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Original Article

Effects of photoperiod on growth and ovarian maturation in female green mud crab, *Scylla serrata* (Forsskål, 1775)

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Abstract
Photoperiod is one of the most significant environmental cues for an organism, which are also essential for controlling its growth, metabolism, and maturity. The current study used an 8-week experiment to examine the effects of photoperiod T1 (12L:12D), T2 (18L:6D), and T3 (6L:18D) on mud crab (*Scylla serrata*) growth performance and gonadal maturation. A total of 36 healthy crabs (average initial weight 416.18±2.42 g) were fed twice daily at 10% of body weight. Crabs cultured under 6L:18D (T3) showed the highest final weight (486.54±4.44 g), weight gain (70.45±3.92 g), specific growth rate (0.26±0.01 % day^{-1}), gonadosomatic index (12.06±0.20%), and hepatosomatic index (5.64±0.35%), significantly higher than T1 and T2. Digestive enzyme activities (amylase, protease, lipase) were highest in the 6L:18D group, indicating efficient nutrient utilization. Ovarian carotenoid levels were significantly ($p < 0.05$) higher in 6L:18D (0.4399±0.0640 µg mg^{-1}). Histology revealed advanced vitellogenesis and a healthy hepatopancreas in the 6L:18D group, while the 18L:6D showed delayed maturation. Overall, the results indicate that among the treatment groups, the 6L:18D improved the growth, digestion, and reproductive performance of female *S. serrata*. This could be used in broodstock maturation, which has a detrimental effect on wild stock, and could offer valuable insights into sustainable broodstock management.

Keywords: digestive enzymes; gonadal maturation; histology; mud crab; photoperiod

1 | INTRODUCTION

The green mud crab *Scylla serrata* (Forsskål, 1775) is one of the most commercially important crustacean species widely distributed in mangrove and estuarine ecosystems of the Indo-Pacific region (Leoville *et al*. 2021). Commonly known as the green or mangrove mud crab, this species holds significant economic and ecological importance, particularly in aquaculture (Rahman *et al*. 2020). With the increasing global demand for mud crabs, India has emerged as a major contributor to the international market, exporting crabs valued at approximately USD 54.26 million during the 2021–2022 fiscal year (Kaippilly *et al*. 2025). Despite its commercial significance, reliance on capture fisheries alone is insufficient to meet the growing demand for *S. serrata* (Rahman *et al*. 2020). Therefore, the development and refinement of aquaculture practices are essential to ensure a sustainable and consistent supply of this species (Sathiadhas and Najmudeen 2004). The species is highly valued due to its large body size, rapid growth rate, and superior meat quality, making it a preferred candidate for aquaculture and fisheries in many Asian countries, including India (Wolff *et al*. 2000).

In hatchery operations, broodstock are predominantly collected from the wild. However, wild-caught brooders are often susceptible to bacterial and viral diseases, and mass mortality events are frequently reported due to Mud Crab Reovirus (MCRV) in India (Ganesan *et al*. 2023). Additionally, over-exploitation of wild stocks has been widely documented (Le Vay *et al*. 2008; Cheung *et al*. 2010; Fondo *et al*. 2010; Mirera *et al*. 2013). These challenges highlight the urgent need to improve broodstock performance under captivity conditions to obtain high-quality ovigerous females (Shelley and Lovatelli 2011).

Reproduction processes in crustaceans are regulated by endogenous and exogenous factors. External factors such as temperature, photoperiod, food availability and salinity play significant roles in influencing reproductive performance (Adiyodi and Adiyodi 1970; Aaqillah-Amr *et al*. 2021). Among these factors, photoperiod has been shown to influence the behaviour and physiology of crustaceans (Bishop and Herrnkind 1976). Photoperiod particularly affects the timing of ovarian growth and maturation (Nagaraju 2007; Chang and Mykles 2011; Hussain *et al*. 2017). Endogenous regulation involves both neuroendocrine and non-neuroendocrine systems, in which hormones secreted from neuroendocrine organs control reproductive maturation in crustaceans (Nagaraju 2007; Chang and Mykles 2011; Nagaraju 2011).

Previous studies on *S. serrata* have mainly focused on growth and development from juveniles to adults (Morales and Barba 2015). However, controlled maturation of female mud crab under captive conditions remains a major bottleneck in hatchery technology. Although nutritional and hormonal approaches have been explored, the role of photoperiod manipulation in regulating ovarian maturation and reproductive performance of *S. serrata* broodstock remains poorly understood and requires systematic investigation.

Therefore, optimizing photoperiod regimes could provide an effective, non-invasive strategy to enhance gonadal development, spawning readiness, and overall broodstock quality in captivity. The present study investigates the effects of photoperiod on female *S. serrata* broodstock without eyestalk ablation. The study aims to establish an optimal photoperiod regime to enhance ovarian maturation and improve broodstock quality under controlled experimental conditions. The findings are expected to provide critical insights for evidence-based management strategies in mud crab hatchery operations, thereby supporting the sustainability and profitability of mud crab aquaculture.

2 | METHODOLOGY

2.1 Experimental design and set up

Female green mud crabs were collected from Pulicat Lake (13.422832°N, 80.296034°E), Tamil Nadu, India. The crabs were acclimatized in 2000 L fibre-reinforced plastic (FRP) circular tanks (2.0 m diameter x 0.76 m height) and fed a natural diet. A total of 9 tanks were used (3 treatments × 3 replicates). The crabs were randomly distributed into three experimental groups. During the experimental periods, crabs were fed twice daily at 09:00 and 17:00 hours, at 5–10% of their body weight. The feeding quantity was adjusted periodically based on routine sampling. Excess feed and fecal matter were siphoned out daily to maintain water quality, and 100% of the water was exchanged every three days. A total of 36 healthy crabs of uniform size and weight, with an average carapace width of 126.13±1.02 mm and a mean body weight of 416.08±2.68 g, and in similar gonadal condition, were selected for the experiment. The experiment was conducted over a period of eight weeks. The three experimental groups were assigned the following photoperiod treatments: T1, which was exposed to a natural diurnal photoperiod; T2, which was exposed to 18 h light: 6 h dark (18L:6D), and T3, which was exposed to 6 h light: 18 h dark (6L:18D). For photoperiod manipulation, crabs were maintained in FRP tanks equipped with LED lighting systems. For dark conditions, tanks were covered with black sheets to ensure complete darkness. A 12 W m^{-2} light source was suspended approximately 7 inches above the surface to provide illumination while minimizing heat transfer to the water

(Chen *et al*.2021).

2.2 Water quality parameters

During the experimental period, key water quality parameters, including salinity, temperature, dissolved oxygen, pH, ammonia-N, nitrite-N, nitrate-N, total hardness, and alkalinity level, were regularly monitored to maintain optimal rearing conditions for the experimental crabs. To ensure good water quality, 100% of the water was exchanged every three days. Salinity, pH, and dissolved oxygen were measured using a laboratory-grade multiparameter water quality meter (Model AZ 86031, India). The concentrations of total alkalinity, ammonia-N, nitrite-N, nitrate-N, and total hardness were determined following standard methods prescribed by the American Public Health Association (APHA 2005).

2.3 Growth and nutrient utilization

At the end of the experimental period, the body weight of all crabs was recorded to evaluate growth performance and nutrient utilization. The parameters assessed included weight gain (WG), weight gain percent (WG%), average daily gain (ADG), specific growth rate (SGR), and survival rate. These parameters were calculated using the following formulas:

WG (g) = Final weight − Initial weight

WG (%) = [(Final weight − Initial weight) / Initial weight] × 100

ADG (g day^{-1}) = (Final weight − Initial weight) / days

SGR (% day^{-1}) = [(ln final weight − ln initial weight) / days] × 100

Survival (%) = (Final number of crab / Initial number of crabs stocked) × 100

In addition to growth parameters, reproductive and physiological indices such as gonadosomatic index (GSI) and hepatosomatic index (HSI) were determined following the method described by Devlaming *et al*. (1982).

GSI (%) = Ovary weight / Body Weight × 100

HSI (%) = Hepatopancreas weight / Body Weight ×100

2.4 Total carotenoids

The total carotenoid content in the ovaries and hepatopancreas of mud crabs was determined following the method of Suhnel *et al*. (2009) with slight modifications. Approximately 50 mg of frozen, freeze-dried tissue was weighed and placed in a 50 mL beaker. The beaker was covered with aluminium foil to protect the samples from photodegradation. Carotenoids were extracted using an acetone: hexane mixture (1:3, v/v), and the samples were incubated in darkness at 23°C with intermittent shaking every 5 min. The samples were then centrifuged at 3000 rpm for 3 minutes, and the supernatant was collected. The extraction was repeated, and the final extract volume was adjusted by adding 3 mL of the solvent mixture. The extract was transferred to a light-protected volumetric flask and flushed with nitrogen gas to prevent oxidation. The carotenoid concentration was measured using a UV-visible spectrophotometer (Model: KLUV-2100) at a wavelength of 460 nm. Astaxanthin was used as the standard reference carotenoid, with a molecular weight (MW) of 596.84 and a specific extinction coefficient (ε) of 124,000 in hexane (Buchwald and Jencks 1968). The total carotenoid content (μg mg^{-1}) was calculated using the following formula:

Total carotenoid (μg mg^{-1}) = (A/ε) × MW × 1000 (V/W)

Where, A = absorbance at 460 nm; MW = molecular mass of carotenoid standard (astaxanthin = 596.84); ε = extinction coefficient of astaxanthin in hexane (124,000); V = sample volume (ml); W = sample dry weight (mg)

2.5 Digestive enzyme analysis

At the end of the feeding trial, three crabs from each treatment were randomly selected and euthanized using an ice slurry (1:1 mixture of crushed ice and water) for approximately 5 minutes until complete immobilization. The hepatopancreas and midgut were carefully dissected and collected for digestive enzyme analysis. The tissues were homogenized in 0.25 M sucrose solution using a homogenizer, and the homogenate was centrifuged at 3,070 × g. The resulting supernatant was collected and used for enzyme assays. The activities of protease, lipase, and amylase were determined. Protease activity was measured using the casein digestion method (Sarath *et al.* 1989), lipase activity by a titrimetric method using stabilized olive oil emulsion as the substrate (Cherry and Crandall 1932), and amylase activity by the dinitrosalicylic acid (DNS) method (Clark 1964). All enzyme activities, except lipase, were quantified spectrophotometrically based on changes in absorbance. Lipase activity was determined by measuring the rate of fatty acid release over the incubation period. Enzyme activities were expressed as units per milligram of protein per minute (U mg^{-1} protein min^{-1}). One unit of enzyme activity was defined as follows: Protease: Amount of enzyme releasing 1 μg of tyrosine per minute. Lipase: Amount of enzyme releasing 1 μg of fatty acid per minute. Amylase: Amount of enzyme releasing 1 μg of maltose per minute.

2.6 Histology

At the end of the experimental period, three crabs from each treatment were randomly selected and euthanized using an ice slurry (1:1 mixture of crushed ice and water) until complete immobilization (approximately 5 minutes). The ovaries and hepatopancreas were carefully dissected, excised, and immediately fixed in 10% neutral buffered formalin (NBF) for histopathological analysis. The fixed tissues were dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax at 58°C. Paraffin-embedded tissue blocks were sectioned at a thickness of 6 µm using a rotary microtome (Leica RM2255, India). The sections were stained with hematoxylin and eosin (H&E) using an automated stainer (Microm HMS7). The stained sections were examined under a light microscope (Leica E200, India) at 20X magnification. Microphotographs were captured for detailed histological analysis and comparison among treatments.

2.7 Data analysis

All experimental data were tested for homogeneity of variance using Levene's test and for normality using the Shapiro–Wilk test. The results are presented as mean ± standard deviation (SD) of triplicate samples. Differences among treatments were analyzed using one-way analysis of variance (ANOVA). When significant differences were observed, Duncan's multiple range test was applied to compare the treatment means. The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). Histological observations of the hepatopancreas and ovary were evaluated qualitatively based on tissue structure, cellular organization, and morphological variations among the treatments.

3 | RESULTS

3.1 Growth performance, GSI and HSI

Growth performance and somatic indices of *S. serrata* reared under different photoperiod regimes are presented in Table 1. One-way ANOVA revealed that photoperiod exerted a significant effect on growth parameters, including final weight ($F_{(2,33)} = 14.918$, $p < 0.001$), weight gain ($F_{(2,33)} = 13.511$, $p < 0.001$), weight gain percentage ($F_{(2,33)} = 12.364$, $p < 0.001$), and average daily growth ($F_{(2,33)} = 13.511$, $p < 0.001$). No significant variation was found in initial weight among the groups ($F_{(2,33)} = 0.003$, $p = 0.997$). At the end of the experimental period, crabs reared under T3 (6L:18D) exhibited the highest final weight (486.54±4.44 g), which was significantly higher than that observed in T1 (12L:12D) and T2 (18L:6D) ($p < 0.05$). Specific growth rate (SGR) also showed significant variation among treatments (One-way ANOVA: $F_{(2,33)} = 12.387$, $p < 0.001$), with T3 (0.26±0.01% day^{-1}) recording higher values than T1 (0.25±0.02% day^{-1}) and T2 (0.23±0.02% day^{-1}).

TABLE 1 Growth performance of *Scylla serrata* reared at different photoperiod levels.

Parameters	T1 (Control)	T2 (18L:6D)	T3 (6L:18D)	*p*-values
Initial weight (g)	416.18±2.51^{a}	416.17±2.62^{a}	416.11±2.42^{a}	0.997
Final weight (g)	482.76±2.48^{b}	478.12±4.16^{c}	486.54±4.44^{a}	0.001
Weight gain (g)	66.58±3.89^{b}	61.95±4.21^{c}	70.45±3.92^{a}	0.001
Weight gain percentage (%)	16.00±1.02^{b}	14.89±1.05^{c}	16.93±0.96^{a}	0.001
Average daily gain (g)	1.11±0.06^{b}	1.03±0.07^{c}	1.17±0.06^{a}	0.001
Specific growth rate (%/day)	0.25±0.015^{b}	0.23±0.015^{c}	0.26±0.014^{a}	0.001
GSI %	10.53±0.59^{ab}	8.80±0.47 ^{c}	12.06±0.20 ^{a}	0.001
HSI %	5.39±0.30 ^{a}	4.78±0.22 ^{b}	5.64±0.35^{a}	0.032
Survival rate (%)	86.11±4.81^{a}	80.56±4.81^{a}	91.67±8.33^{a}	0.171

Values are expressed as mean ± standard deviation. Different superscript letters within the same row indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.05$). Statistical differences among treatments were determined using one-way ANOVA.

Reproductive and physiological indices followed a similar trend. The highest GSI and HSI were recorded in T3 (12.06±0.20% and 5.64±0.35%, respectively), whereas the lowest values were recorded in the T2 group (GSI: 8.80±0.47%; HSI: 4.78±0.22%), with significant differences confirmed among treatments for both GSI (One-way ANOVA: $F_{(2,6)} = 39.693$, $p < 0.001$) and HSI (One-way ANOVA: $F_{(2,6)} = 6.479$, $p = 0.032$). Survival rates ranged from 80.56±4.81% to 91.67±8.33%, showing no statistically significant differences among experimental groups (One-way ANOVA: $F_{(2,6)} = 2.400$, $p = 0.171$).

3.2 Digestive enzyme analysis

Activities of amylase, protease, and lipase in the hepatopancreas and midgut were significantly influenced by photoperiod levels (Figures 1, 2 and 3). One-way ANOVA confirmed significant treatment effects across all measured parameters in the hepatopancreas: amylase ($F_{(2,6)} = 5.437$, $p = 0.045$), protease ($F_{(2,6)} = 14.886$, $p = 0.005$), and lipase ($F_{(2,6)} = 14.671$, $p = 0.005$). Similarly, midgut enzyme activities varied significantly across treatments, as validated by ANOVA for midgut amylase ($F_{(2,6)} = 5.598$, $p = 0.042$), protease ($F_{(2,6)} = 8.544$, $p = 0.018$), and lipase ($F_{(2,6)} = 24.630$, $p = 0.001$).

The highest enzyme activities were observed in the T3 group (6L:18D), indicating improved nutrient digestion and assimilation at this level and in the hepatopancreas, amylase, protease, and lipase activities reached 0.0296±0.0068, 0.0094±0.0015, and 1.1148±0.01159 U mg protein^{-1}, respectively, while midgut activities were

0.0305±0.0062, 0.0089±0.0023, and 1.1907±0.1909 U mg protein^{-1}. Lower enzyme activities were recorded in crabs reared at T2 (18L:6D), particularly for lipase, which showed minimum values in both the hepatopancreas (0.5495±0.1394 U mg protein^{-1}) and midgut (0.3670±0.1394 U mg protein^{-1}). Although the T1 (12L:12D) group maintained moderate amylase and protease activities, lipase activity declined compared with that in T3. These results demonstrate that a 6L:18D photoperiod optimizes digestive enzyme function, ensuring efficient energy utilization during reproductive maturation.

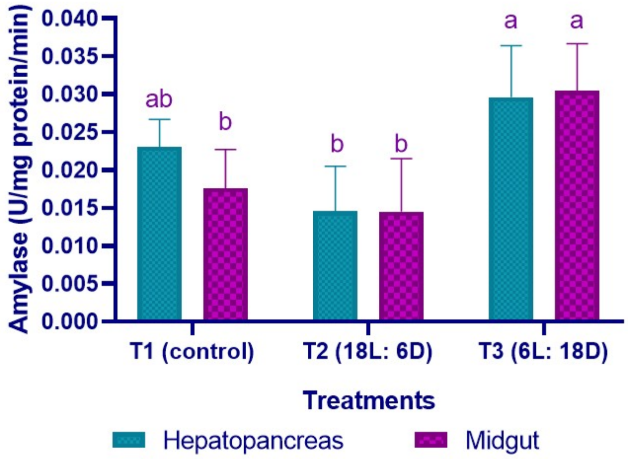

FIGURE 1 Total amylase activity of hepatopancreas and midgut in *Scylla serrata* reared at different photoperiod levels. Values were expressed as mean ± standard deviation (*n* = 3). In the graph, each parameter bar with a different alphabet letter represents significant differences (*p* < 0.05).

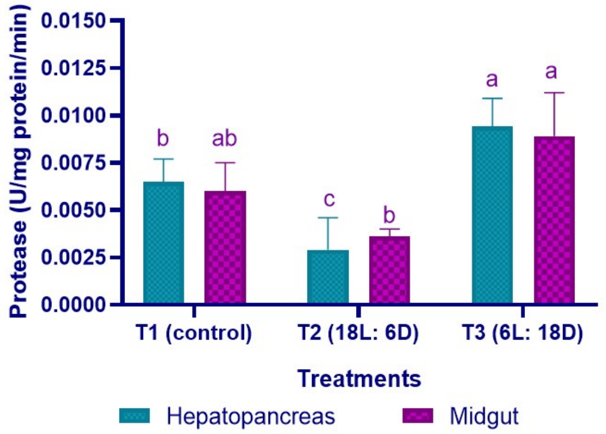

FIGURE 2 Total protease activity of hepatopancreas and midgut in S. serrata reared at different photoperiod levels. Values were expressed as mean ± standard deviation (*n* = 3). In the graph, each parameter bar with a different alphabet letter represents significant differences (*p* < 0.05).

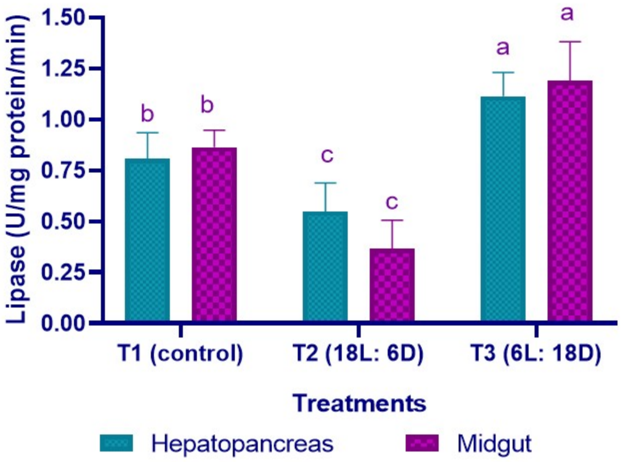

FIGURE 3 Total lipase activity of hepatopancreas and midgut in *Scylla serrata* reared at different photoperiod levels. Values were expressed as mean ± standard deviation (*n* = 3). In the graph, each parameter bar with a different alphabet letter represents significant differences (*p* < 0.05).

3.3 Total carotenoids

Total carotenoid concentrations in the ovary and hepatopancreas of *S. serrata* varied significantly with different photoperiod levels (Figure 4). Significant variations were validated by One-way ANOVA for both ovarian carotenoid deposition ($F_{(2,6)}$ = 8.819, *p* = 0.016) and hepatopancreatic storage ($F_{(2,6)}$ = 8.674, *p* = 0.017). Ovarian carotenoid content was highest in T3 (6L:18D) (0.4399±0.0640 µg mg^{-1}), while the lowest ovarian carotenoid concentration was observed in the 18L:6D group (T2) (0.2870±0.0292 µg mg^{-1}). Similarly, carotenoid accumulation in the hepatopancreas was greatest in the 6L:18D group (T3) (0.0357±0.0129 µg mg^{-1}), followed by the T1 and T2 groups, while the 18L:6D group showed the lowest value (0.0101±0.0029 µg mg^{-1}). These findings suggest a tissue-specific pattern of carotenoid deposition influenced by different photoperiod levels, wherein a 6L:18D photoperiod promotes efficient carotenoid accumulation in the ovary to support reproductive maturation.

3.4 Histological observation

Histological analyses of ovarian and hepatopancreatic tissues revealed distinct structural modifications in *S. serrata* in response to varying photoperiod levels (Figures 5 and 6). In the control group, ovaries exhibited a compact organization with numerous large, uniform vitellogenic oocytes filled with dense eosinophilic yolk granules typical features of the mature or late-vitellogenic stage. Ovaries from crabs reared at (18L:6D) (T2) displayed disorganized lobular architecture, smaller oocytes, darker cytoplasm, and minimal yolk deposition, indicating retarded or incomplete vitellogenesis. The (6L:18D) group (T3) showed well-developed ovarian lobules containing abundant vitellogenic oocytes with distinct yolk globules and prominent nuclei.

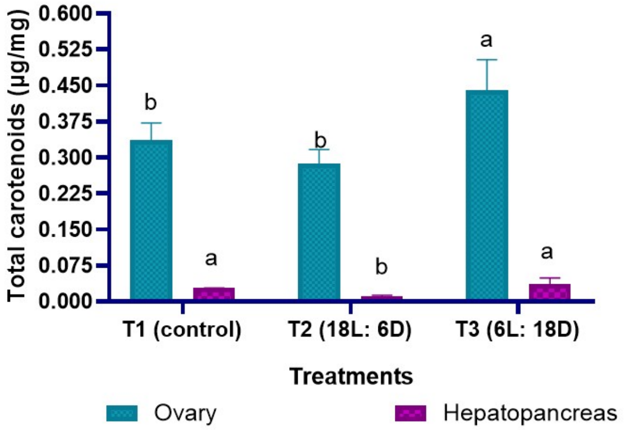

FIGURE 4 Total carotenoids of ovary and hepatopancreas in S. serrata reared at different photoperiod levels. Values were expressed as mean ± standard deviation (*n* = 3). In the graph, each parameter bar with a different alphabet letter represents significant differences (*p* < 0.05).

The hepatopancreas also exhibited marked photoperiod-dependent structural differences. In (6L:18D) groups T3, the hepatopancreatic tubules were well-organized with intact epithelial linings, prominent B- and R-cells, and abundant lipid droplets, indicating active nutrient absorption and efficient lipid storage. However, hepatopancreatic tissues from the (18L:6D) group (T2) displayed degenerated, shrunken tubules with reduced secretory activity, suggesting compromised metabolism due to insufficient lipid intake. Overall, histological evidence substantiates that a (6L:18D) photoperiod level effectively supports gonadal development and hepatopancreatic functionality by maintaining a physiological balance between lipid storage and translocation, critical processes for reproductive maturation in *S. serrata*.

4 | DISCUSSION

4.1 Growth performance, GSI and HSI

The current research has clearly shown that photoperiod plays a significant role in growth performance and reproductive indices of *S. serrata*, and the short photoperiod (6L:18D) has shown better results than the long photoperiod. The final weight, weight gain, specific growth rate (SGR), gonadosomatic index (GSI), and hepatosomatic index (HSI) of crabs raised in darkness (6L:18D) were much higher, which suggests that long dark periods are more favourable to the growth of the crabs as well as their reproductive development. Such results indicate that lower light intensity could be the optimal way to allocate energy to growth and gonadal maturation. Similarly, Morales and Barba (2015) have reported that zero photoperiod increased growth and survival of *S. serrata* and Hussain *et al*. (2017) reported that continuous darkness induced growth and accelerated ovarian maturation in *M. lamarrei lamarrei*. In contrast Chen *et al*. (2023) reported that the highest growth rates were observed in those exposed to 18 L:6 D conditions in *Scylla paramamosain* juveniles.

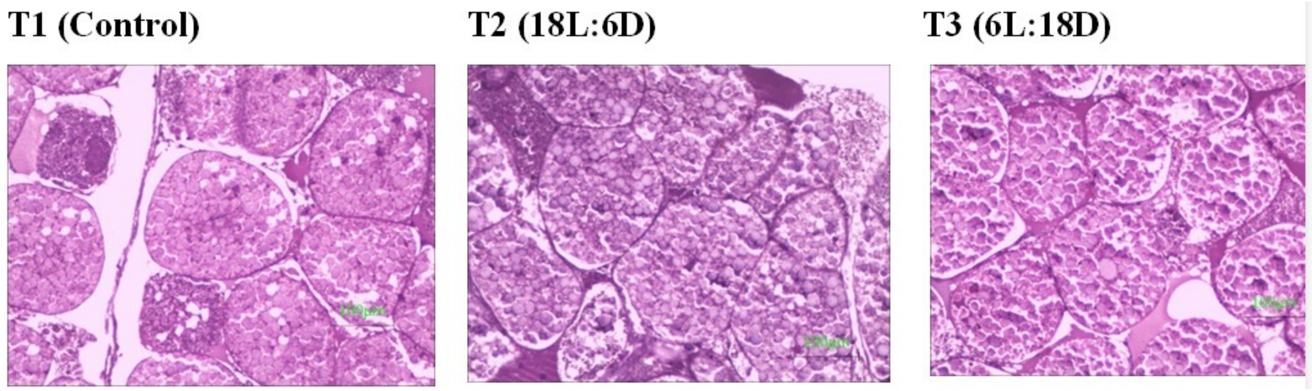

FIGURE 5 Histological section of ovary of the female *Scylla serrata*, reared at different photoperiod levels using 20X magnification. The control crabs had compact ovarian structures with large, yolk-filled vitellogenic oocytes, while the T2 (18L:6D) group had disordered lobules and less yolk deposition. The T3 (6L:18D) group had well-developed ovaries with a large number of vitellogenic oocytes, suggesting better maturation.

The increase in growth performance observed under prolonged darkness could be attributed to improved feeding efficiency and metabolic regulation. Photoperiod is known to influence behavioural rhythms, feeding behaviour, and energy metabolism in crustaceans (Bishop and Herrnkind 1976). Reduced light conditions may decrease stress and unnecessary activity, thereby allowing a greater proportion of dietary energy to be utilized for growth. A similar pattern has been reported in *S. serrata*, where feeding efficiency and weight gain were significantly influenced by environmental factors, including photoperiod (Morales and Barba 2015). Additionally, studies on *Scylla paramamosain* have demonstrated that photoperiod manipulation can significantly affect growth performance and physiological metabolism, particularly lipid utilization (Chen *et al*. 2023).

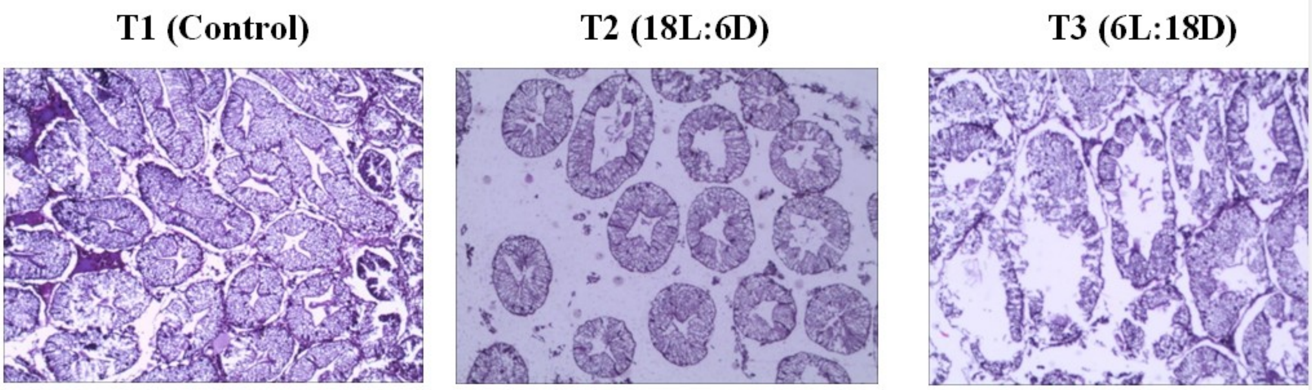

FIGURE 6 Histological section of hepatopancreas of the female *Scylla serrata*, reared at different photoperiod levels using 20X magnification. The tubules in the control and T3 (6L:18D) groups were well organized, had intact epithelium, and had balanced lipid storage. T2 (18L:6D) displayed degenerated tubules with decreased secretory activity.

The significantly higher GSI values observed under the 6L:18D photoperiod indicate enhanced ovarian development and reproductive maturity. Photoperiod regulates reproductive processes through neuroendocrine mechanisms, particularly by modulating the release of gonad-inhibiting hormone (GIH) from the eyestalk (Nagaraju 2007; Chang and Mykles 2011). Reduced light conditions may suppress the release of inhibitory hormones, thereby stimulating vitellogenesis and accelerating gonadal development. This observation is consistent with findings in other crustaceans, where dark conditions promote ovarian maturation and increase gonadal indices. For example, ovarian development in *Macrobrachium lamarrei* was significantly improved under continuous darkness, with higher ovarian indices and advanced stages of oocyte development (Hussain *et al*. 2017).

The hepatosomatic index (HSI) also followed a similar trend, with higher values recorded under the 6L:18D regime, indicating enhanced hepatopancreatic activity and energy storage. The hepatopancreas is a key organ involved in the digestion, storage, and mobilization of nutrients during reproduction, particularly for the transfer of lipids to developing oocytes (Ren *et al*. 2022; Chen *et al*. 2023). Higher HSI values under reduced photoperiod suggest efficient nutrient assimilation and allocation toward reproductive processes. Similar relationships between HSI and reproductive development have been reported in mud crabs and other decapod crustaceans (Wu *et al*. 2007).

In contrast, crabs exposed to longer photoperiods (18L:6D) exhibited reduced growth and reproductive indices, suggesting that excessive light may negatively affect physiological performance. Prolonged light exposure can increase metabolic stress and disrupt endocrine regulation, thereby inhibiting growth and maturation. This is supported by previous studies in crustaceans, where extended light conditions were associated with reduced reproductive performance and delayed ovarian development (Hoang *et al*. 2002).

There were no significant differences in survival rates among treatments, indicating that all photoperiod regimes were within the tolerable range for *S. serrata*. Although prolonged darkness resulted in slightly higher survival, the differences were not statistically significant. Similarly, Chen *et al*. (2023) reported that extended periods of darkness increased survival. In contrast, studies on other decapods have demonstrated that photoperiod can significantly influence larval performance. For example, *Portunus trituberculatus* showed higher survival under 12L:12D and 18L:6D (and natural photoperiods) compared to extreme regimes such as 0L:24D, 6L:18D, or 24L:0D (Xu *et al*. 2022). Therefore, there is no universally optimal photoperiod across species or even among different life stages. However, numerous studies highlight the importance of incorporating a dark phase. In the present study, the superior growth and reproductive performance observed under the 6L:18D regime clearly demonstrate its suitability for broodstock management.

4.2 Digestive enzyme analysis

The present study demonstrates that photoperiod plays a significant role in regulating digestive enzyme activity in *S. serrata*, with the highest activities of amylase, protease, and lipase observed under the short photoperiod (6L:18D). This suggests that reduced light conditions enhance digestive functionality and nutrient absorption, which are essential for promoting growth and reproductive development. The elevated enzyme activities in the hepatopancreas and midgut under the 6L:18D regime indicate a strong physiological capacity to digest carbohydrates, proteins, and lipids, thereby enabling efficient energy utilization.

Photoperiod significantly regulates digestive enzyme activity, carotenoid deposition, growth, and reproductive performance in *S. serrata*. The highest activities of amylase, protease, and lipase observed under the short photoperiod (6L:18D) suggest that extended dark conditions enhance digestive functionality and nutrient absorption. This is consistent with findings in decapod crustaceans, where favourable environmental conditions improved digestive enzyme activities and nutrient utilization efficiency (Han *et al*. 2018; Xu *et al*. 2020). Notably, elevated lipase activity under 6L:18D indicates enhanced lipid digestion and mobilization from the hepatopancreas to the ovary, a key process during gonadal maturation in mud crabs (D'Abramo 2002; Ren *et al*. 2022; Chen *et al*. 2023). In contrast, crabs under prolonged light (18L:6D) exhibited significantly lower enzyme activities, likely due to disruption of circadian rhythms and metabolic homeostasis (Xu *et al*. 2018), while the control (12L:12D) supported only baseline metabolic functions.

4.3 Total carotenoids

The higher carotenoid accumulation in the ovary and hepatopancreas under 6L:18D further reflects enhanced reproductive readiness, as carotenoids are essential for oocyte development and antioxidant protection (Tantikitti *et al*. 2015; Wade e*t al*. 2017; Chen *et al*. 2023). Correspondingly, superior growth performance, GSI, and HSI under 6L:18D indicate that extended dark conditions favour both somatic growth and gonadal development. It has been suggested in the literature that reduced light conditions may influence gonad-inhibiting hormone activity, potentially contributing to enhanced reproductive maturation, though hormonal measurements were not conducted in the present study (Nagaraju 2007). Comparable improvements under darker conditions have been reported in other crustaceans (Bishop and Herrnkind 1976; Morales and Barba 2015). Despite these differences, survival remained unaffected across all treatments, confirming that photoperiod manipulation does not compromise animal health. Overall, a short photoperiod (6L:18D) optimizes nutrient utilization, carotenoid transfer, and reproductive performance, making it a promising strategy for broodstock management in mud crab aquaculture.

4.4 Histological observations

Histological observations in the present study clearly demonstrate that photoperiod significantly influences ovarian maturation and hepatopancreatic functionality in *S. serrata*. Crabs exposed to the short photoperiod (6L:18D) exhibited well-developed ovarian lobules with abundant vitellogenic oocytes containing dense yolk globules, indicating advanced maturation. In contrast, the long photoperiod (18L:6D) resulted in poorly organized ovarian tissue, reduced oocyte size, and limited yolk deposition, suggesting delayed or incomplete vitellogenesis. These findings are consistent with previous studies indicating that reduced light exposure enhances reproductive development in crustaceans by modulating neuroendocrine pathways (Nagaraju 2007; Chang and Mykles 2011). Similar enhancement of ovarian maturation under dark conditions has been reported in *Macrobrachium lamarrei*, where increased vitellogenesis and oocyte growth were observed.

The hepatopancreas also showed pronounced photoperiod-dependent changes. The 6L:18D group exhibited well-structured tubules with prominent B- and R-cells and abundant lipid droplets, reflecting active nutrient absorption and efficient lipid storage. Conversely, degeneration of hepatopancreatic tubules under 18L:6D indicates impaired metabolic activity. This supports earlier findings that the hepatopancreas serves as a key site for lipid accumulation and mobilization during ovarian maturation in mud crabs (Ren *et al*. 2022; Chen *et al*. 2023).

Overall, the results suggest that shorter photoperiods enhance reproductive performance by maintaining a balance between lipid storage and transfer to the ovary, thereby promoting efficient vitellogenesis and broodstock quality in *S. serrata.*

5 | CONCLUSIONS

This study demonstrates that extended scotophase (6L:18D) significantly enhances growth performance, digestive enzyme activity, carotenoid accumulation, and gonadal development in *S. serrata* during reproductive maturation. The comprehensive improvement across biochemical, physiological, and histological parameters indicates that photoperiod manipulation represents an effective, non-invasive strategy for optimizing broodstock conditioning in mud crab aquaculture. These findings contribute to our understanding of environmental regulation of crustacean reproduction and provide a scientific foundation for developing sustainable hatchery management practices.

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ETHICAL APPROVAL

The present experiment was carried out in strict compliance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Environment and Forests (Animal Welfare Division), Government of India, for the care and use of animals in scientific research. Prior to the commencement of the study, approval was obtained from the Institutional Animal Ethics Committee (IAEC) of Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Nagapattinam, Tamil Nadu, India.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Credit authorship contribution statement Manimaran H. - Conceptualization, Investigation, Sampling, Analysis & Writing; Chidambaram P. - Project administration & Validation; Cheryl Antony- Supervision and guidance; Uma A - Supervision and guidance. Selvaraj S. Sampling, data collection, and analysis; Dinesh R.: Data curation, Writing, Review & Editing; Velmurugan R.: Crab procurement and conduct experiment; Yuvarajan P.: Assisted in collecting biological samples and conducting experiment; Joshna M.: Conceptualization, Review & Editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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