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Research Article

SEXUAL REPRODUCTION AND LARVAL DEVELOPMENT OF NEREIDIDAE POLYCHAETA PERINEREIS CULTRIFERA (GRUBE, 1840)

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ABSTRACT

In recent years the polychaete hatcheries has been developed for the production of healthy aquaculture organisms, since polychaetes is being used as live feed due to its has high nutrients compared to artificial feed. In the present study both male and female species *Perinereis cultrifera* were identified and successfully cultured under the controlled condition. The result of the present study clearly shows that the embryogenesis process and larval development of *Perinereis cultrifera* based on their egg embryos cleavages. The developments of setigers and eyes also indicate the lateral larval developments of the larval stages of *Perinereis cultrifera.* The successfully matured juvenile stage of this species was recorded after 20th day with the development of 16-18 setiger. This present study is the first attempt for culturing the *Perinereis cultrifera* under the hatchery condition in India. In future this study may helpful for culturing the other polychaete species in a hatchery system.

INTRODUCTION

Polychaetes tend to form the dominant sediment dwelling fauna of most mud flats, estuaries and sheltered sandy shores and it also play a significant role in ecosystem functioning and services (Baharudin *et al*., 2014). Polychaetes reproductive and developmental modes are conspicuously variable even among morphologically similar congeneric species (Levin & Bridges, 1995). Polychaete worms, community composition and reproductive ability are highly dependent on salinity and temperature gradients (Pardal *et al.*, 1993). These two physical factors may strongly influence species distributions through their impacts on benthic and pelagic stages. Low salinities cause mortality, lower fecundity, and prevent reproduction to varying degrees (Daunys *et al.,* 2000; Pechenik *et al.,* 2000). Polychaetes are commonly divided into three types of reproductive modes, annual species-large reproductive efforts; the perennial species-low reproductive efforts; multi-annual species-high reproductive efforts (Fauchald, 1983).

The majority of larger marine invertebrate species, including *A. marina* and *N. virens* reproduce by releasing their eggs and/or sperm into the water column so that fertilization takes place externally. These spawning episodes can be highly seasonal, with offspring production often confined to just 1–2 weeks of the year (Watson *et al.,* 2000; Guest *et al.,* 2005). Biologists have recognized phenomena like epitokous metamorphosis, semelparity, stolonization, lunar periodicity of spawning, swarming and exchange of pheromone signals between sexual partners, fertilization; development of the feeding larval stage, brooding and metamorphosis in polychaetes. The Nereididae polychaetes exhibit a wide range of reproductive modes, including external brooding, viviparity and hermaphrodtism. Most species undergo morphological and physiological modifications when they become sexually mature, suiting many of them for a brief pelagic existence and improving the chances that sexual partners will find each other (Schottler, 1989; Fischer, 1999). This metamorphosis of the immature worm into a special reproductive form is known as epitoky, a process that is particularly well described in the sandworm (Hoeger, 1991; Breton *et al.,* 2003). The current study aims to focus on sexual reproductive and various larval development stages in the nereididae po1ychaete *Perinereis cultrifera* (Grube, 1840).

MATERIALS AND METHODS

The present study the sexually matured (12.5 to 15.0 cm in length) *P. cultrifera* were selected for culturing purpose (Figure 1). The male and female polychaetes were identified based on their body colour. After identification 5 pairs of both male and female *P. cultrifera* were cultured with the regular water exchange, feeding and close observation for maturation. As this is the crucial step in the polychaete culture, utmost care was taken. After recognizing both male and female sexes were separated from the maturation tank and introduced in to spawning tank with maintenance of filtered sea water with 30 psu salinity and temperature of 27 – 28.5°C. The eggs and larval metamorphosis was photographed using light microscope, photographs and video was made with camera. After fertilization, the larvae were reared through

metamorphosis using standard technique for polychaetes (Strathmann, 1987). Larvae were kept in larval rearing tanks at a density of 2 per ml and gently stirred with plexiglas paddles. The larval culture was maintained and cleaned every day with filtered sea water and fed with *Chlorella sp.* respectively

RESULTS

In the present study, the males *P. cultrifera* were identified based on their white colour and females identified based on their green colour. After identification 5 pairs of both male and female species of *P. cultrifera* were allowed to spawn naturally. 5 pairs were successfully swarmed and released their eggs. After releasing the eggs and sperms the parents died, because they are semelparous. Once the parents died, they were removed from the tank. After fertilization, the egg clutch varied from 10,000 to 15, 000. The ooplasm was relatively opaque, and contained 70 – 80 lipid droplets surrounding a germinal vesicle. A fertilization membrane was elevated ten to twenty minutes after eggs and sperm are mixed. The germinal vesicle disappeard and both polar bodies were extruded 25 to 30 minutes after fertilization. The eggs were carefully scooped out by 10µm mesh screen and transferred to hatching tanks after iodine (10%) wash to avoid bacterial contamination. Eggs were kept in suspension in the hatching tanks by frequent stirring and gentle aeration. The brooders and eggs are shown in Figure 2. Thereafter the eggs were allowed to develop further.

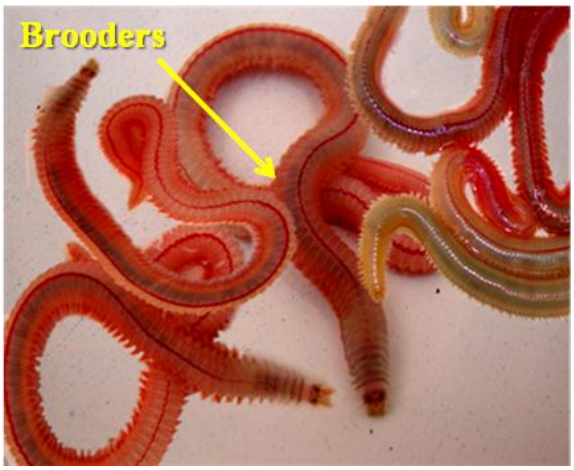


Figure 1: Image of *Perinereis cultrifera*

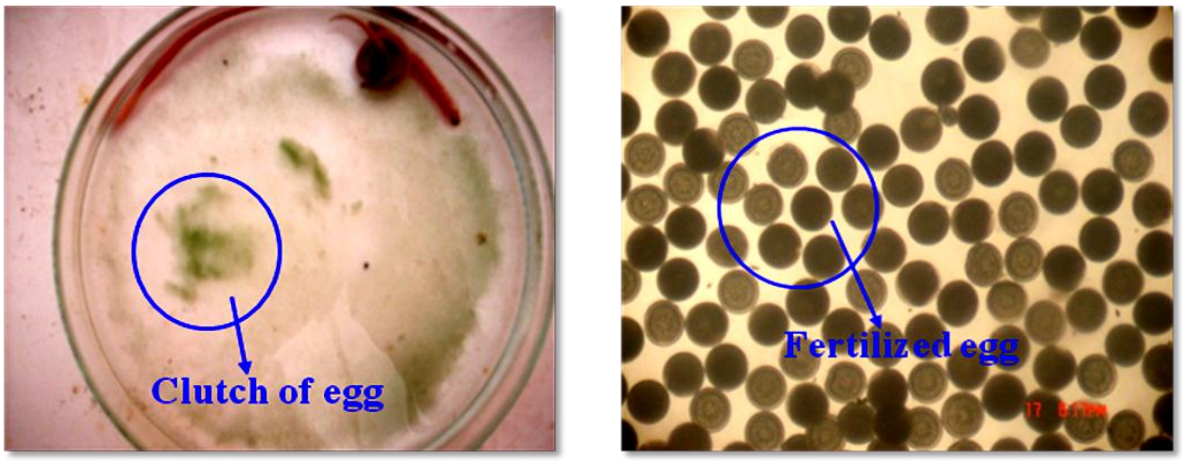


Figure 2: Brooder eggs of *Perinereis cultrifera*

Embryogenesis and early larval development:

The fertilized eggs were round in shape with an outer membrane covering the zygote. The fertilized eggs were turquoise green in colour and measured 280 - 320 µm in diameter enclosed by a very thick gelatinous and very transparent envelope. The eggs contained a large number of oily-like globules were observed to run together to form fewer larger globules. During the period of polar body formation and cleavage, lipid droplets were gradually migrated to the vegetal pole and fused. The first cleavage was unequal and occurred within two hours, followed by the four-cell stage at the internal of three hours and the eight-cell stage at four hours (Figure 3). Cleavage was spiral, and in eight to ten hours a cap of colorless transparent micromeres developed over four macromeres. A band of cilia (prototroch) was appeared 18-24 hr after fertilization and the embryos began to rotate within the jelly layer capsule.

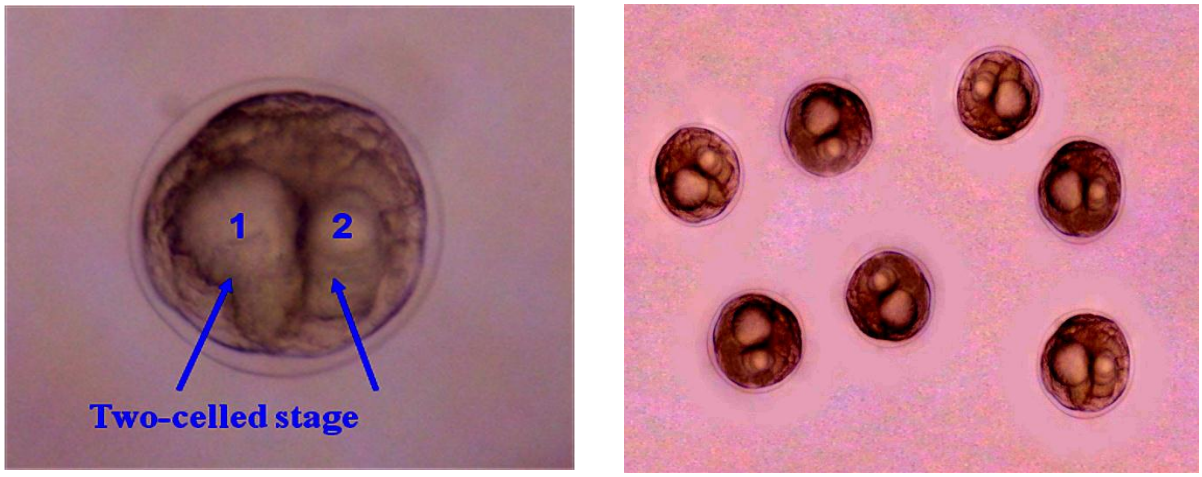


Figure 3: Cleavage of embryonic cell

The micromeres completely overgrew the macromeres in typical epibolic gastrulation within 24 to 30 hours. Further development led directly to a spherical, setigerous embryo by 60 to 63 hours. The embryo devoid of cilia and rotate, but does twitch sporadically. The early trochophore rotating in a jelly layer began to elongate within 60 to 63 hours. In the case of early trochophore at 65 - 70 hours after fertilization all three pairs of parapodial lobes were formed, most of the setae protruded through the cuticle, and the body now consisted of a massive head and three trunk segments (Figure 4).

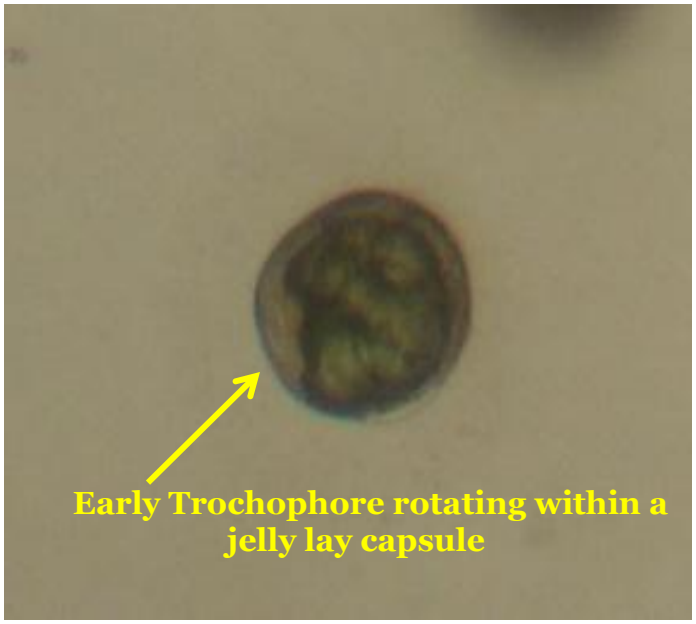
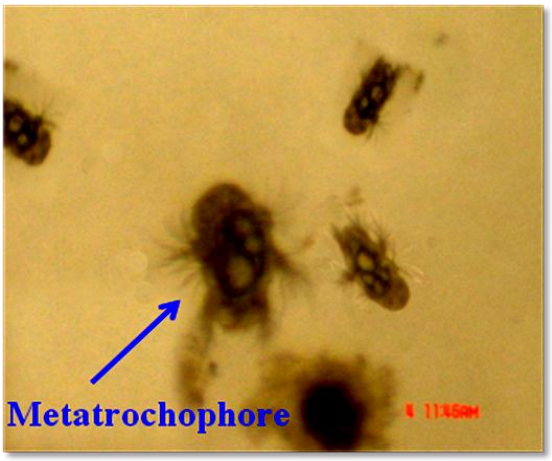


Figure 4: Trochophore development

This stage of development is arbitrarily designated as “early trochophore larva” or “3-setiger larva”. The trochophore began swimming freely after hatching out (65-75 hr after fertilization). Newly hatched larvae at 3-chaetiger stage differed with each other in age by less than 24 h were reared in tanks, in fresh sea water under a constant temperature (27 ± 0.5 °C) and a light/dark photoperiod (12 h/12 h). Further development led to a mid-3-setiger larva by the fall of third day, followed by a late 3-setiger larva on fourth day. After 4th day, the larvae metamorphosed into 3 setiger nectochaetes, the prototroch remained in the larval prostomium and lipid droplets moved to the middle part of the larval body (Figure 5). During this stage, the prostomial tentacle, peristomial cirri and anal cirri began to grow. The 3-setiger larval stage lasted until seventh day, when a rudimentary fourth setiger appeared.



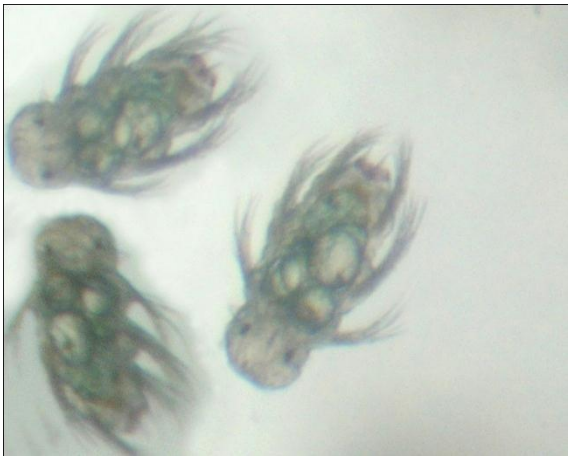
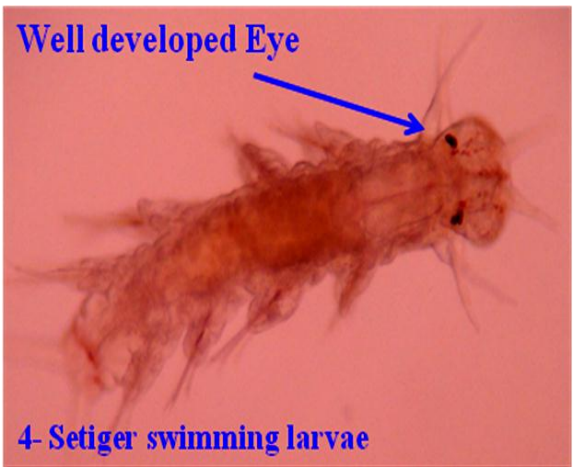


Figure 5: Larval development

Later larval development:

On 8th day fully developed four setiger larvae could be seen. Beyond this, growth was found to increase. The larvae were reared at temperature of 27 ± 1.5°C and salinity of 30psu. Nectochaetes developed the fourth setiger a week after fertilization, when the lipid droplets disappeared and the larvae began to feed. Four pairs of tentacular cirri eventually developed and are positioned with respect to one another, anterodorsally, posterodorsally, anteroventrally, and posteroventrally. Tentacular cirri of the 3-setiger larva represent the definitive anterodorsal tentacular cirri. Figure 5. shows the development 5-setiger larva (12 days).



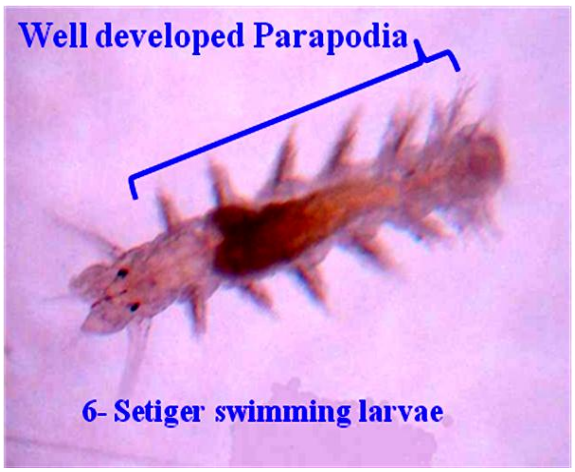


Figure 6: Development of eyes

Figure 7: Development of Parapodia

The first parapodium of the 5-setiger larva acquired a medial digitate lobe which elongated considerably and advanced to 7-setiger stage on 20th day (Figure 7). All nototrochs were present until the juvenile has acquired 16-18 setigers (Figure 8). The ciliated nuchal organs were retained throughout the life

of the worm. The development of larval stages and their size and duration of development are shown in Table 1.

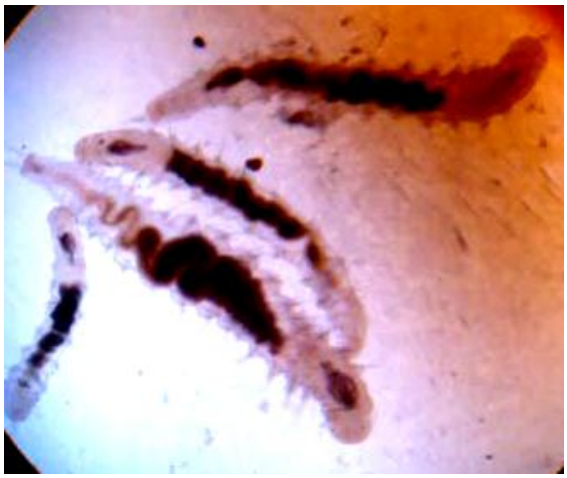


Figure 8: Well developed juvenile

Stages of development	Length (cm)	Age
Fertilization membrane elevated	-	10 – 20 minutes
Polar bodies extruded	-	25 – 30 minutes
2-cell stage	-	1.5 – 2.0 hours
4-cell stage	-	3.0 – 4.0 hours
8-cell stage	-	5.0 – 6.0 hours
16-cell stage		7.0 – 10.0 hours
Early gastrula	-	12.0 – 16.0 hours
Late gastrula	-	24 hours
Early trochophore rotating in a jelly layer	-	60 – 63 hours
Early trochophore	0.27	65 – 70 hours
Meta-trochophore	0.30	3 days
3-setiger larva	0.35	4 days
4-setiger larva	0.42	7 days
5-setiger larva	0.51	12 days
6-setiger larva	0.59	15 days
7-setiger larva	0.85	20 days
8-setiger post larval stage	1.72	30 days
10-setiger post larval stage	3.97	45 days
20-setiger post larval stage	5.86	61 days

30-setiger post larval stage	7.20	82 days
40-setiger post larval stage	8.70	95 days
50-setiger post larval stage	10.56	107 days
60-setiger post larval stage	12.98	125 days
70-setiger post larval stage	15.70	150 days

Table 1: Development and growth of *P. cultrifera* reared in the laboratory and the age in time after fertilization.

DISCUSSION

Polychaetes are one among the marine organisms that have had the greatest evolutionary success. They are very abundant and widely distributed both ecologically and geographically, in marine environments, while a significant component has also colonized brackish water habitats. Being one of the groups best represented in unpredictable environments, they have been widely studied in an attempt to understand what characteristics of their life cycle and life history have enabled them to colonize and survive in such habitats (Wilson, 1991; Hadfield and Strathmann, 1996; Giangrande, 1997). In many species of polychaetes living in coastal environments, systems for the protection of the eggs and embryos are present, while species living in the open sea can spawn freely in the external environment where fertilization and development occur. The forms of protection are strictly related to different types of development and in most cases the forms of protection are present in species with a direct development, while the species that release their gametes directly by external means have a pelagic development (Thorson, 1950; Giangrande, 1997).

The present study on oocyte diameter with respect to seasons revealed that with the increase in maturity stages / size groups the diameter also increases. The observed oocyte diameter value ranged from a minimum of 9.32 µm to a maximum of 325.82 µm. After keen observation of the oocyte diameter of different samples, it was found that the size of oocyte increases with increase in the size of the animals. This is in close accordance to the size reported by Kupriyanova *et al*. (2001) in serpulids. The season wise maturation of oocyte diameter made in the present study, clearly indicated the growth of oocyte as immature, early maturing, late maturing and matured is almost similar to the one made by Olive *et al.* (1998). The fertilization and the production of viable offspring are dependent upon many cellular and molecular processes and one of the most critical is the completion of oocyte maturation. Chausson *et al*. (2004) investigated the effects of cellular and molecular processes involved in oogenesis and oocyte maturation. Betteley *et al*. (2008) reported the oocyte diameter measurements at different times were taken to investigate the gametogenic processes. 5 pairs were successfully spawned their eggs and these eggs were allowed to develop further, after 4 days the 3-setiger larvae were observed.

In a previous attempt to distinguish the epitokous forms of *P. cultrifera* from the Mediterranean Sea and the English Channel, Scaps *et al.* (2000) and Rouabah and Scaps (2003) reported morphological (number and morphology of paragnaths on the proboscis) and biochemical (allozymes, general protein band patterns

obtained after one-dimensional electrophoretic procedure, two-dimensional electrophoresis of proteins) divergence and concluded that *P. cultrifera* is a complex of species. The juvenile, benthic worm of 10 to 11 segments has the same life style as the adult. The absence of larval dispersion or its weak amplitude promotes the maintenance of the larvae in the biotope favorable to the adults and limits the possibilities of colonization of new habitats, and promotes the geographic isolation of individuals.

The mature eggs were green in colour having the size in the range from 250 to 320 µm and contained 10 – 20 droplets surrounding a germinal vesicle. After fertilization, the fertilized eggs were secreted a jelly layer with 150 µm thickness. Herpin (1926) also pointed out in his study, this jelly is not adhesive as in the case with *Perinereis cultrifera* in which, species it sticks the eggs to the side of the bowls and possibly to rocks. Previous studies showed that early development in *Hediste diadroma* is characterized by a small egg size (130 – 170 µm in diameter) and long pelagic larval life (about 1 month in the field) after hatching out at trochophore stage (Sato and Tsuchiya 1987, 1991; Sato, 1999). Likewise, Tosuji and Sato (2006) indicated the early development and gamete compatibility in two sympatric estuarine species of the genus Hediste. Further, they examined the effects of salinity on fertilization and successful early development of *Hediste diadroma* and *Hediste japonica*, most eggs were fertilizable in a wide range of salinities (27-32psu) and developed successfully to embryos with ciliary movement in 22.5-30 psu. True to this, in the present study also the salinity was maintained at the range of 20-30 psu and the temperature of 25 - 28°C.

According to Cazaux (1981), larval development in nereidids is present in four stages; trochophore I, trochophore II, metatrochophore I and metatrochophore II. In *P. cultrifera* and *P. rullieri*, the trochophore and metatrochophore stages were completed inside the gelatinuous egg mass, and the first free-living stage is the nectochaete that has already acquired benthic habits. Most of the species studied, produce lecitotrophic eggs ranging in size up to 370 µm, in the case of *Perinereis rullieri*, to 100 µm, in the case of the female eggs of *D. gyrociliatus*. Egg size is an important life history trait, being an indicator of energy investment per offspring. Large eggs rich in food reserves allow lecitotrophic or direct development, thus avoiding the particularly high risks associated with planktotrophy in brackish environments. Small sized species cannot generate enough energy and do not have enough space to produce a large number of eggs in a single episode; hence they typically produce a small number of large eggs often associated with protective systems (Westheide, 1984; Giangrande, 1997).

CONCLUSION

The present study suggested the optimum salinity (30 psu) and temperature (27-28.5°C) that could be enough for the spawning of *Perinereis cultrifera.* Besides on the above the larval species were cultured with *Chlorella sp.* as the feed material. The Microscopic pictures clearly show the successful embryogenesis process and larval developments. The present study concluded that after 65-75 hr after fertilization the trochophore began swimming freely which evidently indicates the development of segments with pair of setigers. And also the present study clearly shows the conversion of larval stage to juvenile stage with the

development of eyes and 16-18 setigers. We hope that this study can be help full for the researchers working on the aspects of polychaete culture.

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