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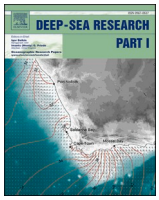

Reproductive traits, dynamics of oogenesis and embryonic developments in the deep-sea shrimp *Plesionika semilaevis* from the southeastern Arabian sea

S. Sreelakshmy [a,b], Rekha Devi Chakraborty [a,*]

[a] Shellfish Fisheries Division, Central Marine Fisheries Research Institute, Ernakulam North P.O., P.B. No. 1603, Cochin, 682018, Kerala, India
[b] Cochin University of Science and Technology, South Kalamassery, Kochi, Kerala, 682022, India

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ABSTRACT

Reproductive traits are a crucial biological indicator of a population's resistance to anthropogenic and environmental pressures. The reproductive biology of *Plesionika* species from temperate and sub-tropical regions is relatively well known. However, a knowledge gap exists regarding the species' reproduction and life strategies in tropical waters like the Indian Ocean. This paper covers the reproductive period, ovarian and embryonic development, embryo size, brood size and size at sexual maturity of *Plesionika semilaevis*, a commercially important species from the tropical waters of the southeastern Arabian Sea. Histological analysis revealed asynchronous oocyte development with multiple oocyte stages present in each ovary sample, although with varying proportions. Maturation of the ovary was seen during the embryo incubation phase, which indicates that females are able to spawn several times during a reproductive cycle. A total of 1802 females of *P. semilaevis* were examined and sorted into ovigerous and non-ovigerous stages, and the embryos were divided into four stages of development. Although ovigerous females were present in every month's samples, the main reproductive season is from November to February, as evidenced by the larger percentage of ovigerous females during this time. Based on the percentage of embryos in various stages, the spawning and hatching periods were estimated to be December to February and March to May, respectively. Embryo size increased with the developmental stage ($p = 0$) but was independent of body size ($p > 0.05$). A positive correlation was observed between brood size and female body size in all four stages of embryo development but, as the embryos developed a significant decrease in brood size was noticed. Based on the size at which 50% of the females had matured ovaries and 50% were ovigerous, the size at sexual maturity, CL_{50ov} and CL_{50em}, was calculated to be 15.51 mm and 15.07 mm in carapace length, respectively. The brood size ranged from 2093 to 12887 embryos with a mean size of 0.51 × 0.40 mm following spawning and 0.66 × 0.50 mm prior to hatching.

1. Introduction

Commercial fisheries are expanding into deeper waters, and the paucity of information on the life histories of the deepwater species being fished is considered a significant barrier to developing and implementing effective management strategies. The specific life-history characteristics of many deep-sea organisms like longer life span, slow growth, late sexual maturity and low fecundity make them especially vulnerable to depletion with a limited potential to rebound from overexploitation (Cheung et al., 2005; Morato et al., 2006). The dearth of life history information for the exploited deep-sea species poses a significant obstacle to the development and use of effective management strategies (Polidoro et al., 2008).

The fecundity, sex ratio, gonadal and embryonic development and periodicity are essential variables to consider when evaluating the recruitment, reproductive potential, biogeographic dispersion, adaptations to specific habitats and connectivity of marine species (Chemel et al., 2023). A species' life strategy is directly linked to its reproductive behavior. Inadequate environmental conditions in deep-sea environments, such as lack of seasonality in temperature, photoperiod, and food availability, result in low reproductive success (Bauer, 2004).

Pandalidae, a family of tropical-water dwelling deep-sea shrimps, have distinctive growth and reproduction habits. Even within the same genus, pandalid species exhibit a diversity of sexual systems (gonochorism and hermaphroditism) and kinds of reproduction (semelparity and iteroparity). As a result, reproductive strategies vary greatly among

* Corresponding author.
E-mail address: rekhadevi7674@gmail.com (R.D. Chakraborty).

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pandalid species (Correa and Thiel, 2003; Bauer, 2004). Genus *Plesionika*, under the family Pandalidae represents a significant group of deep-sea caridean shrimps represented by more than 100 species distributed worldwide. A characteristic feature of many pandalid shrimps is the energy they allocate for egg production (Corey and Reid, 1991; Wehrtmann and Lardies, 1999). The energy and nutrients found in the egg help the embryos survive and develop, thus having a significant impact on the life-history characteristics of invertebrates (Begon et al., 1996; Figueiredo et al., 2008). A fair amount of information is available regarding the reproductive biology of *Plesionika* species found in various temperate and subtropical climates, however, there exists a knowledge gap when it comes to tropical waters. Significant reproductive studies of *Plesionika* species are of *P. heterocarpus. P. edwardsii, P. narval, P. gigliolii, P. martia* and *P. acanthonotus* from the Mediterranean Sea (Company and Sarda, 1997; Colloca, 2002; Maiorano et al. .2002; Anastasopoulou et al., 2017), *P. izumiae* from the Japanese waters (Omori, 1971; Ahamed and Ohtomi, 2011), *P. martia* from Ionian Sea (Chilari et al., 2005) and *P. edwardsii* from the Tyrrhenian Sea and North Atlantic (Possenti et al., 2007; Triay-Portella et al., 2017). Despite the increasing commercial interest in *Plesionika* species in Indian waters, contributing to 21% (*P. semilaevis* and *P. quasigrandis*) of the deep-sea shrimp catches (Chakraborty et al., 2022), aspects of the reproductive biology of these species remain almost unknown.

Plesionika semilaevis (Bate, 1888) is one of the commercially and ecologically important deep-sea shrimp species found at a depth of 250 m and above in the southeastern Arabian Sea, contributing 1.23% of the deep-sea shrimp catches in the south-west coast of India (Chakraborty et al., 2022). It plays a critical role in deep-sea food webs as an intermediate trophic-level organism (Sreelakshmy et al., 2023). A study done by Ohtomi (1997) described the reproductive parameters like brood size and reproductive period of *Plesionika semilaevis* from Kagoshima Bay and is the only report available on the reproductive aspects of this species. Detailed studies on the reproductive biology of *Plesionika semilaevis* including ovarian and embryonic development from Indian waters and any other geographical area with its distribution are lacking.

Information on ovarian maturation, embryo development, reproductive period, and brood size is beneficial from a fisheries perspective for understanding the reproductive biology of the species, estimating reproductive potential, and recruitment for maintaining the species' population stability (Mori et al., 1998; Figueiredo et al., 2008; Kevrekidis and Thessalou-Legaki, 2013). Against this background, this study aims to investigate the detailed aspects of the reproductive biology of *Plesionika semilaevis* along with the developmental stages of the ovaries and embryos by macroscopic and microscopic analysis. The study also sought to determine the species' spawning season, which will give the essential prerequisite for stock assessment and stock sustainability and establish successful fishery management.

2. Materials and methods

2.1. Sampling

Samples of deep-sea shrimps were collected fortnightly between August 2019 and May 2022, except during the monsoon ban period (June to August) from about 5 to 6 deep-sea bottom trawlers during each sampling. The trawlers have an overall length of 15–40 m and are powered with high horse power engine (350–500 hp).They were operated at a depth of 150–250 min the southwest coast of India, with a 25–26 mm mesh size in its cod-end. The samples were transported in fresh condition in an insulated ice box to the laboratory for further analysis. Morphological identification of *P. semilaevis* followed the taxonomic keys of Chace (1985), Ohtomi and Hayashi (1995), Chan and Crosnier (1997), Ahamed et al. (2017), Kuberan et al. (2020) and Chan et al. (2020). By examining the morphology of the endopod of the first pair of pleopods, the sex of an individual was determined (King and Moffitt, 1984).The females were sorted into ovigerous and non-ovigerous states based on the presence of embryos between the pleopods. Carapace length (CL) and total length (TL) were measured to the nearest 0.01 mm using calipers. An electronic digital balance with a 0.001 g precision was used to measure the body weight (BW).

2.2. Gametogenesis and stages of ovarian development

Macroscopic and microscopic examination was used to establish the stages of female gonadal development. For macroscopic analysis, all individual shrimps were dissected and their ovaries were examined under a stereoscope and categorized into five developmental stages based on location in the cephalothoracic cavity, shape, size, and relative intensity of the color of ovary (Maiorano et al., 2002; Anastasopoulou et al., 2017; Triay-Portella et al., 2017). The mature ovary occupied most of the cephalothoracic cavity's dorsal region, extending anteriorly to the orbital margin visible over the carapace as blue head roe.

Microscopic analysis of each development stage of ovaries was carried out by selecting10 specimens from each stage. The ovary was removed and fixed in 5% formalin for 24 h for additional histological examination. The fixed tissues were dehydrated in ethanol solutions, cleared in xylene, embedded in paraffin and sliced into 5–6 μm sections. The sections were stained with hematoxylin followed by eosin (Triay-Portella et al., 2014) and mounted for further microscopic analysis. The developmental changes in the ovary were observed using a compound microscope (Leica DM 750) at a magnification of 4x-100x.The oocytes were classified into different developmental stages based on the size of cells, cytoplasmic appearance and presence of lipid globules and yolk granules.

2.3. Stages of embryo development, brood size and embryo size

All females were examined for the presence of different stages of embryos attached to the pleopods. The embryos were carefully separated from the pleopods and categorized into four stages based on the color of the embryos. These embryos were then observed under a stereoscopic microscope (Nikon SMZ1270, Japan) to observe the different changes in embryos with development.

The total weight of embryos was weighed to the nearest 0.001 gm, a subsample was weighed, and the number of embryos was counted under a binocular microscope. The brood size was estimated for ovigerous females with embryos in different stages of development as follows:

$$\text{Brood size} = \frac{\text{Number of embryos in the subsample} * \text{Total weight of the embryos}}{\text{Weight of the subsample}}$$

The variations in brood size relative to the body size (CL) of females carrying embryos at different developmental stages was also determined. For each stage of embryo development, the sizes of the embryos were calculated using a total of 100 ovigerous females of each stage. The major (length) and minor (width) axes of 10–20 randomly chosen embryos of each individual were measured under a stereomicroscope with an ocular micrometer to the nearest 0.01 mm. Each individual's mean embryo length and width were calculated and finally, mean embryo sizes (length and width) for each embryonic stage were computed. Embryo volume (v) was estimated for each embryonic stage using the formula given by Turner and Lawrence (1979):

Embryo volume (v) $= \pi\, d_1^2 d_2/6$

where d_1 is the minor axis (mm) and d_2 is the major axis (mm).

One-way analysis of variance (ANOVA) was carried out with the help of SPSS statistical software in order to determine the variation in developmental stages with embryo size (length, width and volume) and female body size (CL).

2.4. Reproductive period, sex ratio, GSI and size at sexual maturity

The reproductive period was investigated by considering the

percentage of ovigerous females and their maturity stages of ovaries and embryos. The frequency of females with various stages of embryo development was noted month-wise to estimate the spawning and hatching periods. Monthly variation in the sex ratio (females/males) was also calculated and their deviation from the expected 1:1 were tested using the chi-square analysis.

Gonad weight (GW), of females were taken to the nearest 0.001 g, and the gonadosomatic Index (GSI) was calculated as the percentage weight of the gonad to the total body weight (Grant and Tyler, 1983).

Size at sexual maturity (CL_{50}) was determined based on two factors, i.e., the CL length at which 50% of females are ovigerous (CL_{50em}) and the CL at which 50% of the females have mature ovary (CL_{50ov}) (Triay-Portella et al., 2017). This was estimated by a logistic equation as described by King (1995): $P = 1/1 + \exp(a + b * CL)$, (R Studio, version 4.1.1.).

where P is the predicted proportion of ovigerous females/females with matured ovaries, a and b are the estimated coefficients of the logistic equation, and CL is the carapace length.

3. Results

3.1. Size structure

Males and females are sorted out based on the morphological sexual differences between them. Sex was determined based on the shape of the endopod of the first pleopods. The endopod of females has a pointed leaf-like structure, while that of males has a blunt rectangular shape (Fig. 1). A total of 997 males and 1802 females consisting of 1233 ovigerous and 569 non-ovigerous individuals were examined for this study. Ovigerous females had a CL range of 12–24 mm (TL 72–136 mm) and non-ovigerous females had a CL range of 10–24 mm (TL 67–132 mm).

3.2. Gametogenesis and ovarian development

Ovaries are paired organs with a tubular structure and bilateral symmetry located dorsally in the cephalothoracic cavity overlying the digestive gland. Significant macroscopic variations were observed in the ovaries and categorized into five stages based on the relative intensity of color, size and position of the ovaries (Fig. 2). The GSI values ranged between 0.093 and 4.33. Histologically ovary consists of several layers of developing oocytes encased by a thin gonadal wall. Oogonia, early previtellogenic oocyte, late previtellogenic oocyte, early vitellogenic oocyte, late vitellogenic oocyte and atretic oocyte are the different stages of oocyte development. These phases could be distinguished by their differences in cell size, shape, cytoplasmic appearance, accumulation of yolk granules, and presence of lipid globules in the cytoplasm.

Stage 1 refers to immature females with thin translucent cream-colored gonads that are not visible through the carapace and weigh between 0.002 g and 0.01 g. The GSI values ranged between 0.093 and 0.25. Ovaries at stage 1 present a predominance of oogonia and early previtellogenic oocytes. Oogonia were spherical cells found in clusters in the center of ovaries known as germinal zone (Fig. 3a) and had a diameter of 0.004–0.005 mm, possessed a large and well-defined nucleus, non-evident nucleolus, homogenous thin rim of basophilic cytoplasm without granulation and strongly reactive to hematoxylin (Fig. 3b). Early previtellogenic oocytes are spherical with a diameter of 0.02–0.03 mm were round to elliptical with thin granulation in the cytoplasm (Fig. 3c). They have a smaller nucleus, visible nucleoli and are less basophilic than oogonia. Although follicular cells are present, they aren't associated with the oocytes (Fig. 3d).

Stage 2 refers to developing females with pale creamy blue gonads visible through the carapace weighing 0.009 g–0.06 g. The GSI values ranged between 0.66 and 1.38. The ovaries appear more turgid and histologically disorganized, with abundant connective tissues. Oogonia, early previtellogenic oocytes, late previtellogenic oocytes and follicle cells were observed. Follicle cells do not appear to be associated with the oocytes. Late previtellogenic oocytes are basophilic, semi-spherical, and a little larger, with a diameter of 0.045–0.055 mm. The nucleus is large, with nucleoli visible in the interior (Fig. 3e).

Stage 3 refers to early mature females with light blue ovaries weighing between 0.029 g and 0.12 g, clearly visible through the exoskeleton. The GSI values ranged between 1.67 and 2.43. At stage 3, the ovaries had two distinct zones: the central germinal zone, which contained oogonia, early and late previtellogenic oocytes, and the peripheral growth zone, which included late previtellogenic oocytes, early and late vitellogenic oocytes associated with the follicle cells. During vitellogenesis, the ooplasm becomes acidophilic due to the

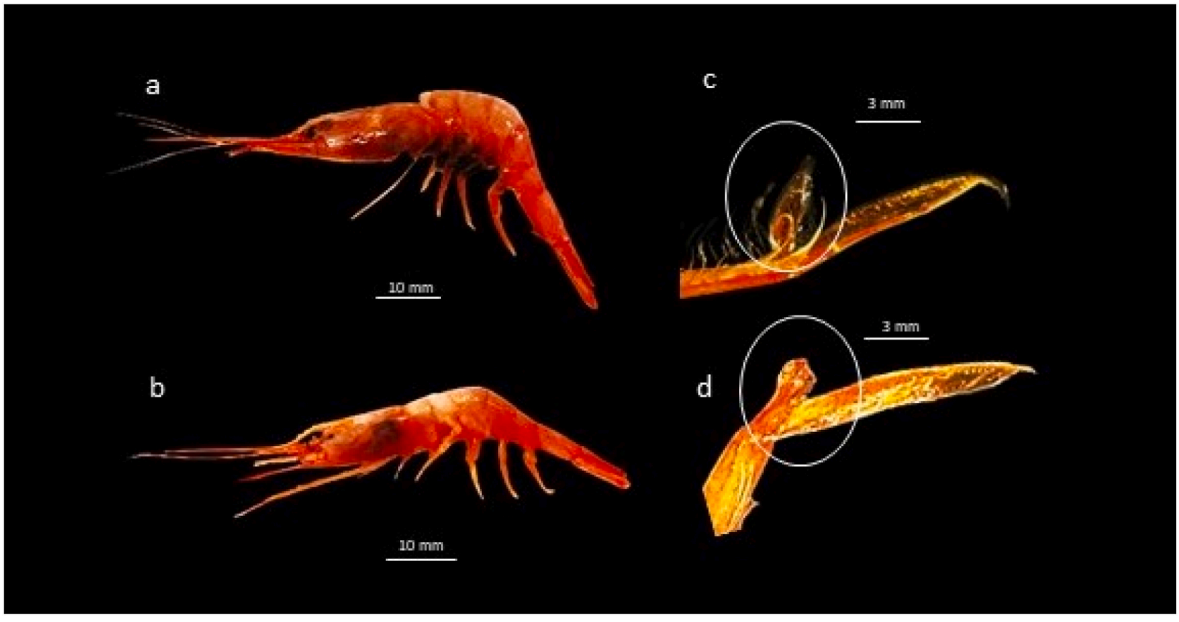

Fig. 1. Ovigerous (a) and non-ovigerous (b) females of *P. semilaevis*, (c) leaf-shaped endopod of first pleopod in females (c) and blunt-shaped endopod of first pleopod in males (d).

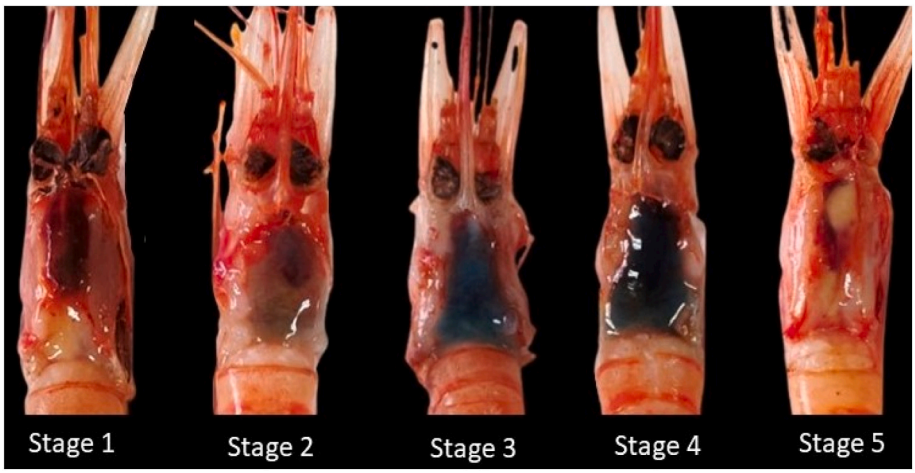

Fig. 2. Macroscopic variation in different stages of ovarian development.

accumulation of yolk granules, which results in a pink stain. Early vitellogenic oocytes had a diameter of 0.1–0.2 mm and were marked by vitellogenic granulations, lipid globules and a thick chorion (Fig. 3f). Lipid globules have a transparent appearance and are primarily observed in the central part of the cell, while yolk granules have an opaque appearance and are distributed evenly (Fig. 3g). Late vitellogenic oocytes are the largest cells with a diameter of 0.3–0.5 mm and a granular appearance due to an abundance of yolk granules and lipid globules.

Stage 4 refers to fully mature females with dark blue head roe. The ovaries are dark bluish-black in color, turgid and occupying the entire space in the cephalothoracic cavity extending up to the abdomen, weighing between 0.08 g and 0.22g. The GSI values ranged between 3.0 and 4.33. At stage 4, ovaries revealed a confined germinal zone enveloped by advanced late vitellogenic oocytes. Late vitellogenic oocytes are large and irregularly shaped, with cytoplasm wholly filled with dense yolk granules and lipid globules, hindering the visualization of the nucleus (Fig. 4a and b). At this point, oocytes have grown to their entire size and are encircled by a layer of flattened follicle cells.

Stage 5 refers to spent females with cream-colored flaccid ovaries. These ovaries have an unorganized appearance and are distinguished by the abundance of connective tissue, follicular cells, and atretic oocytes that are undergoing reabsorption (Fig. 4c). Atretic oocytes in the reabsorption process can be easily identified by vacuolated cytoplasm (Fig. 4d). Even though the immature and spawned stage ovaries have similar coloration, the presence of atretic oocytes is the primary feature that allows for microscopical differentiation between the two stages. The proportion of oogonium also increases in this stage.

3.3. Stages of embryo development, brood size and embryo size

Four different stages of eggs with distinct color variations were observed by morphological examination (Fig. 5). In stage I, the embryos are bright blue in color, lacking eye pigmentation. Yolk filled 80–100% of the embryo volume. In stage II, the embryos have a bluish-green appearance. Minute eye pigmentations are visible under the microscope but not to the naked eye. Ventral cleft starts to appear with the visible demarcation of cephalothorax and abdomen. In stage III, embryos are pale olive-green colored with eye pigmentation clearly visible. Embryos have large cephalothoraces, extended abdomen, visible segmentation, rudimentary appendages and distinctly clear ventral cleft. In stage IV, embryos brown-colored appearance because of the mucous-like substance holding the embryos together. The embryos are ready to hatch, with their segmentations completed and eye spots fully pigmented.

The overall mean brood size (±SD) was 6413 ± 2457 (ranging from 2093 to 12887). The brood size also varied based on the different stages of the development of embryos. The females with stage I and stage II embryos (non-eyed stages) had the highest brood sizes 8324 ± 2076 (4166–12887 embryos) and 6981 ± 1866 (4068–11291) embryos respectively. The embryos were more compact and clustered together tightly. Significant embryo loss was observed during the development of the embryos. In stage III and stage IV (eyed stages), the brood size was reduced to 5179 ± 1265 (3004–8411) and 3449 ± 970 (2093–6048) embryos respectively. Embryo loss was estimated to be 59.6% from stage I to stage IV embryos.

The brood size increased significantly with the female sizes for all the stages of embryo development. The maximum brood size was 7502 for smaller individuals (CL 12–15 mm), 10813 for medium-sized individuals (CL 16–19 mm) and 12887 for larger individuals (CL 20–24 mm). A significant positive correlation was observed between the brood size of embryos at different developmental stages and carapace length (Fig. 6).

Table 1 displays the mean length and width of the embryos and the mean volume of the embryos at each developmental stage. The embryo length increased gradually with each developmental stage. Such a gradual change was not observed for embryo width, but a sharp increase in width was observed from stage II to stage III. The embryo volume remained in the range of 0.043–0.051 mm^3 during the first two stages but started to increase significantly to 0.091 mm^3in the later stages. The embryo's volume increased by 109% from stage I to stage IV. Analysis of variance showed a significant variation in embryo length ($p = 0$; $F = 928$), width ($p = 0$; $F = 1725.5$) and volume ($p = 0$; $F = 2584$) with each developmental stage, however, the embryo developmental stages did not differ much with the size of the female ($p < 0.05$; $F = 2.918$).

3.4. Reproductive period, sex ratio, GSI and size at sexual maturity

Fig. 7 illustrates the monthly variations in the proportion of *P. semilaevis* ovigerous females carrying embryos at various developmental stages. Although ovigerous females were present in every month's sample, their frequency changed on a monthly basis. The percentage of ovigerous females increased from November to February (66.4%–84%) and decreased from March to October (67.7%–45.2%), with the least number of ovigerous females present in September (35.7%).The percentage of ovigerous females incubating stage I embryos peaked in December and January. Ovigerous females with stage II embryos showed a similar peak from November to February. The proportion of embryos with eyed stages i.e., stages III and IV, increased steadily from January to May and April, respectively. The stage IV

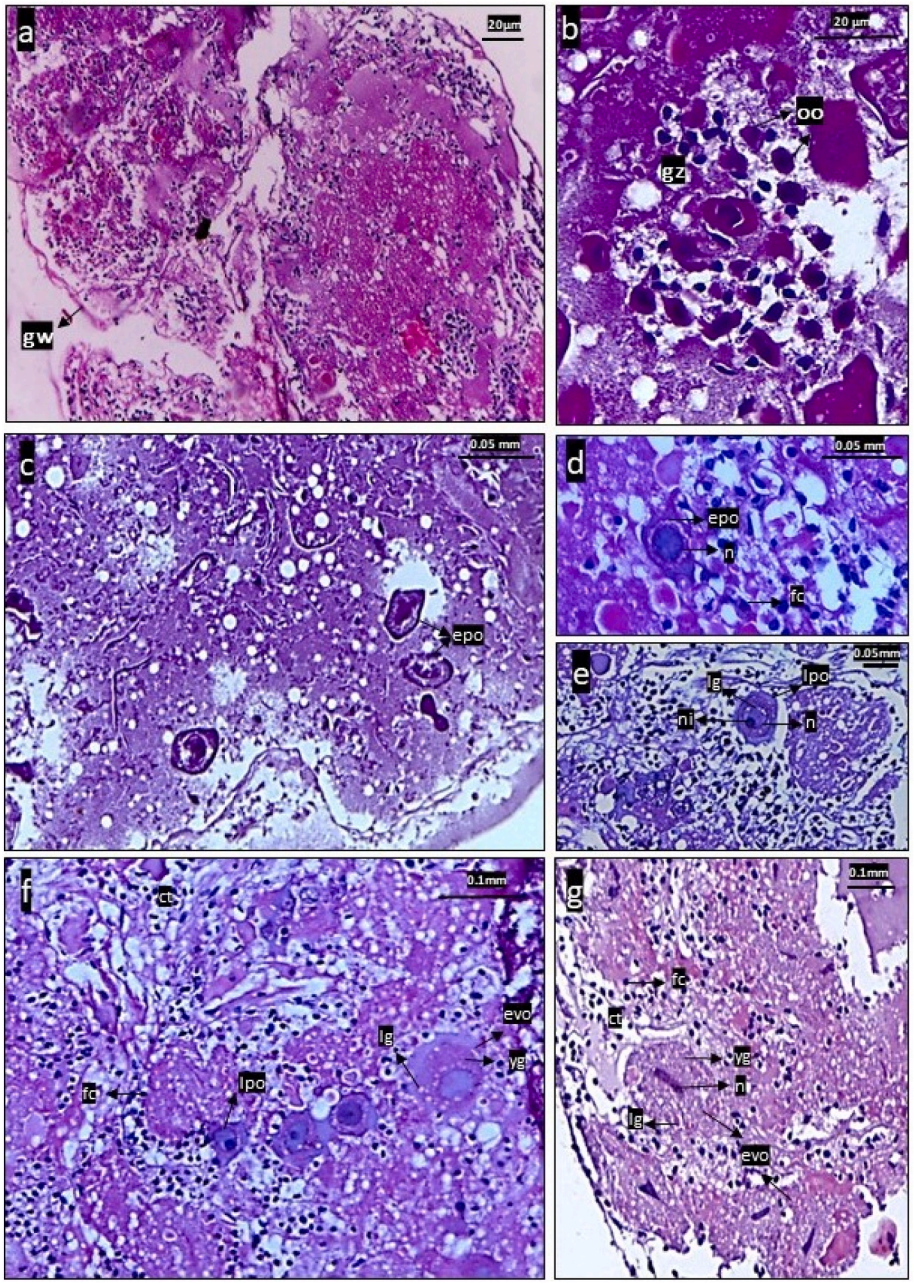

Fig. 3. Histological sections of different stages of ovary of *P. semilaevis*: Stage 1 (a,b), Stage 2 (c,d,e), Stage 3 (f,g); gz, germinal zone; oo, oogonium; epo, early previtellogenic oocyte; n, nucleus; ni, nucleoli; fc, follicular cells; lpo, late previtellogenic oocyte; evo, early vitellogenic oocyte; lg, lipid globules; ct, connective tissue; yg, yolk granules.

embryos, just before hatching, were highest from March to May, suggesting a significant hatching period. All ovigerous females with stage I bright blue embryos had cream-colored ovaries, either immature stage 1 ovaries preparing for the next brood or atretic ovaries with released oocytes. Over half of the females with stage II embryos had pale cream-colored developing ovaries. All mature females with stage IV eggs and more than half of the females with stage III eggs had blue-colored ripe ovaries, indicating upcoming spawning and new brood.

The overall sex ratio (males: females) was 1:1.8, which is significantly different from the expected sex ratio (1:1). Females outnumbered males in all the months studied. The highest percentage of females was observed during February (73.8%) and the least during October (52.7%). Monthly variation in sex ratio and results of chi-square analyses are provided in Table 2.The monthly sex-ratio showed significant variation from the hypothetical ratio 1:1 for all the months except October.

Size at first maturity, determined by the presence of embryos between pleopods (CL_{50em}), was found to be 15.07 mm CL (Fig. 8a). Size at first maturity determined from pooled early mature (Stage 3) and fully mature (Stage 4) ovarian stages (CL_{50ov}) was estimated at 15.51 mm CL (Fig. 8b).

4. Discussion

Several aspects, including reproductive period, ovarian and embryonic stages of development, embryo size, brood size, size at maturity and sex ratio, were studied for *P.semilaevis* from the southeastern Arabian Sea.

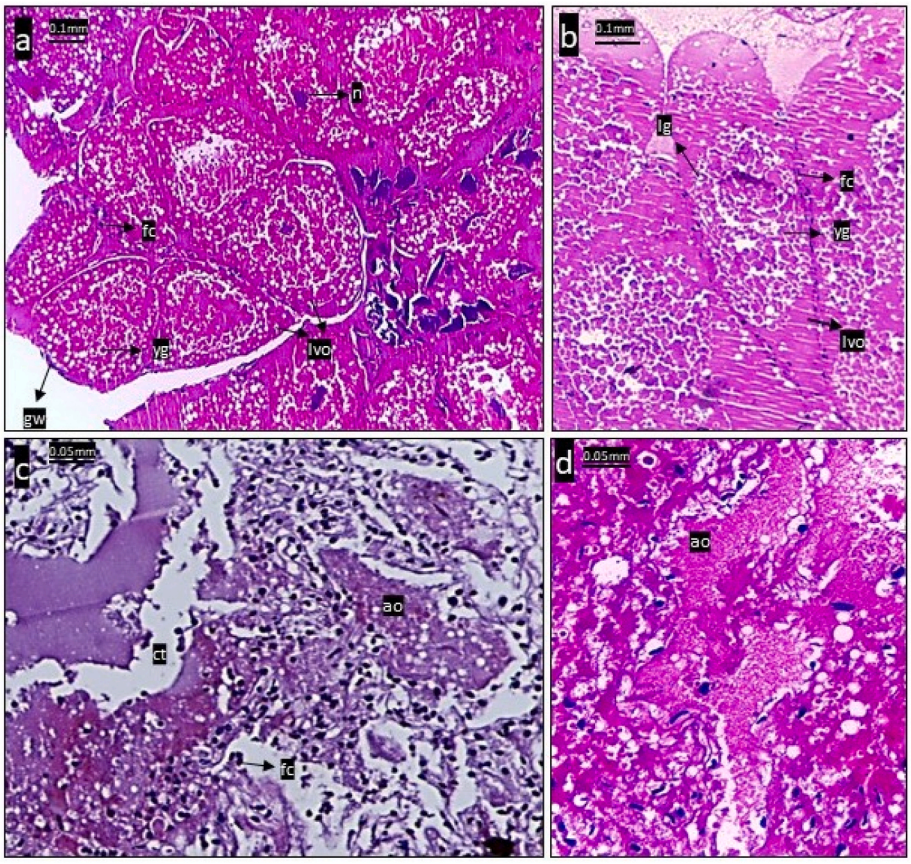

Fig. 4. Histological sections of different stages of ovary of *P. semilaevis*: Stage 4 (a,b), Stage 5 (c,d); fc, follicular cells; gw, gonadal wall; lvo, late vitellogenic oocyte; lg, lipid globules; ct, connective tissue; yg, yolk granules; n, nucleus; ao, atretic oocytes.

Based on the color and position of the ovary in the cephalothoracic cavity, five ovarian maturation stages were recognized in the females of *Plesionika semilaevis*. The classification of ovarian development in detailed stages is essential to gather comprehensive data for future reproductive and population dynamics studies. Mature ovaries are bright dark bluish-black, visible through the carapace as a head roe. Ohtomi and Hayashi (1995) and Ohtomi (1997) described the mature ovary as blue for *P. semilaevis*, Maiorano et al. (2002) as greenish blue for *P. martia*, Colloca (2002) and Possenti et al. (2007), Triay-Portella et al. (2017) as dark blue for *P. edwardsii* and Ahamed and Ohtomi (2011) as dark yellow for *P. izumiae*. Decapod crustacean ovaries change color during the reproductive cycle due to variations in the amount of carotenoids present during oogenesis (Liñán-Cabello et al., 2002; Gregati et al., 2010), accumulation of yolk granules and the decrease of cytoplasmic organelles during vitellogenic stages.

The histological analysis supported the maturity scale based on the morphology of ovaries. The histology of ovarian tissues and the findings of the GSI analysis indicates that changes in the color and form of the ovaries closely correspond to the development of oocytes. The average GSI value increased from 0.093 in females with stage 1 ovary to 4.33 in females with stage 4 ovary. The central region of the ovary showed the presence of oogonia and previtellogenic oocytes. In contrast, more developed vitellogenic oocytes were present towards the periphery of the ovary, corroborating Braga et al. (2009). As observed, the oogonia proliferating in the germinal epithelium develops into previtellogenic oocytes, moving to the growth zone between the large vitellogenic oocytes to form small germinative islets. The high number of nucleoli inside the nucleus of previtellogenic oocytes suggests that they have become quite active. The enlargement of the growing vitellogenic oocytes mainly results from the expansion of the cytoplasm, which has become acidophilic due to the presence of yolk granules and lipid droplets. Follicle cells form a layer around the vitellogenic oocytes. Follicular cells either synthesize vitellogenin and secretes it into the hemolymph, which is later collected by the oocytes (Yano and Chinzei, 1987), or may take part in the collection of yolk proteins into the oocytes. The identification and differentiation of the immature and spawned stages primarily rely on the atretic oocytes observed. Since the ovaries at the immature and spawned stages have the same coloration and the same germinative cells (oogonia and early previtellogenic oocytes) are primarily found in these stages, atretic oocytes were considered to be the key trait in their histological differentiation.

The brood size ranged from 2093 to 12887 embryos. For all four embryonic stages, there was a positive link between shrimp size (CL) and brood size. Ohtomi (1997) observed a smaller brood size (1048–8702 embryos) in *P. semilaevis* from the subtropical waters of Kagoshima Bay, Japan. The physical space between the pleopods available for the attachment of the embryos is the cause of this correlation. A significant decrease in brood size during incubation was observed in the ovigerous females of *P. semilaevis*. Similar observations were reported for the other *Plesionika* species (Ohtomi, 1997; Maiorano et al., 2002; Ahamed and Ohtomi, 2011; Triay-Portella et al., 2017) and other caridean shrimps (Penha-Lopes et al., 2007; Echeverría-Sáenz and Wehrtmann, 2011; deMoraes et al., 2017). In caridean shrimps, embryo loss during incubation is a common observation that can be attributed to a number of biological (parasites, micro predators, etc.) and physical (mechanical stress, increase in embryo size, etc.) factors (Balasundaram and Pandian, 1982; Kuris, 1991; Oh and Hartnoll, 1999; Hernáez et al., 2010). Since no parasites and micro predators were observed in the present study, the embryo loss could be due to the restricted space to carry the embryos beneath the pleon due to increased embryo volume (Suh et al., 2003).

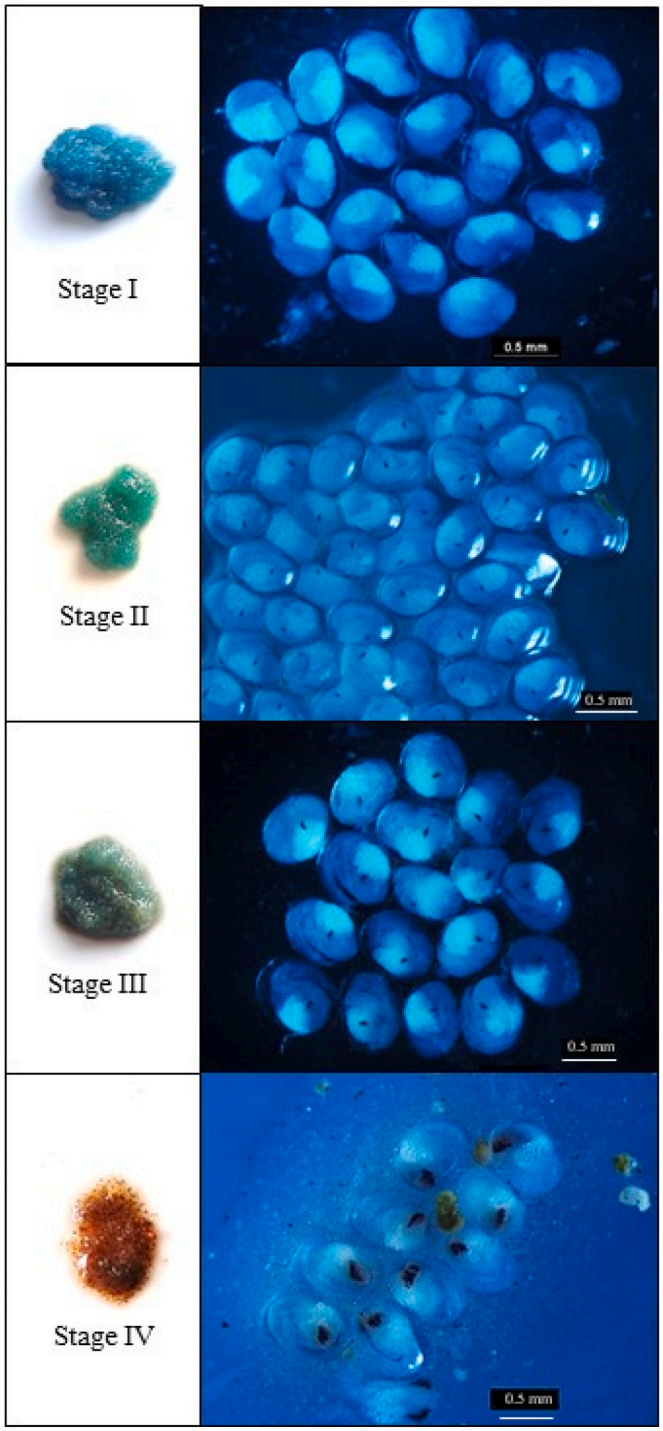

Fig. 5. Macroscopic (left column) and microscopic (right column) variation in different stages (I-IV) of embryo development in *P. semilaevis*.

Fishing methods can also contribute to embryo loss in all stages.

The mean embryo sizes following spawning and prior to hatching were 0.51 × 0.40 mm and 0.66 × 0.50 mm, respectively. Ohtomi (1997) also reported a similar pattern of increasing embryo size with each development stage in *P. semilaevis* from Kagoshima Bay. He observed a mean embryo size of 0.60 × 0.56 mm just after spawning and 0.78 × 0.53 mm prior to hatching, which is higher than our results. The volume of the embryos also increased with each development stage. The increase was very distinct from stage II to stage III (0.05 mm^3–0.085 mm^3) with the development of rudimentary appendages, ventral cleft, and large cephalothoraces. However, this increase in embryo volume during incubation is common in crustaceans due to the rise in water content and biochemical changes in the embryo during development (Lardies and Wehrtmann, 1997; Wehrtmann and Kattner, 1998).

Ovigerous females were present in all the sampling months, with a peak from November to February, indicating this to be the main reproductive period. Ohtomi (1997) reported the occurrence of ovigerous females of *P. semilaevis* in Kagoshima Bay, Japan, from May to December and suggested the influence of photoperiod on species reproduction. According to Omori (1971), *P. izumiae* breeding in Suruga Bay was frequent as the water warmed in the late spring and summer. Studies on the reproduction of caridean shrimps have shown that water temperature significantly influences spawning (Kikuchi, 1962; Allen, 1966; Maiorano et al., 2002; Vafidis et al., 2005). According to Bauer (1992), neritic species of caridean shrimp in temperate waters will have shorter breeding periods than those in subtropical and tropical areas. Due to generally constant and high temperatures, the reproductive cycle is prolonged and year-round in tropical waters (Orton, 1920).The tropical climate prevailing in the southeastern Arabian Sea could be the reason for its continuous reproductive activity.

Stage I and stage IV embryos occurred all months, suggesting that this species is iteroparous. However, the prevalence of ovigerous females carrying stage I embryos rose from November to February, with peaks in December and January indicating their primary spawning season. Stage IV embryos had a higher occurrence from March to May, suggesting this to be their significant hatching period. Ovigerous females carrying stage I embryos lacked mature ovaries, whereas ovigerous females carrying embryos in stage IV, which were close to hatching, had mature ovaries. Additionally, all maturity stages were visible in the ovaries of non-ovigerous females who were larger than the size at sexual maturity, suggesting that once *P. semilaevis* achieves sexual maturity, it spawns multiple times during a year. This reproductive pattern has been described for various tropical and temperate deep-sea shrimps (Bauer, 1989; Company and Sarda, 1997; Ohtomi, 1997; Maiorano et al., 2002; Anastasopoulou et al., 2017; Paramasivam et al., 2018; Kuberan et al., 2021).

The maximum CLs observed by Ohtomi (1997) was 19.25 mm in females, while in this study, the maximum CL observed was 24 mm. With higher latitudes, shrimps tend to have smaller sizes. Geographical differences in the maximum body size may have resulted from differences in longevity, growth rate, and food availability among the populations, or they may have been caused by physiological growth mechanisms (Ohtomi, 1997). Triay-Portella et al. (2017) preferred the calculation of size at maturity based on ovarian maturation rather than ovigerous condition in the case of *P. edwardsii,* as a significant fraction of non-ovigerous females were mature, leading to an overestimation of size at maturity. In the present study, the size at sexual maturity estimated based on ovarian maturation and ovigerous condition showed similar values since the occurrence of ovigerous females was relatively higher than non-ovigerous females.

In fisheries management, the size at which shrimps of a given population become sexually mature for the first time is an important management parameter used to monitor whether enough juveniles in an exploited stock mature and spawn (Beverton and Holt, 1959; Jennings et al., 1998). The size during sexual maturity is especially significant and is frequently used as a criterion for the minimum permissible capture size (Lucifora et al., 1999). Information on the reproductive parameters of deep-sea resources forms the basis for developing and implementing resource management strategies for the sustainable harvest of the deep-sea fishery. The data gathered in this study can be applied to research on the recruitment dynamics of the species, which will help manage resources more effectively and act as a prerequisite for stock assessment and stock sustainability and establish successful fishery management.

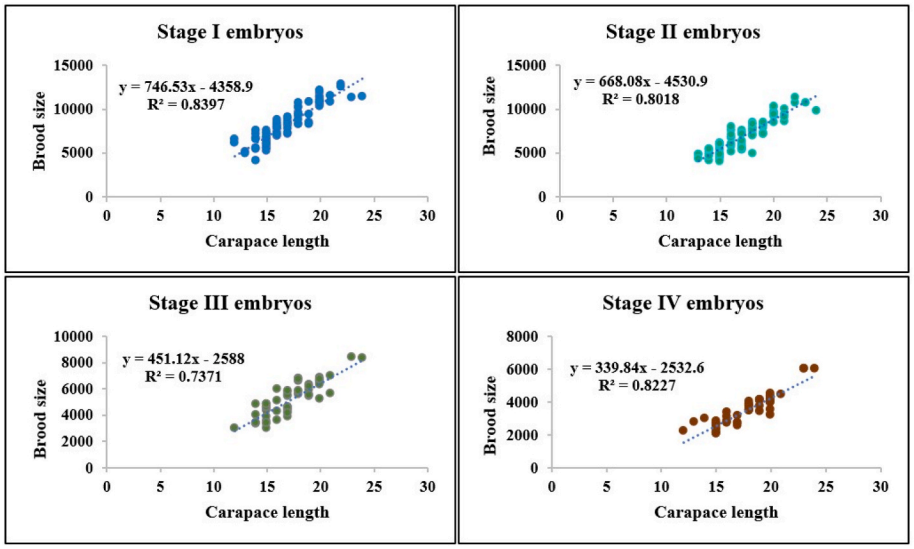

Fig. 6. Relationships between carapace length (mm) and brood size for different stages (I-IV) of embryo development.

Table 1
Mean length, width and volume of embryos of *P. semilaevis* at each developmental stage.

Stage	Embryo length ±SD (mm)	Embryo width ±SD (mm)	Embryo volume ±SD (mm^3)	CL range (mm)	No. of shrimps observed
I	0.511 ± 0.028	0.401 ± 0.024	0.043 ± 0.006	10–23	100
II	0.568 ± 0.019	0.412 ± 0.007	0.051 ± 0.003	12–25	100
III	0.616 ± 0.02	0.513 ± 0.009	0.085 ± 0.003	13–25	100
IV	0.657 ± 0.01	0.51 ± 0.013	0.091 ± 0.005	13–25	100

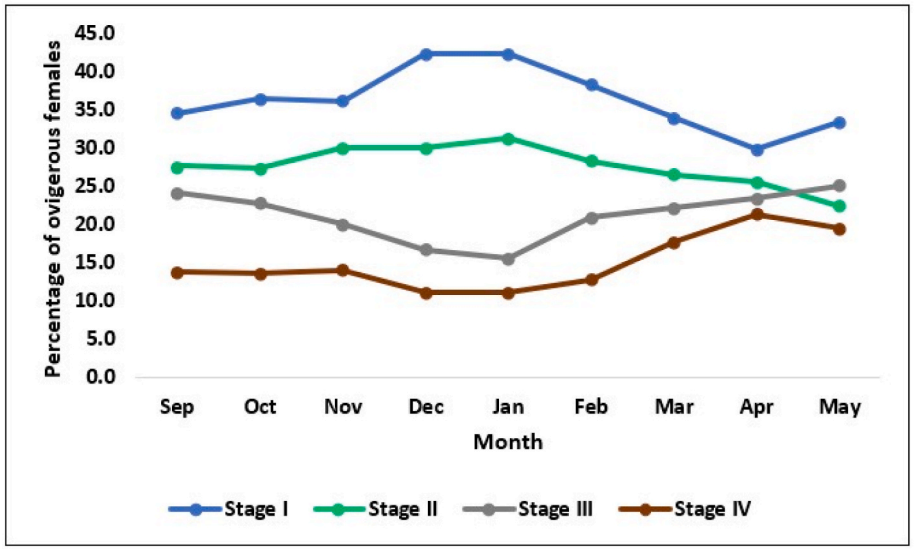

Fig. 7. Monthly changes in percentage occurrence of different developmental stages of embryos in ovigerous females of *P. semilaevis.*

5. Conclusions

This is the first comprehensive study on reproductive parameters, including the ovarian and embryonic development of *P. semilaevis* in Indian waters and other locations where this species is distributed. Females reproduce multiple times a year as evidenced by the presence of ovigerous females in all the sampling months. The major spawning and hatching periods were estimated to be December to February and March to May, respectively. They are sequential breeders i.e., after reaching sexual maturity, they are able to produce subsequent broods, as indicated by their ovarian maturation with embryonic development. The data gathered in this study can be applied to research on reproductive dynamics, which will help manage resources more effectively and advance the ability of the species to reproduce in captivity. These studies are notable for their significance in identifying the different stages of ovarian maturation and embryonic development in detail which helps to obtain accurate information about the length at maturity and the hatching and spawning seasons, which is essential when establishing management measures. They are also significant in determining whether the current closed season is appropriate for the species.

Table 2
Month-wise sex ratio in *P. semilaevis*.

Sl. No	Month	Sex Ratio (Male: Female)	Chi-square value (X^2)	Level of significance
1.	January	1:2.02(100,202)	34.45	$P < 0.01$[a]
2.	February	1:2.82 (142,401)	123.53	$P < 0.01$[a]
3.	March	1:1.44 (178,257)	14.35	$P < 0.01$[a]
4.	April	1:2.16 (45,97)	19.04	$P < 0.01$[a]
5.	May	1:1.81 (37,67)	8.65	$P < 0.01$[a]
6.	September	1:1.55 (74,115)	8.89	$P < 0.01$[a]
7.	October	1:1.11 (123,137)	0.75	$p > 0.05$
8.	November	1:1.61 (146,235)	20.79	$P < 0.01$[a]
9.	December	1:1.91 (152,291)	43.61	$P < 0.01$[a]
10.	Overall	1:1.81 (997,1802)	274.08	$P < 0.01$[a]

[a] Significant.

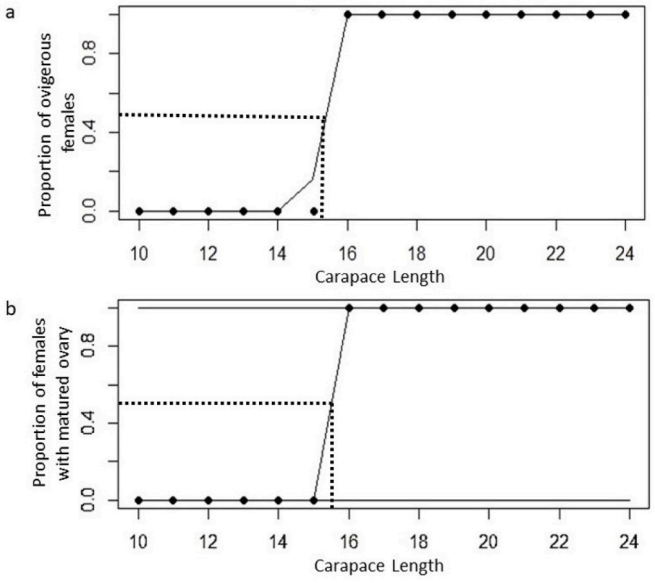

Fig. 8. Graphs for estimating size at first maturity based on the proportion of ovigerous females (a) and matured ovaries (b) of females of *P. semilaevis*.

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Authors' contribution

All the authors participated sufficiently in the work. All authors read and approved the final manuscript.

Sreelakshmy S: Conceptualization, methodology, formal analysis, investigation, writing-original draft.

Rekha Devi Chakraborty: Conceptualization, methodology, resources, supervision, writing-review and editing.

Declaration of competing interest

"The authors declare that they have no conflicts of interest".

Data availability

Data will be made available on request.

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