# Sex Ratio, Gonad and Condition Indices of Mangrove Clam, *Polymesoda (Geloina) erosa* (Bivalvia: Corbiculidae) in Marudu Bay, Sabah, Malaysia: Implication for Broodstock Selection in Artificial Breeding Program

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## ABSTRACT

The mangrove clam, *Polymesoda erosa* is among the commercially important bivalve species in Marudu Bay, Malaysia. However, a recent study has shown that this species experiences a high level of fishing exploitation. To sustain this species, one potential strategy is to restock hatchery-produced seeds through sea ranching. However, this requires the successful artificial production of seeds, which currently has not been achieved. The present study was conducted to determine the sex ratio and maturity level of different shell length classes of the clam to guide the selection of broodstock for artificial propagation in the future. A total of 240 mangrove clam specimens were randomly collected from the mangrove swamps in Marudu Bay and sorted into four shell length classes: 5.50-6.49 cm, 6.50-7.49 cm, 7.50-8.49 cm and 8.5-9.49 cm. The clams were subjected to gonad histological examination and condition analysis. Results showed that the natural stock of clams exhibited close to a 1:1 male-to-female sex ratio with no hermaphroditism observed. Clams within the 5.50-8.49 cm shell length classes had high gonad index, indicating that most of the clams at this size are fully mature. Statistical analysis revealed a significant difference (p = 0.027) in condition index between two shell length classes: 5.50-6.49 cm and 7.50-8.49 cm. This could be attributed to differences in energy utilization; young clams utilize more energy for growth, whereas adult clams utilize energy for growth, reproduction, and metabolic maintenance. This study suggests that using mangrove clams with shell length between 5.50 cm and 8.49 cm will likely result in high spawning success in an artificial breeding program.

**Keywords:** Aquaculture, Maturity, Mollusk, Sea ranching, Stock enhancement

## INTRODUCTION

Marudu Bay is situated in the northernmost part of Sabah (North Borneo), Malaysia. It is located within the Tun Mustapha Park and is part of the Coral Triangle Initiative. The bay is an ecologically important area and one of the biodiversity hotspots in Malaysia. One of the important bivalve species that thrives in the intertidal areas of mangrove swamps in Marudu Bay is the mangrove clam (Corbiculidae). According to Ransangan and Soon (2018), two species of mangrove clams occur in Marudu Bay, namely *Polymesoda erosa* and *P. expansa*; the former was found to be more abundant in their study. The same authors also reported that adult *P. erosa* are often encountered landward of the mangrove swamps, whereas juvenile clams are commonly found in the seaward

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zone. *P. erosa* in the bay was estimated to attain a shell size of 9.46 cm, and the species exhibited a continuous recruitment pattern with two annual peaks: one in June and another in November (Ransangan *et al*., 2019).

This clam is highly sought in local markets and fetches a relatively good price (about 1.5-2.0 USD·kg$^{-1}$). It is commonly served as a grilled dish in many roadside stalls and open-air restaurants throughout Sabah. The clam is also extensively used by hatcheries as a supplemental diet to induce broodstock maturation of mangrove crabs (Thien and Yong, 2017). Demand for these clams has encouraged local fishermen to intensify fishing effort, which, in turn, is expected to impact the natural stock of the clam, not only in Marudu Bay, but also in other mangrove ecosystems along the coastal zone of Sabah. A study by Ransangan *et al*. (2019) showed that the clams, *Polymesoda* spp. in Marudu Bay have already been experiencing high exploitation (E = 0.65) and high fishing mortality (F = 1.44 year$^{-1}$). Over a long period, this could lead to local extinction.

In view of the high level of exploitation, a management plan to sustainably conserve the clam is vital. One of the practical conservation efforts is to breed the clam in hatcheries, and then reintroduce the resulting juveniles into their natural habitats by sea ranching. However, before such a breeding program can be initiated, an understanding of the basic reproductive biology of the clam is necessary. According to Litulo (2005), reproductive patterns such as gonad maturation, production and release of gametes, and fecundity play important roles in the continuity of important fishery species such as mangrove clams. Understanding their reproductive pattern and being able to predict this cycle allows breeding manipulation to be carried out effectively. Hence, this study was conducted to determine which clams, with respect to shell length and gonadal index, are likely to be viable candidates for broodstock selection in an artificial breeding program.

## MATERIALS AND METHODS

### *Sampling*

Sampling of the mangrove clams was done in mangrove swamps at site 1 (N06º32'18.2", E116º 44'15.8") and site 2 (N06º33'39.0", E116º45'51.5") along the inner part of Marudu Bay (Figure 1). The sampling was carried out during low tide using hand rakes during May 2017 to April 2018. Finding the mangrove clams was challenging, as our team had to compete with the local clam collectors. Due to this, we chose two sampling sites where enough clams could be obtained for our study. Unfortunately, the sites were dominated by larger


Figure 1. (a): Map of Malaysia with sampling location circled; (b): Sampling sites (numbered 1 and 2) of mangrove clams in the mangrove swamps of inner Marudu Bay (sources: Expatgo (2020) and Arcgis (2020)).

clams (>5 cm) which were assumed to be already mature (Dolorosa and Dangan-Galon, 2014). However, this did not affect the study objective, as the focus of the present investigation was to determine which sizes of clams have a higher degree of gonad maturation. A total of 240 clam specimens were collected randomly throughout the sampling period. The clam specimens were transported to the Borneo Marine Research Institute, Universiti Malaysia Sabah for species identification following criteria suggested by Hamli *et al*. (2015). The clams were then divided randomly into two groups; one group was subjected to gonad histological examination (76 individuals) and the other was used to determine condition index (164 individuals). Water quality parameters were not measured during the sampling due to low-tide conditions in the collection sites, which were located in the landward regions of the bay.

*Condition index*

At the laboratory, the clams were first washed using clean fresh water and dry-blotted with paper towel before weighing and measuring with an electronic balance and Vernier calipers. Subsequently, the clams were chilled in a refrigerator (4 ºC) for 20 min before dissection. The shells and meats were separated and dried in an oven at 60 ºC until constant weight was achieved. The condition index (CI) was then calculated following Walne (1976), as follows:

$$CI = \frac{\text{Dry meat weight (g)}}{\text{Dry shell weight (g)}} \times 100$$

*Histological examination of gonads*

Gonad developmental stages which consist of (i) inactive or resting, (ii) early gametogenesis, (iii) advanced gametogenesis, (iv) mature, and (v) spawned were determined histologically following Yurimoto *et al*. (2008) and Acarli *et al*. (2018). Briefly, each clam specimen was dissected for gonad retrieval. The gonads were then fixed in Bouin's solution for 24 h, dehydrated in series of varying concentrations (70%, 80%, 90% and 100%) of alcohol for 30 min each, cleared in two 100% xylene solution dips, and then embedded and blocked in paraffin. The paraffin blocks were sectioned by Shandon Microtome (Thermo Scientific, USA) to 7 µm and stained with hematoxylin-eosin following Howard *et al*. (2004). The histological slides were carefully examined under a light microscope (Leica, Germany) at 100X magnification to determine the gonad developmental stages (Sahin *et al*., 2006) and sex (Idris *et al*., 2017). A sex ratio for the entire sample was calculated.

*Gonad index*

Every stage of gonad development observed during histological examination was assigned a numerical ranking score (Table 1) following a scheme suggested by Wilson and Seed (1974) and Buchanan (2001). Finally, the scores derived from Table 1 were used to calculate the Gonad index (GI) of the clams following King *et al*. (1989), as follows:

$$GI = \frac{\text{number in each stage x numerical ranking of that stage}}{\text{number of animals in the sample}}$$

*Statistical analyses*

Differences in sex ratios among shell length classes were analyzed using a weighted Chi-square analysis. Prior to the analysis of condition and gonad indices, the data were first tested for normality using the Shapiro-Wilk test (IBM SPSS Statistics 26). Because the data obtained for both condition and gonad indices deviated from normality, the Kruskal-Wallis test (IMB SPSS Statistics 26) was employed. All the analyses were tested at a 95 % confidence level. Tests were considered significant when the p value was less than 0.05.

## RESULTS

*Sex ratio*

The clam samples in the present study were divided into four shell length classes based on a 1-cm interval: 5.50-6.49 cm, 6.50-7.49 cm, 7.50-8.49 cm and 8.50-9.49 cm (Table 2). The occurrence of larger clams in this study was anticipated due to the

Table 1. Numerical scoring scheme used for the gonad index of the mangrove clam, *Polymesoda erosa* in Marudu Bay.

| | 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).

Table 2. Chi-square analysis of sex ratio by shell length class of mangrove clam, *Polymesoda erosa* in Marudu Bay.

| Shell length class (cm) | Number | | | Sex ratio (M:F %) | Chi-square | p value |
|---|---|---|---|---|---|---|
| | Total | Male | Female | | | |
| 5.50-6.49 | 8 | 6 | 2 | 3:1 (75:25) | 2.000 | 0.157 |
| 6.50-7.49 | 43 | 20 | 23 | 0.87:1 (46.51:53.49) | 0.209 | 0.647 |
| 7.50-8.49 | 20 | 10 | 10 | 1:1 (50:50) | 0.480 | 0.827 |
| 8.50-9.49 | 5 | 1 | 4 | 1:4 (20:80) | 1.800 | 0.180 |
| Total | 76 | 37 | 39 | 0.97:1 (48.68:51.32) | 0.013 | 0.909 |

sampling sites being located at the landward region of Marudu Bay, which is favorable habitat for adult clams (Clemente and Ingole, 2011). It was also observed that the stocks of mangrove clams (*Polymesoda erosa*) in the bay were slightly skewed toward female individuals (0.97:1; M:F). However, weighted Chi-square analysis (Table 2) showed that the gender composition was not significantly different ($p>0.05$).

Interestingly, further investigation into the sex ratios of clams in different shell length classes revealed that small clams (5.50-6.49 cm) were mostly males (3:1; M:F), but as the clams grew in size (8.50-9.49 cm) more females were observed (1:4; M:F). The clams in the middle shell length classes (6.50-7.49 cm and 7.50-8.49 cm) had a similar number of males and females (Table 2).

*Gender determination*

Gender determination of the clams was achieved using microscopic examination of the clam gonads. Figure 2 shows the histological differences between male and female clams. The male clams were identified by the presence of spermatozoa and spermatids (Figure 2d), whereas the female clams were identified by the presence of the follicle wall and mature oocytes (Figure 2e). Maturation stages of male and female gonads are presented in Figure 3. The number of male and female individuals, and the gonad developmental stages according to sex and shell length are summarized in Figure 4.

*Gonad index*

A non-parametric comparison of the gonad maturation stages (Figure 5) showed no significant differences among the males in different shell length classes ($p>0.05$). However, there was a significant difference ($p = 0.046$) between females in the 5.50-6.49 cm and 8.50-9.49 cm groups. Female clams in the 5.50-6.49 cm shell length class showed higher proportion of individuals in developing or redeveloping or spawning stages than the larger females (Figure 5). Irrespective of sex, there was a significant difference in gonad index ($p = 0.036$) between the clams in the shell length classes of 6.50-7.49 cm and 8.50-9.49 cm (Figure 6).

*Condition index*

There was a significant difference ($p = 0.027$) in condition index between the clams in the length shell classes of 5.50-6.49 cm and 7.50-8.49 cm (Figure 7).

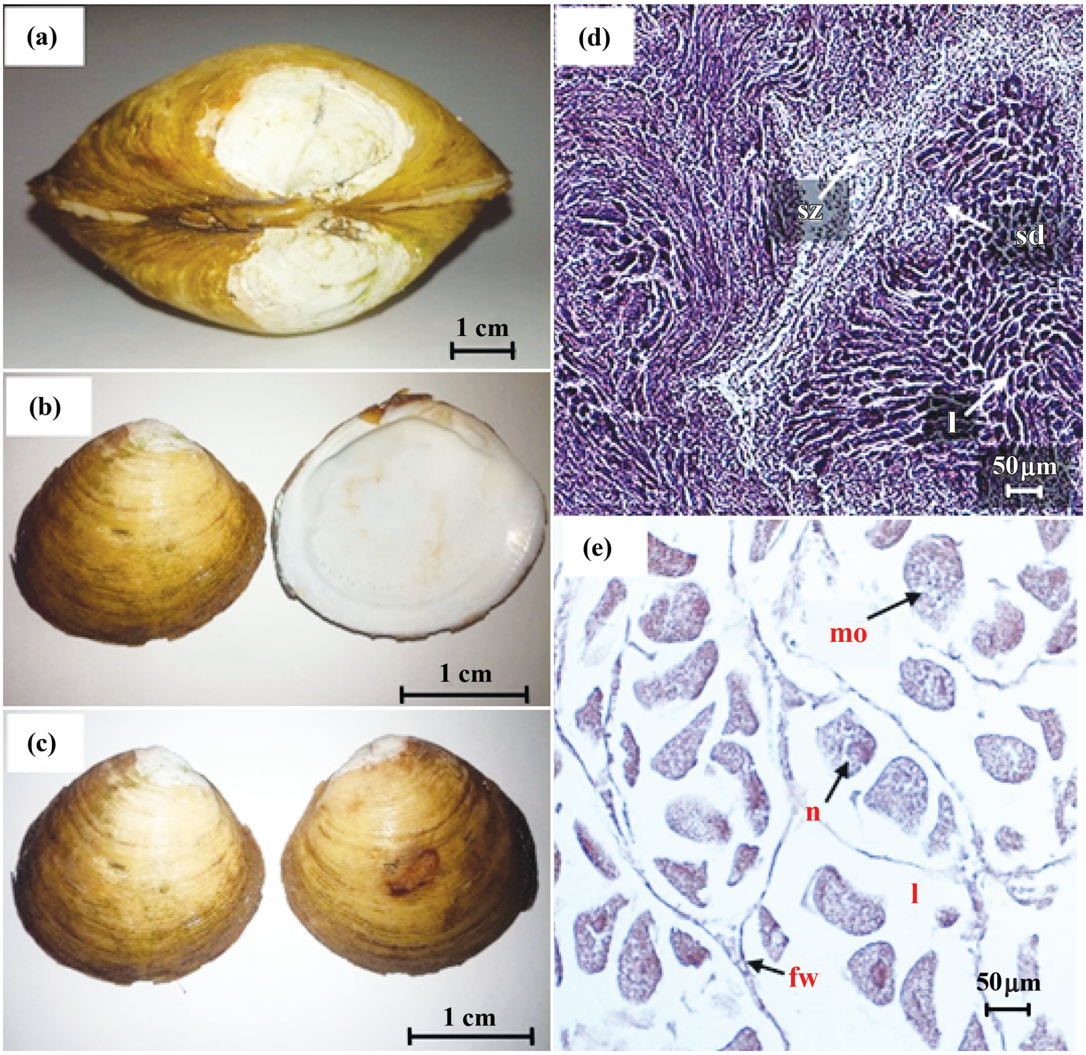


Figure 2. Gender determination of the mangrove clam, *Polymesoda erosa*. (a) intact clam; (b, c) dissected clams; (d) gonad histology (male, 10×); (e) gonad histology (female, 10×); sz: spermatozoa; l: lumen; mo: mature oocyte; sd: spermatid; fw: follicle wall; n; nucleus.

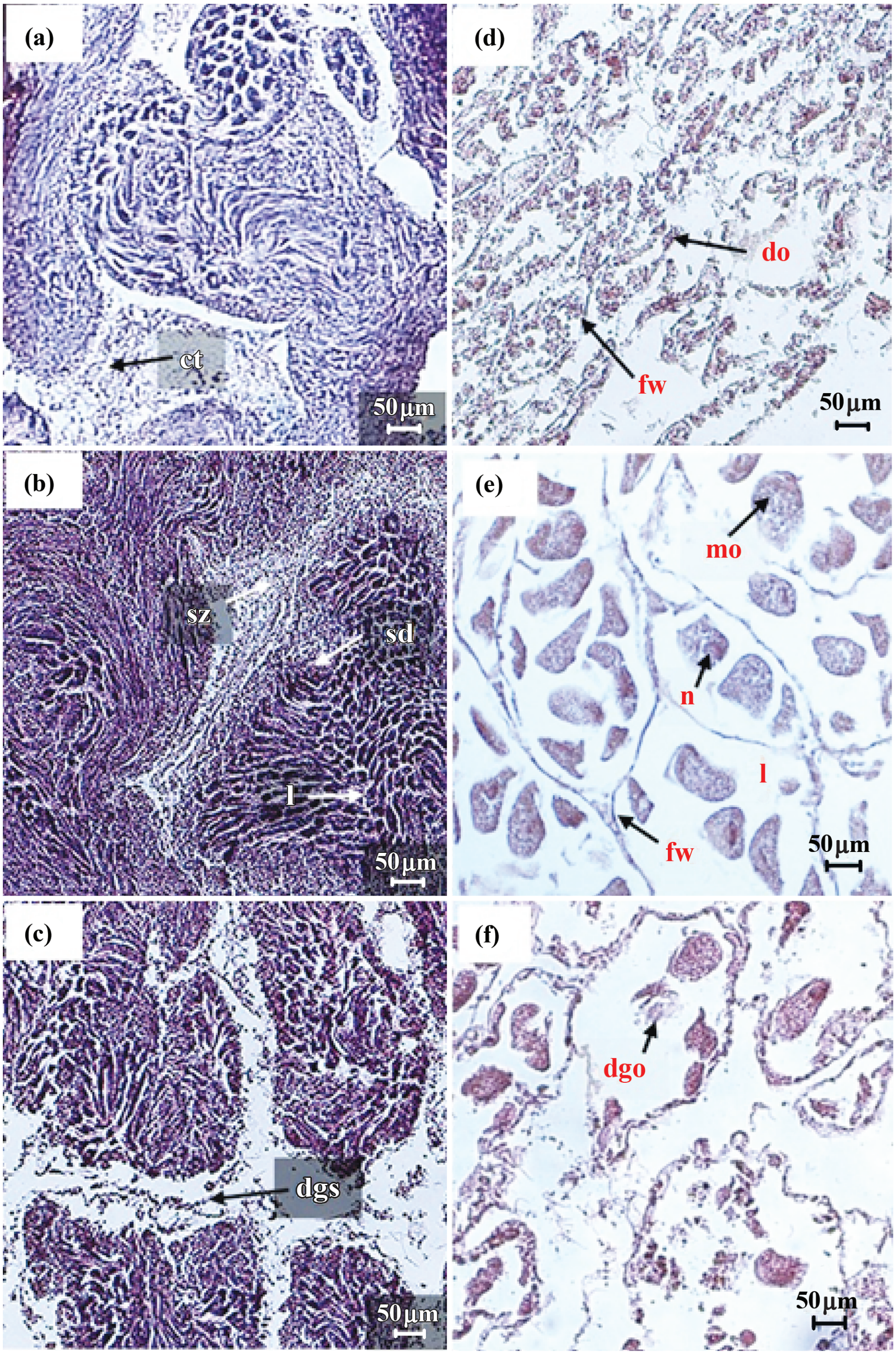


Figure 3. Maturation stages of mangrove clam gonads. (a,b,c) immature, spawning and ripe males, respectively; (d,e,f) immature, spawning and ripe females, respectively; (ct) connective tissue; (sd) spermatid; (sz) spermatozoa; (l) lumen; (dgs) degenerate spermatozoa/ spermatid/ spermatocyte; (fw) follicle wall; (do) developing oocyte; (n) nucleus; (mo) matured oocyte; (dgo) degenerate oocyte.

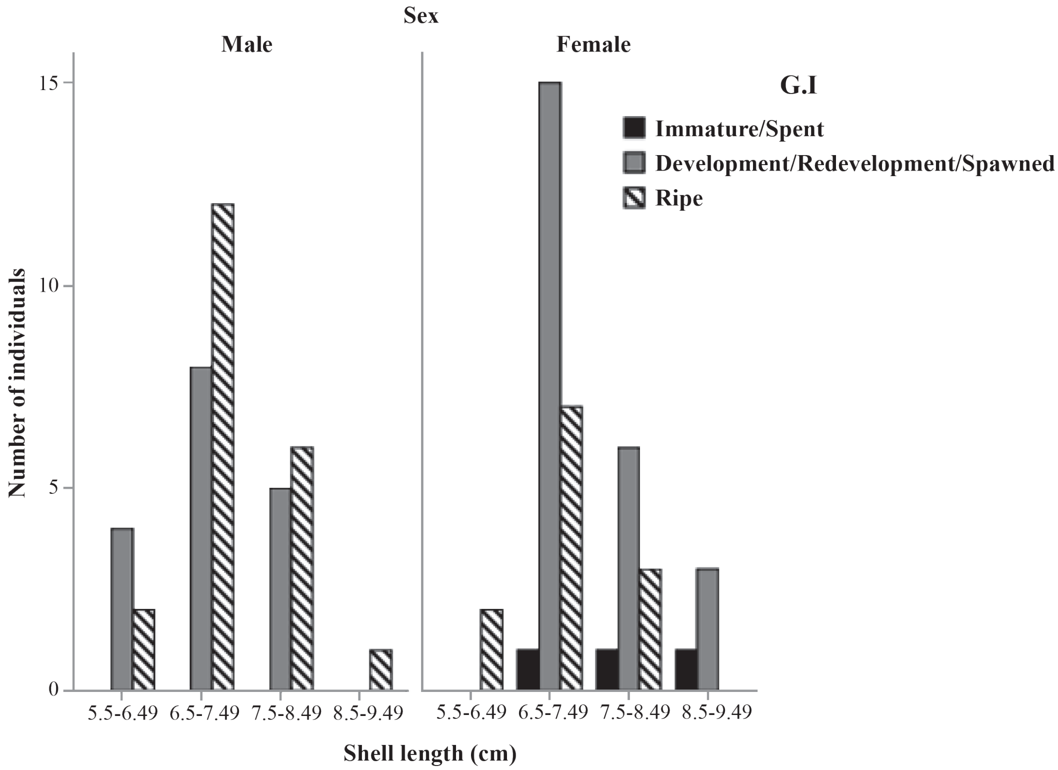


Figure 4. Gonad maturation stages of the mangrove clam, *Polymesoda erosa* by sex and shell length class.

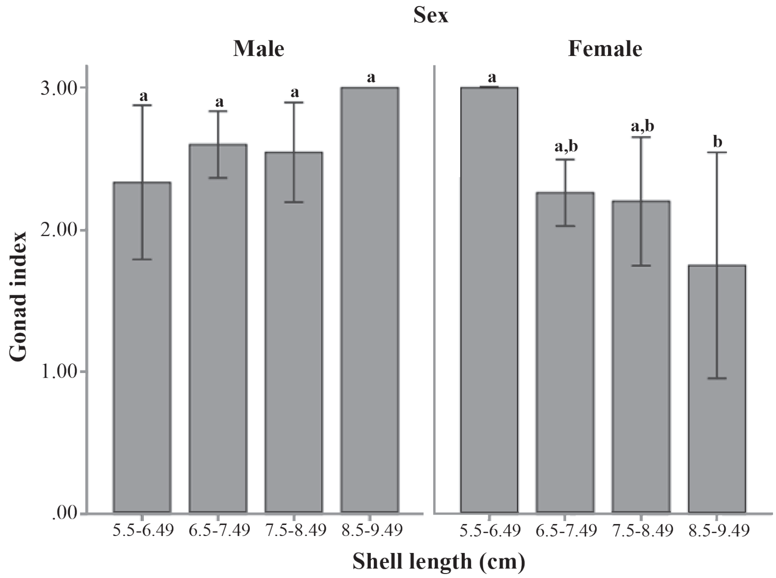


Figure 5. Gonad index of the mangrove clam, *Polymesoda erosa* by sex and shell length class. Error bars represent standard deviation; different letters above bars indicate significant difference ($p<0.05$).

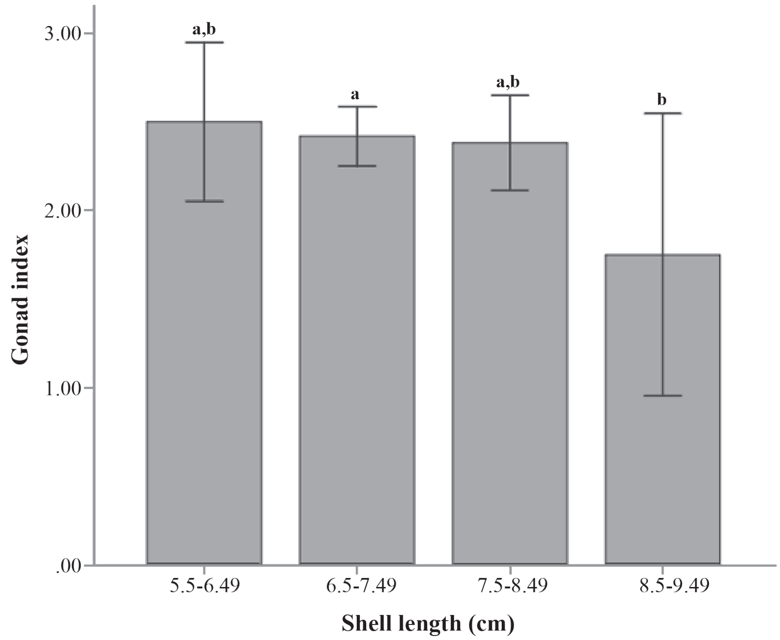


Figure 6. Gonad index of the mangrove clam, *Polymesoda erosa* by shell length class. Error bars represent standard deviation; different letters above bars indicate significant difference ($p<0.05$).

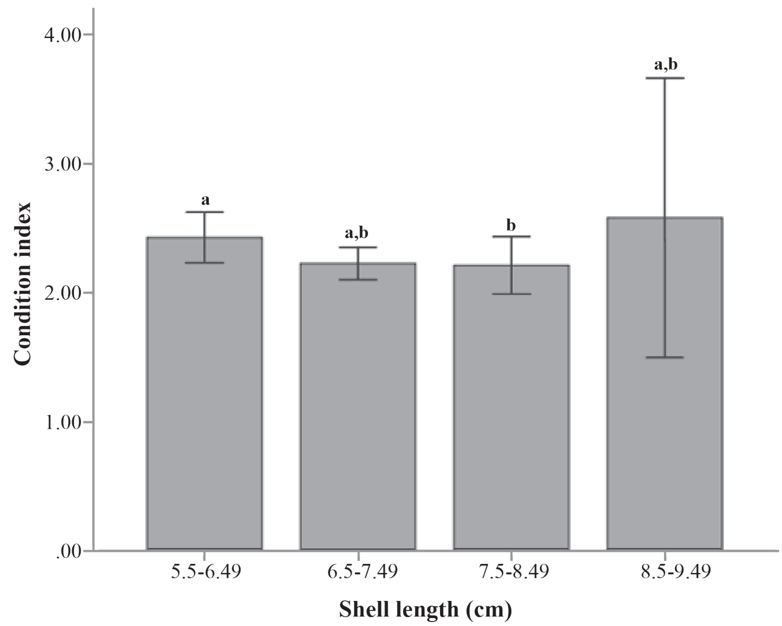


Figure 7. Condition index of the mangrove clam, *Polymesoda erosa* by shell length class. Error bars represent standard deviation; different letters above bars indicate significant difference ($p<0.05$).

## DISCUSSION

*Sex ratio*

*Polymesoda erosa* is among the bivalves popularly harvested by the local fishers in Marudu Bay. The current study reveals that the natural stock of *P. erosa* in Marudu Bay has a nearly 1:1 male-to-female ratio. Similarly, Gimin *et al*. (2005) found *P. erosa* (shell length 5.50-9.00 cm) in northern Australia to exhibit a 1:1 sex ratio. There are various abiotic and biotic factors known to affect the sex ratio in bivalves. This includes temperature, food availability, pollution (Dridi *et al*., 2014; Breton *et al*., 2018) and hormones (Wang and Croll, 2004). It was reported that sex hormones (e.g. testosterone, 17β-estradiol, and progesterone or dehydroepiandrosterone (DHEA)) could induce juvenile bivalves (sea scallops) to become masculine (Wang and Croll, 2004). Occurrence of hermaphroditism in mangrove clams varies by species. However, according to Clemente and Ingole (2009), *P. erosa* is mostly dioecious. We also observed that *P. erosa* in Marudu Bay exhibits unisexual reproduction. Apart from sex hormones, temperature also has been reported to influence sex ratio in many bivalve species, particularly oysters. For example, the European flat oyster (*Ostrea edulis*) was observed to skew toward female in summer (>18 ºC) (Zapata-Restrepo *et al*., 2019). In contrast, the tropical Cortes oyster (*C. corteziensis*), which inhabits waters of northwestern Mexico was reported to become male biased in the warmer season (>18 ºC) and female biased during the cooler season (<9 ºC) (Chávez-Villalba *et al*., 2008).

Food availability has also been reported to affect sex ratio in bivalves. For example, *Mytella charruana* changed their sex from female to male during a malnourishment period (Stenyakina *et al*., 2010). However, *Crassostrea virginica* (Bahr and Hillman, 1967) was observed to become female biased under food starvation. The masculinity in bivalves (e.g., *Gomphina veneriformis* and *Mya arenaria*) can be induced by organotin compounds such as tributylin. Unfortunately, many of these compounds are extensively used in the shipping industry as antifouling agents (Gagné *et al*., 2003; Park *et al*., 2015). High concentrations of heavy metals were also reported to affect sex ratios in bivalves. For instance, Liu *et al*. (2014) reported that the blood cockle (*Tegillarca granosa*) can become male biased in the presence of high concentrations of copper ($Cu^{2+}$ >14.2 μg·L$^{-1}$), zinc ($Zn^{2+}$ >1.68 mg·L$^{-1}$), lead ($Pb^{2+}$ >86.0 μg·L$^{-1}$) and cadmium ($Cd^{2+}$ >110.0 μg·L$^{-1}$). The present study, however, indicated that the natural stock of *Polymesoda erosa* in Marudu Bay exhibited a normal sex ratio. This shows that the mangrove ecosystem of Marudu Bay provides sufficient foods for the clams to thrive (Sing and Ransangan, 2019) and suggests no pollution from heavy metals (Denil *et al*., 2017). Temperature is less concerning because the bay has been reported to have consistently high annual temperature (27-31 ºC) (Taib *et al*., 2016; Tan and Ransangan, 2016).

*Gonad index*

Gonad index has been widely used to study the reproductive cycles of a large range of aquatic species, including bivalves (Ouréns *et al*., 2012). During spawning, gametes are released, which causes the size of gonad to decrease and also the gonad index. In the subsequent gametogenesis, gonad size increases again, and the gonad index is once again gradually increased (Walker *et al*., 2007). Over its entire life-span, a bivalve will continuously experience repeated gametogenesis. Hence, analysis of changes in the gonad index over time allows reproductive capability of the clams to be determined. This makes it easy to manipulate the clams for a breeding program. Several studies have acknowledged that in order for gametogenesis and spawning to occur in bivalves, environmental conditions must be appropriate (Hamli *et al*., 2019), coupled with a sufficient food supply (Rodríguez-Jaramillo *et al*., 2008; Dridi *et al*., 2014; Breton *et al*., 2018; Khafage *et al*., 2019).

Although the difference was not significant ($p>0.05$), we observed that the gonad index of *Polymesoda erosa* with shell lengths of 5.50–6.49 cm was higher than that of larger clams (6.50–7.49 cm and 7.50–8.49 cm). In contrast, the clams in the 8.50–9.45 cm category exhibited significantly lower gonad index. Gimin *et al*. (2005) also found that *P. erosa* in northern Australia achieved gonad maturation in individuals as small as 4.50 cm shell length. We can infer from this study that the mangrove clams in Marudu Bay reach sexual maturity by a size of 5.50 cm shell length.

*Condition index*

Condition index increases when the gonad of a bivalve attains maturation phase as more meat tissue is accumulated and decreases when the gonad is in resting or spent phases (Hamli *et al*., 2019). Hence, the condition index can be used to describe meat quantity (Hassan *et al*., 2017) and growth performance of bivalves (Filgueira *et al*., 2013). Moreover, the gonad represents 59 % of the total weight of soft tissues in bivalves, and thus contributes significantly to the total weight. Besides gonad maturation, differences in shell thickness, feeding, environmental conditions and diseases have also been reported to affect the condition index of bivalves (Duinker *et al*., 2008). The current study revealed that clams in the 7.45-8.49 cm shell length class have lower condition index than other sizes, which indicates that these clams are actively utilizing their energy for reproduction, growth and metabolic maintenance compared to the juvenile clams (5.50-6.49 cm) and adult clams (8.50-9.49 cm), which likely channel more of their energy towards growth and metabolic maintenance, respectively.

## CONCLUSION

The natural stock of the mangrove clam in Marudu Bay has a nearly 1:1 (0.97:1) male to female ratio with no evidence of hermaphroditism. The high gonad index of clams in the 5.5-8.49 cm shell length class implies that the majority of the clams at this size have already fully matured. The lower condition index of clams within the 7.45-8.49 cm shell length class also indicates that these large clams are likely to utilize more energy for growth, reproduction, and cell maintenance. Therefore, random selection of clams with shell lengths between 5.50 and 8.49 cm for use as broodstock in artificial breeding programs will likely result in a balanced sex ratio and high spawning success.

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