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Lunar periodicity in reproduction of two venerid clams *Meretrix meretrix* and *Katelysia hiantina* in Panguil Bay, Northern Mindanao, Philippines

Robert Keith A. Sienes, Rito S. Juranes Jr., Ephrime B. Metillo*

Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines

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Abstract

The venerid clams *Meretrix meretrix* and *Katelysia hiantina* are important component of shallow bentho-pelagic estuarine ecosystems, fisheries and cuisine in the Indo-West Pacific region. The uncontrolled and regular harvesting of these species may lead to population decline. Studies on aspects of growth and reproductive biology are indispensable input to the rational management of the clams. In this study, we examined lunar periodicity in growth through gross morphology measurements and reproductive potential of the two coexisting venerid species *M. meretrix* and *K. hiantina* from inner Panguil Bay, Philippines. For each clam species, thirty live samples per lunar phase sampling were collected during low tide from gleaners *in situ* from March to May 2017. Clams were individually measured for body length, width, inflation, and total weight, and dissected to obtain gonadosomatic index and fecundity that was estimated through volumetric method. Results showed no significant difference in growth for both clam species during each lunar phase. In contrast, significant differences were found for both fecundity and GSI for the two species to lunar phases. Furthermore, both species showed unimodal and bimodal peak for fecundity and GSI, with maximum values of *M. meretrix* peaking during new moon and full moon while those of *K. hiantina* only during full moon. We attribute these differences in GSI and fecundity peaks to reproductive niche differences of the two coexisting species in the brackish area of inner Panguil Bay. Hence, this study gives insight into the reproductive biology and potential for fisheries production of the studied venerid clams.

* **Corresponding Author:** Ephrime B. Metillo ✉ ephrime.metillo@g.msuiit.edu.ph

Introduction

Bivalve molluscs are common and economically important invertebrates in aquatic ecosystems. They largely contribute to aquaculture production and the coastal economy particularly in shallow coastal areas of Asia (Hasan, 2017). Having an Indo-West Pacific tropical to subtropical distribution, the species *Meretrix meretrix* and *Katelysia hiantina* belong to superfamily Veneroidea which is one of the most speciose taxon with 720 species recorded as of 2010 (Huber, 2010).

Studies on the reproductive biology of bivalves have been extensively conducted globally (Drummond *et al.*, 2006; Chung. 2007; Jagadis and Rajagopal, 2007; Nakamura *et al.*, 2010; Sawant and Mohite, 2013; Morillo-Manalo *et al.*, 2016; Idris *et al.*, 2017). Methods like body indices and histological analysis are commonly used to establish gonad cycle (Ojea *et al.*, 2004; Delgado and Camacho, 2005; Sawant and Mohite, 2013; Lagade *et al.*, 2014; Kovalyova, 2017). Several works focused on the seasonality of the gonad development of bivalves (Suja and Muthiah, 2007; Joaquim *et al.*, 2008; Nakamura *et al.*, 2010; Hamli *et al.*, 2015; Idris *et al.*, 2017). Recent findings revealed that spawning of some tropical bivalve species is protracted and that all gonad stages are present each month (Bantoto and Ilano, 2012; Morillo-Manalo *et al.*, 2016; Idris *et al.*, 2017). The continuous spawning and possible occurrence of these stages throughout a month may suggest lunar rhythm. However, the influence of moon phases on the reproductive biology remains a gap for most tropical bivalve species (Tran *et al.* 2011; Hamli *et al.* 2015).

The broader goal of this study is to characterize the gonadal development of *Meretrix meretix* and *Katelysia hiantina* throughout the lunar cycle in Panguil Bay, Southern Philippines. The specific aim of the present study is to determine the changes of size, gonadosomatic index, and fecundity during each lunar phase of the two commercially important bivalves, *M. meretix* and *K. hiantina*, in Panguil Bay.

Materials and methods

Collection of samples was done in the inner part of an exploited, mangrove-dominated and brackish Panguil Bay, Northern Mindanao, Philippines (Metillo *et al.,* 2015). Particularly, sampling was conducted in the intertidal flat of Barangay Pacita, Municipality of Lala, Province of Lanao Del Norte (Fig.1).

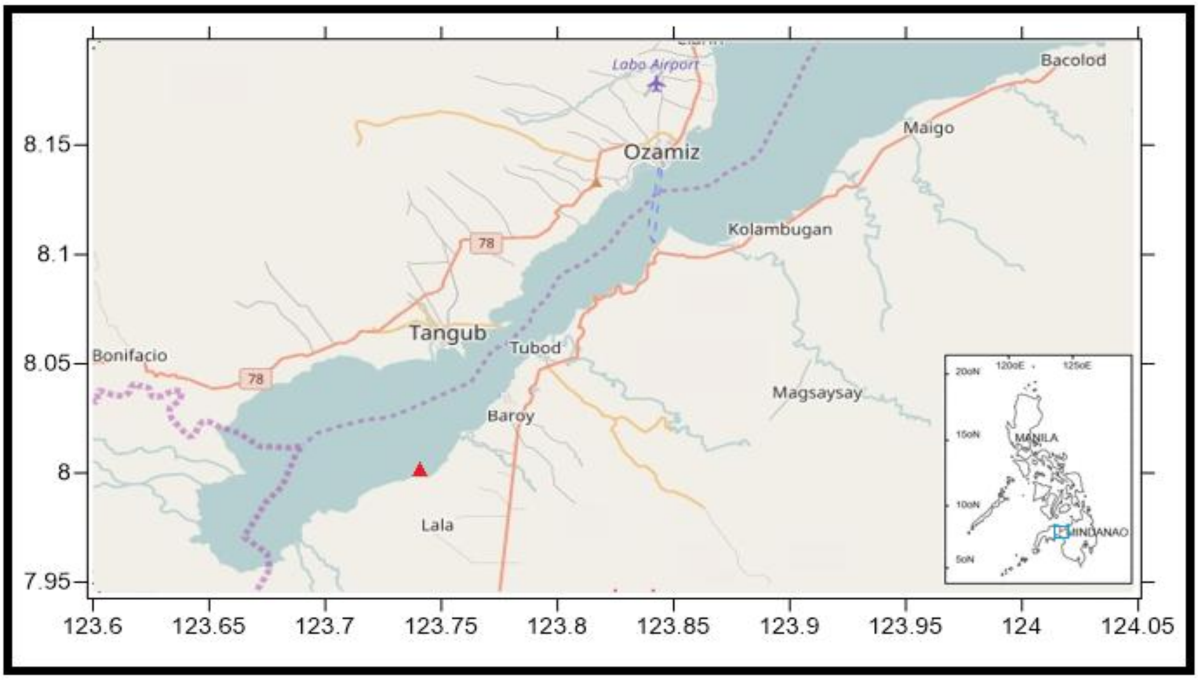

Fig. 1. Map of Panguil Bay showing the sampling station in Lala, Lanao Del Norte (▲) in the inner part of the bay. Insert is the Map of the Philippines with location of Panguil Bay enclosed in a small rectangle.

The sampling site is characterized by a river outlet with fishponds lining towards the river mouth with muddy to sandy substrate. Samples were collected every lunar phase from March to May 2017 during low tide. Measurement of water temperature, salinity and pH were done in-situ using a field mercury thermometer, handheld refractometer and pH meter, respectively.

Thirty live individuals of each species of *M. meretrix* and *K. hiantina* were purchased from local gleaners for every lunar period sampling after making sure all sizes of individuals for each species were represented in the sample. This was achieved by random subsampling of bivalves from the catch of gleaners.

Collection of samples was every moon phase at low tide. Shell length, along the antero-posterior axis, width, in the dorso-ventral axis, and inflation or thickness, maximum distance between the valves, were taken using a vernier caliper to the nearest 0.1 mm.

Individual live bivalves were carefully dissected. Tissue weight was taken before the gonads were removed and weighed. The Gonado-Somatic Index (GSI) was based on the total body tissue and gonad weights measured to the nearest 0.01 g. GSI was computed using the formula (Morillo-Manalo *et al.*, 2016):

$$\text{GSI} = \frac{gonad\ weight\ (g)}{total\ tissue\ weight}\ x\ 100$$

Portion of the gonads were smeared in a glass slide and were observed under a compound microscope for confirmation of mature female samples using Narasimhan *et al.* (1988) and Sawant and Mohite (2013) as guide. Gonads of mature female samples were preserved with 5% buffered formalin in plastic containers for fecundity estimation.

Fecundity estimation was determined by volumetric method. Subsamples of 1 ml were taken in triplicate from each container. Oocytes were counted using a Sedgewick rafter counting chamber under a compound microscope. The total number of oocytes in the gonad was obtained from the equation (Holden and Raitt, 1974):

$$F = {}^{nV}\!/_{v}$$

where F = fecundity, n = number of oocyte in the subsample, V = total volume of the sample, and v = volume of the subsample in the same units. The mean of the three subsamples represented the fecundity of each sample.

Statistical analysis

Prior to analysis of variance (ANOVA), Shapiro-Wilk test was used to ensure data followed the normal distribution. The one-way ANOVA with post hoc Tukey's test was used to test the significant differences among shell length, width and thickness, GSI values, and fecundity among lunar phases. All mean values of measurements had (±) standard deviation as error values. Statistical analyses were done using SPSS v.23.0 software (SPSS 2015).

Results

Environmental parameters

Physico-chemical parameters during sampling did not vary significantly (Table 1) except for salinity which we attribute to freshwater input caused by heavy rains. Salinity ranged from 16 to 25 ppt. Subsurface water temperature ranged from 29.7 to 32 °C, while pH registered a range of 6.9 to 7.6.

Shell measurements

A total of 360 individuals for each venerid clam species were randomly collected from the gleaners during sampling. Morphological measurements of *M. meretrix* ranged from 24.15 – 37.29 mm in length, 24 – 31.82 mm in width and 16 – 19 mm in its thickness with mean values of 32.20±1.91 mm, 27.01±1.34 mm and 19.58±1.02 mm in length, width, and inflation, respectively (Fig. 2).

Morphological measurements of *K. hiantina* showed ranges of 34.00 - 48.33 mm for length, 28.45 - 38.41 mm for width and 20.00 - 26.65 mm for thickness with mean values of 39.97±2.59 mm for length, 32.80±1.95 mm for width and 22.98±1.45 mm for thickness.

Table 1. Physico-chemical parameters recorded during sampling of live *Meretrix meretrix* and *Katylesia hyanthina* in inner Panguil Bay, Philippines.

Parameters	Range
Temperature (°C)	29.9-32.2
Salinity (‰)	16.0-26.0
pH	7.0-7.6

The mean shell dimensions of *M. meretrix* clam species during the four lunar phases did not differ significantly in length ($p > 0.06$), width ($p > 0.07$), and inflation ($p > 0.59$) (Fig. 2). Comparing the sizes among the moon phases for *M. meretrix*, samples collected during full moon has the highest mean length of 32.93±1.87 mm and mean width 27.42±1.17 mm. Highest mean inflation was recorded during new moon with 19.78 ±0.81 mm. Least mean morphological measurements were observed during first quarter with 31.64±1.20 mm, 26.51±0.94 mm, and 19.47±0.98 mm in length, width and inflation, respectively.

On the other hand, for *K. hiantina*, the last quarter phase measurements showed the highest mean length with 40.20±2.34 mm while the lowest mean measured length belonged to first quarter with 39.66±2.58 mm. The width with the lowest mean measurement belonged to the first quarter with 32.27±1.79 mm while the highest mean measurement is held by new moon at 33.20±2.19 mm. First quarter's mean thickness is lowest with only 22.71±1.19 mm while the highest mean thickness was observed during the last quarter with 23.27±1.31 mm. Despite the differences in measurements among the lunar phases, result showed no significant difference in length ($p > 0.23$), width ($p > 1.70$), and thickness ($p > 1.39$).

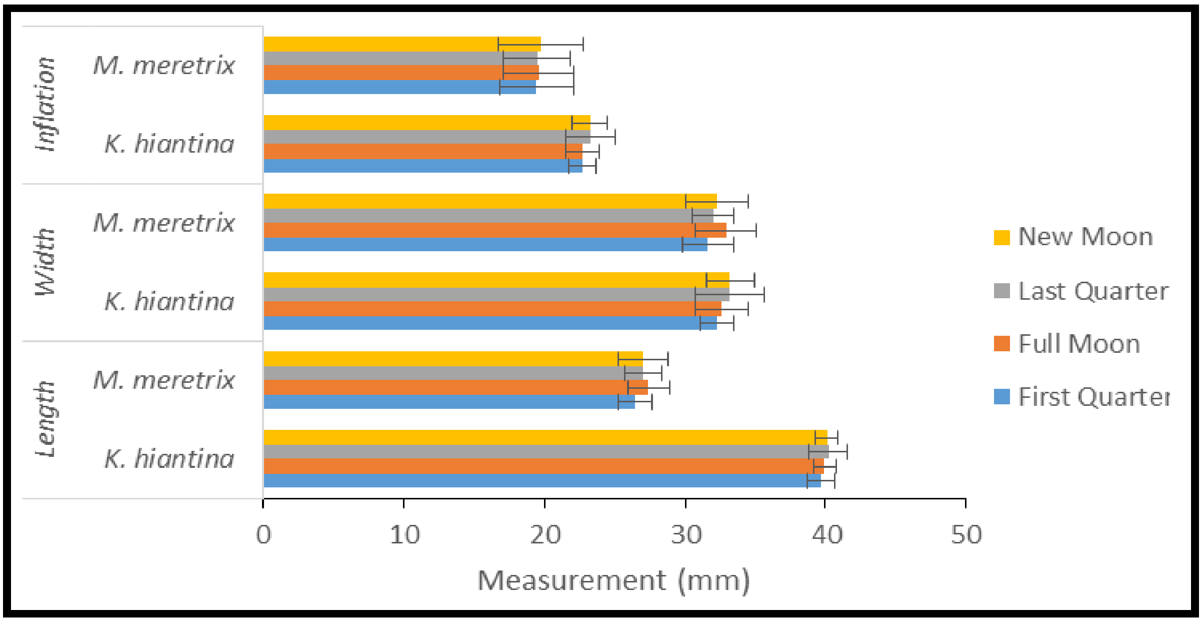

Fig. 2. Mean length, width, and inflation measurements of *K. hiantina* and *M. meretrix* w during four lunar phases in Panguil Bay, Philippines. Error bars = standard deviation.

Gonadosomatic index

Recorded mean GSI values for *M. meretrix* has a value of 15.28±9.40% and ranged from 2.78 to 45.45%. *K. hiantina* displayed a range of 2.13 to 38.71% with a mean value of 12.07±7.17%. Comparing GSI values across lunar phases showed peaks for both of the venerid clams (Fig. 3). Two peaks were observed for *M. meretrix,* a minor peak during full moon (15.26±4.67%) and a maximum peak during new moon (17.82±9.96%). A decrease in GSI was observed during first quarter (11.45±5.23%). The variation in GSI across lunar phases showed significant differences for *M. meretrix* (ANOVA, $F = 3.17$, df = 3, $p < 0.05$). This is further supported by the result of the post-hoc Tukey's test with new moon GSI values differing significantly from other moon phases ($p < 0.05$ for all comparison). The full moon minimum GSI peak, however did not differ significantly from those of first quarter and last quarter values ($p > 0.05$).

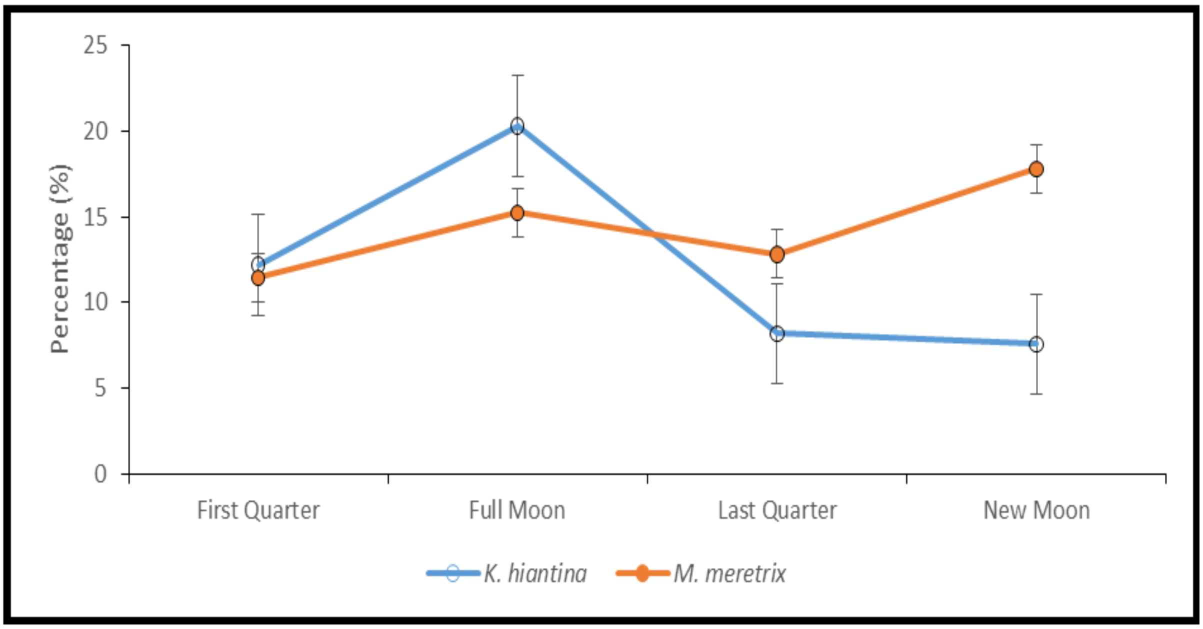

Fig. 3. Comparison of mean GSI for the two venerid clam collected during the four lunar phases with standard error showing a peak during full moon for *K. hiantina* and new moon for *M. meretrix*. Error bars: standard deviation.

The highest mean GSI for *K. hiantina* falls on full moon with 20.33±7.25% then it decreased significantly during the last quarter and the new moon phase with a value of 8.2±2.28% and 7.75±5.24%, respectively. A slight increase was observed during first quarter with 12.18±4.36%. Result also revealed a significant difference in GSI values for *K. hiantina* (ANOVA, $F = 39.63$, df = 3, $p < 0.05$) among lunar phases. Furthermore, post-hoc Tukey's test showed that all moon phases are not significantly different except for the peak value during full moon ($p < 0.05$).

Fecundity

The fecundity of *K. hiantina* ranged from 4.16×10^5 - 2.4×10^6 eggs/female with a mean of $1.12\times10^6\pm4.18\times10^5$ eggs/female. Estimated fecundity for *M. meretrix* has a recorded mean value of $3.15\times10^6 \pm 4.21\times10^6$ eggs/female and ranged from 2.29 to 3.96×10^6 eggs/female.

Fecundity varied from one lunar phase to another for the two clams (Fig.4). The highest average fecundity recorded for *K. hiantina* was during full moon. A peak was observed in the full moon with $1.463\times10^6\pm5.74\times10^5$ eggs/female. The lowest estimation was by individuals collected during new moon with $8\times10^6\pm1.72\times10^5$ eggs/female. Result showed significant difference in fecundity estimation across lunar phase for *K. hiantina* (ANOVA, $F = 6.72$, df = 3, $p < 0.05$). Further, the post-hoc Tukey test affirms the significant differences of all lunar phases ($p < 0.05$) except new moon and last quarter.

Estimation of fecundity for *M. meretrix* also showed trends and variation. Two peaks were observed – a minor peak during full moon ($1.28\times10^6\pm3.44\times10^5$ eggs/female) and a major peak during new moon ($1.58\times10^6\pm1.72\times10^5$ eggs/female). First and last quarter lags behind with mean values in fecundity of $7.71\times10^5\pm2.70\times10^5$ and $8.85\times10^5\pm2.20\times10^5$ eggs/female, respectively. Result showed significant difference in fecundity estimates among lunar phases (ANOVA, $F = 22.42$, df = 3, $p < 0.05$). The result on post-hoc Tukey's test affirms with the significance of fecundity on lunar phases. It showed that in terms of fecundity first quarter has significant difference towards full moon ($p < 0.05$) and new moon ($p < 0.05$). Last quarter is also significantly different to full moon ($p < 0.05$) and new moon ($p < 0.05$).

Discussion

M. meretrix and *K. hiantina* are coexisting bivalves in Panguil Bay (Jimenez *et al.*, 2009). Both are active burrower and suspension-feeding species that grow well in the intertidal areas with muddy or silty substrate type such as mangroves and estuaries (Poutiers, 1998; del Norte-Campos *et al.*, 2006). However, Nayar and Mahadevan, (1974) stated that *M. meretrix* predominantly inhabit sandy bottoms. The distribution of *M. meretrix* and *K. hiantina* is widespread in the Indo-West Pacific, from East Africa to the Philippines, north to Japan and south to Indonesia (Poutiers, 1998). More published information on other relevant characters are available for *M. meretrix* than *K. hiantina*. For instance, the colour of *M. meretrix* varies from creamy white to light and dark brown (Sur *et al.*, 2006).

It can grow up to 75-90 mm in length and an estimated lifespan of 7.8 years (Narasimhan *et al.*, 1988; Nayar and Mahadevan, 1974). The clam *M. meretrix* is dioecious and attains first sexual maturity at 21-26 mm length (Jayabal and Kalyani, 1986).

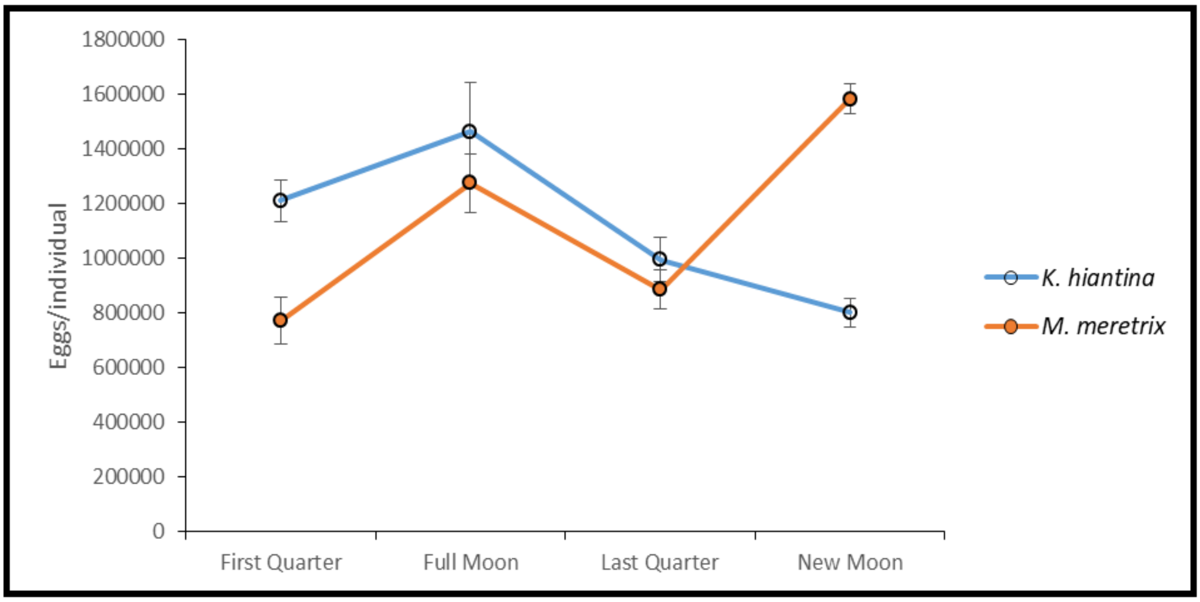

Fig. 4. Comparison of mean fecundity for the two venerid clam collected during the four lunar phases showing a peak during full moon for *K. hiantina* and new moon for *M. meretrix*. Error bars: standard deviation.

The bivalve molluscs, in this study, *M. meretrix* and *K. hiantina* tend to follow a lunar pattern in reproduction. According to McDowall (1970), species in diverse phyla, like Mollusca, mostly exhibit lunar rhythm in reproduction. Bivalves are also influenced by the lunar periodicity when it comes to spawning (Lucas and Southgate, 2012) and growth (Clark, 1974; Tran *et al.*, 2011).

The present study showed that shell measurements do not vary among lunar phases for *M. meretrix* and *K. hiantina*. The high standard deviation values in the present study of shell morphology measurements caused no differences in measurement among samples collected in different lunar phase. The absence of difference in morphological measurements within species could be traced to the overlapping generations common in mollusc populations (Nota, 1991; Cardoso and Veloso, 2003; Kevrekidis *et al.* 2009; Gerasimova and Maximovich, 2013). According to Resgalla *et al.* (2007), growth on bivalves is driven by temperature and salinity. Temperature is directly proportional to metabolism (Widdows, 1973), therefore influencing the clam's growth. Condition index among bivalves decreases with an increase in temperature and could directly affect growth (Hiebenthal *et al.*, 2012). Morever, phytoplankton abundance can greatly influence growth of bivalves (Dauble *et al.*, 1985; Lodeiros and Himmelman, 2000). However, a study by Miyaji *et al.* (2002) on bivalve *Phacosoma japonicum* indicated that a pair of two microgrowth lines and two increments is produced every lunar day. A similar study on the clam *Tapes philippinarum* by Richardson (1988) showed semi-diurnal microgrowth band patterns and that the periodicity of the bands is significant to the shell growth.

Variations in GSI represent the progress of reproduction. Maximal GSI was acquired by *K. hiantina* during full moon while, in *M. meretrix* it was noted during new moon. Lowest GSI value was recorded during new moon in *K. hiantina* while during first quarter for *M. meretrix*. Increase in GSI value means gametogenesis whereas decreasing GSI means spawning (Barber and Blake, 2006). In this study, *K. hiantina* exhibited high gametogenic activity during new moon and progressively spawns from last quarter to new moon. On the other hand, *M. meretrix* showed high gametogenic activity during new moon and spawns until first quarter. A minimal rise of GSI during full moon is observed indicating a possible increase in gametogenesis then spawns again until the last quarter. The GSI provides information on how much the body is investing on the gonads (Giese and Pearse, 1974). Similar increase and decrease in GSI values in maturity and spawning were well documented (Cantillanez *et al.*, 2005; Mouneyrac *et al.*, 2008; Morillo-Manalo *et al.*, 2016). Among bivalves, GSI has been used to assess and monitor the development of gonad (Lucas and Beninger, 1985; Lagade *et al.*, 2014) because it is an easy and fast method to identify gonad stages rather than the use of histological analysis (Mouneyrac *et al.*, 2008; Ben Salah *et al.*, 2012).

Fecundity is the reproductive rate of a female individual or population. In this study, fecundity estimates of *K. hiantina* were similar to the results of Kent *et al.* (1998) in which they used a congenere, *Katelysia scalarina*, as a test subject and the fecundity ranged between 7×10^5 and 2.4×10^6 eggs/female. Comparable to the results of the present study on fecundity of *M. meretrix* were those from a study conducted by Thanh (2013). The study reported values ranging from 318,400 to 3,825,000 eggs/individual, with an average of 1,181,151 eggs/individual. Estimates of fecundity also showed a unimodal peak for *K. hiantina* during full moon while a bimodal peak for *M. meretrix* during full and new moon. Rheman *et al.* (2002) stated that fecundity and GSI has a linear relationship, hence the same peaks were observed for both venerid clams in this study.

Bivalves are known to undergo an annual reproductive cycle that involves gametogenesis followed by either a single or several spawning events, which are in turn followed by a period of gonad reconstitution (Crnčević *et al.*, 2013). Some bivalves have unimodal or bimodal type of spawning which means they could either have a single spawning activity or have two spawning periods within a year (Mouneyrac *et al.*, 2008; Ben Salah *et al.*, 2012; Sawant and Mohite, 2013; Morillo-Manalo *et al.*, 2016). However, Rai (1932) opined that under favourable condition spawning will occur throughout the year. A study on *L. philippinarum* (Bantoto and Ilano, 2012) showed presence of gonads in developing, mature and spawning stages in all months of the year and may suggest that gametogenesis, maturation and spawning of occur throughout the year. Possible occurrence of these stages all throughout the month may suggest lunar rhythm.

Reproductive periodicity in marine invertebrates serves two, mutually compatible, functions: (1) allows individuals of a population to take advantage of the best time of the year for reproduction and (2) ensures reproductive synchrony between conspecifics (Giese and Pearse, 1974). Furthermore, when the environment is seasonal, certain times of the year are bound to be more favorable for the production of offspring; individuals that are able to take advantage of these periods will have a higher probability of propagating their genes. Marine invertebrates tend to swarm and reproduce during last quarter and full moon, and one of the famous species to account for that is the bivalve *Ostrea edulis*. Orton (1926) found out maximum spawning in *O. edulis* at the full moon. Later on, it was confirmed by Koringga (1941, 1947) that a relationship between spawning and moon phases exists. In addition, Tan and Yasin (2001) have observed that the giant clam, *T. squamosa* exhibit lunar patterns of spawning. Lunar periodicity also applies on *Littorina saxatilis* (Berry and Smith, 1987) and *Siphonoria denticulata* (Creese, 1980) where they exhibited spawning during the full moon. Another evidences of lunar periodicity in reproduction among bivalves are demonstrated by *Mytilus edulis* which matures during the new moon period and spawns at the following neap tide (McDowall, 1970). In addition, clams *Chlamys opercularis* (Wilson, 1951) and *Pecten maximus* (Mason, 1958) spawn at about new moon and full moon during spring tides (Korringa, 1957).

Results of this study revealed different reproductive peaks for the two coexisting species in the brackish water of inner Panguil Bay. Similarly, a study conducted by Stead *et al.* (2002) showed different spawning period for two coexisting tellinacean bivalves as a result of different feeding behavior. In addition, Cardoso *et al.* (2006) revealed different timing in gametogenesis between *Cerastoderma edule* and *Mya arenaria* in the Dutch Wadden Sea due to factors like size, predation and food availability. This implies that niche separation/partitioning possibly drives different spawning period for coexisting species. The differences in lunar periodicities for GSI and fecundity can be traced to the two species evolving differences in spawning periods to minimize competition of their larval phases and subsequent development stages.

Conclusion

The result of this study showed that *Meretrix meretrix* and *Katelysia hiantina* exhibited lunar rhythm in reproduction. The clams displayed peaks in GSI and fecundity in different lunar phases. Apart from the temporal ecological niche separation exhibited by the two venerid clam species, the reproductive biology results from the two bivalve mollusc species give us insights into managing the population in the wild and its potential for fisheries production. An annual study of lunar periodicity on gonad development of the two venerid clams is highly recommended.

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