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Original Article

Influence of lunar cycle, moon driven tides and water physicochemical factors on the gonadal maturation of green mussel, *Perna viridis*, in the inner Malampaya sound, Taytay, Palawan, Philippines

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Abstract: This study determined the influence of the lunar cycle, tidal fluctuation, as well as water physicochemical factors on the gonadal maturation of green mussel, *Perna viridis*, in Malampaya Sound, Taytay, Palawan, Philippines. Monitoring and sampling were conducted for the 6 months, from October 2017 to March 2018. It was observed that the lunar cycle has a direct influence on gonadal maturity. Gonadal changes in both male and female *P. viridis* were observed histologically and both exhibit the characteristic of lunar-synchronous pattern. It was observed that a high number of green mussels with spawning stages usually occur during the full moon and new moon. Furthermore, gonadal maturity was also affected by the tidal fluctuation. A higher number of *P. viridis* with spawning stages were recorded during spring tide, whereas the percentage of spent individuals were higher during neap tides. Furthermore, it was observed in the present study that salinity fluctuation could influence spawning of green mussel, wherein the number of spent individuals increased after the sudden drop in water salinity from 30 to 13 ppt after the heavy rainfall, an indication that spawning had occurred. The presence of individuals with spawning stages was also observed throughout of period of sampling which indicates that mussels were continuously breeding in the area. The highest percentage of individuals having spawning stages were recorded in October and November. The information on the factors that affect gonadal maturation and spawning schedule can help mussel growers predict the schedule of spatfall.

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Introduction

Green mussel, *Perna viridis* is one of the commercially important bivalve species and a cheaper source of protein (Korringa, 1972; Tortell et al., 1978). Its cultivation is extensively carried out in tropical countries (Rajagopal et al., 2006; Soon and Ransangan, 2014) in the intertidal and subtidal areas. In the Philippines, green mussel culture started in 1962 in the Cavite Province (Aypa, 1990) and became the most widely cultured bivalve species in the country with estimated production of 26.30 thousand metric tons in 2018 (Philippine Statistics Authority 2018). Currently, the Philippine government is making an effort to improve and expand the culture of this mussel. In 2014, the government initiated to put up the first mussel hatchery in the country for a sustainable spat production (Mero et al., 2019). Furthermore, research efforts to increase the larval production has been conducted (Maquirang et al., 2020) and improvement of the grow-out production is on-going. One of the areas in the country with high potential for grow-out culture of green mussel is Inner Malampaya Sound, Taytay, Palawan, Philippines. The mussel sizes produced in this area were larger compared to other mussels cultured in other locations of the country, thus Inner Malampaya Sound can be an ideal source for breeders.

One of the important considerations of successful cultivation of green mussel is to identify the factors, influencing their reproduction and spawning.

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Knowledge on the reproductive biology of the green mussel can help farmers to predict their spawning and spatfall schedule. On the other hand, the reproductive development of mussels depends on the quality of their environment that varies in different areas. Main factors that affect gonadal development of the green mussel in the different area includes, temperature (Lee, 1988; Shafee, 1989; Rajagopal et al., 1998; Alfaro et al., 2001 Rajagopal et al., 2006), density and composition of food supply (Rajagopal et al., 1998; Lopes-lima et al., 2014; Soon and Ransangan, 2014), and light intensity, and wave action (Wong and Cheung, 2003). There is limited evidence about the relationship between the different lunar phases and the spawning activities of green mussel as reported in other mollusk species. In abalone, *Haliotis asinina*, natural spawning usually occurs during new moon and full moon (Capinpin, 1995; Counihan, 2001). Similarly, giant clams, *Tridacna* spp. spawns during mid to late afternoon that coincides with the full moon and new moon periods (Elis, 2009).

Detailed information regarding the lunar cycle in gonadal maturity can enhance the farming efficiency of green mussel particularly in the Malampaya Sound, Taytay Palawan, Philippines. Thus, this study aimed to investigate the gonadal maturity and spawning of green mussel in relationship to some uncontrolled onsite factors such as the lunar cycle, moon driven tides, and physicochemical parameters.

Materials and Methods

Study area and sampling: The study was conducted at Barangay New Guinlo, Taytay, Palawan, Philippines (Fig. 1) located on the inner Malampaya Sound, Taytay, Palawan, Philippines (10.78272 latitude, 119.437218 longitude). The mussel farms in this area supply most of the green mussel in the markets of Palawan, Philippines. The study was conducted during six months from October 2017 to March 2018. Samplings were done weekly representing the different lunar phases (full moon, new moon, first lunar quarter and last lunar quarter). The daily tidal ranges and lunar cycle were identified using the book of Philippine tide and current tables

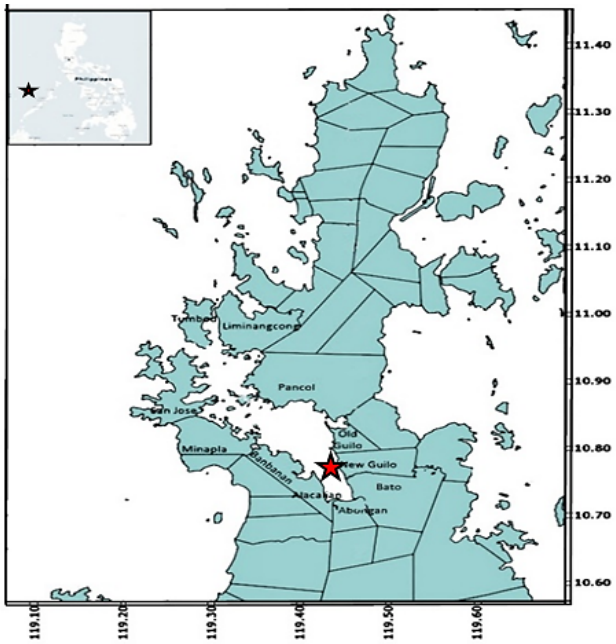

Figure 1. Map of Palawan and Malampaya Sound, Taytay Palawan pointing the location (★) of mussel farms as sampling area.

(2017 and 2018 editions) (NAMRIA, 2017, 2018). Samples were collected regularly from 9 am to 12 noon.

About 50 green mussels (40-60 mm) were randomly collected from three different random points at the mussel farm. Samples were taken from the middle part of the bamboo stakes and then mixed and randomly analyzed for gonadal maturity and gonad index.

Reproductive biology: For every sampling schedule, twenty samples of adult mussel (10 males and 10 females) with shell length of 50-60 mm were sectioned dorsoventrally through the mid-body and gonads were removed from the mantle lobes. The gonads were individually stored in a screw cap container and immediately preserved in 70% Ethanol. Samples were sent to the Microtechnique Laboratory, Institute of Marine Fisheries and Oceanology, University of the Philippines Visayas for further histological analysis. Samples were fixed using Bouin's solution, subsequently dehydrated with the series (80-100%) of ethanol and xylene, embedded in paraffin wax, sectioned (7 µm) and stained with hematoxylin and eosin based on Bancroft and Stevens

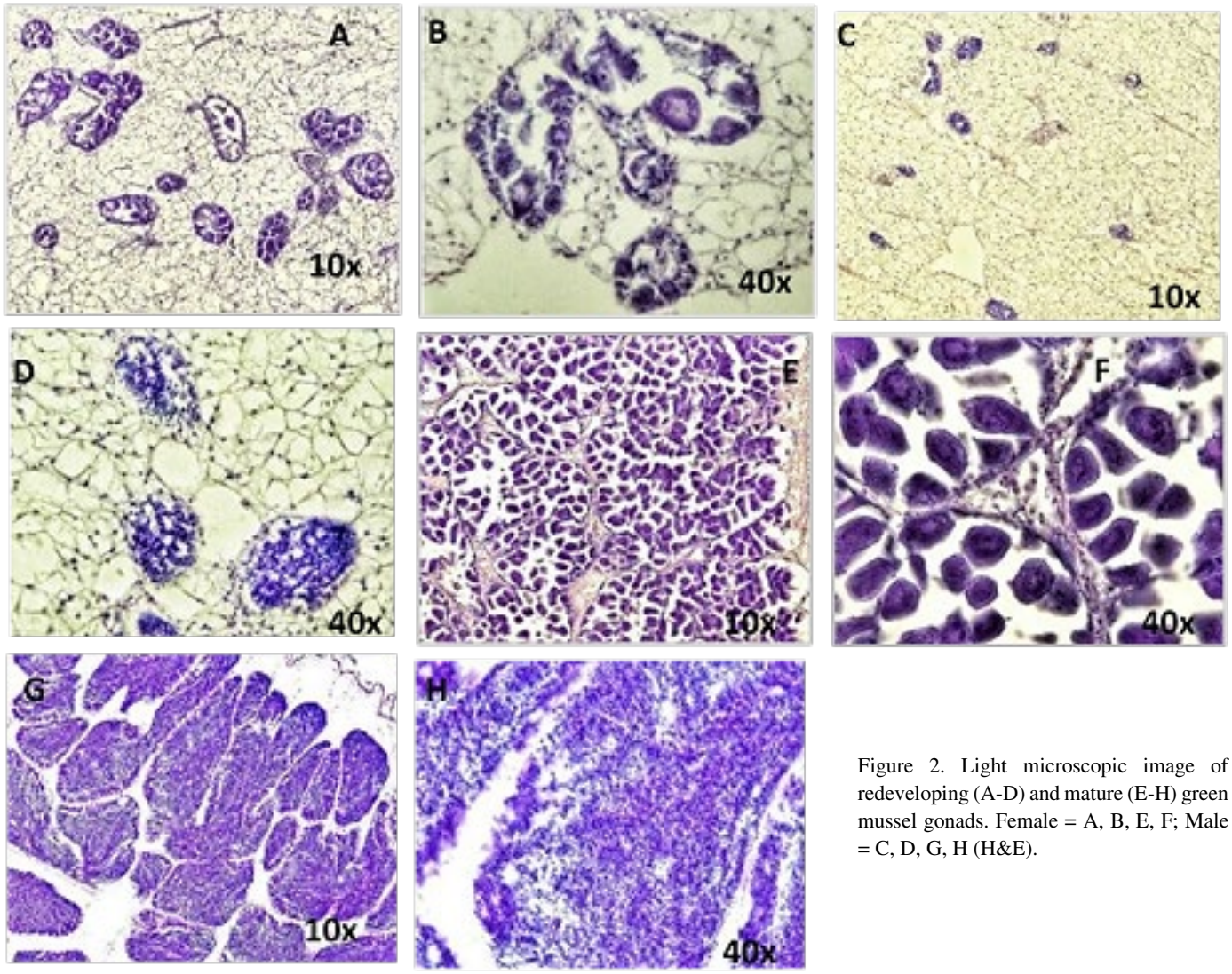

Figure 2. Light microscopic image of redeveloping (A-D) and mature (E-H) green mussel gonads. Female = A, B, E, F; Male = C, D, G, H (H&E).

(1996). Gonad sections were examined under a light microscope, classified, and scored based on the classification scheme based on Shafee (1989) (Figs. 2, 3). After classification of gonads, the gonad index (GI) was computed using the formula below (King et al., 1989):

GI = ((No, in each stage) (rank of stage) / Total sample size) x 100

Water physico-chemical parameters: Water parameters in the study area such as salinity (using Rocker SA10T and RHSN-10ATC refractometer), pH (using Oakton Eco Testr pH1 and Eutech pHtestr20), temperature (using digital thermometers), and dissolved oxygen (kit) and turbidity (using Secchi disk) were measured around 9-12 am during the sampling period. These monitoring covered three strategic points in the area at different depth (surface and bottom (2-3 m below depending on tidal height)). Bottom waters were collected using Niskin water sampler.

Data Analyses: Data were subjected to a normality test using the Shapiro-Wilk test and homogeneity test using Levene's test before further analysis was done. The gonad index and different water parameters were compared monthly, every lunar week, and tidal schedule (spring tide and neap tide), using ANOVA, Brown-Forsythe, and t-test based on the homogeneity of each data set.

Results

Influence of lunar cycle on gonad index and gonadal maturation: Observation of the gonad index (GI) and gonadal maturation was conducted at different lunar phases from October 2017 to March

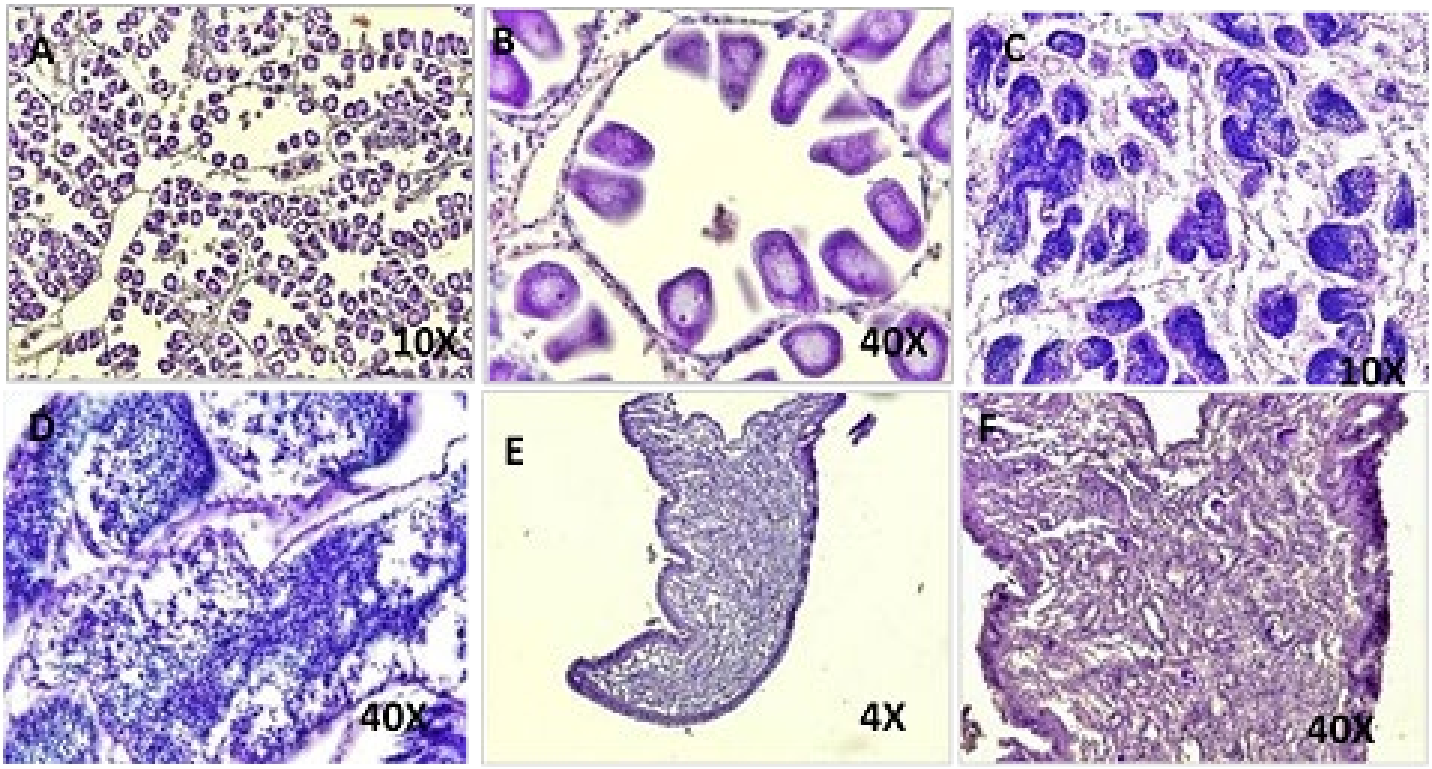

Figure 3. Light microscopic image of spawning (A-D) and spent/resting (EF) green mussel gonads. Female = A, B; Male C, D (H&E).

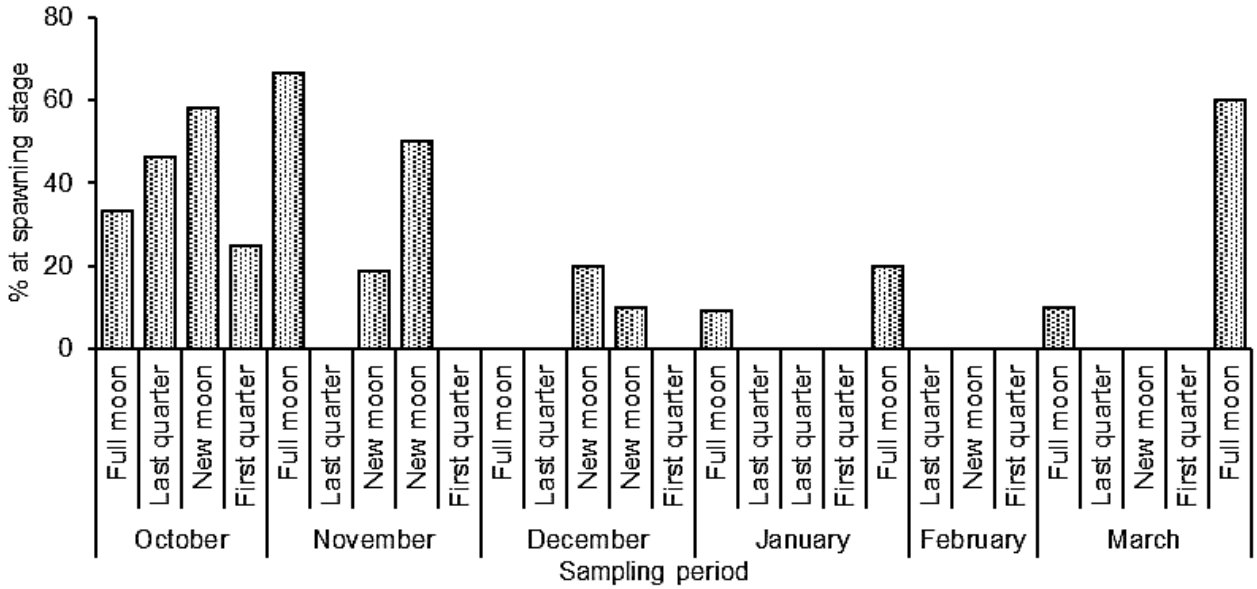

Figure 4. Female green mussels at spawning stage during different lunar phases at the inner Malampaya sound, Taytay Palawan.

2018. Although no significant no significant difference was observed, the highest percentage of individuals with spawning stages were recorded during full moon, while the fewest number was during the first quarter of the lunar cycle i.e. the redeveloping stage was high in almost all the lunar phases (Table 1). Furthermore, the females with spawning stages were observed to have a lunar synchronous pattern during the full moon and new moon which can be noted clearly after the reproductive/breeding/spawning peak (October) (Fig. 4).

Influence of moon driven tides on gonad index and gonadal maturation: Gonad index was found to be significantly higher during the neap tides. However,

Table 1. Gonad maturity stages and gonad index of green mussel in relationship to the lunar phases.

Lunar Phase	Gonad maturity stages (Average % of occurrence)					Gonad Index
	Ripe	Spawning	Spent	Resting	Redeveloping	
Full moon	4.93±3.60	22.97±7.77	3.54±1.76	10.79±8.44	57.77±10.30	184.88±14.96
Last quarter	4.76±4.76	17.51±6.82	14.47±8.56	2.04±2.04	61.21±13.32	189.29±11.41
New moon	2.86±2.86	18.36±6.22	14.64±9.19	3.85±2.41	60.30±14.15	181.43±14.50
First quarter	8.73±8.73	3.17±3.17	11.04±9.34	8.51±5.40	68.54±15.32	184.17±21.61

Table 2. Body indices and gonad index, and gonad stages of green mussel in relationship to tides.

Tide	Gonad maturity stages (Average % of occurrence)					Gonad Index
	Full/ Ripe	Spawning	Spent	Resting	Redeveloping	
Neap	6.56±4.59	10.89±4.33	12.88±6.06	5.03±2.76	64.60±9.70	186.92±11.02
Spring	3.89±2.23	20.66±4.83	9.09±4.75	7.32±4.32	59.04±2.76	183.92±9.231

Table 3. Summary of means of physicochemical parameters and their significant values among different comparisons.

Parameter	Range	Mean ± SE	Monthly	Lunar week	Tides Neap vs Spring
Salinity (ppt)	13-37	29.47±0.31	0.015*	0.002**	0.806
DO (ppm)	4.8-11.2	7.8±0.18	0.976	0.233	0.816
pH	4.5-8.6	8.00±0.05	0.000**	0.000**	0.732
Temperature (°C)	26-33.2	28.20±0.08	0.001**	0.166	0.0.816
Turbidity	0.8-2.5	1.40±0.71	0.257	0.633	0.530

*= significant different, ** = high significant different

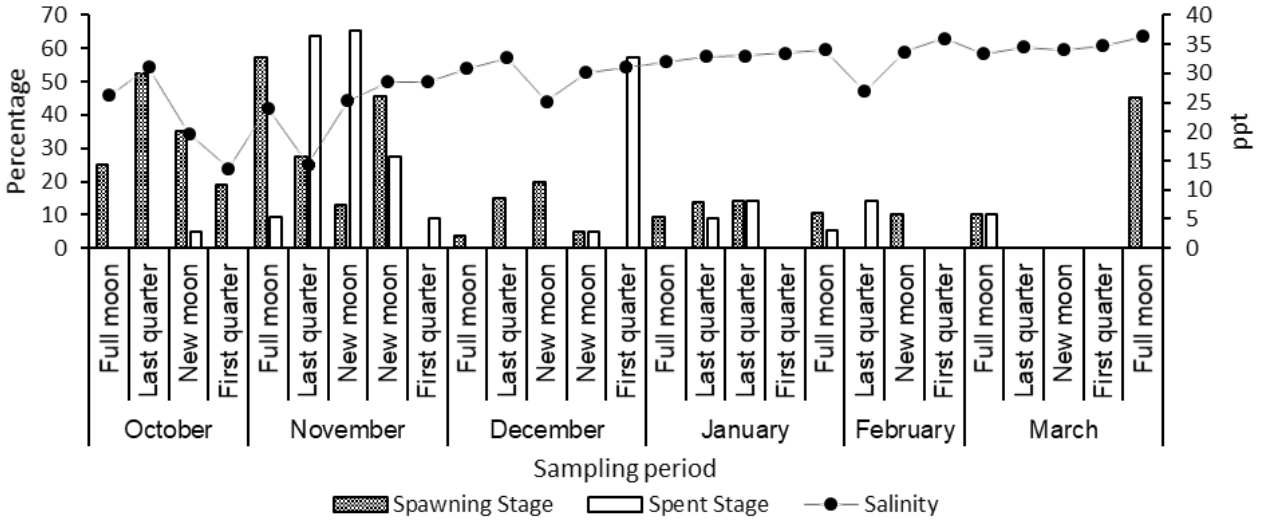

Figure 5. Spawning and spent stages of green mussel in relationship with salinity fluctuations.

the number of individuals with spawning stages were observed to be high during spring tides (Table 2). Whereas, individuals having spent stages were high during neap tides. Thus, these observations indicate that tidal fluctuation at different lunar phases can influence spawning activities of green mussels.

Influence of physicochemical parameters on gonad stages: The physicochemical parameters of the water in the study area were within the range required for mussel culture. Salinity, pH, and temperature levels

Table 4. Monthly body indices, gonadal maturity stages and gonad index of green mussel in the Inner Malampaya Sound, Taytay, Palawan.

Month	Gonad Maturity Stages %					Gonad Index
	Full/ Ripe	Spawning	Spent	Resting	Redeveloping	
October	33.75	31.25	1.25	0.00	33.75	232.50±8.54[a]
November	2.02	31.31	31.31	10.10	25.25	150.00±15.17[b]
December	0.00	8.00	13.00	14.00	65.00	159.00±24.10[ab]
January	0.00	8.08	4.04	1.01	86.87	193.84±3.77[ab]
February	0.00	3.33	5.00	5.00	86.67	185.00±10.41[ab]
March	0.00	12.00	1.00	0.00	87.00	199.00±1.00[ab]

Gonad index with similar superscript = no significant different

have significant variations per month. Significant changes in salinity and pH values were noted during weekly sampling (Table 3). Drastic salinity fluctuations were observed from the third week of October until the third week of November due to constant rain. A high percentage of the spent individuals were observed after the occurrence of a huge drop in salinity in the area. This may indicate that majority of the mature individuals have released their gametes as influenced by sudden changes in environmental factors (Fig. 5).

Monthly trend of the gonadal maturity of green mussel: Mussels with spawning stages were observed throughout the period of sampling (October 2017 to March 2018) as shown in Table 4. An indication that mussel is continuously breeding in the sampling area. However, the highest percentage of spawning stages were recorded during October and November 2017. Whereas the highest percentage of individuals with spent gonads were observed in November. High percentage of mussel having redeveloping stages were observed from December to March (Table 4).

Discussions

Regulation of reproduction in an organism is genetically controlled in response to its environment (Barber and Blake, 2006). One of the abiotic factors that affect gametogenesis and spawning in marine organisms is lunar periodicity. Several studies have shown that reproductive cycles of marine invertebrates are often linked to the lunar phase (Counihan et al., 2001; Collin et al., 2016; Gupta 2017). In the present study, weekly gonadal changes in both male and female *P. viridis* were observed histologically and both exhibit the characteristic of lunar-synchronous pattern. It was observed that a high number of green mussel with spawning stages usually occur during the full moon and new moon and spent gonads were observed thereafter an indication that spawning had happened. The occurrence of spawning during or a few days before the new moon and full moon were also observed in other mollusk species (Castanos, 1997; Capinpin, 1995; Counihan et al., 2001; Elis, 2009). Likewise, some marine teleost showed group synchronous gonadal development in response to the lunar phase. For instance, in rabbitfish, their gonads mature synchronously and showed spawning peaks between full and last quarter moon, after which the gonads return to spent condition (Takemura at al., 2004). Thus, lunar cues play a significant factor in allowing synchrony in the gonadal maturation of the marine organisms to ensure reproductive success (Takemura et al., 2004).

The rhythms of the moon influence the marine environment in the form of tidal rhythm (Gupta, 2017). The tidal cycle or periodic fluctuation of tides can also influence the biological rhythms of the marine organisms (Tran et al., 2011). Those organisms that live in the intertidal environment are greatly affected by tidal variations (Nishida et al., 2006). Thus, these organisms need to synchronize their physiological activities especially their reproduction timing in response to the tidal pattern to ensure better survival of their offspring. Majority of the intertidal animals favored broadcast spawning during night-time and spring tide (Korringa, 1957; Naylor, 1976). This allows better transport and dispersal of gametes, as well as safety of their offspring from predation, which is greater at night time (Berry, 1986). However, lugworm (Duncan, 1960), spirorbid polychaetes

(Rothlisberg, 1974), and larviparous oysters (Knight-Jones, 1952) spawn during neap tides (Berry, 1986). In the present study, it was observed that a higher number of *P. viridis* with spawning stages were recorded during spring tide intervals. Whereas the percentage of spent individuals was higher during neap tides, an indication that tides have a direct influence on the reproduction of green mussel. Moreover, prolonged exposure of green mussel to air during low tide can possibly stimulate mature individuals to spawn. Several reports have shown that bivalves and other invertebrates can be induced to spawn through desiccation (Velasco et al., 2007; Abidin et al., 2016; Arendse et al., 2018).

Gonadal maturation and spawning of *P. viridis* were also influenced by salinity, temperature and food availability (Walter, 1982; Nagabhushanan and Mane, 1991; Rajagopal et al., 1998). Temperature may have direct influence on the development of gametes. The optimal range for *P. viridis* reproduction is 26 to 32ºC, however, an increase in temperature which ranges from 30 to 32ºC accelerates the ripening of gametes (Sreedevi et al., 2014). The temperature level recorded during the duration of the present study is within the optimum requirement for gonadal development. Therefore, the temperature in the inner Malampaya sound, Palawan, Philippines provides a suitable temperature requirement for the reproduction of *P. viridis*. However, temperature fluctuation throughout the day is not very evident in the present study since the monitoring of temperature was done only in the morning. Therefore, it is inconclusive if the temperature has a direct effect on the spawning activities of mussels. However, based on the observation of Walter (1982) temperature fluctuation had no obvious relationship with the spawning of mussel in the Philippines. Further, Stephen (1980) also noted that changes in temperature have little importance in determining spawning in Indian oyster. On the other hand, it was observed in the present study that the number of spent individuals increased after a sudden drop in salinity from 30 to 13 ppt after the occurrence of heavy rainfall. The sudden change in salinity may have influence mature individuals to release their gametes and the presence of spent individuals is an indication that spawning has occurred. The observation was in accordance with the study of Stephen and Shetty (1981) that rapid salinity change stimulates the spawning of bivalves in Indian coastal water. Thus, salinity is one of the key factors in influencing spawning activities of marine invertebrates in the coastal and estuarine environment.

The *P. viridis* in the Philippines is continuously breeding throughout the year as observed by Walter (1982). The continuous gonadal development and spawning of *P. viridis* in the Philippines is a likely occurrence since the country is situated in the tropical region thus *P. viridis* are exposed to a stable warm condition. The presence of individuals with redeveloping stages was high throughout the study an indication that mussel in the area is continuously undergoing gonadal maturation. The occurrence of individuals with spawning stages was also evident throughout the period of sampling (October to March). Furthermore, the highest percentage of individuals having spawning stages were recorded in October and November. Although green mussels in the country are continuously breeding, they have also shown reproductive or spawning peaks. These were confirmed by the occurrence of spat fall in some areas in the country. For instance, the peaks of spat fall were observed in Cañas Bay, Iloilo during May and November; December to May for Sapian Bay, Capiz; October to December and April to June in Maqueda Bay, Samar; December to January and March to April for Silanga Bay, Samar; and November to December and April to May for Cambautatay Bay, Samar (Baylon et al., 2016). Furthermore, PCARR (1977) reported that the settlement and spat fall of green mussel in Bacoor Bay, Cavite occurred in April to May and October to November. Moreover, spawning peaks of green mussel may also vary in other countries depending on the season. For instance, spawning occurs during June and October in Kalpakkam, east cost of India (Rajagopal et al., 1998) and April and November in Eastern Johore Strait water, Singapore (Low et al., 1991; Wong and Cheung, 2003; Soon et al., 2016). On the other hand, Lee (1988) reported a

single breeding period that extended from June to September in the Victoria Harbor, Hong Kong. While, Yoshiyasu et al. (2004) reported spawning from summer to early autumn in the Sagami Bay, Japan.

Conclusion and recommendations

Detailed reproductive pattern is an important aspect in biology, conservation, and aquaculture of green mussels. Information obtained in the present study showed that the lunar cycle, tidal fluctuation, and changes in physicochemical parameters in water can affect the gonadal maturation and spawning of green mussels in inner Malampaya Sound, Taytay, Palawan, Philippines. Further investigation on the effect of these factors on condition index, meat yield on the period not covered by this study is recommended.

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