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Breeding Biology and Spawning Seasons of the Orange Mud Crab (Scylla olivacea) in a Coastal River of Bangladesh

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Running head: Breeding biology and spawning seasons of orange mud crab in Bangladesh

Breeding Biology and Spawning Seasons of the Orange Mud Crab (*Scylla olivacea*) in a Coastal River of Bangladesh

Abstract

Background: As crucial export commodities with high prices and high demand in the international market mud crab (*Scylla olivacea*) farming is booming rapidly in Bangladesh. Many mud crabs are captured arbitrarily from nature and hence place exquisite stress on their wild stock, which is a serious conservation deal. Enforcing bans during the peak breeding season and fixing a legitimate harvest size are considered rational safeguards for the gentle migration and spawning of berried females. Consequently, there is a dire need to diagnose the breeding peak and to establish conservation policies for berried crabs. Live samples of *S. olivacea* were collected throughout the year from the adjacent river Rai sweeping landmass of the southwest Sundarbans mangrove.

Results: The peak breeding season was identified from March-April despite reproduction throughout the year. A second peak was found in July-August. The availability of crablets (1-2 months of age) was highest in April-May but lowest in January-February. Additionally, the greatest number of berried broods was found in March (39%) in captivity. The maximum GSI (12.09) was recorded in February-March, which implies that the breeding time is on the verge. The minimal size at first maturity (MSFM=M_{50}) was assessed as 87.3 mm CW which fell into the size class 80-89 mm (CW).

Conclusion: The findings of this study will aid in imposing a forbidding period of capture and thus shielding the mother crabs. Furthermore, it will facilitate in policy making to conserve adolescent crabs in recognition of the minimum authorized catch size that will ascertain adult intercourse and beg once anyhow before their catch.

Key words: Berried brood, Histology, Reproductive biology, Sexual maturity, Spawning

Introduction

The orange mud crab, *Scylla olivacea* is a valuable and uppermost export-oriented nonconventional aquatic commodity after tiger shrimp [1, 2], resulting from pleasant food when it is used as a food source [3]. Worldwide, evolved mud crab aquaculture practices are dependent solely on natural repositories collected annually [4] by artisanal fishermen [1]. In Bangladesh, mud crabs are found in southeastern and southwestern coastal districts, but are abundant in the Khulna, Bagerhat, and Satkhira districts of southwestern coastal regions adjacent to the Sundarbans mangrove forest [5]. It is being caught from intertidal to subtidal wetland ecosystems of mangrove forests and is sold in the depot in Bangladesh [6]. On the other hand, crabs that are unfit for export are hardened and fattened for a short period of time to improve the muscles and gonads, respectively [7, 8], and from Bangladesh, mud crabs are exported in live forms [6, 9]. However, increased international demand and price [10, 11] triggered the crab fishers to harvest all size groups and over exploitation [12] to maximize their income [13]. Approximately 80% of the crab populations are harvested before they reach the first size of maturity [14], thus hampering their regular recruitment and threatening the natural stocks associated with the severe pressure on the wild crab population [15] in Southeast Asian countries, including Bangladesh.

Understanding the biological features such as reproductive biology and spawning seasons is a basic tool for developing authoritative management strategies and planning to safeguard imminent breeders [16-18], which guarantees intermittent recruitment in the wild stock of commercially exploited species such as the orange mud crab. In addition, assessment of existing stock sizes and breeding seasons deserves research priority for the implementation of legal harvest sizes, restrictions on catch areas and seasons for sustainable management purposes. However, to the best of our knowledge, information on the biological features of the mud crab is still limited to zero in

Bangladesh. Research has yet to identify the breeding biology and spawning seasons of the mud crab in Bangladesh. Reporting without proper identification will be abandoned in the near future. Therefore, understanding the aspects of the breeding biology, size at maturity, and peak reproduction season of *S. olivacea*, a mud crab in Bangladesh, is necessary.

Materials and methods

Study area and duration

This study focused on three potential sites of River Rai [S1 (22°17'49.79"N, 89°18'38.40"E); S2 (22°16'36.72"N, 89°19'43.23"E); S3 (22°15'52.82"N, 89°20'39.20"E)] located beside the Koyra subdistrict sweeping across the fringe of the Sundarbans mangrove forest in Khulna district, Bangladesh (Fig. 1) from July 2022 to June 2023.

Sample collection and processing

Crabs in the live state were collected (total=325) during each full moon period from the adjacent River Rai of the Sundarbans mangrove forest flowing beside the Koyra subdistrict under the Khulna district, Bangladesh (Fig. 1). The identification of the breeding biology and spawning season was carried out with three-dimensional (3D) observations *namely*, (a) the availability of gravid broods (Observation of the gonadal maturity stage; estimation of the GSI and determination of the minimal size at first maturity (MSFM=M_{50})), (b) the abundance of crab larvae/crablets in the Mangrove River and (c) the attainability of berried broods in captivity.

Determination of the gonad development stage or maturity status of gravid broods

For the observation of gravid broods, crab samples were collected from the sampling sites, and carried out in the laboratory, graded and sexually mature females were dissected, and the presence or absence of gonad mantle and its proportion were measured. Gonad samples were selected and categorized following standard protocol [19, 20]. The gonadosomatic index (GSI) of females was

84 calculated via the following formula [21]:

85 GSI = 100×GW/TW, where GW is the gonad weight (g) and TW is the total body weight.

86 **Sampling larva or crablets (C1) for estimating abundance**

87 A 50-meter distance from the river flowing beside the mangroves at all the sampling sites during
88 every full moon period was sampled with a 1-meter mouth opening push/drag net. The entire
89 sample was preserved in 10% formalin and subjected to laboratory/hatchery. The plethora of small
90 crabs, *e.g.,* Megalopa, crab instars, and crablets (1-2 months of age) were separated and counted,
91 after which the peak of the abundance was recorded. The absolute breeding season was scrutinized
92 by the back-computation of crablet age [22].

93 **Assessing the size at maturity**

94 Probit and logit analyses were carried out to measure the 50% sexual maturity (M_{50}) of females
95 [23, 24]. The sampled crabs in the 10 mm carapace width (CW) size groups were allotted for this
96 analysis. For the purpose of probit scale conversion, percentage data from the mature female crabs
97 of all size classes were computed. After the probit data were plotted upon CW, a regression line
98 was affixed to the data points. The median size (M_{50}) at sexual maturity was calculated by
99 extrapolating the value of CW corresponding to probit 5 [24]. With respect to logit analysis, the
100 percentage of adult females in every size class was estimated and converted to the logit ranking. A
101 regression line was fitted when the logit value was plotted against CW. The 50% maturity (M_{50})
102 or MSFM was assigned at a value coequal to logit 0.

103 **Estimating the highest occurrence of berried broods**

104 A total of 60 gravid broods were reared in sand bed concrete tanks supplied with seawater. Once a
105 crab turned into berried, it was removed and recorded for the corresponding months. Similar-sized
106 gravid broods were introduced to maintain their number and density. The process was carried out

throughout the year.

Histology of gonads

The histological work was performed at the Laboratory of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202. The 'animal tissue technique' method established by [25] Humason, (1972) was followed in this study. The histological analysis and the classification of the type of spawning were performed according to [26] Islam *et al.*, (2010). Fragments of the ovary were fixed in Bouin's fixative for 24 h and processed according to the standard histological methods mentioned above. The tissues were sectioned into slides via a rotating microtome (Leica RM2255, Leica Biosystem, Germany). For the staining of permanent slides, hematoxylin and eosin (HE) was used. Following this procedure, the material was mounted in Canada balsam, then were examined, selected, photographed (OLYMPUS BX 53), and described.

Statistical analysis

The data computation, analysis, and presentation of the graphs and table were created by employing Microsoft Office Professional Plus 2016. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Software (version 20.0, IBM Corporation, Armonk, NY, USA).

Results

The highest abundance of crab larvae/instar was recorded from 170-210 crablets/CPUE; in the months of April and May, whereas the lowest was found between January and February (9-16 crablets/CPUE), suggesting that the previous month (March) might be a peak of spawning/breeding (Fig. 2). Moreover, another intermediate peak was explored around September - November, thus attributing a second peak of spawning (July - August).

130 The monthly fluctuations in the gonadosomatic index (GSI) of *S. olivacea* are displayed in Fig. 3.
131 The GSI values reached a maximum in February and March, ranging from 9.22 to 12.09 (% of
132 body weight). The loftiest level of the GSI indicates that the breeding season is approaching close,
133 which means that they might breed either at the end of February or during the time course of either
134 March or April. From the pause of March to June, the GSI values steered in a descending order,
135 but a second pinnacle of the GSI (8.92) was recorded in July - August. However, the trends of the
136 GSI at the broad scale varied slightly ahead from July, indicating that August - September would
137 also be another peak breeding time. A comparatively smaller GSI was reflected from October to
138 December (1.25 - 4.6). Monthly surveillance of gonadal development revealed that, mud crabs (*S.*
139 *olivacea*) are the prolific breeders with all maturity stages during the year but with a significant
140 seasonal oscillation.
141 The monthly changes in maturity stages are shown in Fig. 4; laying out that the highest volume of
142 fully gravid crabs (41.93-45.65%) was observed from February - March indicating that the
143 breeding season was approaching. A comparative percentage of mature crabs began depleting after
144 March and continued to deplete until the end of May. An additional peak of maturity was traced
145 during the end of July, which suggests that the second peak of breeding might be in August. These
146 findings also indicate that a large proportion of crabs are in an immature state. Since mature crabs
147 were observed each month, they are year-round breeders.
148 The relationships between the gonadosomatic index (GSI) and each ovarian development stage are
149 shown in Table 1. The GSI was lowest at Stages I and II and commenced to increase after yolk
150 aggregation initiated at Stages III. The ovaries in the mature state had the highest mean of GSI
151 approximately12 %. The changing patterns of the maturation stages of various size classes of
152 female mud crabs are delineated in Fig. 5. The figure shows that the size class with a CW of greater

than 100 mm presented the highest proportion of fully mature crabs (50%). The results also revealed that the mud crab reached full maturity only when it reached 70 mm CW.

As depicted in Fig. 6, berried broods were available under hatchery conditions, and the highest proportion (39%) of berried broods were available in March, followed by April, May, and June (33%, 28%, and 28%, respectively). A second narrow peak was recorded in September (11%).

The maturity size of *Scylla olivacea* (M_{50}) was ascertained as 87.3 mm CW through probit analysis (Fig. 7A), and 50% maturity was found at 87.4 mm using logit analysis (Fig. 7B). The probit analysis yielded a result almost identical to that of the logit analysis.

Oocytes encounter different developmental stages and turn into fully mature eggs. However, the process of oocyte development was asynchronous in *S. olivacea*. On the basis of the external shape and size of the oocytes, the formation and accumulation of yolk granules, the status of the nucleus and cytoplasm, and the presence of advanced oocytes via histology, the oocytes were classified into five distinct gonad development stages (Table 2, Figs. 8-9).

The immature or proliferative ovary (stage I) has a vitreous to translucent color, making it difficult to distinguish from the digestive gland. Stage I ovaries embodying with oogonia were largely located on the border of the ovary (Fig. 8A). The shape of each oogonium (5-10 μm) is spherical, with barely noticeable cytoplasm (Fig. 9A). The developing or previtellogenic ovary (stage II) consists of double lines on the top part of the digestive gland and can be segregated simply from the gland (Fig. 8B). The ovaries at the stage II are characterized by the formation of primary oocytes including vacuolated globules in the cytoplasm and follicle cells lining the edge of the cytoplasm (Fig. 9B). The inception of vitellogenesis occurs in the early development or primary vitellogenesis stage. The ovary becomes yellow in color (Fig. 8C). The vacuolated globules were no longer observed in the cytoplasm. Small yolk globules develop from the edge of the oocyte

cytoplasm but they occupy a portion of the entire cytoplasm (Fig. 9C). At the late-maturing or secondary vitellogenic (stage IV) stage, lobules appeared in the sterno-carapace and upper digestive gland (Fig. 8D). The prompt growth of oocyte occurs in this stage (120-200 µm), and toward this stage or at the end of primary vitellogenesis, the nucleus becomes extravagant in size (30-40 µm). The follicle cells were hardly discernible, and the cytoplasm was irregular with larger globular inclusions near the periphery (Fig. 9D). In the mature or tertiary vitellogenesis stage (stage V), the ovary expanded to its maximum size, finally enveloping the hepatopancreas and the greater portion of the cardiac stomach (Fig. 8E). The yolk globules were partially fused together (Fig. 9E).

Discussion

A clear understanding of breeding biology, the breeding season, and availability is considered basic for efficient management of natural stocks and planning effective strategies for protecting potential breeders [16, 17] and vulnerable offspring to ensure regular recruitment in natural stocks of commercially exploited species. However, mapping and assessing the stock status of regularly exploited species might forecast the judicial harvest for sustainable production.

However, through careful and intense observations of the abundance, GSI, maturity stages, and availability of berried crabs, two distinct breeding peaks were determined, with the principal peak occurring from March - April and July-August as the secondary peak (Figs. 2-4 and 6). Two breeding peaks are not surprising because two breeding peaks are recognizable in other marine and brackish crabs [19, 27]. *Scylla serrata* inhabiting in India has two breeding peaks: one from December - March, and the other between September and November [28].

In India, the flower crab (*Portunus pelagicus*) spawns from February to March [29], and in South Australia, it grows for 3-4 months through the summer and autumn seasons [30]. This ordinary

discrepancy between that study and ours could be associated with differences in geographical areas and species, since the reproductive biology of species fluctuates with the season and environment [31, 32]. Various studies have established that mud crabs give birth to their lineage in the sea and that young crablets drift to inshore waters or quiescent water for aliment. In addition, the breeding season varies widely depending on the topographical properties and running hydrological status [33]. The findings of our study confidently assured that February, March, and April are the breeding months of the mud crab, whereas the time frame between March and April is the peak of spawning. Like some other studies [19, 27, 28], crablets (1-2 months of age) and mature females, even berried broods, were found throughout the year (Figs. 2-3 and 6), which suggests that the mud crab (*S. olivacea*) is a persistent breeder. The findings of our study are also consistent with the results in the blue swimming crab *P. segnis, Leptodius exaratus* crab, xanthoid crab *Eriphia smithii*, and portunid crab *Thalamita pelsarti* [34-38].

The GSI and size of a species indicate the maturity status of that species. The results of this study revealed that at stages I and II, the GSI remained low, bearing female gonads with a white translucent color, and started to increase when yolk aggregation progressed from a light orange color to stage III (Table 2, Fig. 4). The fully mature crabs with dark orange gonads had the highest mean of GSI (>12%). The results suggest that, at a GSI less than 4, the female *S. olivacea* yield either immature or developing gonads; whereas at a GSI above 8, they enter the late mature or fully mature stage. The changes in the maturation stages of various size classes of female crabs demonstrated that the size class nearest 100 mm CW contained the highest percentage of fully mature crabs (50%). From a conservation perspective, this size is crucial since it is the minimum allowable size at which female mud crabs can be captured.

Consistent with the present study of histological assessment, [18, 39] Ikhwanuddin *et al.* (2014)

222 and Amran *et al.* (2018) reported that oocyte diameter increased linearly as ovarian maturity
223 accelerated, but oocyte diameter differed significantly (Table 2). Oocytes and follicle cells
224 constitute the major cell types in the ovarian lobes of *Scylla* species. In this study, immature gonads
225 had larger and explicit follicle cells, which is analogous to those observed in *S. olivacea* by Islam
226 *et al.* (2010) [26].
227 The external morphology of the gonads revealed that the color of the immature ovary is watery to
228 translucent and turns yellow-orange to red-orange in the mature state. These phenomena coincide
229 with the results of Fahimi *et al.* (2017) and Ali *et al.* (2020) [19, 40] but contrast with those of *S.*
230 *paramamosain* [26]. This color variation could be caused by diet intake by the crab [41]. However,
231 the accumulation of lipids in the form of yolks in the oocytes may influence the degree of ovarian
232 color fluctuation [39].
233 Generally, the mean size at first maturity (MSFM) is often used as a legal minimum size for
234 decapod species retention. Many open-water mud crab fisheries use 50% mature size as the
235 ordinary smallest ethical size exclusively for female crabs [24, 42]. Most of the *S. olivacea*
236 captured from the mangrove region of the Khulna division consisted of small crabs. In this study,
237 87.3 mm CW was estimated as the MSFM (M_{50}) of female *S. olivacea*. The closest MSFM value
238 of 95.5 mm CW for *S. olivacea* was reported by [40] Ali *et al*. (2020) who studied the southwest
239 mangrove River Chuna in Shyamnagar, Satkhira. This slight discrepancy in the onset of maturity
240 within the same species might be related to the facts of habitat location (latitude), ecological
241 conditions (food abundance), environmental factors (salinity and temperature), changes in
242 molting, and the increase of molting events [27, 43, 44]. Despite geographical differences, our
243 results strongly matched with the M_{50} values of *S. olivacea* (84.63 mm CW and 86.00 mm CW)
244 from the mangrove areas of Sundarban in the southwest coastal region of Bangladesh [45] and

Sarawak coastal waters of Eastern Malaysia [46], respectively. Additionally, [14] Islam & Kurokura (2012) reported a slightly higher M_{50} (100 mm CW) of female *S. olivacea* from the mangrove swamp of the Thailand coast. In support of our results, [1] Macintosh et al. (2002) suggested that the mud crabs (*S. serrata*) should be no smaller than 100 mm CW in the Ranong fishery located on the western coast of Thailand (1). The differences in the results might be caused by species diversity. As the M_{50} for *S. olivacea* (87.3 mm CW) in the mangrove region of Khulna lies within the size class of 80-89 mm CW, the minimum valid harvesting size of female *S. olivacea* should be at least 90 mm CW, which will be beneficial for the protection and conservation of young crabs.

This research revealed that maturity size does not vary greatly, but it may fluctuate depending on the species within a particular area. As long as this study was confined to a single place, the results in the deep sea and other areas may disagree. In view of this, additional research on mud crab sexual maturity is warranted before any legal mud crab capture size is established in Bangladesh. Since young mud crabs constitute more than 70% of the population, scientists should administer concrete management and systematic conservation schemes for the mud crab population in Bangladesh.

Conclusion

This study revealed that the orange mud crab (*Scylla olivacea*) breeding season lasts between February and April, whereas the peak period of spawning occurs from March to April. Nevertheless, to be safe, the month of February should be included in the peak breeding season, as the availability of fully mature crabs tends to increase from late February onward. The present study revealed that female mud crabs should be captured at least at 90 mm CW, which will exonerate and protect young crabs. Prior to setting any size limit for mud crab catches in

Bangladesh, more research on the sexual maturation of male crabs is warranted. By and large this study represents an authentic and rational scientific account of peak breeding span and the size of earliest maturity for the mud crab (*S. olivacea*). Thus, the results of this study could be invaluable for establishing any laws or policies pertaining to the conservation and management of the natural population in Bangladesh.

Declarations

Ethics approval and consent to participate

The authors were aware of animal ethics clearance from the concerned authorities. However, the protocol of the experiment was approved by Bangladesh Fisheries Research Institute (BFRI), and the research was conducted by considering the guidelines of the 'Animal Welfare Act 2019' approved by the National Parliament of Bangladesh.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Shawon Ahmmed: Data curation, formal analysis, investigation, resources, software, validation, visualization, writing-original draft, and writing-reviewing & editing. Mizanur Rahman Washim: Data curation, formal analysis, investigation, methodology, software, validation, writing-original draft, and writing-reviewing & editing. Mollah N.S. Mamun Siddiky: Investigation, resources, and writing-reviewing & editing. Md. Latiful Islam: Conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, and writing-review & editing.

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Figure Captions

Figure 1. Sampling sites at Koyra subdistrict under Khulna district of Bangladesh.

Figure 2. Month wise variation in plentitude of crab larvae/instar. The thin black line diagram illustrating the actual number of crablets. The shadow with blue-colored solid graph in back of the line implies the forecasted birth-month of the crablets. CPUE means: The number of larvae/Instar^{-1} mouth opening push or drag net during single high tide, approximately 1-2 h of hauling.

Figure 3. Changing pattern of GSI at successive months. The data are presented as mean of GSI. The solid line manifesting the average GSI of the month, and the dashed line represents the general data trend.

Figure 4. Proportion of different gonad maturation stages of mud crab in the sample.

Figure 5. Frequency of maturation stage in different size classes for female *Scylla olivacea*.

Figure 6. Monthly availability of berried mud crab in the hatchery condition.

Figure 7. Probit values of mature female crab percentage vs. carapace width (mm) **(A)** and Logit values of mature female crab percentage vs. carapace width (mm) **(B)**.

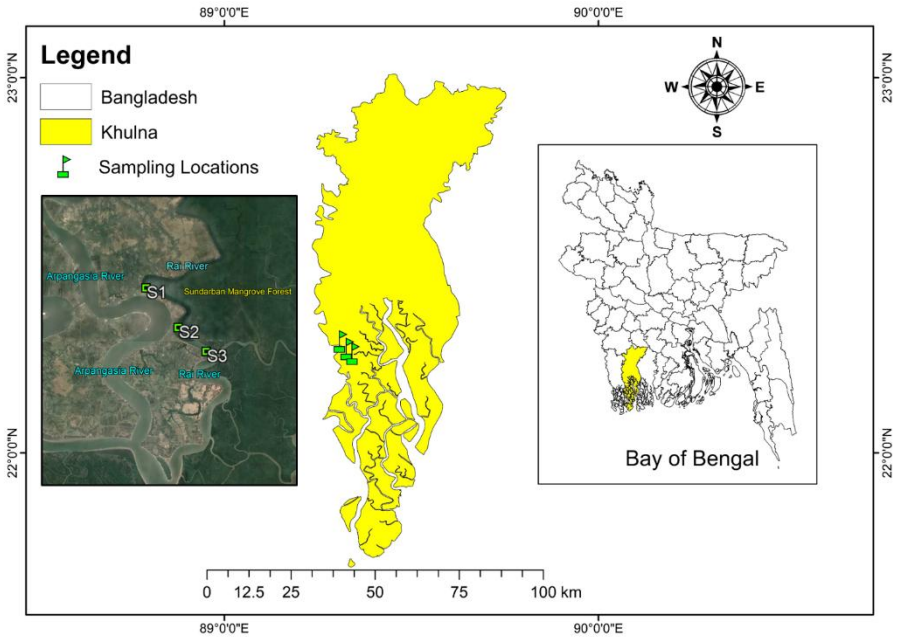
Figure 8. Anatomical observation of ovary development stage of *Scylla olivacea* collected from the mangrove river Rai; Proliferation **(A)**; Pre-vitellogenesis **(B)**; Primary vitellogenesis **(C)**; Secondary vitellogenesis **(D)**; Tertiary vitellogenesis **(E)**. [†Note: O= Ovary; HP= Hepatopancreas; I=Intestine; H= Heart; Sp= Spermatotheca; Cs= Cardiac stomach; Rs= Respiratory organ.]

Figure 9. Histological micrographs of different gonadal development stages of wild caught female mud crab. Proliferation **(A)**; Pre-vitellogenesis **(B)**; Primary vitellogenesis **(C)**; Secondary vitellogenesis **(D)**; Tertiary vitellogenesis (mature) **(E)**. [†Note: O= Oogonium, F= Follicles, Lu= Lumen, N= Nucleus, Nu= Nucleolus, P= Primary oocytes, Yg= Yolk globules, V= Vacuolated globules, Vt= Vitellus].

Table Captions

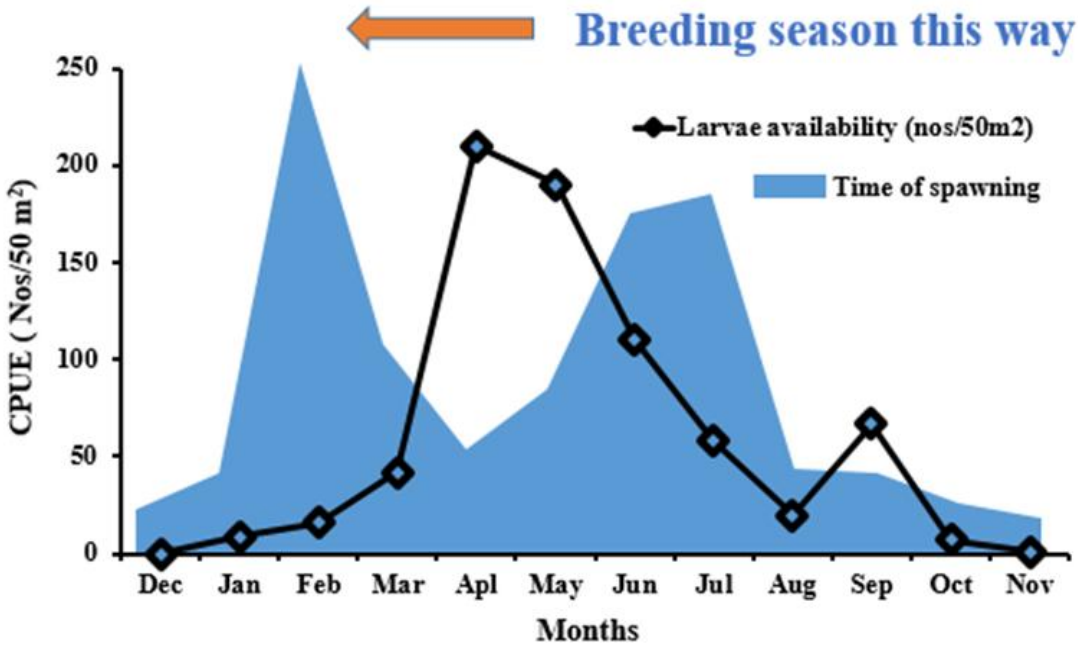
Table 1. Frequency distribution of GSI for every development stage of female gonad.

Table 2. The macroscopic and microscopic (histological) characteristics of ovarian developmental stages of *Scylla olivacea*.



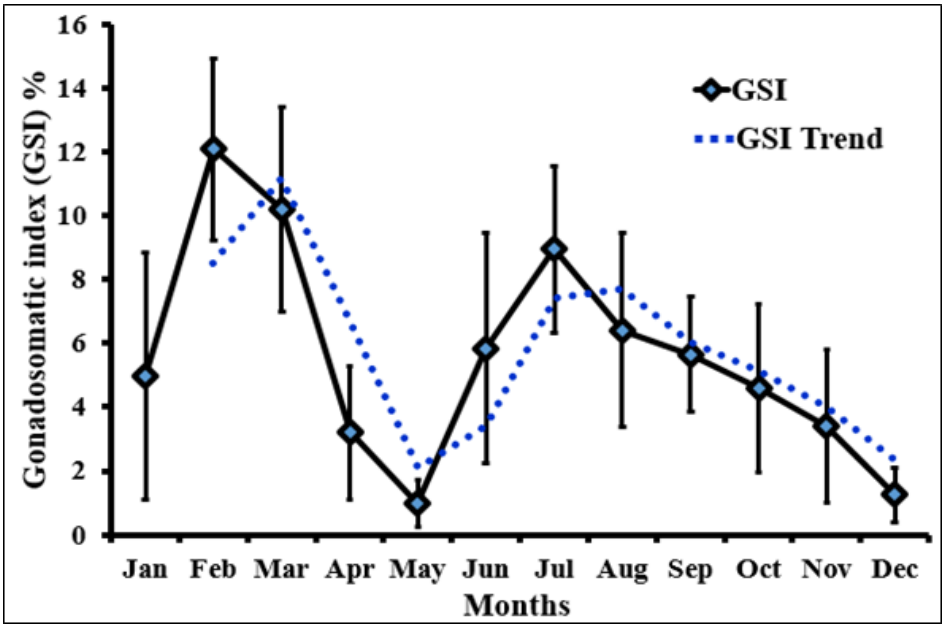
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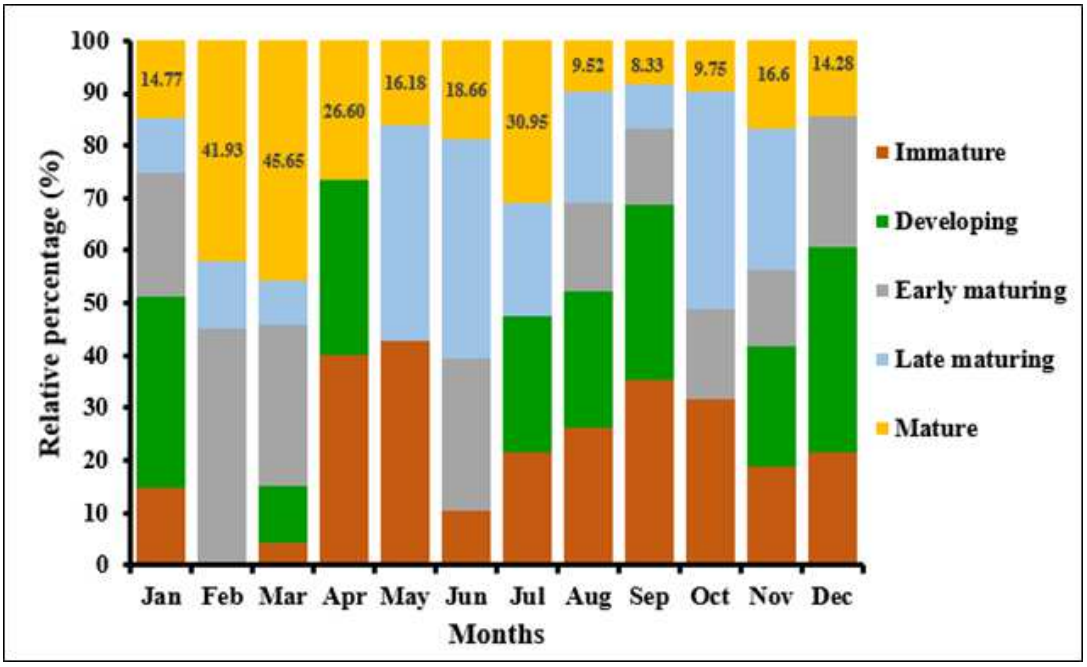
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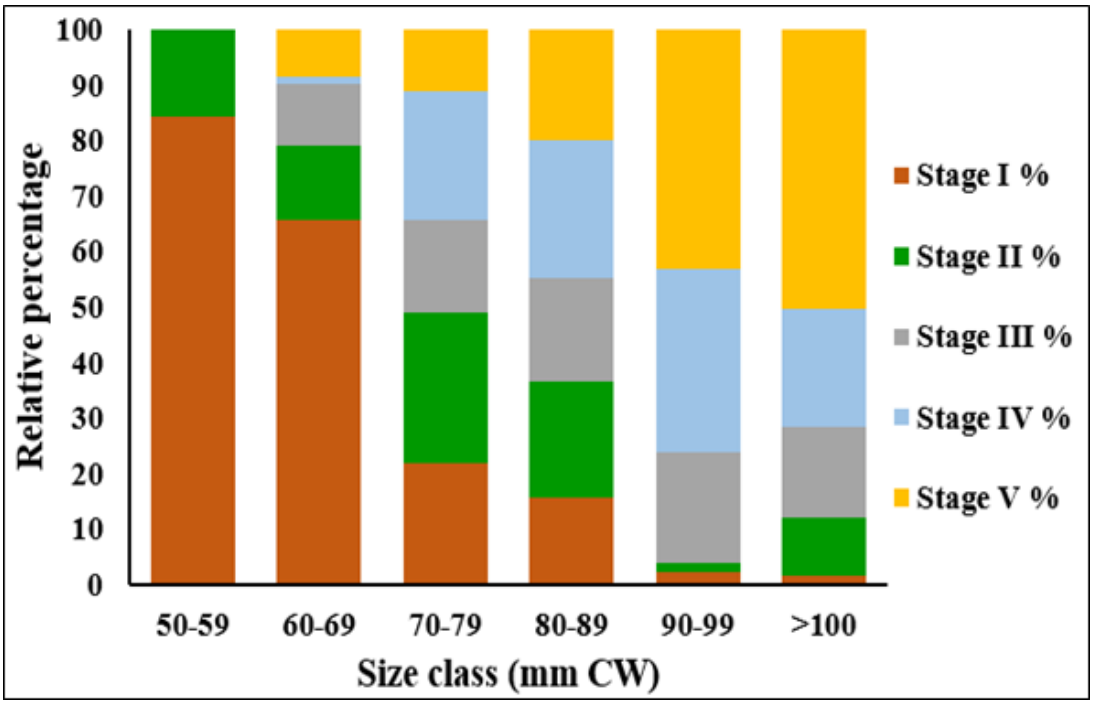
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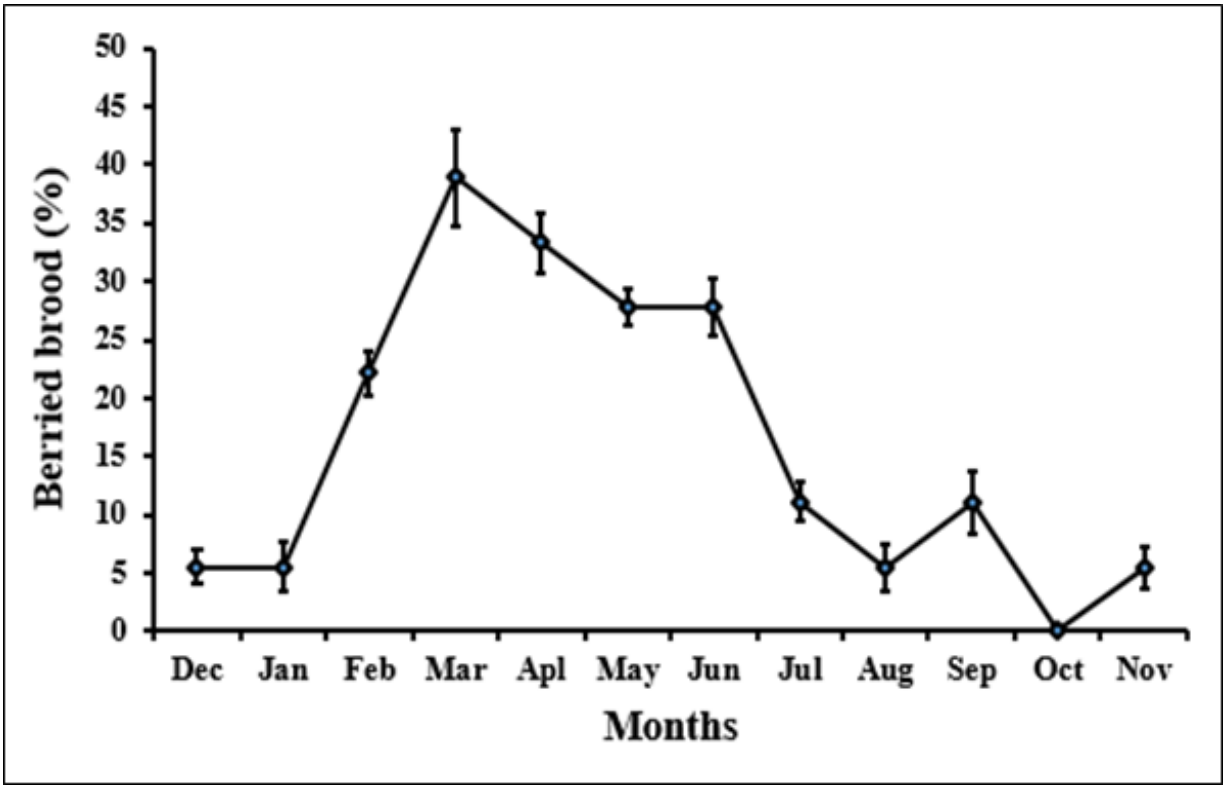
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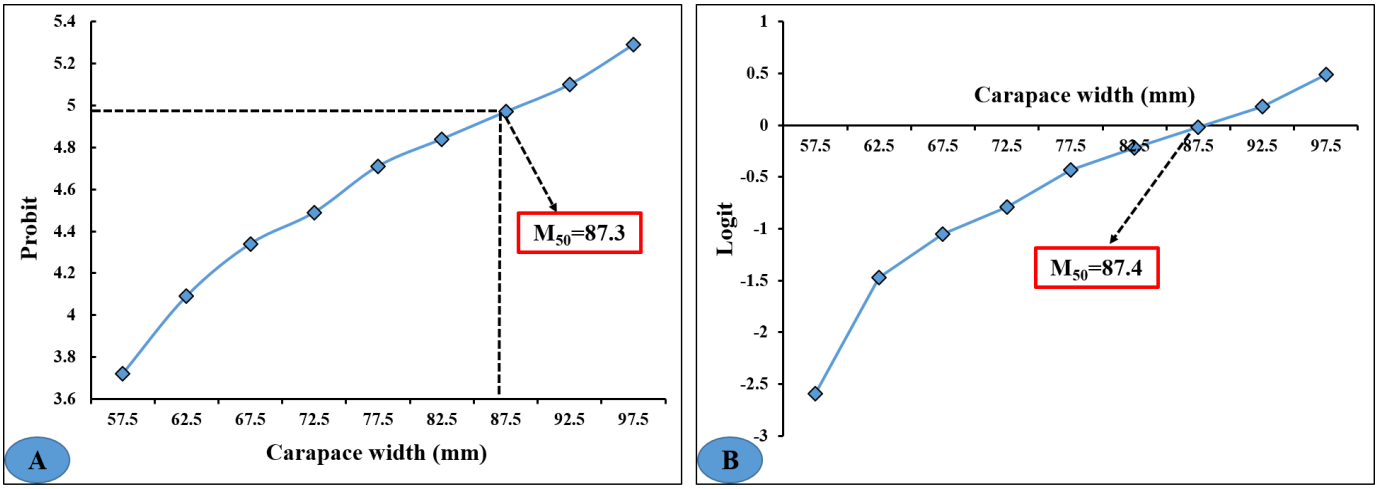

Relative percentage
100
90
80
70
60
50
40
30
20
10
0
50-59
60-69
70-79
80-89
90-99
>100
Size class (mm CW)
Stage I %
Stage II %
Stage III %
Stage IV %
Stage V %

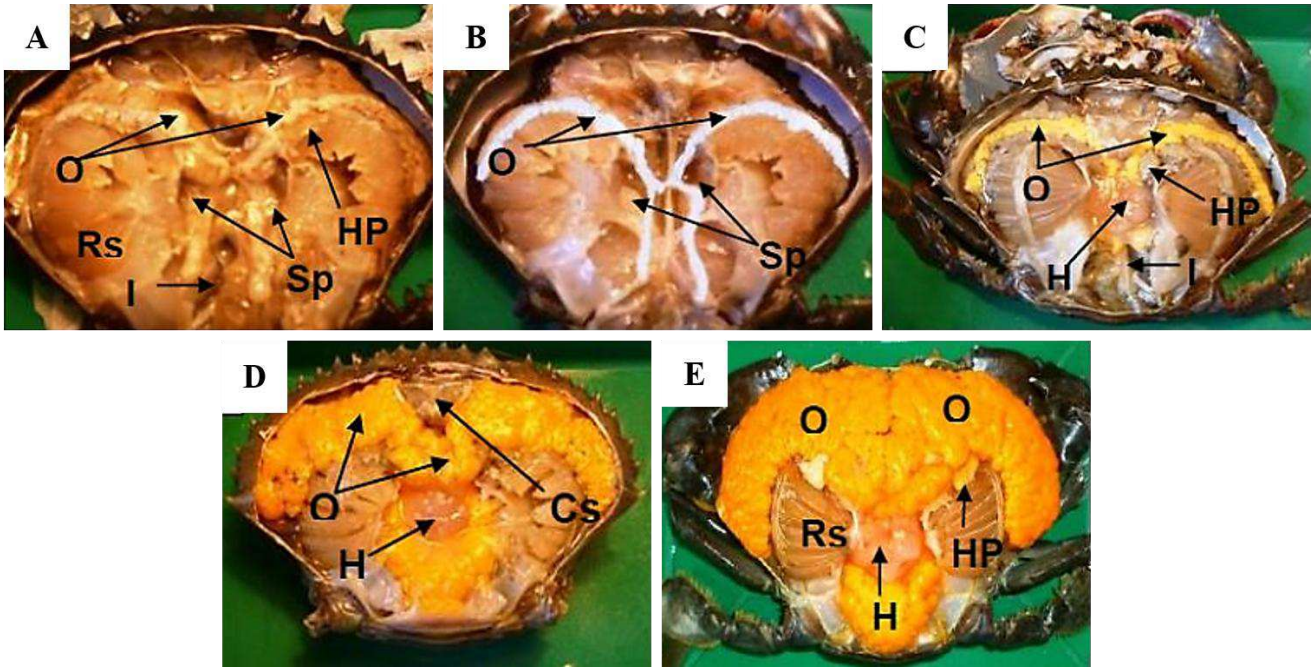
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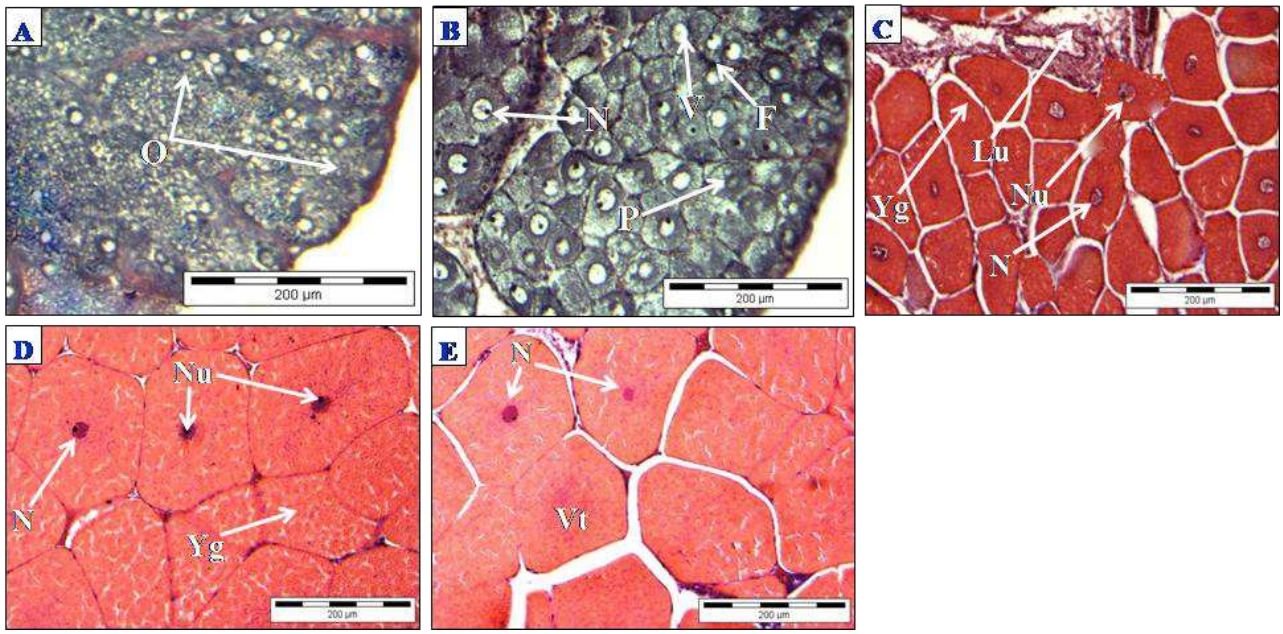
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Berried brood (%)
50
45
40
35
30
25
20
15
10
5
0
Dec
Jan
Feb
Mar
Apl
May
Jun
Jul
Aug
Sep
Oct
Nov
Months

494


A
Probit
Carapace width (mm)
M50=87.3
B
Logit
Carapace width (mm)
M50=87.4


A
O
Rs
I
Sp
HP
B
O
Sp
C
O
HP
H
I
D
O
H
Cs
E
O
O
Rs
HP
H


A
O
200 µm
B
N
V
F
P
200 µm
C
Lu
Yg
Nu
N
200 µm
D
Nu
N
Yg
200 µm
E
N
Vt
200 µm

501

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