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Research Article
Determination of Gonad Development of Mangrove Clam *Polymesoda expansa* (Mousson 1849) by Histological Classification

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Abstract

Background and Objective: Mangrove clams are considered as food, collected from the nature. These species deserve special attention for its potentiality. This study was aimed to determine the generative activities of the mangrove clam *Polymesoda expansa* (Mousson, 1849) and relationship with water quality parameters. **Materials and Methods:** The reproductive biology of mangrove clam *Polymesoda expansa* (Mousson 1849) was investigated from October, 2010 to November, 2011 in Kelulit, Miri, Malaysia. Different gonad development stages were classified through histological method which was later on correlated with environmental variables through Pearson Correlation analysis. **Results:** It was observed that *P. expansa* is a dioecious clam. Five different gonad development stages in both males and females were identified. The Gonad Index (GI) indicated that gametogenesis began in November and by January onwards mature clams were found. Spawning season was identified all the year around mainly during September-December. The highest Gonad Index (GI) value was recorded in September, 2011 for females with 2.0 and it was 1.7 for males in March and November 2011. However, there was no significant difference ($p>0.05$) in Gonad Index (GI) between the males and females when it was confirmed by Kruskal-Wallis non parametric test. The monthly recorded physico-chemical parameters of estuarine water at sampling site were 2.79±3.15 psu, 5.46±1.34, 29.4±1.3°C, 2.73±1.09 mg L^{-1} and 283.5±150.5 mm for salinity, pH, temperature, Dissolved Oxygen (DO) and rainfall, respectively. **Conclusion:** The information gathered from this study indicated that *P. expansa* is dioecious animal and has continuous gametogenic development through a year. Therefore, *P. expansa* is able to spawn all the year around regardless on water parameters changes in the habitat.

Key words: Gonad development, histology, gonad index, polymesoda expansa, dioecious animal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The mangrove clam *Polymesoda expansa* (Mousson 1849) is a deep burrowing bivalve distributed widely across the Indo-Pacific mostly in the tidal flat of Southeast Asia[1-3]. Three species of the mangrove clams were reported from the Indo-Pacific region; *Polymesoda erosa*, *P. bengalensis* and *P. expansa*[4]. The distribution of *P. bengalensis* is restricted to the Bay of Bengal, whereas *P. erosa* and *P. expansa* are known to have a wider, somewhat overlapping distribution in the Indo-Pacific from India to Vanuatu; North to Vietnam and South to Eastern Java[1]. It has been speculated that *P. expansa* is less tolerant of colder waters of the Northern and Southern extremities of its distribution. This species is also reportedly found in man-made prawn ponds[3]. The mud clam *Polymesoda* spp. from the family Corbiculidae, lives semi-infaunally on the soft sediment that is accumulated around the roots of the mangrove trees and spends a considerable portion of its life exposed to air in mangrove swamps where salinity fluctuates greatly[4-6]. In Sarawak, Corbiculidae family was recorded from all divisions where *P. expansa* is one of the most common and frequently abundant species found after *P. erosa* in the mud flat habitat[7]. The coastal area of Kelulit (study area) is located at Miri division in the Northern side of Sarawak facing South China Sea at the West. This coastal area is characterized by mud flat and mangrove forests with abundant natural invertebrates and widespread local fisheries. Although the clam is an indigenous bivalve, is collected for food, yet has not being cultured on a commercial scale. The production of this species from natural habitat in this area is still unknown and unreported.

Generally, the reproductive cycle of marine invertebrates, mainly bivalves are mostly influenced by adjacent environmental parameters and their gonads could vary from place to place over year[8]. Inter-area differences in reproductive cycle and breeding patterns have been noticed in bivalve communities and the differences emerge to be linked with variations in food and temperature[9]. However, the variations of metal levels in sediments could also affect on reproduction of clams elsewhere[10].

A histological preparation of gonad on reproductive cycle studies of clams to date is the most reliable where detailed information can be obtained[11]. In view of the fact that the method is laborious, when it is carried out at regular periods throughout the year, consequently, it can give detailed information of the timing and duration of the gonad cycle[12]. In bivalves mollusc, the gonad may be well-differentiated and clearly visible as in Pectinidae, or undifferentiated and encircling the digestive gland[13]. However, gonad in Corbiculidae is difficult to distinguish especially in female during the off breeding season[14]. As they undergo for external fertilization, males will produce sperms, while the females lay their eggs into the water column during the spawning time.

The marine mollusc study in Malaysia is normally carried out as a side product of other projects. Researcher's record was mostly derived from what they seen and collected during other parallel field survey[15]. Consequently, the basic information on reproductive pattern, fecundity and growth of bivalves is relatively poor in Sarawak coast area and mud flats. Again the reported study on the gonad development of *P. expansa* related to environmental parameter in Malaysia is still scarce. Besides, the information on reproductive cycle and breeding biology of *P. expansa* is important to interpret the influence of environmental parameter in the field. Therefore, the objective of the study was to investigate the gonad development and yearly reproductive cycle of *P. expansa* at Kelulit, Sarawak, Malaysia.

MATERIALS AND METHODS

Study area and sampling procedure: The study area is situated in the mangrove site of Kelulit river (03°58.649' N, 113°44.244' E) Miri, Sarawak, Malaysia. Samples were collected monthly in random over a period of 14 months from October, 2010 to November, 2011. However, no samples were collected in February, April and August, 2011 due to over exploitation by the locals. Physico-chemical properties of adjacent estuarine water such as temperature, salinity, pH and dissolved oxygen were measured and recorded using WQC-24P Multi-parameter during sampling. Live clam were transferred to the laboratory for further processing.

Gonads collection and index values calculation: A small part of the gonad from the mesosoma and mantle lobe from each dissected clams were taken out and processed following histological techniques[16].

Monthly Gonad Index (GI) was computed utilizing a numerical grading system to obtain a quantitative value that represents the reproductive activity. From the results of the histological studies, the sexes of the specimens were further confirmed with the initial visual identification. Later, macroscopic gonad stages of each sample were assigned and gonad index were calculated[17-19]:

$$GI = \frac{\text{Number in each stage} \times \text{numerical ranking of that stage}}{\text{Number of animals in the samples}} \quad (1)$$

The stages of gonad developmental were classified as rest (indifferent), develop (immature), mature, spawn and spent [20].

Histology: Histology was observed following histological techniques[16].

Statistical analysis: Pearson correlation analysis was implied to find any correlation between GI and physico-chemical properties of estuarine water parameters including rainfall in Kelulit, Miri[17].

RESULTS

Environmental parameters: Study showed that small differences among monthly measurement for salinity and DO for water quality (Table 1). However, temperature assessment indicated month of September, 2011 as the highest and the lowest recorded in January. May, 2011 has as the highest pH value and November, 2010 as the lowest. The data on monthly rainfall from October, 2010 to November, 2011 was collected from the Department of Meteorology of Malaysia (Fig. 1). Rainfall found fluctuated throughout the year with highest in January, 2011 and lowest in October, 2011.

Gonad development stages: Five stages of gonad development were identified in *P. expansa* both for males and females are resting, developing, mature, spawning and spent (Plate 1, 2). The colors of gonads were grayish in females whereas, whitish in males and no hermaphrodite found in *P. expansa* at Kelulit. Different gonad development stages for both sexes are summarized as in Table 2.

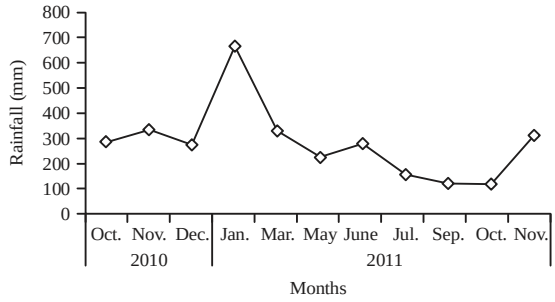

Fig. 1: Rainfall data collected taken from Kelulit, Miri, Malaysia, during the sampling period from October, 2010 to November, 2011 (Source: Malaysia Meteorology Department)

Table 1: Physico-chemical properties of estuarine water (Mean±SD) measurement from October, 2010 to November, 2011

	Parameters			
Months	Salinity (psu)	Temperature (°C)	pH	Dissolved oxygen (mg L^{-1})
October, 2010	2.0±0.26	31.3±0.70	4.98±0.25	5.00±0.12
November, 2010	0.3±0.10	29.3±0.17	3.45±0.24	2.74±0.12
December, 2010	0.8±0.17	30.4±0.56	3.52±0.37	1.88±0.04
January, 2011	0.6±0.17	27.7±0.26	6.12±0.10	3.28±0.02
March, 2011	0.1±0.06	28.2±0.17	6.83±0.25	2.62±0.03
May, 2011	8.6±0.26	29.5±0.26	6.87±0.41	2.62±0.03
June, 2011	2.8±0.52	28.8±0.10	6.60±0.14	3.37±0.03
July, 2011	3.9±0.10	27.9±0.17	6.34±1.01	1.98±0.03
September, 2011	8.9±0.20	31.6±0.30	6.11±0.10	0.67±0.02
October, 2011	1.4±0.26	29.4±0.20	3.72±0.05	2.49±0.01
November, 2011	1.3±0.46	29.8±0.10	5.57±0.09	3.40±0.02

Table 2: Description and criteria for the gonadal stages of *Polymesoda expansa*

	Histological characteristics	
Gonadal stages	Females	Males
Resting	Fully covered by connective tissue (ct) and less empty acini (ea) appeared (Plate 1a)	Gonad area is fully covered by the connective tissue (ct) and present of lumen (L) (Plate 2a)
Developing	Acinus visible and the diameter increased. When ripe, acinus size increases and oocytes are getting filled (Plate 1b-c)	Ascinus forming stage, intensive spermatocytes (ec) and spermatogonia (eg) stages. When ripe, ascinus size increases and connecting tissue disappeared (Plate 2b-c)
Mature	Filled with packed ocytes (po) and nucleus was able to seen (Plate 1d)	Ascinus reach to maximum size and visible of spermatozoa (ez) (Plate 2d)
Spawning	Emission of ovules (ov) and ascinus structure started to loose in present of lumen (L) (Plate 1e)	Structure deformities and ascinus walls are getting thinner (Plate 2e)
Spent	Connective tissue (ct) re-formed and some residual ovules (ov) present (Plate 1f)	Complete walls destruction thus making empty acini (em) and covered by connecting tissue (Plate 2f)

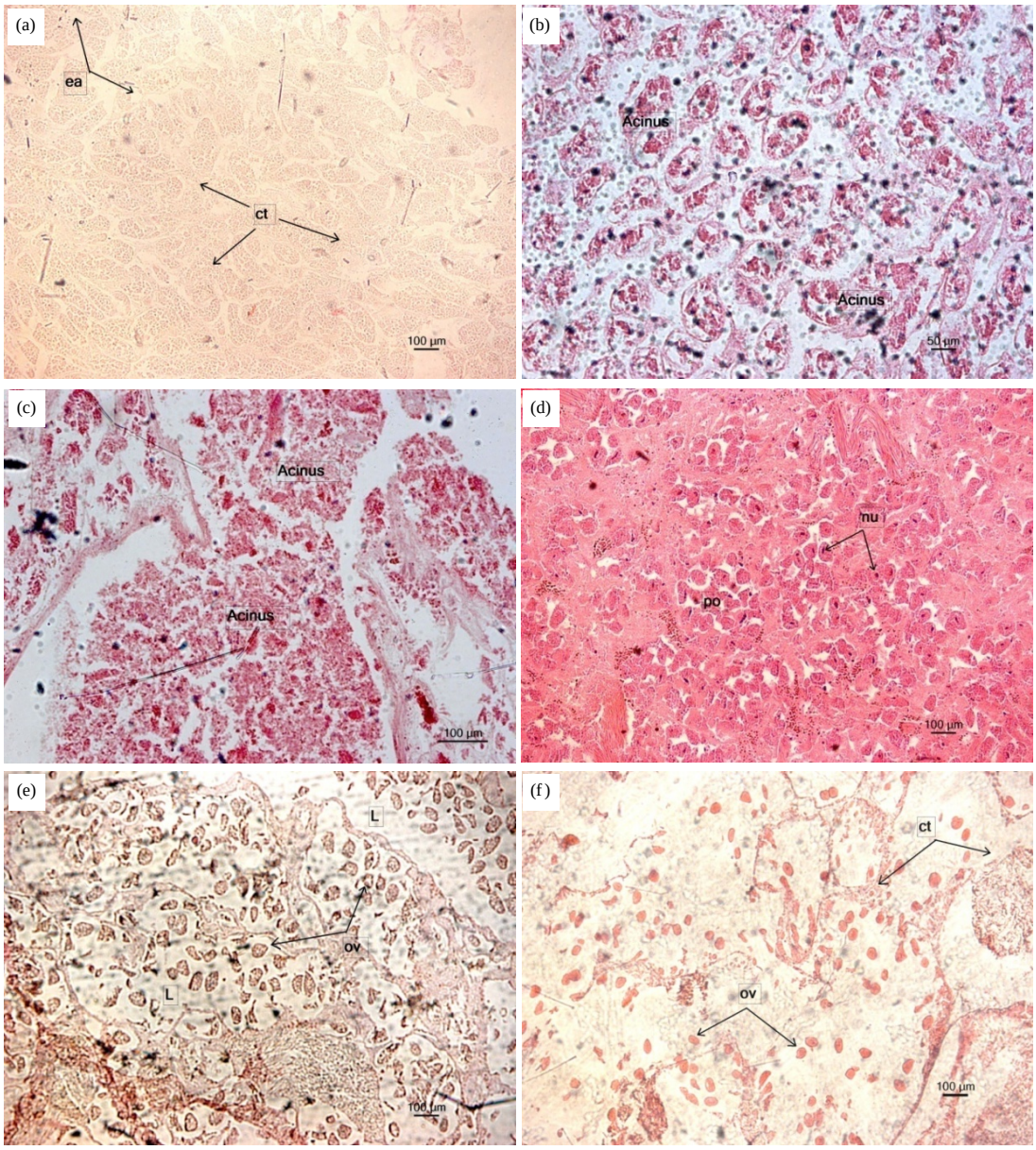

Plate 1(a-f): Gonad development stages in females of *P. expansa* (a) Resting, (b-c) Developing, (d) Mature, (e) Spawning, (f) Spent

Reproductive cycle: Since no hermaphrodites found, the reproductive cycle on *P. expansa* were easier to determine. Active gametogenesis was recorded throughout the study period with all stages of development usually occurring simultaneously within the same sample population. Relative frequencies of the maturity stages for *P. expansa* at Kelulit are illustrated in Fig. 2.

Though gametogenesis started early of January and the clams developed gametes, some of the gonads were seen still in resting stage (Fig. 2). Active maturation was observed in the month of January with gametogenesis where gametes matured slowly and from March-October, mature gametes were found less than 25% until it became higher in November (Fig. 2). From March to November, the gonads were maturing and the spawning started. Gonads were observed to be spawned in September and continuously throughout the year it became less in percentage.

Spermatogenesis and oogenesis appeared to continue to some extent through the breeding season in parallel to spawning. From December until June, a small percentage of the gonads were spent, which was the follicle progressively

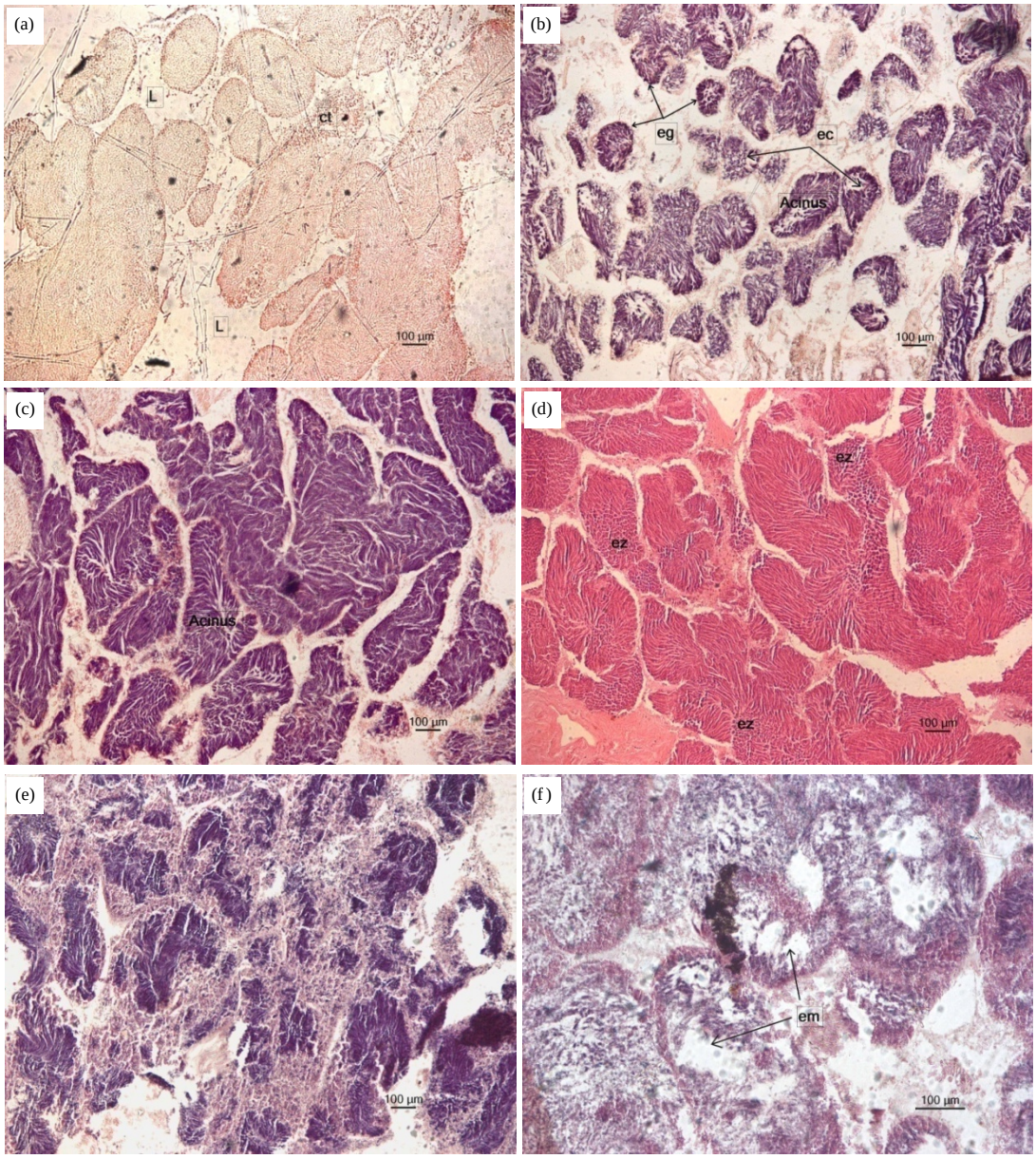

Plate 2(a-f): Gonad development stages in males of *P. expansa* (a) Resting, (b-c) Developing, (d) Mature, (e) Spawning, (f) Spent

occurring with fewer and fewer mature gametes. Spawning occurred all year around. However, in January, when water temperature were at their lowest (Fig. 2), no spawning was recorded. *Polymesoda expansa* population at Kelulit, Sarawak was thus characterized by all year around spawning, although only in September spawning occurred showing maximum frequency.

Monthly gonad index: Gonad index results represented that spawning in *P. expansa* was year around except in December and January. Spawning between sexes was fairly synchronized in November and May (Fig. 3). Between January and July, spawning activity was occurred mainly in males. Kruskal-Wallis non-parametric test showed no significant differences at $p>0.05$ in Gonad Index (GI) between the males and females.

The GI showed a seasonal trend along the year, with high values related with mature individuals while the fall of values were due to spawning activity. The GI values for pooled of *P. expansa* were higher in November and March and were lower from December to January (Fig. 4). The GI values indicated that the reproduction was inactive from December to January and gametogenesis started in March and continued until October.

Table 3: Pearson Correlation analysis of Gonad Index (GI) of *P. expansa* with different physico-chemical properties of estuarine water parameters in Kelulit, Miri

Parameters	Temperature (°C)	pH	Salinity (psu)	DO (mg L^{-1})	Rainfall (mm)
r	0.181	-0.114	-0.110	0.132	-0.126
Significance (2-tailed)	0.594	0.739	0.747	0.699	0.712
N	11.00	11.00	11.00	11.00	11.00

*Correlation is significant at the 0.05 level (2-tailed), r: Pearson correlation coefficient, N: number of sample, DO: Dissolved oxygen

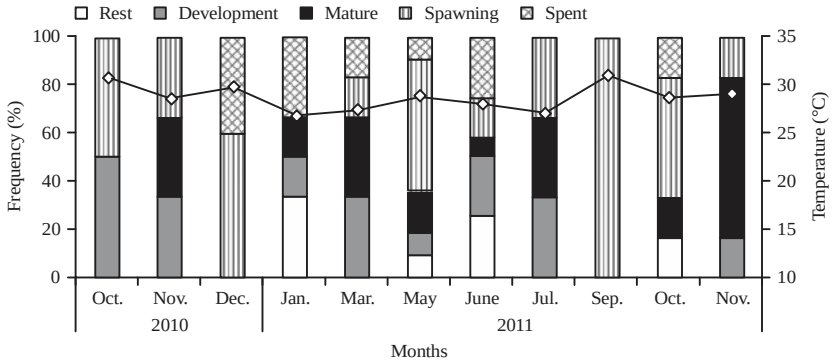

Fig. 2: Reproductive stages of *P. expansa* in Kelulit, Miri (n = 67)

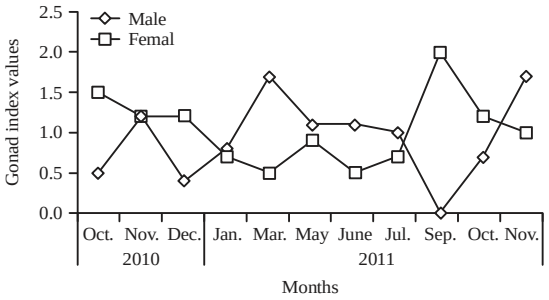

Fig. 3: Gonad Index (GI) values for both male and female of *P. expansa*

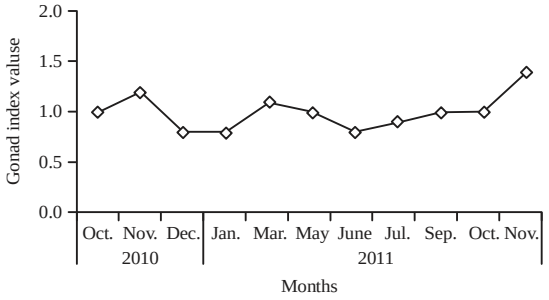

Fig. 4: Gonad Index (GI) values for pooled of *P. expansa*

A Pearson correlation analysis test (Table 3) was applied between GI and the different physico-chemical properties of estuarine water parameters from Kelulit, Miri, however, no significant correlation ($p>0.05$) was found. The GI presented a positive correlation only in temperature and dissolved oxygen while other physico-chemical properties of estuarine water parameters showed negative correlation.

DISCUSSION

Environmental parameters: Water temperature showed considerable seasonal variation with extreme values of 27.7°C in January, 2011 and 31.6°C in September, 2011 when monthly temperature was recorded with the mean of 29.4±1.3°C while salinity was 2.79±3.15 psu. Temperature is the most important parameters, which influenced on bivalve reproduction regulation[21]. Besides that, changes in water salinity affect a wide variety of biochemical, physiologic processes and growth in marine bivalves[22,23].

Different temperature and sampling dates indicate synchronous gonad maturation in male and female gonad of bivalve[24]. This is due to the faster gonad maturation rate that was observed at 25-30°C rather than at 20°C which is possibly because of the higher metabolic rates found at higher temperature in this species. When the variation in temperature and the gonadosomatic index examined, water temperature at 24°C seems to be the lower threshold for gonad development[25]. However, present study on reproductive cycle was found neither temperature nor salinity showed a clear relation with their reproductive cycle since spawning appear during the annual cycle.

Gonad developmental stages: The sexes were determined for each individual by the presence of egg and sperm in the tissue section under a microscope. Many schemes for observation of histological samples for classifying gonad developmental stages in mollusc can be found[26]. From the microscopic observations of gonadal sections it was determined that *P. expansa* is a dioecious (separate sex) species.

Five stages of gonad maturation of *P. expansa* were clearly described. Resting stage represented minimum gonadal proliferation where gonad and interfollicular areas were fully covered by connective tissue[27]. No oocytes, spermatocytes or later stages recognizable and sex undetermined[28]. Gametogenesis started in developing stage and was characterized by a rapid thickening of the gonad and branches of the developing follicles[29]. Follicle size increased, the first spermatocytes were observed in males and the first previtellogenic oocytes appeared on the follicular wall in female[30].

Mature stage was characterized by over 90% of the volume of the gonad being occupied by mature follicle with well-developed gametes for most individuals[31]. The inter follicles connecting tissue disappeared and follicles reached to maximum size[20]. In male, acinus seemed full of spermatozoa, with their tails towards the acinus lumen while in female, this stage was at the end of vitellogenis process. Spawning in male, the lamellar arrangement of spermatozoa was disappearing and follicles were less densely packed[32]. Empty spaces were observed in the follicular lumen and a low density of free ripe oocytes with a broken germinal vesicle was observed in female[31]. Finally, complete destruction of the follicle walls and gonad volume fast covered by the connective tissue in male spent stage[20]. Whereas, follicules were getting smaller, connective tissue re-formed and oocytes in some follicules absorbed.

Reproductive cycle: The reproductive strategy found in *P. expansa* was clearly observed as dioecious. The existence of dioecious within the family Corbiculidae was observed for *P. erosa*[33]. The two sexes can be separated from very small sizes on 14 mm in *P. solida*[34]. Annual gametogenic cycle was exhibited throughout the study period, which began in October to November with rapid proliferation of gametes especially in November to July.

Most of the marine bivalves in Malaysian coastal waters showed a reproductive pattern that is active all year around. Therefore, the extensive culture system is only practiced for bivalve molluscs on coastal mudflats[35]. Continuous spawning activity all year around was reported on *Perna viridis* from Penang[13]. Similar observations were made for *P. viridis* at Quezon, where the mussels undergo gametogenesis throughout the year with no repetitive during two years of study[36].

Monthly gonad index: Numerical grading system from the sectioning of the gonads tissue was used to calculate the Gonad Index (GI)[19]. The objective of the analysis was to find the spawning period or seasonal variations of *P. expansa*. Continued gonad developmental and spawning throughout the year in *P. expansa* in the region was an expected trend. This was an acceptable trend since they occur in a tropical condition where they are subjected to stable and warm temperature all year around. However, the Pearson correlation analysis indicated that an environmental factor was not influencing the gonad growth.

Determination of first maturity length and spawning period are the basic requirements for protection and conservation of the species. Several methods might be used to study the gonad development and reproductive cycle; however, the most precise results were obtained through histological technique. This was because, other approaches such as metabolomics and genetic methods were not being able to determine an absolute gender in some gonad developmental stages[37].

CONCLUSION

Polyemesoda expansa from Kelulit, Miri was observed dioecious and no hermaphrodite individual was found. Through histological classification, five stages of gonad development had been carried out and clearly identified for both male and female which were resting, developing, mature, spawning and spent. Reproductive cycle of *P. expansa* showed gametogenesis that continuously spawned all the year round since they occur in tropical conditions where most of the physical and chemical parameters are fairly stable throughout the year. The GI was found not to be influenced by any of the physico-chemical parameters recorded in Kelulit, Miri, Malaysia. It was proven by the pearson correlation analysis that showed no significant correlation ($p>0.05$) between GI and physico-chemical parameters of estuarine water in the study area.

SIGNIFICANCE STATEMENTS

This study discover, characteristic of the gametogenic development in the *P. expansa* through one year observation. *Polymesoda expansa* is categorized as dioecious and able to spawn every month. This study will help the researcher to uncover the critical area especially in the artificial breeding and larvae rearing for *P. expansa* under laboratory condition that many researchers were not able to explore. Thus, new technique in artificial breeding and rearing may be discovered.

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