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Sex ratio, gonadal and condition indexes of the Asiatic hard clam, *Meretrix meretrix* in Marudu Bay, Malaysia

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Abstract. *Duisan L, Salim G, Ransangan J. 2021. Sex ratio, Gonadal and Condition indexes of the Asiatic Hard Clam,* Meretrix meretrix *in Marudu Bay, Malaysia. Biodiversitas 22: 4895-4904.* Asiatic hard clam, *Meretrix meretrix* is one of the important shellfishery resources in Marudu Bay, Sabah, Malaysia. It is among the most popular clam species being widely traded in the local wet markets around Sabah, Malaysia. Unfortunately, the shellfishery management for this species has not been well established. In addition to overexploitation, habitat destruction is also one of the significant threats to this species due to the extensive land use of the coastal areas in Sabah. Hence, conservation and breeding efforts for this species are greatly required. Therefore, the current study was conducted to examine the sexual maturity of the clam with respect to shell length classes for artificial seed production purposes. For this study, a total of 86 clam specimens were randomly collected from mudflats in Marudu Bay. The specimens were utilized for gonad histological and condition analyses. The clams were grouped into three shell length classes; (3.00-4.99) cm, (5.00-6.99) cm, and (7.00-8.99) cm prior to the analyses. Results showed the natural stock of the Asiatic hard clams in Marudu Bay was dominated by females (1.39:1) over males with no hermaphroditism observed. The gonadal index was recorded higher among clams with shell lengths between 5.00 and 6.99 cm. The condition index analysis also recorded high (>4.0) for clams in all the shell length classes. The findings of this study suggest that the clams with shell lengths between 5.00 cm and 7.00 cm are already fully matured and can be utilized as a broodstock candidate for an artificial breeding program in the hatchery.

Keywords: Aquaculture, maturity, Veneridae, venus clam

INTRODUCTION

Shellfish is an important source of livelihood in coastal communities worldwide (Oliveira et al. 2013; Rohmah and Muhsoni 2020). Generally, it is affordable (Lagade et al. 2013) and contains similar amounts of protein, glycogen, and minerals as other kinds of seafood (Sundaram and Deshmukh 2011; Jaya et al. 2017). Marudu Bay, Sabah, Malaysia is surrounded by pristine mangrove forest which provides suitable habitats for breeding and nurseries of both fishes and shellfishes (Zakaria and Rajpar 2015; Mojiol et al. 2016). Furthermore, one of its important shellfishery resources is the Asiatic hard clam (*Meretrix meretrix*) which inhabits the intertidal zones and supports artisanal fishery (Hamdan et al. 2019). However, recent studies showed that this species is overexploited (Tan et al. 2017; Admodisastro et al. 2021). Despite the significant economic contribution, little is known about the sexual maturity of this clam that creates challenges for the fishery management, conservation, and stock restoration of this species.

Knowledge about the sex ratio is important for fishery management and the conservation of economically important bivalve species because it provides information about the proportion of males and females in a population (Adebiyi 2013; Martínez et al. 2014). Furthermore, it is used to verify the capability of the bivalve concerned in undergoing sex reversal (Guo et al. 1998). Finally, such knowledge helps select the right proportion of male and female brood-stocks in an artificial breeding program.

The gonadal analysis (e.g., gonadal index) has been used to determine the gonad maturation in bivalves. Unfortunately, the determination of sex via histological examination usually requires the sacrifice of clam (Chávez-Villalba et al. 2011; Sawant 2012; El-Deeb et al. 2018). Furthermore, this analysis enables an accurate forecast of timing for the spawning season (Hamli et al. 2015), thereby significantly improving resource management for bivalve fisheries. These data are also exploited in the selection of bivalve brood-stock for the artificial breeding program. Similarly, the condition index does not only verify the maturation phases of the gonad (Acarli et al. 2018) but also serves as an indicator of the overall health status of a bivalve, and it is often utilized in the verification of meat quality, as well as to predict the harvest time for farmed bivalves (Acarli et al. 2018). Hence, this study aims to determine the sex ratio, gonadal and condition indexes of the natural stock of the Asiatic hard clam, *Meretrix meretrix* in Marudu Bay in view of the need to conserve and restore the stock of this species through aquaculture activities in the future.

MATERIALS AND METHODS

Sample collection

Specimens of Asiatic hard clam, *Meretrix meretrix* were collected at the four mudflats (A, B, C and D) within the inner part of the Marudu Bay, Sabah, Malaysia (Figure 1). These sites were selected because they are the common fishing grounds for hard clam in the bay. The clams were collected using the traditional fishing gear locally called "kerek" or hand dredging tool following the technique described by Tan and Ransangan (2019). A total of 86 live clam specimens with different shell length sizes were successfully collected between May 2017 to April 2018. In every sampling trip, clam specimens were kept moist in Styrofoam boxes and transported to the Borneo Marine Research Institute, Universiti Malaysia Sabah, for laboratory analyses.

Condition index

At the laboratory, clam specimens were cleaned and dissected. Then, the meats and shells were separated and dried in a drying oven at 60ºC until a constant weight was achieved. The condition index (CI) was then calculated following Walne (1976) as follow:

$$CI = \frac{\text{Dry Meat Weight}}{\text{Dry Shell Weight}}\, x\ 100$$

Gonad histological examination

Gonad development stages ((i) inactive or resting, (ii) early gametogenesis, (iii) advanced gametogenesis, (iv) mature, and (v) spawned) and the sex ratio was determined by the examination of the histological slides of gonad tissues. The histological examination of gonad tissues followed the methods described by Yurimoto et al. (2008) and Acarli et al. (2018). Briefly, each clam specimen was dissected to obtain the gonad. The gonad samples were then fixed in Bouin solution for 24 hours and dehydrated in a series of alcohol solutions (70% to 100%), cleared in two dips of 100% xylene and then embedded in paraffin. The paraffinized gonad tissues were mounted on wooden blocks and sectioned by the Shandon Microtome (Thermo Scientific, USA) into 7µm thick and stained with hematoxylin-eosin staining following Howard et al. (2004). The slides were then observed under a light microscope (Leica, Germany) at 10X magnification to determine the gender and the gonad developmental stages following the criteria suggested by da Silva et al. (2009).

Gonad index

The gonad development stages observed under a microscope were ranked into 4 categories following Buchanan (2001) and Wilson and Seed (1974), as given in Table 1.

Finally, the gonadal index (GI) was then calculated according to the formula suggested by King et al. (1989) as follows:

$$GI = \frac{\Sigma nR}{N}$$

Where, *n* denotes number of each stage; *N* denotes total sample size and *R* denotes rank of stage.

Statistical analyses

The significant difference of sex ratio among clams with different shell length classes was analyzed using the weighted chi-square analysis. As for condition and gonadal indexes, Kruskal-Wallis test (IMB SPSS Statistics 26) was employed. All analyses were tested at 95% confidence level. Prior to analysis of the condition and gonadal indexes, data were first tested for normality using the Shapiro-Wilk test (IBM SPSS Statistics 26). Tests were considered significant when the p-value was less than 0.05.

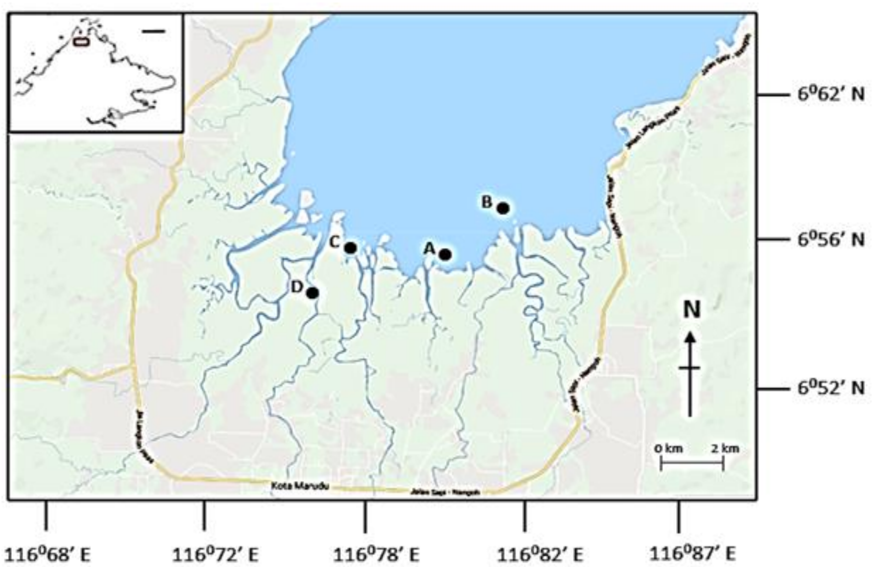

Figure 1. Sampling sites (A, B, C and D) of the Asiatic hard clam, *Meretrix meretrix* in Marudu Bay, Sabah, Malaysia

Table 1. Description and the numerical scoring given to each of the gonad developmental stages

	Stage	Score	General description
0	Resting	0	Animal has completed spawning. Gonad is mostly comprised of storage cells. Sex determination cannot be done.
1	Immature	1	Gametogenesis has started, follicles visible but no mature gametes apparent. Small clusters of germinal cells are scattered throughout the connective tissue. Oogonia and spermatogonia fanned from the germinal epithelium line the walls of the follicles. Sex determination is still difficult, especially in the early phases of this stage.
2	Developing	2	The follicles in both males and females occupy a large part of the mantle. In males, masses of primary and secondary spermatocytes and spermatids fill the follicles while small darkly staining nuclei of spermatozoa are scattered among the larger cells. In females, oocytes have begun to accumulate yolk and have grown considerably. Some of the larger oocytes are still attached to the follicular epithelium by a slender stalk of cytoplasm which eventually ruptures to leave the oocyte free within the follicle.
3A	Ripe	3	The gametes are now morphologically ripe. In males, the follicles are packed with spermatozoa arranged in lamellae converging towards the center of the lumen. A few residual spermatocytes and spermatids may still be present. In females, majority of the oocytes have reached their maximum size and are packed tightly together in the follicles. The pressure within these follicles compresses the oocytes into polyhedral forms. The connective tissue has lost most of its reserves of glycogen and lipid, which may be almost completely obscured by the swollen follicles.
3B	Spawning	2	Gametes have begun to be released. Large numbers of ripening oocytes are still present in the follicles. Residual oocytes tend to be spherical as the reduction in numbers greatly reduces compaction. Large numbers of spermatozoa line the follicles.
3C	Redeveloping	3	Rapid proliferation and growth of oocytes and a densely staining band of spermatids has given rise to new lamellae of spermatozoa. Gametogenesis continues until a new stage 3A is reached prior to further spawning.
3D	Spent	1	After the final spawning, the follicles have begun to collapse and degenerate. A small number of unspawned gametes are rapidly broken down by amoebocytes and the animal again enters the neuter stage (resting).

Note: The numerical rank scoring scheme was adopted from Wilson and Seed (1974) and Buchanan (2001)

RESULTS AND DISCUSSION

Sex ratio

Sex determination of hard clam (*Meretrix meretrix*) based on external appearance is not possible. Thus, microscopic examination of the gonads was carried to determine the clam sex. Figure 2 shows the histological differences between female and male clams. Male clams were identified by the presence of spermatozoa and spermatids (Figure 2D), while the female clams were identified by the presence of the follicle wall and mature oocytes (Figure 2E). Stages implying the maturation of gonad are presented in Figure 3. Figure 4 summarizes the number of female and male individuals and the gonad developmental stages concerning sex and shell length. Histological analysis revealed that the natural stock of *M. meretrix* in Marudu Bay, although not significantly deviated from the 1:1 ratio (c2=22.79, df=2, P>0.131), it was dominated by females at the ratio of 1.39 to 1.0 over the male (Table 2). Interestingly, younger specimens comprised of more females, but larger clams (>7.00 cm) were dominated by males. In terms of gonad maturation, more males were observed to have matured gonads than females (Figure 2). The number of ripe male and female specimens within the 5-6.99 cm shell length class was higher than that in the other classes (Figure 3). The natural stock of *M. meretrix* in Marudu Bay was dominated by females at a ratio of 1.39 to 1 male. Furthermore, these results are in line with Narasimham et al. (1988), which stated that the sex ratio of *M. meretrix* in Korampallam creek, India was also dominated by females at the ratio of 1.36:1 over males. However, it differs from a closely related species, *M. lyrata* which exhibits more males than females (Jayabal and Kalyani 1986; Hamli et al. 2015). According to (Hamli et al. 2015), most *Meretrix* species are dioecious, however, Chu and Kumar (2008) stated that 6% of *M. lyrata* are hermaphrodites. Regrettably, no hermaphroditism was observed in this study.

The numerous abiotic and biotic factors such as sex hormones (Wang and Croll 2004; Aji 2011), temperature, food availability, and pollution (Dridi et al. 2014; Breton et al. 2018) are known to influence sex ratio in bivalve species (Table 3). According to Wang and Croll (2004), sex hormones such as 17β-estradiol, testosterone, progesterone, or dehydroepiandrosterone (DHEA) induced juvenile sea scallop to masculinity.

Temperature also affects the sex ratio in many oyster species. For example, *Crassostrea virginica* in North Carolina was discovered to become female-biased and protandric during warmer and cool-season, respectively (Coe 1936). Similarly, the Pacific oyster, *Crassostrea gigas* in France, was found to become female and male-biased during summer and winter respectively (Lango-Reynoso et al. 2006). However, Tropical Cortes oyster, *C. corteziensis* in north-western Mexico was observed to become male and female-biased in warm (>18°C) and cool (<9°C) seasons respectively (Chávez-Villalba et al. 2008).

Food has also been stated to influence the sex ratio of bivalve species. For example, *Mytella charruana* changed sex from female to male due to starvation (Stenyakina et al.

2010). Also, *C. gigas* becomes female-biased as a result of food starvation (Egami 1953). Food deprivation is sometimes caused by a high stocking density of a population in an area (Ahmad et al. 2019).

Table 2. Chi-square analysis of the sex ratio with shell length, *Meretrix meretrix* in Marudu bay, Sabah, Malaysia

Shell length (cm)	Number			Sex ratio (M: F, %)	Chi-square	*P* value
	Total	Male	Female			
3.00-4.99	38	14	24	1:1.71 (36.8:63.2)	2.632	0.105
5.00-6.99	38	16	22	1:1.38 (42.0:58.0)	0.947	0.330
>7.00	10	6	4	1:0.67 (60.0:40.0)	0.400	0.527
Total	86	36	50	1:1.39 (42.0:58.0)	2.279	0.131

Table 3. Factors which can affect sex ratio in bivalve species

Bivalve species	Location	Factors affecting sex ratio	Sex ratio (M: F)	References
Mya arenaria	Baie du Moulin à Baude, Tadoussac, QC, Canada	Tributylin contamination	1.04:1	Gagné et al. 2003
Mya arenaria	Anse de Saint-Étienne, Canada	Tributylin contamination	1.73:1	Gagné et al. 2003
Mya arenaria	Baie-Sainte-Catherine, QC, Canada	Tributylin contamination	1.13:1	Gagné et al. 2003
Gomphina veneriformis	South Korea	Exposure of tributylin (0.8 µg/L) for 36 weeks	Initial; 1:1 Final; 1.37:1	Park et al. 2015
Mya arenaria	Bannow bay, southeast coast Ireland	Possible Climate change Narrow temperature difference between summer and winter (2.6 cm-11.6 cm shell length)	 1.15:1	Cross et al. 2012
Tegillarca granosa	Jangsu Bay, Korea	Sequential hermaphrodites	Initial; 2.25:1 Final; 1.85:1	Lee et al. 2014
Crassostrea corteziensis	Central Mexican Pacific coast	Hermaphroditism (1.3%) 2-10 cm in shell length	0.32:1	Mena-Alcantar et al. 2017
Sinanodonta woodiana	Cieply channel, north-west Poland	Hermaphroditism (2.34%) 3-16 cm shell length	0.56:1	Labecka and Domagala 2018
Mytella charruana	Southeastern, United States	Food availability	Initial; 0:1 Final; 1.33:1	Stenyakina et al. 2010
Placopecten magellanicus	Chester, Nova Scotia, Canada	Sex steroid: Estradiol Testosterone Progesterone Dehydroepiandrosterone	 6.75:1 6:0 8.67:1 5.25:1	 Wang and Croll 2004
Ostrea virginica	Milford, Connecticut, United States	Breeding season: Beginning sample (July) Second sample (Aug) Third sample (Sep)	 17.72:1 7.06:1 4.38:1	 Coe 1936
Crassostrea gigas	Hatchery of Coast Seafood Company, Quilcene, Washington, United States	Environmentally determined: One year old Two years old Three years old	 1.7:1 0.82:1 0.33:1	 Guo et al. 1998
Ostrea edulis	Sweden, Europe	Increase of temperature	> proportion of male individuals	Joyce et al. 2013
Tegillarca granosa	Yueqing, Wenzhou, China	Exposure of major heavy metal (Cu ≥14.2 µg/L, Pb ≥86.0 µg/L, Cd ≥110.0 µg/L and Zn ≥ 1.68 mg/L	> proportion of male individual	Liu et al. 2014
Gomphina veneriformis	South Korea	Exposure of 0.8 µg/L of Tributylin for 36 weeks	Initial; 1:1 Final; 1.37:1	Park et al. 2015
Anadara indica	Semarang, Indonesia	Sex reversal: < 35 mm shell length > 35 mm shell length	 1:1 0.25:1	Afiati 2016

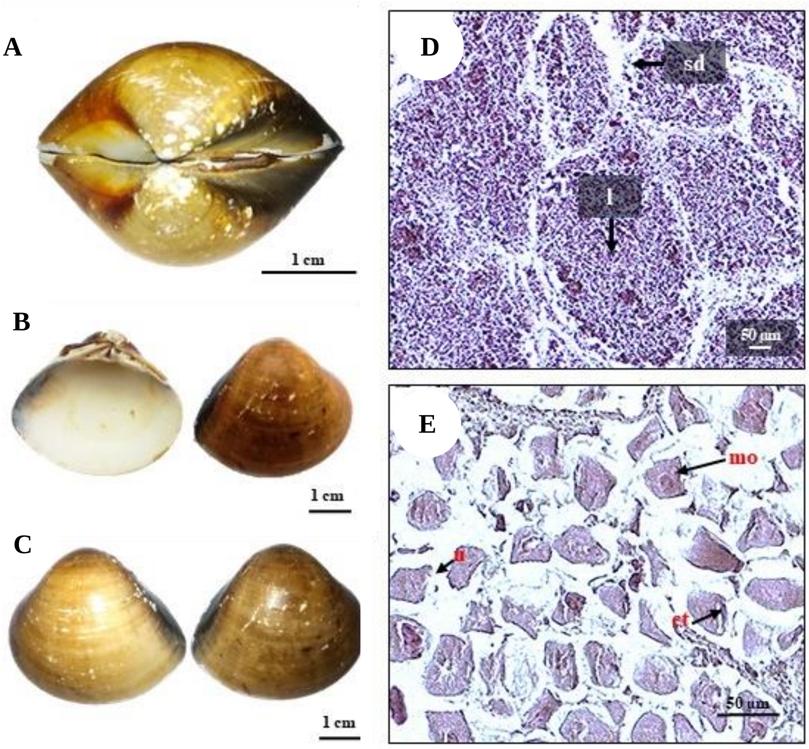

Figure 2. Sex ratio of *Meretrix meretrix* in Marudu Bay, Sabah, Malaysia. Note: A. Intact clam; B, C. Bisected clams; D. Gonad histology (male, 10x); E. Gonad histology (female, 10x); *sd:* spermatid; *l:* lumen; *mo:* mature oocyte; *ct:* connective tissuu; *n:* nucleus

Organotin compounds (e.g., tributyltin), which are widely used as antifouling agents in the shipping industry, have been reported to induce masculinity in bivalves (e.g., *Gomphina veneriformis* and *Mya arenaria*) (Park et al. 2015). Furthermore, investigations were conducted on the effect of heavy metals on sex ratio, however, Liu et al. (2014) showed that the blood clam (*Tegillarca granosa*) skewed towards the male in the presence of a high concentration of copper (Cu^{2+}, >14.2 µg/L), zink (Zn^{2+} >1.68 mg/L), lead (Pb^{2+} >86.0 µg/L) and cadmium (Cd^{2+} >110.0 µg/L), respectively. Nevertheless, this study fails to establish the factors responsible for the female-biased sex ratio of the *M. meretrix* in Marudu Bay. Starvation and chemicals pollution do not influence the gander skewness of *M. meretrix* because the bay has high chlorophyll-a content (Sing and Ransangan 2019) and no excessive contamination from heavy metals (Denil et al. 2017). Whether or not the high temperature (28^{O}C-31^{O}C) of the bay (Taib et al. 2015; Tan and Ransangan 2016) influences this observation was not analyzed in this study. Therefore, correct estimation of sex ratio is particularly important for artificial breeding in species like bivalves, which gander are difficult to identify morphologically, and also single-pair mating is often unproductive.

Gonadal index

The non-parametric analysis of gonad maturation showed a significant difference ($P<0.05$) among the males in the 3.00-4.99 cm and 5.00-6.99 cm shell length classes. While for females, it was noted no significant difference ($P>0.05$) within the three shell length classes (3.00-4.99 cm, 5.00-6.99 cm and >7.00 cm). Clams with shell lengths between 5.00 and 6.99 cm, irrespective of sex, appeared to have a higher gonadal index than the clams in the other two shell length classes (Figure 5). There was a significant difference ($P<0.05$) in terms of gonadal indexes between clams in the 5.00-6.99 cm and 3.00-4.99 cm shell lengths classes. However, no significant difference ($P>0.05$) of gonadal indexes was noted among big-sized clams (>7.00 cm) compared to clams in the other shell length classes (Figure 6).

The gonadal index is defined as the ratio of the gonad to body size, which has been used to describe and analyze the reproductive cycle of marine species (Idris et al. 2017). Furthermore, the temporal variations in gonad size reflect the phases of the reproductive cycle (Erickson et al. 1985). Accumulation of nutrients in the gonad before gametogenesis and gametes production leads to gonadal growth, which increases the gonadal index. The release of gametes during spawning decreases the gonad size and index. However, in subsequent gametogenesis, the gonad size and index increase gradually (Walker et al. 2007). Results of the current study showed that bivalves require satisfactory environmental conditions (Hamli et al. 2019) and availability of foods (Dridi et al. 2014; Chilmawati 2016; Breton et al. 2018; Khafage et al. 2019) for gametogenesis and spawning to occur. The temperature was stated to influence the spawning activity of bivalves (Aji 2011; Sreedevi et al. 2014). Also, a previous study by Tomatala (2011) stated that the spawning of the pearl oyster (*Pinctada maxima*) was affected by the change in the temperature of the water, while Ouréns et al. (2012) highlighted the limitations of using the gonadal index to predict the reproductive cycle of marine species. However, there is a strong optimism on using the gonadal index to estimate the sexual maturation of tropical bivalves such as *M. meretrix*, where age determination is difficult.

Some temperate bivalves (e.g., *Donax trunculus*) become sexually matured with age (Gaspar et al. 1999), while tropical bivalves (e.g., *Arca granosa*) mature beyond a certain length (Maske and Muley 2015). This study observed that the gonadal index of *M. meretrix* with shell lengths between 5.00-6.99 cm was higher than other classes, though not significantly different from those larger clams (>7.00 cm). In contrast, the clams within the 3.00-4.99 cm shell length category have a significantly lower value of the gonadal index. In India, *M. meretrix* was reported to have attained first sexuality maturity at a shell length of 2.1-2.6 cm (Jayabal and Kalyani 1986). This is true in Marudu Bay because many clams were observed to have matured gonads at 3.0 cm. However, the low number of small clams which have matured gonads is explained by the fact that there use more energy for growth rather than for gonad development (Zarnoch and Schreibman 2008), while larger clams (>7.00 cm) are considered old and require a lot of energy to maintain the declining metabolic rate.

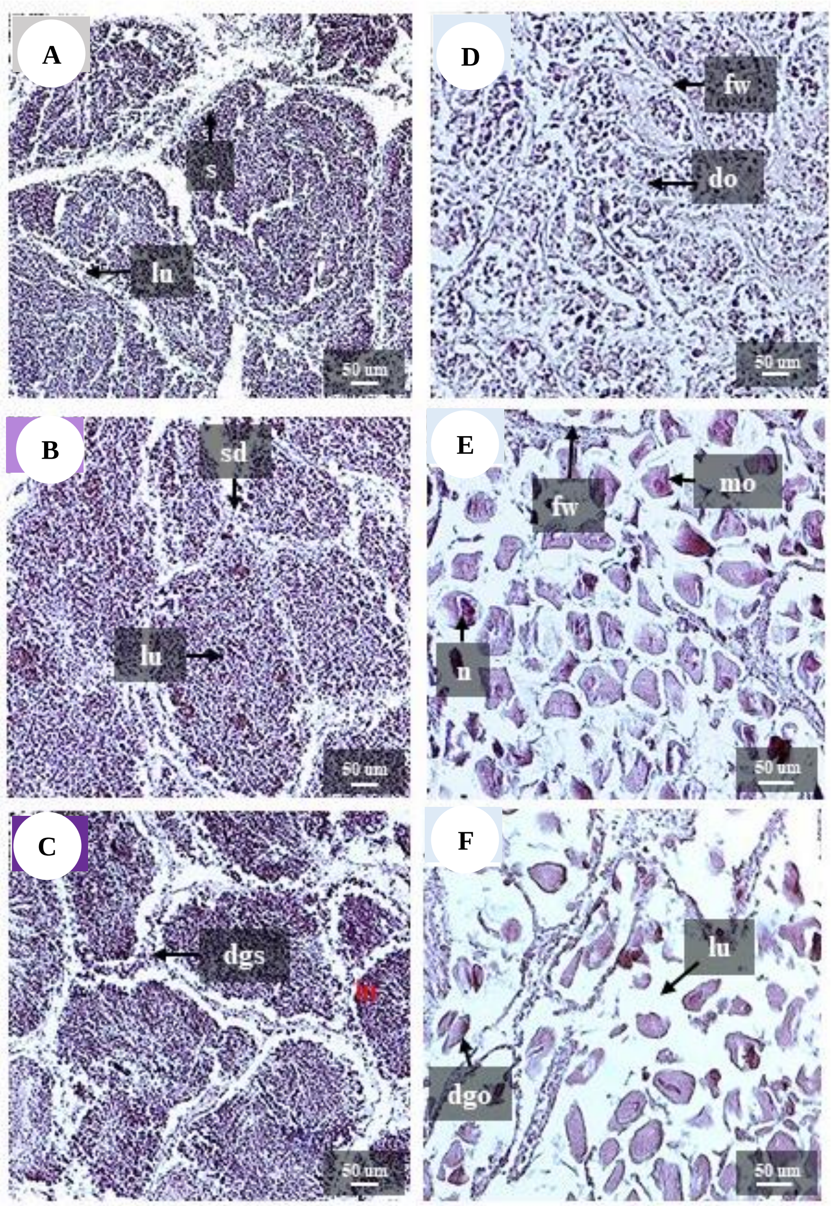

Figure 3. Maturation stages of the *Meretrix meretrix*. A, B, C. Immature, spawning and ripe males, respectively; D, E, F. Immature, spawning and ripe females, respectively; ct: connective tissue; sd: spermatid; sz: spermatozoa; l: lumen; dgs: degenerate spermatozoa/ spermatid/ spermatocyte; fw: follicle wall; do: developing oocyte; n: nucleus; mo: matured oocyte; dgo: degenerate oocyte

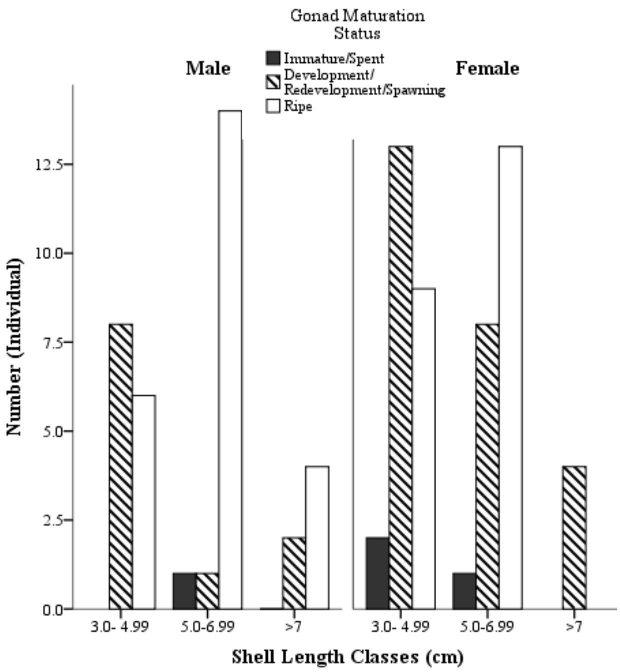

Figure 4. Gonad maturation stages of the Asiatic hard clam, *Meretrix meretrix* with respective to gander and shell length

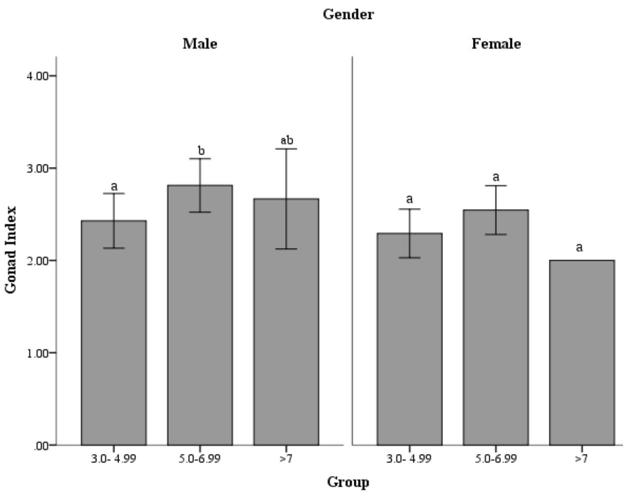

Figure 5. Gonadal index of *Meretrix meretrix* in Marudu Bay according to sex and shell length classes. Note: *a* different from *b*; *ab* not different from *a* and *b*

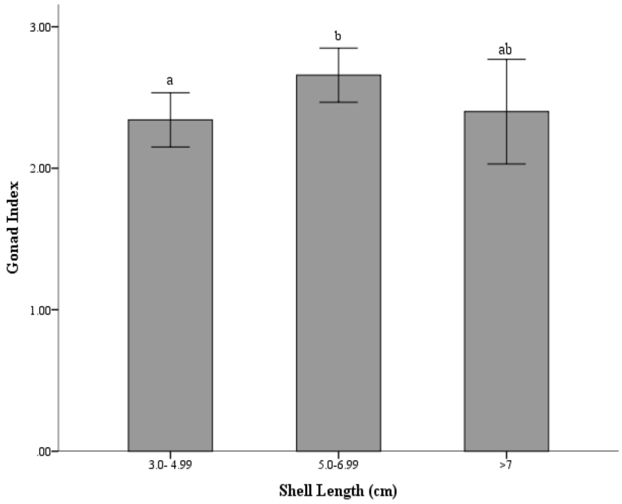

Figure 6. Gonadal index of *Meretrix meretrix* in Marudu according to shell length classes. Note: *a* different from *b*; *ab* not different from *a* and *b*

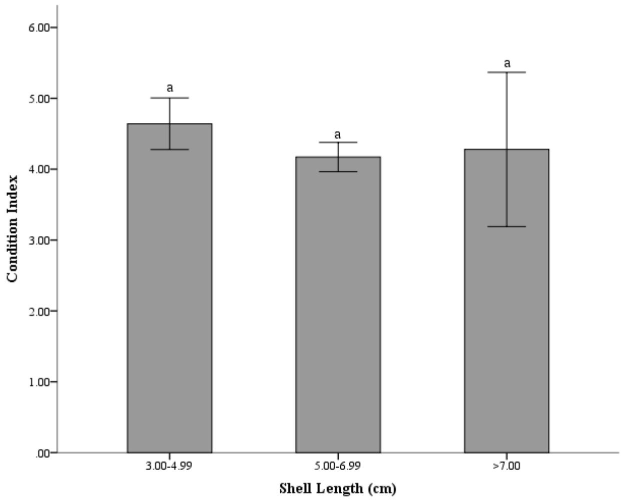

Figure 7. Condition index of *Meretrix meretrix* in Marudu Bay according to sex and shell length classes

Condition index

Statistical analysis showed there is no significant difference (P>0.05) in condition indexes among clams in all shell length classes (Figure 7).

The condition index increases when the gonad reaches the maturation phase, as more meats are produced or accumulated, and decreases when the gonad is in spent or resting phases (Hamli et al. 2017). According to Ismail (2012), the consummation of shellfish that were exposed to contamination such as mercury can result in life-threatening conditions. Hence, the condition index is often used as an indicator of meat quality (Yenni and Nurjanah 2011; Teguh et al. 2016; Hassan et al. 2017; Arifin 2019) and performance of farmed bivalves (Filgueira et al. 2013). The gonad tissue represents 59% of the total soft tissues weight, thus contributing significantly to the total weight of the individual bivalve. However, differences in shell thickness, fecundity stages, feeding rates, and biomass density also influence the condition index of bivalves (Duinker et al. 2008).

Environmental conditions influence the condition index of clams (Tanyaros and Tongnunui 2011; Kasmini et al. 2018; Sinaga et al. 2018). Other previous studies also showed that temperature, salinity, and photoperiod affect the gonad development of bivalves (Zarnoch et al. 2008;

Hamli et al. 2019). However, Joyce et al. (2013) reported that the gametogenesis and sex ratio of bivalve (e.g., *Ostrea edulis*) remain unaffected by temperature and photoperiod. Furthermore, gametogenesis requires high energy as a result of this it exhausts the energy storage and the condition index of animals (da Silva et al. 2009; Hassan et al. 2017; Hamli et al. 2019). The study showed that the energy expenditure in clams could be influenced by salinity, temperature and food availability (Miller et al. 2014). According to Lucas and Beninger (1985), bivalves with a high condition index could indicate that they do not experience high energy-consuming processes such as stresses due to poor environmental conditions, illnesses, gametogenesis, or spawning. Meanwhile, it was observed that clams within the class of 5.00-6.99 cm shell length had a lower condition index. This implies that the clams utilized more energies for biological efforts compared to those in lower (3.00-4.99 cm) and higher (>7.0 cm) classes of shell length, respectively.

In conclusion, the Asiatic hard clam (*Meretrix meretrix*) is an important species for artisanal shellfishery in Marudu Bay, Malaysia. Furthermore, the sex ratio of the clam in the bay was skewed toward the female population and exhibited a high condition index. It was also observed that clams with a shell length of 5.00-7.00 cm have a high gonad index, implying that sexual maturity has been fully attained. Therefore, this basic biological information be utilized in the future for a successful artificial breeding program for this clam.

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