

Original Article

Reproductive biological characteristics of the polychaete *Dendronereis chipolini* distributed in the Mekong Delta, Vietnam

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Abstract: The reproductive biology of the polychaete *Dendronereis chipolini*, collected from extensive shrimp ponds in coastal areas of the Mekong Delta, was investigated to provide baseline data for future efforts to artificially reproduce the species. Live specimens were examined to determine sex differentiation, fecundity, gonadal development, and patterns of embryonic and larval development. Sex was primarily distinguished by body coloration: mature males exhibited a bright milky-green hue, whereas mature females displayed a dark moss green coloration. Absolute fecundity averaged $185,773 \pm 76,352$ eggs per female, while relative fecundity was $209,520 \pm 31,414$ eggs per gram of female body weight. Histological analysis revealed four distinct stages of oocyte development, characterized by increasing egg diameters ranging from 40-80 μm (Stage I) to 100-120 μm (Stage IV). Embryonic development, from fertilization to hatching, was completed within 7 hours and 30 minutes at a temperature range of 28-30°C. Larval development progressed through three stages: Trochophore, Metatrochophore, and Nectochaete, with the final stage exhibiting morphological features resembling those of adult worms. These findings contribute to the foundational knowledge required for developing reproductive protocols for *D. chipolini* in aquaculture systems.

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Introduction

Polychaetes, commonly referred to as bristle worms, constitute the most diverse and ecologically significant class within the phylum Annelida, with more than 10,000 known species distributed globally (Jumars et al., 2015). Predominantly marine, polychaetes inhabit a broad range of environments, from intertidal zones to deep-sea sediments, where they perform critical roles in the structure and functioning of benthic ecosystems. Their abundance, functional diversity, and adaptability enable them to contribute substantially to essential ecological processes, including bioturbation, nutrient cycling, and trophic interactions. One of the most notable ecological functions of polychaetes is bioturbation—the reworking of sediments through burrowing and feeding activities. This behavior enhances oxygen penetration into sediments, stimulates the decomposition of organic matter, and facilitates

nutrient regeneration (Kristensen et al., 2012). Through these mechanisms, species such as *Arenicola marina* and *Nereis diversicolor* significantly influence sediment chemistry and microbial activity, thereby promoting overall benthic productivity. Additionally, polychaetes contribute to nitrogen and phosphorus cycling by processing organic detritus and releasing waste products that support remineralization and primary production in coastal and estuarine ecosystems (Aller, 1994).

From a trophic perspective, polychaetes occupy various niches—as detritivores, suspension feeders, or predators—and serve as a fundamental food source for higher-trophic-level organisms such as fish, crustaceans, and seabirds (Jumars et al., 2015). This versatility makes them a key component of many marine food webs. Their sensitivity to environmental disturbances also makes them reliable bioindicators in benthic monitoring programs. Shifts in polychaete

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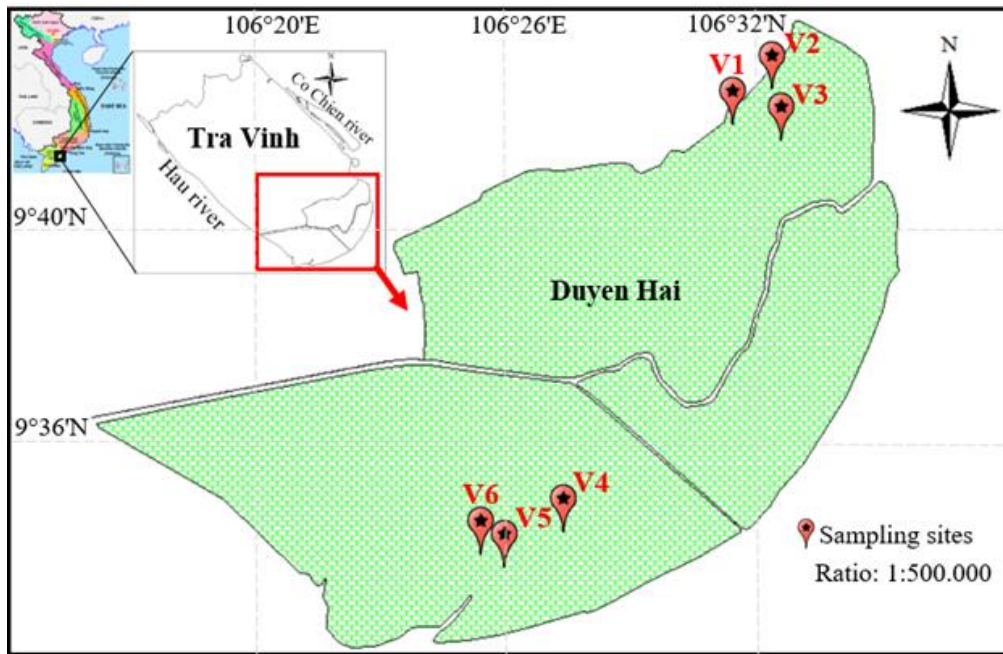


Figure 1. Sampling sites for the polychaetes *Dendronereis chipolini* at Duyen Hai, Tra Vinh.

community composition is widely used to assess anthropogenic impacts such as eutrophication, pollution, and habitat degradation (Borja et al., 2000).

Polychaetes are also noted for their reproductive plasticity and seasonal breeding cycles. Many nereidid species, such as *Perinereis nuntia* var. *brevicirris*, undergo epitokous metamorphosis and synchronized spawning events, often triggered by environmental cues like photoperiod and temperature (Bessie, 1996). Understanding such reproductive patterns is essential for domestication and controlled cultivation. Species with predictable gonadal development are ideal candidates for mass rearing as a biosecure feed source in aquaculture.

Beyond their ecological roles, polychaetes have garnered increasing attention in aquaculture, particularly as a natural, nutritionally rich feed for broodstock. Species such as *Nereis virens* and *Perinereis* spp. are routinely used in hatcheries for shrimp *Litopenaeus vannamei*, crabs, and marine fish due to their high protein content, balanced amino acid profiles, and abundance of essential fatty acids—especially highly unsaturated fatty acids (HUFA) such as EPA and DHA (Pezeshki et al., 2017). These nutrients are essential for broodstock development, gamete quality, and larval survival. Polychaetes also

provide key micronutrients, including vitamins (A, D, and E) and sterols, which contribute to immune function, stress resistance, and reproductive performance (Sánchez-Muros et al., 2020). Broodstock shrimp exhibit a strong preference for polychaetes, particularly during their reproductive phases, as these worms support improved fecundity, egg viability, and larval fitness (Coman et al., 2007; Huynh and Vu, 2018).

However, reliance on wild polychaete populations raises concerns regarding biosecurity, supply consistency, and ecological sustainability. Wild-caught polychaetes are often collected from estuarine or coastal sediment-rich environments, which are prone to contamination by pathogenic microorganisms. Pathogens such as *Enterocytozoon hepatopenaei* (EHP), acute hepatopancreatic necrosis disease (AHPND/EMS), and Decapod iridescent virus 1 (DIV1) have been associated with polychaetes and pose significant risks to shrimp hatcheries and broodstock health (Merican 2022). Additionally, environmental degradation and overharvesting of natural stocks further limit the availability and quality of wild-sourced polychaetes.

To address these challenges, there is an urgent need to develop a domesticated, pathogen-free source of

polychaetes for aquaculture use. A deeper understanding of the reproductive biology and life history traits of candidate species is essential for establishing closed-cycle culture systems. Among potential candidates, *Dendronereis chipolini*, a benthic polychaete species native to tropical estuarine systems, is promising owing to its ecological relevance, dietary suitability, and reproductive potential. Therefore, the present study aims to investigate the reproductive biological characteristics of *D. chipolini* as a foundational step toward its domestication and mass production. Insights from this research will contribute to the development of a sustainable, biosecure source of polychaetes for shrimp broodstock conditioning, ultimately supporting hatchery productivity and reducing reliance on wild-caught resources.

Materials and Methods

Sample collection and handling: Polychaete specimens were collected using a Petersen grab from improved extensive shrimp ponds in the coastal area of Duyen Hai district, Tra Vinh Province, in the Mekong Delta, Vietnam (Fig. 1). Upon collection, the samples were rinsed through a sieve of 500 μm to remove mud and debris. A total of 2,375 worm specimens were collected over a period of 12 months. The worm specimens used for artificial breeding were transferred live to the laboratory under aerated conditions. Those used for other biological reproductive characteristics were fixed in 10% formalin and transported to the College of Aquaculture and Fisheries, Can Tho University, Vietnam, for further analysis.

Sex identification and fecundity assessment

Sex determination: Sex of polychaetes was initially determined by external coloration at the maturation stage: males were milky white, whereas females were moss green. For further confirmation, dissection was performed to observe the presence of testes and ovaries. The male-to-female sex ratio was calculated as described by Bessie (1996).

Fecundity analysis: Fecundity was assessed in 35 mature female individuals at stage IV, collected from

the sampling sites. Two types of fecundity were recorded, including:

Absolute fecundity (S): Total number of eggs present in stage IV ovaries.

Relative fecundity (s): Number of eggs per gram of female body weight, calculated using the formula: $s = S/W$, where S is the absolute fecundity, and W is the body weight of the female (g).

To estimate egg number, all eggs were extracted from each female, diluted in 10 mL of water, and homogenized. A 100 μL subsample was taken and examined under a microscope. This step was repeated ten times per individual. The total egg count was calculated by multiplying the mean number of eggs per 100 μL by 100. The individual worm was weighed using a 4-digit balance (Sartorius, model CPA224S, Germany).

Gonadal development staging in females: The ovaries were fixed in 10% formalin, then processed, sectioned, and stained for histological analysis to determine gonadal development stages according to Bessie (1996). Oocyte diameters were measured microscopically, and maturation was categorized into four stages based on the oocyte diameter determined by Bessie (1996) as follows: *Stage I:* Oocyte diameter 40-80 μm ; *Stage II:* Oocyte diameter 80-120 μm ; *Stage III:* Oocyte diameter 120-160 μm ; *Stage IV:* Oocyte diameter 160-200 μm .

The samples were fixed in 10% neutral-buffered formalin and processed for histological examination. Samples were dehydrated using a series of graded ethanol solutions (70-99.8%), cleared in xylene, and embedded in paraffin blocks. Sample blocks were mounted on a rotary microtome (Eprexia HM340E), trimmed, and sectioned at 5 μm . All tissue sections were stained with haematoxylin and eosin and permanently mounted in Entellan (Merck Millipore) (Suvarna et al., 2019). Slides were examined under light microscopy (Nikon E200, Japan) at magnifications of 10X, 20X, and 40X, respectively.

Embryonic and larval development observations: Mature live worms collected from the field were selected and artificially spawned in the laboratory. Fertilized eggs were obtained following successful

spawning and were monitored to characterize embryonic development. Eggs were incubated at a salinity of 20‰, temperature of 28-30°C, and pH of 7.0-8.0, with a density of approximately 30,000 eggs per liter. Embryonic development was recorded from fertilization until hatching. Morphological changes were observed and photographed using a compound microscope. After hatching, thirty larvae were randomly sampled from each nursery tank to monitor larval development. Morphological features and pigmentation were used to identify larval stages. A developmental stage was considered achieved when more than 50% of the observed larvae had progressed to that stage. The duration of each stage was recorded in hours, and larval development was monitored until the formation of three body segments.

Results

Sex distinction: *Dendronereis chipolini* exhibits sexual dimorphism with distinct male and female individuals. However, prior to sexual maturation, the sexes cannot be distinguished by external morphological characteristics (Fig. 1). At maturation stages III and IV, males and females become distinguishable by body coloration visible to the naked eye (Figs. 2, 3). Mature males display a bright milky green hue, whereas females present a darker moss green coloration over the entire body.

Histological and microscopic analyses confirmed the presence and morphology of gametes. Oocytes were spherical in shape and distributed throughout the body cavity (Fig. 4A, C). Spermatocytes appeared as numerous minute, dot-like structures under light microscopy (Fig. 4B, D). These findings provide a reliable basis for sex identification and reproductive status assessment during advanced gonadal development.

Fecundity: Fecundity analysis was conducted on ten mature female specimens at stage IV. The mean absolute fecundity (Fa) was estimated at $185,773 \pm 76,352$ eggs per female, while the mean relative fecundity (Fr) was $209,520 \pm 31,414$ eggs per gram of body weight (Table 1). The smallest female (0.39 g) produced 79,208 eggs, whereas the largest



Figure 2. Immature polychaete *Dendronereis chipolini*.

female (1.86 g) yielded up to 372,460 eggs. These results indicate a positive correlation between female body weight and fecundity, suggesting that reproductive potential is size-dependent in *D. chipolini*.

Ovarian development stages: Oocyte development in female *D. chipolini* was classified into four distinct stages based on oocyte diameter and external morphological and histological changes (Figs. 5, 6):
Stage I: Individuals were small, with light-red bodies and undeveloped gonads. Oocytes were indistinct and measured 40-60 μm in diameter.

Stage II: The body became bright red with increased size and mass. Oocytes grew to 60-80 μm in diameter.

Stage III: Significant somatic and gonadal development was observed. Oocyte diameters increased to 80-100 μm , with external visibility of eggs distributed throughout the body. The coloration ranged from pink to orange-red.

Stage IV: This stage was marked by pronounced external features and full sexual maturity. Females had a dark moss-green body, whereas males were bright milky green. Oocytes reached their maximum size of 100-120 μm .

These observations provide a morphological and histological framework for assessing reproductive maturity and optimizing broodstock selection in polychaete aquaculture.

Characteristics of embryonic and larval development stages

Embryonic development duration: The embryonic development of *D. chipolini* was observed under



Figure 3. Male and female of *Dendronereis chipolini*.

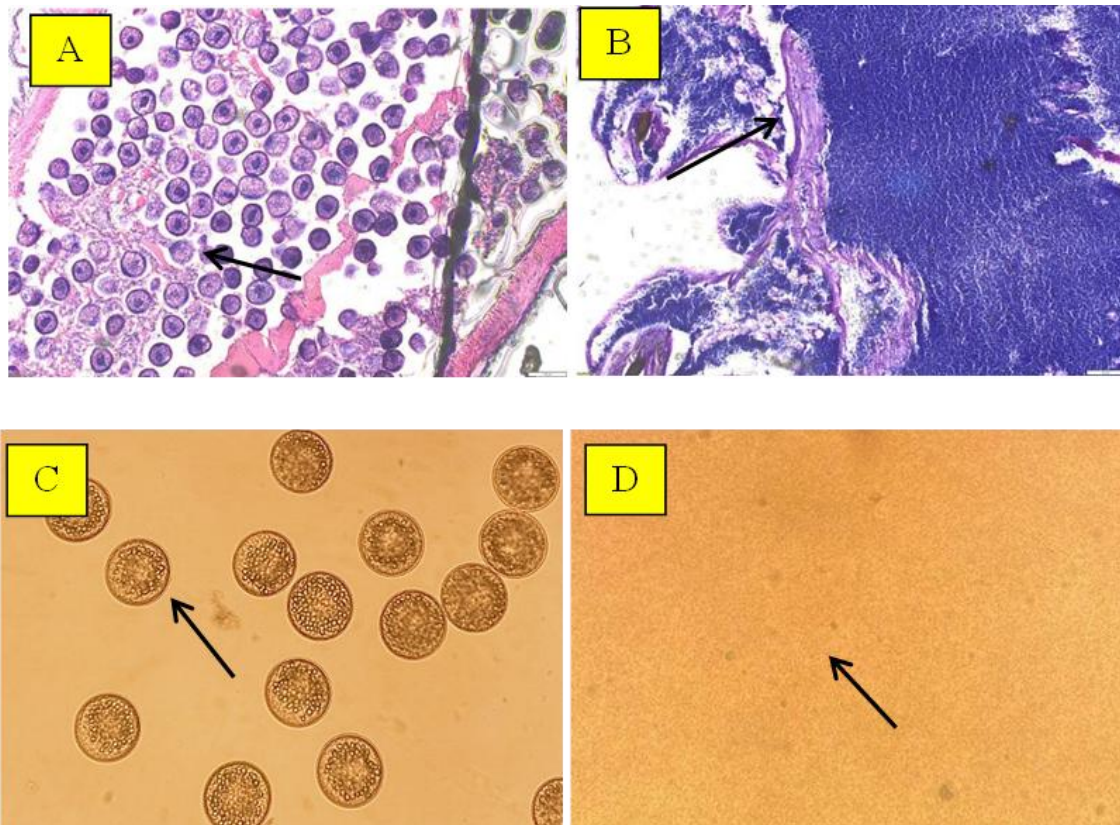


Figure 4. Histomorphology of eggs (A) and sperm (B) of *Dendronereis chipolini*, and eggs (C) and sperm (D) observed under a light microscope. Black arrows indicate the positions of eggs or sperm in each panel.

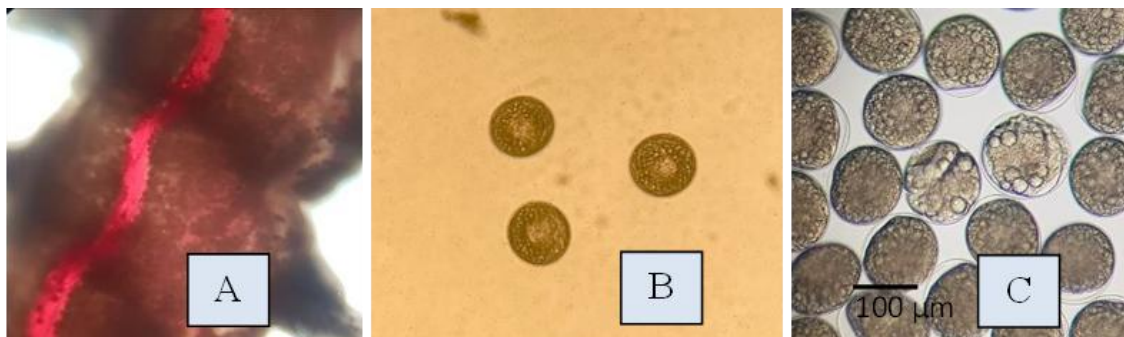


Figure 5. Polychaete egg within the female body (A), unfertilized egg (B), and fertilized egg (C) observed under a light microscope (scale bar = 100 µm).

Table 1. Absolute and relative fecundity of mature female *Dendronereis chipolini* (Fa (absolute fecundity) and Fr (relative fecundity)).

Individual	Weight of female (g)	Fa (Eggs/female IV stage)	Fr (Eggs/g of female)
1	1.86	372,460	200,247
2	0.7	126,430	180,614
3	0.96	271,450	282,760
4	0.66	135,468	205,255
5	0.89	185,840	208,809
6	1.4	321,670	229,764
7	0.9	190,452	211,613
8	0.69	156,452	226,742
9	0.76	162,380	213,658
10	0.96	198,250	206,510
11	1.36	397,600	292,353
12	0.84	156,240	186,000
13	0.75	135,240	180,320
14	0.64	121,450	189,766
15	1.11	245,730	221,378
16	0.96	191,203	199,170
17	0.76	164,520	216,474
18	1.11	255,710	230,369
19	0.96	204,270	212,781
20	0.76	174,350	229,408
21	0.56	101,825	180,862
22	0.90	180,125	199,695
23	0.62	123,052	199,436
24	0.52	109,532	209,831
25	0.39	79,208	205,735
26	1.35	261,472	193,683
27	1.3	246,580	189,677
28	0.97	178,360	183,876
29	0.89	157,840	177,348
30	0.73	157,820	216,912
31	0.9	174,590	193,989
32	0.49	86,430	176,029
33	0.7	131,340	187,629
34	0.84	261,530	312,835
35	0.47	85,201	181,666
Mean±SD	0.88 ± 0.30	185,773 ± 76,352	209,520 ± 31,414

controlled laboratory conditions at 28-30°C, 20‰ salinity, and a pH range of 7.5-7.7. Developmental progression and timing are summarized in Table 2 and illustrated in Figure 7. Fertilized eggs exhibited clearly distinguishable polar bodies and egg membranes approximately 40 minutes post-fertilization. The first cleavage occurred after an additional 50 minutes, resulting in a two-cell stage. Successive divisions produced four-cell and eight-cell stages at approximately 1 hour and 2 hours post-fertilization, respectively. The embryos then entered the morula stage, which persisted for approximately 4 hours.

Hatching into Trochophore larvae occurred at an

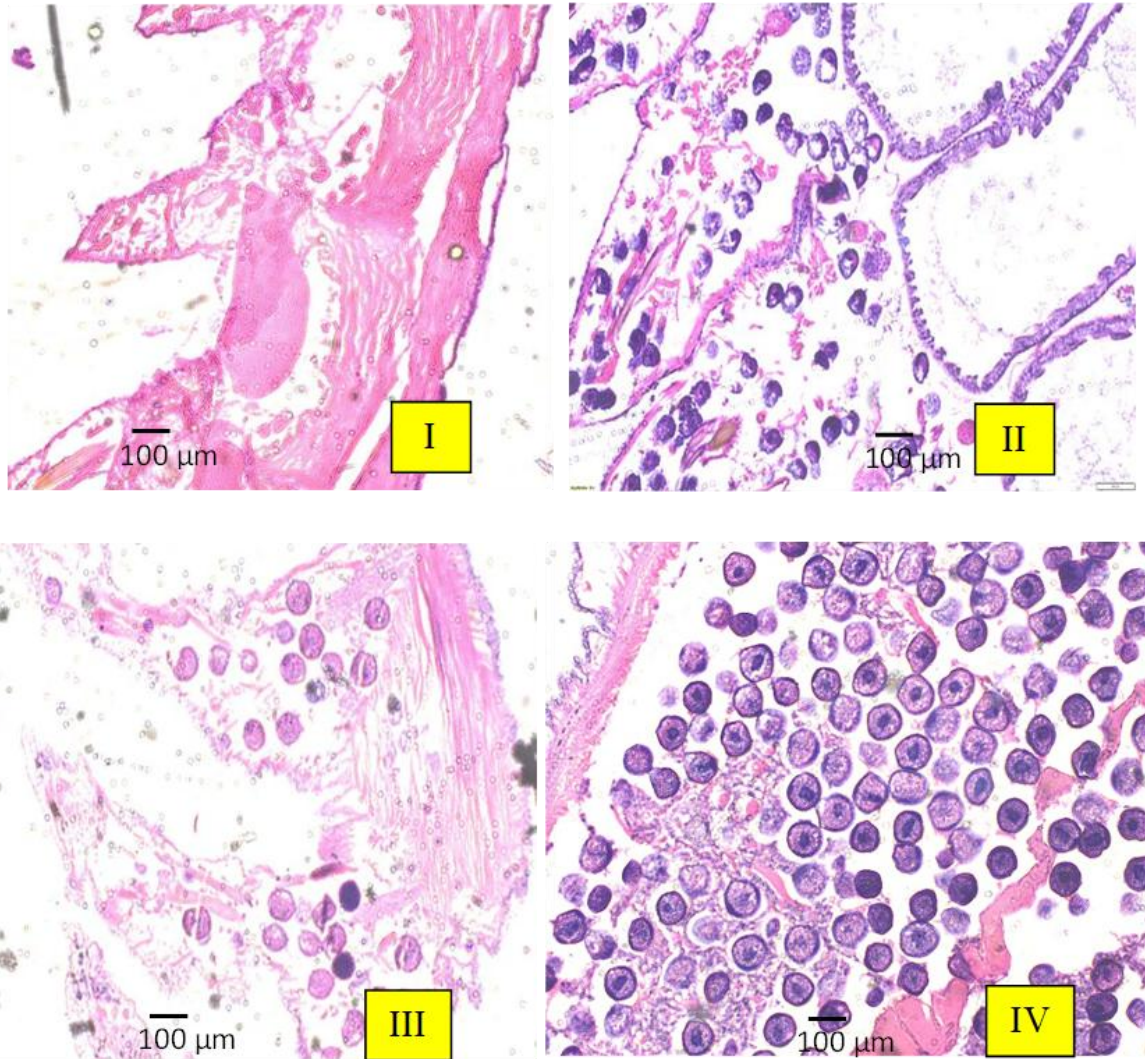
average of 7 hours and 30 minutes post-fertilization, with more than 50% of embryos having completed embryogenesis by this time. The newly hatched Trochophores were characterized by the formation of a ciliary band and observable rotational movement, indicating the onset of active larval motility and external development.

Larval development stages: Larval development of *D. chipolini* proceeds through three distinct stages, Trochophore, Metatrochophore, and Nectochaeta, under controlled environmental conditions (temperature: 28-30°C; salinity: 20‰; pH: 7.5-8.0) (Table 3, Fig. 8).

Trochophore stage: Approximately 7 hours and 30

Table 2. Duration of embryonic development in the polychaete *Dendronereis chipolini*.

Embryonic development stages	The average transition
Fertilized oocyte with polar body	40±14
2 cells	50±14
4 cells	60±0
8 cells	25±7
16 cells	35±7
Morula	240±14

Figure 6. Micrographs of histological sections (10X) showing developmental stages (I-IV) of the ovary in *Dendronereis chipolini*.

minutes post-fertilization, fertilized eggs hatched into free-swimming Trochophore larvae. These larvae were characterized by the development of a ciliary band (prototroch), which facilitated rotational swimming. The average body length was 120.3 µm. At this stage, larvae relied entirely on endogenous reserves from the yolk sac for nutrition.

Metatrochophore stage: Following the trochophore phase, larvae transitioned into the Metatrochophore

stage, lasting approximately 17 hours. This stage was marked by the appearance of two pairs of elongated chaetae (bristles), signaling early parapodial development. Larvae maintained an average length of 121.2 µm and continued to utilize internal yolk reserves, as external feeding had not yet commenced. **Nectochaeta Stage:** Approximately 23 hours and 24 minutes after the onset of the Metatrochophore stage, larvae advanced to the Nectochaeta stage. This phase

Table 3. Duration of larval developmental stages of *Dendronereis chipolini* under controlled laboratory conditions (temperature: 28-30°C; salinity: 20‰; pH: 7.5-8.0). Values are presented as mean \pm standard deviation (SD).

Developmental transition	Duration (hours \pm SD)	Duration (minutes \pm SD)
Fertilization \rightarrow Trochophore	7.5 \pm 0.7	450 \pm 42
Trochophore \rightarrow Metatrochophore	17 \pm 0.3	1020 \pm 15
Metatrochophore \rightarrow Nectochaeta	23.4 \pm 0.7	1404 \pm 42

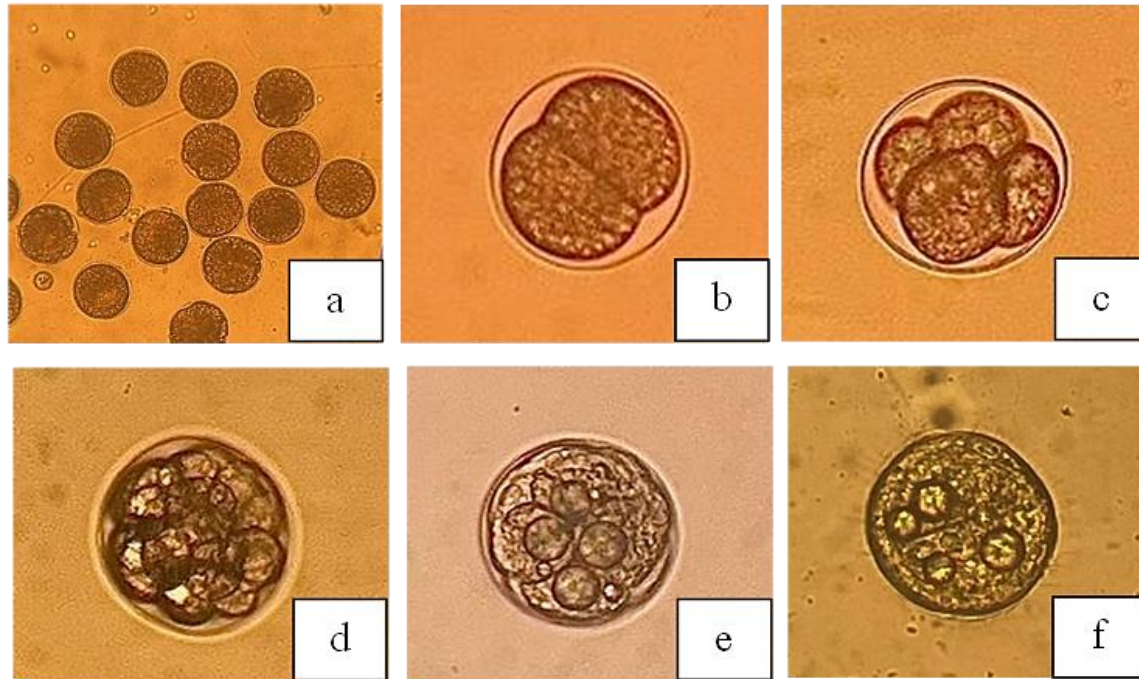


Figure 7. Embryonic developmental stages of the polychaete *Dendronereis chipolini* observed under a light microscope: (a) Fertilized egg, (b) 2-cell stage, (c) 4-cell stage, (d) 16-cell stage, (e) Morula, and (f) Trochophore larvae.

was characterized by visible body segmentation, the emergence of eye spots, and the development of well-defined parapodia. Larvae began external feeding and displayed more complex locomotor behaviors, alternating between swimming near the water surface and crawling along the substrate. The average body length was 146.8 μm at this stage.

Discussions

The reproductive biology of *D. chipolini* observed in this study aligns with general patterns previously reported in polychaetes. As noted by Olive (1983), most polychaetes possess a relatively simple reproductive system, often lacking specialized gonoducts. Gametes are typically released into the coelomic cavity and expelled through rupture of the body wall, leading to post-spawning mortality. This mode of reproduction was also evident in *D. chipolini*, in which gametes were distributed extensively

throughout the body, including the parapodia, and were released externally at maturity.

Sexual dimorphism in *D. chipolini* becomes distinguishable only at advanced stages of gonadal development, consistent with descriptions of external morphological differentiation in mature polychaetes (Bessie, 1996). In our observations, mature males exhibited a bright milky-green coloration, whereas females displayed a darker moss-green hue. Such coloration differences are useful for rapid sex identification in broodstock selection under aquaculture conditions.

The mean absolute fecundity recorded for *D. chipolini* was 185,773 \pm 76,352 eggs/female, with a maximum of 397,600 eggs in a 1.36 g individual. Relative fecundity averaged 209,520 \pm 31,414 eggs/g of body weight. These values are comparable to or exceed those reported for other polychaete species used in aquaculture. For example, *Perinereis nuntia*

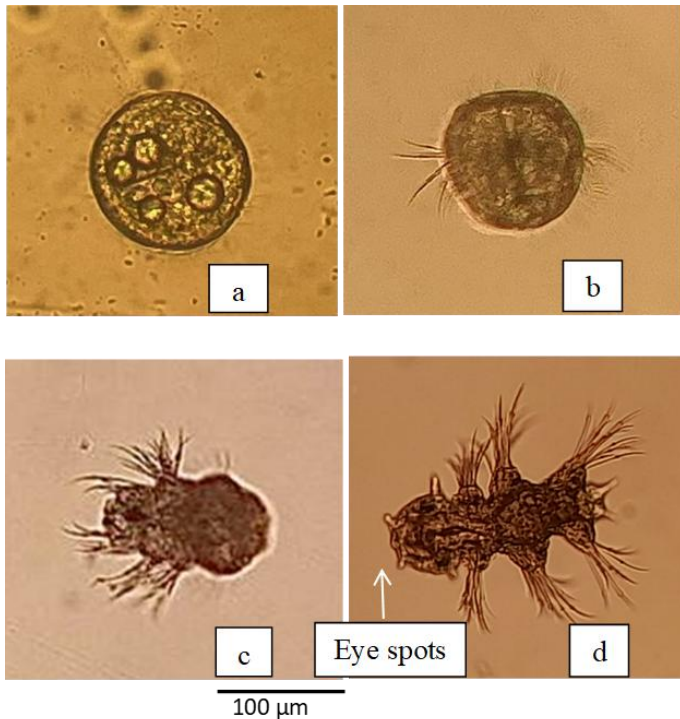


Figure 8. Larval developmental stages of the polychaete *Dendronereis chipolini*: (a) Trochophore, (b) Metatrochophore, (c) Nectochaete with 2 segments, and (d) Nectochaete with 3 segments (Scale bar = 100 μ m).

var. brevicirris has a mean absolute fecundity of 208,358 eggs/female (Okada and Yamaguchi, 1997), and *Marphysa mossambica* was reported to produce only 4,073.9 eggs/female with a relative fecundity of 565.7 eggs/g (Prevedelli et al., 2007). The notably higher fecundity of *D. chipolini* relative to its body size highlights its potential as a broodstock feed candidate for shrimp hatcheries that require high-nutrient, dense live feed.

Oocyte size in *D. chipolini* ranged from 40 μ m in early development to approximately 120 μ m at full maturity. This is relatively small when compared to *P. nuntia var. brevicirris*, whose mature oocytes reach 200 μ m (Okada and Yamaguchi 1997), and *T. heterochaetus*, which also produces larger gametes (Bessie 1996). Smaller egg size in *D. chipolini* may be attributed to its overall smaller body size and potentially shorter larval developmental cycles.

The embryonic development of *D. chipolini* under laboratory conditions was rapid. Trochophore larvae emerged approximately 7.5 hours post-fertilization at 28-30°C, which is significantly faster than many other

polychaete species. For instance, *N. virens* requires about 15 hours to reach the trochophore stage (Bass and Brafield 1972), and *P. nuntia var. brevicirris* takes 30 to 60 hours at 26°C (Liu 1980). Variability in developmental timing has also been observed in *Tylorrhynchus heterochaetus*, with first cleavage occurring 2 hours post-fertilization and larval formation taking up to 24 hours (Yasunori et al. 2003). These differences highlight species-specific developmental rates and their sensitivity to environmental conditions such as temperature and salinity.

Larval development of *D. chipolini* progresses through trochophore, metatrochophore, and nectochaeta stages. Early stages are characterized by endogenous feeding, whereas external feeding commences during the nectochaeta stage, when parapodia and segmentation become apparent. The rapid, clearly defined larval development, combined with high fecundity and distinct morphological indicators of maturity, supports the feasibility of culturing *D. chipolini* in hatchery conditions for use as live feed in shrimp aquaculture. These findings provide a clear developmental timeline for the early life stages of *D. chipolini*, which is essential for designing effective larval-rearing protocols and optimizing hatchery production.

Conclusions

The study of *D. chipolini* has provided valuable insights into the reproductive biology and developmental stages of polychaetes. Sexual dimorphism in this species is evident: mature males display a bright milky-green coloration, whereas females exhibit a distinct dark moss-green hue. Fertility metrics indicate a high reproductive capacity, with females producing an average of 185,773 eggs per individual and a relative fecundity of 209,502 eggs per gram of body weight. Gonadal development in females progresses through four distinct stages, characterized by a range of egg sizes from 40 to 120 μ m. Embryonic development occurs rapidly under controlled conditions (28-30°C), with larvae hatching approximately 7.5 hours post-fertilization and

progressing through three distinct metamorphic stages: Trochophore, Metatrochophore, and Nectochaete, the latter resembling adult polychaetes. The findings from this study contribute significantly to the understanding of polychaete reproductive strategies and developmental biology. These insights not only enrich current knowledge of *D. chipolini* but also enhance its potential as a live feed source for shrimp broodstock in aquaculture systems.

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