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Geometric morphometric analysis on the shell of green mussel *(Perna viridis)* from two culturing sites in Cavite province

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ABSTRACT
Green mussel (*Perna viridis*), locally known as 'tahong', has been a valuable source of food and livelihood in the country. However, environmental stress is also continually paving its way in posing a threat to the marine environment especially in most culturing sites of green mussel. Morphological plasticity could be used as an indicator of the changes in the marine environment. Thus, the study determined morphological variations on green mussel's shell using geometric morphometric analysis and their possible relationship to the physicochemical parameters from two culturing sites in Cavite Province. The geometric morphometric analysis was used to statistically analyze shape variation within and among samples based on their digitized landmarks and the Procrustes superimposition to remove size difference. In addition, the physicochemical parameters such as temperature, pH, salinity, and turbidity were determined. Results showed that the dimension of the shell in terms of shape was relatively similar to both sites. Also, the physicochemical parameters from both culturing sites were relatively similar. Moreover, the Principal Component Analysis, Canonical Variate Analysis, and Discriminant Analysis showed that there was no morphological variation and no significant difference in the digitized landmarks on the green mussel shells from both collecting sites in one collection *($p<0.0001$)*. Furthermore, there was no relationship between the geometric morphometrics of *P. viridis* and the physicochemical parameters from the two culturing sites. This implies that the shell morphology of green mussels in terms of shape might have a high tolerance to the different parameters in the marine environment.

Keywords: aquaculture, environmental stress, shape variation , shell morphology, tahong.

1. Introduction

P. viridis, commonly known as green mussel, is one of the most beneficial mariculture organisms produced with the largest quantity and lucrative products [10]. It is frequently found in densities in any underwater marine object. They typically glue themselves to hard substrata and able to settle in an artificial environment such as boats, bridges, seawalls, pier pilings, etc. they can also be found attached to red mangrove roots [4]. In the Philippines, green mussels were originally regarded as by oyster farmers and bamboo fish-trap operators in Cavite Province. It was only in the early 1950s that their value as a primary crop was recognized and since then the industry has proliferated [13].

It is considered a valuable food source for human consumption, due to its high-quality protein and well-balanced nutritional make-up [16]. They can rapidly make solid colonies in a range of environmental settings [11]. Green mussels form the basis for the important aquaculture industry in its natural tropical waters in Asia-Pacific and Indo -Pacific regions making it widely-distributed and available [3].

It is also known to have the capability of bioaccumulating toxins which allows it to be utilized as a bioindicator in signaling pollution caused by different compounds and substances such as organochlorides, petroleum products, and heavy metals [4].

Meanwhile, Geometric morphometrics (GM) has progressively become a valuable means in studying and evaluating shape variation in a broad diversity of taxa [5]. It is a powerful statistical and analytical tool for shape analysis that has become increasingly widespread in anthropological research [1]. It has been utilized in several fields where quantifying morphological variation is of concern and relevance (e.g. biology, engineering, medicine, and paleontology) [5]. The diverse and extensive scope of questions such as assessment of phenotypic differences amongst taxa [12], the examination of relationships between phylogeny and shape [12, 7], the distribution and shape throughout geographic space [6], and the making of ancestral morphotypes' reconstructions [22]. With these, geometric morphometrics processes information on the patterns of variations and the relationships in individual variables.

In mussels, the shape of the shell is greatly affected by density and growth rate [9]. In the study by [17, 23], showed that environmental factors such as density, trophic conditions, wave impact, and water depth influenced bivalve shape. Studies showed that the shell shape in the clam is density-dependent wherein the dorso-ventral shell axis or height is mostly influenced by space competition [12, 14]

These variations can be also effectively validated through geometric morphometrics. Thus, determining the variation in the geometric morphometrics could give us information on the effects of these factors on the development of the shell.

This study investigated the morphometric shell shape variations on the green mussel shells from culturing sites in Bacoor and Cavite City by geometric morphometric analysis. In addition, attempts were made to determine the relationship between the geometric morphometric analysis of the shells of green mussels and the physicochemical parameters from the two culturing sites.

2. Materials and methods

2.1. Sampling Sites

The study was conducted within October 2018 in two culturing sites in Cavite namely in Bacoor and Cavite City .

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Figure 1. Map of Cavite Province showing the two sampling sites in Bacoor (blue pin) and Cavite City (green pin).

Site 1 is situated in Sineguelasan, Bacoor, Cavite (N14° 28.315'E120° 56.159). There are several residents and privately-owned areas of mussel culture. The water was greenish to brownish with a lot of debris and plastics due to many residential houses nearby. Plastics and other pollutants were almost everywhere. On the other hand, Site 2 is located in Binakayan, Cavite City (N14° 28.475'E120° 55.032). It is near the Binakayan Demonstration Farm in which green mussels are present. The water in Site 2 was green in color. It was clearer and has less debris and plastic than Site 1. Few water lilies can be seen or noticed.

2.2. Sampling collection

The collection was in October 2018. One collection trip was done for a month. A total of 300 samples were collected from the two sites each having three stations. On each station, a total of fifty mussels were collected randomly by hand (n = 50). Sampling tools included a collecting net and cooler. Immediately after the collection, the muscle abductor was all removed. The collected shell samples were cleaned off the epiphytes, debris, and epizoans. In the laboratory, samples were further cleaned under running freshwater and air-dried before further processing.

2.3. Geometric morphometric analysis of green mussel shells

2.3.1. Photo documentation of samples

Collected shell samples were labeled according to their site and date of collection. Each shell was photography photographed using a digital camera (Sony DSC-W800, RS Components, Midrand, South Africa) with known calibration (10 cm). Photographs were then process using ImageJ® 1.49 version computer.

2.3.2. Digitization of landmarks and removing size differences

After this, procedures of the digitization of landmarks on the photographs followed [18]. Digitization includes the actual laying of landmarks (the first landmark was the highest tip of the rounded part; the second landmark was the aligned part with ligament; the third landmark was the lowest end of the beak and the fourth landmark was the hinge of each green mussel shell samples) using tpsUtil and tpsDig programs. After this, size differences were then removed by the Procrustes superimposition using PAST (Paleontological Statistics) software [8]. The digitized landmarks from each shell sample were considered as principal component scores and/or coordinate points which record the relative positions anatomically-definable locations in the Cartesian plane.

2.4. Physicochemical characterization

In each site, temperature (°C), salinity (psu), and pH were measured using a handheld multiparameter probe meter (YSI, Yellow Spring, Ohio, USA) and turbidity (m) was measured using Secchi disc.

2.5. Statistical analysis

Principal component analysis (PCA) was used to determine the similarity or difference in their morphology-based on landmarks generated by the Geometric Morphometrics. The same data set was subjected to discriminant analysis (DA) to determine the morphometrical shell shape variation among the collected green mussel shells and canonical variate analysis (CVA) to determine if there is a significant difference among collected green mussel shells. Furthermore, the relationship between physicochemical parameters and the data from the Procrustes superimposition were analyzed using PCA. The statistical analysis above was done using PAST (Paleontological Statistics) software [8].

3. Results and discussion

3.1 Geometric morphometric analysis of green mussel shells (*P. viridis*) from the two culturing sites

Most of the digitized green mussel landmarks overlap each other with regards to convex hulls and 95% confidence ellipses (Figure 2a and 2b, respectively). This might indicate that almost all of the collected green mussel shells from the two culturing sites in Cavite Province are very similar to each other. This clustering was mainly due to landmarks on the hinge and dorso-distal areas with the moderately positive loadings for the X and Y axis of landmark 2 and 3 (0.4773 and 0.4217 loadings, respectively) and moderately negative loading for the X and Y axis of landmarks 2 and 4 (-0.4896 and -0.5565 loadings, respectively). Using PCA, there was no morphological shell shape variation in the collected green mussel shells within the two culturing sites in Cavite Province because both the convex hulls and 95% confidence ellipses overlapped each other.

Moreover, 100% of the total variance can be explained by the first principal component (PC 1) alone (eigenvalue = $9.53711x10^{-5}$). This might indicate that the shape variation within the two culturing sites at Cavite Province during the sampling collections was low thus shell shape between the two sites was similar.

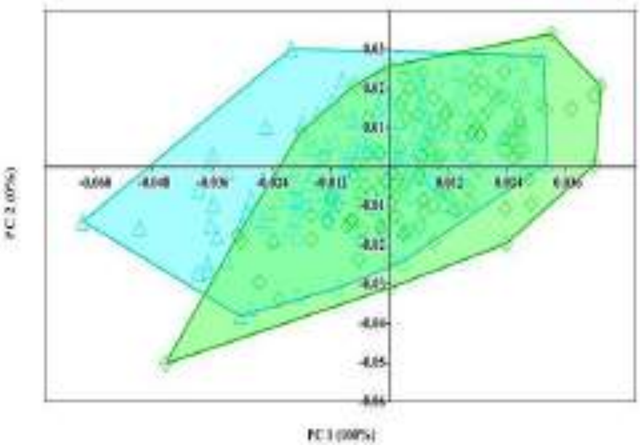
In CVA, the results show that almost all the bars overlap each other indicating that there were no significant shell shape changes among the collected green mussel shells from the culturing sites both in Bacoor and Cavite City ($p<0.0001$) (Figure 3). It can be inferred that there was no significant shell shape variation among the collected individuals with 10, 000 permutation tests done for pairwise distances.

Furthermore, DA showed that there were no significant differences among populations with a discriminant score of 68.79% wherein, almost all of the bars in the histogram are overlapping (Figure 4). It may also imply that the variance is low among the studied green mussel shells between the two culturing sites.

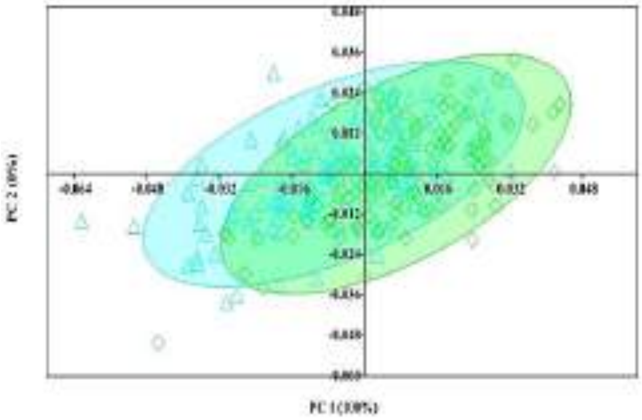
Results were in contrast with [15], wherein it showed significant difference among collected mottled rabbitfish, *Siganus fuscescens,* where all pairwise comparisons between microsatellite Clusters 1, 2A, and 2B were significant (Wilk's lambda = 0.114; Pillai trace = 1.320; $p< 0.0001$).

4.2. Relationship between the geometric morphometric analysis of the shells of green mussels (*Perna viridis*) and the Physicochemical Parameters from the Two Culturing Sites

The physicochemical parameters from Bacoor, in terms



a) Convex hulls



b) 95% ellipses

Figure 2. Scatter plot diagram in (a) convex hulls and (b) 95% ellipses of digitized green mussel landmarks from Bacoor and Cavite City Collections, showing Principal Components 1 and 2.

of temperature, was 29.24±0.36°C, pH was 8.38±0.1, salinity was 24.08±1.42 psu and turbidity was 2.58±0.74 m. While Cavite City in terms of temperature was 21.93±0.32°C, pH was 7.98±0.09, salinity was 21.52±0.00 psu, and turbidity was 3.27±1.07 m. It shows that Bacoor recorded the highest readings of physicochemical parameters than in Cavite City.

In the study of [21], a modified category of a farming site for green mussels with different physicochemical parameters was evaluated. From their results, the temperature ranges 28.5 -30.3°C, pH 7.67-8.46, salinity 24.49-27.31 psu, and turbidity 1.9 to 4.15 m. This kind of farming site was suitable for green mussel farming and highly recommended.

Perna viridis exhibit fast growth and early onset of maturity and temperature and salinity are factors that were crucial for population dynamics [20, 19]. It can be implied that changing pH and salinity levels might contribute to the elongated shell shape of green mussels because of the calcium carbonate ($CaCO_3$) present both in the sediments and/or the water affecting and constituting their shell shape build-up [2].

Moreover, the biplots of physicochemical parameters showed to be located in different coordinates with the digitized landmarks of the shells from both culturing sites (Figure 5). This might imply that physicochemical parameters might not affect the shell morphology in green mussels at the two culturing sites in the province of Cavite. As green mussels might have a wide tolerance for changing marine environments.

The researchers strongly recommend additional sites in the area to be studied, nutrient water analysis, and additional landmarks on the green mussel shell. Further study on the presence of water lilies might affect the growth of green

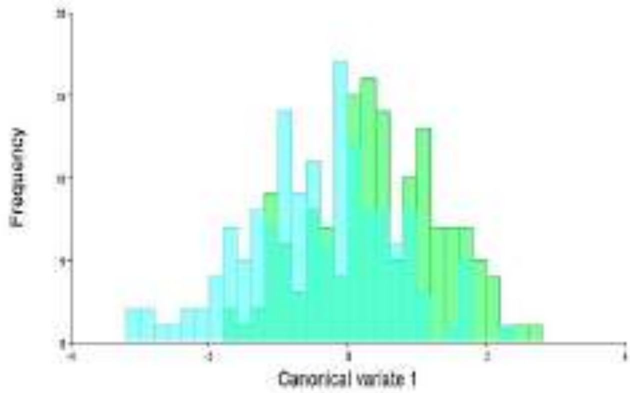


Figure 3. Canonical Variate Analysis of the digitized landmarks from the collected individuals from both Bacoor and Cavite City.

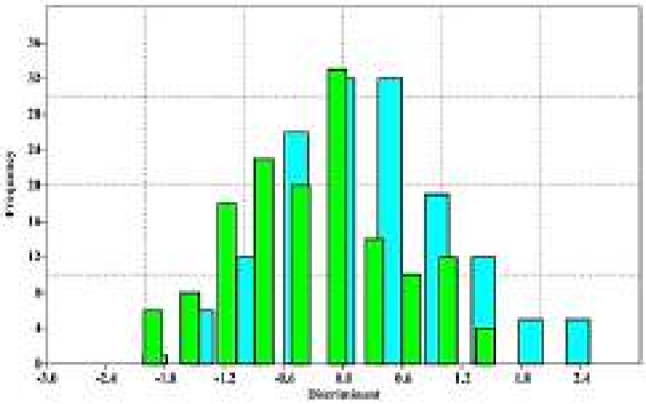


Figure 4. Discriminant Analysis of the digitized landmarks from the collected individuals from both Bacoor and Cavite City.

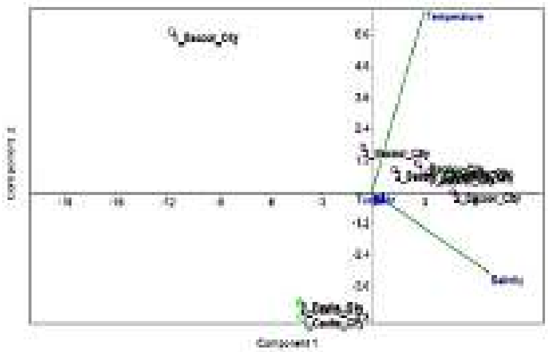


Figure 5. Principal Component Analysis of the Physicochemical Parameters and the Digitized Landmarks of *Perna viridis* Collected from the Two Culturing Sites in Cavite Province.

mussels. In addition, comparative growth analysis on both green and brown mussels as possible resource competition should be recommended.

4. Conclusions

The geometric morphometric analysis of green mussels between two culturing sites appeared to have low variance having minimal differences.

The results of PCA showed no morphological differences based on the digitized landmarks on the shape of the green mussel shells from the two culturing sites. Validating by, CVA and DA, it showed no significant differences in shell shape variation among the collected green mussel shells from culturing sites *(p<0.0001)*.

The physicochemical parameters between the two culturing sites in Cavite Province were relatively similar. There is no relationship between the geometric morphometrics of green mussels and the physicochemical from the two culturing sites. This might imply that the shell morphology of green mussels might have a high tolerance to the different physicochemical parameters in the marine environment.

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