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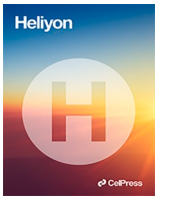

Research article

Annual gametogenic phenology of oyster, *Magallana bilineata* (Röding, 1798) collected from the west coast of Moheshkhali Island, Cox's Bazar, Bangladesh

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ABSTRACT

The current study aimed to describe the annual gametogenic phenology of the oyster *Magallana bilineata* (Röding, 1798) (=*Crassostrea madrasensis*), which is found on the west coast of Moheshkhali Island, Cox's Bazar, Bangladesh. Samples were drawn monthly from the intertidal region during low tide, from which 20 adult individuals were selected at random for biometry and histology. The mean condition index (CI), a ratio of tissue wet weight (g) to shell length (cm), varied from 0.58 ± 0.08 to 1.32 ± 0.36. Histology revealed two spawning cycles in the habitat of *M. bilineata* over the 12 months of the study. Gametogenesis initiated in December and May, and ripe animals principally occurred from July to September and February to May. In the habitat, *M. bilineata* exhibited two spawning peak periods: April to June and August to October. The undifferentiated stage as a preparatory step for the next spawning extended from November to February for the first spawning cycle and for a brief period in June for the next spawning cycle. The initiation of spawning in March could be associated with the high-level decline of salinity and increased temperature between February and March, in association with the annual rainfall start, whereas the next spawning cycle could be associated with a gradual increase in salinity. No spawning activity was reported from December to February, when the water temperature remained below 22 °C. Further study could be undertaken on the timing of spatfall of *M. bilineata* in the habitat to harvest spats for commercial farming of this promising species.

1. Introduction

Oyster farming is a proven industry in many parts of temperate, subtropical, and tropical oceans around the world. The oyster species Magallana bilineata (Röding, 1798) is widely available along the coasts of India, Sri Lanka, and Bangladesh [1–3]. They naturally settle on hard substrates, such as jetties, bridge pylons, sluice gates, and concrete blocks used for shoreline protection [3]. Understanding the reproductive cycle of bivalves is crucial for both proper management of wild stock and aquaculture development [4–7]. Information on annual gametogenic phenology is crucial for understanding gonadal development, spawning time, optimum

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harvesting time, and overall management of any bivalve species. When the reproductive cycle and environmental cues governing the maturation process are known, artificial breeding techniques can be developed for a timely supply of adequate spats at the farming level.

Reproductive phenology is influenced by genetic and evolutionary variables, as well as extrinsic factors such as water temperature, pH, salinity, food availability and other chemical and physical stimuli [5,6,8,9]. For instance, it has been reported that temperature and food availability mainly affect gonadal development and spawning in bivalves [9–12]. Similarly, both endogenous and exogenous factors influence the maturation of oyster brood stock, and the most important external elements are temperature and diet [11,13,14]. Once oysters attain full sexual maturity, slight variations in environmental factors, such as a rise or decline in salinity or temperature, will trigger spawning [15].

Among various techniques, histology is the most reliable approach for measuring the progress of the reproductive cycle in bivalves [9,16–18]. Histological methods classify the oyster's reproductive cycle based on the characteristics of gamete cells at various stages of development in the follicles [5], and gametogenesis can be ordered based on the microscopic appearance of gonadal tissue from histological preparations [19,20].

Studying the oyster reproductive cycle allows us to learn important information about population structure, spawning time, sexual maturation stages, egg production, and favorable ecological conditions for reproduction, all of which aids in predicting the feasibility of oyster farming in any specific geographic region. However, due to either a lack of understanding about the timing of spatfall from wild stock or a lack of oyster hatcheries, no farming system for *M. bilineata* has yet been developed in many parts of the world. All the necessary data could be furnished by studying the reproductive cycle of this commercial species in its natural habitat. Therefore, it is high time to investigate the gonadal histology of *M. bilineata*, which may help us breed them artificially for commercial farming. To that end, the current study aimed to investigate the annual reproductive cycle of the oyster *M. bilineata* collected from the west coast of Moheshkhali Island, Cox's Bazar, Bangladesh, based on biometry and histology.

2. Materials and methods

2.1. Sampling activities

Samples of the Indian backwater oyster *M. bilineata* were collected from Ghotinganga on the west coast of Moheshkhali, Cox's Bazar, Bangladesh (21.65°N and 91.88°E; Fig. 1). Samples were drawn from a bridge pylon on a monthly basis for 12 months, from July 2018 to June 2019. After collection, the samples were brushed and washed with fresh and clean water to remove algal biomass, mud, and other waste material. Next, 20 adult individuals with shell lengths greater than 41.00 mm were randomly selected from the sample group for biometry and histology. Shell length, width, and height were measured using digital calipers. After dissection, the soft tissue wet weight was measured using an electronic balance. Biometric measurements of the analyzed samples are shown in Table 1. The condition index (CI) was calculated as the ratio of wet tissue weight (g) to shell length (cm) [21]. A cross section of 2–3 mm thickness was cut through the middle of the soft body for histology.

2.2. Histology

Routine histological procedures were followed in the current study. Briefly, after fixation in Davidson's solution, the tissue samples were placed in an automatic tissue processor for dehydration, clearing, and infiltration. After embedding, tissue sectioning was

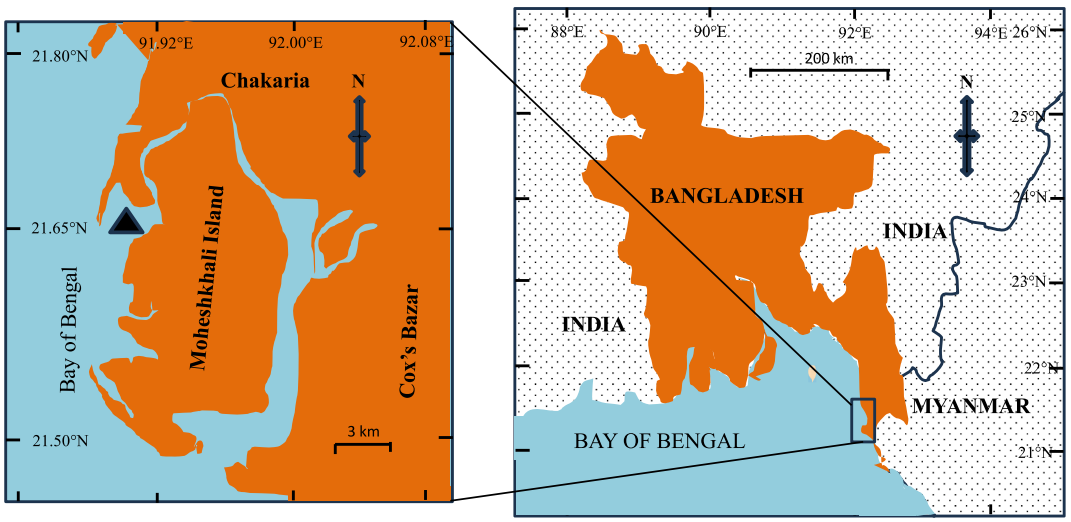

Fig. 1. Map showing sampling location (▲).

Table 1
Summary of sampling efforts of oyster, *Magallana bilineata*. The values represent monthly mean and standard deviation. SH, shell height in mm; SL, shell length in mm; SW, shell width in mm; and TWW, tissue wet weight in g.

Month	N	SL	SW	SH	TWW
Jul '18	20	57.40 ± 13.15	40.90 ± 5.11	22.00 ± 3.59	3.43 ± 1.34
Aug '18	20	58.78 ± 11.48	37.83 ± 9.65	28.36 ± 5.62	7.28 ± 2.48
Sep '18	20	97.98 ± 20.03	67.87 ± 12.73	39.43 ± 13.35	10.38 ± 4.95
Oct '18	20	103.56 ± 16.84	80.91 ± 12.70	34.41 ± 9.13	13.66 ± 4.04
Nov '18	20	89.66 ± 21.44	72.60 ± 22.17	35.74 ± 8.15	12.31 ± 6.99
Dec '18	20	90.28 ± 11.86	68.06 ± 7.96	26.84 ± 8.01	6.82 ± 3.36
Jan '19	20	70.76 ± 20.46	47.90 ± 5.36	21.68 ± 3.18	4.18 ± 1.79
Feb '19	20	67.57 ± 6.89	44.02 ± 5.97	20.21 ± 3.73	4.53 ± 1.91
Mar '19	20	77.91 ± 13.08	51.34 ± 10.42	32.22 ± 10.71	7.55 ± 5.54
Apr '19	20	61.10 ± 11.81	38.41 ± 12.93	21.94 ± 8.41	3.85 ± 2.52
May '19	20	81.20 ± 19.04	51.90 ± 19.84	27.70 ± 6.43	7.80 ± 4.53
Jun '19	20	83.40 ± 30.37	60.00 ± 19.19	33.50 ± 8.77	9.66 ± 7.95

performed in the microtome at 6 μm thickness. The section was stained with Harris hematoxylin, followed by eosin Y, and then mounted on a slide. The histological slides were examined under a microscope to determine the sex and reproductive maturity of the oysters. A compound microscope fitted with a camera (ZEISS Primostar; Carl Zeiss AG, Baden–Württemerg, Germany) and connected to a computer was used to take photomicrographs of the stained sections. The reproductive maturity of the gonads was categorized into six stages [22]: 1) early developing; 2) late developing; 3) ripe; 4) spawning; 5) spent; and 6) undifferentiated (i.e., sexually indifferent). The descriptions and criteria of each gametogenic stage for males and females are summarized in Table 2 and Fig. 2 (A-L).

2.3. Maturity index (MI)

The gonad maturity stages were numerically ranked on a scale of 0–5, where the undifferentiated stage scored 0, spent stage scored 1, spawning stage scored 2, early developing stage scored 3, late developing stage scored 4, and ripe stage scored 5 [23]. The maturity index (MI) was calculated by dividing the sum of the numerical scores of the monthly gonadal stages by the total number of oyster samples analyzed in the respective month. The formula is therefore as follows:

MI = Sum of numerical scores of all stages for a month/number of samples analyzed in respective months.

2.4. Monitoring of waterquality parameters

Water quality parameters, including temperature, pH, and salinity, were monitored monthly. The water temperature at the sampling site was measured three times on each sampling date using a Celsius thermometer. The salinity of the seawater was determined during sampling with a portable refractometer (HI 96822; Hanna Instruments, Smithfield, Rhode Island, USA). A portable direct reading pH meter (HI 98107; Hanna Instruments) was used to measure the pH of the surface seawater during sampling. Monthly rainfall data for the Cox's Bazar region during the study period were obtained from recorded data provided by the Bangladesh Meteorological Department.

2.5. Statistical analysis

All collected data were analyzed statistically and expressed as mean (±SD) using SPSS statistical software version 23.0 (IBM Corp., Armonk, NY, USA). A chi-square test (χ^2-test) was performed to test the null hypothesis of a 1:1 sex ratio between males and females. Furthermore, a Pearson correlation analysis was performed to analyze the degree of relationships among the environmental parameters, MI, and CI.

Table 2
Criteria for identification of maturity stages of *Magallana bilineata* modified from Barman et al. (2022) [22].

Maturity stage	Male	Female
Early developing	Primary and secondary spermatogonia develop along the base of the follicle wall.	Small oocytes develop along the base of the follicle wall.
Late developing	Centripetal arrangement of the spermatozoa, spermatocytes and spermatids in the follicle with few spermatogonia.	Around 50% oocytes attached to the follicle walls by slender peduncles; eggs have vitelline membrane with nucleus at the center.
Ripe	Enlarged follicles with their lumen tightly packed with spermatozoa forming plums at many places.	Many mature ova free from the lumen with a large nucleus at the center of the ovum.
Spawning	Follicle collapsed; large lumina with few spermatozoa loosely filled the follicles.	Germinal vesicle broken; acini look partially empty due to release of some ova.
Spent	Follicles contain very few relict spermatozoa; follicles begin to degenerate.	Acini collapsed and empty; some residual broken oocytes present with clear signs of cytolysis.
Undifferentiated	Sex indistinguishable; many empty spaces between and within the follicles; phagocytic cells abundant inside and outside the follicles.	

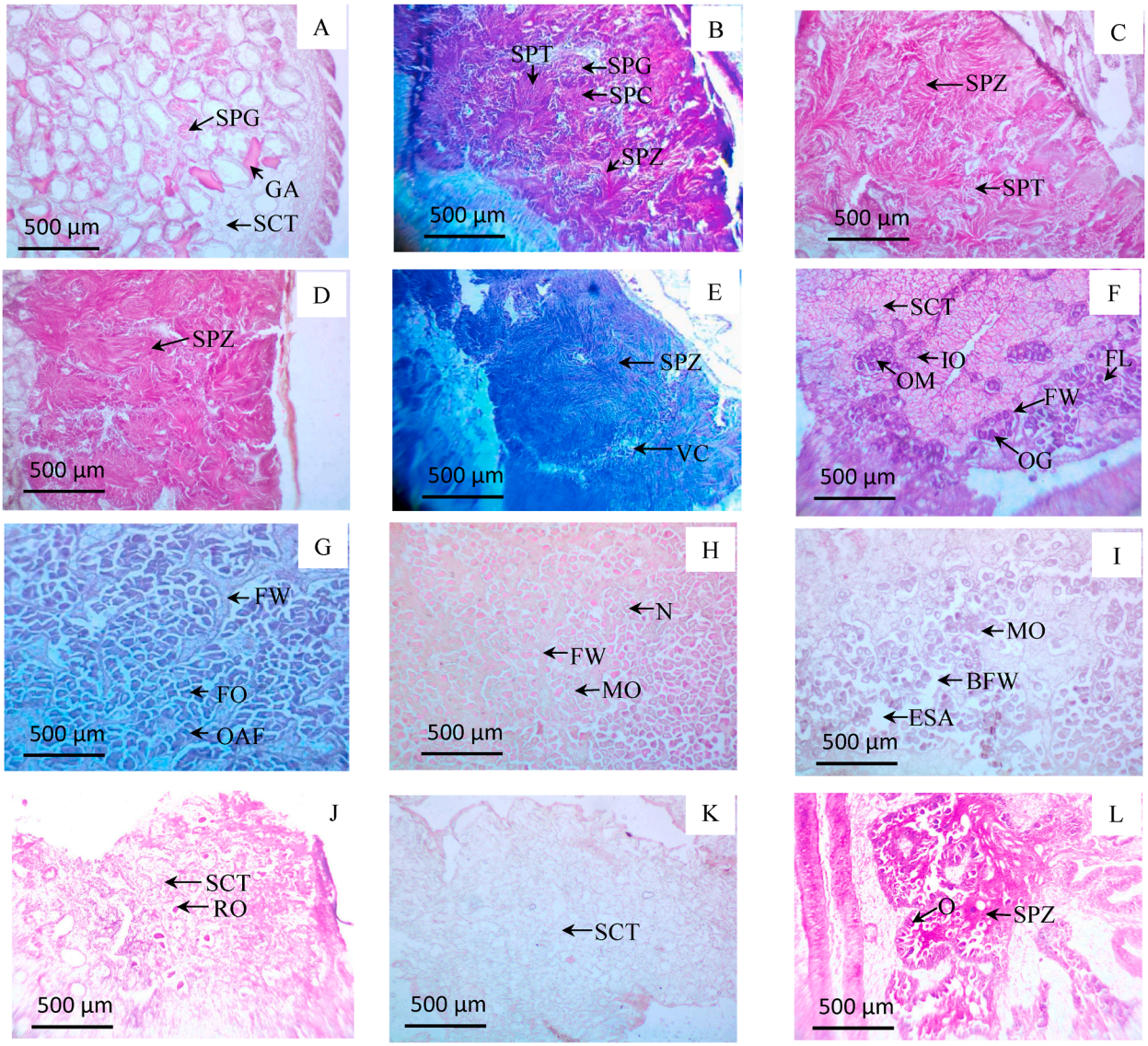

Fig. 2. Photomicrographs of different gametogenic stages of the oyster (*Magallana bilineata*) in gamete development. A) Early developing male; B) Late developing male; C) Ripe male; D) Spawning male; E) Spent male; F) Early developing female; G) Late developing female; H) Ripe female; I) Spawning female; J) Spent female; K) Undifferentiated; and L) Hermaphrodite. Here, GA = gonadal acinus; SCT = storage connective tissue; SPG = spermatogonia; SPC = spermatocyte; SPD = spermatid; SPZ = spermatogonia; VC = void cells; FL = follicle lumen; FW = follicle wall; OG = oogonia; IO = immature oocyte; OM = maturing oocyte; MO = mature oocyte; FO = free oocyte inside lumen; O = oocyte; RO = residual oocyte. Mag. 10×, Scale bar: 500 µm.

3. Results

3.1. Environmental parameters

The monthly recorded environmental parameters of water temperature, salinity, rainfall, and pH at the sampling site from July 2018 to June 2019 are shown in Fig. 3. The average water temperature recorded in the study area was 25.84 ± 3.07 °C. The lowest water temperature was recorded in January (20.13 °C), and the highest temperature was recorded in May (28.53 °C). Over the 12-month study period, temperatures varied by 8.4 °C.

The pH at the sampling site ranged from 7.37 to 8.77. The minimum pH was recorded in November, and the maximum pH was found in February. Over the 12-month study period, rainfall occurred from March to October. No rainfall was reported during November or February. During the rainy months, the lowest rainfall amount was reported in March (46 mm), and the highest rainfall was recorded in October (391 mm). The mean pH of the sample location was 8.10 ± 0.34 during the study period. The minimum value

of salinity was recorded in April (22.86 ppt), whereas the maximum value was recorded in February (33.67 ppt). The mean value of salinity over the study period was 29.28 ± 3.56 ppt.

3.2. Sex ratio

The sexes of *M. bilineata* were identified as male, female, hermaphrodite, or undifferentiated stage by microscopic investigation of the histological preparations. Out of 240 specimens analyzed, histology revealed 107 males (44.6%), 100 females (41.6%), 3 hermaphrodites (1.3%), and 30 resting (undifferentiated) individuals (12.5%). The χ^2-test conducted for the study showed that the calculated value of χ^2 (0.2368) was less than the tabulated value (3.841) at degree of freedom 1, which indicates that there was no significant deviation in the sex ratio of males to females from the expected 1:1 (Table 3). We report 30 sexually undifferentiated individuals among the 240 oysters examined, where an ovarian or testicular gamete was completely absent in the histological preparation. We also identified three hermaphrodite specimens in June samples, in which oocytes were noted in follicle walls and spermatozoa were found in the lumen. This could represent a typical transition period from male to female.

3.3. Gametogenic cycle

Temporal changes in the gametogenic phenology of *M. bilineata* collected from the west coast of Cox's Bazar are shown in Fig. 4(A and B). The gonadal development, maturation, and spawning patterns of the oysters were relatively similar in males and females. *M. bilineata* exhibited two spawning cycles over the 12-month study period.

The first spawning cycle initiated in December, when 14.3% of the males and 16.7% of the females were in the early developing stage. The late developing stage continued from December to March in males and from December to February in females. Ripe animals first appeared in February, when 55.6% of males and 44.4% of females were categorized as in this stage. Ripe oysters occurred from February to April. It was possible to affirm that the first reproductive period began in March, represented by 27.3% of males and 50% of females in the spawning stage. The first reproductive stage finished in May. The initiation of spawning in March could be associated with the steep decline in salinity (29.0–21.7 ppt) and the increasing temperature (24.9–28.2 °C) between February and March, concurrent with the start of annual rainfall.

Another spawning cycle began in May, immediately after the first spawning cycle, as reflected by the presence of the early developing stage once again, when 18.2% of males and 22.2% of females were found to be in that stage. The late developing stage was reported from June to July in males and from June to August in females. Ripe animals reappeared in July and persisted until October. More than 30% of the animals were ripe from July to September. Spawning resumed again in July and continued until November, with

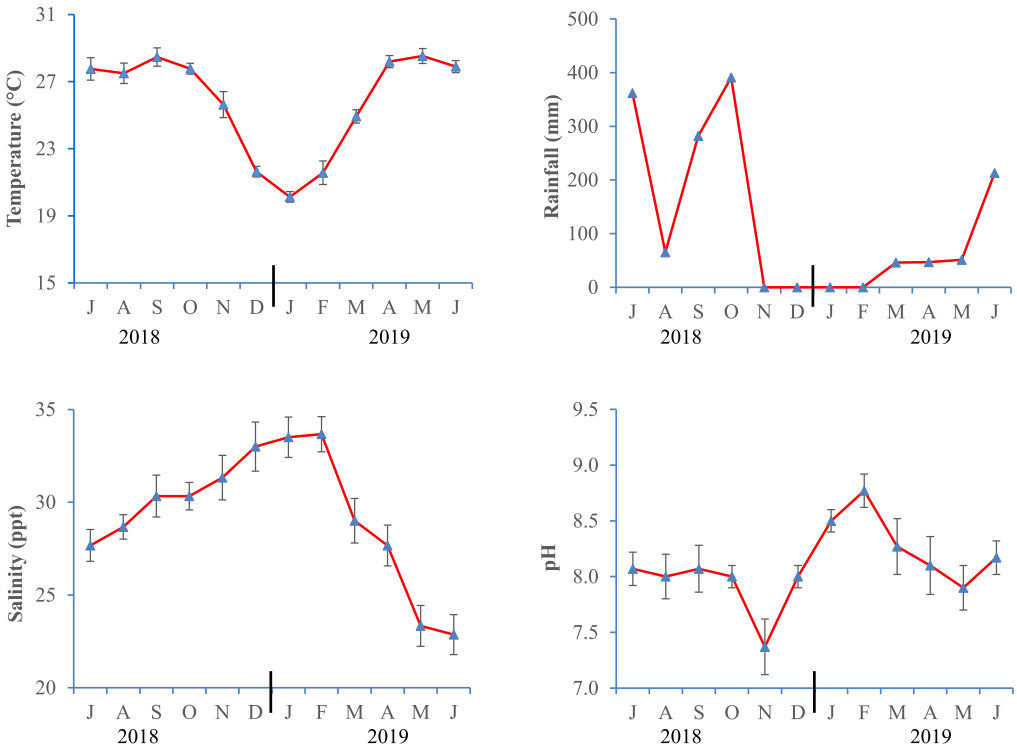

Fig. 3. Monthly variation of environmental parameters (mean ± SD) in the study area.

Table 3
Chi-square (χ^2) test on sex ratio of *Magallana bilineata*.

Sex	Observed No. (O)	Expected No. (E)	O - E	$(O - E)^2$	$(O - E)^2/E$	$\sum(O - E)^2/E$	Calculated χ^2	Tabulated χ^2
Male	107	103.5	3.5	12.25	0.1184	0.2368	0.2368	3.841
Female	100	103.5	−3.5	12.25	0.1184			
Sex ratio (M:F)	1.07:1.00	1:1						

the peak spawning period reported from August to October, with more than 38% of the animals identified in that stage. Sexually indifferent animals were principally reported in June and from November to January.

3.4. Maturity index (MI)

The temporal dynamics of MI in *M. bilineata* calculated from the gametogenic stages are shown in Fig. 5. The MI ranged from 0.90 to 4.35, with two distinct peaks in July 2018 (4.05) and February 2019 (4.35). During the highest peak in February, 90% of the oysters were in the late developing and ripe stages, whereas 80% of the animals were in the late developing and ripe stages during the second peak in July. Spawning activity of *M. bilineata* was noticed from July to November, when the MI decreased consistently, from a high in July (4.05) to a low in November (0.90). The MI then increased persistently from November until February, indicating the gametogenesis of the species in the studied habitat. A drastic decrease in MI from February to May was associated with the next spawning pulse of the oysters. MI accelerated again from May (1.45) to the end of the study in June (3.2), which could be associated with the initiation of gametogenic activity for the next spawning cycle. The MI had a moderately positive linear correlation with water pH ($r = 0.725$) and weak linear correlations with the other environmental variables analyzed.

3.5. Condition index (CI)

Monthly variations in the mean CIs of *M. bilineata* are presented in Fig. 6. The CIs of the oysters increased gradually from July to October and then decreased consistently until January. The maximum mean CI was noted in October (1.32 ± 0.36), and the lowest mean was reported in January (0.58 ± 0.08). CIs accelerated again beginning in January, with a minor peak in March. The indices dropped again in April and then increased steadily until the end of the study in June. The CIs peaked three times over the 1-year investigation, in October, March, and June. CI exhibited moderate positive linear correlations with water temperature ($r = 0.542$) and rainfall ($r = 0.502$) but showed a moderate negative correlation with water pH ($r = -0.639$).

4. Discussion

This is the first report of gametogenic phenology in the backwater oyster *M. bilineata* from Bangladesh waters, although research on gametogenic phenology of this species has been widely conducted along the east and southwest coasts of India [1,24–26]. The shell length of *M. bilineata* samples analyzed in the current study that were considered sufficient for studying gonadal histology ranged from 41.00 mm to 124.94 mm. The shell length at first maturity for the species were reported as > 12 mm in males and >24 mm in females [27].

In the present study, the sex ratio of males to females was 1.07:1.00, indicating an almost equal sex ratio in the *M. bilineata* population collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Almost equal sex ratios have also been reported for *Megallana* (=*Crassostrea*) *angulata* from Taiwan [28] and *M. belcheri* from Thailand [29]. In contrast, several other species of *Megallana* (=*Crassostrea*), including *M. brasilliana*, *M. cortezieensis*, *M. gigas*, *M. irhizophorae*, and *M. aidii*, have been reported to have a female bias [22,30,31]. However, some of these species have been reported with either equal sex ratios [32–34] or male-biased ratios in different countries [35].

We reported three hermaphrodite animals (1.3%) from the June sample in our study. Hermaphrodite animals are not uncommon in oysters, and the frequency may differ with age, shell height, and environmental conditions [1,19,22,33,35]. The percentages of males, females, hermaphrodites, and indeterminates were 50.9%, 42.5%, 1.3%, and 5.3%, respectively, in Ostrea madrasensis from Ennur Backwater, Madras, India [36]. *M. bilineata* is an alternate hermaphrodite on the east coast of India and is dioecious on the west coast of India [27]. It has been suggested that the *M. bilineata* population could be unisexual in a favorable environment to support rapid growth and reproduction, and hermaphroditism is an exception that could occur when the animals are exposed to environmental stress. Adult larviparous oysters are always hermaphrodites, whereas oviparous oysters are separate sexes [37].

The reproductive cycle of bivalve mollusks usually involves the following steps: 1) an undifferentiated stage as a preparatory step for the next gonadal development stage; 2) differentiation of gonads and progressive development to the ripe stage; 3) spawning by releasing the gametes; 4) the spent stage, in which some residual gametes are subjected to phagocytosis; and eventually 5) the undifferentiated condition for initiating the next cycle [9,38]. However, an undifferentiated stage is often lacking in tropical bivalves [7,39].

In our study, we observed year-round gametogenic activity of *M. bilineata*. This could be possible in tropical areas, where the environmental temperature never drops below the certain levels required for bivalve gonadal activity [1,36]. We report two breeding seasons of *M. bilineata*, from July to November and from March to May. Gametogenesis commenced at least twice, in December and May. The spawning periods of *M. bilineata* reported from different locations are listed in Table 4. Studies in the literature have

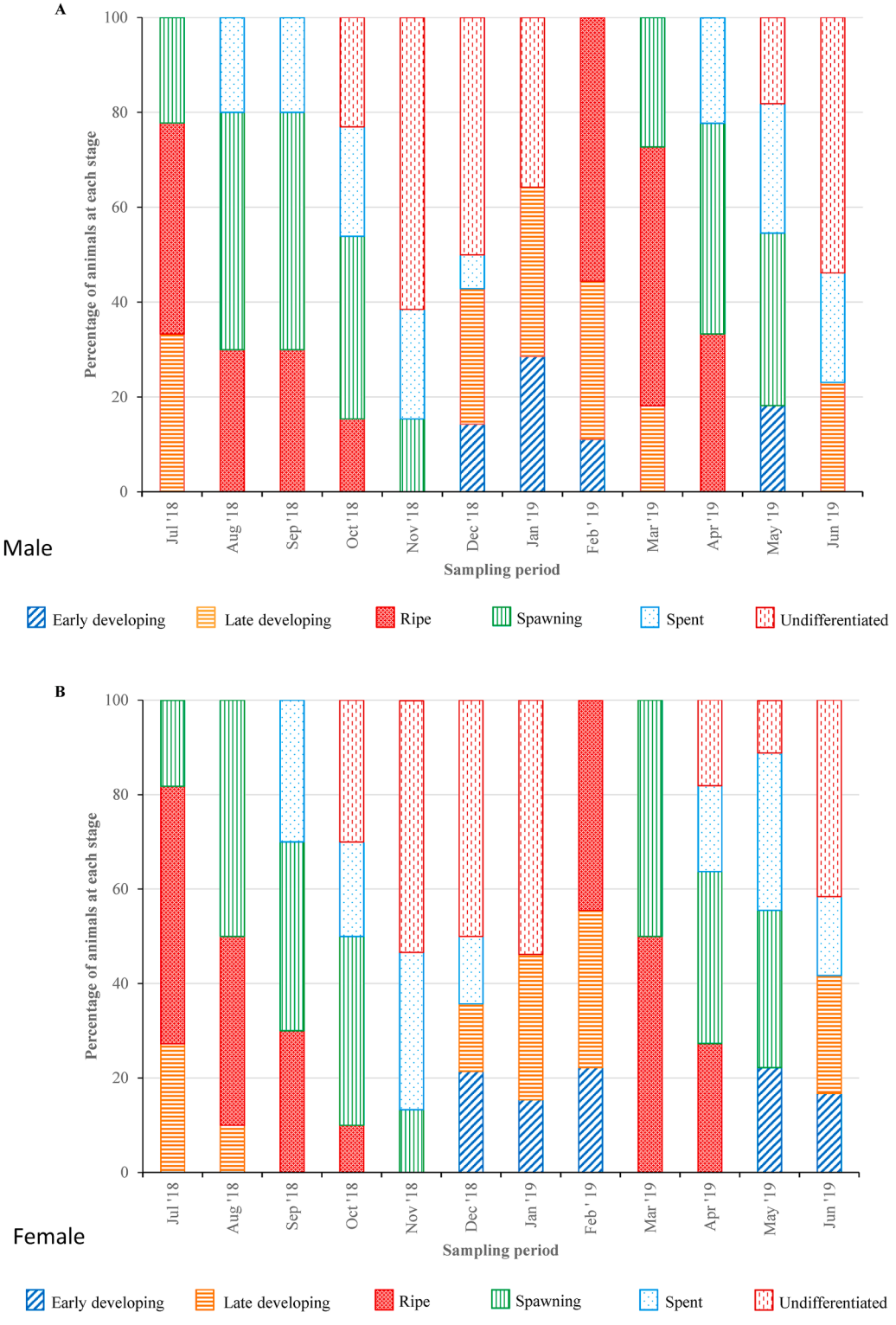

Fig. 4. A. Temporal distribution of spermatogenic stages of *Magallana bilineata* collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh, **B.** Temporal distribution of oogenic stages of *Magallana bilineata* collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh.

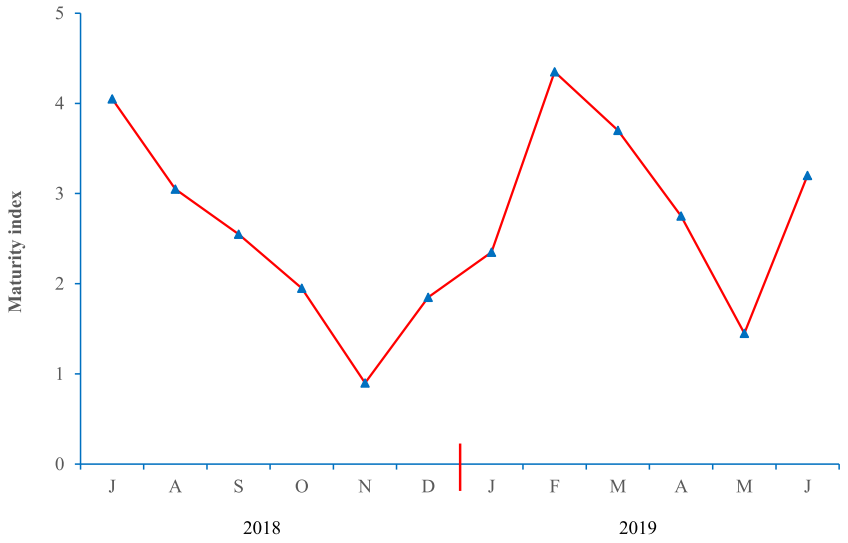

Fig. 5. Monthly changes in the maturity index of *Magallana bilineata* over the study period.

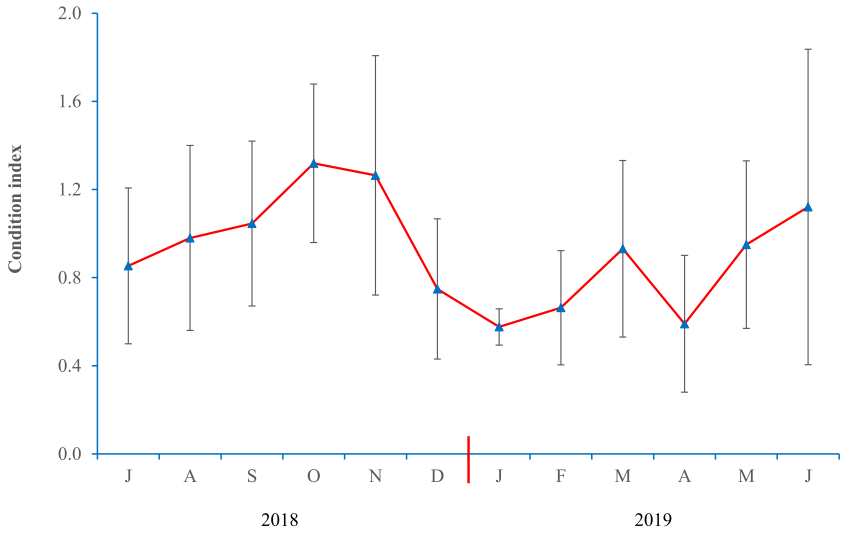

Fig. 6. Monthly changes in the condition index (mean ± SD) of *Magallana bilineata* over the study period.

suggested a single prolonged spawning season [1,26,27] or two separate spawning seasons [24,25,40] of this species in various locations. A prolonged spawning season of this species was reported from January to November in a study on the northwest coast of India, with two peaks, in March to April and September to October [26]. However, the spawning activity of *M. bilineata* principally occurred from December to May, with two peaks from December to January, and April to May, in a study from Mulki Estuary, India [27]. In contrast, the spawning period of this species was noticed from April to November in another study in Mulki Estuary, India [24].

Table 4
Spawning periods of *Magallana bilineata* from different locations.

Locality	Spawning period	Reference
Moheshkhali, Cox's Bazar, Bangladesh (21.65°N; 91.88°E)	Two spawning seasons: March to May and July to November	Current study
Madras Harbour, India	Round the year; 2 peaks: November to December and May to August	[1]
Mulki Estuary, India (13°5′N; 74°46′E)	Two spawning periods: mid-April to June and mid-September to November	[24]
Thoothukudi (Tuticorin), Tamil Nadu, India	Two spawning periods: March to April and August to September	[25]
Mulki Estuary, India (13°5′N; 75°47′E)	Early September to early May; 2 peaks: December to January and April to May	[27]
Muttukadu, Tamilnadu, India	January to November; 2 peaks: February to March and September to October	[26]
Korampallam, Tamilnadu, India	Two spawning periods: March to April and September to October	[40]

In contrast, a year-round spawning of *M. bilineata* was reported in a study at Madras Harbor, with two peak periods of maximum sexual activity in November to December and May to August [1]. The gametogenic cycle and spawning seasons of bivalves often vary with latitudinal differences [20,41], although interannual variation could also be possible, given that the environmental variables may vary from year to year [24]. In particular, the duration of the spawning period is often negatively correlated with latitude [13,42]. In our study, we recorded a shorter spawning duration of 8 months (July to November and March to May), in comparison to the spawning period for this species reported from India [1,26]. This could be due to the current study's site being located at a higher latitude (21.65°N) than Madras Harbor (13.42°N) and Muttukudu (12.82°N), Tamilnadu, India.

The seasonality of the CI of bivalves provides useful information for managers because the market price of and demand for bivalves mostly depend on it [7]. CI is recognized as a quick, inexpensive, and responsive tool for evaluating the physiological state of oysters. It is also applied as a measure of the apparent health and commercial quality of bivalves [21,43]. The monitoring of CI to assess growth in oysters has been widely documented [44,45]. As a physiological index, CI is highly influenced by gonadal development and the age of the oyster. In the present study, the CIs increased consistently from July to October with the advancement of maturation of *M. bilineata* in the habitat. The highest peak CI reported in October (1.32 ± 0.36) did not match with the gametogenic cycle. This discrepancy could be explained by other factors, such as food availability and the corresponding somatic growth of the oysters, although we did not monitor plankton abundance or chlorophyll data from the water. The declining CIs from October on were persistent with the spawning event of the oysters in the habitat. CIs exhibited the lowest value in January, when a majority of the animals lacked gonadal materials. The increment in CIs from January to March was in accordance with the gonadal proliferation. A drastic decline in CIs from March to April was consistent with the initiation of spawning of *M. bilineata* in the habitat. A decline in CI after spawning of this species has also been reported on Indian coasts [36,46]. However, in our study, we did not observe a close relationship between CIs and the maturation of oysters. This discrepancy could be associated with the CI formula used in the current study, which might not be sensitive enough to quantify the soft tissues in relation to the whole body due to the irregular shape of the oysters.

Environmental factors, such as temperature, salinity, dissolved oxygen (DO), pH, and food availability, play a significant role in the development of bivalves [46,47]. Among them, temperature and salinity are two key environmental variables affecting various aspects of oyster biology, including gonadal development, time of spawning, feeding, growth, respiration, parasite–disease interactions, predation rates, and the subsequent distribution of the oysters [48]. A rise in temperature and both rise and fall in salinity have been shown to have a great influence on gamete development and spawning of *C. madrasensis* [25,44,49]. In our study, no spawning activity of *M. bilineata* was reported from December to February, when the environmental temperature remained below 22 °C. The maturation of oysters and their peak spawning activity occurred in a 25 °C–30 °C temperature range. In our study, we reported two spawning seasons for *M. bilineata*: March to May and July to November. The drastic increase in water temperature from February to March (24.9 °C–28.2 °C) and the reduction in salinity (29.0 ppt–21.7 ppt), along with the commencement of annual rainfall, could be associated with the beginning of spawning in March. Another spawning pulse might be associated with heavy rainfall and a considerable increase in salinity. Our findings are in agreement with Stephen [24], who reported that the spawning event of *M. bilineata* correlated with the salinity seasons and decreasing or rising trends on the southwest coast of India. The decrease in salinity due to heavy rainfall may have triggered gamete release in *M. rhizophorae* in Camamu Bay, Brazil, when oocytes are exposed to conditions outside tolerance levels [50]. In our study, the mean salinity was 29.60 ± 3.32 ppt at the sampling site and ranged from 21.67 ppt to 35 ppt. The salinity ranges observed in the current study are in conformity with the salinity ranges reported from the southeastern coast of Bangladesh [51]. Salinity plays a vital role in various physiological functions in *Crassostrea* spp., including growth [52], oxygen consumption and clearance rate [53], and the gametogenic cycle [54,55]. As the temperature remains relatively high in the tropics throughout the year, sudden changes in salinity in connection with rainfall and evaporation could act as a natural stimulus for gametogenesis and spawning in tropical bivalves. An important parameter for bivalves is pH, given that they prefer alkaline water for better growth of the calcareous shell [56]. In the present study, the mean pH was 8.10 ± 0.34, which indicates alkaline water at the sampling site. A moderate positive linear correlation was reported between the MI of M. bilineata and water pH ($r = 0.725$). In contrast, reduced pH (7.5–7.7) increased the rate of reproductive development in both males and females of *C. virginica* [57]. However, no noticeable influence of dropping pH on sperm mobility or fertilization was observed in *C. gigas* [58,59].

In conclusion, monthly sampling of *M. bilineata* from the west coast of Moheshkhali, Cox's Bazar, Bangladesh, revealed two gametogenic cycles over the course of a year: one from December to May, with peak spawning periods from March to May, and one from May to November, with peak spawning periods from August to October. The spawning peak periods and spawning cycles for *M. bilineata* in the habitat can guide the timing of seed collection and the development of hatchery systems for artificial propagation. It is therefore possible to develop a farming calendar for *M. bilineata* at the study site based on these findings.

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Intellectual property

√ We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

4. Research Ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

IRB approval was obtained (required for studies and series of 3 or more cases)

Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

√ The design and utilization of animals in current research have been approved by the Ethical Standard of Research Committee of Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University, Mymensingh-2202, Bangladesh (Ref./BAURES/ESRC/FISH/74).

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Data will be made available on request.

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CRediT authorship contribution statement

Md. Jasim Uddin: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation,

Funding acquisition, Formal analysis, Conceptualization. **Md. Shamsur Rahman:** Writing – review & editing, Software, Methodology, Formal analysis, Conceptualization. **Saima Sultana Sonia:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Sheikh Khadijatul Kubra:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Md. Sujon Mia:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Selina Yeasmine:** Writing – review & editing, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28753.

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