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UTILIZATION OF BIVALVE TROCHOPHORES AS LIVE FEED TO LARVAE OF LITOPENAEUS VANNAMEI, PENAEUS MONODON, MACROBRACHIUM ROSENBERGII AND LATES CALCARIFER

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ABSTRACT

The four bivalves, *Perna viridis*, *Crassostrea madrasensis*, *Anadara granosa* and *Meretrix casta* were collected from Kakinada Bay during January 2014 - December 2015. These bivalves were used for mass scale production of trochophores by induced breeding, which were used as live feed to the larval forms of fish and shrimp. The success rate of induced breeding technique was achieved by employing thermal stimulation (42.87%), salinity shock (30.77%) and stripping method (66.67%). Average fecundity estimated was 5.6, 6.4, 2.8 and 2.6 millions in *P. viridis*, *C. madrasensis*, *A. granosa* and *M. casta* respectively. The rate of trochophore development was 66% in *P. viridis*, 65% in *C. madrasensis*, 57% in *A. granosa* and 54% in *M. casta*. The rate of feeding in post larvae (PL fed with trochophore of bivalves) of *L. vannamei*, *P. monodon*, *M. rosenbergii* and *L. calcarifer* ranged between 40.0% and 90.0%. The survival rate ranged between 40.0% and 85.0% in post larvae of *L. vannamei*, *P. monodon*, *M. rosenbergii* and *L. calcarifer* fed with trochophores of four bivalves during experimental conditions.

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INTRODUCTION

India is endowed with rich and diverse bio-resources and the molluscs are not an exception. Molluscs are a heterogeneous group of animals both in shape and diversity and are represented by polyplacophores, gastropods, bivalves, cephalopods and scaphopods. Most of the molluscs inhabit the marine environment and very few dwell in the terrestrial and freshwater habitats. About 8,000-10,000 species of molluscs were recorded from the world waters and a total of 3,271 species are reported from India (Subba Rao, 1991). They are represented in 220 families and 591 genera and the spectrum comprises 190 gastropods, 1,100 bivalves, 210 cephalopods, 41 polyplacophores and 20 scaphopods. Molluscs were exploited for edible, industrial and ornamental purposes and the history of exploitation way back to the time immemorial. About 28 species of bivalves are exploited at present in India. About 70,705 tones of bivalves were landed in Kakinada Bay from 1996-2000 (Appukuttan *et al.*, 2002). The aquaculture industry has expanded very fast in India and the basic need is live food organisms particularly for the hatcheries of fish and prawn. Besides microalgae, live zooplankton feed are also equally important to meet the demand at present in hatcheries. *Artemia*

nauplii are widely using as live zooplankton feed in hatcheries in India. *Artemia* cysts importing from other countries and it is more expensive. In order to avoid the huge amount of expenditure towards procurement of *Artemia* cysts from foreign countries, bivalve's larvae serve as one of the alternate live feed to hatcheries for development of fish and prawn larvae.

Trochophore-stage of bivalve larvae have frequently been used as a live first feed in marine fish larviculture, particularly with species whose larvae are very small or have small mouths. At approximately 50-70 µm, the bivalve trochophore would appear to be an ideal first prey item and movement in the water column is sufficient to attract the attention but not so rapid as to make the trochophore impossible for young larvae to catch. Nutritionally, trochophores present an excellent HUFA profile (Whyte, 1992).

The south-east Asians are widely using bivalve larvae as live feed for rearing of larvae of economically important fishes like milk fish, mullet, yellow tail, groupers, sea breams, tilapia etc. in their hatcheries (Pillay, 1990). According to literature survey rearing of larvae of Asian sea bass, tiger shrimp, Pacific white shrimp and fresh water prawn by feeding bivalves trochophores

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is meager. The present work was an attempt to mass scale production and utilization of bivalve (*Perna viridis*; *Crassostrea madrasensis*; *Anadara granosa* and *Meretrix casta*) trochophores as live feed to larvae of economically important fish and prawn.

MATERIALS AND METHODS

The samples were collected from Kakinada Bay, near Chollangi village in the stretches of Coringa estuary, East coast of India during January 2014 - December 2015. Geographically, Coringa estuary extends from Kakinada in the north to the Gautami Godavari River in the south. It is located between 16°-30' to 17°-00' N latitudes and 82°-14' to 82°-23'E longitudes (Plate 1). Kakinada Coringa estuary was situated in Andhra Pradesh, India. It is the second largest stretch of Mangrove forests in India with 35 species belonging to twenty four families and more than 120 bird species. It is home to the critically endangered white backed vulture and the long billed vulture.

Bivalves (*P. viridis*; *C. madrasensis*; *A. granosa* and *M. casta*) were collected randomly on every month from study area by hand picking at low tide time (Approximately 1 m depth) by using motor boat. The bivalves were packed in wet gunny bags and then brought to the laboratory as early as possible. After proper washing and brushing the mud, epifauna and flora from the animals, transferred to brood stock tank. Feed was given to the bivalves after 24 hours of acclimatization to laboratory conditions. This study was undertaken in the Department of Marine Living Resources and Ocean, Atmospheric Science and Technology Cell (OASTC) laboratories, Andhra University, Visakhapatnam, India.

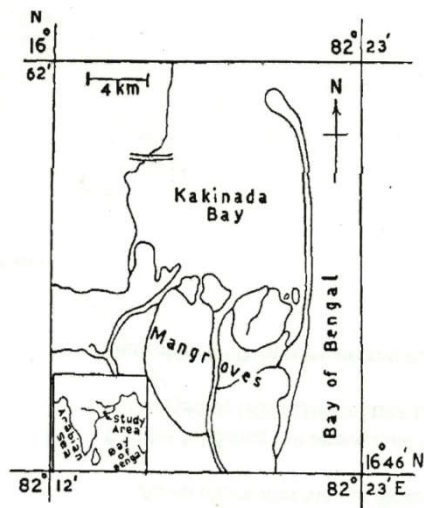


Plate 1 Study area

Physical and chemical parameters in brood stock tank

Physical and chemical parameters i.e. Temperature, Salinity, pH and Dissolved Oxygen (DO) were recorded daily. Temperature was recorded by thermometer, Salinity recorded by Refractometer (Salinometer), P^H recorded by Elico Digital P^H meter, DO recorded by C.P Aqua India D.O Kit. The physico-chemical parameters in the brood stock tank maintained as follows: temperature ranged from 25°C-32°C. Salinity ranged from 9 to 20 ppt. pH ranged from 7.9-8.7. DO ranges from 4.5 to 6mg/l.

Algal feeding to brood stock

The two species *Chaetoceros calcitrans* and *Isochrysis galbana* were selected for algal culture. The algae was cultured in autoclaved sea water enriched with Conway medium (Walne, 1970) and F2 medium (Guillard, 1975) separately. The algal culture was harvested at Exponential phase (Growth phase) for feeding. It was used as live feed to the brood stock of bivalves. The cells of *Isochrysis galbana* harvested ranged from 3.5 to 4.2 million cells with an average 3.84 million cells/ml. The cells of *Chaetoceros calcitrans* harvested ranged from 3.1 to 4.3 million cells/ml with an average of 3.73 million cells/ml during study period. About 20 l (two equal halves) of these algae culture was given as daily feed to each FRP tank (100 l) containing bivalves brood stock.

Induced spawning techniques

P. viridis in the size range of 5.0-15.0 cm and weight 18-225 g; *C. madrasensis* in the size range of 6.0-20.2 cm and weight 40-750 g; *M. casta* in the size range of 3.0-5.8 cm and weight 17-80 g; *A. granosa* in the size range of 3-5.9 cm and weight 25-102 g were selected for induced breeding.

Thermal shock

For thermal shock of induced spawning, the spawning tank was filled with sea water at salinity 18-20 ppt and room temperature at 23-25°C. Condition the organisms for about one to two weeks. Then the temperature of the sea water was first raised to 35°C with heated sea water and maintained for 45 minutes. After heat treatment, cooled sea water was used to cool down the temperature of sea water to 25°C for a period of 30-45 minutes before another heat treatment process begins. The thermal shock procedure was repeated until the first animal starts spawning, followed by Stephen and Shetty, (1981); CMFRI, (1982); Alfaro *et al.*, (2010); Nur Leena and Aziz, (2013).

Salinity shock

Induced breeding achieved by changing of salinity. For this method, bivalves were maintained at salinity of 30 ppt for about 2 months then decreasing the salinity to 24 ppt. sudden salinity change leads to the stimulus of mature specimens to spawning. The technique was adopted for all four species, followed by Stephen and Shetty (1981).

Stripping method

The ripe eggs and sperms were squeezed carefully from the ripe gonads into shallow jar and mixed together for fertilization. This method of fertilization in bivalves was achieved successfully in the present study following the technique adopted by Nelson *et al.*, (2006).

Fecundity and larval counting

Fecundity was based on number of fertilized eggs of individual species present in spawning tank. The fertilized eggs were passed through an 80 µ mesh screen to remove excess debris. These eggs were diluted to 10 l of sea water. After proper agitation of the eggs, 1 ml sample is taken and further diluted to 100 ml. This sub sample is agitated and 1 ml sample was taken and placed in a small dish. The eggs in this sample were counted using a microscope. Two or more samples are counted

and averaged. The average count was multiplied by 1×10^6 to obtain total number of eggs (Wilbur and Robert, 1975). Bivalves larvae were also counted by this method, but the second dilution may not be necessary.

Embryonic development

The fertilized eggs were immediately transferred to 500 l rectangular FRP tanks containing 250 l filtered sea water and subsequent embryonic developments were monitored periodically. The embryonic developmental stages were photographed using an optical microscope (Olympus-CH20i) at 40x magnification.

Rearing of trochophore

Mass scale rearing of trochophore of *P. viridis* and *C. madrasensis* (60-70µ) & *M. casta* and *A. granosa* (40-60 µ) was undertaken in filtered sea water at suitable environmental conditions i.e. Temperature: 27°C, Salinity: 20-25 ppt, pH: 8 and DO: 5-6 mg/l. These trochophores were used as live feed to larvae of fish and prawns.

Shrimp/Prawn/Fish larval sampling

Post larvae (1-3 days old) of *Litopenaeus vannamei* were brought from BMR hatchery, Uppada, Visakhapatnam. Post larvae (1-3 days old) of *Penaeus monodon* were brought from JK hatchery, Kakinada. Three days old larvae of *Macrobrachium rosenbergii* were brought from CIFA, Bhubaneswar, Odisha. Three to four days old larvae of *Lates calcarifer* were brought from RGCA, Chennai. Larvae were packed in aerated polyethylene bags maintained at proper salinity (27-28ppt) and temperature (25-27°C) at the rate of 500 no's per bag (2 l capacity).

Feeding and survival rate

Feeding rate was calculated by feeding activity and percentage of shrimp/fish larvae with full gut. Survival rate was calculated by percentage difference between initial density before feeding and density after 3 days feeding.

Post larvae (PL1-3) of *L. vannamei* and *P. monodon*, 3 days old larvae of *M. rosenbergii* and 3-4 days old larvae of *L. calcarifer* were brought to the laboratory and maintained in two separate tanks (each 50 l capacity) at the rate of 500 no's of PL (post larva) per tank. These experiments were undertaken on all four bivalves. Same water quality parameters were maintained in the laboratory as in the case of trochophore larval rearing, but the salinity in the larval tank of *L. calcarifer* maintained at 30 ppt. The PL's were starved for 1 h and then they were fed with trochophores at the rate of 30-50 no./ml for 3 times daily. In the other tank, artemia nauplii were added at the rate of 3 no./ml (because of large size) as control feed. After 2 hours of each feeding, the gut of PL was observed under the microscope for gut fullness.

RESULTS

Induced spawning technique

The success rate of induced spawning by thermal stimulation was high (42.87%) in *M. casta* followed by *A. granosa*, *C. madrasensis* and *P. viridis*. The success rate of induced spawning by salinity shock was high (30.77%) in *A. granosa* followed by *M. casta*, *P. viridis* and *C. madrasensis*. The

success rate of induced spawning by stripping method was high (66.67%) in *P. viridis* followed by *C. madrasensis*, *A. granosa* and *M. casta*. Among the three experiments, the highest success rate of induced spawning was achieved in four bivalves by stripping method followed by thermal stimulation (Table 1).

Table 1 Percentage success of induced spawning in four bivalves.

Species	Thermal stimulation	Salinity shock	Stripping method	Natural
<i>P. viridis</i>	22.22	11.11	66.67	-
<i>C. madrasensis</i>	27.27	9.09	54.55	9.09
<i>A. granosa</i>	30.77	30.77	38.46	-
<i>M. casta</i>	42.87	14.28	35.71	7.14

Fecundity (includes all experiments)

The average fecundity estimated was 5.6, 6.4, 2.8 and 2.6 millions in *P. viridis*, *C. madrasensis*, *A. granosa* and *M. casta* respectively (Table 2).

Table 2 Average fecundity in four marine bivalves

Species Name	Average fecundity/animal (millions)	Average no. of trochophore developed (millions)	Rate of trochophore developed (%)
<i>P. viridis</i>	5.6	3.7	66
<i>C. madrasensis</i>	6.4	4.0	65
<i>A. granosa</i>	2.8	1.6	57
<i>M. casta</i>	2.6	1.4	54

Embryonic Development

After 20 minutes of post fertilization, the first polar body started developing. Fertilized eggs have undergone first cleavage within 45 minutes. Rapid cell division occurred within 4 hours after fertilization. The trochophore larva appeared after 6-7 hours of fertilization in four bivalves (Plates 2-5).

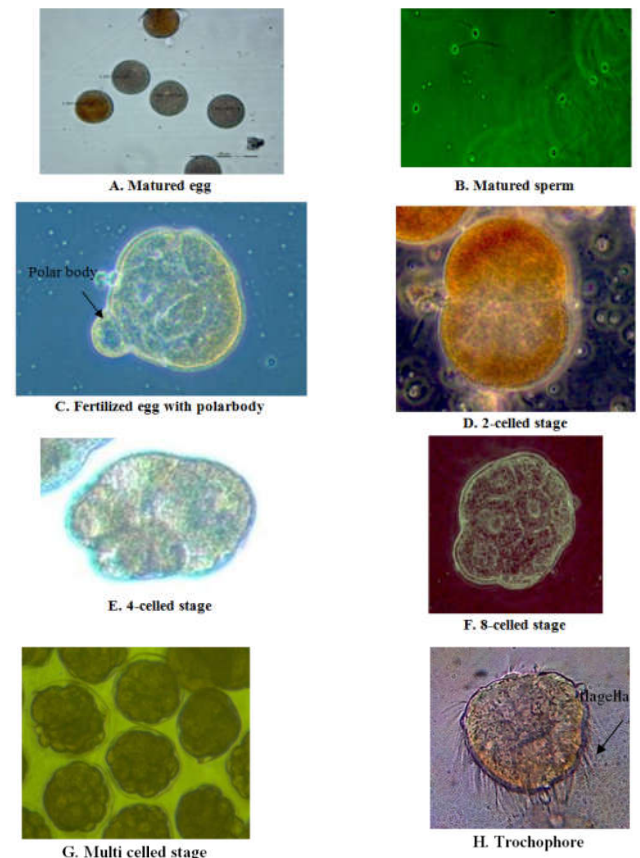


Plate 2 Embryonic development of *Perna viridis*

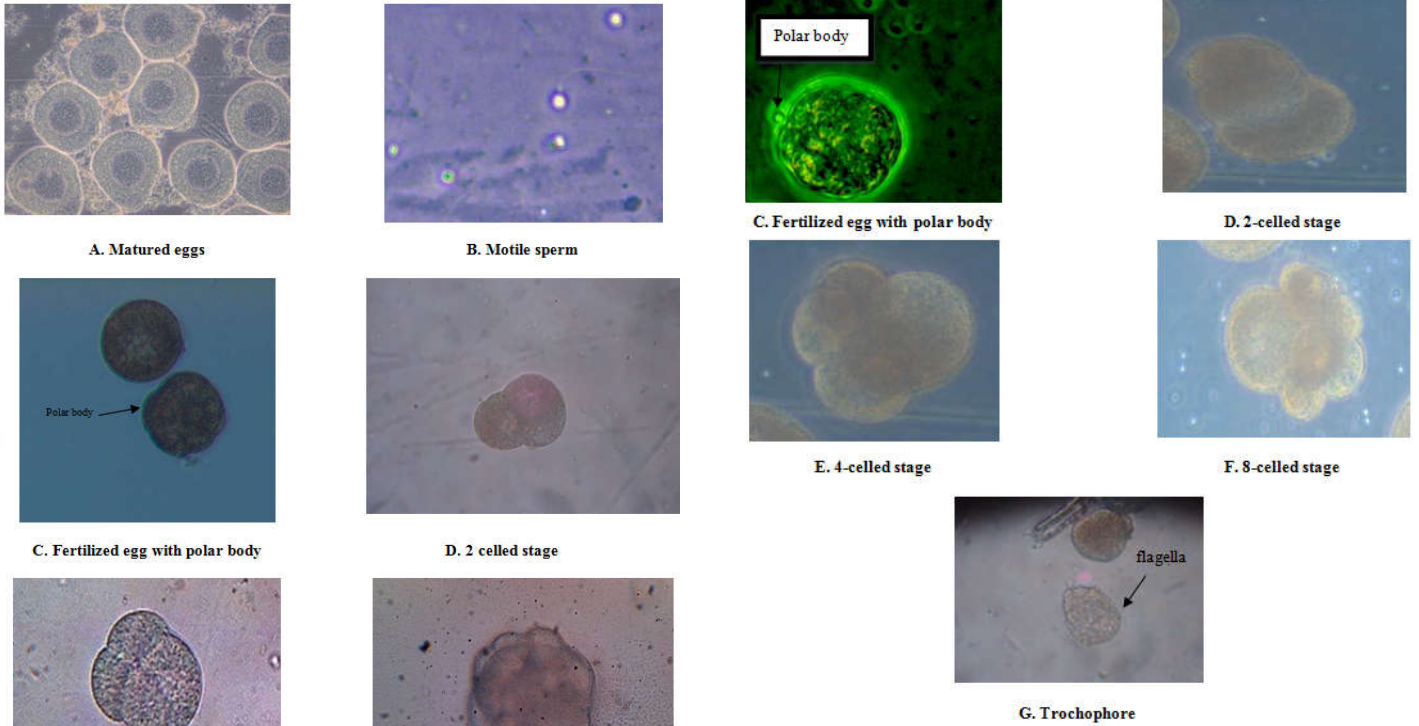


Plate 4 Embryonic development of *Anadara granosa*

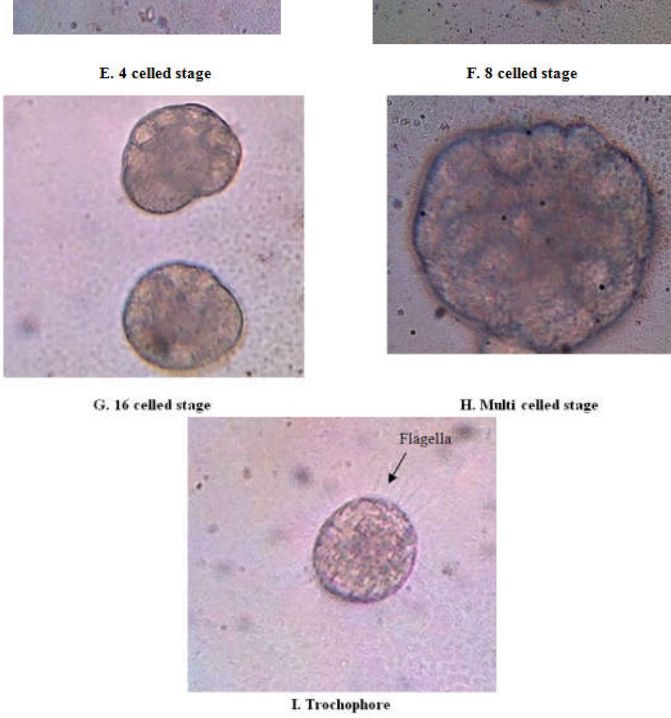


Plate 3 Embryonic development of *Crassostrea mandrasensis*

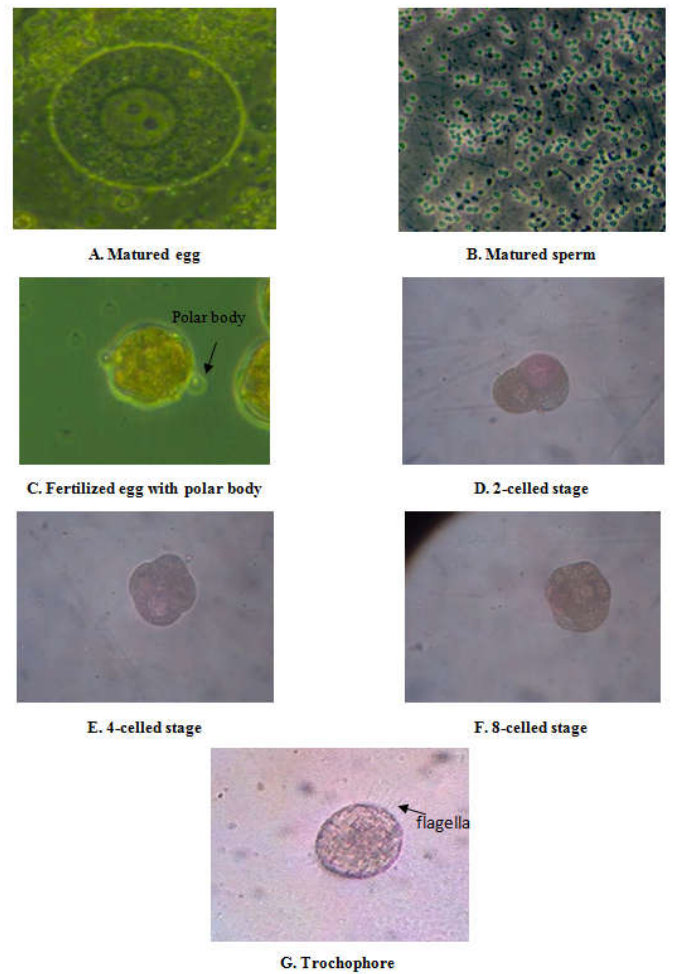
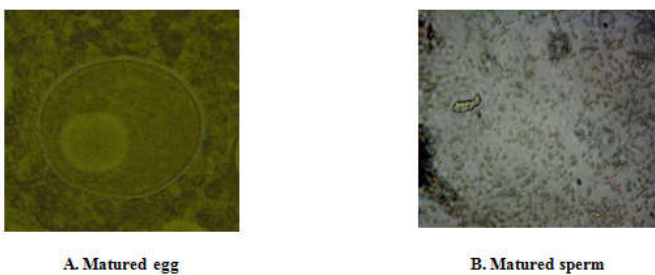


Plate 5 Embryonic development of *Meretrix casta*

Rate of trochophore development

The average rate of trochophore development recorded was 66% in *P. viridis*, 65% in *C. madrasensis*, 57% in *A. granosa* and 54% in *M. casta* (Table 2).

Feeding and survival rate

Litopenaeus vannamei

The rate of feeding observed was 80%, 90%, 60% and 60% in *L. vannamei* PL fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively, where as 90% of feeding observed in *L. vannamei* PL fed with *Artemia* nauplius. The rate of survival noticed was 80%, 85%, 50% and 50% in *L. vannamei* PL fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively, where as 85% of survival reported in *L. vannamei* PL fed with *Artemia* nauplius (Figure 1, Plate 6).

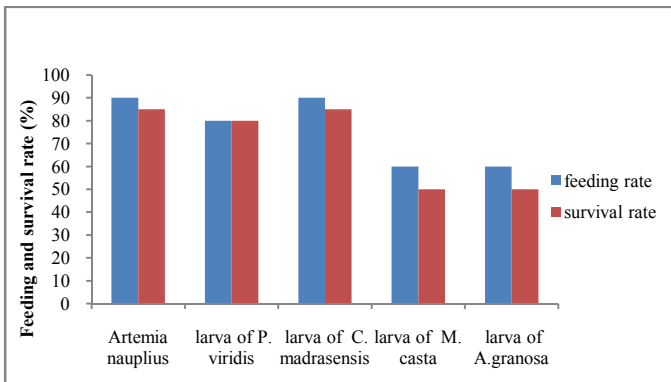


Figure 1 Rate of feeding and survival in post larvae of *L. vannamei* fed with artemia nauplius and trochophore of bivalves

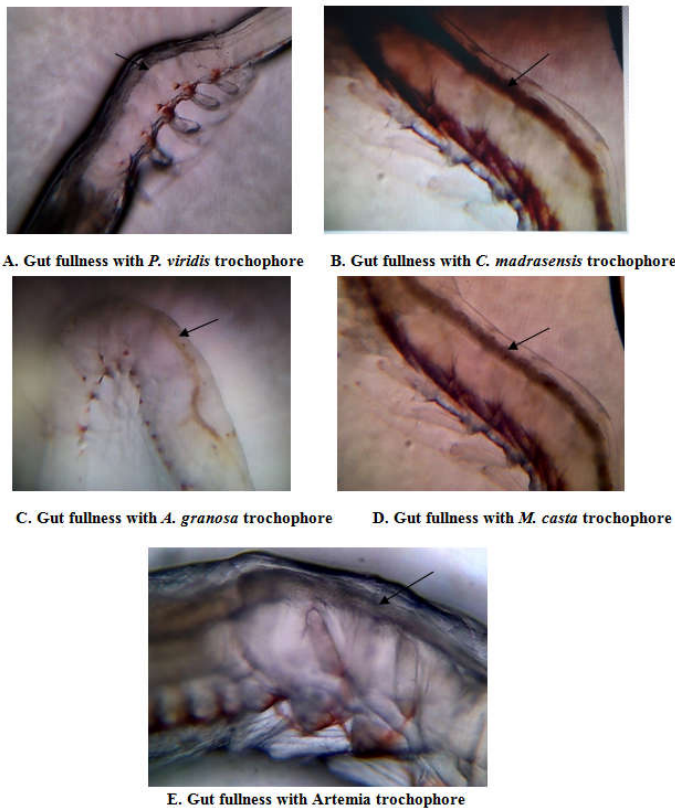


Plate 6 *L. vannamei* post larval feeding

Penaeus monodon

The rate of feeding observed was 80%, 90%, 45% and 50% in *P. monodon* PL fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively, where as 90% of feeding observed in *P. monodon* PL fed with artemia nauplius. The rate of survival noticed was 80%, 80%, 45% and 50% in *P. monodon* PL fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively, where as 85% of survival reported in *P. monodon* PL fed with artemia nauplius (Figure 2, Plate 7).

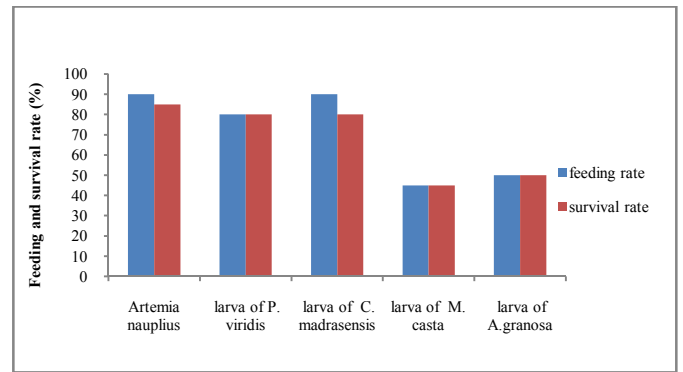


Figure 2 Rate of feeding and survival in post larvae of *P. monodon* fed with artemia nauplius and trochophore of bivalves

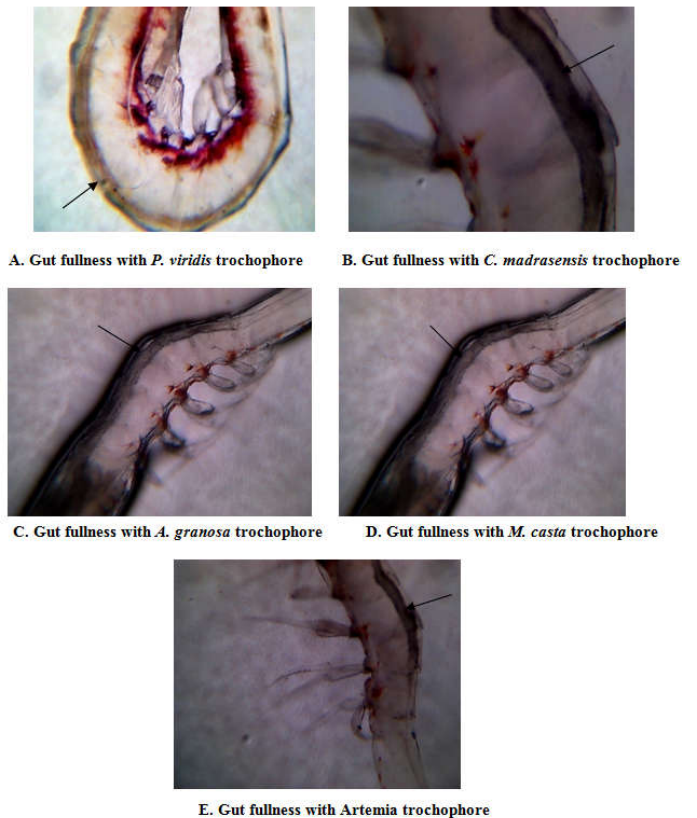


Plate 7 *P. monodon* post larval feeding

Macrobrachium rosenbergii

The rate of feeding observed was 75%, 80%, 40% and 40% in *M. rosenbergii* larvae fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively, where as 90% of feeding observed in *M. rosenbergii* larvae fed with artemia nauplius. The rate of survival noticed was 70%, 75%,

40% and 40% in *M. rosenbergii* larvae fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively where as 85% of survival reported in *M. rosenbergii* larvae fed with artemia nauplius (Figure 3, Plate 8).

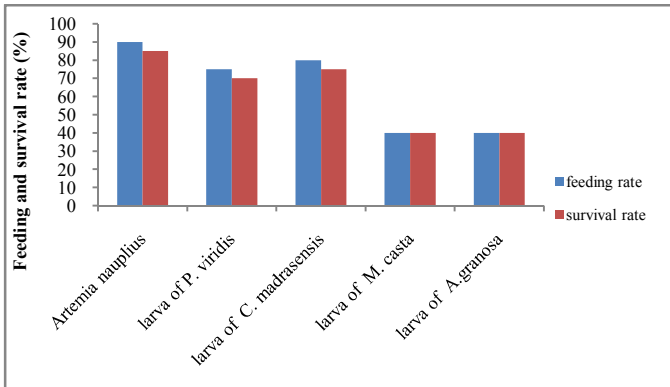


Figure 3 Rate of feeding and survival in post larvae of *M. rosenbergii* fed with artemia nauplius and trochophore of bivalves



Plate 8 *M. rosenbergii* post larval feeding

Lates calcarifer

The rate of feeding observed was 75%, 75%, 40% and 50% in *L. calcarifer* larva fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively. The rate of survival noticed was 70%, 75%, 40% and 50% in *L. calcarifer* larvae fed with trochophores of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* respectively. 3-4 days old larvae of *L. calcarifer* did not take artemia nauplius as food in the present study (Figure 4, Plate 9).

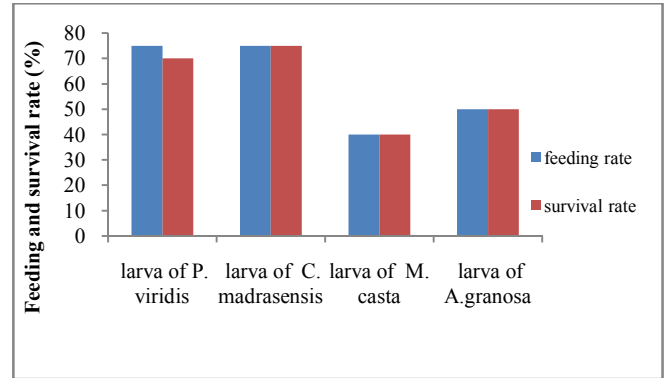


Figure 4 Rate of feeding and survival in post larvae of *L. calcarifer* fed with trochophore of bivalves



Plate 9 *L. calcarifer* larval feeding

DISCUSSION

Some of the techniques developed for induced breeding in large number of molluscs have been reviewed by Ino (1973) which involve physical, electrical, mechanical, chemical and biological stimulations. For induced breeding, thermal shock (temperature changes from 20-25°C to 35°C), salinity shock (salinity changes from 30ppt to 20-25ppt) and artificial propagation (squeezing of male and female gametes) were used in the present study. Induced breeding in bivalves by physical, chemical and biological stimulations was also successfully conducted by Alagar Swami 1980; Nayar *et al.*, 1982; Virabhadra Rao 1983; Wong and Ghee 1985; Wong *et al.*, 1986; Nayar *et al.*, 1987 & 1988; Appukuttan *et al.*, 1988; Narasimham *et al.*, 1988; Lian 1991; Muthiah *et al.*, 1992; Adly *et al.*, 1994; Muthiah *et al.*, 1988; Muthiah *et al.*, 2002; Madhu and Madhu 2009; Leroy *et al.*, 2010; Jagadis 2011; Ludi 2011; Nur Leena Wong and Aziz Arshad 2013. Earlier observations indicated that thermal stimulation was effective method for induced spawning in bivalves.

Fertilized eggs were immediately transferred to hatching tank containing filtered seawater with aeration. After 20 minutes of post fertilization, the first polar body started developing, 2nd cleavage occurred within 50 minutes and 3rd cleavage occurred within 60 minutes. Multi celled stage was developed within 2-3 h and blastula stage appeared within 3-4 h. The trochophore appeared after 6-7 hours of fertilization in four bivalves in the present study. Almost similar results were observed by earlier

authors in various bivalves (Rao *et al.*, 1976; Nayar *et al.*, 1982; Virabhadra Rao 1983; Wong and Ghee 1985; Wong *et al.*, 1986; Nayar *et al.*, 1987; Appukuttan *et al.*, 1988; Narasimham *et al.*, 1988; Muthiah *et al.*, 1992; Adly *et al.*, 1994; Muthiah *et al.*, 1988; Muthiah *et al.*, 2002; Dharmaraj *et al.*, 2004; Madhu and Madhu 2009; Leroy *et al.*, 2010; Jagadis 2011; Nur Leena Wong and Aziz Arshad 2013; Tankarsoon and Ransangan 2014). On contrary the trochophore was observed after 19 hours of fertilization in *Enis arcuatus* (Fiz Da Costa *et al.*, 2008). Similarly the trochophore appeared at 16-18 hours in *C. iredalai* (Nor Idayu *et al.*, 2015). The variation may be attributed to environmental conditions, water quality parameters, geographical and species variations. The rate of development of trochophore noticed was 66%, 65%, 57% and 54% in *P. viridis*, *C. madrasensis*, *A. granosa* and *M. casta* respectively in the present study.

Mass scale rearing of trochophore of *P. viridis* and *C. madrasensis* (60-70 μ) & *M. casta* and *A. granosa* (40-60 μ) was undertaken in filtered sea water at suitable environmental conditions i.e. Temperature: 27°C, Salinity: 20-25 ppt, pH: 8 and DO: 5-8 mg/l. These trochophores were used as live feed to larvae of shrimp. Similar water quality parameters were also maintained in the bivalve larval tank by Virabhadra Rao 1983; Adly *et al.*, 1994; Nur Leena Wong and Aziz Arshad 2013; Huo Zhongming 2014. The low salinity in the larval tank in the present study indicated that the condition provided as a natural habitat of bivalves.

There are several advantages of using bivalve trochophores for larval fish feeds (Adly *et al.*, 1994). In addition trochophores are simple to produce using sudden temperature and salinity shock. Trochophores are small in size, usually less than 60 μ (Alagaraswamy, 1980). They have cilia and flagellates for locomotion. Live foods are able to swim in water column and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response (David, 2003). A common procedure during the culture of both larvae of fish and prawns is to add microalgae to intensive culture system together with the zooplankton has become a popular practice (Tamaru *et al.* 1994). Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998) and hence are known as "living capsules of nutrition". Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish. For some marine fish species (groupers, siganids, snappers) very small zooplankton, such as trochophore larvae need to be used as a starter feed, since the commonly used rotifers are too big. Trochophore larvae of the Pacific oyster, *Crassostrea gigas* are 50 μ m in size and free-swimming (slow circular swimming pattern) ciliated organisms which have a high nutritional value for marine fish larvae (Xing and Long, 1991; Harvey, 1996). Trochophore larvae may contain upto 15 % (of total fatty acid) of both EPA and DHA (FAO Corporate Document Repository). Veliger larvae (60-80 μ m) can also be given as initial feed for fish larvae with particularly small mouths (Lim *et al.*, 1982; Whyte, 1992; Marliave *et al.*, 1994). The rate of feeding in post larvae (PL fed with trochophore of bivalves) of *L. vannamei*, *P.monodon*, *M.rosenbergii* and *L. calcarifer* ranged between 40.0% and 90.0%. The survival rate ranged between 40.0 and 85.0% in post larvae of *L. vannamei*,

P. monodon, *M. rosenbergii* and *L. calcarifer* during experimental conditions. Observations on feeding and survival of shrimp and prawn post larvae and fish larvae fed with trochophore larvae of *P. viridis*, *C. madrasensis*, *M. casta* and *A. granosa* indicated that these trochophore of bivalves are another alternative live feed to shrimp and fish hatcheries.

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