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RESEARCH ARTICLE

First Record of Hermaphroditism in Green Mussels (*Perna viridis*) in the Philippines

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ABSTRACT

This paper reports an incidence of hermaphroditism in green mussel (*Perna viridis*) in Inner Malampaya Sound, Taytay Palawan, Philippines, through histology. One hermaphrodite specimen (0.18% of the total samples) was recorded. The sample was macroscopically classified as a male. However, histology showed the presence of both male and female gametes inside the gonad with the dominance of male gametes. The total male to female (1:0.86) sex ratio in this study was statistically different (P = 0.002) from the expected binomial distribution of the 1:1 sex ratio. Despite its currently low occurrence, hermaphroditism in green mussels could have significant effects on reproduction, population dynamics, and aquaculture if its incidence increases in the future.

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1. INTRODUCTION

Commercial cultivation of mussels is extensively carried out in tropical countries (Rajagopal et al. 2006; Soon and Ransangan 2014). In the Philippines, mussels were originally present in farms as a "nuisance" to oyster farmers. Later, the country became one of the first non-European countries to explore the intensive mussel culture somewhere in the late 1950s (Guerrero et al. 1983). At present, the green mussel, *Perna viridis*, is widely distributed and cultivated in the Philippines, both for commercial purposes and subsistence consumption. Its ability to tolerate a wide range of environmental conditions allows it to grow rapidly in dense colonies on various hard substrates (Toralde et al. 2021). However, despite the modernization of culture practices and the number of studies conducted about green mussels, there was still no recorded incidence of hermaphroditism in green mussels in the country prior to this study.

Hermaphroditism is common to mollusks (Valdes et al. 2010). On the other hand, only about 13 of 117 bivalve families (Vaught 1989) and about 400 of 10,000 species of bivalves are believed to be hermaphroditic (Coe 1943). Some of the known hermaphrodites include scallops (Pectinidae), freshwater clams (Pisidiidae) and Giant clams (Tridacnidae) (Britton and Morton 1989, Lee 1988).

Green mussels (*Perna viridis;* Family Mytilidae) or *tahong* in the Philippines are considered a dioecious species with no distinguishing external features to differentiate males from females (Yap et al. 1979). Petes et al. (2008) found that while field biologists commonly use gonadal tissue color—white for males and orange for females—to identify mussel sex, this method is unreliable, as their study showed both sexes with orange tissue and noted that environmental stress, especially in the intertidal zone, influences tissue color patterns. Furthermore, hermaphrodites present variations in coloration consistent with common knowledge (Montenegro et al., 2010). Several cases of hermaphroditism have been reported in tropical regions like India (Nagabhushanan et al. 1992) and Malaysia (Al-Barwani et al. 2013), as well as in subtropical areas such as Hong Kong (Lee, 1988) and Florida (Barber et al. 2005). Despite these discoveries, however, many argue that this species must be classified as gonochoristic since the percentage of hermaphroditism is very low (>0.01) (Lee 1988; Ragone Calvo et al.1998) and other researchers failed to find similar incidence in their respective study areas (Huang et al. 1985; King et al. 1989; Buchanan 2010). Montenegro et al. (2010) concluded that hermaphroditism in marine mussels could be an accidental phenomenon, but they do not discount the effect of pollution, and endocrine disruption.

Studies about hermaphroditism in green mussels *(Perna viridis)* is significant for its potential effects on reproduction, population dynamics, and aquaculture (Levitan and Petersen, 1995; Ghiselin, 1969). It could impact genetic diversity, species adaptability, and serve as a survival strategy in low-density populations (Avise and Mank, 2009). Hermaphroditism may disrupt sex ratios (Luttikhuizen et al. 2011), threaten species viability (Sousa et al. 2009), signal environmental stress (Jobling et al. 1998), and pose challenges for aquaculture, potentially leading to economic consequences (Lucas and Southgate 2012, Bertolini et al. 2009). In addition, studying the sex ratio in green mussels (*Perna viridis*) is critical for understanding population dynamics and promoting sustainable aquaculture practices. The sex ratio plays a key role in reproductive success, genetic diversity, and the overall health of mussel populations. A skewed ratio, particularly one with a female bias, can lead to reduced fertilization rates, which may compromise both mussel production and the stability of natural populations (Waples, 2002). Additionally, insights into how environmental factors such as temperature and food availability affect sex ratios can guide the management and optimization of aquaculture conditions, resulting in more efficient and sustainable production practices (Sará et al., 2013). For instance, A recent study in the Philippines by Toralde et al. (2021) examined the sex ratio of green mussels and found that females consistently dominated, accounting for 59% of the population compared to 41% males in Laute Bay, Villareal Bay, and Concabato Bay. This female-biased sex ratio may be influenced by environmental factors such as temperature, food availability, and the presence of exogenous steroids.

Earlier studies have suggested a correlation between sex ratios and the potential incidence of hermaphroditism in green mussels. However, while identifying sex ratios is important for understanding the reproductive aspects of green mussels, this alone may be insufficient due to differences in the developmental patterns of male and female gonads (Lee, 1988). In contrast, other studies have utilized histological methods to provide more reliable evidence of hermaphroditism in mussels (Shafee, 1989; Nagabhushanan et al., 1992; Alfaro et al., 2001; Barber et al., 2005). This study aimed to provide a preliminary report on the incidence of hermaphroditism and to identify the sex ratio of green mussels in farms located within Inner Malampaya Sound, Taytay, Palawan.

2. METHODOLOGY

Sample Collection

A total of 1,890 green mussels (*Perna viridis*), ranging in size from 40 to 60 mm, were collected from green mussel farms in the inner Malampaya Sound, Taytay, Palawan, Philippines (10.78272° N, 119.437218° E) between October 2017 and March 2018 (Figure 1). Each week, seventy samples were randomly collected and manually removed from the stakes, ensuring the byssus threads remained intact.

Processing and Analysis of Samples

` Mussels measured along their maximum anterior-posterior axis for body length (BL) using a vernier caliper. Wet whole-body weight (BW) was measured using (i200) digital weighing scale (In 0.01 g sensitivity). The mussels were initially sexed macroscopically, based on the color of the gonad and mantle, following the descriptions provided by Vakily (1989) and Rosell (1991) (Figure 2). Each week, twenty green mussels, consisting of ten males and ten females as identified macroscopically, were collected for histological analysis (totaling 540 specimens over the study period) following the number of samples on the study of Baldevieso et al. 2024. Mussels were cleaned

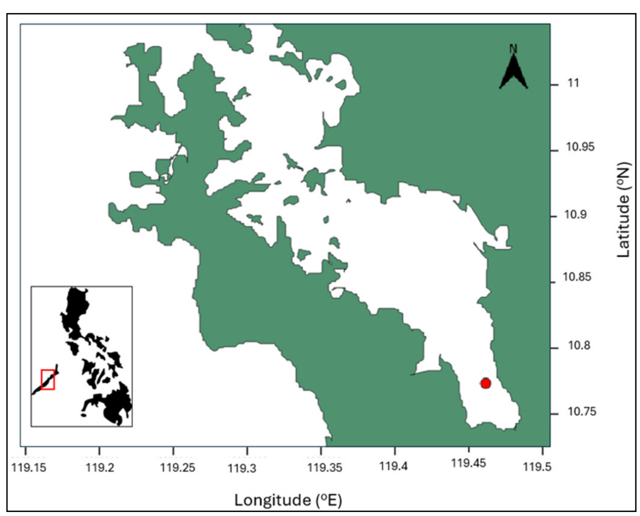

Figure 1. Map of Taytay, Palawan, Philippines showing the sampling site

using clean sea water before dissecting. Gonads were carefully excised and preserved in 70% ethanol before being sent to the histological laboratory for further examination. The specimens were prepared according to the standard protocol of Bancroft and Stevens (1990): they were fixed in Bouin's solution, dehydrated through a graded series of ethanol (80-100%) and xylene, embedded in paraffin wax, sectioned at 7 µm thickness, and stained with hematoxylin and eosin.

Water Monitoring

Salinity and temperature were monitored during sampling using a handheld refractometer (Rocker SA10T and RHSN-10ATC) and digital water thermometer. Average Salinity (29.47 ± 0.31 ppt) and temperature (28.20 ± 0.08 °C) levels are at optimum levels.

Data Analysis

The macroscopic sex ratio deviation from the expected 1:1 ratio was analyzed using a Chi-square test, conducted with SPSS software (version 24). This statistical method was employed to determine whether the observed sex ratio significantly deviates from the expected equal distribution between males and females.

3. RESULTS

The total male: female (1:0.86) macroscopic ratio in this study was significantly different (P = 0.002) from the expected binomial distribution of 1:1. Males dominated the population significantly in the months of October (sex ratio = 1:0.73; P = 0.009) and December 2018 (sex ratio = 1:0.72; P = 0.002) (Figure 3). There were slightly more females in November (sex ratio =1:1.05) and March 2018 (sex ratio = 1:1.03 ;) but a high number of uncategorized individuals were recorded in November also. This might be due to complete spawning during this month.

Out of the 540 samples subjected to histological analysis, only one was preliminary identified as a hermaphrodite (0.18%). This specimen was collected in October 2017, with a shell length of 60

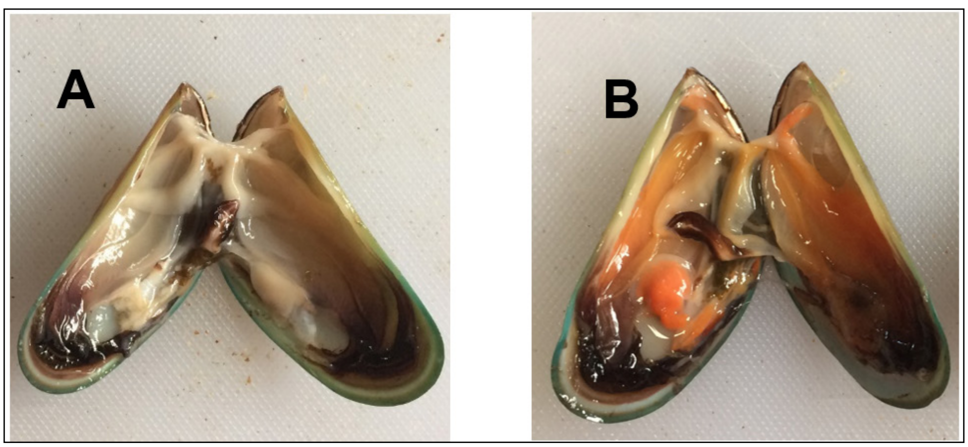

Figure 2. Visual differentiation of male and female green mussel (*Perna viridis*); A = Male with milky creamy white gonad and mantle; B = Female with orange to red-orange gonad and mantle.

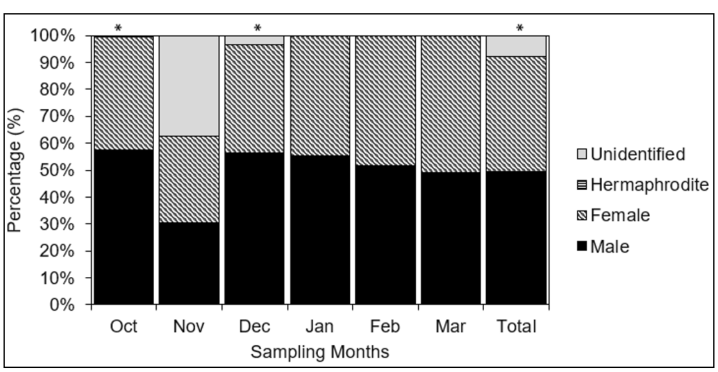

Figure 3. Monthly sex ratio of green mussel from October 2017 to March 2018;
'*' = Chi-Square P value <0.05 as comparing to 1:1 male: female sex ratio.

mm and a total wet weight of 11.32 g. Macroscopically, it appeared male, characterized by the milky creamy white coloration of its gonad and flesh. However, histological examination revealed the presence of both male (spermatocytes and spermatozoa) and female gametes (vitellogenic oocytes) within separate acini, each at different stages of gametogenesis (Figure 4).

4. DISCUSSION

The sex ratio of the green mussels from Malampaya Sound, Taytay, Palawan fluctuates seasonally, as reflected by the findings of Walter (1982). The sex ratio was statistically different from the expected sex ratio of 1:1. Although the same result was reported by Barber et al. (2005), Lee (1988) and Al-Barwani et al. (2013), reported no statistical differences in sex ratio as for other species of mussels, no statistical difference from the expected 1:1 sex ratio was recorded for *Perna canaliculus (*Buchanan 2010; Alfaro et al. 2001), *Perna picta* (Shafee, 1989), *Mytella guyanensis* (Erote et al. 2009) and *Brachidontes pharaoensis* (Abdel et al. 2017). Toralde et al. (2021) examined the sex ratio of green mussels and found that females consistently dominated, accounting for 59% of the population compared to 41% males in Laute Bay, Villareal Bay, and Concabato Bay, Philippines

Previous study related to the reproductive biology of green mussel in the Malapmaya Sound, Taytay Palawan revealed that gonad maturation and spawning of green mussels were influenced by lunar cycle, tidal fluctuations as well as changes in the physicochemical properties of water (Baldevieso et al. 2021). Aside from these, changes in the temperature can affect the sex determination of invertebrates (Korpelainen 1990). For instance, an increase in the number of female individuals of *Crassostrea virginica* (Coe 1963) and *C. gigas* (Lango-Reynoso et al. 2006) was reported during high-temperature seasons (summer). Furthermore, Lango-Reynoso et al. (2006) observed an increase in the number of individuals during winter. However, the effect of temperature on the sex ratio and hermaphroditism in the present study is not clear since only one hermaphrodite sample was recorded and the duration of the study is limited to 6 months.

The rarity of hermaphroditism (1 of 540 samples or 0.18%) in this study closely resembles the findings in Hongkong (<0.1%) (Lee 1988), India (0.8%) (Nagabhushanan et al. 1992), Florida (0.85%) (Barber et al. 2005) and Malaysia (Al-Barwani et al. 2013) for *Perna viridis*. Hermaphroditism was also recorded for other Mytilid species such as *Perna canaliculus* (1 of 6720) (Alfaro et al. 2001), *Mytella* guyanensis (0.2%) (Carpes-Paternoster 2003), (0.67%) (Erote et al. 2009) *Brachidontes pharaonis* (0.33%) (Abdel et al. 2017) and *Perumytilus purpuratus* (1.12%) (Montenegro et al. 2010).

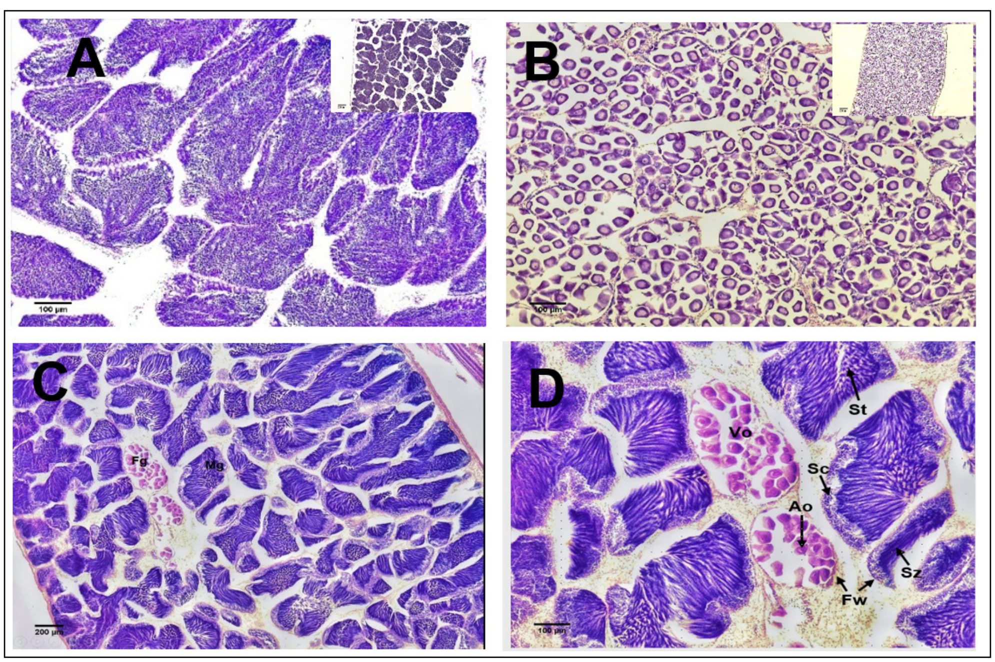

Figure 4. Photomicrograph of normal mature male gonad (A), normal mature female gonad (B), and hermaphrodite gonads of **P. viridis** (C & D); Fg = female gametes, Mg = male gametes, St= spermatids, Sc = spermatocytes, Sz= spermatozoa, Fo = Follicle wall, Vo = Vitellogenic oocyte.

The distribution of gametes in the hermaphrodite sample in this study falls under "Type 1" of Soria et al. (2002), where the gonad contains distinct female and male acini and varying in proportion. This trait together with the dominance of the male part is similar to the findings of Al-Barwani et al. (2013). On the other hand, other mussel species such *Perumytilus purpuratus* (Lamarck 1819) (Montenegro et al. 2010) and *Brachidontes pharaonis* (Abdel et al. 2017) falls under "Type 2" category, where both male and female gametes are present in the same acini. This proves that the type of gamete arrangement on the gonads of hermaphrodite mussels varies among different species.

Gastropods are reported to be simultaneous hermaphrodites with only a few species showing sequential hermaphroditism (Jarne et al. 2006). In comparison, bivalve species showed evidence of sex-change pattern. For instance, Lubet (1959) found that the scallop *Chlamys varia* (Pectinidae) is a protandric species. On the other hand, protogyny was recorded in few species such as the galeommatoids *Kellia suborbicularis* (Laseidae), *Montacuta substriata* (Montacutidae) (Oldfield 1961), freshwater Corbicula clams (Corbiculidae) (Pigneur et al. 2012) and Pacific oysters (*Crassostrea gigas)* (Zhang et al. 2014). In addition, Ruaza 2019 reported the incidence of pseudohermaphrodism *Canarium urceus urceus*.

The sample in the study might be a potential sequential hermaphrodite since the female gametes are atretic or inactive compared to the male gametes. However, there was no conclusive evidence to determine whether this hermaphroditism falls into protandry or protogyny since it is not clear which sex cells became active first. Furthermore, in Parasan Coast, Indonesia, Noor et al. (2019) reported that gonadal tissue development in mussels was first observed at a length of 6.5 mm. By three months of age, the average shell length was 32 mm, which increased to 57 mm by six months. *Perna viridis* in the Philippines were reported to be sexually mature at a shell length of 20-30mm, which was attained after 2-3 months (Yap et al. 1979) of culture. Therefore, the possibility of a series of sex change might have happened several times before the discovery of the sample.

The occurrence of hermaphroditism in green mussels (*Perna viridis*) carries important implications for their reproduction, population health, aquaculture practices, and ecological roles. When mussels can produce both male and female gametes, it has the potential to impact genetic diversity and the population's ability to adapt to environmental changes. In some cases, hermaphroditism might act as a survival strategy, especially in environments where mates are hard to find, helping to ensure the species continues to reproduce even when population numbers are low (Levitan & Petersen, 1995; Ghiselin, 1969). In terms of population dynamics, the presence of hermaphrodites could disrupt the typical balance between males and females, which might affect how successfully the population grows. If the sex ratio becomes skewed, it could lead to a smaller effective population size, putting the genetic health and long-term survival of the species at risk (Luttikhuizen et al. 2011). However, this trait could also help the species remain resilient, allowing it to keep reproducing even under stressful conditions or when mates are scarce (Avise & Mank, 2009). Ecologically, finding hermaphroditic mussels might signal environmental stress, such as pollution or temperature changes. Such conditions can lead to intersex traits, which might indicate underlying environmental problems (Jobling et al. 1998). Moreover, changes in how green mussels reproduce can have ripple effects throughout the ecosystem, potentially altering predator-prey relationships and competition among other filter-feeding species (Sousa et al., 2009). In aquaculture, the presence of hermaphrodites could present challenges. It might introduce unexpected genetic variability, complicating selective breeding programs and making it harder to achieve consistent traits like growth rate and meat quality (Lucas & Southgate, 2012). Additionally, hermaphroditism could affect when and how mussels spawn, potentially impacting the quality and yield of farmed mussels, with possible economic consequences for producers (Bertolini et al. 2009).

5. CONCLUSION AND RECOMMENDATION

Despite the rarity of hermaphroditism in green mussels, this study successfully documented preliminary evidence of its occurrence in the Philippine population. However, further research is needed to understand the underlying causes of hermaphroditism. Given its infrequent nature, ongoing monitoring and sampling of mussel populations are essential to identify and study these rare individuals. If possible, future research should aim to determine whether the hermaphroditism observed is simultaneous or sequential. To obtain a comprehensive understanding, it is also recommended to conduct similar studies from April to November, covering a full year. Additionally, expanding research to other regions in the Philippines could provide insights into how environmental factors, such as water quality, and production practices influence the occurrence of hermaphroditism.

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AUTHORS CONTRIBUTION

The main author, Mr. Adzel Adrian G. Baldevieso, was in charge of the conceptualization, proposal writing, data gathering, laboratory analysis data analysis and drafting of final manuscript. Ms Ma. Shirley M. Golez and Dr. Fiona L. Pedroso contributed to the conceptualization, laboratory analysis, and drafting of final manuscript.

CONFLICT OF INTEREST

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. Furthermore, we know of no conflict of interest associated with this publication, and there has been no significant final support for this work that could influence its outcome. As Corresponding author, I confirm that the manuscript has been read and approved for submission by all named authors.

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