

**BREEDING OF SELECTED SPECIES OF  
INDIAN FRESHWATER PEARL MUSSEL,  
*Lamellidens marginalis* (L.)**

A THESIS SUBMITTED TO  
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY  
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
**MASTER OF FISHERY SCIENCE**  
IN  
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By

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BHUBANESWAR

**1996-97**

*Dedicated to my*

**BELOVED PARENTS**



केन्द्रीय मीठजल जीवपालन अनुसंधान संस्थान

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This is to certify that the thesis entitled " Breeding of selected species of Indian Freshwater pearl mussel, *Lamellidens marginalis* (L.)," submitted for the Degree of Master of Fishery Science in the subject of Aquaculture of the Orissa University of Agriculture and Technology, Bhubaneswar, is a faithful record of bonafide and original research work carried out by **Mr. Bijay Kumar Behera** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the work of investigation have been fully acknowledged.


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Major Advisor.

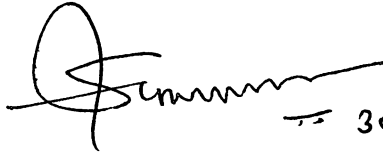
## CERTIFICATE-II

This is to certify that the thesis entitled "Breeding of selected species of Indian Freshwater pearl mussel, *Lamellidens marginalis* (L.)," submitted by Mr. Bijay Kumar Behera to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the Degree of Master of Fishery Science in the subject of Aquaculture has been approved by the Students Advisory Committee after an oral examination on the same in collaboration with an External Examiner.

  
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*Bijay Kumar Behera*  
**BIJAY KUMAR BEHERA**

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## INTRODUCTION

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Pearls, especially have been among man's favourite gems since ancient times. The pearl is both mystic and beautiful with soft colour and cool lustre. According to the Indian system of gemology, the pearl is only next to diamond in importance. Perhaps this is the only gem which do not require the treatment by lapidary as in the case of other precious stones.

Theoretically any shelled mollusc can produce a pearl of some sorts, but only those mulluscs which have a pearl lining or pearl nacre on the interior of the shell surface can produce lustrous pearls. Thus, pearl formation can be regarded as an abnormality in the normal biological processes which build up the shell - the protective covering of the animal. This abnormal responses of the mantle epithelium in selected marine oysters and some freshwater mussels forms the basis for culture of pearls, a regulated commercial biomineralisation process.

The genesis of modern freshwater pearl culture can be traced to the traditional practice existing from the 12th century in lake Tahu by China of producing pearl coated Buddha images in the mussel *Cristaria plicata*.

During the 18th century, the Swedish naturalist Carl. Von Linneaeus experimented with freshwater mussels for production of culture pearls but the results were not of any commercial importance.

Finally, it was the Japanese who succeeded in solving the mystery of pearl. In 1907, Tochiki Nishikawa gave the most possible scientific explanation on the origin of pearls which heralded the pearl empire in Japan. His theory, explains that a pearl is formed when the pearl secreting cells of the mantle migrate into the body of the mussel under the stimulus of a foreign body and form a pearl-sac by cell division around the foreign body; the pearl-sac secretes nacre which gets deposited on the foreign body and in course of time a pearl is produced. Japan, has developed this scientific principle into a commercial proposition by 1935. The Japanese mussel, locally called ike-chogi (*Hyriopsis schlegeli*), abounds in the freshwater Biwa-Ko (Ko means lake) in Shiga province in Honshu of Central Japan and has also been transplanted to the north-eastern lake Kasumiga-Ura. Commercial freshwater pearl culture is flourishing in lake Biwa from 1935; while the activity began in lake Kasumiga-Ura in 1963.

In USA, freshwater pearl merchandise was well developed in the early and mid forties in the Mississippi river basin into which flow the rivers Tennessee, Kentucky and Arkansas. In addition to the natural freshwater pearls popularly called "balls", "Pears" and "buttons" in different shapes like "petal", "Lily", "leaf" and "arrow head", huge quantities of shell pieces of pig-toe mussel (*Quadrula undata*) were also exported to Japan for manufacture of shell bead nuclei (Pennak, 1953). The principal species for pearl production in the USA being the niggerhead (*Quadrula ebena*), threeridge (*Quadrula undulata*), bullhead (*Pleurobema oesopus*), buckhorn (*Tritogonia veruicosa*), *Unio* sp. and *Megalansis* sp..

In Europe, the freshwater pearl mussel *Unio* sp. is well distributed. The Scotch pearls were traditionally fished from rivers Tay, Earn and Teith and others in Scotland and North Wales. The mussel, *Margaritifera margaritifera* in the river Conway has historical importance in pearl fishing industry in Wales (Jones, 1973). Pearl fishing was once flourishing in Vologne river in France, German and Austrian rivers, and Bavarian streams and in particular the Bavarian river Isar were the famous haunts of the mussel *Unio* sp.. The streams of Lunenburg Heath and rivers of the Russian and Scandinavian countries were also once inhabited by pearl mussels.

Among South East Asian countries, Bangladesh had started a pilot project on culture of pearl mussel *Lamellidens marginalis* and *Parreysia corrugata*, during 1975-80 (Ahmed, 1982). In recent years, Vietnam has also succeeded in the production of culture pearls from the mussel *Hyriopsis cumingii* (Janaki Ram and Tripathi, 1992).

In India, marine pearl culture had had its beginning in the early seventies and the theory of natural or culture pearl formation in marine oysters is now fairly known (Alagarwami, 1991). But freshwater pearl culture, on the other hand, remained as an unexploited area despite the vast freshwater resources.

At present, freshwater pearl culture by virtue of having comparative cost effectiveness, wider scope and ease of operations

is a developed industry in China with an output upto 200t and in Japan, 5t to 10t of culture pearls per year. The world trade of pearls, reportedly over US \$3 billion per year is constituted in bulk by freshwater culture pearls (Fassler, 1991). It is reported that in Japan there are 47 culture farms with 110 hectares of water spread in lake Biwa and 16 farms with a water stretch of 13 hectares in lake Kasumiga-Ura in operation. (Kafuku and Ikenoue, 1983). In recent years China has made tremendous progress in freshwater pearl culture, employing the mussel *Hyriopsis cumingii* in an artificial lake dug for the purpose near Shanghai (Ward, 1985). China has also introduced the Japanese pearl mussel *Hyriopsis schlegeli* in 1982 for quality upgradation of the exported pearls. In USA, four pearl culture farms, one in California and two in Tennessee are involved in the production of freshwater culture pearls and the first batch cultured pearls came to the markets by 1987 (Ward, 1987). In India, the traders at Bombay and Hyderabad are importing more than Rs. 300 million worth of pearls every year to meet the ever increasing domestic demand and for partly over-seas trade. The Central Institute of Freshwater Aquaculture (CIFA), realising the scope and importance of Inland pearl culture in India took the lead and evolved an indigenous system of culturing pearls from common freshwater mussels *Lamellidens marginalis*, *Lamellidens corrianus* and *Parreysia corrugata* (Janaki Ram, 1989; Janaki Ram and Tripathi, 1992). Suitable surgical grafting and culture procedures have been

developed for different pearl products like:

1. Regular round pearls.
2. Irregular baroque pearls.
3. Half-round shell-attached pearls.
4. Design pearls.

Now, the Institute has started imparting training in pearl culture to farmers and entrepreneurs for commercialisation of the freshwater pearl culture technology in the country.

These mussels besides yielding the cash crop of pearls are also important in more than one way. The soft mussel meat after processing can be exported due to its high demand in the international market. In India, the mussel meat is also consumed by poorer sections of the people particularly in the North-Eastern states. Mussel meat can also be utilised as a cheap protein ingredient in the feed formulations for aquaculture of shrimps and catfishes. The pearl and shell powder are known to be useful in the traditional systems of medicine in China and India during, pregnancy and for weak constitution, tooth cavities, hyper acidity, allergies etc. (Ward, 1985). The pearl and shell products are also used in the manufacture of cosmetics and toothpaste in Japan and China (Ward, 1985). The mussel shells are used for manufacture of fancy items such as handicrafts, ear tops, rings, pendants etc are manufactured in addition to shell buttons (Banerji and Satish, 1988). The shell dust accumulated in the process of manufacture of different

shell products is used as "shell grit," as a poultry feed component (Banerji and Satish, 1988). Again mussel shells are a rich natural resource for commercial slaked lime production. The thick, solid shells of certain mussel species are utilised for the manufacture of pearl bead nuclei, the essential input material for pearl culture industry (Janaki Ram and Tripathi, 1992).

In this backdrop, there is tremendous scope for development of freshwater mussel culture in the country. It can be said that wider adoption of freshwater mussel culture can serve the dual role of import substitution and foreign exchange earnings. Further this can generate economic and employment opportunities particularly in the rural areas of the country.

Availability of pure mussel seed in mass scale assumes critical importance for realising the potential benefits of this important group of aquatic organisations. The aquatic environment is dynamic and ever changing and plays the key role in the dispersal and distribution of the mussel population and the success or failure of a brood in reaching its appropriate destinations. This explains the uncertainty of availability of seed mussels for freshwater pearl and mussel culture and there is imperative need for scientific efforts to ensure the seed production through captive culture and breeding techniques.

Hitherto research attention has been concentrated on technology evolution, development and standardization of the

freshwater pearl culture at CIFA, however, not much of attention could be paid on the important aspect of mussel breeding and seed production. Culture of freshwater pearl mussels, *Lamellidens marginalis*, *Lamellidens corrianus*, *Parreysia corrugata* is one of the areas where considerable advances have been made on the farming techniques and the seed mussels have always been obtained from the wild. The natural mussel seed resources may not be adequate to support the freshwater pearl culture industry in the future. Thus it is imperative to develop indigenous techniques for seed production under captive conditions of the important common freshwater pearl mussels. It is pertinent to note that a majority of freshwater mussels have a indirect larval development with a mandatory parasitic glochidial stage making the seed production under controlled conditions all the more difficult.

Considering the scope and importance of evolution of freshwater mussel seed production technology, the present studies have been undertaken with the following objectives.

1. To examine the egg to juvenile stage of the life history of freshwater mussels in captive conditions.
2. To find out the fish host mussel larval relationship and target organs of the host.

## CHAPTER-II

# REVIEW OF LITERATURE

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## REVIEW OF LITERATURE

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### SYSTEMATIC POSITION

The taxonomic status of the common freshwater mussel *Lamellidens marginalis* is as follows:

Phylum	:	Mollusca.
Class	:	Bivalvia
Sub class	:	Palaeoheterodonta
Order	:	Unionoida
Family	:	Unionidae
Sub-family:		Unioinae
Genus	:	<i>Lamellidens</i> ( Simpson, 1900)
Species	:	<i>Lemellidens marginalis</i> (Lamarck,1819).

### DISTRIBUTION

The species *Lamellidens marginalis* is well distributed in nearly all States of India, such as Andhra Pradesh, Orissa, West Bengal, Uttar Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Bihar, Kerala, Tamilnadu, Rajsthan and Tripura (Thomas, 1974). The mussel is also recorded in the adjoining countries, Pakistan, Bangladesh, Sri Lanka and Burma (Subba Rao, 1989).

### GENERAL ECOLOGY AND BIOLOGY

The freshwater mussels constitute an important group of the benthic invertebrate population in freshwater ecosystem. Species

of *Lamellidens* are normally distributed in stagnant to slow flowing habitats like ponds, tanks, lakes and reservoirs at a depth of 0.5m and beyond (Janaki Ram & Radha Krishna, 1984). It may be said that *Lamellidens marginalis* is a typical pond water species. All the species in general thrive well in neutral to slightly alkaline water and occur more abundantly in medium hard waters. Generally the mussels live nearly buried in the mud or in sand at the bottom, with the posterior side of the shell valves exposed. They may also occur wedged in between the rocks and stones, with the valves slightly spread and the two siphons exposed. The mussels crawl slowly by extending their large muscular foot between the two shell valves and leave a furrow like mark on the sediment. They may migrate to shallow places by night and retire to deeper places by day and may change the habitats with the seasons (Thomas, 1974).

Freshwater mussel are considered as mucoid filter feeders. The mussels generally feed on minute organisms such as diatoms, desmids, algal filaments, spores and protozoans. (Churchill & Lewis, 1924). In the alimentary canal of freshwater mussels, algal forms like the loricae of rotifers, active *Euglina* like forms and fungal spores along with muddy matrix have been recorded (Clark and Wilson 1912). This muddy matrix has been termed as dust fine detritus (Baker 1916). Allen (1912 and 1914) stated that mussels exercise considerable choice in ingestion of food material. According to Kellogg (1915) there is no selection or separation of food organisms from other water borne particles. It has been described that the

ingestion of food material in freshwater mussels is mainly due to the action of cilia of the gill filaments causing a current of water which enters in the mantle cavity through the inhalant aperture. It is also reported the presence of monaxon spicules in the stomach content of mussel collected from the riverine source indicates that there is no selection or separation of food materials by the mussel. Thus the detritus, diatoms, fragments of animal remains, algal filaments, desmids constitute the major food items of the mussels (Patil, 1974). The information on supplementary feeding of freshwater mussels is virtually non-existing, It was observed that the Indian freshwater pearl mussels (*Lamellidens marginalis*) under captive culture conditions can be maintained on diets such as cultured green algae (*Kirchneriella* spp., *Scenedesmus* spp. and *Chlorococcum* spp.) blue-green alga (*Spirulina* spp.) and conventional fish feeds ( rice bran and ground nut oil cake) with growth increment of 0.07 cm and 0.586g; 0.11cm and 1.018g; 0.13cm and 3.99g respectively in a period of 30 days (Gayatri Mishra, Kuldeep Kumar and Janaki Ram, 1995).

The sexes are separate in the *Lamellidens marginalis*(Thomas, 1974). The species is a prolific breeder with spawning peak during August and September (Ghosh and Ghosh, 1972). The histology of testes, ovary, fertilization and embryonic development have been reported by Kotpal (1995).

The testes are a pair of large, simple racemously branched structures lying among the intestinal coils in the visceral

mass just above the foot. During breeding seasons, the testes becomes greatly enlarged and conspicuously whitish in colour. The coelomic epithelium, lining the tubules of testes gives rise to the spermatozoa. The testes of each side was a short duct, known as vas deferens. Each sperm duct opens by a genital aperture below and a little in front of the renal aperture into the suprabranchial canal of the inner demibranch of its side.

The ovaries are a pair of large, simple, racemously branched structures lying among the intestinal coils in the visceral mass just above the foot. During breeding season the ovary becomes greatly enlarged and conspicuously pinkish in colour. The coelomic epithelium lining of the tubules of the ovary, gives rise to ova. The mature ovum is large, rounded, filled with a finely granular cytoplasm, rich in yolk and containing avescicular nucleus with nucleolus. The ovary of each side has a short duct, the oviduct opening into the suprabranchial chamber of the inner gill lamina by a genital aperture, just in front of the renal aperture of the ureter.

In male, the sperms are shed through the genital aperture into the supra-branchial chambers and released from the body with the outgoing water through the exhalant siphon. Some of these sperms will find entry into inhalant siphon of a female. The eggs, shed through the genital aperture into the suprabanchial chambers, do not leave the body but are carried through the ostia into the water tubes of outer gill lamellae,

where they are held by mucus and fertilized by sperms from other mussel, drawn in by water current, thus forming zygotes (Kotpal, 1995).

However, Thomas (1974) stated that the matured ova from the ovarian follicles pass through the oviducts into the suprabranchial canals of the two inner demibranchs and are fertilized there by the spermatozoa coming from outside in the stream of respiratory water. After fertilization the ova pass along the inner suprabranchial canals of the outer demibranchs and enter the water tubes where they develop into minute glochidia. The demibranchs containing ova in various stages of development are called marsupia or brood pouches. In *Lamellidens marginalis*, it is usually the two outer demibranchs that serve as marsupia, although sometimes all the four demibranches may act as such. The females are easily recognized at this stage by the swollen appearance of their outer gill plates. The embryonic development of the fertilized ovum up to glochidial larval stage in some of the freshwater mussels such as *Anodonta* (Kotpal, 1995), *Unio* (Lillie, 1893 and 1895), *Cyclas* (Ziegler, 1885) and *Lamellidens* (Kotpal, 1995; Prasad, 1918; Thomas, 1974) have been described earlier. The glochidia are incapable of movement and independent feeding. In *Anodonta*, the glochidia can open or close their shells, but cannot move independently. The larvae must now come in contact with a freshwater fish and live as an ectoparasite, in the absence of which larvae die. When the glochidium larvae come in contact with the proper fish host, the larvae seize hold of host's skin, fin, gills by closing their hooked valves and penetrating the host tissue by larval threads (Kotpal, 1995). The closure of valves seems to be due to

chemical stimulation, by salts escaping from the tissues of the fish. In this position they become encysted by a pathological over growth of the skin or mucous membrane of the host. The skin of fish thus growing around the glochidia forms the "worms" or "black heads" while thus embedded they lead truly ectoparasitic life, absorbing nourishment through processes of mantle from its host for 10 weeks, during which period they undergo a peculiar metamorphosis to develop into young mussel (Kotpal, 1995). After the metamorphosis, the cyst weakens and breaks, liberating the miniature mussel or juveniles stage, which opens and closes its valves. It extends its foot and sinks to the bottom to grow upto the adult form and mode of life. The adult mussel lives for about 5 years. Kafuku and Ikenoue (1983) opined that the spawning of freshwater mussel, *Hyriopsis schlegelii* takes place from April to August. The peak of spawning season is in May and June when the water temperature is 20-25°C. The mussel attains sexual maturity at 4 years old when the shell length becomes 10-11 cm. It was reported that the longevity of the mussel exceeds 40 years (Kafuku and Ikenoue, 1983). It has been reported that *Lamellidens marginalis* is a prolific breeder and attains sexual maturity when it is over two years old. The mussels breed almost round the year with a peak in August-September. Fertilization is external and takes place in branchial cavity and the fertilized ova pass into the space of gill lamellae. In course of development, the glochidial larva hatch out from the fertilized eggs and are passed out by the exhalant siphon. The newly hatched larvae gets attached to fish for further development. The parasitic life is about 10 weeks after which it fully metamorphose into an adult and detaches from fish (Anonymous, 1990).

Woody and Holland (1993) examined monthly and age-specific gametogenic development of the wash board mussel, *Megalonaias nervosa*. It has been found that the species is a late tachytictic breeder. Female marsuipa contained eggs or glochidia primarily from August (17°C) through October (9°C). Males were mature from July through October. Most females released their glochida in October. In host specificity studies, three fish species were verified as hosts for the glochidial stage. Green sunfish (*Lepomis cyanellus*), black bullhead (*Ictalurus melas*), and channel catfish (*Ictalurus punctatus*) produced juveniles after 26-28 days at 17°. White suckers (*Catostomus commersoni*) and yellow perch (*Perca flavescens*) have retained glochidia from 23 up to 26 days, without resulting in juveniles. Glochidia remained attached to common carp (*Cyprinus carpio*) and fathead minnows (*Pimephales promelas*) for shorter periods of less than or equal to 3 days. Channel catfish were retested at 12°C the juveniles were recorded after 56 days (Woody and Holland, 1993).

Thus in the life cycle of a majority of freshwater mussels, the mandatory parasitic glochidium larval stage on a fish host, ensures certain advantages. Besides affording protection and a means of nourishment, the fish host ensures a far and wide and rapid dispersal of the mussel species. A case of concurrent mutual aid and injury of give and take is demonstrated by a freshwater fish, *Aplodinotus grunniens*. The fish, heavily infected by numerous glochidia, not only distributes the larval mussels but also unwittingly helps to grow its own food supply (Kotpal, 1995).

## CHAPTER-III

# MATERIALS AND METHODS

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## MATERIALS AND METHODS

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The present study has been carried out at the Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, Orissa (Late-20°11'6"-20°11'45" N; long. 85° 50'52"-85°51'35"E) during the period May to October, 1996.

### EXPERIMENTAL SET UP

The experiments were designed to study the certain life history stages of the freshwater mussel *Lamellidens marginalis* (Plate-1) and the fish host-mussel larval parasitic relationship. The experiments were set up in a laboratory room of the Institute. Round plastic tubs of 50cm diameter and 20cm depth were employed for the brood mussel rearing (Plate-4). Rectangular FRP tanks (100cm x 50cm x 45cm) were used for rearing the different fish host species (Plate-6 and 7). All the FRP tanks and plastic tubs were cleaned with detergents and disinfected with 2% KMNO<sub>4</sub> solution as a prophylactic measure before starting the experiment.

### COLLECTION AND REARING OF DIFFERENT FISH HOST SPECIES

The fish species of different sizes were collected from the CIFA fish farm. The live test fishes were brought to the laboratory and given a dip treatment in 0.5% KMNO<sub>4</sub> solution for about 1-2 minutes prior to the experimentation. Then the test fishes as listed below were released in equal numbers into the fish holding tanks

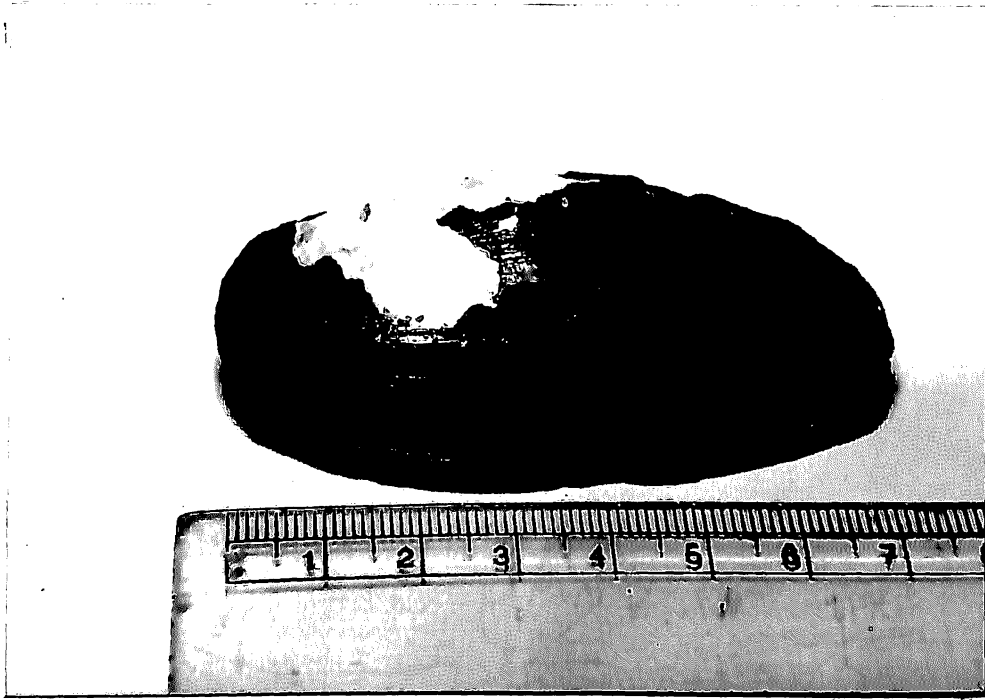


Plate I. Indian pearl mussel, *Lamellidens marginalis*.



Plate II. Indian pearl mussel, *Lamellidens corrianus*.



Plate III. Riverine mussel, *Parreysia corrugata*.



Plate IV. The spawning mussels in a tub.



Plate V. Experimental set up for hatching the eggs of pearl mussels.



Plate VI. Experimental set up indicating mussel spawning tubs and fish hosts rearing containers.



Plate VII. Close-up of the fish host rearing tanks.

(30/tank) filled with aged tap water.

Host fish species	Length size (cm)
<i>Cirrhina mrigala</i>	11-15
<i>Labeo rohita</i>	11-15
<i>Glossogobius giuris</i>	7-11
<i>Catla catla</i>	13-15
<i>Hypophthalmichthys molitrix</i>	13-15
<i>Cyprinus carpio</i>	10-15
<i>Ctenopharyngodon idella</i>	9-11

#### COLLECTION AND REARING OF MATURE MUSSELS

The live healthy mussels, *Lamellidens marginalis* of 8cm to 10cm in shell length were collected from CIFA fish farm and from near by village ponds and brought to the laboratory for the experimental trials. The mussels were cleaned thoroughly with tap water to remove the mud and epiflora and fauna of the shell surface. These mussels were kept in a bucket without water for about 1 hour and the resultant stress conditions induced the spawning activity. The mussels were released into the plastic tubs @ 20 mussels/tub containing aged tap water and eggs and glochidium larvae were collected by pipetting. The experimental fish tanks and mussel tubs were aerated continuously.

## FOOD AND FEEDING

The fishes were fed with a formulation containing locally available quality ingredients such as,

Groundnut oil cake	-	40%
Soybean oil cake	-	20%
Rice bran	-	18%
Fish meal	-	20%
Vitamins and mineral additives	-	2%

The feed in the form of pellets (1.0cm dia) was provided to the following fishes at the rate of 2% of the total body weight on alternate days during the duration of the experiment.

1. *Cirrhina mrigala*
2. *Labeo rohita*
3. *Hypophthalmichthys molitrix*
4. *Cyprinus carpio*
5. *Ctenopharyngodon idella*

The fishes *Glossogobius giuris*, *Catla catla* were fed ad libetum with zooplankton collected from CIFA ponds.

It have been observed that provision of feed in the mussel spawning tanks resulted in pollution and affected survival of the released eggs and larvae. Hence the experimental mussels were not

fed during the period of spawning till collection of the fertilized eggs and glochidial larvae. The glochidial larvae of the freshwater pearl mussels are ectoparasite in nature. The larval obtained nourishment from the body fluid and blood of the fish host till metamorphosis. The juvenile mussels (5mm in shell length) were fed with *Chlorella* for optimum survival and growth. The pure cultures of *Chlorella* were obtained from the Microbiology laboratory of the Institute.

### **CLEANING AND WATER EXCHANGE**

For maintaining the optimum water quality conditions in the experimental tanks, the unutilized feed, metabolites and offal settled at the tank bottom were siphoned out daily until the glochidial larval infection was noticed on the fish hosts. When once glochidial larval incidence was recorded on the fish host, then only the supernatant water in the tanks was decanted without disturbing the juvenile mussel settlement on the tank bottom. Generally, 75% of the water was exchanged at 3 days interval in all the experimental tanks to maintain the water quality.

The mussel spawning tubs were cleaned daily and 100% of the water exchanged.

### **HATCHING AND COLLECTION OF MUSSEL LARVAE**

Generally, the hatching of the larvae takes place in the gill marsupium of the female mussel and are released into the ambient water through the exhalant siphon. It was observed that with

the glochidial larvae, fertilized and unfertilized eggs were also released into the water. The brood mussels were removed after the release of glochidium larvae and eggs. The aeration was suspended in the mussel spawning tanks till the collection of larvae and eggs. After settlement, the excess water in the spawning tubs was carefully siphoned out without disturbing the settled mussel larvae and eggs.

The whitish fertilized eggs were observed to be agglutinated in a mass of 300-500 eggs. These were collected by pipetting carefully from the bottom of the mussel spawning tubs and 1000-1500 eggs were distributed in the glass jar (11) containing aged tap water with feeble aeration till hatching (Plate-5). The mussel larvae released in the spawning tubs were pipetted out and directly placed in the glass jar (11).

### **ESTIMATION OF LARVAL NUMBERS**

The small volume of the collected glochidial larvae with water from the spawning tub was made upto 1 liter in a glass jar. Then estimation of larvae was done by taking 10 random samples of the thoroughly mixed larval suspension in the jar. Each sample volume was 10ml. The number of larvae in each sample was counted under a binocular stereo zoom microscope and the total number of larvae in the sample computed.

The total number of larvae in the tub was estimated adopting the following formula.

$$\begin{array}{rcl} \text{Total number} & & \text{Mean number} \\ \text{of larvae in} & & \text{of larvae in} \\ \text{the tub} & = & \text{10 samples} \\ & & \text{-----} \times \\ & & \text{Sample volume} \\ & & \text{(10ml)} \end{array} \quad \begin{array}{l} \text{Total volume of} \\ \text{the water in the} \\ \text{glass jar being 1l.} \end{array}$$

### SCREENING OF FISH HOSTS

The fish hosts were depurated for a period of 30 days in the tanks to avoid earlier glochidial infections before start of the experiments. After ascertaining that the experimental fishes are devoid of mussel larval infection, approximately 3000 numbers of newly collected/hatched glochidial larvae were transferred into the fish holding tanks.

### OBSERVATION OF THE LARVAL STAGE IN THE FISH HOST

After every 7 days interval after the introduction of mussel larvae, one fish from each tank was sacrificed and the infection rate and the different parts of the body like skin, gills, fins for glochidial larval attachment were examined. The observations were continued for 1 month to note the different stages of larval metamorphosis in the fish host. The gills and fins were removed from the sacrificed fish and observed individually under the microscope.

### OBSERVATION OF JUVENILES AND ENUMERATION

The glochidial larvae after reaching the juvenile stage falls from the fish host to the bottom. The bottom sediment from each

operational tank was collected at regular intervals and examined under a microscope to record the presence of juveniles and their developmental stage.

The number of the juveniles present in each fish holding tank was assessed following the sampling method as described earlier.

Microphotographs were taken using a Leitz DMIL microscope of the glochidia, developing glochidia in the fish host and juvenile mussels after metamorphosis.

#### **PHYSICO-CHEMICAL PARAMETERS**

Important physico-chemical parameters such as temperature, pH, dissolve oxygen, total alkalinity, ammonical nitrogen ( $\text{NH}_4^+\text{-N}$ ), total hardness and calcium hardness of the water used for the rearing of brood mussels and host fishes were recorded following the standard methods (APHA, 1985).

## CHAPTER-IV

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# RESULTS AND DISCUSSION

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## RESULTS AND DISCUSSION

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### RESULTS

The freshwater mussel *Lamellidens marginalis* is commonly encountered in stagnant to feebly flowing freshwater bodies. The available information pertaining to reproduction, spawning and larval cycle of the species in the country is scanty. An attempt is made in the present investigations to elucidate mode of reproduction, spawning, larval cycle and juvenile production of the species.

The freshwater mussel *Lamellidens marginalis* is a prolific breeder and it spawns round the year with a peak spawning activity from July to September. During the present study it was observed that the testes of matured mussel is white to cream in colour while the ovary is dull red to conspicuously pinkish in colour. The fertilization was observed to be internal.

#### Structure of sperm and ovum of *Lamellidens marginalis*

The sperm of *Lamellidens marginalis* is not clearly divided into head, mid part and tail. Generally the sperm is elliptical with a rudimentary tail part (Plate-8).

The matured ovum is large compared to spermatocyte, round and filled with finely granular cytoplasm with avescicular nucleus. The fertilized eggs are agglutinated together to form plate like structures of

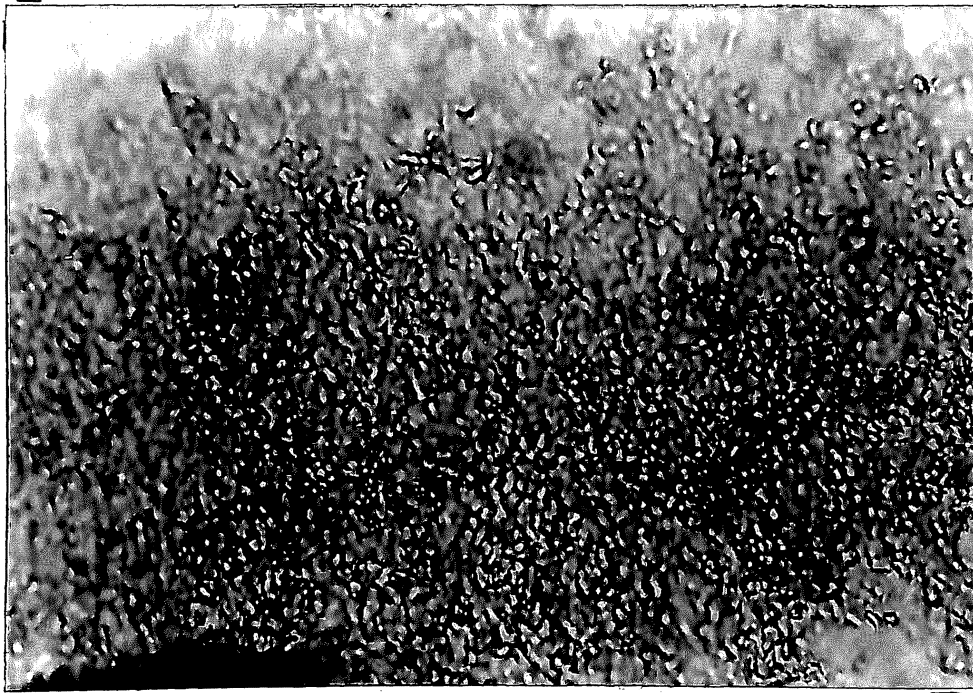


Plate VIII. Smear preparation of sperms aspirated from testes of the mussel.

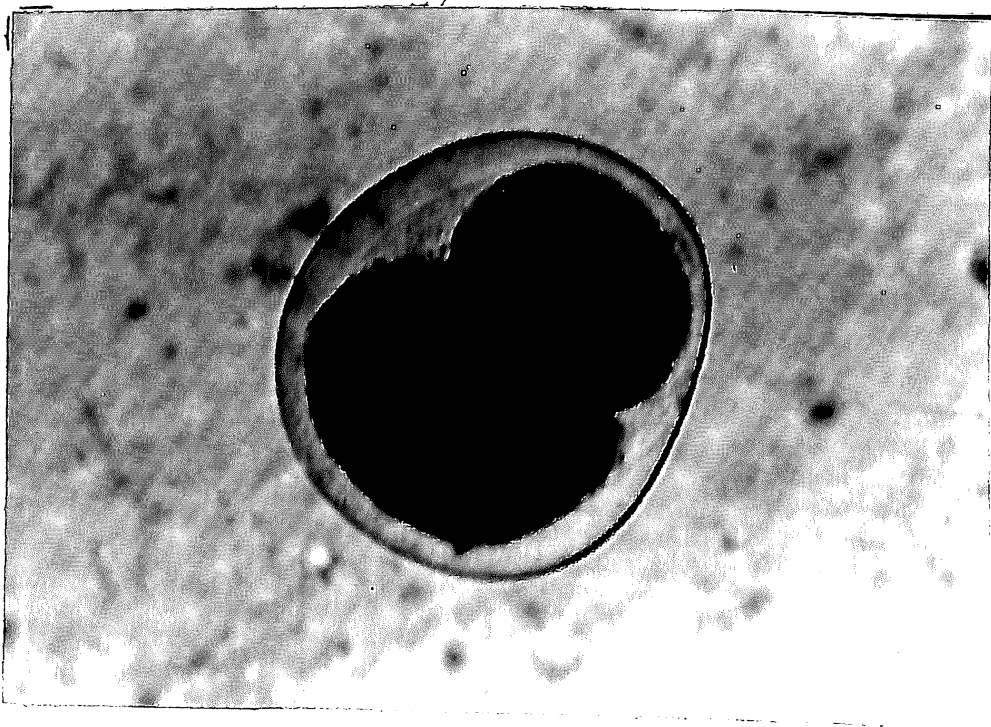


Plate IX. Embryonic development of the fertilized egg of the mussel.

various shapes and sizes and the eggs undergo further development and reach glochidial larval stage (Plate 9 and 10).

### **Glochidium larva**

The glochidium larvae are expelled into the ambient water from the gill marsupia of the spawning females through the exhalant siphon. Initially the glochidium larva is minute (0.22mm) and resembles the letter 'D' in shape with two shell valves (Plate-11). The shell valves are thin, brittle, brown and translucent with pitted appearance on the surface. On both the valves, tiny spines are present in the interior towards the ventral aspect of the shells. The larva is provided with a long convoluted tube. The larva is active in first 12 hrs. after hatching constantly opening and closing the shell valves. The larva is normally benthic in nature and the swimming movement appears to be very much restricted. The larva when comes in contact with fish host instantly clings to the peripheral margins of the fins by employing the spine like structures. In due course of time the larva enters into the subcutaneous space of the fin and gradually loses its 'D'-shape and becomes round. Finally on the 9th to 10th day, the larva becomes completely round and encapsulated (Plate-12) inside the fin tissue and are referred as "advance stage". The long convoluted tube penetrates into the nearby blood capillary and there by the larva derives nourishment from the fish host. It has been observed that the larva spends nearly 30-40 days for metamorphosis before dropping out from the fish host as a juvenile.

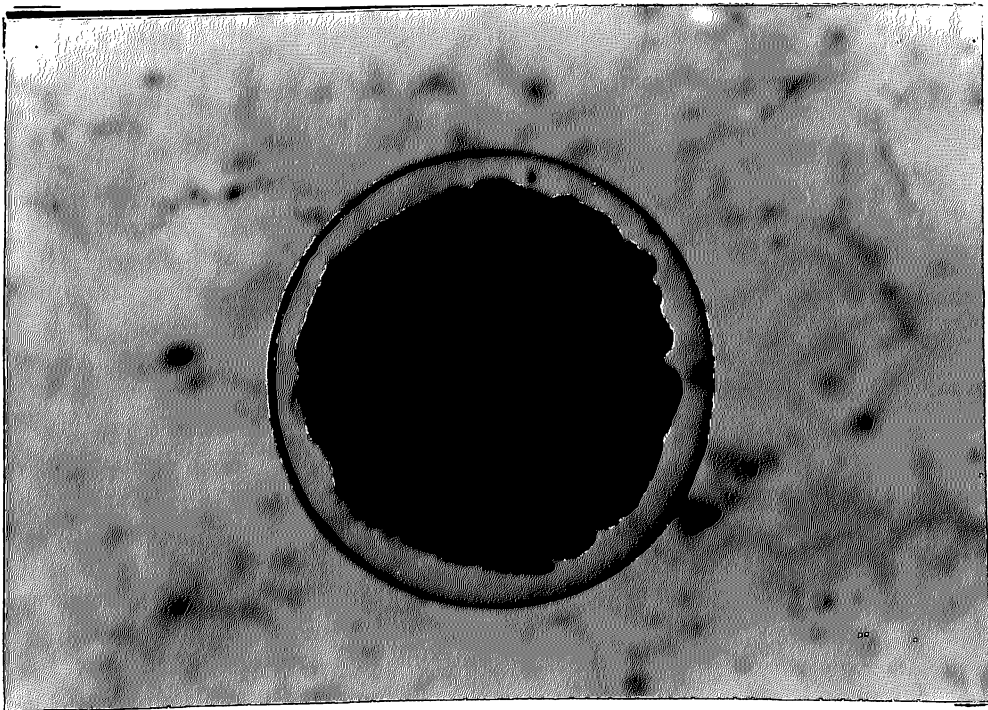


Plate X. The advanced stage of the egg of the mussel before hatching.

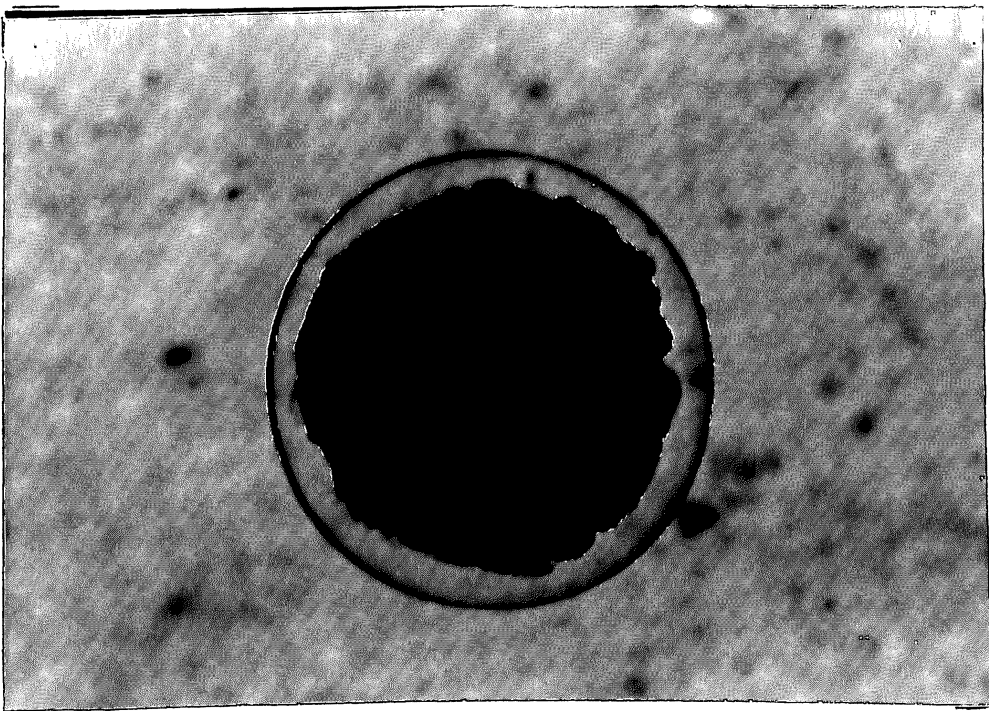


Plate X. The advanced stage of the egg of the mussel before hatching.



Plate XI. Glochidial larvae of the pearl mussel,  
*L. marginalis*.

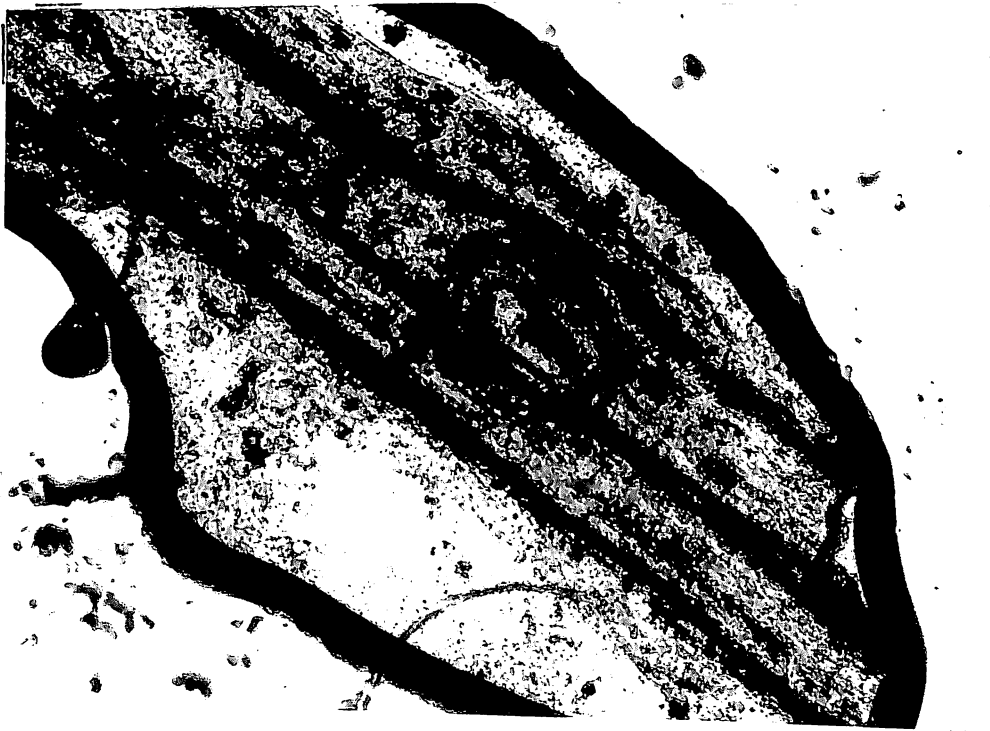


Plate XII. An encapsulated glochidium larva in  
the fin tissue of the fish host.

### Juvenile mussel

It has been observed that the juvenile mussel, a miniature adult in appearance and function drops out of the host on to the bottom of the experimental tanks and started growing further. The just metamorphosed juvenile mussel is small and the shells are comparatively thicker with greenish brown colouration. (Plate-13). The juvenile mussel is capable of restricted movements with periodic closing and opening of the two shell valves. The juvenile mussels were observed to readily accept the natural food like *Chlorella* for growth and for further development into the adult (Plate-14).

### Fish host-glochidial larval parasitic relationship

The experiments were aimed at finding the specificity, if any, between fish host and larvae of *Lamellidens marginalis*. A number of fish hosts like *Cirrhina mrigala*, *Labeo rohita*, *Glossogobius giurius*, *Catla catla*, *Hypophthalmichthys molitrix*, *Cyprinus carpio*, *Ctenopharyngodon idella* were screened. Individual species of the fish hosts were maintained separately in the experimental tanks @30 fishes/tank. Further, approximately equal number of glochidium larvae were released into each tank. During the experiment a total of 3250, number of glochidial larvae were released to the fish host (*C. mrigala*) tank whereas a total of 3200 number of glochidial larvae were released to the fish host (*L. rohita*) tank. In the case of *G. giurius*, the released rate of glochidial larvae was 3150 in the tank. A number of glochidial larvae, i.e. 3250, 3050, 3200 and 3100 were released into the other fish host species (*C. catla*,

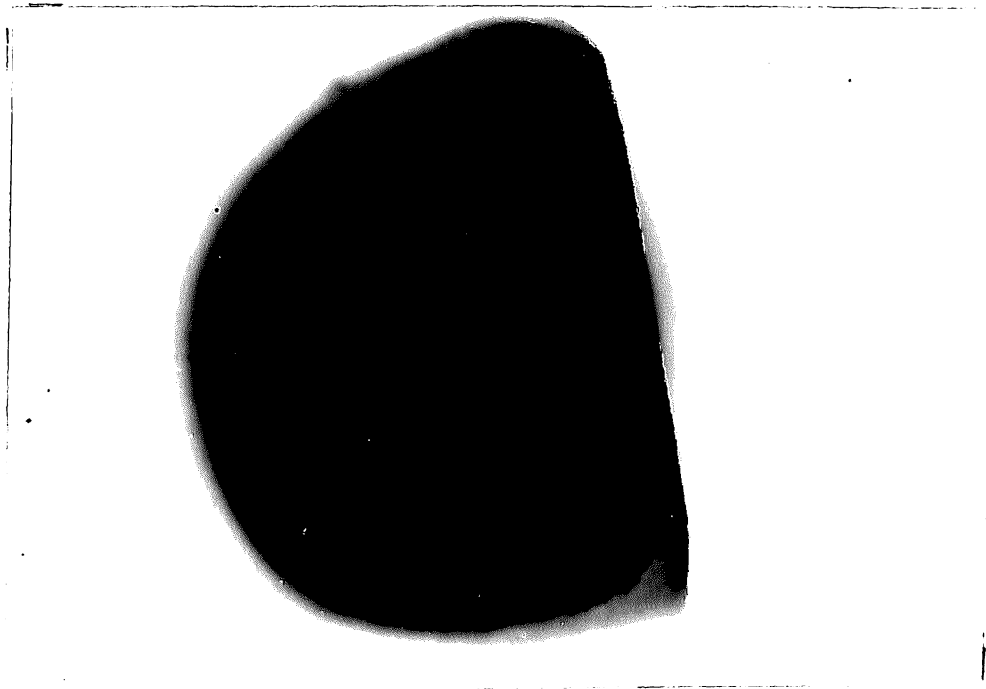


Plate XIII. Recently metamorphosed juvenile mussel.



Plate XIV. Grown up juvenile mussels under captive culture conditions.

*H. molitrix*, *C. carpio* and *C. idella*) tanks respectively during the period of May-August, 1996. The results of the experiment are given in the table-1. From the table it can be observed that, the average rate of infection of glochidial larvae to different fish hosts were as follows; in case of *C. mrigala* the average number of infection was 108, in *L. rohita* it was 106, in *G. giurius* the average infection rate was 105. In the case of *C. catla*, *H. molitrix*, *C. carpio*, *C. idella*, the average rate of glochidial larvae infection were, 108, 102, 107 and 103 respectively.

After noting the incidence of infection of glochidium larvae on the fish host species, some of the host species were randomly sacrificed to record the rate of infestation. The tissues such as skin, gill and different fins like dorsal, pectoral, pelvic, anal and caudal fins were examined minutely under the microscope to ascertain the suitable target organ for glochidial larval attachment. It was observed that, the skin and the gills were not the target organs for attachment of glochidial larvae in all the fish host species screened. The glochidial larval infestation was recorded only on the different fins of the experimental fish. It was observed that an average number of 22 glochidial larvae infested the host fish *C. mrigala*. In the case of *L. rohita* and *G. giurius*, the average glochidium infestation was 21 and 54 respectively, while an average of 25 glochidial larvae were infested on the fish host, *C. catla*. As far as the exotic carps are concerned it was observed that in *H. molitrix*, *C. idella* and *C. carpio*, the average rate of infestation were 9, 7 and 6 respectively.

It can also be observed from the table-2a - 2g that, *G. giurius* harboured maximum infection of the glochidial larvae followed by *C. catla* and *L. rohita*.

TABLE-1 Details of the experimental setup on fish host-mussel larval parasitic relationship.

Sl. No.	Fish host (*n=30)	Average size of the fish host (cm)	Date of release of mussel larvae	Total no. of mussel larvae released	Average no. of mussel larvae released
1	<i>C. mrigala</i>	13	1.5.96 to 15.5.96	3250	108
2	<i>L. rohita</i>	13	16.5.96 to 31.5.96	3200	106
3	<i>G. giurius</i>	9	1.6.96 to 15.6.96	3150	105
4	<i>C. catla</i>	14	17.6.96 to 30.6.96	3250	108
5	<i>H. molitrix</i>	14	1.7.96 to 15.7.96	3050	102
6	<i>C. carpio</i>	12.5	16.7.96 to 31.7.96	3200	107
7	<i>C. idella</i>	10	1.8.96 to 16.8.96	3100	103

\*n = number of fish hosts.

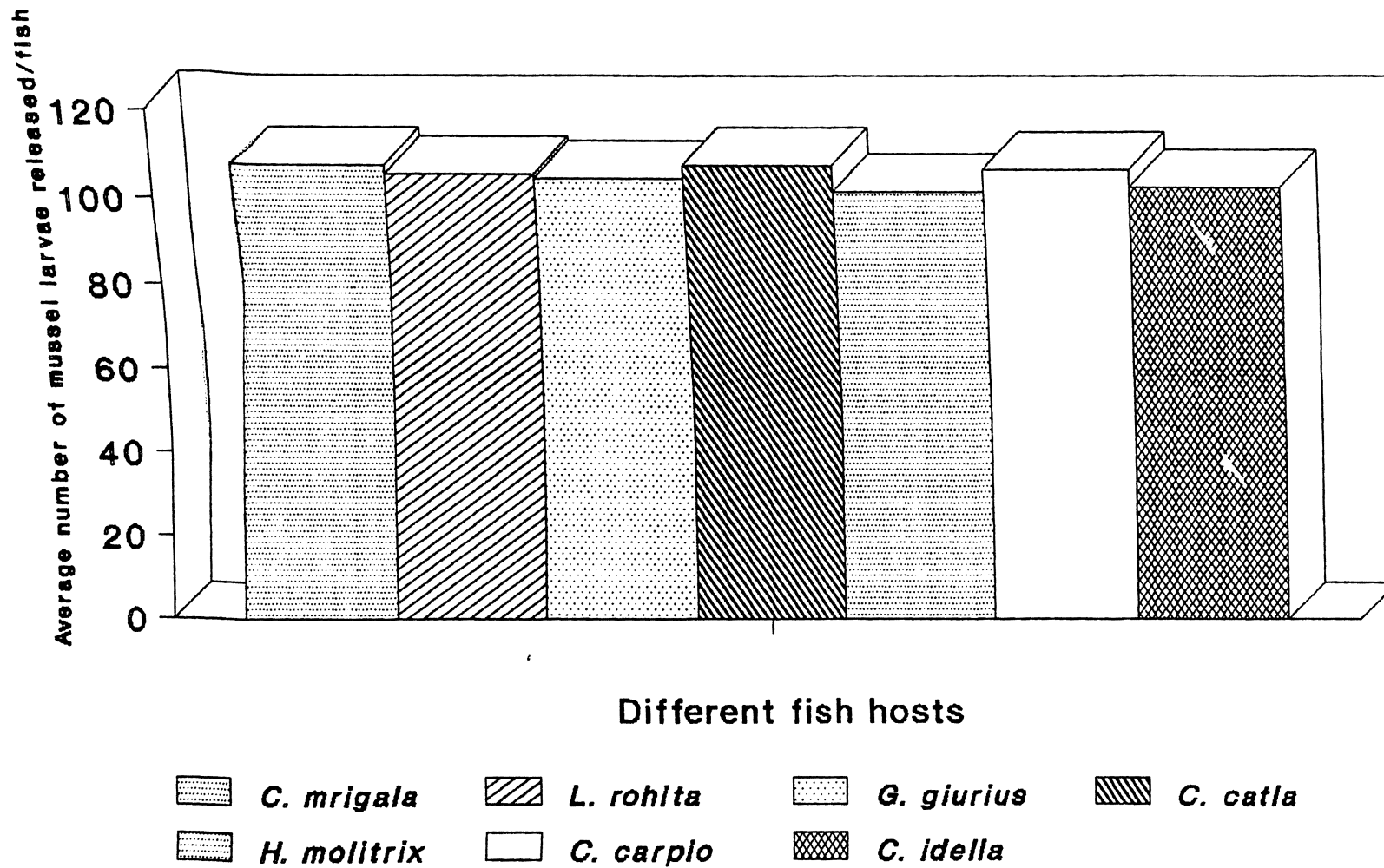


Fig.1 Average number of mussel larvae released to different fish host.

With respect to the suitable target organ for glochidial larvae attachment, it was observed that the glochidial larvae attaches it self to all the fins, however, more number of glochidium larvae were recorded on the pelvic, anal and caudal fins followed by pectoral fin in all the fish host species except *G. giurius*. In *G. giurius*, it was observed that, almost all the fins were highly infested with glochidial larvae (Table-2).

### Juvenile recovery

The number of juveniles recorded from the fish host tanks were; *G. giurius* -540 juveniles, *C. mrigala*- 270 juveniles, *L. rohita* - 230 juveniles followed by *C. catla* -220 juveniles. However, in the case of *H. molitrix*, *C. idella* and *C. carpio* the juvenile recovery was 110, 90 and 80 respectively. (Table -3). From the table it can be observed that, the average rate of juvenile recovery was 23, in *G. giurius*, in *C. mrigala* it was 12, in *L. rohita* the average juvenile recovery was 10. In the case of *Catla catla*, *H. molitrix*, *C. carpio*, *C. idella*, the average rate of juvenile recovery was 10, 5, 4 and 4 respectively.

From this experiment, it is clear that, among the different fish hosts tried, *G. giurius* proves to be the ideal fish host for production of maximum number of juveniles followed by *C. mrigala* and *L. rohita* (table-3)

TABLE-2a Details of glochidial infection on fish host (*C. mrigala*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (g)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	22.5.96	15.1	17.2	—	—	1	—	2	2	3	8	
2	29.5.96	14.8	16.8	—	—	3	4	5	6	4	22	
3	4.5.96	14.5	16.7	—	—	4	5	7	4	8	28	
4	11.5.96	13.9	14.2	—	—	2	3	5	5	4	19	22
5	18.5.96	14.7	16.6	—	—	2	3	9	5	8	27	
6	25.5.96	11.8	14.3	—	—	3	4	6	8	7	28	

TABLE-2b Details of glochidial infection on fish host (*L. rohita*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (g)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	7.6.96	12.7	14.7	—	—	1	3	6	8	4	22	
2	14.6.96	14.3	15.7	—	—	3	6	5	7	7	28	
3	21.6.96	12.4	13.7	—	—	1	3	4	6	4	18	
4	29.6.96	12.5	15.2	—	—	2	2	—	5	4	11	21
5	6.7.96	12.7	14.8	—	—	2	5	8	4	7	26	
6	15.7.96	11.4	12.8	—	—	1	6	7	3	4	21	

TABLE-2c Details of glochidial infection on fish host (*G. giurius*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (g)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	22.6.96	10.5	4.5	—	—	6	13	20	6	6	51	
2	29.6.96	7.5	2.6	—	—	5	10	26	7	10	58	
3	6.7.96	7.8	2.9	—	—	5	4	8	3	2	22	54
4	12.7.96	8.2	3.1	—	—	28	22	47	27	20	144	
5	19.7.96	8.1	3.2	—	—	5	4	2	2	2	15	
6	26.7.966	10.7	5.9	—	—	3	3	11	8	9	34	

TABLE-2d Details of glochidial infection on fish host (*C. catla*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (g)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	6.7.96	14.7	17.3	—	—	1	3	—	8	11	23	
2	12.7.96	15.2	18.7	—	—	—	—	5	7	12	24	
3	19.7.96	13.4	15.2	—	—	1	2	4	6	5	18	
4	26.7.96	13.5	15.7	—	—	3	4	6	2	13	28	25
5	2.8.96	15.8	19.3	—	—	2	1	12	1	5	21	
6	9.8.96	14.5	16.8	—	—	4	—	11	12	9	36	

TABLE-2e Details of glochidial infection on fish host (*H. molitrix*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (g)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	22.7.96	15.1	18.5	—	—	1	—	2	3	1	7	
2	30.7.96	14.8	17.3	—	—	—	2	1	—	5	8	
3	7.8.96	14.7	17.2	—	—	2	1	1	3	3	10	
4	14.8.96	13.8	16.9	—	—	1	—	3	2	5	11	9
5	21.8.96	13.5	16.2	—	—	—	1	—	1	3	5	
6	30.8.96	13.4	16.5	—	—	3	4	1	2	3	13	

TABLE-2f Details of glochidial infection on fish host (*C. carpio*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (g)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	7.8.96	12.3	14.7	—	—	1	1	—	1	1	4	
2	16.8.96	10.8	11.3	—	—	1	—	2	2	3	8	
3	23.8.96	14.5	15.8	—	—	—	—	3	1	2	6	
4	30.8.96	12.8	14.9	—	—	—	1	—	1	1	3	6
5	7.9.96	12.4	14.8	—	—	2	3	2	3	1	11	
6	13.9.96	13.2	15.1	—	—	—	—	2	2	—	4	

TABLE-2g Details of glochidial infection on fish host (*C. idella*) and the target organs.

Specimen No.	Date of observation	Length of fish (cm)	Weight of fish (gm)	Different target organs of the body							Total	Average infection
				Skin	Gill	Dorsal fin	Pectoral fin	Pelvic fin	Anal fin	Caudal fin		
1	23.8.96	10.2	11.3	—	—	—	1	3	4	4	12	
2	30.8.96	10.5	12.2	—	—	1	—	1	—	2	4	
3	7.9.96	9.8	10.7	—	—	—	1	1	1	—	3	
4	13.9.96	10.8	12.7	—	—	1	2	—	—	—	3	7
5	20.9.96	10.7	12.5	—	—	2	1	4	2	4	13	
6	27.9.96	10.3	12.2	—	—	1	—	3	2	1	7	

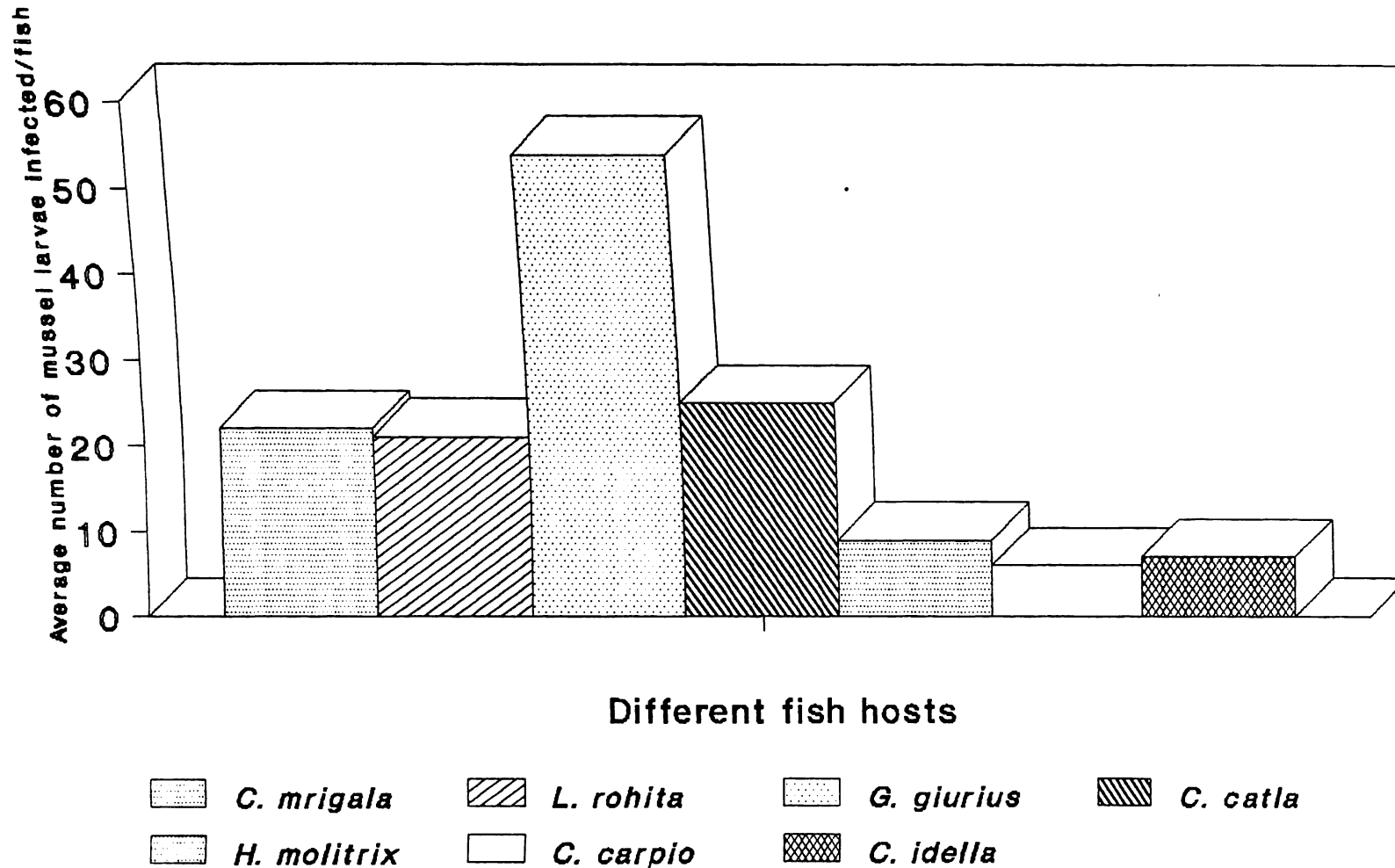
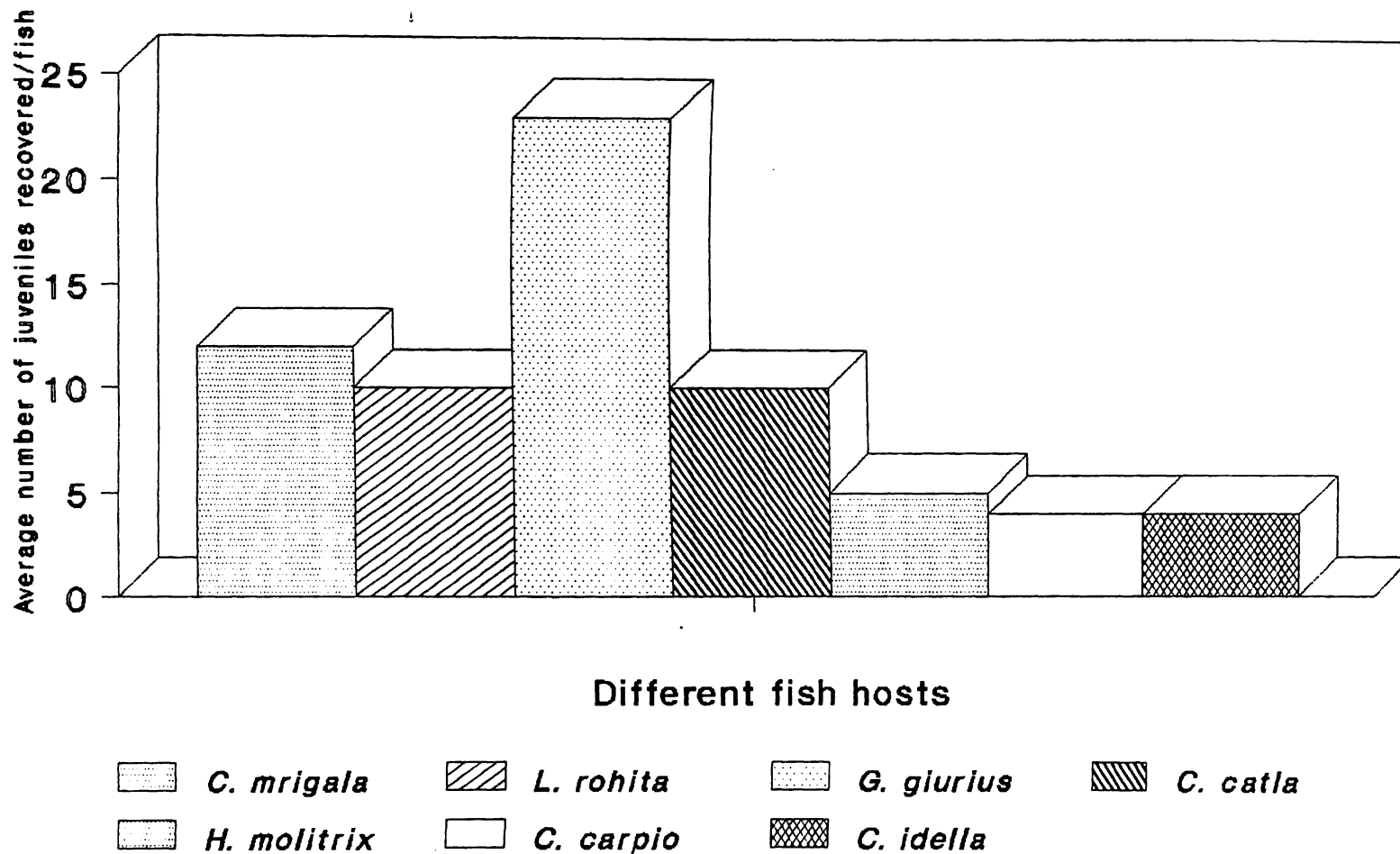


Fig.2 Average number of mussel larvae infected on different fish hosts.

TABLE-3 Results of the fish host-mussel larval parasitic relationship studies.

Sl. No.	Fish host species	Total no. of glochidium larvae released per fish	Av. no. of glochidium larvae released per fish	Av. no. of glochidium larvae infected	Total no. of juveniles recovered per fish	Av. no. of juveniles recovered
1	<i>C. mrigala</i>	3250	108	22	270	12
2	<i>L. rohita</i>	3200	106	21	230	10
3	<i>G. giurius</i>	3150	105	54	540	23
4	<i>C. catla</i>	3250	108	25	220	10
5	<i>H. molitrix</i>	3050	102	9	110	5
6	<i>C. carpio</i>	3200	106	6	80	4
7	<i>C. idella</i>	3100	103	7	90	4



**Fig.3 Average number of juveniles recovered from different fish hosts.**

It could be observed from the table-3 that though the initial number of infestations by the larvae were more, the progression to advanced larval stage and juvenile stages were by far limited. This may be attributed to the failure of glochidium larvae to penetrate the fish host's fin tissue due to nonspecific nature of the fish host.

The present preliminary experiments have also indicated that the duration of larval metamorphosis was different with respect to different fish hosts tried. However, further detailed studies are required to establish the host related factors influencing the period of mussel larval metamorphosis.

The physico-chemical parameters of the water in the fish host and mussels spawning tanks are provided in the table-4.

**TABLE-4:** Physico-chemical parameters of water in the experimental tanks.

Parameters	Range	Average
Temperature	28°C-31°C	29°C
pH	6.8-7.5	7.15
Dissolved oxygen(ppm)	5.4-7.6	6.5
Total alkalinity (ppm)	134-160	147
Total hardness (ppm)	58-70	64
Calcium hardness(ppm)	16.032-24.048	20.04
Ammonical-nitrogen(ppm)	0.2432-0.4630	0.3531

## DISCUSSION

Success in seed production of the freshwater mussels largely depend on a number of intrinsic and extrinsic factors. This assumes critical significance due to the fact that the mussels require a mandatory glochidial larval stage on certain host species. The life history stages of the freshwater mussel *Lamellidens marginalis* passes through various critical stages including the development of glochidial larva and its host specificity to become a juvenile to adulthood. The information available on the biological aspects like reproduction, spawning and larval development, juvenile production are rather scanty in the country. The species *Lamellidens marginalis* is though unisexual, the secondary sexual characters are not clearly defined (Kotpal, 1995).

It has been reported that, the sexes are separate in the Indian freshwater mussel *Lamellidens marginalis* (Thomas, 1974). The microscopic analysis of the gonad of 95 specimen of *Anodonta cygnea* recorded that 18 were hermaphrodites. It is also recorded that some individuals of *Lamellidens thwaitesii* are hermaphroditic in nature (Bloomer, 1930). In the present investigations sexual dimorphism has not been recorded in the species *Lamellidens marginalis*.

It was reported earlier that the peak spawning season of *Lamellidens marginalis* is during August to September (Ghosh and Ghosh, 1972). It was recorded that the species *Hyriopsis shlegelli*

spawns from April to August (Kafuku & Ikenoue, 1983). However, in wash board mussel, *Melalonias nervosa* the peak spawning activity occurs during August to October (Woody & Holland, 1993). The present investigations are though short term in nature (May to October, 96), the preliminary studies on the maturation and the spawning activity of the *Lamellidens marginalis* indicated that the peak spawning season probably correspond with the monsoon season (July-September).

It was reported that the size of glochidia of freshwater mussels in general ranges between 0.1 and 0.5 mm (Kotpal, 1995). It was also reported that the larval size of the *H. schlegelii* is 0.3 mm (Kafuku and Ikenoue, 1983). In the current studies the size of the glochidium larva of *Lamellidens marginalis* was observed to be 0.22mm.

It was observed that, the glochidium larvae of *Lamellidens marginalis* take about 10 weeks for larval metamorphosis to reach juvenile mussel stage (Kotpal, 1995). However, in the present investigation it has been observed that, the larva takes 30-40 days of duration in the appropriate fish host to reach the juvenile mussel stage. The difference in duration of larval life cycle in different fish hosts as observed in the present studies needs further investigations.

After the metamorphosis, the juveniles falls from the fin tissue to bottom and starts feeding from external sources. (Kotpal, 1995). Similar

observations have been made in the present study i.e. the juvenile mussels fed with *Chlorella* have registered good growth.

It has been stated that, when glochidium larvae come in contact with proper host it clings to the skin, gill and fins by closing their hooked valves (Thomas, 1974). It was also reported that in the case of *H. schlegelli*, the glochidium larvae clamp to the gill filaments of the fish being brought through the respiratory water currents (Kafuku and Ikenoue, 1983). In the present studies, infection of glochidial larvae in the gills and skin of the fish host were not recorded while the glochidia could only be seen on the different fins of the fish host. It has been observed that the glochidial larvae do possess certain specificity for infection on fins such as pelvic, anal and caudal fins. The reason for this selective infection on the fins could be that pelvic, anal and caudal fins normally come in contact with the sediment on which the benthic glochidial larvae are dispersed.

Some species of mussels require certain species of fish as a specific host while in some mussel species larvae, several kinds of fish will serve as a hosts (Kotpal, 1995).

It was earlier reported that three fish species, Green sunfish (*Lepomis cyanellus*), Black bullhead (*Ictalurus melas*) and channel catfish (*Ictalurus punctatus*) as fish hosts for the glochidial stage of washboard mussel *Megaloniais nervosa* in host specificity studies (Woody & Holland, 1993). It

was mentioned that mussel larva requires 26-28 days for larval metamorphosis at 17°C. In another trial it was shown that the glochidia requires 56 days for larval metamorphosis in channel cat fish, *Ictalurus punctatus*. White suckers (*Catostomus commersoni*) and yellow perch (*Perca flavescens*) retained glochidia upto 23 to 26 days, however no juveniles were produced (Woody & Holland, 1993). In the present host specificity study, seven fish species such as *Cirrhina mrigala*, *Labeo rohita*, *Glossogobius giurius*, *Catla catla*, *Hypophthalmichthys molitrix*, *Cyprinus carpio*, *Ctenopharyngodon idella* were screened for the infection of the glochidial larvae of *Lamellidens marginalis*.

In the present investigation it is recorded that, *Lamellidens marginalis* larvae probably have some selection to specific fish hosts and reached the juvenile stages. During the present experiment, a maximum glochidial larva attachment of 144 on the host species *Glossogobius giurius* of the size of 8.2cm in length was recorded. It has been also observed that heavy infection of the glochidial larvae is injurious to hosts. Therefore, size of the fish host vis-a-vis the density of glochidial infection assumes significance in the future mussel breeding and seed production technology development. In the present study it has been observed that the fins of the fish *Glossogobius giurius* were infected by glochidial larvae at an average of 54 per fish and after a duration of 30-40 days an average number of 23 juveniles per fish were recovered at 28-31°C. In a similar way, the *Cirrhina mrigala* were infected at an average of 22 per fish

and produced 12 numbers of juveniles. In case of the fish host *Labeo rohita*, the average larval infection rate was 21 per fish and the juvenile recovery was 10 per fish host. Again, the fish *Catla catla* were infected by glochidial larvae at an average of 25 per fish produced 10 number of juvenile mussels. The average infection rate in case of *Hypophthalmichthys molitrix* was 9 per fish which produced an average of 5 juveniles per fish. *Cyprinus carpio* was infected with an average of 6 glochidial larvae per fish and an average 4 juveniles per fish were recovered. Similarly, the fins of the fish *Ctenopharyngodon idella* were infected by glochidial larvae at an average of 7 per fish and after a duration of 30-40 days an average of 4 juveniles were produced at 28-31°C.

From the present investigation, it may be concluded that among all fish species screened for host specificity study, *Glossogobius giuris* was found to be the ideal fish host followed by *Cirrhina mrigala* and *Labeo rohita* respectively. These studies have tremendous application value in commercial mussel seed production technology not only to cater to the needs of the pearl industry for pearl seed mussels but also in rehabilitation of endangered freshwater mussel populations in the natural environment.

## **CHAPTER-V**

# **SUMMARY**

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## SUMMARY

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Success of seed production of the freshwater pearl mussels mainly depends upon a number of intrinsic and extrinsic factors. The mussels require a mandatory glochidial larval stage on certain host species to reach the juvenile stage.

The fertilised eggs of the freshwater mussels *Lamellidens marginalis* (L.) undergoes several embryological developments to reach the glochidial larval stage. At initial stages the glochidium larvae are 'D' shaped, minute with thin, brittle, brown and translucent shell valves having pitted appearance on the surface. The larvae thus produced are benthic in nature with restricted swimming movement and are parasitic in nature. In the presence of suitable host, the larvae immediately attaches to the fins of the host fish species with the help of convoluted tube. In due course of time, the larvae enters into the subcutaneous space of the fin, loses its original 'D' shape, becomes round and finally becomes encysted within 9 to 10th day of development from the fertilised eggs stage. As the larvae are parasitic in nature during nourishment from the host fish and after spending really 30 to 40 days, the larvae drops out from the host as juveniles and grows further to adult stage within two years.

In order to have an concise idea about the fish host - glochidial larval parasitic relationship, a number of fish hosts like

*Cirrhina mrigala*, *Labeo rohita*, *Glossogobius giurius*, *Catla catla*, *Hypophthalmichthys molitrix*, *Cyprinus carpio*, *Ctenopharyngodon idella* were exposed to approximately equal numbers of glochidium larvae to study the rate of further infection.

After 7 days, the infected host fish specs were randomly selected and sacrificed to record the rate of infestation in tissues such as skin, gill and different finsh like dorsal, pectoral, pelvic, anal and candel finsh. The observation are made under microscope to find out the suitable target organ for glochidial larval attachment.

It was observed that, an average number of 22, 21, 54, 25, 9,7 and 6 glochidial larvae were infested to the fish hosts *C. mrigala*, *L. rohita*, *G. guirius*, *C. catla*, *H. molitrix*, *C. idella* and *C. caprio* respectively. It was further observed that, the skin and the gills were not the target organs for attachment of glochidial larvae in the above mentioned fish host species, whereas the glochidial larval infestation was recorded only on the different fins of the fish host specious under investigation.

It was further observed that, *G. giurius* harboured maximum number of infection of the glochidial larvae followed by *C. catla* and *L. rohita*. As far as the suitable target organ for glochidial larval are concerned, it was observed that, more number of glochidicum larval were recorded on the pelvic, anal and caudal fin followed by pectoral fin in all the fish host species tried with an

exception to the fish host species *G. giurius* in which all the fins were heavily infested with glochidium larvae.

With regards to the average rate of juvenile recovery from different fish host species, it was observed that, maximum number of juveniles were recorded from *G. giurius* (23. nos) and was ideal fish host species followed by *C. mrigala* ( 12. nos) *L. rohita* (10 nos) and *C. catla* (10 nos), whereas the exotic fish host species tried yielded very low juvenile recovery.

## CHAPTER-VI

# CONCLUSION

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## CONCLUSION

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It is evident from the present investigation carried out that, the glochidial larvae of the freshwater pearl mussel have a mandatory parasitic existence. Results of the present investigation suggests that, *G. giurius* proves to be the best suited fish host species for the attachment, infection and recovery of the glochidial larvae and juveniles followed by *C. mrigala* and *L. rohita*. These observations have tremendous applied value in developing natural spawning and seed production techniques for the freshwater pearl mussel *Lamillidens marginalis* for burgeoning pearl culture industry.

**CHAPTER-VII**

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