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REPRODUCTIVE BIOLOGY AND SPAWNING PATTERN OF OYSTER *Magallana bilineata* IN MENGKABONG BAY, SABAH, MALAYSIA

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ABSTRACT

Magallana bilineata is a commercially important oyster species in Malaysia. Despite its economic significance, the biology and ecology of this species, particularly its reproductive cycle, are poorly understood in the region. Therefore, this study aimed to investigate the reproductive cycle of *M. bilineata* in Mengkabong Bay, Tuaran, Sabah and its correlation with environmental factors. A total of 105 oyster specimens were collected and examined to determine the sex ratio, gonad developmental stages, maturity index (MI), and condition index (CI). The results revealed a sex ratio of 1:2.36 (♂:♀) in the oyster population of the bay, with a significantly higher proportion of females ($P < 0.05$). Hermaphroditism was detected in 1.90% of the samples. *M. bilineata* was found to spawn throughout most months in the bay, with the highest MI and CI values recorded in November 2019 when most oysters were in mature or maturing stages. Salinity was found to be the primary factor influencing male gonad maturation, while temperature was the primary factor influencing female gonad maturation. Understanding the reproductive biology of this species is crucial for predicting its future survival and facilitating assisted spawning and hatchery spat production, thereby ensuring the conservation and sustainability of the oyster fishery in Malaysia.

How to Cite

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INTRODUCTION

Oyster aquaculture is an appealing industry that has demonstrated large-scale production success (Gibbs, 2004). It requires simple technology and relatively low financial investment, incurs no feed costs (Garen et al., 2004; Ferreira et al., 2009), and utilizes well-established processing and marketing networks through the wild harvest fishery (Nowland et al., 2020). Additionally, it can enhance water quality in marine systems as an essential bio-extractive species (Ferreira et al., 2009; Bricker et al., 2020), while providing an affordable source of protein and income for coastal communities (Ruesink et al., 2005).

Magallana bilineata is a commercially significant species in Malaysia (Nor Idayu et al., 2015), primarily found in the South China Sea, Andaman Sea, and Gulf of Thailand (Yoosukh and Duangdee, 1999), with production restricted mainly to Malaysia and the Philippines (Suzana, 2011). *M. bilineata* is native to the east coast of Peninsular Malaysia, particularly in Kelantan and Terengganu, along the coast of Sabah (Doinsing and Ransangan, 2022), and a few sites in Sarawak (Zulfigar and Aileen, 2000). Mengkabong Bay, Tuaran, Sabah is an important site for *M. bilineata* aquaculture (Doinsing and Ransangan, 2022), but its reproductive pattern in the area is poorly understood. Reproduction and settlement processes play a vital role in establishing sustainable populations (McFarland and Hare, 2018). By understanding the reproductive biology of *M. bilineata*, future assisted spawning and hatchery spat production can aid conservation efforts and ensure the fishery's sustainability.

This study presents the first information on the reproductive pattern of *M. bilineata* in Mengkabong Bay, Tuaran, Sabah. Mengkabong Bay, Tuaran is subject to substantial rainfall because of the influx of moist air originating from the South China Sea during the Northeast Monsoon season, which spans from November to March. Conversely, the Southwest Monsoon season, occurring from August to September is characterised by drier conditions and reduced precipitation in the bay (Malaysian Meteorology Department, 2019). A histological technique was used to visually examine the gonadal growth. The maturity index (MI) was calculated to provide insights into the oyster's reproductive cycles and forecast spawning seasons (Etchian et al., 2004). The condition index (CI) was also determined to provide a rapid measure of the reproductive conditions of *M. bilineata* and estimate the optimal harvesting time from an economic and nutritional standpoint (Barman et al., 2022).

MATERIALS AND METHODS

Study Area and Sampling Schedule

The study was conducted in Mengkabong Bay (06° 08’ 038” and 116° 12’ 004”), located on the northern part of Kota Kinabalu on the west coast of Sabah, facing the South China Sea (Fig. 1).

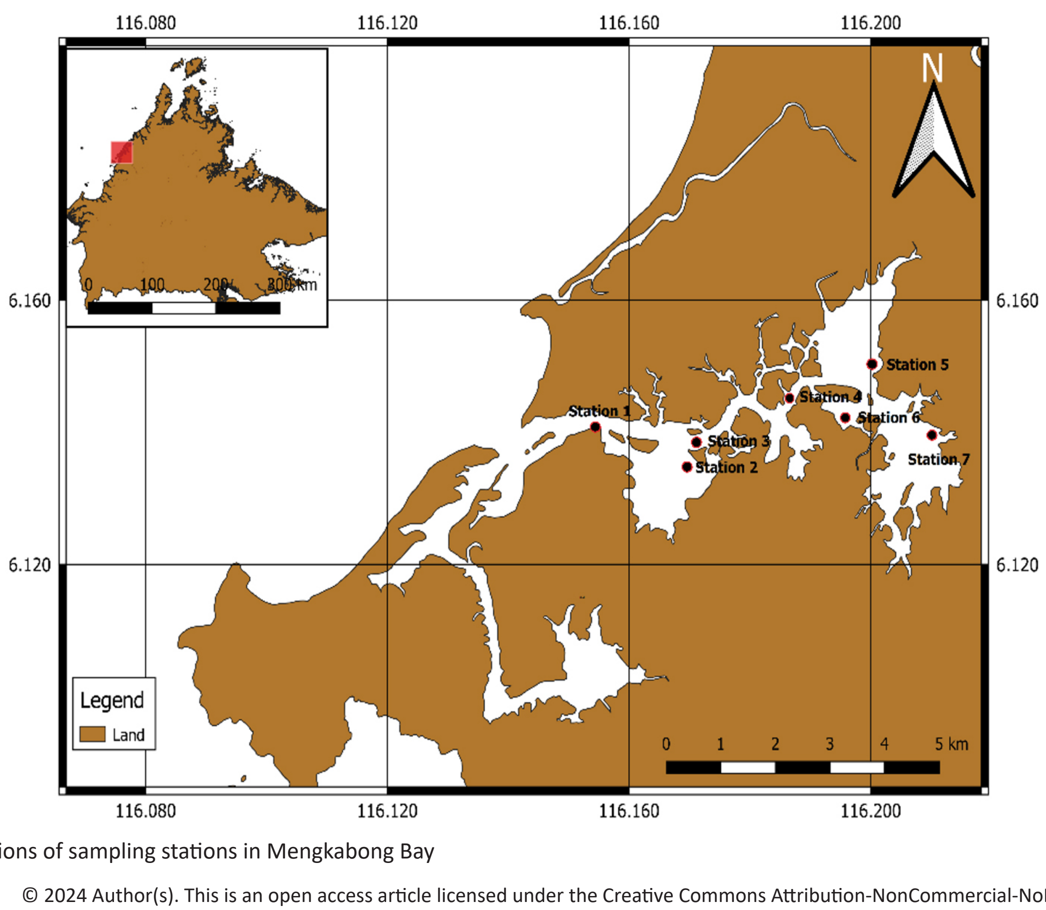

Fig 1. Locations of sampling stations in Mengkabong Bay

Sampling for environmental parameters was performed at stations 1 through 7, while sampling for oysters was carried out only at two *Magallana bilineata* farms, located at stations 2 and 6, from September 2019 to March 2020. The precise location of each sampling site was recorded using a Global Positioning System (GPS), accounting for anthropogenic influences. The distance between stations ranged from 1 to 3 km. Stations 5 and 7 were adjacent to high human density areas, while stations 3 and 4 were primarily surrounded by mangroves, with a graveyard situated at station 4. The oyster farms were located at stations 2 and 6. Station 1, located in front of the Anjung Gayang Jetty, near the Mengkabong River Bridge and Jambatan Gayang, was the primary fishing ground. An industrial zone surrounded the southern spur of the Mengkabong mangrove forest, the Kota Kinabalu Industrial Park (KKIP), while the northern spur was covered by pristine mangrove trees (Environmental Impact Assessment, 1992; Praveena et al., 2007). Mengkabong Bay experiences heavy rainfall during the Northeast Monsoon from November to March every year and limited rainfall during the Southwest Monsoon from August to September, which brings drier air through the bay (Malaysian Meteorology Department, 2019).

Environmental Parameters

Water temperature (°C), salinity (psu), pH, and dissolved oxygen (mg/L) were measured at a depth of 0.5 m below the water surface using a YSI multi-function environmental sensor (Loveland, Co, USA). Water depth (m) was measured using a Hondex depth sounder, while water transparency was measured using the Secchi disc method to determine the Secchi depth (m). Monthly rainfall data were obtained as secondary data from the Malaysian Meteorology Department in Kota Kinabalu.

Histological Studies of Gonads

Fifteen (15) oysters were collected monthly at each station for histological examination of the gonads. Oysters were cleaned of sediment and fouling organisms, and shell height was measured to the nearest 0.01 cm using a vernier caliper (Fig. 2). Shell height was measured since it is the primary measurement used in most individual-based models (Velonia, 2018). The oysters were opened using an oyster knife by separating the hinge, and the adductor muscle was carefully severed to avoid injury to internal tissues. The gonads were removed from the mantle lobes, fixed, and preserved in 10% neutral buffered formalin until analysis (Howard and Smith, 1983).

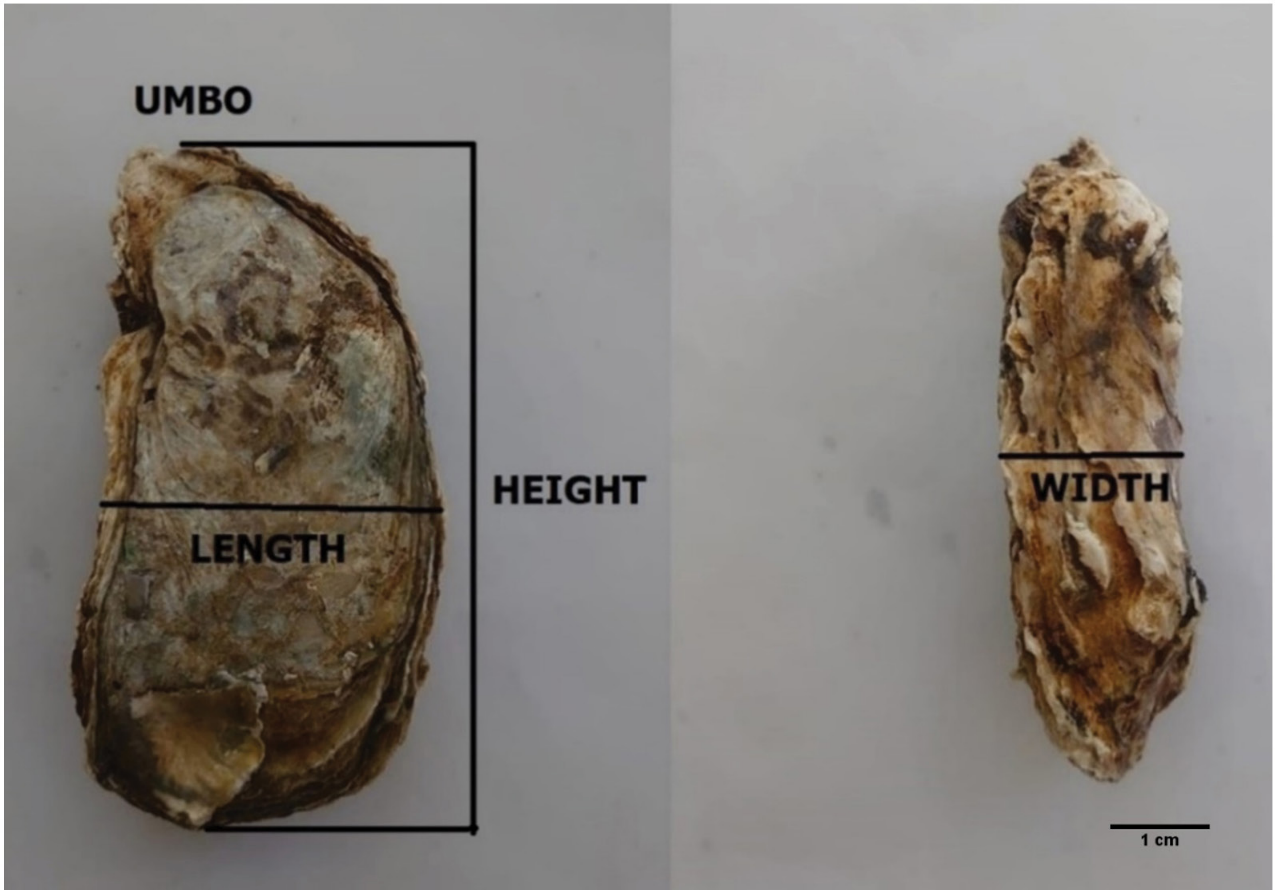

Fig 2. Diagram of the height, length, and width of *Magallana bilineata* specimen. Scale bar = 1 cm.

The gonad was cut into 3-5 mm pieces and placed in a cassette for histology. The samples were dehydrated, cleared, and embedded in paraffin wax using a HistoCore PEARL tissue processor (Leica Biosystems, Wetzlar, Germany). The gonad sections were sliced to 6 µm thickness using the Shandon Retraction Rotary Microtome (AS25, Thermo Scientific, Bremen, Germany) and stained with Harris haematoxylin and eosin. The sections were then mounted with DPX and examined under an inverted microscope (CKX41, Olympus) at 40x to 100x magnifications to determine the sex ratio and gonad developmental stages. Six stages (inactive, early active, late active, ripe, spawning, and spent) were identified following the scale proposed by Ezgeta-Balić et al. (2020), and the numerical values and descriptions of the six stages for male and female gonads are presented in Table 1 and Fig. 3 and Fig. 4. The maturity index (MI) was calculated for female, male, and total oyster samples (excluding hermaphrodite individuals), following the formula proposed by Niogee et al. (2019):

$$\text{Maturity Index (MI)} = \frac{\text{sum of the numerical score of all stages for a month}}{\text{number of monthly samples analysed}}$$

Fifteen oyster specimens were randomly sampled each month at each station for condition index (CI) analysis. The oysters were dissected to separate the flesh from the shell and then oven-dried at 105 °C for 24 hours before weighing. The *CI* were calculated as a percentage of dry tissue weight (DTW) over dry shell weight (DSW) using the formula recommended by Davenport and Chen (1987) and Rahim et al. (2012):

$$CI = (DTW/DSW) * 100$$

The *CI* were classified into three fatness categories following Rahim et al. (2012) as follows:

$CI \leq 2$ (thin); $CI = 2$ to 4 (moderate); $CI \geq 4$ (fat).

Statistical Analysis

Environmental parameters, difference of shell height between hermaphrodite and non-hermaphrodite oysters, maturity index (MI), and condition index (CI) were analyzed using SPSS Windows Statistical Package (version 26). The variables were tested for normality and variance homogeneity, with tests considered significant at $P < 0.05$. For data that met the assumptions of normality, one-way ANOVA followed by Tukey multiple comparison tests were performed. For data violating the assumption of variance homogeneity, Welch's ANOVA followed by Games-Howell multiple comparison tests were conducted. Non-parametric Kruskal-Wallis followed by Dunn's multiple comparison tests were performed otherwise. Independent sample T-tests and Mann-Whitney tests were used to significantly contrast the shell height of hermaphrodites and non-hermaphrodites for data that met or violated the

Table 1. Numerical score and description of the gonad developmental stages in *Magallana bilineata* (adapted and modified from Ezgeta-Balić et al. (2020))

Numerical Score	Description	Male	Female
0	Inactive (sexual rest)	There was no evidence of follicles or only a few small follicles with stem cells. It was impossible to determine the sex.	
3	Early active	There are numerous small follicles. There are many spermatogonia and spermatocytes, but no spermatozoa. There is an abundance of connective tissue.	Oogonia develops from stem cells along the follicles but lacks free oocytes. Connective tissue is plentiful.
4	Late active	Follicle cells are primarily made up of spermatids and spermatozoa. In the centre of the follicle, there is a distinctive swirling pattern of spermatozoa with tails toward the follicle lumen.	Both free and attached oocytes are present, with distinct nuclei that stain lighter than the cytoplasm.
5	Ripe	The germinal epithelium and interfollicular tissue are barely noticeable. Follicles are totally filled with spermatozoa with their tails facing the follicle lumen, generating a swirling pattern.	There are free vitellogenic oocytes with a distinct nucleus and nucleolus.
2	Partially spawned	Follicles are partially empty, with a large number of spermatozoids but they are not densely packed.	The centre of the lumen in the follicle is filled with a large number of free oocytes but they are not densely packed.
1	Spent	Most follicles are empty or partially empty, with some individuals having visible sperm in their sperm ducts.	The walls of the follicles appear to be broken, and they are empty. Oocytes that are still present are being cytolyzed.

normality assumption, respectively. Pearson's correlation was applied to determine the degree of association between MI (total, male, and female), CI, environmental parameters, and monthly rainfall. The significance level ($P < 0.05$) of the sex ratio was tested using a weighted chi-square analysis.

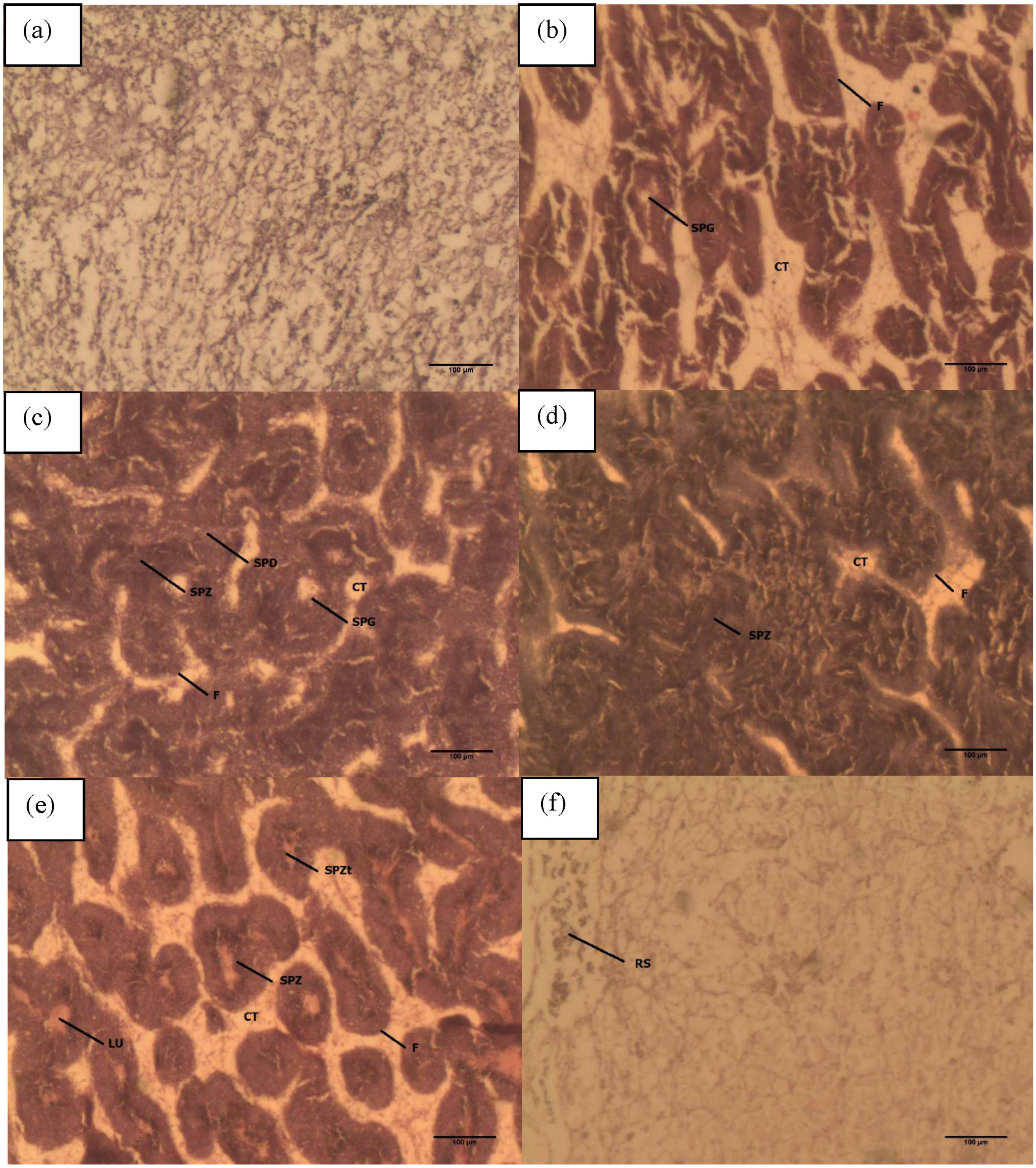

Fig 3. Gonad histological examination of male oyster. Inactive oyster (a) and gonad developmental stages of male (b-f) *Magallana bilineata*: (b) early active; (c) late active; (d) ripe; (e) partially spawned and (f) spawned. SPG: Spermatogonia; SPZ: Spermatozoa; SPZt: Spermatozoa tail; LU: Lumen; RS: Residual spermatozoa; F: Follicle; CT: Connective tissue. Scale Bar = 100 µm.

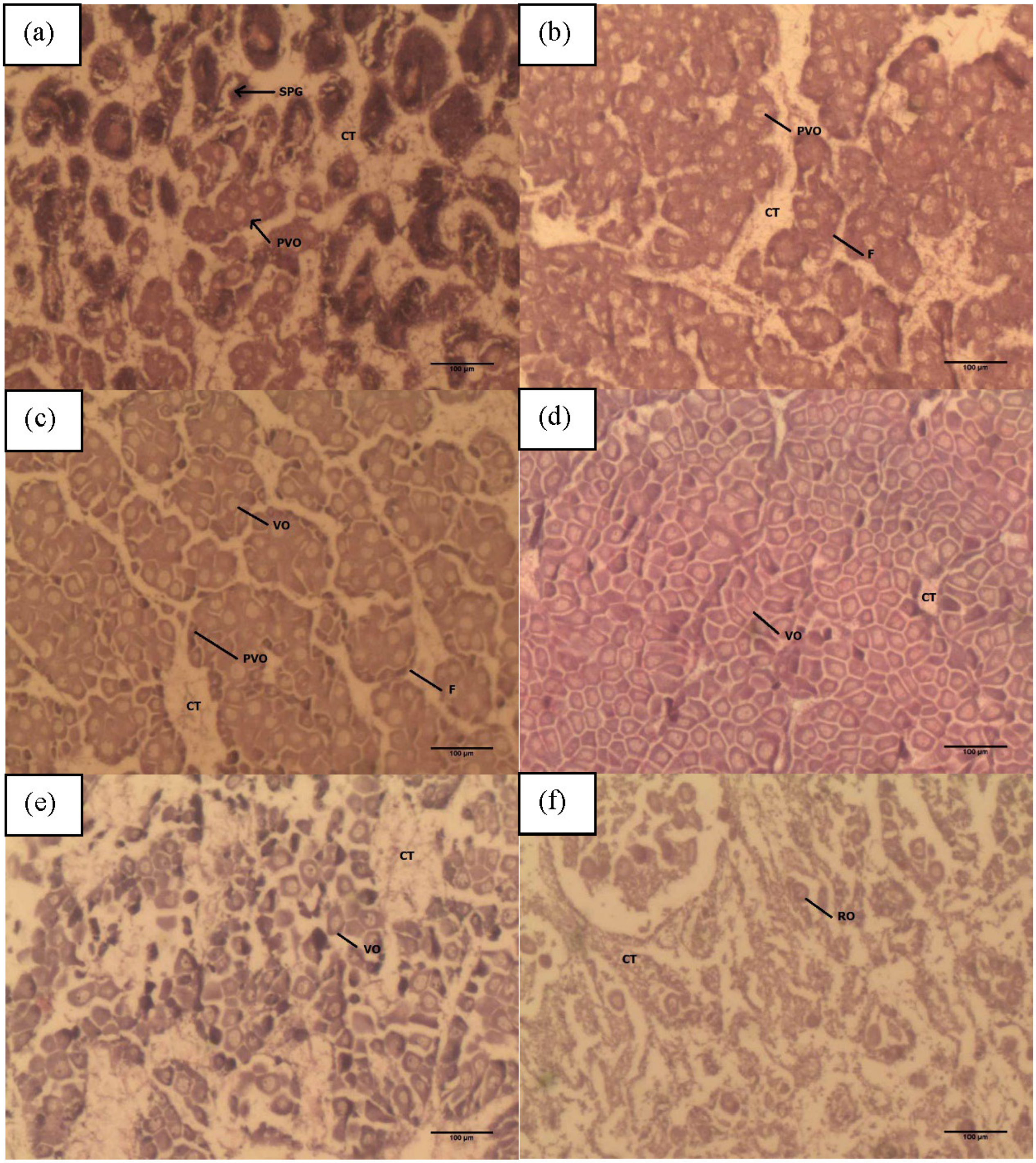

Fig 4. Gonad histological examination of female oyster. Hermaphrodite oyster (a) and gonad developmental stages of female (b-f) *Magallana bilineata*: (b) early active; (c) late active; (d) ripe; (e) partially spawned and (f) spawned. PVO: Previtellogenic oocyte; VO: Vitellogenic oocyte; RO: Residual oocyte; F: Follicle; CT: Connective tissue. Scale Bar = 100 µm.

RESULTS

Environmental Parameters

Environmental parameters in Mengkabong Bay exhibited variations (Fig. 5). Temperature was significantly lower (Games-Howell, $P < 0.05$) in September 2019 and from December 2019 to February 2020, ranging from 29.56 to 30.14 °C, while significantly higher (Games-Howell, $P < 0.05$) from October to November 2019 and March 2020, ranging from 31.33 to 31.74 °C. Water salinity was significantly lower (Games-Howell, $P < 0.05$) from September to December 2019, ranging from 26.58 to 29.14 psu, while significantly higher (Games-Howell, $P < 0.05$) from January to March 2020, ranging from 31.49 to 35.32 psu. Dissolved oxygen was significantly higher (Games-Howell, $P < 0.05$) in March 2020 (4.65 mg/L), but lower (Games-Howell, $P < 0.05$) in October 2019 and from December 2019 to February 2020 than in other months, ranging from 3.34 to 3.73 mg/L. The pH value in March 2020 (7.74) was significantly higher (Dunn's, $P < 0.05$) than from September to February 2020, ranging from 7.19 to 7.45. Secchi depth and water depth did not exhibit significant differences throughout the months (Kruskal-Wallis, $P > 0.05$). Rainfall data indicated heavy rainfall in Mengkabong Bay in December 2019 (377.8 mm), whereas the lowest rainfall of 23.9 mm was recorded in February 2020 (Fig. 6).

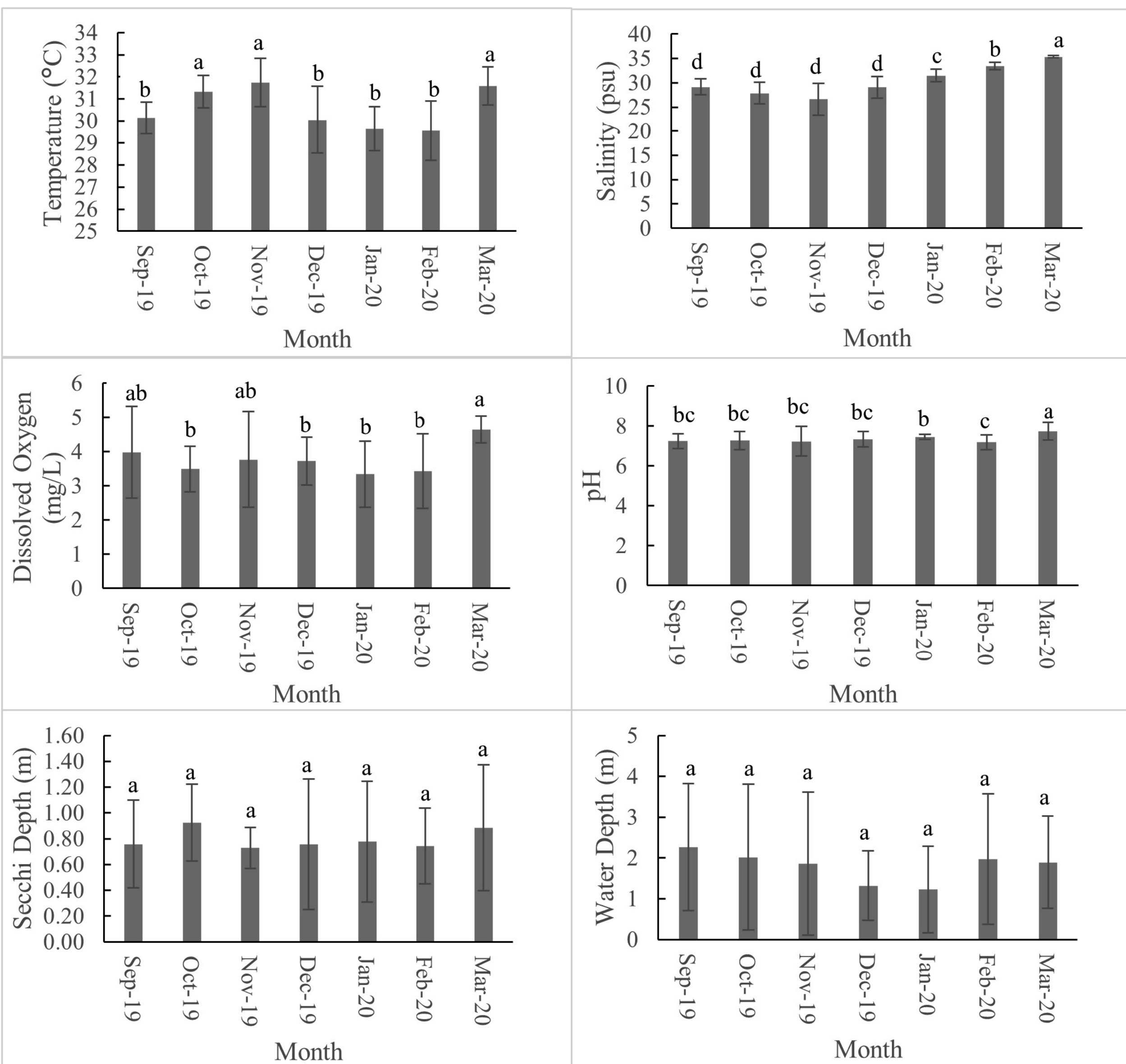

Fig 5. Environmental parameters (mean ± SD) in Mengkabong Bay from September 2019 to March 2020

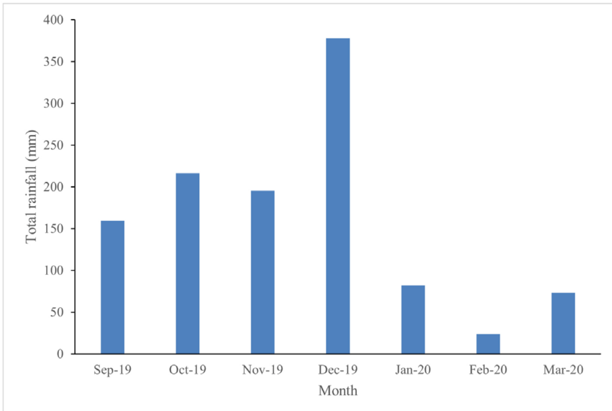

Fig 6. Monthly rainfall in Mengkabong Bay from September 2019 to March 2020

Histological Studies of Gonads

A total of 210 *Magallana bilineata* oyster specimens, ranging in size from 3.20 to 10.20 cm, were randomly collected from Mengkabong Bay for gonadal histological analysis (Fig.7). Of these, 139 (66.19%) were females (mean shell height ± SD = 6.90 ± 1.20 cm), 59 (28.1%) were males (mean shell height ± SD = 6.89 ± 1.13 cm), and 4 (1.90%) were hermaphrodites (mean shell height ± SD = 6.60 ± 0.22 cm) (Fig. 8). During the seven-month study period, hermaphroditic individuals were observed only in October, December 2019, February, and March 2020 (Table 2). The male-female sex ratio was 1:2.36, with a significant female bias (X^2 = 38.6294, *df* = 1, $P < 0.01$). No significant difference in shell height was observed between hermaphrodites and non-hermaphrodites (t (7.621) = 2.152, P = 0.065).

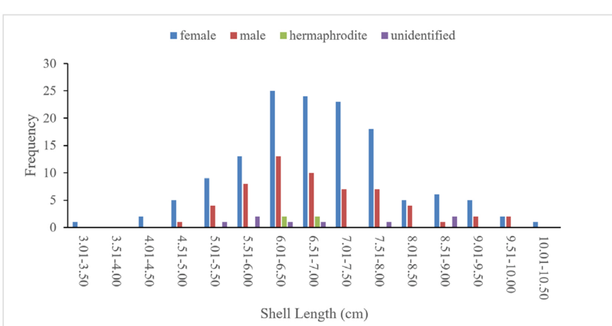

Fig 7. Size distribution of *Magallana bilineata* used in histological analysis from September 2019 to March 2020

Throughout the seven-month study period, male and female oysters exhibited similar gametogenic stages, with temporal variations (Fig. 9). Resting stages were observed in a small number of individuals in October 2019, December 2019, and from February to March 2020. Gametogenesis occurred all year round, with peaks observed in September 2019, December 2019, and January 2020, indicated by higher frequencies of early-developing gonads.

Table 2. The monthly histological sex ratio of *Magallana bilineata* in Mengkabong Bay from September 2019 to March 2020

Month	Total	Male	Male (%)	Female	Female (%)	Hermaphrodite	Hermaphrodite (%)	Undifferentiated	Undifferentiated (%)	Ratio (m:f)	Chi-square	df	P value
Sep-19	30	11	36.67	19	63.33	0	0.00	0	0.00	1:1.73	2.2967	1	0.1297
Oct-19	30	9	30.00	17	56.67	1	3.33	3	10.00	1:1.89	3.3072	1	0.0690
Nov-19	30	4	13.33	24	80.00	2	6.67	0	0.00	1:6	29.1667	1	0.0000
Dec-19	30	12	40.00	15	50.00	1	3.33	2	6.67	1:1.25	0.3375	1	0.5613
Jan-20	30	7	23.33	23	76.67	0	0.00	0	0.00	1:3.29	11.9255	1	0.0006
Feb-20	30	8	26.67	20	66.67	0	0.00	2	6.67	1:2.5	6.3000	1	0.0121
Mar-20	30	8	26.67	21	70.00	0	0.00	1	3.33	1:2.63	7.2932	1	0.0069
Total	210	59	28.10	139	66.19	4	1.90	8	3.81	1:2.36	38.6294	1	0.0000

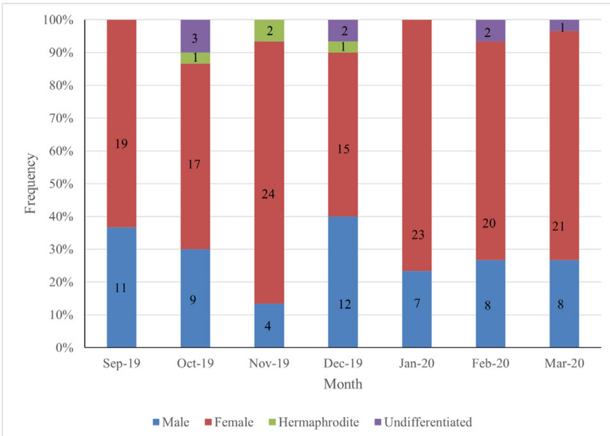

Fig 8. Frequency of females, males, hermaphrodites, and undifferentiated individuals of *Magallana bilineata* from September 2019 to March 2020. The number of specimens analysed is indicated in the graph.

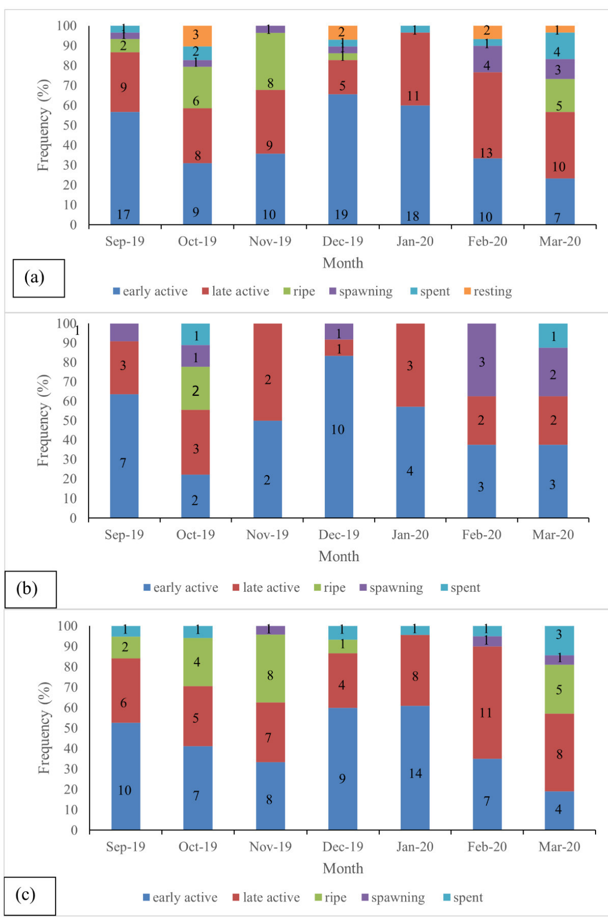

Fig 9. Monthly variation in the frequency of different gametogenic stages of (a) total specimens, (b) males and (c) females of *Magallana bilineata* from September 2019 to March 2020

Early-developing males and females were observed in all seven months, with peaks in September 2019 and December 2019. Late-developing males and females were recorded throughout the study period, with a higher percentage of late-developing males observed than late-developing females. Ripe males were observed in October 2019, while ripe females were recorded in most months except from December 2019 to January 2020. The percentage of ripe males was 22.22% in October 2019, and the highest percentage of ripe females was recorded in November 2019 at 33.33%. Spawned males were observed in most months, except in November 2019 and January 2020, while spawned females were recorded in November 2019 and from February to March 2020. Spent females were found all year round, except in November 2019, while spent males were observed only in October 2019 and March 2020.

The total maturity index (MI) values for *M. bilineata* ranged from 3.15 to 3.86 without a prominent peak (Fig. 10).

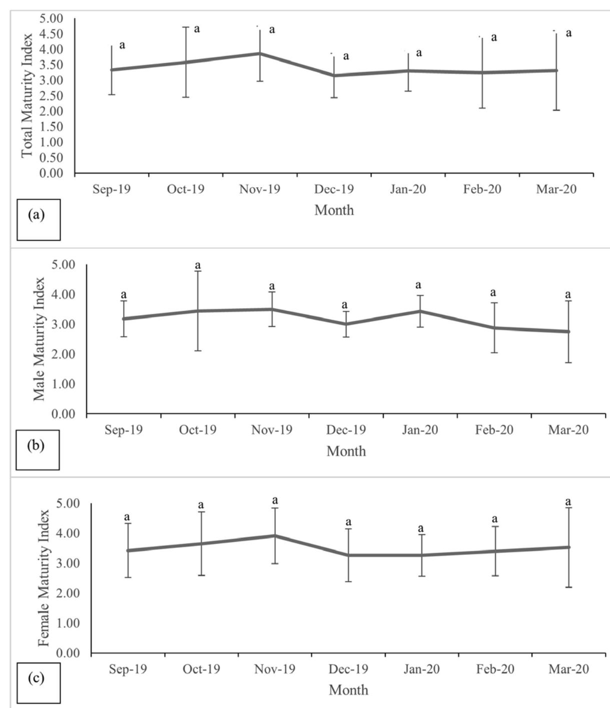

Fig 10. Monthly variation of maturity index of (a) total specimens, (b) male and (c) female (mean ± SD) of *Magallana bilineata* in Mengkabong Bay from September 2019 to March 2020

A weak peak was observed in November 2019, with late active and ripe individuals accounting for 60.71% of the samples. Male, female, and total MI exhibited a similar pattern, consistent with the condition index (CI) during the same period (Fig. 11).

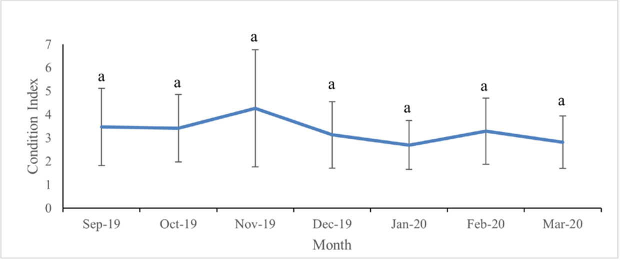

Fig 11. Monthly variation of condition index of *Magallana bilineata* in Mengkabong Bay from September 2019 to March 2020

Correlations were identified between MI, CI, salinity, and temperature (Fig. 12). Female MI and total MI exhibited a significant strong positive correlation (*r* (5) = 0.946, *P* = 0.001). CI demonstrated a strong positive correlation with female MI (*r* (5) = 0.811, *P* = 0.027) and total MI (*r* (5) = 0.813, *P* = 0.030). Male MI was significantly negatively correlated with salinity (*r* (5) = -0.778, *P* = 0.040), indicating that salinity is vital for male gonad maturation. Conversely, female MI was significantly positively correlated with temperature (*r* (5) = 0.839, *P* = 0.018), indicating that temperature plays a critical role in female gonad maturation.

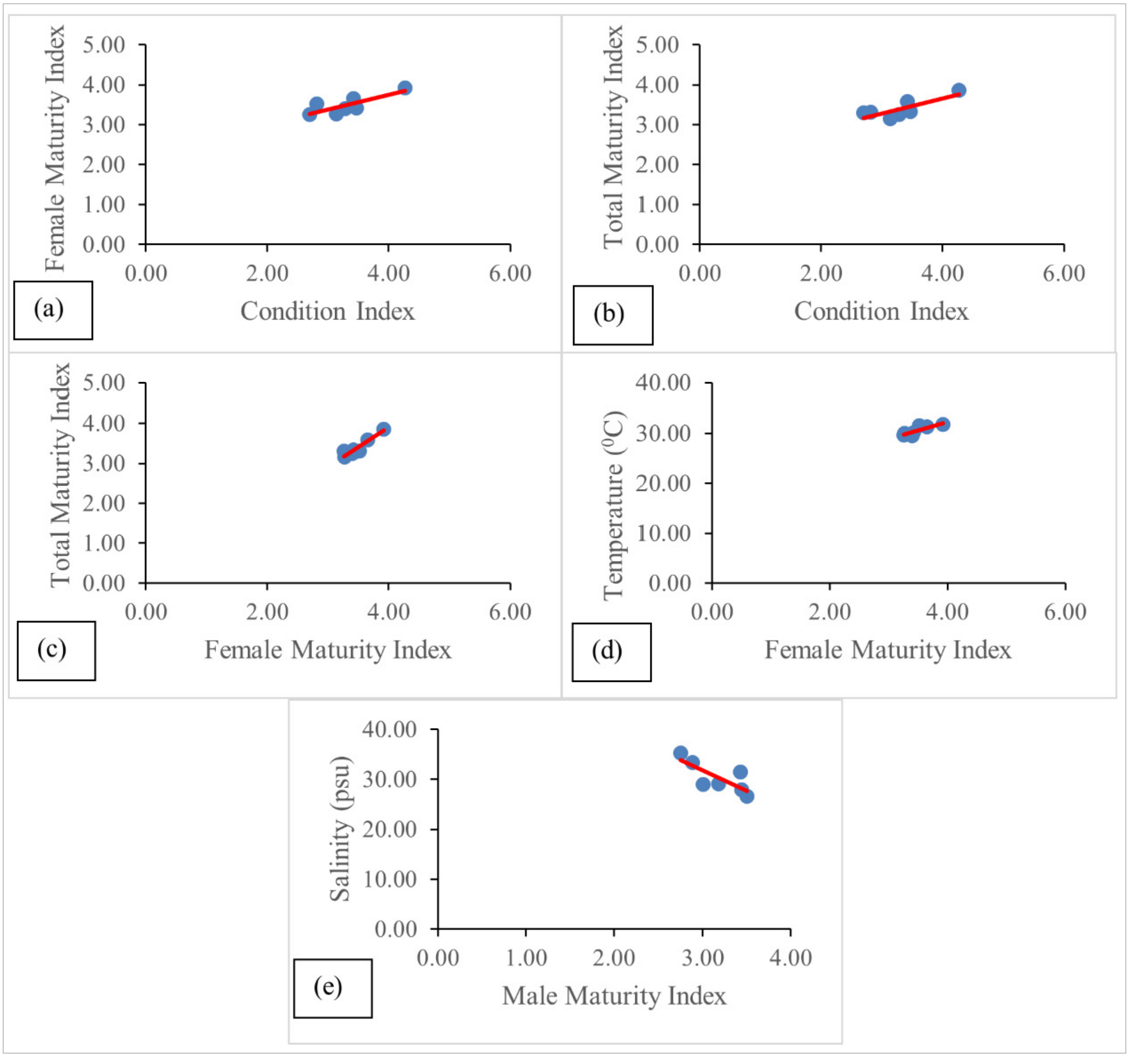

Fig 12. Pearson's correlation coefficient analysis of (a) condition index and female maturity index (*r* (5) = 0.811, *P* = 0.027), (b) condition index and total maturity index (*r* (5) = 0.813, *P* = 0.030), (c) female maturity index and total maturity index (*r* (5) = 0.946, *P* = 0.001), (d) female maturity index and temperature (*r* (5) = 0.839, *P* = 0.018) and (e) male maturity index (*r* (5) = -0.778, *P* = 0.040)

DISCUSSION

Environmental Parameters

The rainfall regime during the monsoon season significantly influences the temporal fluctuation of environmental indicators in Mengkabong Bay. The Northeast Monsoon (from November to March) brings heavy rainfall due to the transport of moist air from the South China Sea, whereas the Southwest Monsoon (from August to September) results in dry weather and less precipitation (Malaysian Meteorology Department, 2019). The water temperature in the bay is influenced by the climate of the estuary, with the lowest temperature recorded in September 2019 and from December 2019 to February 2020, which coincides with the monsoon seasons. Similarly, the lowest water salinity was recorded during the monsoon season from September to December 2019. Changes in salinity in brackish water ecosystems, such as estuaries, backwaters, and mangrove waters, are mainly caused by freshwater input from land runoff driven by monsoon or tidal variations (Rahaman et al., 2013). Dissolved oxygen concentrations and pH remained relatively constant throughout the study period, with only small differences observed.

Histological Studies of Gonads

Gonad histological examination can identify the sex of *Crassostrea* oysters at a size of 2.20 cm (Castilho-Westphal et al., 2015), and oysters can attain sexual maturity at a size from 50 to 80 mm (Rosell, 1990). The size of oyster samples included in this study ranged from 3.20 cm to 10.20 cm, which is considered a suitable size for researching gonadal development. The *Magallana bilineata* population in Mengkabong Bay was found to be female-biased at a ratio of 1:2.36. These findings are consistent with those of several other *Crassostrea* species, including *Crassostrea (M.) saidii*, *C. brasilliana*, *C. cortezieensis*, *C. gigas*, and *C. rhizophorae* (Castilho-Westphal et al., 2015; Ezgeta-Balić et al., 2020; Antonio et al., 2021; Barman et al., 2022). Conversely, several *Crassostrea* species have been reported to be male-biased (Rodríguez-Jaramillo et al., 2008) or have similar or almost equal sex ratios (Castaños et al., 2009; Vaschenko et al., 2013; Dridi et al., 2014).

Although males in this study reached sizes of up to 9.51-10.00 cm, the observed ratio could result from collecting larger specimens, which led to the capture of primarily females. According to Fakhrina et al. (2018), most of Malaysia's tropical oysters are protandric hermaphrodites, maturing first as males before changing to females in later years. This female-biased population may also indicate the ample food supply in Mengkabong Bay. In regions with a good food supply, mature oyster sex ratios typically exhibit a predominance of females, whereas in locations with a restricted food supply, males predominate because bivalves channel more energy reserves to produce the sex that is energetically less expensive (FAO, 2019). Additionally, as indicated by Fabioux et al. (2005), high water temperatures in the surrounding water may contribute to the population's predisposition toward females. A female predominance in a population is ecologically desirable because a species' reproductive capacity is determined by the number of mature females and their ability to produce viable eggs (Murua and Saborido-Rey, 2003).

Of the 210 specimens examined in this study, 1.90% were hermaphrodites. This is higher than the recorded hermaphroditism rates for *C. (M.) saidii* (Barman et al., 2022), *C. rhizhophore* (Castilho-Westphal et al., 2015), and *C. gigas* (Ezgeta-Balić et al., 2020), but lower than those reported for *C. angulata* (Vaschenko et al., 2013) and *C. rhizhophore* (Antonio et al., 2021). There was no significant difference in shell height between hermaphrodite and non-hermaphrodite specimens in this study, suggesting that the presence of hermaphroditism could represent a transitional stage between sexes (Barman et al., 2022) with a strong predisposition to become female (Bayne, 2017). However, the prevalence of hermaphroditism may vary with age and the environment (Galtsoff, 1964).

Throughout the sampling period, *M. bilineata* was observed to spawn in most months in Mengkabong Bay. This finding is consistent with previous studies suggesting that spawning may occur year-round in tropical regions (Castilho-Westphal et al., 2015; Antonio et al., 2021). The relatively constant temperature and salinity conditions in Mengkabong Bay (from 26.58 to 29.14 psu and from 29.56 to 30.14 °C, respectively) appear to be the primary drivers of continuous *M. bilineata* reproduction (Tan and Ransangan, 2014; Cárdenas and Aranda, 2000). The *M. bilineata* spawning peak in Mengkabong Bay was found to occur during the Northeast Monsoon season. The results of the current study are consistent with those of Sivalingam (1977) and Tan and Ransangan (2014) who found that *Perna viridis*, another bivalve species found in Malaysia, has its peak spawning period from March to April and from October to November, which are strongly associated with the monsoon seasons. Furthermore, spent females were observed in the majority of sampling months, while spent males were recorded in only one of the seven sampling months, indicating that males recover from spawning more quickly than females (Barman et al., 2022).

The maturity index (MI) is a valuable tool for understanding the reproductive cycle (Etchian et al., 2004). As gonads progress from premature to mature, MI values increase and decrease during the emptying and reabsorption processes (Gosling, 2003; Gauthier-Clerc et al., 2006). In this study, the MI values of *M. bilineata* showed seasonal consistency, with a weak peak in November 2019 when a higher proportion of oysters were ripe or mature. MI values decreased after December 2019 when a higher percentage of oysters were in the partially spawned, spent, and resting stages.

In bivalve mollusk farming, the condition index (CI) is frequently used to assess meat quality and productivity (Rebelo et al., 2005). While high values indicate gonad maturation (Hamli et al., 2017), a decrease in CI often indicates the bulk release of gametes through spawning (Uddin et al., 2007). The CI values of *M. bilineata* followed a pattern similar to that of MI values, with a higher value obtained in November 2019 when most oysters were mature or maturing and then declining in the ensuing months, indicating coordinated spawning episodes.

The highest MI and CI values were recorded in November 2019 when temperatures were high, and salinities were low. Similar findings were reported by Gomes et al. (2014) who showed that increasing temperature and reduced salinity influence the reproductive development of *C. gigas*. Gomes et al. (2014) also reported higher CI values during months with higher seawater temperatures. The strong positive correlation between CI and female MI indicates that females contributed higher CI than males. This finding may be attributed to female oysters consuming available food more efficiently than males (Dove and O'Connor, 2012). A higher CI value also indicates that the oyster cup was fuller; therefore, the cups of matured female oysters were well-filled with oyster meat (Futagawa et al., 2011), resulting in better economic values than matured male oysters. The strong positive correlation between female MI and temperature may be due to the strong tendency toward becoming female in environments with high temperatures (Fabioux et al., 2005). Similarly, a strong negative correlation between male MI and salinity may be associated with male population domination, as bivalves direct more energy reserves to produce the energetically cheaper male (Saucedo and Southgate, 2008) during extreme low salinity or rapid salinity change. Oysters may endure extremely low salinity or rapid salinity change by shutting their valves and separating their tissues from the surrounding water (Zhang and Zhang, 2012), allowing them to endure short periods of harsh conditions (Lowe et al., 2017).

CONCLUSION

In conclusion, this study provides important insights into the reproductive biology of *Magallana bilineata* in Mengkabong Bay, Sabah, Malaysia. The population of *M. bilineata* in the bay is predominantly female, and hermaphroditism was observed in a small percentage of specimens. *M. bilineata* was observed to spawn year-round, with a peak during the Northeast Monsoon season. The reproductive cycle of the oyster in the bay is strongly influenced by environmental factors, particularly temperature and salinity. The highest maturity index (MI) and condition index (CI) values were observed in November 2019 when most oysters were in matured or maturing stages. These findings could be useful in developing strategies for assisted spawning and hatchery spat production to ensure the conservation and sustainability of the oyster fishery in Malaysia.

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REPRODUKTIVNA BIOLOGIJA I CIKLUS MRIJEŠĆENJA KAMENICE *Magallana bilineata* U ZALJEVU MENGKABONG, SABAH, MALEZIJA

SAŽETAK

Magallana bilineata komercijalno je važna vrsta kamenica u Maleziji. Unatoč gospodarskom značaju, biologija i ekologija ove vrste, posebice njezin reproduktivni ciklus, slabo su poznati u regiji. Stoga je ova studija imala za cilj istražiti reproduktivni ciklus *M. bilineata* u zaljevu Mengkabong, Tuaran, Sabah i njegovu korelaciju s čimbenicima okoliša. Sakupljeno je i ispitano ukupno 105 jedinki kamenica je kako bi se utvrdio omjer spolova, stadiji razvoja spolnih žlijezda, indeks zrelosti (MI) i indeks kondicije (CI). Rezultati su pokazali omjer spolova od 1:2,36 (♂:♀) u populaciji kamenica u zaljevu, sa značajno većim udjelom ženki (P < 0,05). Hermafroditizam je otkriven u 1,90% uzoraka. Utvrđeno je da se *M. bilineata* mrijesti većinu mjeseci u zaljevu, a najviše vrijednosti MI i CI zabilježene su u studenom 2019. kada je većina kamenica bila u fazi zrelosti ili sazrijevanja. Utvrđeno je da je salinitet primarni čimbenik koji utječe na sazrijevanje muških spolnih žlijezda, dok je temperatura primarni čimbenik koji utječe na sazrijevanje ženskih spolnih žlijezda. Razumijevanje reproduktivne biologije ove vrste ključno je za predviđanje njezina budućeg preživljavanja i olakšavanje potpomognutog mrijesta u proizvodnji na mrijestilištima, čime se osigurava očuvanje i održivost ribolova kamenica u Maleziji.

Ključne riječi: Bivalvia, Molusca, reproduktivni ciklus, ekološki čimbenici, sezona monsuna.

REFERENCES

Antonio, Í., Sousa, A., Lenz, T., Funo, I., Lopes, R., Figueiredo, M. (2021): Reproductive cycle of the mangrove oyster, *Crassostrea rhizophorae* (Bivalvia: Ostreidae) cultured in a macrotidal high-salinity zone on the amazon mangrove coast of Brazil. Acta Amazonia, 51, 2, 113–121.

Barman, A.C., Wong, N.L.W., Abd Karim, M.M. (2022): Reproductive cycle of the oyster *Crassostrea (Magallana) saidii* (Wong and Sigwart, 2021) from Southeast Asia. Aquaculture and Fisheries, doi:10.1016/j.aaf.2022.05.007.

Bayne, B.L. (2017): Biology of oyster. Developments in aquaculture and fisheries science, Vol 41. Elsevier Science, Academic Press, London, 860 pp.

Bricker, S.B., Grizzle, R.E., Trowbridge, P., Rose, J.M., Ferreira, J.G., Wellman, K., Zhu, C., Galimany, E., Wikfors, G.H., Saurel, C., Landeck, M.R., Wands, J., Rheault, R., Steinberg, J., Jacob, A.P., Davenport, E.D., Ayvazian, S., Chintala, M., Tedesco, M.A. (2020): Bioextractive removal of nitrogen by oysters in Great Bay Piscataqua River estuary, New Hampshire, USA. Estuaries Coast, 43, 1, 23–38.

Cárdenas, E.B., Aranda, D.A. (2000): A review of reproductive patterns of bivalve mollusks from Mexico. Bulletin of Marine Science, 66, 1, 13–27.

Castaños, C., Pascual, M., Camacho, A.P. (2009): Reproductive biology of the nonnative oyster, *Crassostrea gigas* (Thunberg, 1793), as a key factor for its successful spread along the rocky shores of northern Patagonia, Argentina. Journal of Shellfish Research, 28, 4, 837–847.

Castilho-Westphal, G.G., Magnani, F.P., Ostrensky, A. (2015): Gonad morphology and reproductive cycle of the mangrove oyster *Crassostrea brasiliana* (Lamarck, 1819) in the baía de Guaratuba, Paraná, Brazil. Acta Zoologia, 96, 1, 99–107.

Davenport, J., Chen, X. (1987): A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). Journal of Molluscan Studies, 53, 293–297.

Doinsing, J.W., Ransangan, J. (2022): Population dynamics of the tropical oyster *Magallana bilineata* (Mollusca, Bivalvia, Ostreidae) in Mengkabong Bay, Tuaran, Malaysia. Aquaculture and Fisheries, doi:10.1016/j.aaf.2022.04.005

Dove, M.C., O'Connor, W.A. (2012): Reproductive cycle of Sydney rock oysters, *Saccostrea glomerata* (Gould 1850) selectively bred for faster growth. Aquaculture, 324, 218–225.

Dridi, S., Romdhane, M.S., Elcafsi, M. (2014): Gametogenic cycle of *Crassostrea gigas* in contrasting Mediterranean habitats: Marine (Gulf of Tunis) and continental (Bizert lagoon) culture sites. Journal of Biological Research Thessaloniki, 21, 1, 1–11.

EIA (1992): Proposed mangrove paradise resort complex on LA 91040377 Tuaran, Sabah. Perunding Sekitar, Kota Kinabalu.

Etchian, O.A., Pellerin, J., Audet, C., Mathieu, M. (2004): Sexual maturation and related changes in aspartate transcarbamylase activity of gonad tissues in the soft shell clam (*Mya arenaria*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 139, 2, 287–297.

Ezgeta-Balić, D., Radonić, I., Bojanić Varezić, D., Zorica, B., Arapov, J., Stagličić, N., Jozić, S., Peharda, M., Briski, E., Lin, Y., Šegvić-Bubić, T. (2020): Reproductive cycle of the non-native Pacific oyster, *Crassostrea gigas*, in the Adriatic Sea. Mediterranean Marine Science, 21, 1, 146–156.

Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., Pouvreau, S. (2005): Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. Aquaculture, 250, 458–470.

Fakhrina, M.N., Christianus, A., Ehteshamei, F. (2018): Production of tropical oyster seed in hatchery. Journal of Survey in Fisheries Sciences, 5, 1, 7–19.

FAO (2019): Cultured aquatic species information programme *Crassostrea gigas* (Thunberg, 1793). http://www.fao.org/%0Afishery/culturedspecies/Crassostrea_gigas/en. Accessed 03 January 2023.

Ferreira, J.G., Sequeira, A., Hawkins, A.J.S., Newton, A., Nickell, T.D., Pastres, R., Forte, J., Bodoy, A., Bricker, S.B. (2009): Analysis of coastal and offshore aquaculture: Application of the FARM model to multiple systems and shellfish species. Aquaculture, 89, 32–41.

Futagawa, K., Yoshie-Stark, Y., Ogushi, M. (2011): Monthly variation of biochemical composition of Pacific oysters *Crassostrea gigas* from two main cultivation areas in Japan. Fisheries Science, 77, 4, 687–696.

Galtsoff, P.S. (1964): The American oyster *Crassostrea virginica* Gmelin. Fishery Bulletin, 64, 480.

Garen, P., Robert, S., Bougrier, S. (2004): Comparison of growth of mussel, *Mytilus edulis*, on longline, pole and bottom culture sites in the Pertuis Breton, France. Aquaculture, 511–524.

Gauthier-Clerc, S., Pellerin, J., Amiard, J.C. (2006): Estradiol-17β and testosterone concentrations in male and female *Mya arenaria* (Mollusca bivalvia) during the reproductive cycle. General and Comparative Endocrinology, 145, 2, 133–139.

Gibbs, M.T. (2004): Interactions between bivalve shellfish farms and fishery resources. Aquaculture, 240, 267–296.

Gomes, C.H.A.M., Silva, F.C., Lopes, G.R., Melo, C.M.R. (2014): The reproductive cycle of the oyster *Crassostrea gasar*. Brazilian Journal of Biology, 74, 4, 967–976.

Gosling, E. (2003): Bivalve molluscs: Biology, ecology and culture. Blackwell Publishing Ltd, Oxford, 443 pp.

Hamli, H., Idris, M.H., Rajaee, A.H., Kamal, A.H.M., Hoque, M.N. (2017): Condition index of *Meretrix lyrata* (Sowerby 1851) and its relationship with water parameter in Sarawak. Sains Malaysiana, 46, 4, 545-551.

Howard, D.W., Smith, C.S. (1983): Histological techniques for marine bivalve mollusks. NOAA Tech Memo NMFS-F/NEC-25. Oxford Lab, National Marine Fisheries Service, Oxford, 97 pp.

Lowe, M.R., Sehlinger ,T., Soniat, T.M., La Peyre, M.K. (2017): Interactive effects of water temperature and salinity on growth and mortality of eastern oysters, *Crassostrea virginica*: A meta-analysis using 40 years of monitoring data. Journal of Shellfish Research, 36, 3, 683–697.

Malaysian Meteorology Department (2019): Annual Report 2019. In: Malaysian Meteorological Department. www.met.gov.my. Accessed 08 January 2023

Mcfarland, K., Hare, M.P. (2018): Restoring oysters to urban estuaries: Redefining habitat quality for eastern oyster performance near New York City. PLoS ONE, 13, 11, e0207368.

Murua, H., Saborido-Rey, F. (2003): Female reproductive strategies of marine fish species of the North Atlantic. Journal of Northwest Atlantic Fishery Science, 33, 23–31.

Niogee, S.R., Tonni, K.F., Barman, A.C., Tanu, M.B., Sku, S., Uddin, M.J. (2019): Ovarian cycle of freshwater Pearl Mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a culture pond in Bangladesh. Asian Fisheries Science, 32, 3, 117–123.

Nor Idayu, A.W., Mohd Saleh, M.T., Zainodin, J., Natrah, F.M.I., Annie, C., Cob, C.Z., Aziz, A. (2015): Early development of tropical oyster *Crassostrea iredalei* (Faustino 1932). Advances in Environmental Biology, 9, 21, 1–8.

Nowland, S.J., O'Connor, W.A., Osborne, M.W.J., Southgate, P.C. (2020): Current status and potential of Tropical rock oyster aquaculture. Review in Fisheries Science and Aquaculture, 28, 1, 57–70.

Praveena, S.M., Ahmed, A., Radojevic, M., Abdullah, M.H., Aris, A.Z. (2008): Heavy metals in mangrove surface sediment of Mengkabong Lagoon, Sabah: multivariate and geo-accumulation index approaches. International Journal of Environmental Research, 2, 2, 139-148.

Rahaman, S.M.B., Golder, J., Rahaman, M.S., Hasanuzzam, A.F.M., Hug, K.A., Begum, S., Islam, S.S., Bir, J. (2013): Spatial and temporal variations in phytoplankton abundance and species diversity in the Sundarbans Mangrove Forest of Bangladesh. Journal of Marine Science: Research and Development, 3, 2, 126.

Rahim, A.A., Idris, M.H., Kamal, A.H.M., Wong, S.K., Arshad, A. (2012): Analysis of condition index in *Polymesoda expansa* (Mousson 1849). Pakistan Journal of Biological Sciences, 15, 13, 629–634.

Rebelo, M.F., Amaral, M.C.R., Pfeiffer, W.C. (2005): Oyster condition index in *Crassostrea rhizophorae* (Guilding, 1828) from a heavy-metal polluted coastal lagoon. Brazilian Journal of Biology, 65, 2, 345–351.

Rodríguez-Jaramillo, C., Hurtado, M.A., Romero-Vivas, E., Ramírez, J.L., Manzano, M., Palacios, E. (2008): Gonadal development and histochemistry of the tropical oyster, *Crassostrea corteziensis* (Hertlein, 1951) during an annual reproductive cycle. Journal of Shellfish Research, 27, 5, 1129–1141.

Rosell, N.C. (1990): Slipper-shaped oyster (*Crassostrea iredalei*) in the Philippines. In: Menzel, W. (Ed.), Estuarine and marine bivalve mollusk culture. CRC Press, Taylor & Francis Group, Boca Raton, 308–313

Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E., Kay, M.C. (2005): Introduction of non-native oysters: Ecosystem effects and restoration implications. Annual Review of Ecology, Evolution and Systematics, 36, 643–689.

Sivalingam, P.M. (1977): Aquaculture of the green mussel, *Mytilus viridis* Linnaeus, in Malaysia. Aquaculture, 11, 297–312.

Saucedo, E.P., Southgate, C.P. (2008): Reproduction, development and growth. In: Southgate, P.C., Lucas, J.S. (Eds.), The pearl oyster. Elsevier Press, Oxford, 131-186.

Suzana, M. (2011): Population genetics and molecular taxonomy of two important cultured oyster species in Malaysia, *Crassostrea iredalei* (Faustino, 1932) and *C. belcheri* (Sowerby, 1871). Dissertation, Universiti Sains Malaysia

Tan, K.S., Ransangan, J. (2014): A Review of feeding behavior, growth, reproduction and aquaculture site selection for green-lipped mussel, *Perna viridis*. Advances in Bioscience and Biotechnology, 5, 462–469.

Uddin, M.J., Park, K.I., Kang, D.H., Park, Y.J., Choi, K.S. (2007): Comparative reproductive biology of Yezo scallop, *Patinopecten yessoensis*, under two different culture systems on the east coast of Korea. Aquaculture, 265, 1-4, 139-147.

Vaschenko, M.A., Hsieh, H.L., Radashevsky, V.I. (2013): Gonadal state of the oyster *Crassostrea angulata* cultivated in Taiwan. Journal of Shellfish Research, 32, 2, 471–482.

Velonia, C. (2018): Oyster growth: Tagging and measuring individuals. University of Rhode Island.http://ahumphrieslab.com/kelp-blog/2018/10/14/oyster-growth-tagging-and-measuring-individuals. Accessed 05 January 2021.

Yoosukh, W., Duangdee, T. (1999): Living oysters in Thailand. Phuket Marine Biological Center Special Publication, 19, 2, 363-370.

Zhang, Z., Zhang, Q. (2012): Molecular cloning, characterization and expression of heat shock protein 70 gene from the oyster *Crassostrea hongkongensis* responding to thermal stress and exposure of Cu^{2+} and malachite green. Gene, 497, 2, 172–180.

Zulfigar, Y., Aileen, T.S.H. (2000): A review of oyster culture in Malaysia. In: Tiensongrasmee, B., Tedengren, M., Jarayabhand, P., Tandavanitj, N., Popongviwat, A., Nujsawat, A., Soisodri, S. (Eds.), Mollusk research in Asia, Thailand Research Fund, Bangkok, pp 75–79.