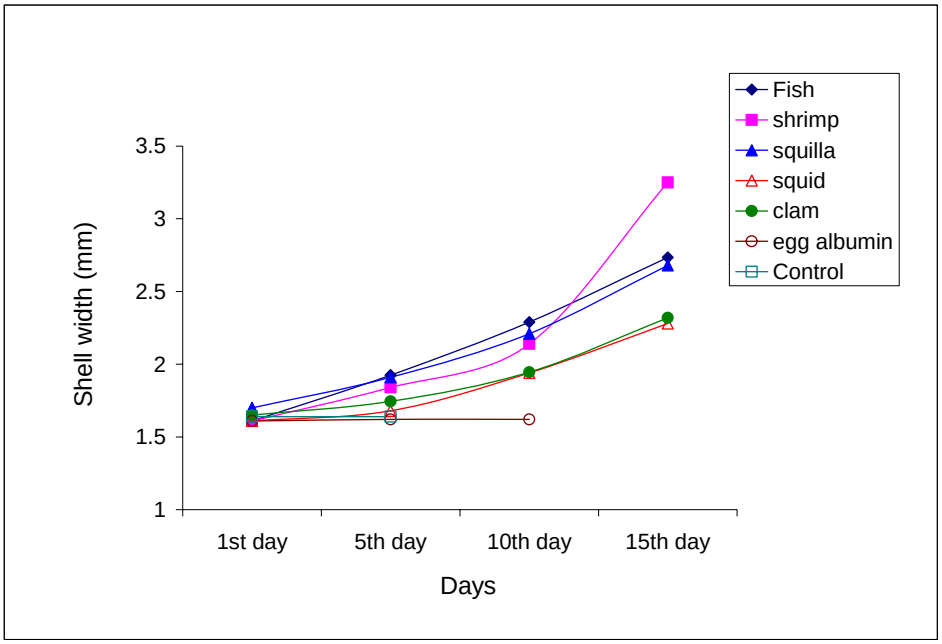


Fig 7.3. The average shell width (mm) of the juvenile *B. spirata* in different feeds during the experiment

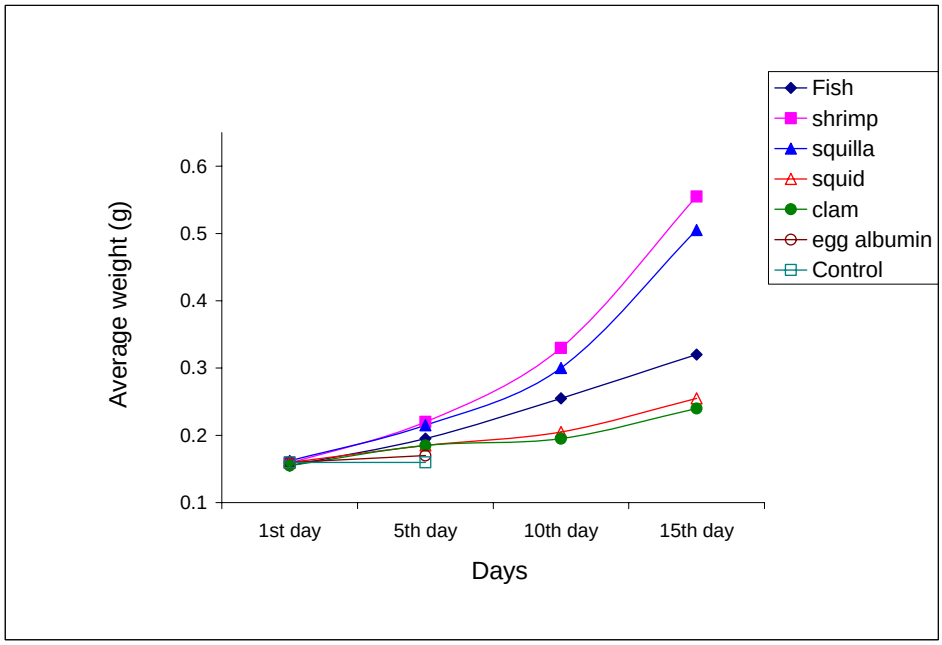


Fig 7.4. Total weight (g) of the juvenile *B. spirata* fed with different feeds

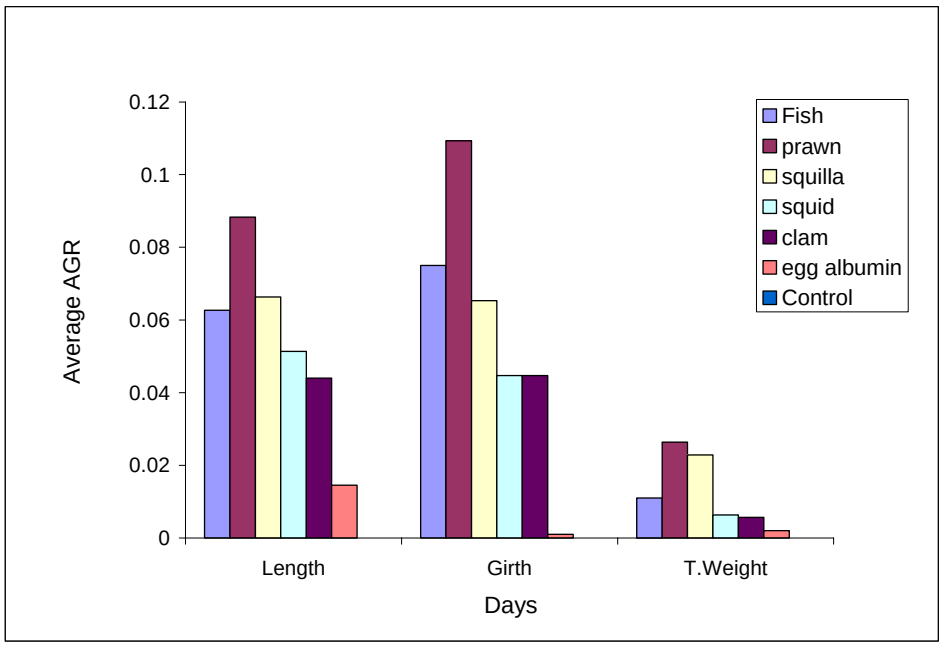


Fig 7.5. The average AGR of the *B. spirata* juvenile fed with different feed

7.2. 2. Effect of size variation on the consumption rate of juvenile *B. spirata.*

The average SL of the juvenile of the size class 1-5 mm during the initiation of the experiment was 3.56 ± 0.07 mm which increased to 5.40 ± 0.11 mm and 11.12 ± 0.14 mm on 15th and 30th day respectively. The average feed consumption per day during this period was 0.03 ± 0.03 g. The average SL of 5-10 mm size class group on the first day of the experiment was 7.13 ± 0.06 mm increased to 10.89 ± 0.13 mm and 13.10 ± 0.43 mm on 15th and 30th day respectively. The average feed consumption on the experimental period was 0.05 ± 0.04 g for this size class. The average SL of 10-15 mm size class increased to 14.26 ± 0.25 and 16.41 ± 0.21 on 15th and 30th day. The average feed consumption also increased to 0.09 ± 0.04 g during this period. The average SL of the size class 15-20 mm was 16.68 ± 0.20 mm during the initiation of the experiment increased to 19.90 ± 0.18 mm on the end of the experiment. During this period the average food consumption per individual was 0.21 ± 0.11 g. The average SL of 24.31 ± 0.43 mm during the initiation of the

experiment increased to 25.05 ± 0.64 mm and 25.57 ± 0.53 mm respectively on 15th and 30th day of the experiment. The average food consumption for this size class was 0.39 ± 0.11 g.

The growth rate was found to be different for length, width and weight of *B. spirata* juveniles of different size classes. The AGR for length was maximum (0.44) for the smallest size class (1-5 mm). As the size increased the AGR for length was found to decrease. The AGR for 5-10, 10-15, 15-20, and 20-25 mm size class of the juvenile whelk were 0.20, 0.12, 0.11 and 0.04 respectively. The AGR for width showed an increase initially (0.18 for the 5-10 mm size class) and thereafter declined. AGR for width was lower for the higher groups. In contrast to the length and width, the AGR for weight was found to increase for the higher size classes of the juveniles. The maximum AGR was recorded for the 20-25 mm size group. For the smallest group (1-5 mm) it was only 0.02 while for the higher size class it was three times higher at 0.06. The AGR for length, width and weight for different size groups for the period of 30 days are represented in the Fig 7.6.

The percentage feed consumption was maximum in younger juveniles than in larger size groups. Feed consumption as % of body weight of juveniles in different size groups are shown in the Fig 7.7.

7.2.3. Preference of substratum by juvenile *B. spirata*

The response of whelks to different substratum varied during the experimental period. The average percentage of occurrence of *B. spirata* in different types of substratum is shown in the Fig 7.8. The highest percentage of occurrence was in the silt substratum with an average of 42.5 during the experimental period of 20 days. The average % of occurrence of the whelks in the fine sand substratum was 41.5 while in the coarse sand and gravel it was low, 10 and 6% respectively.

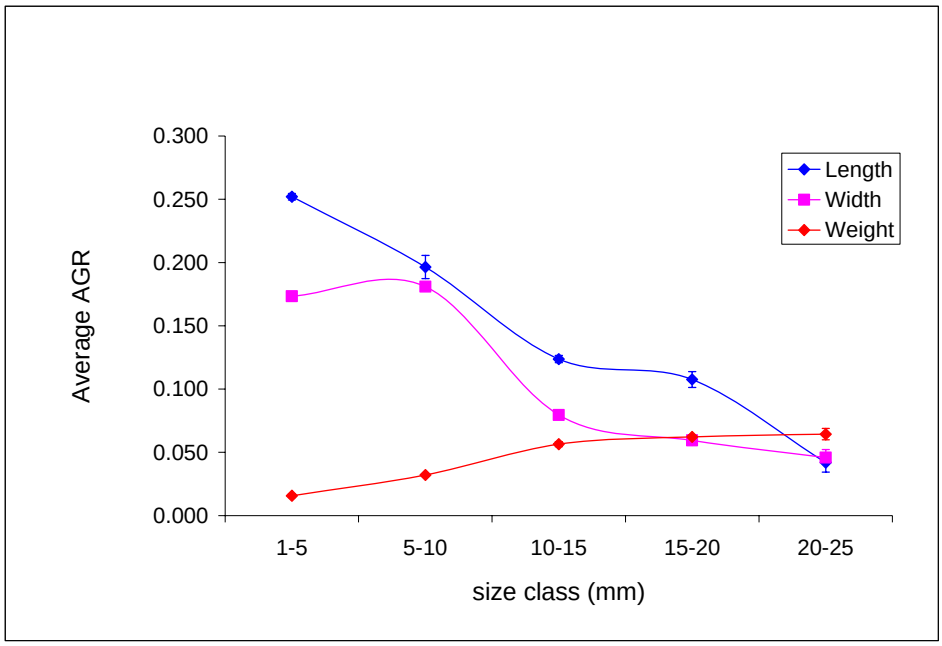


Fig 7.6. Average AGR for length, width and weight of *B. spirata* juvenile of different size classes

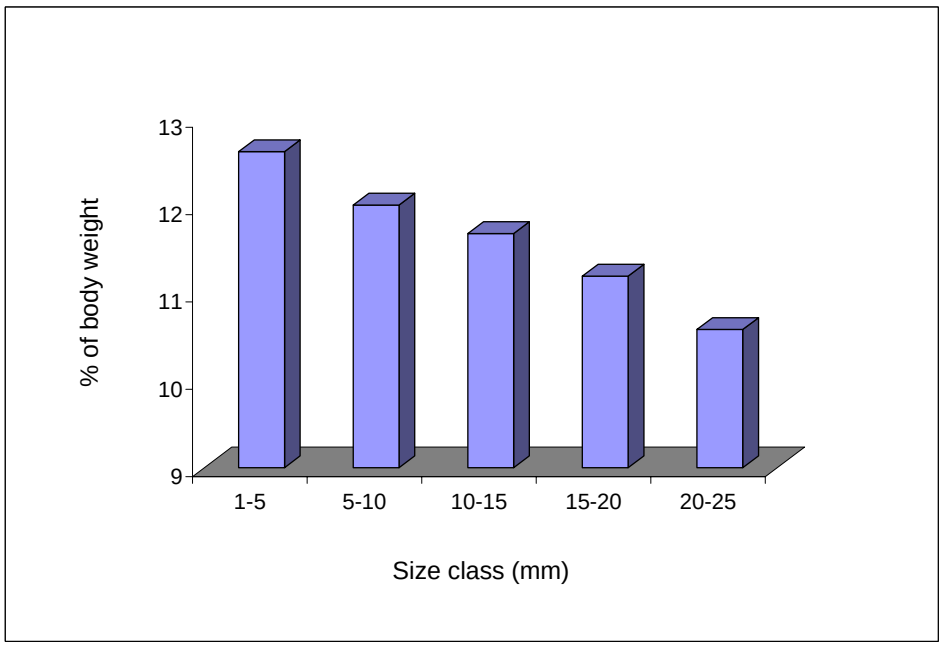


Fig. 7.7. % Feed consumed as % of body weight in *B. spirata* juvenile

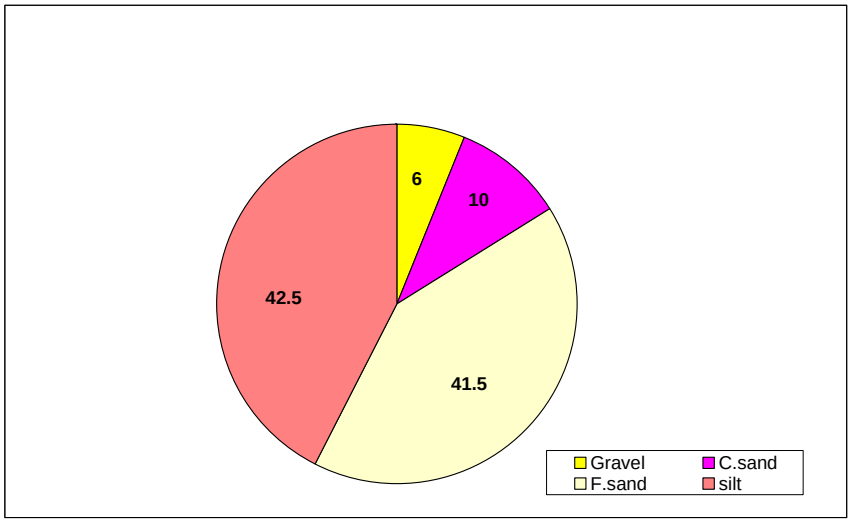


Fig. 7.8. The percentage of occurence of juvenile *B. spirata* in different substrates

The substratum preference study clearly indicated that *B. spirata* preferred silt and fine sand substratum and the variation in the occurrence of whelks in each substratum was significant ($P<0.05$).

Feed acceptance: All the experimental animals consumed feed within 10-15 minutes when feed was provided and were found to move to the preferred substratum after feeding.

7.2.4. The effect of substratum on the growth of the juvenile *B. spirata.*

The survival percentage after 60 days of the experiment was 100 in TRT-1 and TRT-2. No significant ($P>0.05$) variation was observed for the AGR after 60 days in both treatments. The AGR length after 60 days of the experiment in TRT-1 was 0.03 while in TRT-2 it was 0.04. The average AGR for width was identical in TRT-1 and TRT-2 respectively. The summary of the experiment is represented in Table 7.2. The average AGR after 30 and 60 days of the experiment in both treatments is represented in the Fig 7.9.

Behavioural Pattern: The juveniles in TRT-1 responded more slowly to feed than those in the TRT-2. In TRT-1, the juveniles took 15-25 minutes for sensing the presence of feed in the culture tank while in TRT-2 they responded to the feed within 10-15 minutes. In the absence of a substratum, the juveniles vigorously moved throughout the culture tank. In TRT-2 the juveniles occasionally crawled above the

level of the water in the tank. However they could not return to the bottom of the tank along the same path. Desiccation was a threat to the juveniles in TRT-2. Presence of substratum provided the whelk a shelter and all of them lay buried in it.

Table- 7.2. Growth of the *B. spirata* juvenile in substratum experiment.

Growth parameters	**TRT-1**	**TRT-2**
Initial SL	9.54 ± 0.63	9.67 ± 0.02
Initial SW	6.17 ± 0.22	6.40 ± 0.44
Initial weight	0.18 ± 0.00	0.14 ± 0.04
Average AGR for SL after 60 days	0.03	0.04
Average AGR for SW after 60 days	0.03	0.03
Average AGR for shell weight after 60 days	0.002	0.001
Survival percentage	100	100

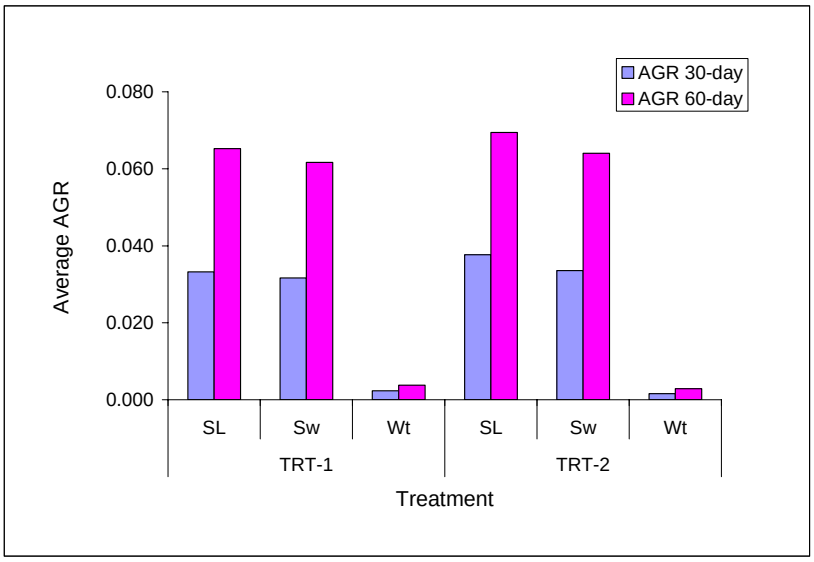


Fig. 7.9. The average AGR of *B. spirata* juvenile on treatment with substratum

7.2.5. Salinity tolerance of juvenile B. *spirata*

Complete mortality was recorded in 15 and 20 ppt on the second day of the experiment. In lower salinities the juveniles completely closed their operculum and remained in the tank bottom in a lethargic state. No movement was observed and they did not accept feed on the first day of the experiment. The siphonal canal was also pulled inside the shell cavity. In 25 ppt salinity 60% mortality was recorded on the second day and those which survived also behaved in the same manner as

observed in lower salinities. On the third day complete mortality was recorded. In 30, 32 and 35 ppt 100% survival was recorded during the 60 days of the experiment. The juveniles were very active in 32 and 35 ppt salinities. In these salinities the juveniles crawled along the rearing tank and responded to feed as soon as it was provided. In 40 ppt salinity, 50% mortality was recorded after 30 days of the experiment. Juveniles who survived were less active than those juveniles in 32 and 35 ppt salinities. Response to feed was noticed, but movement was less.

The average SL of the juvenile after 60 days growth was found to be maximum (17.54 mm) in 32 ppt salinity. In 35 ppt and 30 ppt it was 15.78 mm and 15.01 mm respectively. The growth in length and width was low in higher salinities. The average SL during the experiment in different salinities is represented in Fig 7.10. Maximum SW was recorded in 32 ppt salinity (12.42 mm) and it was 10.28, 11.33 and 10.76 mm in 30, 35 and 40 ppt respectively. The average SW of the juvenile whelks in different salinities is represented in Fig 7.11. The average individual weight of the juvenile obtained in different salinities at the termination of the experiment was 0.52, 0.63, 0.57 and 0.52 mg in salinities 30, 32, 35 and 40 ppt respectively. The average individual weight during the experiment in different salinities is represented in the Fig 7.12

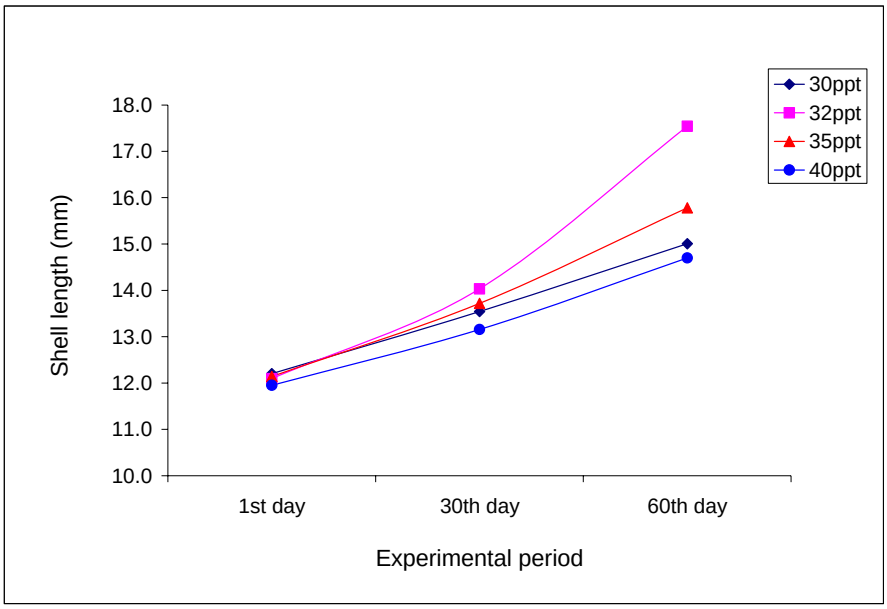


Fig. 7.10. Average shell length of the juvenile reared in different salinities

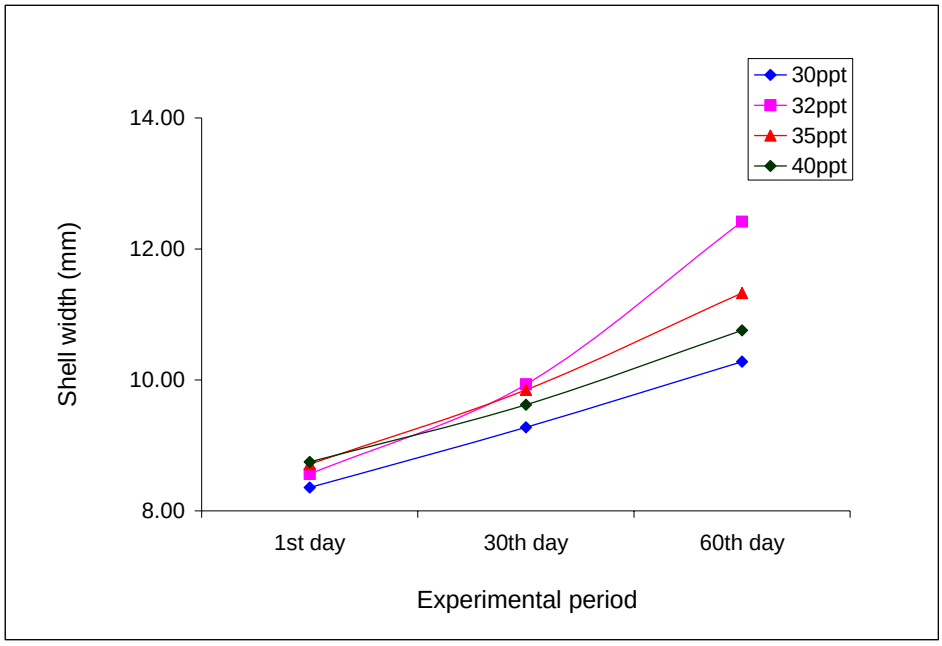


Fig. 7.11. Average shell width of the juvenile reared in different salinities

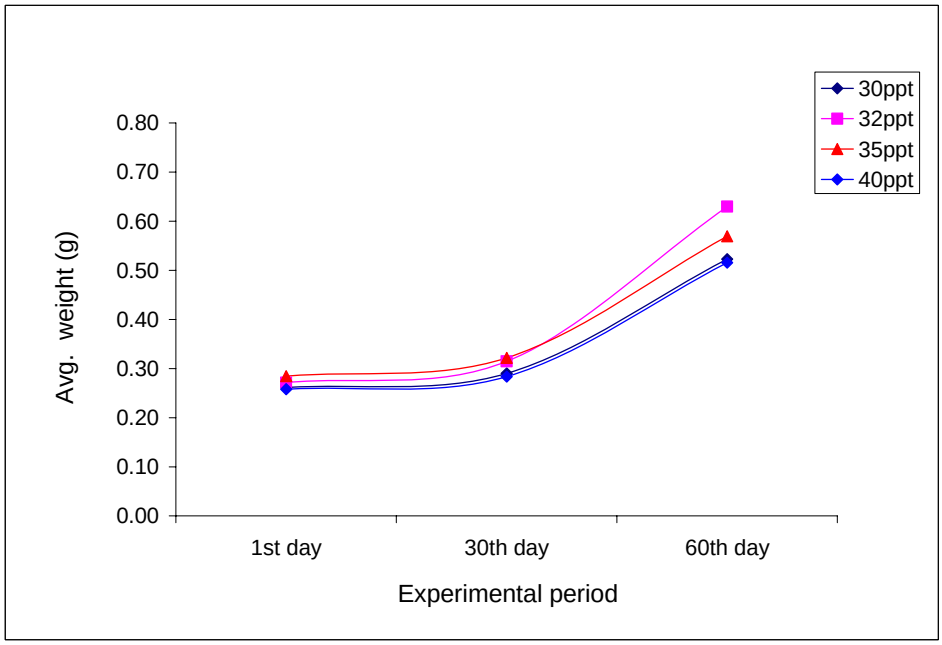


Fig 7.12. Average weight of the juvenile reared in different salinities

7.2.6. Temperature tolerance of juvenile *B. spirata*

The juvenile *B. spirata* could not survive in lower temperatures in the hatchery. In lower temperatures from 20-24^{0}C they closed their operculum tightly and the body

was withdrawn completely into the shell. They did not respond to feed and no movement was observed. Complete mortality was recorded after three days from the initiation of experiment. None of the juveniles could survive the temperature range 20-24^{0}C. In 26- 30^{0}C, 100% survival was recorded and the activities of the juveniles were found to be normal. They moved in the rearing tank in search for feed as soon as it was provided and immediately accepted it. At 32^{0}C no mortality was recorded, but the juveniles were in an inactive state, with partially closed operculum and no response to feed. In 34^{0}C, 50% mortality was recorded after three days from the date of experiment.

After 60 days on termination of the experiment, maximum SL (17.44 ± 1.72mm), SW (12.32 ± 1.61mm) and individual weight (0.62 ± 0.17g) was recorded in 28^{0}C than other lower or higher temperatures. Decrease in growth was observed as the temperature of the rearing medium increased. The growth in terms of SL in 30, 32 and 34^{0}C was 15.68 ± 1.43, 14.6 ± 0.88 and 13.37 ± 0.85 mm respectively. The average growth measurements in different temperatures are given in the Table 7.3.

Table-7.3. Average SL, SW and weight of the *B. spirata* juveniles at different temperature.

Temp ^{0}C	Avg. SL (mm)	Avg. SW (mm)	Avg. weight (g)
26	14.91 ± 1.36	10.18 ± 1.44	0.51 ± 0.12
28	17.44 ± 1.72	12.32 ± 1.61	0.62 ± 0.17
30	15.68 ± 1.43	11.23 ± 1.59	0.56 ± 0.15
32	14.60 ± 0.88	10.66 ± 1.09	0.51 ± 0.04
34	13.37 ± 0.85	9.93 ± 0.78	0.30 ± 0.06

7.2.7. Growth rate of juvenile *B. spirata* in the hatchery

The growth of the juveniles was recorded for the period of 18 months. Initially the juveniles were cultured at the stocking density of 1 individual 2cm-^{2} upto 5th month. After that the stocking density changed to 1 whelk 3cm^{-2} till the 10th month and finally as the individual grows, it was maintained 1 whelk/ 4cm^2. The initial SL, SW and weight of the of the juvenile was 1.55 mm, 2.2 mm and 0.01 g respectively. After 6 months growth the average growth in SL, SW and weight became 6.34 mm, 4.99 mm and 0,192 g respectively. During this period the average growth rate,

month^{-1} was 2.15 mm, 1.28 mm and 0.15 g for SL, SW and weight respectively. During the next 6 months (6-12months) the growth rate month^{-1} was 2.12 mm, 1.39 mm and 0.92 g for SL, SW and weight respectively. During the period of 12-18 month the average growth rate recorded was 0.643 mm, 0.512 mm and 0.595 g for SL, SW and weight respectively. The growth rate varied during different months. The average growth measurement during the culture period is shown in the Fig.7.13. Maximum growth rate/month for SL (5.77 mm) and SW (3.73 mm) was recorded during the 6th month. But maximum growth rate for weight was recorded during the 11th month (1.68 g). The average growth rate during the period of culture is shown in the Table 7.4.

Mortality was negligible during the period of culture. During the first month only 5% mortality was recorded. Thereafter no mortality was observed during the entire period of culture. The hatchery produced juveniles of *B. spirata* is shown in the Fig.7.14.

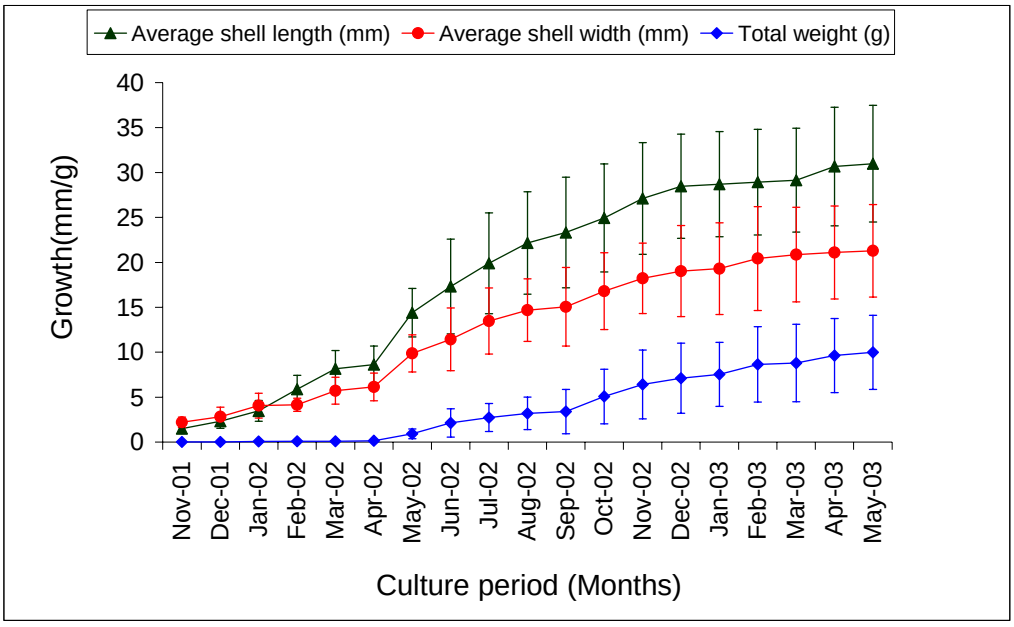


Fig. 7.13. Average growth of the juvenile *B. spirata* during the culture period

Fig. 7.14 . Hatchery produced juveniles of *B. spirata*

Table- 7.4. Average growth /month (mm month^{-1}) of the juvenile *B. spirata* during the culture period

Culture period (Month)	Shell length AGR (mm)	Shell width AGR (mm)	Total weight AGR (g)
1	0.82	0.62	0.01
2	1.16	1.24	0.05
3	2.4	0.09	0.02
4	2.29	1.57	0.01
5	0.47	0.42	0.05
6	5.77	3.73	0.77
7	2.91	1.57	1.22
8	2.58	2.04	0.59
9	2.26	1.21	0.47
10	1.17	0.37	0.2
11	1.62	1.74	1.68
12	2.17	1.42	1.34
13	1.35	0.81	0.69
14	0.23	0.27	0.42
15	0.23	1.13	1.12
16	0.22	0.44	0.15
17	1.53	0.24	0.83
18	0.3	0.18	0.36

7.3. DISCUSSION

The experiment conducted to study the effect of different feeds indicated that the juveniles of *B. spirata* grow best when fed with shrimp meat. Complete mortality of the juveniles when fed with egg albumin indicates that it cannot be used as feed in whelk hatcheries. However the growth of juvenile whelks performance when fed with squilla and fish meat were better than its performance with clam meat. However there was 45% mortality when fish meat was provided as feed. In *B. areolata* hatcheries in Thailand, the juveniles are fed with fish meat. In the laboratory trials on the effect of different diets on the growth and survival of the common whelk *B. undatum* juveniles fed with blue mussel had highest survival rates (67%) than with cod-waste (53%) and fish- feed pelletes (46%) (Nasution and Roberts, 2004). Starving of the juveniles in the present study resulted in complete mortality. Chaitanawisuti *et al*., (2001b) have inferred that growth, survival and FCR of the juvenile spotted babylon are influenced by feeding levels. The mean growth rate of *B. areolata* held at feeding levels of 3% and 5% body weight (BW) were lower than those at 10% and 15% BW. Best growth performance of *B. areolata* was attained when snails were given daily ration at 10-15% BW. In the present study also it was observed that the juvenile *B. spirata* consumed feed @ 10.5 % to 12.6% of BW. This agrees well with the observation on optimal growth and feeding levels by Chaitanawisuti *et al*., (2001b) on *B. areolata*.

In the present study it was observed that juvenile *B. spirata* preferred silt and fine substratum to coarse sand and gravel. Chaitanawisuti *et al.*, (2002) observed no difference in growth and survival among juvenile of *B. areolata* reared in fine sand, coarse sand, mud and small shells. However Chaitanawisuti and Kritsanapuntu (1998) reported that the highest growth rate in shell length and body weight of *B. areolata* was obtained from the culture system with sand substrate and flow through sea water where as the lowest growth rate was recorded in the no sand substrate and static seawater. Accordingly the culture method followed in Thailand for *B. areolata* is that with fine substratum (Chaitanawisuti and Kritsanapuntu, 1999b). Another disadvantage when the juveniles were not provided with substratum has the mortality due to dehydration when the juveniles crawled above the water level in the tank.

Such behaviour has been reported for juveniles of *Chicoreus ramosus* (Steenfeldt, 1995). In such instances mortality has also been reported (Steenfeldt and Bussarawit, 1992). Steenfeldt (1995) has inferred that the climbing of tank sides may because of the limited space and lack of shelter in the tank, but the high number of snails that climbed the elevation showed that there is a negative geotactic behaviour involved.

A different type of hiding behaviour has been observed among the juveniles of *Chicoreus ramosus* (Steenfeldt, 1995). The juvenile *C. ramosus* are rarely found in nature. Their small size and hiding behaviour makes them difficult to spot, but juveniles have been found hiding among the spines of *Spondylus versicolor* which lives attached to corals (Steenfeldt, 1995). He has inferred that the locomotive behaviour in relation to light, current and bottom structure stimulate juvenile *C. ramosus* to climb corals and hide among species of bivalves. Chaitanawisuti and Kritsanapuntu (1997b) have reported that the metamorphosed larvae of *B. areolata* are benthic and spend most of their time immobile and partially buried in the sand, although they are capable of movements when offered a prey or confronted by a predator. Spotted babylon spend much of their life buried in sand and their distribution is limited by the substrate in the natural habitat (Panichasuk, 1996).

The experiment on the salinity tolerance of the juvenile *B. spirata* showed that they are basically stenohaline with very low survival in low salinities. Their growth performance and survival were high in 30 to 35 ppt. The salinity was similar to that observed in the natural habitats (Mohan, 2007) of adult *B. spirata* along Kollam coats from where the broodstock was collected. The salinity tolerance of adult (broodstock) as observed in the present study (section 2.2.4) is also similar to that observed for juveniles. Sokolova (2000) observed that optimum growth conditions of juveniles were dependent on the salinity regime in their original habitat. He observed that when the juveniles of *Littorina saxalitis* obtained from progenic females collected from estuarine, intermediate and marine habitats were studied, the progeny of females from the marine site showed maximum survival at higher salinity which corresponded to the normal salinity in their native habitat during the breeding

period. At the same time juveniles originating from estuarine habitats were able to grow considerably faster in low salinities.

Significant variation in growth and survival were obtained when the juvenile *B. spirata* were reared in different temperatures. The best growth and survival was obtained in 26 to 30^0C. Their survival and growth were very poor in low and high temperatures. Their behaviour itself indicated that their physiological processes were disturbed. Such inactive behaviour was also observed in adult *B. spirata* in the present study (Section 2.2.5.) It is well known that temperature is one of the key factors for governing the physiological process of all organisms. It acts at cellular level by increasing or decreasing the catalytic activity of metabolic and digestive enzymes (Hochachka and Somero, 1984). It also maintains a direct relationship with growth rate and other whole-body functions involved in energy metabolism (respiration, food consumption, excretion, etc.) of invertebrates (Prosser, 1991).

In the present study it was observed that *B. spirata* attained average shell length of 6.3 mm, 22.4 mm and 29.49 mm at the end of 6, 12 and 18 months indicating an average growth rate of 2.15 mm month^{-1} during the first 12 months and 0.64 mm month^{-1} during the 12 to 18 month period. For the same species Patterson *et al.*, (1995) obtained a much lower growth rate (1.22 µm day^{-1} and 0.05 mg day^{-1}) when fed with oyster and crab. These values are much lower than that observed by Chaitanawisuti and Kritsanapuntu (2001a) for *B. areolata*. They obtained an average growth rate of 3.86 mm month^{-1} and 1.47 gm month^{-1} at a stocking density of 300 snails m^{-2} for eight months in a flow through system and 3.21 mm month^{-1} and 1.10 gm in a semi- enclosed rearing system. Still higher growth rates (4.26 mm month^{-1}) were obtained when cultured at 100 individuals m^{-2} (Chaitanawisuti and Kritsanapuntu, 1997a). Raghunathan *et al.,* (1994) found that *B. spirata* fed with clam grew in weight from 0.51 gm day^{-1} to 1.9 gm day^{-1} during final growth. The slow growth rates in the present system may be due to the static system. Almost similar growth rates have been reported for *T. nioloticus* (2.6 mm month^{-1}) (Bech, 1997) and for donkeys ear abalone *H. asinina* (2.42mm month^{-1}) (Pommtong *et al*, 1997). For *Busycon carica* growth obtained was low, 14.4 mm year^{-1} for the first

year. This may be because of the slower growth rates observed in temperate region. Since the preliminary studies conducted in Thailand in culturing *B. areolata* were encouraging (Chaitanawisuti and Kritsanapuntu, 1999b; Chaitanawisuti *et al.*, 2001a, 2002, 2006) targeted studies were conducted to evaluate the growth of *B. areolata* in monoculture and polyculture system using large scale operation. There results were also encouraging and now there are well defined protocols for culturing *B. areolata* in earthen ponds and flow through system. The results of the present study form a baseline data and can be used for development of improved and modified rearing systems.

Summary

SUMMARY

- Among the three methods of packing (wet, moist and dry packing) moist packing (1whelk 10 cm^{-2}) in low temperature (24^0 C) was found to the best and reliable method for transportation of brooders of shell length 41.3 ± 2.1 mm and shell width 28.5 ± 0.7 mm and total weight 18 ± 2.5 g. .

- *B. spirata* preferred silt substratum (73% occurrence) followed by fine sand (25%) and coarse sand (2%). Complete avoidance of gravel substrate was observed.

- In feeding *B. spirata* has a long extendable proboscis and it could sense the feed by chemoreception within 10 min.

- *B. spirata* showed significant preference to squilla (40%) and shrimp (35% meat). Fifteen percent of the whelks accepted mussel meat, 10% clam meat while only 5% fed on squid meat. Whelks refused to accept pellet feed and polychaete worms as their feed.

- The feed consumption rate was considerably higher (9.2 and 9.5% by body weight) for the preferred feed of squilla and shrimp respectively. While for the bivalve feed the value was 7.9% for both mussel and clam. Fish and squid feed had little acceptance and the consumption rate was only 3.9 and 3.8 respectively.

- The whelks exhibited active and passive feeding on alternate days, therefore the recommended rearing protocol for broodstock feeding on alternate days with preferred feed such as shrimp or squilla meat @ of 9.2 to 9.5% of body weight.

- *B. spirata* was found to tolerate salinities greater than 26 ppt. In low salinities high mortality (80% in 25 ppt) occurred. In the present study 28 ppt salinity was found to be threshold salinity of *B. spirata*.

- The broodstock rearing salinity recommended is 28- 35 ppt in which 100% survival was recorded.

- The temperature regime recommended for maintaining broodstock of *B. spirata* is 26 to 30^oC based on their behavioural responses and ready acceptance of feed and high feeding rate.

- *B. spirata* exhibited spawning behaviour activities like half buried nature instead of complete burying, spawning aggregation and pair formation.

- During the spawning period, they did not accept feed even if the feed was placed near the spawner. Complete starvation was observed till spawning and the normal feeding behaviour was resumed after spawning.

- During copulation, the female positioned herself on the right side of the male, and was mounted slightly by the male. The copulation process was observed both during day and night hours, lasting for 2-3 h.

- The erect posture of by pressing the foot down and lifting the shell and body whorl up exposing the neck region was found to be an indication of the spawning activity and any slight disturbance halted the spawning activity.

- The process of egg laying in *B. spirata* occurred mostly during night and early morning hours.

- *B. spirata* took 5-10 minutes to release a single capsule, the interval between each laying varied slightly. It took an average of 1.5 to 2 h for the deposition of 30-35 egg capsules.

- Brooding behaviour was not observed in *B. spirata*. After laying the eggs, the females moved away from the egg mass and subsequently the females scattered to different region of the tank and resumed normal behaviour by burrowing themselves in the sand substratum and accepting the feed.

- Year round spawning was observed for *B. spirata* with peak in during the month of December with maximum number of egg capsules. (850 during 1st year and 450 during 2nd year)

- Significant variation ($P<0.5$) in capsular size and number of egg per egg capsule was observed. Monthly variation in fecundity was observed.

- Larger egg capsules were laid by larger female whelks. As the capsular size increased, the number of eggs in the capsule (fecundity) also increased

- Provision of sand substratum (10 cm thickness) was found to be essential for brood stock maintenance. In the tank provided with sand substratum, it was observed that the females moved to a common area (spawning aggregation) and egg capsules were deposited by all females in the same region.

- Delayed spawning and the lesser number of egg capsules were obtained from the spawning tank without substratum. When egg capsules were laid on the tank without substrate, the capsule stakes were bent resulting in fungal infection

- The egg capsules were transparent, inverted triangular in appearance with a double layered wall with varied thickness. The apical portion of capsular wall was thin. The peduncle of the capsule rested on a flaccid membrane (with an average diameter of 6.26 cm) on which the sand particles adhered to. The average thickness of the peduncle was of 340 ± 55 μm.

- The average percentage of larvae released was above 90% irrespective of the number of eggs in the capsule and size of the egg capsule.

- Salinity of the rearing medium affects the metamorphosis of the egg inside the egg capsule. The egg capsules when reared below 30 ppt salinity, the influx of low saline water into the egg capsule resulted complete mortality. The optimum salinity for incubation of the egg capsule was found to be 32 ppt.

- Exchange of water on alternate day was found to be necessary during the incubation of the egg capsule. The rearing medium Treatment (35 ppt chlorination followed by de-chlorination) of water used for rearing of the egg capsule was found to be essential. Fungal infection and non-viability was observed in the egg capsules which were placed in the seawater without any prior treatment.

- Shell width and length of veligers hatched from 30, 35 and 40 ppt salinities were not significantly different ($P > 0.05$) indicating that *B. spirata* egg capsule can tolerate variations in higher salinity range than in the lower range. Below 30 ppt salinity, the eggs cannot divide, develop and grow.

- Temperature of 26 to 28^0C was found to be optimum for the successful development of the veliger inside the capsule with maximum percentage of hatching of the veliger (95%).

- Low temperature prolonged the development of embryo inside the egg capsule, while higher temperatures induced the faster development, but both were lethal to the embryo.

- Mild aeration provided during the incubation period of the egg capsule helped to maintain the water circulation resulting in the development of healthy larvae.

- Light had no influence on the hatching process of the egg capsule. Release of larvae in both the treatments with light and darkness occurred on the 7^{th} day after spawning. In both treatments the larvae were of the same size.

- The albuminous fluid inside the egg capsule was found to become thin as the development progressed. Gradual decrease in the weight of the egg capsule was observed till the 4^{th} day. Developments of the velar structures were observed from the 5^{th} day and thereafter the capsular weights were found to increase

- All the eggs inside an egg capsule were spherical in appearance and had an average diameter of 240 μm irrespective of the size of the egg capsule, spawner size and season of spawning.

- The embryonic development from egg the veliger was completely studied and recorded. The egg development initiated the metamorphosis by the release of the polar body, which was followed by the cleavage. In the 'indirect mode of

development' small planktonic veligers which were released from the egg capsule undergo further metamorphosis and attained the juvenile stage.

- On day 7th the apical aperture of the egg capsule opened and 60% of veliger larvae were released on the first day and the remaining larvae on the next day. They had a SL 460 ± 27 μm SW 315 ± 28 μm.

- From the 1st day to the 8th day after hatching the eye spot of the larvae was clearly visible. The healthy larvae were found on the surface of the water with fast ciliary movements. They moved as a single mass. The veliger at this stage had a SL 476 ± 24 μm and SW 330 ± 24 μm.

- On day 13th the transparent colour of the velum changed gradually to a light brown as a result of pigmentation. The margins pigmented and thickened. The shell attained the SL 670 ± 59 μm and SW 480 ± 53 μm.

- On the 14th day well differentiated morphological changes were observed, larval foot was developed and a transverse groove began to develop through the centre of the velum.

- On day 16th the pelagic life of the larvae ceased and they gradually entered a creeping stage. The larvae possessed rudimentary velar parts. They remained in this stage for a period of 30-40 h.

- On 18th day complete resorption of the velar lobes occurred during the final stage of metamorphosis. The larvae possessed a well defined metapodium with the operculum attached to the posterior end and had a SL 820 ± 420 μm and SW 740 ± 30 μm.

- On 19th day the larvae completely metamorphosed to young juvenile. The juveniles possessed a well defined siphon, tentacles, foot, operculum and a larval shell (protoconch II) and a transparent body whorl. The juvenile shell was smooth and thin without sculptures. On settlement the juvenile had a SL 920 ± 80 μm and SW 803 ± 70 μm.

- 32 ppt salinity found to be the optimum required for larval rearing of *B. spirata* which gave high growth rate (Average IGR for SL and SW 0.03 and 0.04 respectively), survival (76%) and settlement (58%).

- Salinity above or below 32 ppt delayed the settlement (20-28 days) and the percentage settlement decreased (57-75%). Salinity had positive correlation with the days required for the veliger larvae to settle (r=0.888).

- Supplement of algal feed was necessary for the survival of the veliger from the first day of hatching. Starvation resulted in the mortality from the first day and complete mortality was recorded on 3rd day.

- With the increase of the larval size, the average ingestion rate (IR) increased to 8195 ± 124 to 18,858 ± 631 cells ml^{-1}larvae^{-1}. During the final stage of metamorphosis, when the velum size reduced and cilia on the velum became reduced in number, then the IR decreased to 9635 ± 651 cells ml^{-1} larvae^{-1}. Larvae were fed with algal diet till the day of settlement.

- Difference was also observed in feeding rates during the day time and night hours. Higher rate of feeding was noticed immediately after the water exchange during day time (20977 ± 1198 cells ml^{-1} h^{-1} larvae^{-1}) and it decreased during night hours (11731 ± 821 cells ml^{-1} h^{-1} larvae^{-1}).

- Complete mortality was found when larvae were provided with *Nannochloropsis salina (Ns)* and *Tetraselmis gracil*is *(Tg).* The larvae could survive and settle only on a diet of *Isochrysis galbana (Ig)* or *Chaetoceros calcitrans (Cc).* In both of this feeds 59% settlement was observed and settlement was on 18th day.

- The average feeding rate for *Ns, Tg,Ig and Cc* was 1485 ± 645, 6,577 ± 1618, 15,632 ± 694 and 16,379 ± 536 cells ml^{-1} h^{-1} larvae^{-1} respectively.

- Though the IR decreased in mixed diet than monoalgal diet, there was no significant variation in the survival, growth and number of days required for the larvae to settle as juvenile.

- Low feed resulted in low growth rate, survival and it took nearly 10 days more to settle than in optimum feeding.

- In high concentration though the settlement was earlier by 2 days the survival was 5-9% less than the optimum. The thin shells of the larvae were badly fouled with the debris.

- Supply of optimum level of feed *Chaetoceros calcitrans* (17786 ± 5527 cells ml^{-1} h^{-1}) to the metamorphosing veliger resulted in better survival and settlement percentage (60%).

- The optimum temperature required for the larvae to metamorphose was 27^{0}C. Temperature had significant effect on growth and settlement of the veliger, low temperature delayed settlement while at high temperature, metamorphosis and settlement was faster.

- Higher temperature was more detrimental than low temperature. The metamorphosis was faster and settlement earlier, but subsequently resulted in complete mortality.

- In lower temperature, though the percentage of settlement was lower (40-45%) the juveniles settled were larger than those settled at higher temperature and the settlement survival was higher.

- The ingestion rates at higher temperature were comparatively more than at low and ambient temperature

- Optimum stocking density was found to be 150 larvae L^{-1} and above this metamorphosis and settlement was found to be delayed.

- The duration of pelagic life also varied in different stocking densities, It was much prolonged (17-23rd day) in higher stocking densities when compared to optimum density (17th day), while in lower densities it was on 19-23rd day.

- A substratum was not essential for settlement. There was no difference in the percentage of settlement and growth of the larvae in the treatment with substratum and without substratum. The larvae started to settle on 18th day of pelagic phase and completed the settlement on 22nd day.

- Experiments on water quality maintenance indicated that it is optimal to change the water on alternate days.

- Aeration was found to be essential for larval rearing. Survival and settlement was found to be low in the larval rearing tank which was not provided with aeration. The percentage of settlement in the aerated treatment was 60%, while in the non-aerated treatment it was 36%. Similarly, growth was also retarded in the non aerated culture tank.

- The average ammonia content in the tank provided with aeration was low (7 ± 0.1 µmol l^{-1}) while in the non-aerated treatment it was high (16 ± 0.2 µmol l^{-1}). The amount of ammonia in the rearing tank in both treatments was found to increase with the progression of metamorphosis.

- Light had no significant effect on the growth and metamorphosis of the larvae.

- The experiment conducted to study the effect of different feeds indicated that the juveniles of *B. spirata* grow best when fed with shrimp meat. Their growth in length and weight were comparatively higher. The absolute growth rate (AGR) for length, girth and total weight was higher when shrimp was provided as feed.

- Complete mortality of the juveniles occurred when fed with egg albumin indicating that it cannot be used as feed. However the growth performance when fed with squilla meat and fish meat were better than its performance with clam meat.

- The percentage of feed consumption was higher for younger juveniles than larger groups. The percentage of feed consumption per body weight of the juvenile for different size groups is different. The juvenile *B. spirata* consumed feed @ 10.5% to 12.6% of body weight.

- The growth rate was found to be different for length, width and weight of *B. spirata* juveniles of different size classes.

- The juvenile *B. spirata* preferred silt and fine sand substratum to coarse sand and gravel.

- All the experimental juveniles consumed feed within 10-15 minutes when feed was provided and were found to move to the preferred substratum after feeding.

- The survival after 60 days of the experiment was 100% in the treatments with and without substratum and no significant variation ($P>0.05$) was observed for the AGR after 60 days in both treatments.

- When the juveniles were not provided with substratum in the tank there were chances for dehydration of juveniles by crawling above the water level in the tank.

- Juvenile *B. spirata* were found to be stenohaline with very poor survival in low salinities.

- Similar to adult whelks the juveniles also exhibited the behavioural responses by firm closure of the operculum and being inactive in low and high salinities.

- In 32 ppt salinity the juvenile attained maximum growth in shell length (17.54 mm), shell width (12.42 mm) and weight (0.63 g) in 60 days.

- Best growth and survival of juveniles was obtained in 26 to 30^0C and it was considered as the optimum temperature required for juvenile rearing. Their survival and growth were very poor in low and high temperatures. .

- In the present study it was observed that *B. spirata* attained average shell length of 6.3 mm, 22.4 mm and 29.49 mm at the end of 6, 12 and 18 months indicating an average growth rate of 2.15 mm $month^{-1}$ during the first 12 months and 0.64 mm $month^{-1}$ during the 12 to 18 month period. The growth rate varied with age. Maximum growth rate $month^{-1}$ for shell length (5.77 mm) and shell width (3.73 mm) was recorded during the 6^{th} month. But maximum growth rate for weight was recorded during the 11^{th} month. Mortality was negligible during the period of culture.

SUMMARY OF HATCHERY PROTOCOL

1. BROODSTOCK ACCLIMATISATION

Sl.No.	Requirements	Optimum level/ Most favourable condition
1	Tanks	FRP tank of size 2.5 x 1.5 x 1m. (inner black in colour)
2	Stocking density	1 whelk per 200 cm^2
3	Transportation of brooders	Moist packing at a density of 1 whelk 10 cm^{-2}, in low temperature (24^0 C).
4	Period of acclimatization	2-3 days
5	Feeding during acclimatization	Not required
6	Substratum during acclimatization	Not required
7	Salinity	30-32 ppt
8	Temperature	26^0C to 28^0C
9	Aeration	Essential for 48-72h (continuous)
10	Water exchange	Complete water exchange every 3 days
11	Filtration of water	Not required

2. BROODSTOCK MAINTANANCE

Sl.No.	Requirements	Optimum level/ Most favourable condition
1	Tanks	FRP tank of size 2.5 x 1.5 x 1m or round tank of 1 t capacity (inner side black or white in colour)
2	Stocking density	1 whelk per 100 cm^2
3	Substratum for rearing	Silt substratum of 10 cm thickness
4	Feed requirements	Shrimp or squilla meat as feed @ of 9.2 to 9.5 % of body weight.
5	Feeding schedule	Once in a day at morning,
6	Salinity	28- 35 ppt
7	Temperature	26-30^0C
8	Aeration	Continuous except during feeding
9	Water exchange	50% water exchange every day
10	Filtration of water	Not required

3. SPAWNING

Sl.No.	Requirements	Optimum level/ Most favourable condition
1	Tank	Perspex tank of 100 l capacity with bottom area 3500 cm^2
2	Brooder size	SL 40-45 mm, SW 28-30 mm and TW 18-20 gm
3	Sex ratio (M:F)	1:1
4	Substratum	silt substratum 10 cm thickness
6	Salinity	30-32 ppt
7	Temperature	28-30^{0}C
8	Aeration	Essential, continuous for 24 h
9	Feeding of brooders during spawning	Not required

4. INCUBATION OF EGG

1	Salinity	32 ±1 ppt
2	Aeration	24 h mild aeration
3	Temperature	26-28^{0}C
4	Water treatment	Essential with 30 ppm sodium hypochlorite solution and de-chlorination
5	Filtration of water	Sand filtered water
6	Water exchange	Alternate days
7	Light for incubation of the egg and hatching	No effect. Cover with black cloth reduce dust

5. LARVAL REARING

1	Salinity	32 ±1 ppt
2	Aeration	24 h mild aeration
3	Temperature	26-28^{0}C
4	Water treatment	Essential with 30 ppm sodium hypochlorite solution and de-chlorination.
5	Filtration of water	Sand filtered water
6	Water exchange	Alternate days
7	Light for incubation of the egg and hatching	Not required
8	Light for larval rearing	10 h light and 14 h darkness
9	Feed species	*Isochrysis galbana/ Chaetoceros calcitrans* (unialgal)
10	Stocking density of larvae	150 larvae l^{-1}
11	Feeding rate	Maintain 9,000 to 10,000 cells ml^{-1} h^{-1} $larvae^{-1}$ always in the larval rearing tank

6. JUVENILE REARING

Sl.No.	Requirements	Optimum level/ Most favourable condition
1	Salinity	30-32 ppt
2	Temperature	26-29^{0}C
3	Water	30 ppm sodium hypochlorite treated, dechlorinated water.
4	Aeration	24 h essential except during feeding
5	Water exchange	Every day after feeding
6	Feed	Squilla/ shrimp made into paste
7	Substratum	Not essential for growth up to 5 mm size
8	Substrate preference	Silt/ fine sand of 3 cm thickness
9	Feed consumption rate	@ 10.5 % to 12.6 % of body weight

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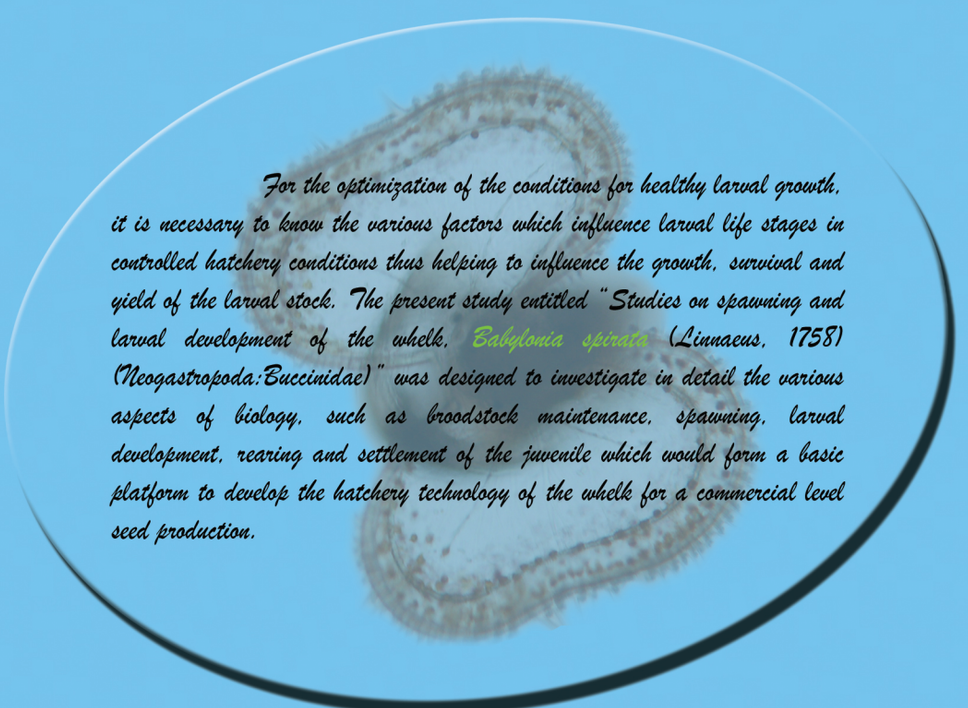

For the optimization of the conditions for healthy larval growth, it is necessary to know the various factors which influence larval life stages in controlled hatchery conditions thus helping to influence the growth, survival and yield of the larval stock. The present study entitled "Studies on spawning and larval development of the whelk, Babylonia spirata (Linnaeus, 1758) (Neogastropoda:Buccinidae)" was designed to investigate in detail the various aspects of biology, such as broodstock maintenance, spawning, larval development, rearing and settlement of the juvenile which would form a basic platform to develop the hatchery technology of the whelk for a commercial level seed production.

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