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Reproductive Cycle of Hard Clam, *Meretrix lyrata* Sowerby, 1851 (Bivalvia: Veneridae) from Sarawak, Malaysia

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Abstrak: Kajian ke atas kitaran pembiakan kerang keras *Meretrix lyrata* adalah berdasarkan kaedah histologi dan Indeks Gonad (GI). Sampel telah dikumpulkan dari muara Sungai Buntal di Kuching, Sarawak, Malaysia Gonad *M. lyrata* telah bermula berkembang semasa September 2013. Gametogenesis terus berkembang sehingga semua individu mencapai peringkat kematangan dan bertelur pada Februari hingga April 2014. Corak GI untuk kitaran setahun telah menunjukkan hubungan yang ketara dengan klorofil *a*. GI yang sepadan dengan klorofil *a* telah mencadangkan bahawa perkembangan kitaran pembiakan *M. lyrata* memerlukan jumlah makanan yang tinggi untuk meningkatkan gametogenesis.

Kata kunci: Klorofil *a*, Gametogenesis, Indeks Gonad, Histologi, *Meretrix lyrata*

Abstract: A study of the reproductive cycle of the hard clam, *Meretrix lyrata,* was documented based on histological observation and Gonad Index (GI). Samples were taken from estuarine waters of the Buntal River in Sarawak, Malaysia. The gonad of *M. lyrata* started to develop in September 2013. Gametogenesis continued to develop until the maturation and spawning stage from February to April 2014. The GI pattern for a one-year cycle showed a significant correlation with chlorophyll *a*. The corresponding GI with chlorophyll *a* suggested that the development of the reproductive cycle of *M. lyrata* required a high amount of food to increase gametogenesis.

Keywords: Chlorophyll *a,* Gametogenesis, Gonad Index, Histology, *Meretrix lyrata*

INTRODUCTION

The hard clam, *Meretrix lyrata* (Sowerby 1851), belongs to the Veneridae family. This species is found at several locations in Sarawak, Malaysia, such as Buntal Village (Kuching District) and Kabong (Betong District). *M. lyrata* inhabits seashore and estuarine areas. According to Hamli *et al*. (2012), bivalves, including *M. lyrata*, are commonly consumed by the local people as a protein source. Moreover, it has economic value and is sold at market with a price range of RM 2 to RM 20 kg^{-1}.

It is difficult to discern male and female individuals of *M. lyrata* based solely on morphology characteristics. Furthermore, the verification of the spawning activity of bivalves in nature is tremendously difficult (Camacho-

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Mondragon *et al.* 2012). Therefore, an accurate method to determine sex and gametogenic development is through the histology procedure. This procedure requires the gonad tissue to be stained before the determination of the gametogenic stage. Gamete developments occur in phases until maturity is reached and before individuals of *M. lyrata* undergo the spawning stage. This can be monitored through, or elucidated by, the reproductive cycle pattern of the bivalve. The understanding of hard clam gametogenic development, especially from the wild population, can be used as baseline information for management strategies in fisheries (e.g., spawning stock protection, determination of larval settlement period) (Gribben 2005; Camacho-Mondragon *et al.* 2012).

The Gonad Index (GI) is the most appropriate method to evaluate the gamete development of bivalves based on histological observation. The GI is the number of individuals in every gametogenesis phase multiplied by the numerical ranking of every phase and then divided by the sample size (Gosling 2003). The gametogenic stage of other bivalves, such as venerid clams (*Meretrix lusoria* and *Meretrix petechhialis*) (Nakamura *et al*. 2010; Jun *et al*. 2012), Snout Otter Clam (*Lutraria philippinarum*) (Bantoto & Ilano 2012), baby clam (*Marcia opima*) (Suja & Muthiah 2007) and mud clam (*Polymesoda erosa*) (Clemente & Ingole 2009) were determined using quantitative and qualitative methods.

Habitat condition is one of the important parts that regulate and influence the biological function in molluscs (Wahi *et al.* 2009; Hookham *et al.* 2014). Moreover, previous works attempted to relate environmental factors, such as salinity, temperature and food, as indicators that influenced the gametogenic development of clams (Saxby 2002; Chu & Kumar 2008).The main aims of this study are to (i) identify the stages of gametogenic development in *M. lyrata* and (ii) determine whether environmental factors, such as temperature, salinity, dissolved oxygen and phytoplankton, could influence sexual development in this bivalve.

MATERIALS AND METHODS

Sampling and Study Area

Approximately 30 mature *M. lyrata* individuals, with shell lengths between 36.0 mm and 76.0 mm, were collected at the Buntal estuary, Kuching, Sarawak, Malaysia site (N 01° 42’ 18.6”; E 110° 22’ 03. 6”) (Fig. 1) every month from May 2013 to April 2014. The environmental parameters were also recorded *in situ* and *ex situ* simultaneously with the collection of *M. lyrata*. Samples of *M. lyrata* were brought to the Aquatic Ecology Laboratory (Universiti Putra Malaysia, Bintulu, Sarawak) and cleaned to remove fouling organisms and sediment.

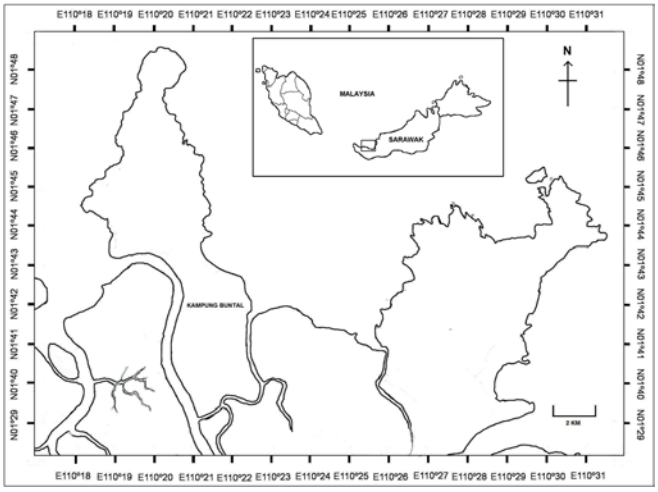

Figure 1: Study site for monthly *M. lyrata* collection from Buntal estuary, Sarawak.

Histological Analysis

Gonad tissue from the samples was removed and fixed in Bouin's solution (Sigma-Aldrich Co., Saint Louis, USA). The tissue was dehydrated using several series of ethanol and xylene before being infiltrated with paraffin wax (Humason 1972). Tissue samples were then sliced into 7–10 µm sections and stained with haematoxylin and eosin. The tissue samples were observed under a microscope (Leica Microsystem Inc., Buffalo Grove, USA) at 400× magnification, with each gametogenesis stage recorded according to the following gametogenic stages: rest, early development, late development, mature, spawning and spent, based on Wilson and Seed (1974).

Gonad Index

The gonad developmental stage of *M. lyrata* was categorised according to Ceballos-Vazquez *et al*. (2000), and each value for every stage was calculated for the GI as follows:

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

The gonad developmental stage was numerically ranked as follows: 1 = rest and spent stages, 2 = development and 3 = mature and spatial spawn.

RESULTS

Gonad Development

The reproductive stages of *M. lyrata* were identified by the presence of spermatogonia, spermatocyte, and spermatid for males and, oogonia, oocytes

and ova in females. There were several stages for male and female reproductive development in the present study, such as rest, development, mature, spawning and spent stages (Figs. 2 and 3).

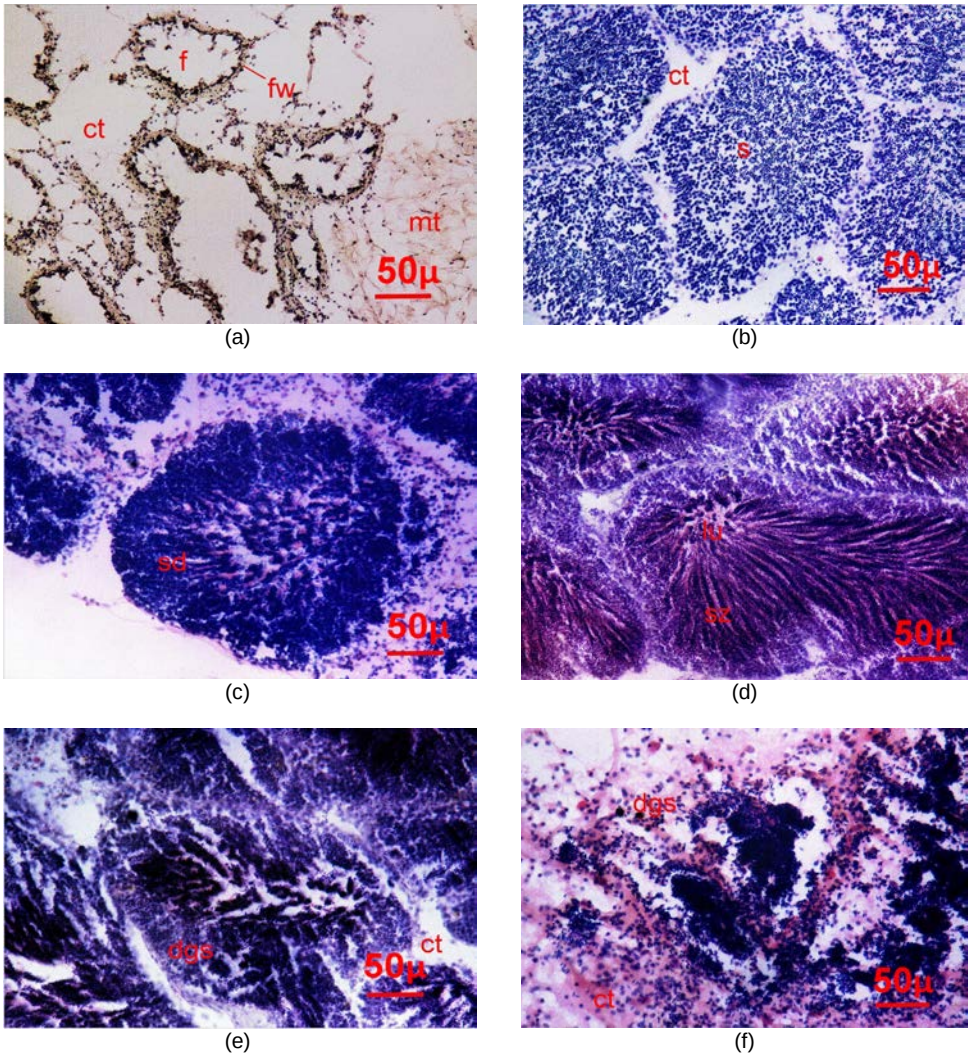

Figure 2: Male gonad stages for *M. lyrata* from Buntal estuary, Sarawak: (a) rest; (b) early development; (c) late development; (d) matured; (e) spawning; (f) spent.

Notes: Scale: 50 µm, ct = connective tissue; dgs = degenerate spermatozoa/spermatid/ spermatocyte; f = follicle; fw = follicle wall; lu = lumen; mt = muscular tissue; sz = spermatozoa; sd = spermatid; s = spermatocyte.

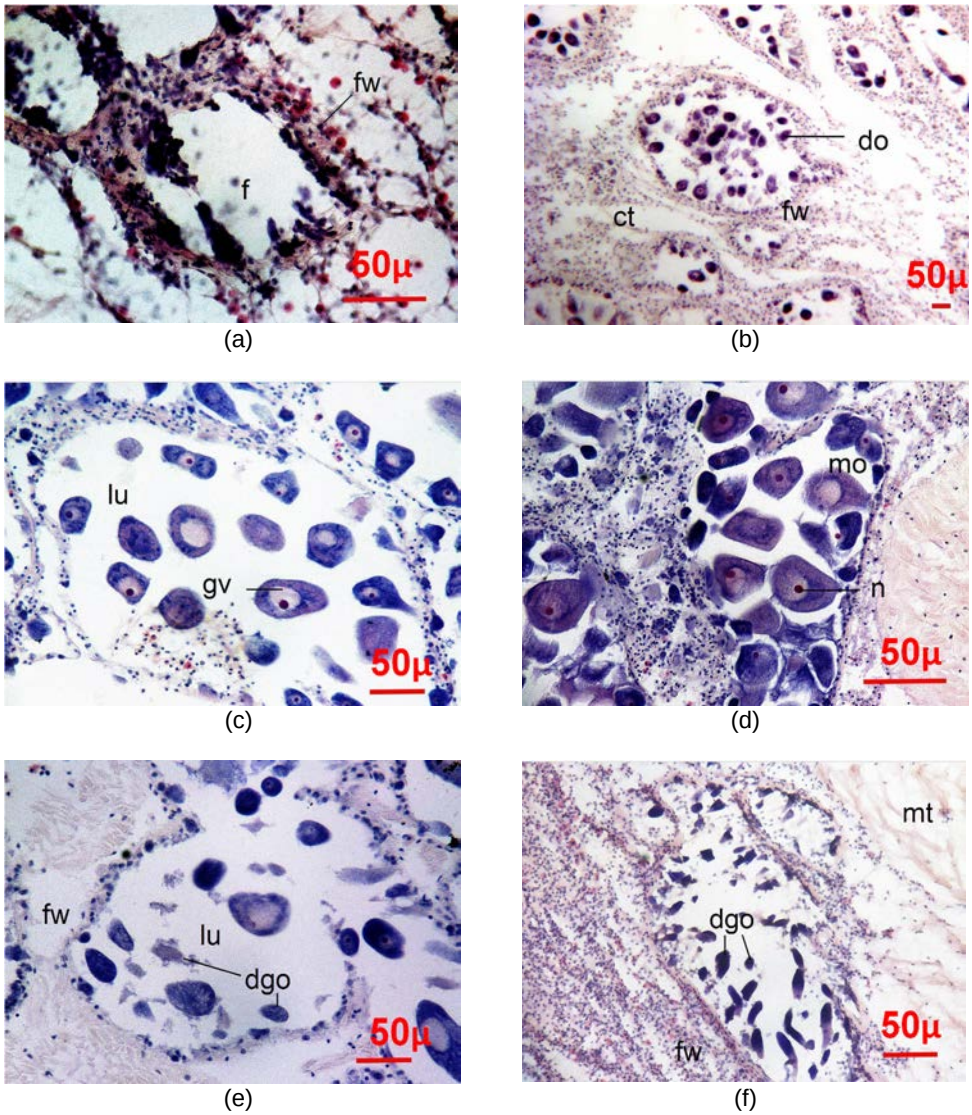

Figure 3: Female gonad stages for *M. lyrata* from Buntal estuary, Sarawak: (a) rest; (b) early development; (c) late development; (d) matured; (e) spawning; (f) spent.

Notes: Scale: 50 µm, dgs = degenerate oocyte; do = developing oocyte; gv =germinal vesicle; mo = matured oocyte; m = muscular tissue; n = nucleus; ct = connective tissue; lu = lumen; f = follicle; fw = follicle wall.

Rest Stage
It is difficult to differentiate between the male and female gonads; however, the degraded gamete was still visible in a few of the tissue samples. Most of the follicles were empty, and some follicles were degraded (Figs. 2[a] and 3[a]). The connective tissue was filled with lipid and glycogen.

Developmental Stage
The follicles in *M. lyrata* were filled with spermatogonia and spermatocytes in males and with oogonia and oocytes in females. A follicle filled with spermatogonia help make the sexual identification easier. During this stage, the connective tissue started to decrease in size. There was no presence of matured gametes during the early developmental stage (Figs. 2[b] and 3[b]). At the late developmental stage, male (spermatocytes) and female (oocytes) gametes were abundant, and several mature spermatids (male) and ova (female) were identified. The follicles became more compact with spermatocytes, spermatids and ova (Figs. 2[c] and 3[c]). The increase in the follicle size occurred with a decline in the size of the connective tissue.

Mature Stage
The gonad was fully filled and compact with matured spermatid (male) and ovum (female). Flagella (spermatozoa) were oriented towards the lumen of the follicle in male gonads (Fig. 2[d]). In the female gonad, the nucleus and germinal vesicle in the ova were visible (Fig. 3[d]). The increased follicle size possibly affected the connective tissue, which decreased in size.

Spawning Stage
Gametes were secreted from the follicles and entered the active phase. The number of spermatozoa and ova in the lumen was reduced, which exposed the empty area (Figs. 2[e] and 3[e]). The follicles started to decrease in size, and the loose connective tissue started to increase in size. Degradation and cytolysis occurred in a few of the unspent gametes.

Spent Stage
The majority of the gametes were used during spawning, while the unused gametes rapidly degraded. The size of the degraded follicles diminished, but some of the follicles maintained their shape. Connective tissues started to be filled with lipid and glycogen. The gonad was ready for the resting stage (Figs. 2[f] and 3[f]).

Sex Ratio
During the study period, a total of 157 (56.07%) males and 123 (43.93%) females of *M. lyrata* were identified through the histological procedure (Table 1). The ratio of male:female was calculated based on the abundance of *M. lyrata* collected from May 2013 to April 2014. The chi square result revealed a male:female ratio of 1:0.783, which was significantly different ($p<0.05$). Male *M. lyrata* was dominant during the pooled months in the present study. Males of *M. lyrata* dominated the sampled population during May (60.87%), June (71.43%), July

(80%), September (63.64%), November (52%) and December (75%) 2013, as well as February (58.07%) and April (73%) 2014. However, females were dominant during August (57.14%) and October (66.65%) 2013 and during January (53.33%) and March (60%) 2014. The highest male dominance was in July 2013, with 80% male and 18.75% female.

Table 1: Monthly distribution of males and females of *M. lyrata,* Sarawak, Malaysia.

Year	Month	Male	Female	Total	Percentage of male (%)	Percentage of female (%)	Sex-ratio Male:female
2013	May	14	9	23	60.87	39.13	1:0.643
	Jun	15	6	21	71.43	28.57	1:0.400
	Jul	12	3	15	80.00	18.75	1:0.250
	Aug	9	12	21	42.86	57.14	1:1.333
	Sep	7	4	11	63.64	36.36	1:0.571
	Oct	9	18	27	33.33	66.65	1:1.999
	Nov	13	12	25	52.00	48.00	1:0.923
	Dec	12	4	16	75.00	25.00	1:0.333
2014	Jan	14	16	30	46.67	53.33	1:1.143
	Feb	18	13	31	58.07	41.94	1:0.722
	Mar	12	18	30	40.00	60.00	1:1.500
	Apr	22	8	30	73.33	26.67	1:0.364
Grand total		157	123	280	56.07	43.93	1:0.783

Monthly Variation in *M. lyrata* Gametogenesis

The gonad development completed the stages of *M. lyrata* gametogenesis, rest, development, mature, spawning and spent, in October, November, and December 2013 and January 2014, respectively (Fig. 4). In comparison, during June 2013, the *M. lyrata* population was characterised by the lowest number of gametes associated with the matured and spawning stages. The spawning stage for the *M. lyrata* population occurred in every month, except for September 2013.

Monthly GI

The monthly GI of *M. lyrata* showed that the total GI was the highest in June 2013, with a GI value of 3, and the lowest GI value was in September 2013 (GI = 1) (Table 2; Fig. 5). The GI between male and female showed a simultaneous maximum value (GI = 3) in June 2013, which indicated the maturity phase. Females frequently reached the maturity phase in four months, and males reached the maturity phase in one month. The GI pattern for both sexes gradually decreased from July to August 2013, when spawning occurred. Both sexes reached the minimum GI = 1 in September 2013 as reproductive sex in the resting phase. The increased GI pattern observed between October 2013 and January 2014 for females and in April 2014 for males occurred during gametogenesis.

Correlation of *M. lyrata* GI with Environmental Factors

Among the 15 environmental variables, there was a positive correlation (p<0.05) between the *M. lyrata* monthly GI and the chlorophyll *a* concentration (Table 3). Other environmental variables, such as the hydrogen ion concentration (pH), total dissolved solids, salinity, water and air temperature, turbidity, conductivity, dissolved oxygen, total rain fall, ammonia, nitrite, nitrate, phosphate and total suspended solids (TSS) were not correlated with the monthly GI (p>0.05).

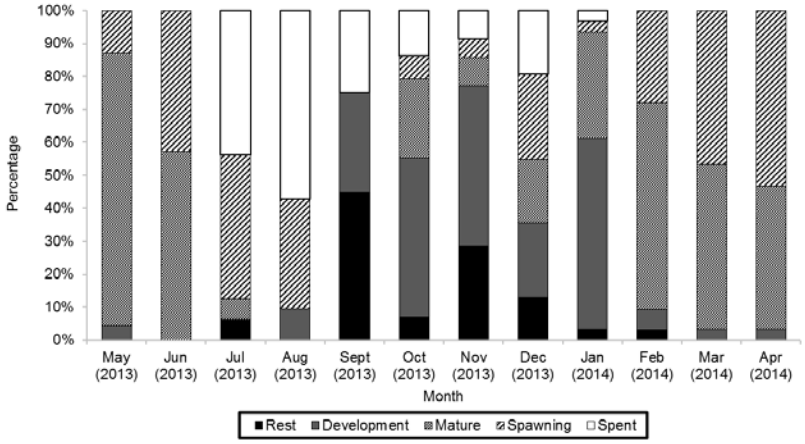

Figure 4: Reproductive stages of *M. lyrata* from Buntal estuary, Sarawak.

Table 2: Monthly male and female GI values for *M. lyrata* from Buntal estuary, Sarawak.

Year	Month	Male					GI
		Rest	Development	Mature	Spawning	Spent	
2013	May	0	0	14	0	0	3.00
	June	0	0	12	3	0	3.00
	July	1	0	1	6	5	2.08
	Aug	0	0	0	2	7	1.44
	Sept	5	1	0	0	1	1.14
	Oct	3	3	3	0	0	2.00
	Nov	3	6	2	1	1	1.92
	Dec	0	0	3	8	4	2.47
2014	Jan	0	6	8	0	0	2.79
	Feb	0	2	15	1	0	2.89
	Mar	0	1	6	5	0	2.91
	Apr	0	1	11	10	0	2.96

(continued on next page)

Table 2: *(continued)*

Year	Month	Female					GI	Total GI
		Rest	Development	Mature	Spawning	Spent		
2013	May	0	1	5	3	0	2.89	2.96
	June	0	0	0	6	0	3.00	3.00
	July	0	0	0	1	2	1.67	2.00
	Aug	1	1	0	5	5	1.92	1.71
	Sept	0	0	0	0	4	1.00	1.00
	Oct	6	2	4	2	4	1.78	1.85
	Nov	7	1	1	1	2	1.42	1.68
	Dec	0	7	3	0	2	2.08	2.30
2014	Jan	0	12	2	1	1	2.13	2.33
	Feb	0	0	5	8	0	3.00	2.45
	Mar	0	0	9	9	0	3.00	2.97
	Apr	0	0	2	6	0	3.00	2.47

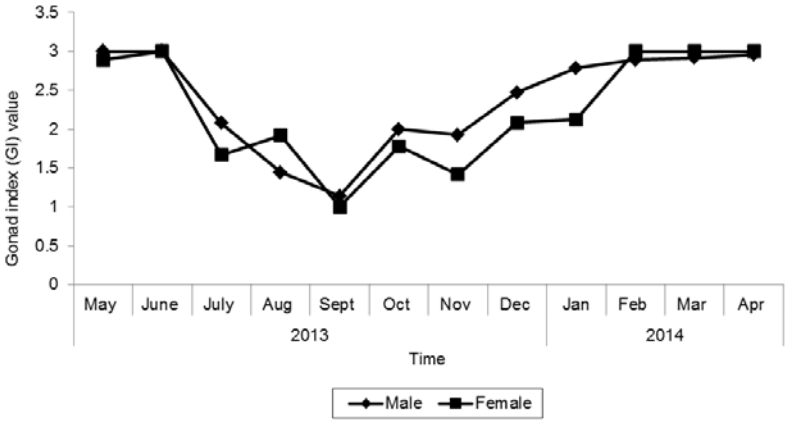

Figure 5: Male and female GI values for *M. lyrata* from Buntal estuary, Sarawak.

The positive correlation between GI and chlorophyll *a* is shown in Figure 6. The GI value increased with the increased concentration of chlorophyll *a.* This pattern of corresponding increases in both GI and chlorophyll *a* was observed from November 2013 to March 2014. Another clear pattern was identified during the months of May to September 2013 and April 2014, which consisted of a decrease in the GI and a decrease in chlorophyll *a*.

Table 3: Pearson correlation analysis of the GI values of *M. lyrata* with diferent environment parameters at the Buntal estuary, Sarawak, Malaysia.

Parameters	R	Sig. (2-tailed)
pH	0.321214	0.308648
TDS (mg/L)	–0.16303	0.612671
Salinity (PSU)	–0.2174	0.497309
Water Temperature (°C)	–0.42124	0.172638
Air temperature (°C)	0.213435	0.505374
Turbidity (NTU)	0.289421	0.361539
Conductivity (µS/cm)	–0.22748	0.477047
Dissolved oxygen (mg/l)	0.300205	0.343093
Total rainfall (mm)	0.040292	0.901058
Ammonia nitrogen (mg/l)	–0.11724	0.716697
Nitrite (mg/l)	0.400778	0.196666
Nitrate (mg/l)	–0.25463	0.424485
Phosphate (mg/l)	–0.10092	0.754996
TSS (g/l)	–0.2734	0.389883
Chlorophyll *a* (mg/m^3)	0.649531*	0.0222585*

Notes: *Correlation is significant at the 0.05 level (2-tailed)
TDS - total dissolved solid

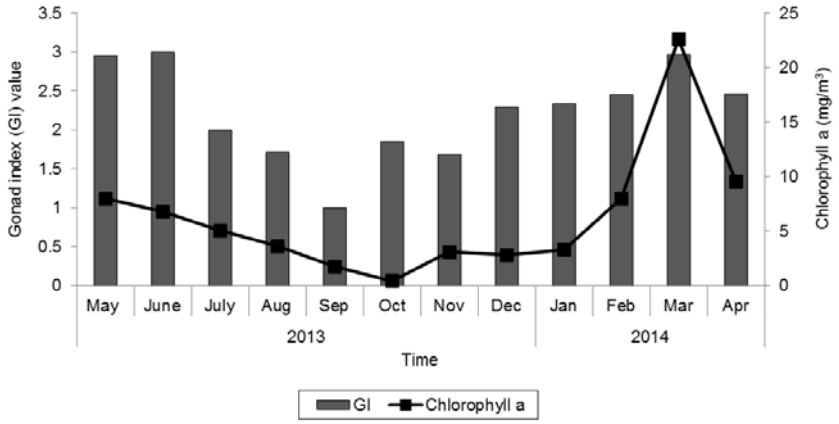

Figure 6: Monthly correlation graph between *M. lyrata* GI and chlorophyll *a* from Buntal estuary, Sarawak.

DISCUSSION

The present study on the reproductive biology of *M. lyrata* showed that this clam was diecious and dominated by male individuals. According to Haley (1979), the sex of bivalves was determined based on a minimum number of three genes with male and female alleles. The study suggested that bivalves with high frequencies of males consisted of a genotype distribution towards maleness. Previous work by Chu and Kumar (2008) reported that *M. lyrata* from Vietnam had another sex mode, which was a hermaphrodite. However, marine bivalves had the dominant diecious sex mode (Barnes 1980). Invertebrates with the diecious sexual mode had high levels of genetic variation (Zierold *et al.* 2009). Hence, they had a high potential to respond to environmental changes and a higher rate of survival (Nguyen *et al.* 2006). Moreover, environmental factors were expected to play a significant role in the sexual determination of bivalves (Morton 1991).

Based on the histological observation, maturity was reached at an early stage, which facilitated spawning during May – June 2013 and February – April 2014. Therefore, *M. lyrata* in this study spawned during the second month (February) to middle (June) of the year, which indicated that the optimal conditions were present for gametes to develop and for *M. lyrata* to successfully propagate. According to the previous research, gametogenesis in bivalves corresponded to environmental variables, such as temperature, salinity, food, nutrients and geographic location (e.g., Saxby 2002; Philippart *et al.* 2003; Dridi *et al*. 2007; Suja & Muthiah 2007; Chu & Kumar 2008; Enriquez-Diaz *et al*. 2009; Serdar & Lok 2009).

The present study recorded high GI values for males and females in June 2013 and low GI values in September 2013. The time periods indicated as the mature and spawning seasons were similar to those reported by Chu and Kumar (2008) for similar species. However, females reached a maximum GI value frequently when compared to males in the study period. This might be due

to chlorophyll *a* concentration in the environment, which was suggested by Perez Camacho *et al.* (2003). The biochemical composition between different sexual modes in clams was related to the sexual maturation process (Perez Camacho *et al.* 2003). GI values in the present study were consistent with the chlorophyll *a* pattern. According to Serdar *et al. (*2010), the carpet clam reached maturity each month, except in November, December and January, which corresponded to low concentrations of chlorophyll *a*.

The significant relationship between chlorophyll *a* and the GI of *M. lyrata* was not previously recorded in the waters of Sarawak (Son & Tung 2011). Environmental variables may have significant influences on bivalves. However, this study showed that, in tropical marine ecosystems, chlorophyll *a* was the most important variable influencing the reproductive pattern of diecious bivalve populations (Adjei-Boateng & Wilson 2013). Therefore, chlorophyll *a* was a significant form of nourishment needed by the bivalves to achieve reproductive success, as evidenced by the success of gametogenesis in the maturation and spawning stages in the presence of abundant particulate matter and chlorophyll *a* in the water (Perez Camacho *et al.* 2003; Serdar *et al.* 2010).

CONCLUSION

The reproductive biology of *M. lyrata* from Sarawak in the present study was dominated by males. The majority of *M. lyrata* matured during the early months of the year, and spawning occurred during July to September. The gamete cycle started to redevelop on October and into the early part of the subsequent year. This knowledge regarding the *M. lyrata* reproductive cycle and the influencing factor can help researchers and aquaculturists to select a suitable period to collect broodstock for artificial breeding and culture. Furthermore, culture development may help increase the economic value, source of protein and conservation of this species that was previously exploited in the wild by local people.

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