

ORIGINAL ARTICLE

Reproductive biology of mud crabs (*Scylla olivacea*) collected from Paikgachha, Khulna, Bangladesh

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ABSTRACT

Objective: This study was carried out to estimate the sex ratio, maturity size, gonadosomatic index (GSI), and peak breeding season of mud crabs.

Materials and Methods: Samples were collected randomly from the estuary and river of the study area. Sampling was carried out monthly from April to September at every full moon during one high tide. A total number of 240 specimens were sampled, where 53 individuals were hermaphrodite. The crabs were shifted alive to the biology and histology lab for detailed biological study. Sex was determined. Male and female sex ratio and breeding season were also investigated.

Results: The male:female ratio was 1:0.96 and the ovarian development was categorized into five stages based on internal observations, viz. immature (stage I), underdeveloped (stage II), early developed (stage III), late developed (stage IV), and mature (stage V). The maturity percentages were 37%, 19%, 13%, 11%, and 20%, respectively. 50% maturation was estimated at 82.36 mm internal carapace width (ICW). The highest mean GSI value was 7.97 ± 3.03 . The mature stage was found in all the working periods. This shows that females have activated ovaries in all the working months, and the species are continuous breeders. A higher frequency of vitellogenic ovary and higher GSI value were found in September. The maximum GSI value was found in the size group 70–79 mm.

Conclusion: The study shows that the capture from the wild sources of mud crabs without any regulation can threaten the population structure. The capture of female mud crabs should be more than 82.36 mm ICW, which will help conserve and protect young crabs.

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Introduction

Bangladesh is the world's largest deltaic country and is enriched with a large number of aquatic species. Because of Bangladesh's geographic condition, these resources provide a feasible circumstance to develop fisheries [1]. The fisheries sector's national economic contribution is 3.69% to the Gross Domestic Product (GDP) and 22.60% to the agricultural GDP [2]. There were 60% of the country's exported mud crabs coming from the mangrove forests of the Sundarbans [3]. In Bangladesh, 60% of people's daily need for animal protein comes from supplementary fish [4]. Bangladesh's coastline has 710 km associated with 618,780 ha of mangrove tidal flats and 80,000 ha of surrounded areas suitable for coastal aquaculture [5].

Recent information from the Department of Fisheries of Bangladesh states that about 300,000–400,000 people are directly or indirectly associated with mud crab aquaculture for their livelihood [6]. Mud crabs (*Scylla* spp.) are one of the most delicious seafood items and attain high economic value worldwide, especially in tropical and subtropical countries [7]. The growing demand for mud crabs in Asia, Europe, and America has led to increased mud crab production [7,8]. It is a euryhaline species found in coastal waters with a 2–30 ppt salinity and is particularly dominant in the mangrove area. Biochemically, mud crabs are enriched with 15%–25% protein, 1% fat, and 2%–3% minerals [8,9] that make them a popular food item globally and with high market prices. For these reasons, mud crab *Scylla olivacea*

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is a highly exploited species associated with mangrove ecosystems because of over-harvesting. In the southeast and southwest regions of Bangladesh, Chittagong, Cox's Bazar, Noakhali, Bhola, Potuakhali, Barguna, Khulna, Bagerhat, and Satkhira, mud crab aquaculture has been practiced for many years [10]. According to Export Promotion Bureau 2001 [11], crab export earned \$33 million in 2018–19, which was \$23.28 million in 2015–16 [12]. Around 99% of crabs are exported from Bangladesh that is harvested from the Sundarbans [13].

Due to the increasing demand for mud crabs in the local and international markets, most families in the greater Khulna area have chosen to grow crabs as their primary income source [14,15]. Crab collection and fattening are alternative sources of income and sustainable livelihood for the disadvantaged people in southwest Bangladesh. Despite tremendous opportunities, the mud crab trade is subject to wild capture, mostly from coastal mangroves, and there is no absolute procedure for hatchery production [7]. As a result, the natural populations of mud crab are declining throughout southeast Asia due to over-exploitation, loss of mangrove habitat, water pollution, and coastal environment degradation. Approximately, less than 80% of the crab populations are being caught before reaching the first size of maturity [16]. For this cause, extensive management activities are speedily needed for the mud crab to sustain its population through over-exploitation. It is essential to know the biological aspects about the mud

crab such as maturity size, peak season, and other biological parts. This study aims to identify the maturity size, ovarian development stages, gonadosomatic index, and breeding season of *S. olivacea*.

Material and Methods

Ethical approval

The Research Ethics Committee of the Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore, Bangladesh, had given ethical approval (approval number: FBST/JUST/AECA-4012/2018 Date: April 02, 2018). All procedures carried out involving fish during the present study followed the institutional guideline, following the international and national guidelines.

Study site

S. olivacea crabs were collected primarily from estuaries and rivers near Paikgachha upazila in the Khulna district (Fig. 1). For further study, mainly breeding aspects were studied in the Fisheries and Marine Bioscience laboratory at Jashore University of Science and Technology.

Study period

The study was carried out over a calendar year from April to September 2018, and samples were collected each month from the study site.

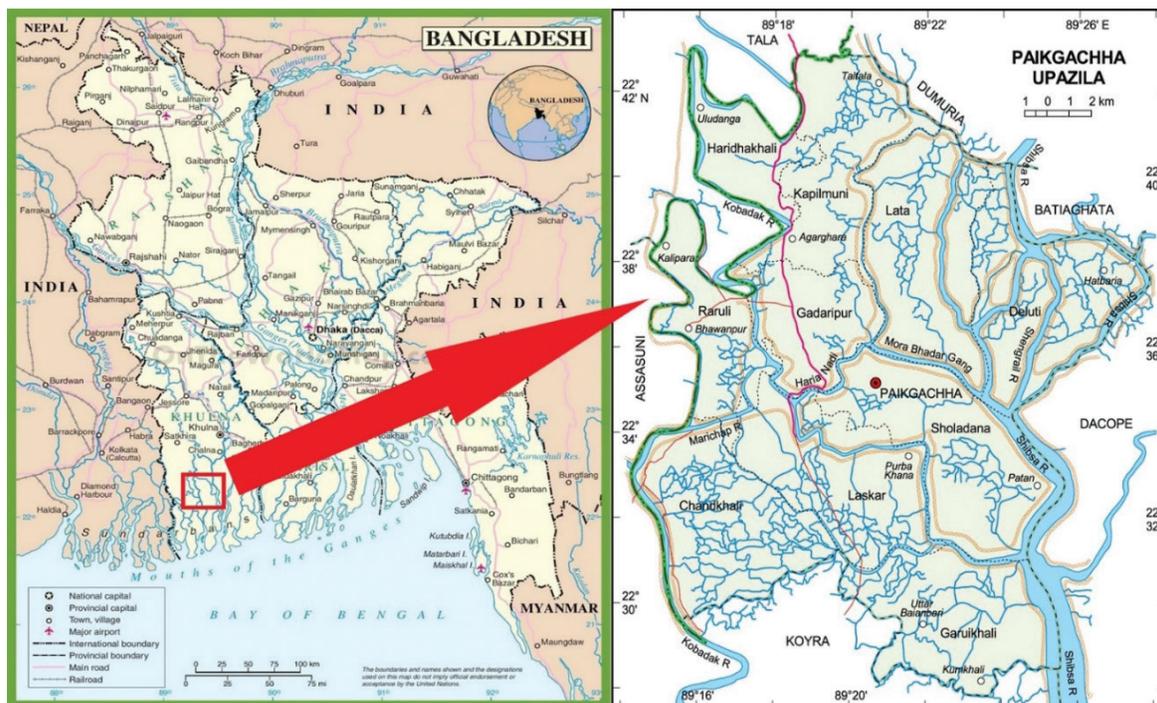


Figure 1. Study area (Paikgachha upazila in Khulna district).

Sample collection

Live crabs were collected randomly from the estuary and river of Khulna and Shatkhira regions. The sampling was carried out monthly from April to September on each full moon during high tide. A total of 240 specimens were sampled where 53 individuals were hermaphrodites. The crabs were then transported alive to the Biology Laboratory of the Department of Fisheries and Marine Biosciences at Jashore University of Science and Technology for detailed biological study [17].

Sex identification

The abdominal flap of each crab was observed to determine the sex. In male *S. olivacea*, the abdominal flap was narrower like a “V” shape, female crabs had a wider “U”-shaped abdomen, and hermaphrodite mud crabs had irregular abdomens (Fig. 2) [18]. The female crab's abdominal flap is a little bit narrower at the first stage of life, and gradually the shape changes with its life stages.

Determination of sex ratio

The ratio of males to females of the collected samples was estimated by the following formula [19]:

$$\text{Sex Ratio} = \frac{\text{Female crab (Nos)}}{\text{Male crab (Nos)}}$$

Where GSI = gonadosomatic index, GW = gonad weight, and TW = total body weight [17].

Investigating the breeding season

Breeding season was determined by counting the mature females monthly, which was expressed as %.

Data analysis

Collected data were carefully summarized before actual tabulation. Some data were found in local units when collected, and these data were converted into international

units during data processing. The processed data were then transferred to a master sheet from which classified tables were prepared to reveal the study's findings. Preliminary data sheets were correlated with computer spreadsheets to ensure data accuracy.

Results and Discussion

Sex ratio

The sex ratio was analyzed based on 187 specimens of *S. olivacea* collected from the months of April to September 2018. Out of 187 specimens of *S. olivacea*, 95 specimens were male and 92 specimens were female. The total male:female ratio was determined to be 1:0.96 (Table 1). The highest number of females was found in August, while the lowest was in September. Viswanathan et al. [23] recorded the sex ratio of males and females as 1:0.87, which is quite similar to the present study. Kannathasan and Rajendran [24] recorded the sex ratio as 1:1.01 in the southeast coast of the Bay of Bengal of India. Ali et al. [25] observed the sex ratio of males and females as 1:0.94, which is in agreement with the present study.

Maturity size

The common minimum legal size used in many open water mud crab fisheries only for female crabs was at least 50% maturity (M_{50}) [26,27]. The current study found that the size at initial maturation (M_{50}) of female *S. olivacea* was estimated at 82.36 mm (ICW) (Fig. 3). Ali et al. [17] detected that the size at first maturity (M_{50}) to be approximately 95.5 mm CW, which lay at a size class of 91–100 mm (CW). Similarly, M_{50} at ICW of 10.3 cm (103 mm) for wild *Scylla paramamosain* was witnessed in Thailand [18]. Khor et al. [28] reported that CW varies from 47 to 134 mm for *S. olivacea*, which is similar to the present study. Islam et al. [29] also reported that 50% of female individuals get mature at the size of 84.63 mm ICW. Prasad and Neelakantan claimed that the estimated M_{50} size of females was 91–100 mm ICW on the coast of Karwar, India [30].



Figure 2. Different sexes of crab. (a) Male crab with V-shaped carapace, (b) female crab with U-shaped carapace, and (c) hermaphrodite mud crab with an irregular carapace. Photos taken from Piakgacha crab market by Sumona Khatun, JUST, Bangladesh.

Table 1. Monthly variations in the sex ratio of *S. olivacea* randomly collected from Paikgachha, Khulna.

Months	Total no. of specimens	Total male	Percentage of male	Total female	Percentage of female	Sex ratio
April	28	10	35.71	18	64.29	1: 1.8
May	42	28	66.67	14	33.33	1: 0.49
June	30	13	43.33	17	56.67	1: 1.30
July	37	20	54.05	17	45.95	1: 0.85
August	22	5	22.73	17	77.27	1: 3.4
September	28	19	67.86	9	32.14	1: 0.47
Total	187	95		92		1: 0.96

$p > 0.05$ is not significantly different.

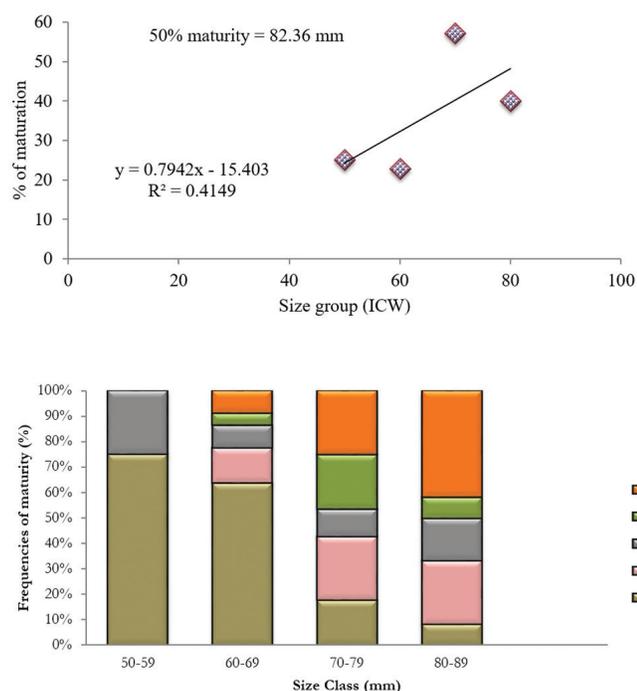


Figure 3. Relationship between initial carapace width (ICW) and maturation percentages (top) and frequencies of maturity size in different size classes for female *S. olivacea* (below). The estimation of 50% maturation of *S. olivacea* from Paikgachha, Khulna. 50% maturity was 82.36 mm.

Ovarian development

The present study investigated the ovarian development stages of *S. olivacea* based on internal observation. Internal observation occurred through dissection of the species. The process of maturation of ovaries was classified into five phases (stages): immature (stage I; color: transparent), underdeveloped (stage II; color: off white), early maturing (stage III; color: yellow), late maturing (stage IV; color: orange), and mature (stage V; color: deep orange). The classification was carried out based on the external

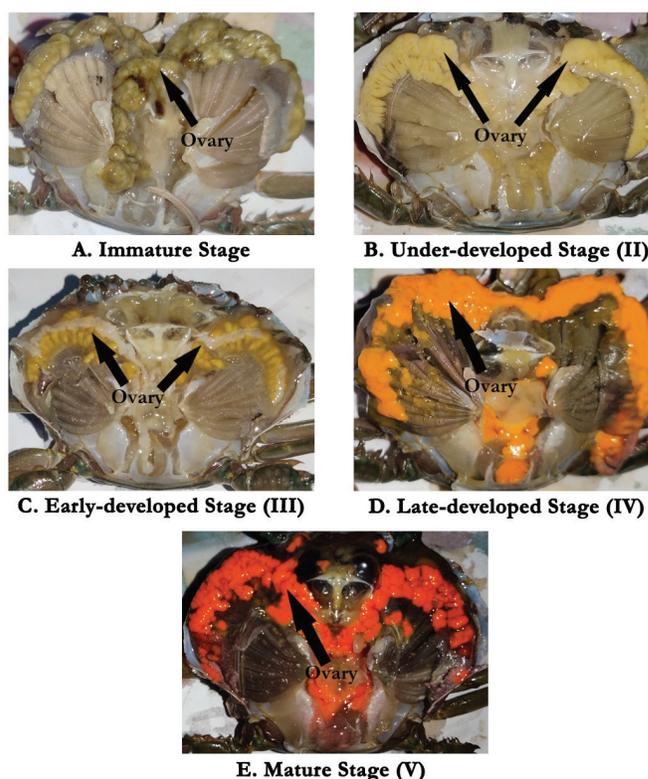


Figure 4. Ovarian development stages of *S. olivacea* collected from Paikgachha, Khulna, Bangladesh.

characteristics and observed color of the ovaries through dissection of the crab [22]. Among the sample population, 37% belonged to the immature stage, 19% to underdeveloped, 13% to early developed, 11% to late developed, and 20% to the mature stage (Fig. 5). Islam and Yahya [31] reported that the mean GSI value was highest (over 10%) at the advanced/mature stage (stage V) and the mature stage ranged from 60– to 69 mm ICW (Fig. 6). Immature ovaries showed a color from translucent to yellow and then were orange when matured. In the present study, the maximum underdeveloped stage (II) color was found to be

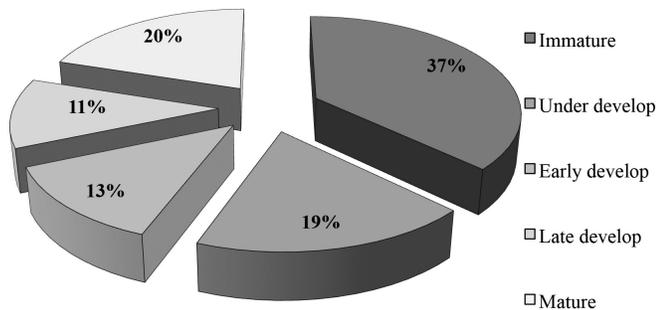


Figure 5. Ovary development stages after observation through the dissection of crab.

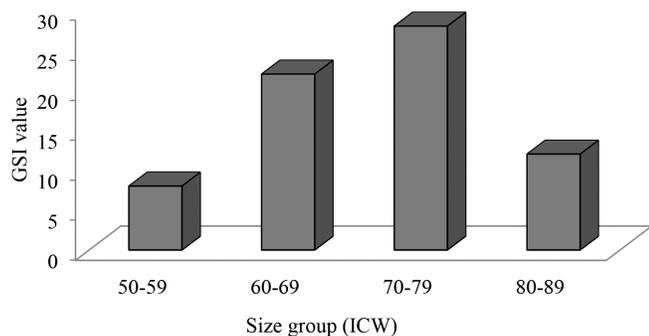


Figure 6. Gonadosomatic index of female *S. olivacea* by size from Feb to Sep 2018 from Pacikgachha, Khulna.

white to creamy white, and the mature stage (V) color was yellow to deep orange (Fig. 4). Farizah et al. [32] observed that the yolk globules showed up and started to appear macroscopically when the ovaries entered the maturation stage (also known as the secondary vitellogenesis stage); the color of the ovary was orange to a deep orange with a tissue thickness of 10–20 mm and the cardiac stomach was eventually covered (>75%). Islam et al. [18] reported that the color of mature or tertiary vitellogenesis stages was yellow to orange, not deep or black orange; the only exception is *S. paramamosain* species. In accordance with Quintio et al. [33], this color variation may happen due to the diet intake of the crab; however, Ikhwanuddin et al. [34] elaborated that the changes of ovarian coloration may occur due to the accumulation of lipid in the form of yolks in the oocytes.

Gonadosomatic index (GSI)

The ratio of crab GW to total body weight is the gonadosomatic index or GSI, primarily used to detect the age and percentages of gonad maturation for reproduction purposes. The relationship between GSI and each ovarian developmental stage is presented in Table 2. Mature ovaries at stage V indicate the highest mean GSI of more than 10% and that was 47.6%. The highest mean of GSI

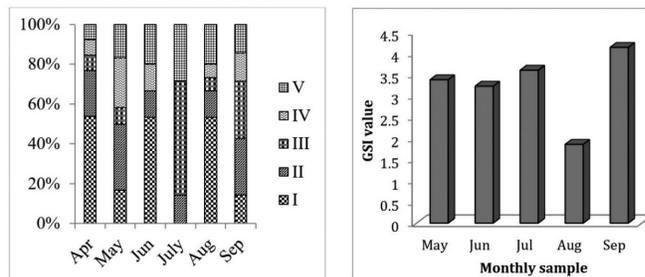


Figure 7. Monthly changes of ovaries at the five development stages of female *S. olivacea* (left) and GSI variation of *S. olivacea* from April to September 2018 (right).

Table 2. Frequency distribution of GSI for each development stages of female *S. olivacea*.

GSI %	Frequency at each development stage %				
	Stage I	Stage II	Stage III	Stage IV	Stage V
<1	6.44	4.91	0.00	0.00	0.00
1–5	0.00	7.66	22.85	11.44	4.36
5–10	0.00	0.00	0.00	32.64	54.41
>10	0.00	0.00	0.00	0.00	47.6
No. of samples	26	13	9	8	14
Mean GSI	0.25	0.96	2.54	5.51	7.97
SD	0.23	0.52	1.3	1.57	3.02

(7.97) was found in stage V (Table 2). The maximum GSI was found in the 70–79 mm size group (Fig. 6). Islam and Yahya [31] observed that GSI was very low at maturing stages (I and II), and as the yolk formation began (stage III), the GSI started to rise subsequently. At the advanced stage (V), maximum average GSI values of over 10% were recorded. The highest mean of GSI (10.35) was found in stage V, supporting the present study's finding. The present study also relates with Islam et al.'s [18] study, which found the highest mean of GSI (10.7). Ali et al. [17] found the low values of GSI at stages I and II which simultaneously began to escalate once the yolk accumulation began at stages III, which rose the mean GSI value up to >11% in the matured ovaries.

Breeding season

The percentage frequencies of maturity stages of ovaries in different sampling months are shown in Figure 7. Mature stages are found for all the working months. The vitellogenic ovary stages III–V were found every month. A high frequency of vitellogenic ovary was noted in September (Fig. 7). The GSI value was high in the month of September (Fig. 7). The peak period for ovary and spawning maturation was found from November to December [35]. The peak period may vary due to the various geographical

areas and different species, and also the biology of the species may be varied with seasons and environment [25]. Ali et al. [17] found that the river adjacent to the southwest part of the Sundarbans is the primary source of live crab collection. Although there is no recorded breeding season of mud crab, March–April was marked as the peak breeding season. The observed second peak breeding season is in August–September, similar to the present study. In the present study, the mean size during maturity was determined only for females, so there should be further research on the sexual maturity of male mud crabs before setting up any legal capture size of mud crabs in Bangladesh. This experiment was conducted only in one area, so the result in deep sea or other areas may vary.

Conclusion

The present study was designed to determine the reproductive biology of the mud crab *S. olivacea* by measuring the body weight, ovary developmental stage, maturity size, and breeding season. Among the sampled population, 44% were matured. For crab culturing, juvenile crabs were used which can be a threat to the population structure. The M_{50} was found at ICW82.36 mm ICW indicates early maturity. Therefore, some conservation measures should be taken by the government to conserve and manage this sector. This study of the reproductive biology of *S. olivacea* can help to build a proper management strategy for crab population conservation.

List of abbreviations

ha = Hectares, GSI = Gonadosomatic index, ICW = Internal carapace width, GDP = Gross Domestic Product, Nos = Numbers, GW = Gonad weight, TW = Total body weight, M_{50} = 50% maturity, CW = Carapace Width.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Authors' contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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