Indian Journal of Geo Marine Sciences
Vol. 50 (10), October 2021, pp. 795-801

# On captive breeding, spawning, embryonic and larval development in Horse conch, *Pleuroploca trapezium* (Linnaeus, 1758) from Southeast coast of India

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*Received 14 April 2020; revised 31 August 2021*

Captive breeding, spawning and larval development of endangered gastropod, horse conch, *Pleuroploca trapezium* (Linnaeus, 1758) was studied and reported from Thoothukudi, Southeast coast of India for the first time. Wild collected brooders of *P. trapezium* were spawned after 10 months of rearing under captivity as a cluster of egg cases on the wall of the tank and also above the shell of the other horse conch. Each egg case had 150 to 460 numbers of eggs. The incubation period ranged from 23 to 30 days and 91 % of hatching was recorded. Detailed observations were made on the embryonic development and larval rearing until 40 days of post hatch (dph). The day one larvae measured 750±14.15 µm and reached an average shell size of 1830±37.48 µm after 40 dph. After 30 dph, though most of the pre-juveniles settled and crawled at the bottom of the rearing tank, the presence of active velum indicated that the competency to metamorphosis for the development of juvenile was not fully attained and complete mortality occurred. Therefore, further study is warranted using different cues to promote the metamorphosis of *Pleuroploca trapezium* for successful life cycle closing and juvenile production.

[**Keywords**: Captive breeding, Endangered, Larval rearing, Marine gastropod, Metamorphosis, Threatened and protected species]

## Introduction

Controlled breeding of marine gastropods has received only a little attention worldwide. In temperate waters few researchers have achieved success in *Strombus gigas*[1,2], *Trochus* sp.[3] and Muricids[4,5]. Research on marine gastropod breeding and seed production in India has started gaining momentum in the past decade by the earnest efforts of Central Marine Fisheries Research Institute, India on few marine gastropods especially on Abalone *Haliotis varia*[6] and *Babylonia spirata*[7]. Considerable success has been achieved on the broodstock maintenance, controlled breeding and larval rearing of commercially important species of Muricids and Strombids such as *Lambis lambis*[8], *Chicoreus virgineus*[9], development of post settled juveniles of *Chicoreus ramosus* and *Lambis lambis* and captive breeding in *Cypraea tigris*[10].

The horse conch, *Pleuroploca trapezium* (Linnaeus, 1758) belongs to the family Fasciolariidae, one of the few marine gastropods listed under Indian Wildlife Protection Act (IWPA) 1972 (Schedule IV, 19. Mollusca, iv. *Fasciolaria trapezium*) and considered as an Endangered, Threatened and Protected (ETP) species. Reports on this species are limited to that of the hatching and development of wild collected eggs of Indo-Pacific *Pleuroploca trapezium*[11-13]. In this context, the knowledge on captive breeding and intracapsular development and larval rearing of the horse conch is very scanty, an attempt was made to breed under captive condition at Tuticorin Research Centre of ICAR-CMFRI. This study is aimed to give the details about the brood collection, spawning, detailed embryonic development, larval rearing up to the metamorphic stage.

## Materials and Methods

### Broodstock collection and maintenance

The horse conches were found in stray numbers with other commercial gastropod catches. Ten numbers of live horse conches, *P. trapezium* with the average length and weight of 173.27±11.94 mm and 679.4±76.96 g, respectively were collected and transported to the Shellfish hatchery in an aerated seawater container. The collected brooders were not sexed and maintained at the rate of 10 brooders in one tonne capacity Fibre Reinforced Plastic (FRP) tank (2×1×0.5 m; area 2 $m^2$). The bottom of the brood maintenance tanks were filled with fine sand (sieved using 500 µm sieve) collected from beach of Hare Island, Thoothukudi to a height of 10 cm as described by Jagadis *et al.*[10]. Horse conches are carnivores and were fed with any live clams such as *Paphia malabarica*, *Marcia opima*, *Meretrix meretrix*

and *M. casta* at the rate of 4 numbers animal$^{-1}$ day$^{-1}$ (average meat weight = 8 g). Sufficient numbers of clams were replaced in the broodstock tank every day. Water quality parameters in the brood maintenance tank were monitored on weekly basis and recorded. Water temperature, Dissolved Oxygen (DO) and pH were recorded using thermometer, digital DO meter (MERCK, Germany) and digital pH meter (LABINDIA, India), respectively. Total ammonia was determined by using standard protocols of APHA[14].

**Spawning, egg case morphology and fecundity**

Horse conch brooders were maintained over a period of ten months. During the period, the activities of the conches were closely monitored through visual observation and photographs were taken for mating, egg laying and brooding behaviour and recorded. Spawned female conches were tagged. From each spawning, egg cases were randomly taken from different clusters (approximately 10 % of total egg cases) and measured using digital vernier callipers to 0.01 mm accuracy for their egg case length, stalk length and capsular width. The egg case numbers and colour were observed and recorded. The number of eggs per case was counted by cutting open randomly selected 3 egg cases from each cluster and counted under the microscope for calculating the fecundity of a single female horse conch per spawning.

**Embryonic development**

Embryonic development from day one post spawn (dps) was monitored '*in situ'* by leaving the egg cases in the spawning tank till the complete embryonic development has taken place as evidenced by the developed embryos concentrating at the apical region of each egg case. Then they were carefully detached from the spawning tank and transferred to 100 L FRP incubation tank with filtered and aerated sea water. At regular interval, the developmental stages within the egg case was closely followed by cutting open one egg case from each cluster on each observation day and the development was observed and photographed at a standard magnification using a stereozoom microscope (Leica, S8 APD Stereozoom®, Switzerland). Size of the egg and developing larvae were measured, calculated and recorded using a calibrated ocular micrometer fitted in the microscope.

**Hatching and larval development**

Hatching rate of the spawned egg cases was studied by rearing till complete hatching of randomly selected 20 egg cases; each kept in 5 L glass beaker with 3 L filtered seawater (using 10 µm pore size filter bags filled with cotton) with adequate aeration and daily water exchange. Incubation duration was monitored for complete hatching of all the 20 egg cases selected which is ensured by complete release of larvae from each egg case and become empty. After complete hatching was ascertained, the larvae in the water column were gently filtered with a fine hose and nylon bolt sieve (300 µm) and 50 numbers of larvae L$^{-1}$ were stocked in 50 L FRP tank containing 30 L of filtered seawater with aeration and fed with *Isochrysis galbana* at the rate of 10,000 to 20,000 cells mL$^{-1}$ day$^{-1}$. Daily water exchange was carried out in the larval rearing tanks and filled with filtered seawater using 10 µm pore size filter bags filled with cotton. After 25 days of post hatch (dph), the larvae were fed with finely crushed clam meat sieved through 40 µm sieve at the rate of 2 mL per tank. No substrate was provided in the larval rearing tanks. A sample of 5 larvae were taken at 5 days intervals from the water column of each tank and placed in 5 mL of water in an embryo cup and observed under the stereoscopic microscope. The stages were photographed and recorded.

**Statistical analysis**

The statistical analysis of this study was done using the descriptive analysis of excel data analysis for the estimation of arithmetic mean and standard error.

## Results

**Broodstock maintenance and spawning**

The brooders were maintained and fed with live clams at the rate of 4 clams brooder$^{-1}$ day$^{-1}$ (8 g meat weight). The brooders actively feed on the clam by opening the shell with their powerful foot. During the period of maintenance, animals had a survival of 100 %. The water quality parameters in the broodstock maintenance and incubation tank were given in Table 1. Three females spawned after 10 months of rearing under captivity. Spawning started during 18$^{th}$ December 2017 and continued till February 2018. The spawning was

Table 1 — Water quality parameters maintained in the hatchery for broodstock and larval rearing

| Water quality parameters | Values |
|---|---|
| Temperature (°C) | 28.0±0.57 |
| Salinity (g L$^{-1}$) | 32.0±1.15 |
| pH | 8.1±0.04 |
| DO (mg L$^{-1}$) | 5.0±0.36 |
| Ammonia (mg L$^{-1}$) | 0.1±0.01 |

The data is expressed as arithmetic mean of six replications ± SE

observed in both day and night time. The female laid egg cases as a cluster on the wall of the tank and also above the shell of other conches (Plate 1a). The spawning process lasted for 5 – 58 h. The time gap between successive laying of eggs as cluster by the same individual ranged between 1 and 10 days.

**Egg case morphology and fecundity**

The egg case was vase-shaped, faint pink in colour (Plate 1b), containing gelatinous fluid inside the egg case that was translucent and gummy, allowing the embryos to maintain a constant position within the egg case and also for the protection of developing eggs (Plate 1c). Egg cases had semi transparent wall which was linked by a peduncle to a common basal membrane. The apical plate was convex, surrounded by an apical ridge and a strong horizontal ridge was present between apical plate and stalk of the egg case. The length of the egg cases ranged from 16 to 27 mm (24.0±1.6 mm); stalk length from 7 to 10 mm (9.0±0.51 mm) and average apical width was 5.0±0.3 mm. The size of the egg case varied between brooders as well as in different clusters. The average size of the egg case by a single brooder was larger at the beginning of the spawning, which got reduced at the end of the spawning. Brooders of *P. trapezium* spawned in multiple clusters of various numbers having 17 to 85 (59±10.2) egg cases and the total egg cases laid ranged from 340 – 458 (393.7±34.5) numbers in single spawning. The larval escape aperture was covered by a transparent membrane at the centre of the apical area and measured to 0.9 to 1.2 mm (1.1±0.04 mm) in diameter. The colour of the eggs inside the capsule varied from light orange to red. The number of eggs in the egg cases ranged between 150 and 460 (376±46.2) from all the sizes of egg case. The details of egg mass of *P. trapezium* were compared with earlier studies (Table 2).

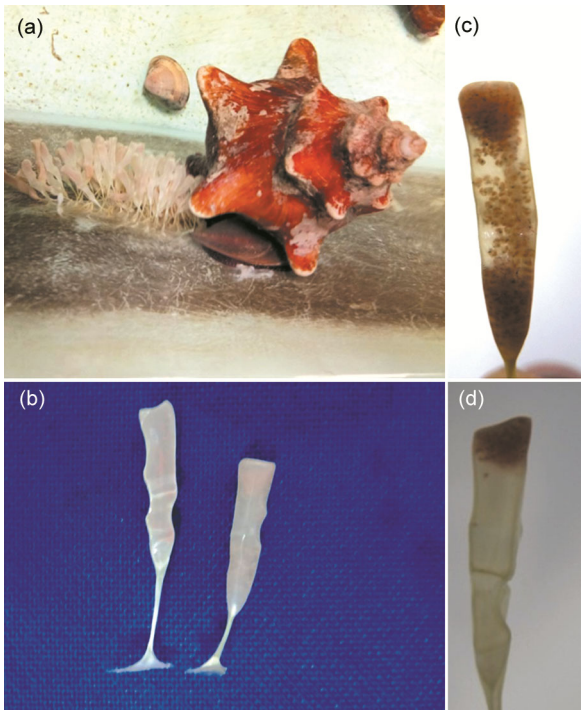


Plate 1 — Horse conch brooder egg laying and egg cases: a) Egg laying by female *P. trapezium*, b) Egg case of *P. trapezium*, c) Embryos within egg case of *P. trapezium*, and d) Egg case of *P. trapezium* prior to hatching

**Embryonic development**

Eggs of *P. trapezium* were spherical in shape and had an average size of 359±10.36 µm (Plate 2a). Nearly all the eggs were fertilized (98±0.51 %). Cell division in the fertilised eggs does not begin immediately after spawning. The first cell division was observed after 3 h of spawning and the embryo reached two celled stage (Plate 2b). In the two celled stage the egg was elongated and the first cleavage was vertical which divides the egg into two nearly equal size blastomeres. Further, it divided into four cell stage within the first day of post spawning (1 dps) (Plate 2c). The divided blastomeres were arranged

Table 2 — Egg case of *Pleuroploca trapezium* in the present study compared with the earlier studies

| Location | No. of egg case/ spawn | No. of eggs/ egg case | Egg case length (mm) | Egg case width (mm) | Embryo colour | Development type | Egg case source | Reference |
|---|---|---|---|---|---|---|---|---|
| India | 340 -458 (393.7±34.5) | 150-460 (376±46.2) | 24.0± 1.6 | 5.0 ± 0.3 | Pale orange & Pale red | Planktonic veliger | Captive bred | Present study* |
| India | 105 – 130 | 90 – 110 | 29.0 | 5.0 | Pale violet | Not reported | Wild | Raghunathan & Ayyakkannu[13] |
| Japan | 32 | Not determined | 16.2 ± 1.9 | 6.0 ± 0.5 | - | Planktonic veliger | Wild | D'Asaro[17] |
| Sri Lanka | 110-140 | Not determined | 26.6 ± 0.2 | 4.8 ± 0.2 | Red | Planktonic veliger | Wild | D'Asaro[17] |

*The data for this current study is expressed as arithmetic mean of six replications ± SE.

symmetrically and joined by their inner surfaces. On second day of post spawning (2 dps), embryo was in non-motile condition and had an average size of 325±12.15 µm (Plate 2d). On 4 dps, the size of the micromeres considerably increased and embryo grew to a size of 362±7.53 µm (Plate 2e). On 9 dps, the size was 425±15.02 µm. By 11 dps, the average size increased to 510±14.99 µm (Plate 2f). Concurrently, the thickness of the intracapsular fluid present inside the egg case got reduced. On 13 dps, the average size of the embryo was 560±10.85 µm (Plate 2g).

The embryo transformed into veliger on 15 dps and the shell length was 600±10.98 µm (Plate 2h). On 21 dps, the larvae grew to a size of 710±11.03 µm (Plate 2i). The larval heart became distinctly visible through the transparent thin shell and it beats on an average 82 times/minute. The viscosity of the intra-capsular fluid greatly reduced and all the larvae move upwards and concentrated at the apical portion of the egg case near the escape aperture (Plate 1d). During this stage, almost all the larvae were able to swim in the intracapsular fluid which indicated that the larvae were ready for hatching. The detailed embryonic stages with respect to days were given in Table 3 and its measurements in Table 4.

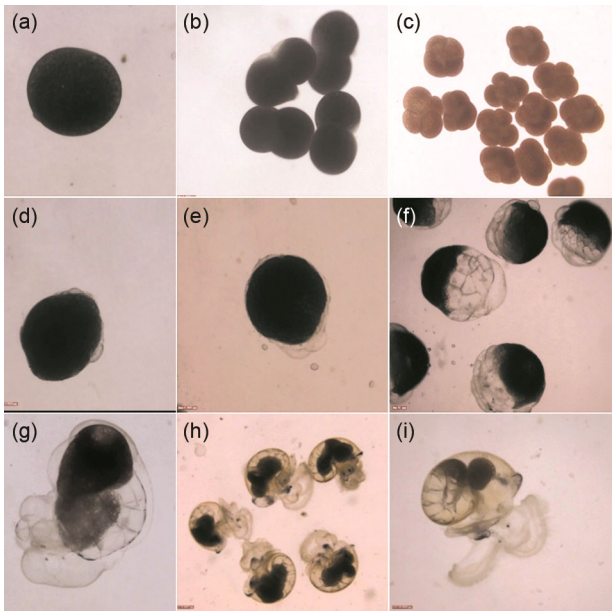


Plate 2 — Intra-capsular development in horse conch, *P. trapezium*: a) Fertilised egg, b) 2 celled stage embryo, c) 4 celled stage embryo, d) 2 dps embryo, e) 4 dps embryo, f) 11 dps embryo, g) 13 dps embryo, h) 15 dps embryo, and i) 21 dps embryo

Table 4 — Measurements of intra-capsular developmental and larval stages of the horse conch, *P. trapaezium*

| Stage | Period | Size (µm) |
|---|---|---|
| Embryo | 2 dps | 325±12.15 |
| | 4 dps | 362±7.53 |
| | 9 dps | 425±15.02 |
| | 11 dps | 510±14.99 |
| | 13 dps | 560±10.85 |
| | 15 dps | 600 ±10.98 |
| | 21 dps | 710±11.03 |
| Larvae | 1 dph | 750±14.15 |
| | 10 dph | 875±15.07 |
| | 20 dph | 1080±24.93 |
| | 30 dph | 1400±51.05 |
| Pre-juvenile | 35 dph | 1650±41.58 |
| | 40 dph | 1830±37.48 |

The data expressed as arithmetic mean of six replications ± SE.

Table 3 — Intra-capsular developmental stages of the horse conch *P. trapaezium*

| Period (dps) | Intra-capsular developmental stages |
|---|---|
| 1 dps | Cell division started after 3 hrs of spawning. Completed two celled stage and four celled stage on the first day. |
| 2 dps | The cell divisions were completed and morula stage was formed. A transparent micromere appeared at the anterior marginal region and the embryo was in non-motile condition. |
| 4 dps | The size of the micromeres considerably increased and short cilia had appeared near the upper portion of the embryo. |
| 9 dps | The micromere portion extended up to more than half the portion of the embryo. Slow rotation of the embryo was observed inside the egg case. |
| 11 dps | The embryo became elongated, developed fine cilia around the anterior side and rotation became faster. |
| 13 dps | The embryo reached an undefined shape. Ciliary movement was observed along with developing velum and eyes developed. |
| 15 dps | The embryo transformed into veliger with small bi-lobed velum, short and active cilia. The shell was very thin, highly transparent with faint brown pigmentations. The developed metapodium (posterior foot) had very thin and transparent operculum. |
| 18 dps | The size of bi-lobed velum increased allowing the larva to move rapidly and mostly in rotational movements but still unable to swim. |
| 21 dps | First whorl started developing and the brown pigmentation on the shell surface became more prominent. The internal features of the larvae can be clearly seen through the thin transparent larval shell. The larval heart became distinctly visible through the transparent thin shell. The intracapsular fluid greatly reduced and all the larvae moved and concentrated at the apical portion of the egg case near the escape aperture. |
| 23 dps | The apical aperture of the egg case opened and the free swimming larvae hatched out. |

**Hatching and larval development**

Hatching of the egg cases were observed from 23 dps and continued till 30 dps. In all the spawning over 90 % hatching was observed. The hatched larvae were actively swimming to the surface of the water with fast beating of cilia on the velar lobes (Plate 3a). Newly hatched larvae had a thin, brown pigmented faint brownish shell with ¾th developed whorl and measured about 750±14.15 µm (Table 4). It had a fully developed well extended bi-lobed velum with two rows of cilia and light brown pigmentation at the margin. A pair of prominent eye at the base of the transparent antennae was developed and the internal organs were clearly visible through the transparent shell. Foot was very active with translucent oval shaped operculum. The bi-lobed velum further divided into four lobes on 4 days post hatch (4 dph).

On 10 dph, the larvae had a four lobed velum which is greatly increased in size with very active ciliary movement. Shell was translucent and light brown in colour with fully developed single whorl and had a shell size of 875±15.07 µm (Plate 3b). Eyes were more prominent and both the antennae were equal in length. Dark black colouration appeared on the margin of the outer lip and also increased black pigmentation noticed below the metapodium. The siphonal canal had begun to develop at this stage and the four lobed velum got enlarged.

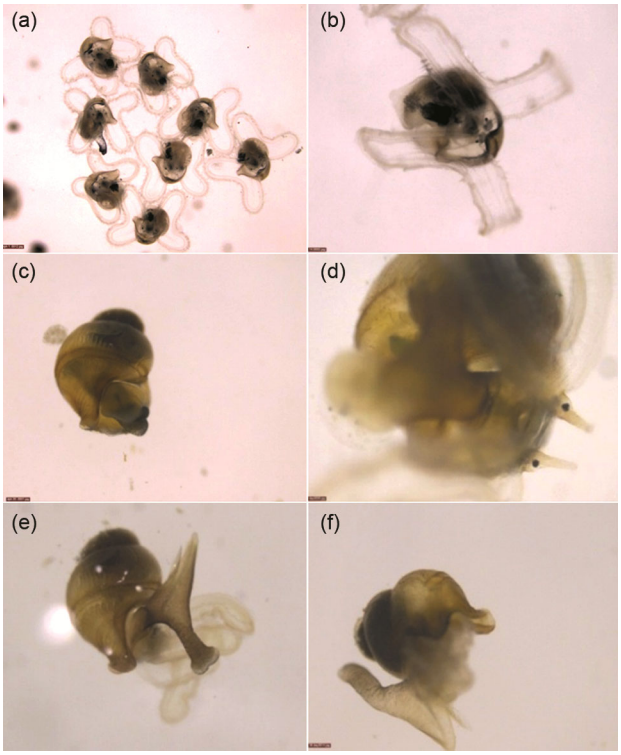


Plate 3 — Larval development in *P. trapezium*: a) 1 dph larvae, b) 10 dph larvae, c) 20 dph larvae, d) 30 dph larvae (pre-juvenile stage), e) 35 dph larvae, and f) 40 dph larvae

After 20 dph, the larvae reached the shell size of 1080±24.93 µm (Plate 3c) and colour of the shell turns from light brown into dark brown. Four-lobed velum was still remained with active cilia. At this stage, the eyes were bigger, prominently bulging at the base of the antennae which now is pigmented with black spots. Two thick growth lines were observed in the middle of the body whorl region and diminutive vertical striae observed in the middle part of the whorl. The length of the siphonal canal increased and spiral striae were noticed in the outer lip of the shell. The foot became very active, heavy and dark black pigmentation was observed on the metapodium. The size and thickness of the operculum increased. Nearly 50 % of the larvae were found at the bottom of the rearing tank.

On 30 dph, the larvae attained pre-juvenile stage. The size and activity of the foot significantly increased; however, velum was intact with very active ciliary movement. The shell was darker in colour with partially developed second whorl. The length of the siphonal canal increased and the spiral striae developed in the outer lip became thick. The pre-juveniles attained the shell size of 1400±51.05 µm (Plate 3d). At this stage, most of the pre-juveniles settled at the bottom of the rearing tank. From 35 dph onwards the pre-juveniles started creeping at the bottom of tank with well developed foot; however, still had active four-lobed velum. Eye balls were bulged and antennae became equal in length and relatively thick. The vertical striae in middle of the body whorl and spiral striae in the outer lip of the shell became more prominent and increased in numbers. Average shell size of the pre-juveniles at this stage was 1650±41.58 µm (Plate 3e).

On 40 dph, the pre-juveniles reached an average size of 1830±37.48 µm (Plate 3f). There were no morphological differences noticed between day 35 and 40 except for increase in size. Most of the pre-juveniles settled and crawled at the bottom of the rearing tank. The presence of active velum even at this stage of development indicated that the competency to metamorphosis was not fully attained. After this, they became feeble and complete mortality occurred in few days.

## Discussion

**Broodstock maintenance and spawning**

The spawning in *P. trapezium* is observed from January to April by studying its gonad index from the Gulf of Mannar region[13]. This is in agreement with the

present study, where *P. trapezium* spawned during the month of December – February. Jagadis[8-10] had observed similar spawning season for other three large gastropods which indicates that this season provides a suitable environment for gastropod breeding in this region. During the study, communal spawning behaviour was observed in *P. trapezium* where multiple female brooders laid egg cases as a cluster on the hard substratum which is common among other fasciolarid gastropods as reported by several authors[15,16] and in *P. trapezium*[13].

**Egg case morphology and fecundity**

D'Asaro[17] has stated some variation in the egg case structure within the Fasciolariinae. *Fasciolaria* and *Pleuroploca* have almost conical vasiform cases tapering to a narrow stalk. He also observed that the egg cases laid by *Fasciolaria* spp. have species-specific apical ridges and shows a variation in the structure of the escape aperture, but in *Pleuroploca*, observed conical egg cases with simple apical ridges and distinctive horizontal ridges on the sides. Observations on the egg case morphology such as egg case length and egg case width of *P. trapezium* in the present study (captive bred) were comparable with the reports (wild spawned) of Raghunathan & Ayyakannu[13] and D'Asaro[17]. The egg case number and fecundity of *P. trapezium* recorded in the present study was higher than that reported by Raghunathan & Ayyakkannu[13] from the same region. D'Asaro[19] has reported a much smaller egg case length in sub tropical region.

**Intracapsular development**

Intracapsular metamorphosis (direct development) is the common feature among Fasciolariids[17,18]. Most of the *Pleuroploca* species such as *P. aurantiaca*, *P. gigantean*, *P. lignaria*, *P. lugubris* and *P. salmo* completes their development within the egg capsule *i.e.* intracapsular metamorphosis (with no planktonic stage), through the embryo ingesting nurse eggs and hatching as crawling juveniles[15-19]. But, the present study revealed that embryonic development of *P. trapezium* was indirect (pelagic development) with hatching of numerous typical planktonic veliger larvae that is normally unnoticed for species of this family. Embryonic growth of *P. trapezium* occurs through a series of cell divisions with simultaneous morphological changes. On 15 dps, the embryo transformed into veliger stage and on 21 dps the larvae were able to swim in intracapsular fluid which indicated that larvae were ready for hatching. This phenomenon is in agreement with Gohar & Eisawy[12] who stated that *Pleuroploca trapezium* is the only reported *Pleuroploca* species that hatches as veliger larva. Further, D'Asaro[17] also reported that the embryonic development process of *P. trapezium* as planktonic veliger. Similarly, some authors reported direct planktonic veliger from other genus of Faciolariidae such as *Dolicholatirus cayohuesonicus*, *Latirus infundibulum* and *Latlrolagana smaragdula*[17].

**Larval development**

Feeding the gastropod larvae with *I. galbana* is a common practice[8-10]. The larval density in the rearing tank varies according to the researchers for most of the marine gastropod larval rearing. Ballantine & Appeldoorn[20] reported an optimal growth rate of *Strombus gigas* at the density of 100 larvae $L^{-1}$. Similarly, in this study, the larvae were reared at a density of 100 nos/L. The larvae reached metamorphic competence on 30 dph by developing antennae of equal length and prominent bulging of the eye ball. This is in agreement with Jagadis *et al*.[10] who observed similar changes in *C. ramosus*. From 35 to 40 dph, the pre-juveniles only increased in shell size, settled and crawled at the bottom of the rearing tank, however, the presence of active velum indicating that though they had reached the metamorphic competence on 30 dph there was no sign of velar resorption for complete metamorphosis. Authors working on gastropod larval rearing have considered that larvae become competent to metamorphose into juveniles only when the velar lobes are resorbed[8,10,12,21]. In the present study no spontaneous metamorphosis was observed and hence it is assumed that it requires some kind of cue to induce metamorphosis to become juveniles. Cob[21] reported that larvae of *Strombus canarium* reached metamorphic competence at 17 – 23 dph and metamorphosed only when settlement cues were sensed. However, the findings made by Jagadis[8,10] on *Lambis lambis* and *Chicoreus ramosus* indicates that there is a possibility of very meagre percentage of larvae settling and developing into juvenile naturally. But for large scale successful settlement they do require an external 'cue' at an appropriate time. In the present study, presence of active velum even after 40 dph indicated that the competency to metamorphosis was not reached by the pre-juveniles that became feeble and complete mortality was occurred thereafter which strongly suggest the need for a suitable settlement cue. Similar to the present findings, Roller & Stickle[22] recorded mortality after 53 days of larval development without showing signs of metamorphosis in a muricid gastropod *Stramonita haemastoma haemastoma* which also points to the need for an appropriate 'cue' at appropriate time for complete metamorphosis and settlement as juveniles.

## Conclusion

Studies related to controlled spawning, intracapsular and post hatch larval development of *P. trapezium* is scanty. The present attempt is first of its kind in tropical waters and throws light on the broodstock maintenance, broodstock feed, successful captive spawning and larval rearing of *P. trapezium* under controlled condition for this important species. It warrants, further research focussing on improved larval rearing ambience, larval nutrition, pathology and emphasis on developing an appropriate 'cue' for juvenile settlement that might culminate in development of a technology package for controlled breeding of Endangered, Threatened and Protected marine gastropods.

## Acknowledgements

The authors are thankful to the Director, ICAR-CMFRI, Kochi for constant encouragement. Thanks are due to the Scientist-in-Charge, Tuticorin Research Centre, Thoothukudi for his continued support and encouragement during the period of research. Our sincere thanks are also due to Dr. K. S. Mohammed, Head, Molluscan Fisheries Division, CMFRI, Kochi for his constant support and critical reviewing of the manuscript.

## Conflicts of Interest

All authors of this manuscript are declaring that no conflict of interest present in this manuscript.

## Author Contributions

IJ: Concept, designing of experiment, data assessment, interpretation and writing. MK: Collection, experimental monitoring, data generation, compilation and drafting. DLP: Experimental monitoring, data generation, compilation, micro photographing and drafting. JP: Technical assistance in collection, experiments and documentation of data.

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