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# Pollutant exposure in Manila Bay: Effects on the allometry and histological structures of *Perna viridis* (Linn.)

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ABSTRACT

**Objective:** To determine the effects of the water quality of Manila Bay on allometric parameters and histological biomarkers of selected organs of *P. viridis*.

**Methods:** Green mussels were collected from two coastal sites of Manila Bay, Las Piñas – Parañaque (LPP) and Bacoor, Cavite (BC). Twenty-four green mussels from each site were used for the assessment of allometric parameters, and six green mussels from LPP and eight from BC were used for the assessment of histological structures of gonads, gut, and digestive glands. Gonad development was categorized into five stages, whereas gut and digestive glands were scored into four categories.

**Results:** Allometric parameters that include shell height, weight, and total wet and dry soft tissue weight were significantly different between LPP and BC. It was also observed that exposure to the pollutants in Manila Bay resulted to delays in gonadal development, and detrimental changes and lesions in the histostructure of digestive gland and gut.

**Conclusions:** Pollutants in Manila Bay have detrimental effects to the growth, reproductive development, and histological structure of digestive organs of *P. viridis*.

## 1. Introduction

Water pollution is one of the biggest problems that our world is facing. Water is considered polluted when certain substances or contaminants are present and makes it unfit for specific purposes. These pollutants are mostly products of anthropogenic activities, specifically as agricultural, domestic and industrial wastes. Common pollutants include heavy metals such as lead, copper, chromium, cadmium, and mercury [1,2]. Moreover, pesticides used in protecting crops and other plants are also considered pollutants once they are introduced in the water system [3].

In the Philippines, many bodies of water are now classified as massively polluted. One of which is Manila Bay, a semi-enclosed marine inlet surrounded by Metro Manila and different municipalities of Cavite, Bulacan, Bataan, and Pampanga [4–6]. At present, it has deteriorating water quality because of the intensified disposal of human wastes [5,6]. Water samples collected from the coastal lagoon of Manila Bay have varying levels of heavy metals [6,7], polycyclic aromatic hydrocarbon (PAH) [8], benzotriazole ultraviolet stabilizers and organophosphorus flame retardants and plasticizers [9,10], and tributyltin (TBT). These water pollutants directly endanger the health of aquatic animals [2] resulting to a decline of marine resource production.

*Perna viridis* (*P. viridis*), commonly known as Asian green mussel or tahong, is one of the marine resources that are harvested in the coastal areas of Manila Bay. Green mussels generally grow on hard surfaces, and is said to be invasive for its wide range of tolerance. However, a recent report showed that there is a decline in the population of this aquatic species [6]. According to DENR (2004), the decline in mollusk production is attributed to the high levels of heavy metals, oil and grease, and suspended solids in Manila Bay. This

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problem in green mussel population tremendously affects the livelihood of people living in the coastal areas of the bay which rely mostly on fisheries and aquaculture [9]. As a common and affordable food and rich source of iodine, green mussels are available in the local markets in the cities and municipalities around Manila Bay.

Aside from the alarming state of *P. viridis* population, this mollusk species is considered as a bioindicator responding well to environmental changes. It was reported that green mussel is an organism suited for biomonitoring studies of coastal waters because of their widespread geographical distribution, sedentary mode of life, and filter feeding mechanism [11,12]. As a filter feeder, they have the ability to store and accumulate several organic and inorganic contaminants [13], which is used for studies in detecting chemical pollutants in the coastal waters and their possible effects to the aquatic organism. Pollution impacts to organisms can be assessed or measured through biomarkers at the cellular, histological, molecular, biochemical or physiological level [11]. The abundance of *P. viridis* in Manila Bay is a good criterion for its use as a bioindicator, specifically in determining the effects of chemical pollutants present in the bay on bivalves. This will clarify and explain the reports on the gradual decline of mollusk production, which vehemently results to economic losses in the aquaculture sector. At present, no other studies were conducted on determining the health and growth status of green mussels in Manila Bay.

Hence, this study was conducted to determine the effects of the water quality of Manila Bay on allometric parameters and histological biomarkers of selected organs of green mussels (*P. viridis*). Specifically, this study assessed shell and soft tissue allometric parameters and histological structures of the gonads, gut and digestive glands of *P. viridis* collected from Manila Bay.

## 2. Materials and methods

### 2.1. Sampling of green mussels (*P. viridis*)

Green mussels (*P. viridis*) were collected from two coastal sites of Manila Bay, Las Piñas – Parañaque (LPP) and Bacoor, Cavite (BC). Form each site, 30 green mussels were randomly collected by the local fisherman. The collected samples were placed in a container with water from the Bay, and were immediately transported to the Science Laboratory of Las Piñas National High School (LPNHS) for assessment.

### 2.2. Taxonomic identification of *P. viridis*

The taxonomic identification of green mussels collected from two sampling sites of Manila Bay was based on the certification of the Zoology Division, National Museum of the Philippines.

### 2.3. Assessment of allometric parameters

Twenty-four green mussels from each site were used for the assessment of allometric parameters. Using a ruler, the following shell dimensions were measured in centimeters: shell length (SL) measured from anterior to posterior; shell width (SWI) measured in the lateral edge; and shell height (SH) measured from dorsal to ventral [14]. Shell volume (SV) was computed using this formula:

$$\text{Shell volume (SV)} = \text{SL} \times \text{SWI} \times \text{SH}$$

Using an electronic weighing scale, shell weight (SWE), total wet (WWE) and dry soft tissue weights (DWE) were measured in grams [14]. The wet soft tissue was weighed 10 min after it was removed from the container full of water [15]. The dry soft tissue on the other hand, was weighed after the soft tissues were dried for at least 60 °C for 48 h [16].

Consequently, condition index (CI) was computed by dividing total dry soft tissue weight in grams to shell volume in cubic centimeters multiplied to a constant 1 000 [14].

$$\text{CI}\left(\text{g/cm}^3\right) = \frac{\text{Total dry soft tissue weight (g)}}{\text{Shell volume (cm}^3\text{)}} \times 1\,000$$

### 2.4. Histopathological assessment

Six green mussels from LPP and eight from BC were used for the assessment of histological structures. Gonads and guts with digestive glands were dissected out and were placed in 10% buffered formalin. The samples were brought to the Philippine Kidney Dialysis Foundation Institute for histopathological slide preparation. Each organ was stained with hematoxylin and eosin. Histological slides were observed under an inverted microscope at 200× and 400×.

The histopathological structure of gut epithelium and digestive gland were scored based on the following observed

**Table 1**

Allometric parameters of *P. viridis* collected from two sampling sites in Manila Bay ($n$ = 24).

| Allometric parameters | Sampling sites (Manila Bay) | |
|---|---|---|
| | Las Piñas – Parañaque Coastal side (LPP) | Bacoor, Cavite Coastal side (BC) |
| Shell length (SL) (cm) | 5.54 ± 0.47[a] | 5.50 ± 0.99[a] |
| Shell width (SWI) (cm) | 1.80 ± 0.21[a] | 1.79 ± 0.38[a] |
| Shell height (SH) (cm) | 2.56 ± 0.25[a] | 2.74 ± 0.25[b] |
| Shell volume (SV) ($cm^3$) | 25.61 ± 5.53[a] | 26.86 ± 8.24[a] |
| Shell weight (SWE) (g) | 5.51 ± 1.10[a] | 6.66 ± 1.93[b] |
| Total wet soft tissue weight (WWE) (g) | 3.81 ± .071[a] | 4.43 ± 0.78[b] |
| Total dry soft tissue weight (DWE) (g) | 2.78 ± 0.52[a] | 3.17 ± 0.66[b] |
| Condition index (CI) ($g/cm^3$) | 113.01 ± 30.48[a] | 131.98 ± 64.89[a] |

Values are given as mean ± SD. $n$ = Number of *P. viridis*. Values in the same row with different superscript letters are significantly different ($P$ < 0.05).

**Table 2**

Percentage of green mussels in each gonad development stages collected from two sampling sites in Manila Bay (%).

| Gonad developmental stages | Male | | Female | |
|---|---|---|---|---|
| | LPP ($n$ = 3) | BC ($n$ = 3) | LPP ($n$ = 3) | BC ($n$ = 5) |
| Stage 1 = unidentified | 0 | 0 | 0 | 0 |
| Stage 2 = developing | 33.33 | 0 | 66.67 | 20.00 |
| Stage 3 = mature | 0 | 0 | 33.33 | 0 |
| Stage 4 = spawning | 66.67 | 100.00 | 0 | 60.00 |
| Stage 5 = post-spawning | 0 | 0 | 0 | 20.00 |

LPP: Las Piñas – Parañaque Coastal side; BC: Bacoor, Cavite Coastal side. $n$ = Number of P. viridis.

characteristics: 0 – no recognizable lesions; 1 – few lesions; 2 – adequate number of lesions; and 3 – several/many lesions [17].

Furthermore, gonads were assessed based on the following developmental stages: Stage I – undifferentiated gonad; Stage II – early development stage; Stage III – mature stage; Stage IV – spawning stage; and Stage V – post-spawning stage [18].

### 2.5. Statistical analysis

Statistical differences of data on allometric parameters among groups were analyzed using $t$ test. Data were presented as mean ± SD (standard deviation), and were significant at $P$-values < 0.05. The statistical test was conducted using Microsoft Excel.

## 3. Results

### 3.1. Allometric parameters

Table 1 shows that there are no significant differences in the shell length (SL), shell width (SWI), shell volume (SV), and condition index (CI) of green mussels in Las Piñas – Parañaque (LPP) and Bacoor, Cavite (BC) coastal side. However, significantly lower mean shell height (SH), shell weight (SWE), total wet soft tissue weight (WWE), and total dry soft tissue weight (DWE) were recorded in green mussels collected from LPP compared to samples collected from BC.

### 3.2. Histopathological assessment

The percentage of green mussels in each gonad developmental stages is shown in Table 2. There is a higher percentage of male green mussels with advanced gonad developmental stage from BC compared to samples from LPP. It was recorded that 100% of male green mussels from BC is on the spawning stage (Stage 4), whereas 33.33% of males from LPP are in developing stage (Stage 2) and only 66.67% are in spawning

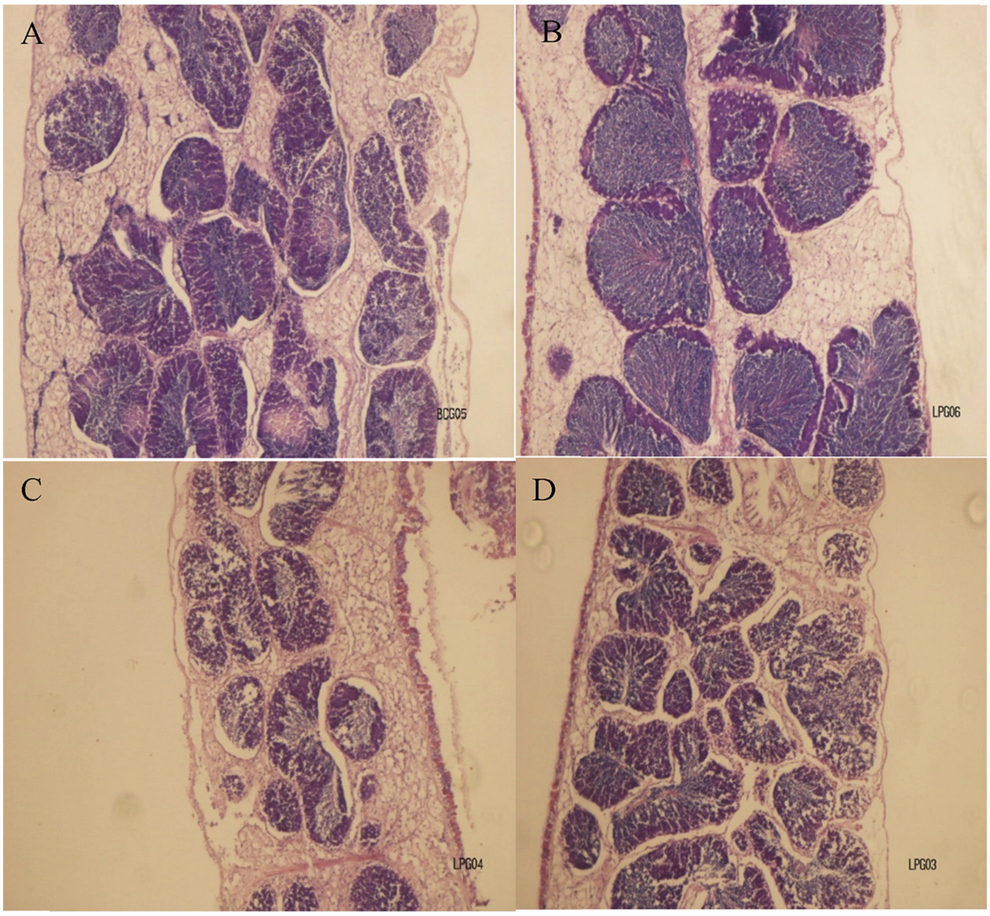


**Figure 1.** Male gonads of green mussels from two sampling sites at 200× magnification.
Bacoor, Cavite sampling site: (A) Stage IV gonad. Las Piñas – Parañaque sampling site: (B) Stage II gonad; (C & D) Stage IV gonad.

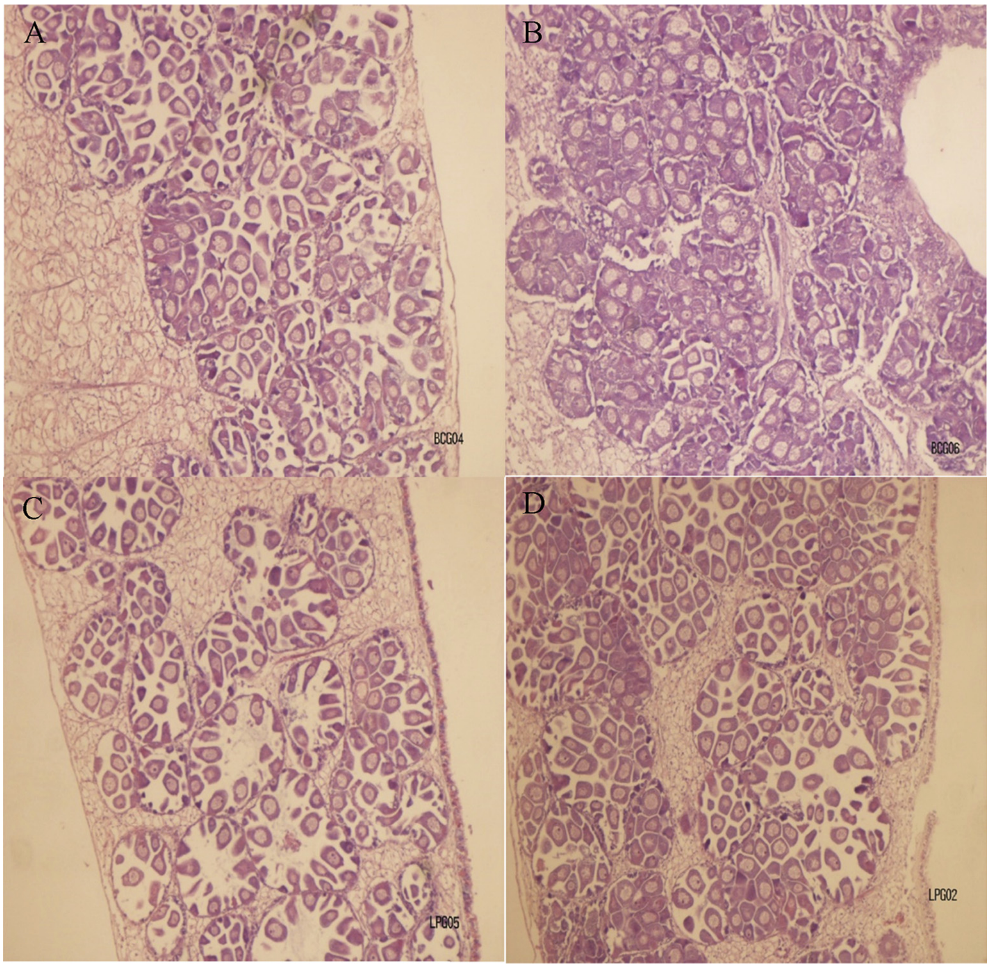


**Figure 2.** Female gonads of green mussels from two sampling sites at 200× magnification.
Bacoor, Cavite sampling site: (A) Stage II gonad; (B) Stage IV gonad. Las Piñas – Parañaque sampling site: (C) Stage II gonad; (D) Stage III gonad.

stage (Stage 4). Male green mussels in the developing stage have gonads in early spermatogenesis [18]. Representative photomicrographs of male gonads from each sampling sites are shown in Figure 1.

Same observations were also recorded in female green mussels. Among the collected samples from BC, about 20% of females are in developing stage (Stage 2), 60% in spawning stage (Stage 4), and 20% in post-spawning stage. In LPP samples, 66.67% of females are in developing stage (Stage 2), and only 33.33% are matured (Stage 3). Females in stage 2 have gonads in early vitellogenesis, and mussels in Stage 3 have gonads in late vitellogenesis [18]. Representative photomicrographs of female gonads from each sampling site are shown in Figure 2.

Table 3 shows the percentage of green mussels in each histopathological scoring of gut epithelium and digestive gland. Samples from both Manila Bay coastal areas have lesions in the gut epithelium and digestive glands. However, there is a higher percentage of green mussels from BC with gut epithelium having lesions and abnormal histological structure compared to samples from LPP. About 33.33% of green mussels from BC have gut epithelium with adequate number of lesions (score 2) and 16.67% of mussels with several lesions (score 3). No green mussels from LPP have several lesions, however the percentage of samples with adequate number of lesions from this sampling site is similar to BC. Representative photomicrographs of gut epithelium from each sampling site are shown in Figure 3.

In contrast to the findings in gut epithelium, severe cases of lesions and necrotic tissues were observed in the digestive glands of green mussels from LPP. About 83.33% of green mussels from this site have digestive glands with several lesions (Stage 3) compared to the 60% of green mussels from BC.

**Table 3**
Percentage of green mussels in each histopathological scoring of gut epithelium and digestive gland (%).

| Histopathological scoring | Gut epithelium | | Digestive gland | |
|---|---|---|---|---|
| | LPP ($n$ = 6) | BC ($n$ = 6) | LPP ($n$ = 6) | BC ($n$ = 5) |
| 0 = no recognizable lesions | 66.67 | 50.00 | 16.67 | 0 |
| 1 = few lesions | 0 | 0 | 0 | 0 |
| 2 = adequate number of lesions | 33.33 | 33.33 | 0 | 40.00 |
| 3 = numerous/many lesions | 0 | 16.67 | 83.33 | 60.00 |

LPP: Las Piñas – Parañaque Coastal side; BC: Bacoor, Cavite Coastal side. $n$ = Number of *P. viridis*.

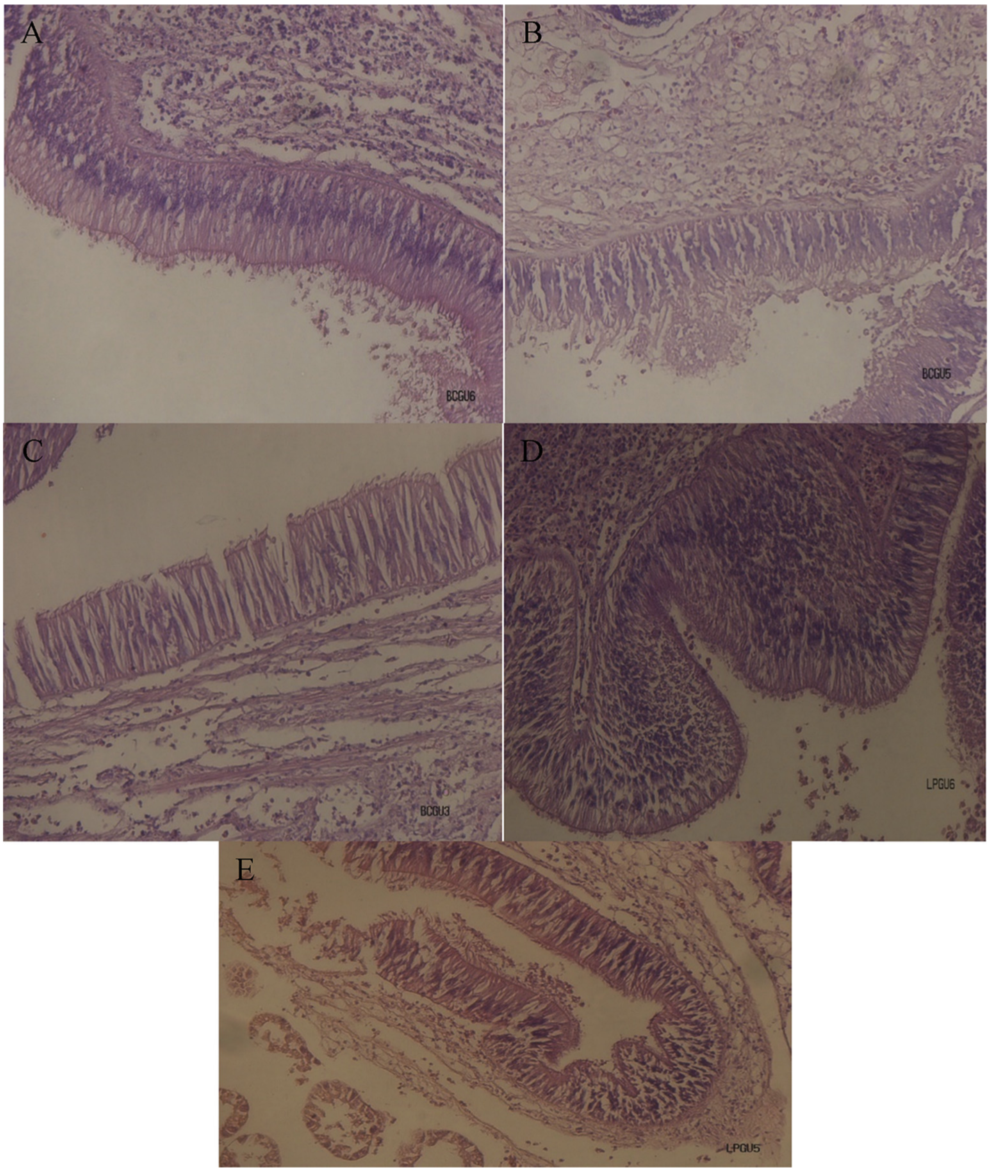


**Figure 3.** Gut epithelium of green mussels from two sampling sites at 200× and 400× magnification.
Bacoor, Cavite sampling site: (A) Gut epithelium with no lesions (Score – 0); (B) Gut epithelium with adequate number of lesions (Score – 2); (C) Gut epithelium with several lesions (Score – 3). Las Piñas – Parañaque sampling site: (D) Gut epithelium (Score – 0); (E) Gut epithelium (Score – 2).

However, about 40% of green mussels from BC have digestive glands with adequate number of lesions. Representative photomicrographs of digestive glands from each sampling site are shown in Figure 4.

## 4. Discussion

Growth was seen affected by the water quality of Manila Bay, specifically shell and total soft tissue weight. One of the factors that tremendously affected growth and development of mussels and other bivalves is oxygen depletion in the water. Substantial increments in organic loads entering the bay through excessive urban emissions of nutrients (nitrogen and phosphorus), could lower the concentration of dissolved oxygen at the bottom, specifically in the inner bay area [5]. Other factors that cause hypoxia and anoxia include heavy metals incidences, frequent blooms of harmful microalgae and persistent red tides caused by dinoflagellates [19]. The lower allometric parameters of *P. viridis* from LPP compared to BC can be due to a more hypoxic and anoxic marine environment.

High levels of heavy metals in the bodies of water can be the major factor for the decreased growth of green mussels. According to a study, *P. viridis* collected from Muar, Peninsular Malaysia had lower total dry weight of soft tissues and condition index than those collected from Sebatu, Peninsular Malaysia [14]. These results could probably be connected to the higher levels of cadmium, copper, and lead in the coastal sediments of Muar compared to Sebatu. Moreover, a reported study mentioned that the sublethal exposure of the freshwater swan mussel (*Anodonta cygnea*) to chromium, copper, and lead resulted to severe changes in shell composition and morphology [20]. This report strengthens the results of this study, because the heavy metals that were mentioned are detected in Manila Bay, which could probably alter the shell dimensions of mussels.

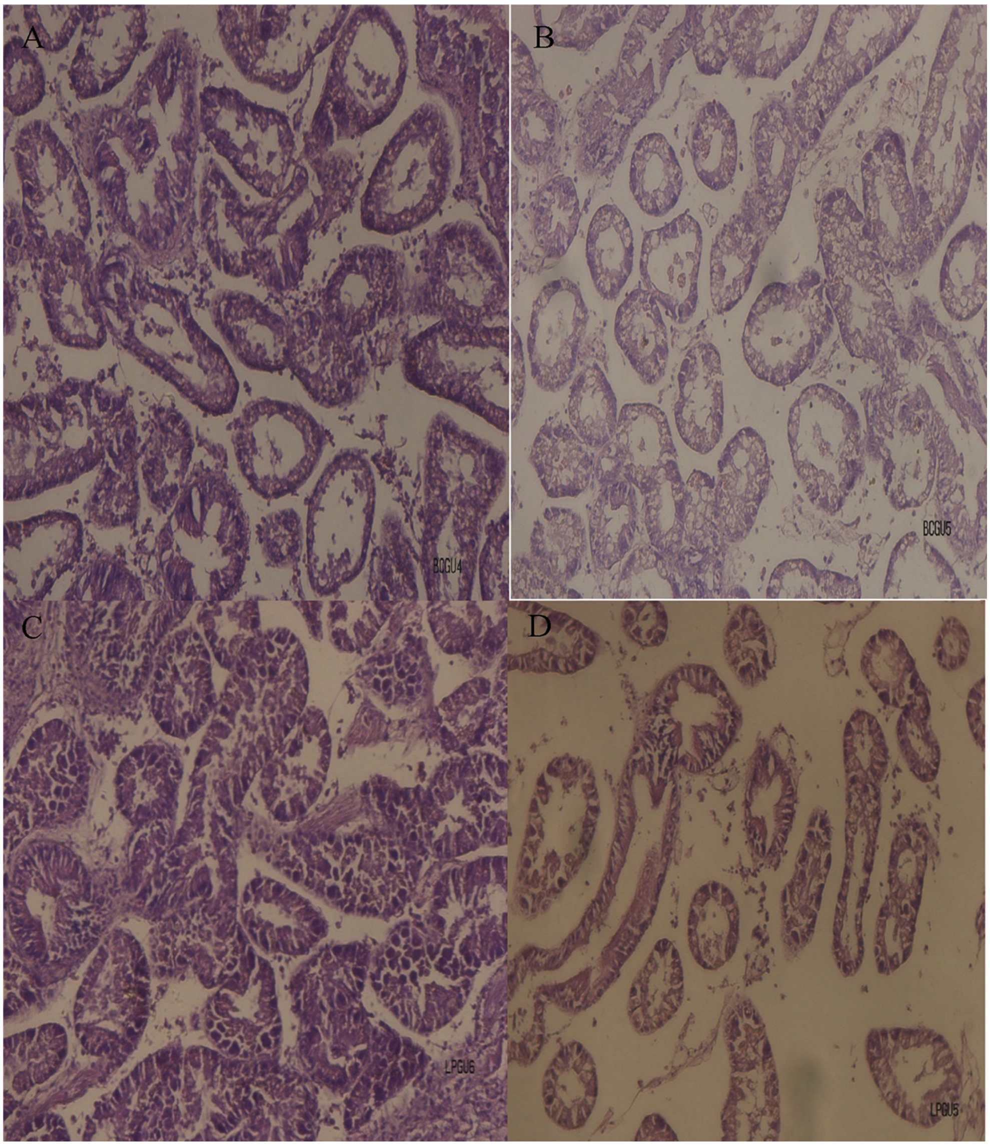


**Figure 4.** Digestive glands of green mussels from two sampling sites at 400× magnification.
Bacoor, Cavite sampling site: (A) Digestive glands with adequate number of lesions (Score – 2); (B) Digestive glands with many lesions (Score – 3).
Las Piñas – Parañaque sampling site: (C) Digestive glands with few lesions (Score – 1); (D) Digestive glands (Score – 3).

Organic contaminants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), which are present in Manila Bay, were also reported to affect the bivalve's growth and development. These contaminants affect primarily the rates of respiration and carbon turnover in mussels, which have high correlation to inhibited growth [21].

Pollutants in both sampling sites of Manila Bay can cause endocrine disruption. These chemicals that include heavy metals and organic pollutants disrupt the activities of androgen and estrogen. Delays in the gonad developmental stages of mussels in this study are the result of the irregular activities of estrogen and androgen receptors [22]. It must be noted that the effects on hormonal activity depends upon the level of chemical pollutants in the environment.

It was reported that aside from the adverse effects of organic contaminants to growth, these pollutants disrupt normal reproduction, fecundity and embryonic development of blue mussel (*Mytilus edulis*) and soft shell clam (*Mya arenaria*) [21]. Heavy metals, which are endocrine disruptors, accumulate in the gonads, adductor muscle, mantle, and foot of mussels [23]. It was mentioned in a study that the mussels (*Mytilus edulis*) collected from the polluted and contaminated waters of Bothnian Sea had abnormal histopathological alterations in the gonads, resulting to abnormal reproduction and spawning [24]. Specifically, it was observed that follicles were in low quality and sperm cells undergo cytolysis. These reports support the findings of this study that pollutants have deleterious effects to gonadal development of mussels.

Aside from gonads, the gut and digestive glands are the most delicate organs to pollutant exposure. Pollutants and contaminants in Manila Bay cause degeneration of cells resulting to lesions and abnormal histological structure of these organs [17]. The digestive glands and gut are the major organs of metabolism and detoxification. The lysosomes in the cells of digestive glands act as major defense lines against the toxic effects of pollutants. However, if the digestive glands cannot manage massive levels of contaminants, defects are observed on the functions of lysosomes [25].

Aside from delayed and abnormal gonadal development, mussels collected from the polluted waters of Bothnian Sea were reported to have abnormal histopathological alterations in the digestive diverticula [24]. Cytoplasmic erosions were observed in the digestive cells of these mussels. Furthermore, mussels collected from the polluted water of Basque Coast, which has recorded levels of inorganic and organic contaminants, had inflamed and damaged digestive glands [26]. These reports support the findings of this study that pollutants could affect the histostructure of the gut and digestive gland.

In general, this study demonstrated that the chemical pollutants and contaminants in Manila Bay, which were already identified by other research studies, have detrimental effects to some of the growth parameters of green mussels that include shell height, weight, and total soft tissue weight. Moreover, pollutant exposure resulted to delays in gonadal development, and caused lesions and abnormal histological structure of digestive glands and gut in green mussels.

## Conflict of interest statement

We declare that we have no conflict of interest.

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