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MORPHOLOGICAL VARIATIONS IN MUD CLAM *Polymesoda erosa* (Lightfoot) USING FLUCTUATING ASYMMETRY ANALYSIS IN LA UNION, CABADBARAN CITY, AGUSAN DEL NORTE, PHILIPPINES

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ABSTRACT

A geometric morphometric study was conducted on the population of *Polymesoda erosa* in La Union, Cabadbaran City, to investigate the morphological variations using fluctuating asymmetry (FA) analysis. FA acts as a reliable indicator of ecological stress and developmental instability since it detects genetic or environmental changes that an organism experiences. A total of ninety (90) individual mud clams were randomly collected and analyze. Water and soil samples were also collected to gather data on the degree of body variations and the impact of external factors on *P. erosa.* The result of the analysis using the SAGE software revealed a highly significant fluctuating asymmetry (P 0.0001). Significant variations in individuals, sides, and the interaction between individuals and sides symmetry were mostly responsible for the substantial variances seen in mud clam species. Particularly, landmarks 10 (dorsal margin maxima) and 13 (anterior margin maxima) exhibited notable variations. For the water quality results, the mud clam's internal valve was likely to be relatively unaffected or minimally impacted, but the level of nickel was high. In conclusion, the fluctuating asymmetry of *P. erosa* can be attributed to a stressed environment. Unfavorable environmental factors cause an organism's morphology to change, affecting its symmetry.
Keywords: fluctuating asymmetry, morphological variation, mud clam, *Polymesoda erosa*

Variasi Morfologi Klam Lumpur Polymesoda erosa (Lightfoot) menggunakan Analisis Fluctuating Asymmetry di La Union, Cabadbaran City, Agusan Del Norte, Filipina

ABSTRAK

Kajian morfometri geometri dilakukan pada populasi Polymesoda erosa *di La Union, Kota Cabadbaran, untuk mengetahui variasi morfologi dengan menggunakan analisis fluktuasi asimetri (FA). FA bertindak sebagai indikator yang dapat diandalkan mengenai tekanan ekologi dan ketidakstabilan perkembangan karena mendeteksi perubahan genetik dan lingkungan yang dihadapi suatu organisme. Sebanyak sembilan puluh (90) individu kerang lumpur dikumpulkan dan dianalisis secara acak. Sampel air dan tanah juga dikumpulkan untuk mengumpulkan data mengenai derajat variasi tubuh dan dampak faktor eksternal terhadap P. erosa. Hasil analisis menggunakan software SAGE menunjukkan adanya fluktuasi asimetri yang sangat signifikan (P 0,0001). Variasi yang signifikan dalam simetri individu, sisi, dan interaksi antara individu dan sisi sebagian besar bertanggung jawab atas variasi substansial yang terlihat pada spesies kerang lumpur. Khususnya, landmark 10 (dorsal margin maxima) dan 13 (anterior margin maxima) menunjukkan variasi yang mencolok. Untuk hasil kualitas air, katup internal kerang lumpur kemungkinan besar tidak terpengaruh atau terkena dampak minimal, namun kadar nikelnya tinggi. Kesimpulannya, fluktuasi asimetri* P. erosa *dapat dikaitkan dengan lingkungan yang tertekan. Faktor lingkungan yang tidak menguntungkan menyebabkan morfologi organisme berubah sehingga mempengaruhi simetrinya.*
Kata Kunci*: asimetri fluktuasi, variasi morfologi, kerang lumpur, Polymesoda erosa*

INTRODUCTION

The mud clam (*Polymesoda erosa*) is a commercially important bivalve species in the Philippines, providing livelihood opportunities for coastal communities and serving as a key component of estuarine ecosystems (Borlongan *et al.*, 2017). However, little is known about the morphological variations of this species, which can provide valuable information on its biology and ecology.

Science has been able to explain how species have changed over time by using certain theories. The morphological characteristics have an impact on how a species grows and develops in relation to its surroundings. Geometric morphometrics (GM) is one of the recognized scientific methods for linking environmental conditions to a species' morphology (Jumawan *et al.*, 2016). It is the quantitative representation and analysis of morphological shape using geometric coordinates instead of measurements (Polly, 2012). The foremost goal of GM is to measure morphological similarity and difference (Polly, 2012).

There has long been interest in biology on the connections among an organism's morphology, development, and phylogeny. The phenotypic aspects of species generally vary in terms of morphology that includes size and shape. These two elements were biologically important in determining morphological array. Environmental conditions such as anthropogenic activities, agricultural runoff and disposing of large quantities of waste pose detrimental to environment specifically aquatic system (Natividad *et al.*, 2015). Despite the growing concerns towards the ecological status of aquatic environments, there is an increasing trend in the number of pollutants and their intensity in various kinds of water bodies (Rathinam *et al.*, 2022). These have a direct impact on certain aquatic animals' physical characteristics. The fluctuating asymmetry (FA) is one component of geometric morphometry that can be used to detect deviations in morphological traits. It refers to all deviations from a prior expectation of symmetric development in morphological traits (Ludwig, 1932). These morphological asymmetries were assumed to result from imperfect development. It identifies the causative influence of environmental stress and provides advantages over other manifestations of stress since FA is practical and easy to measure (Clarke, 1993). Furthermore, FA has long been regarded as an indicative tool in determining the quality and condition of organisms. As supported by the study of Clarke (1993), the environmental impacts of a fertilizer production facility were evaluated using populations of the blood worm *Chironomus salinarius* and the shrimp *Palaemon elegans* in the nearby freshwater and marine ecosystems, respectively. Elevated levels of asymmetry were observed in both species' populations compared to control populations, suggesting that the facility was significantly impacting the development of the organisms. In addition, the study of Ducos and Tabugo (2015) found that *Gafrarium tumidum (*ribbed venus clam) had a higher FA. Furthermore, a general loss in habitat quality and/or pollution may be the cause of the stress that is present. Therefore, a change in FA should be biologically relevant and an important application (Sommer, 1996).

Few studies have been done on the freshwater systems in the southern Philippines, particularly the Cabadbaran River. Studies that evaluate its water quality have not yet been conducted, which would enable us to ascertain its current state.

The commonly caught bivalve in the river is *Polymesoda erosa* known as mud clam. The mud clam has economic significance in the area because it is consumed and sold in the marketplaces by the local communities. The mud clam was known to be capable of tolerating varying environmental conditions (Chiaravalle, 2013). However, agricultural runoffs and other pollutants from the river can also add up to the stresses that freshwater organisms might experience (Velichović, 2004). These agricultural runoffs are a significant source of Nitrogen, Phosphorus, and heavy metals (Eriksson *et al.,* 2005; Petersen *et al.,* 2007). The two main nutrients that cause eutrophication are nitrogen and phosphorus, which can affect the quality of water and interfere with the normal functioning of water bodies (Liu and Qiu 2007; Le *et al.,* 2010).

The study primarily aims to determine the morphology of *Polymesoda erosa* using a landmark-based analysis collected in La Union, Cabadbaran City, Agusan del Norte, Philippines. Specifically, the study aims to analyze the fluctuating asymmetry of the interior valve of Mud clam in the site.

This study will contribute to the understanding of the biology and ecology of *P. erosa*, as well as provide insights into the health of estuarine ecosystems in the Philippines. The findings of this study may also have implications for the management and conservation of this important bivalve species.

RESEARCH METHODS

Time and Place of Research

The study was conducted in a river located in La Union, Cabadbaran City, Agusan del Norte, Philippines (Figure 1). La Union Cabadbaran City was selected as the study site due to its notable population of mud clams in the river, making it an optimal location for investigating the ecological aspects, behavior, and distribution patterns of these specific organisms. The mud clam collection was performed on April 16, 2023, using a simple random sampling approach.

Materials and Data

Ninety mud clam individuals were randomly collected and thus carefully placed in a plastic container filled with fresh water to ensure their survival during transportation to the laboratory. Upon arrival at the Biology Laboratory of Caraga State University, the samples were meticulously measured and sorted to ensure uniformity in size. Each

Figure 1. Map of the sampling location: La Union (9°05’04N, 125°32’ 20E) Cabadbaran City, Agusan del Norte, Philippines.

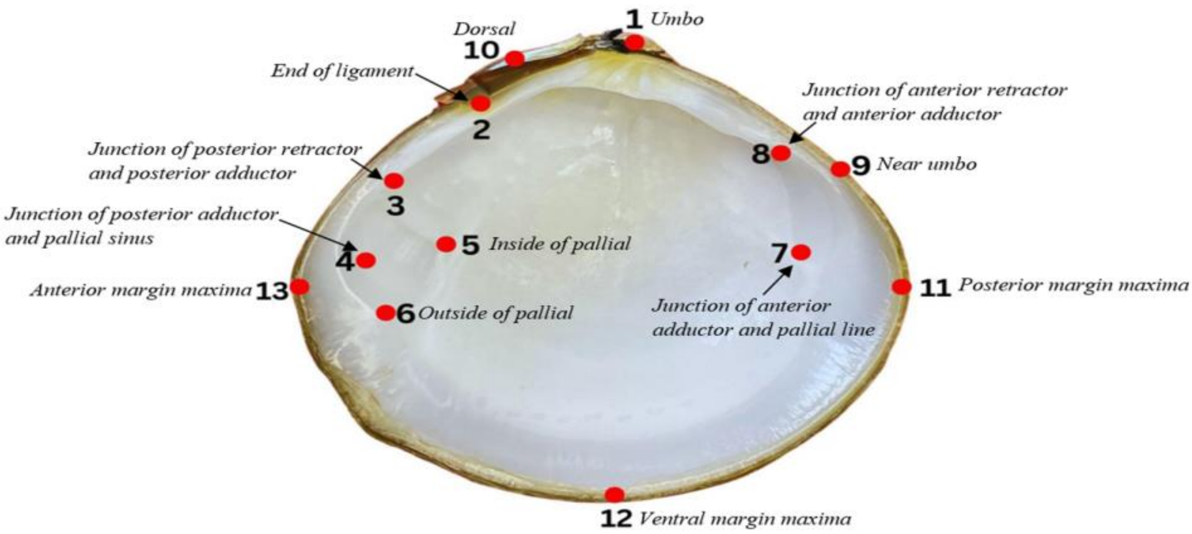


Figure 2. Location of the13 landmarks on the interior valve of the *P. erosa* (mud clam).

sample was documented using a mobile phone for digital records.

Landmark Points

The two-dimensional Cartesian coordinates of 13 landmarks for the interior valve was based on the defined landmarks of Ducos and Tabugo, 2015 as shown in Figure 2. Sample specimens were digitized by TpsDig version2 software (Rohlf, 2017). These landmarks were chosen as an initial observation for analyzing characters which have object type of symmetries and differences between the left and right sides, that can often be seen. As physical points that are constantly exposed to environmental pressures, thus the landmarks are a credible entity to study morphological fluctuations. The descriptions of the landmarks in *P. erosa* (mud clam) are presented in Figure 2.

Shape Analysis

The coordinates data taken from both sides of the interior valve of the *P. erosa* (mud clam) was then subjected to SAGE software to get the principal components which implies the deformation grid of individual asymmetry and also the data of procustes ANOVA was also be obtained for further analysis.

Water Analysis

The determination of pH was conducted using a pH meter. The determination of salinity was conducted using a Salt Refractometer. The determination of turbidity was conducted using La Motte Multi-parameter Meter. The determination of Dissolved Oxygen was conducted using a Dissolved Oxygen meter. The determination of Total Suspended Solids was conducted using gravimetric method dried at 103-105 °C.

Soil Analysis

Microwave-assisted aqua regia digestion was used to determine metal content, while Inductively Coupled Plasma-Optical Emission Spectrometer was used for metals and a Visible Spectrophotometer was used for P.

RESULTS AND DISCUSSION

The analysis considered individuals, sides, and the interaction of individuals and sides. Procustes ANOVA was employed to illustrate and compare the individual symmetry in the ventral shape fluctuations of *P. erosa* (mud clam), as presented in Table 2. Both factors and Procustes ANOVA were applied to both left and right sides of the interior valve. The analysis of the three factors revealed significant fluctuating asymmetry ($P < 0.0001$). The results indicate that the mud clam species exhibited considerable fluctuations, primarily attributed to highly significant differences in individual symmetry, sides, and the interaction between individuals and sides.

The data showed indicates fluctuating asymmetry of the mud clam species that can be attributed to a stressed environment most likely to the presence of pollutants. The stressors can be associated to cause deformities to their novel morphological traits. Many studies had tries to connect that fluctuation is the result of inbreeding, fitness and adverse conditions (Palmer and Strobeck, 1986; Parson, 1990; Palmer, 1994; Moller and Swaddle, 1997; Klingenberg, 2002; Palmer and Strobeck, 2003; Klingenberg, 2015). Thus, fluctuating asymmetry is capable of measuring the developmental instability of every species that encounters or exposes to different adverse conditions and genetic challenges (Klingenberg, 2015). Moreover, FA serves as a reliable indicator of ecological stress and developmental instability, as it detects genetic or environmental changes affecting an organism (Palmer and Strobeck 1986; Leary and Allendorf 1989; Leung and Forbes 1997; Polak and Taylor 2007; Jumawan *et al.*, 2016). Based on observations at the study site, it is hypothesized that the observed morphological changes may be attributed to improper waste disposal practices and the proximity of a pig farm in the area.

The physico-chemical analysis of water revealed only dissolved oxygen exceeded the standard at 5.0 mg L^{-1} set by the DENR for Class C freshwater with the highest recorded value of 6.12 mg L^{-1} (Table 3). Based on the analyzed results from the DOST (Appendix C) revealed the maximum value of 8.23 for

Table 2. Results of the Procrustes ANOVA for interior valve of Mud Clam *P. erosa*

Effect	SS	dF	MS	F	P-value
Individuals	1.2348	1958	0.0006	1.8092	0.0001**
Sides	1.2261	22	0.0557	159.8833	0.0001**
Individual x Sides	0.6825	1958	0.0003	3.4053	0.0001**
Measurement Error	0.8107	7920	0.0001	--	--

** highly significant levels, ^{ns} not significant

Table 3. Water physico-chemical parameters measured in the river of La Union, Cabadbaran City compared with the Department of Environment and Natural Resources (DENR) water quality standards

Parameter (unit)	Results	DENR Class C Standards*
pH	8.23	6.5 – 9.0
Salinity (ppt)	35 ppt	-
Turbidity (NTU)	0.76 NTU	-
Dissolved Oxygen (mg L^{-1})	6.12 mg/L	5.0
Total Suspended Solids (mg L^{-1})	9 mg/L	80.0

pH, 0.76 NTU for turbidity, 6.12 mg L^{-1} for DO, 35 ppt for salinity, and 9 mg L^{-1} for TSS, respectively.

A pH level of 8.23 indicates alkaline or basic conditions in the water. According to Roegner and Mann (1991), mud clams can generally tolerate this pH range without significant negative effects on their internal valve. However, as stated by Maoxiao *et al.,* (2018) long-term exposure to extreme alkaline conditions may still impact the clam's physiology and overall health.

A salinity level of 35 parts per thousand (ppt) suggests that the water is moderately saline (Table 3). According to conservation physiology for the Anthropocene - Issues and applications (2022), mud clams are euryhaline organisms, meaning they can tolerate a wide range of salinities. A salinity of 35 ppt falls within the acceptable range for mud clams, and it should not have a substantial impact on the internal valve (Kim *et al.,* 2001).

Turbidity refers to the clarity or cloudiness of the water caused by suspended particles. A turbidity value of 0.76 NTU indicates relatively clear water. Mud clams generally prefer cleaner, less turbid environments. Low turbidity levels are beneficial as they allow for better light penetration and reduce the risk of sediment accumulation that could negatively affect the clam's feeding and respiration (Henley *et.al.,* 2000).

A dissolved oxygen (DO) level of 6.12 mg/L indicates a moderate oxygen concentration in the water similar results of the study of Sandstrom and Wilde (2014). While this DO level is lower than the DENR Class C standard (typically above 6 mg/L), it is still within a tolerable range for mud clams. However, as stated by the research of Yin *et al.,* (2017), prolonged exposure to lower DO levels may result in physiological stress and reduced growth or survival rates of the clams.

A TSS value of 9 mg/L suggests a relatively low level of suspended solids (Table 3). According to Henley *et al.* (2000), lower TSS levels are generally favorable for mud clams as excessive sedimentation can clog their feeding and respiratory structures. Naturally, suspended solids in aquatic environments can provide food sources for filter-feeding organisms like mud clams. Based on the provided water quality results, the internal valve of the mud clam is likely to be relatively unaffected or minimally impacted. Mud clams have adaptations to tolerate a range of water conditions, and the given parameters fall within acceptable limits for their internal valve health. However, it is important to continue monitoring these parameters over time to ensure the long-term well-being of the mud clam population.

The result on the level of Ni in sediments is alarming (Table 4, Appendix D), and it needs to have local intervention to regulate the mining activities along La Union river such operation of San Roque Metal Incorporated located in Tubay, Agusan del Norte. Mitigation measures are necessary to reduce the environmental impact that may lead to fishery resources of Butuan Bay depleted (Bernales *et al.*, 2022).

According to Leibvitz (2009), high concentrations of Nickel can potentially impact the integrity and growth of the clam's internal valve (shell). Nickel is a toxic metal and can interfere with calcium metabolism,

Table 4. Heavy metal detected in the sediments of La Union River, Cabadbaran City compared with the Department of Environment and Natural Resources (DENR) sediments quality standards

Heavy Metal (mg/Kg)	Results	DENR Class C Standards
Nickel (Ni)	496	80

affecting shell formation and structural integrity. It may result in shell deformities, thinning, or weakening, making the clams more vulnerable to predation, disease, or other stressors (Thain *et al.*, 2019).

The feeding habit of *P. erosa* that filters sediments could be the main reason these species accumulate nickel in their muscles. Because of their capability in filtering water and being persistent in the same place makes them good bio-indicator of marine ecosystems (Chiaravalle, 2013).

Using the symmetry and asymmetry scores obtained from the Principal Component Analysis, affected landmarks in the developmental instability of mud clam species was observed (Table 5). Principal Component Analysis (PCA) was performed to visualized the covariance shape change and evaluate direction and magnitude of fluctuations in each landmark (Ducos and Tabugo, 2015). The upper 5% effective PCA was obtained using SAGE and were considered in the results. Using the symmetry and asymmetry scores obtained from Principal Component Analysis (PCA), affected landmarks in the developmental instability of mud clam species was observed.

In interior valve, the five (5) PCA constitute 73.14% of cumulative variation with the PCA1 being the highest variation accounted with 35.24%. This indicates that PCA1 had the most significant impact on shaping the morphological variation observed in the interior valve. Among the analyzed principal component scores (PCA) in the study, the most frequently affected landmarks were identified as landmark 10 and landmark 13, as shown in Table 5. Landmark 10 corresponds to the dorsal margin maxima, which represents a specific point on the upper edge of the measured region. Landmark 13, on the other hand, represents the anterior margin maxima, indicating a specific point on the front edge of the measured region.

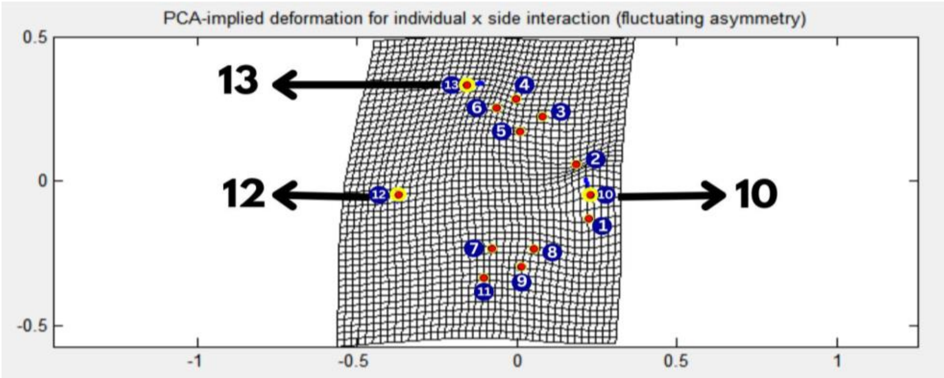
These landmarks, particularly landmark 10 and 13, consistently exhibited notable variations or changes in their positions across the principal component scores. This suggests that these specific regions of the morphological structure, namely the dorsal margin maxima and anterior margin maxima, were highly susceptible to alterations or influences that caused variations in the overall shape or configuration of the analyzed samples.

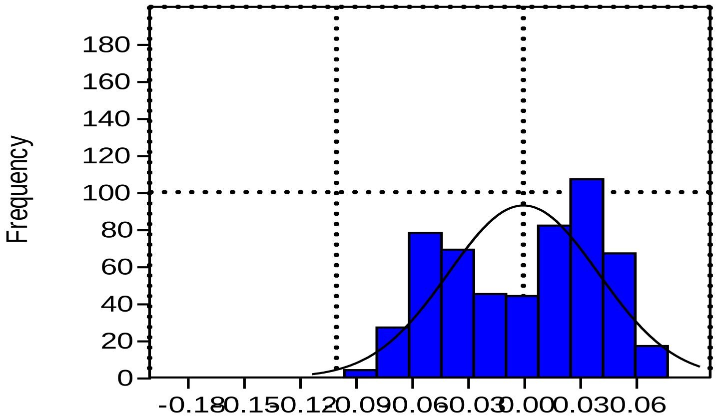
According to Argente *et al.* (2021), these landmarks may be associated with specific functions or adaptations in an organism's environment. For example, in aquatic organisms, dorsal margin maxima and anterior margin maxima could be related to swimming efficiency, predator avoidance, or feeding mechanisms.

Table 5. Principal component series showing the values of symmetry and asymmetry scores with the summary of the affected landmarks

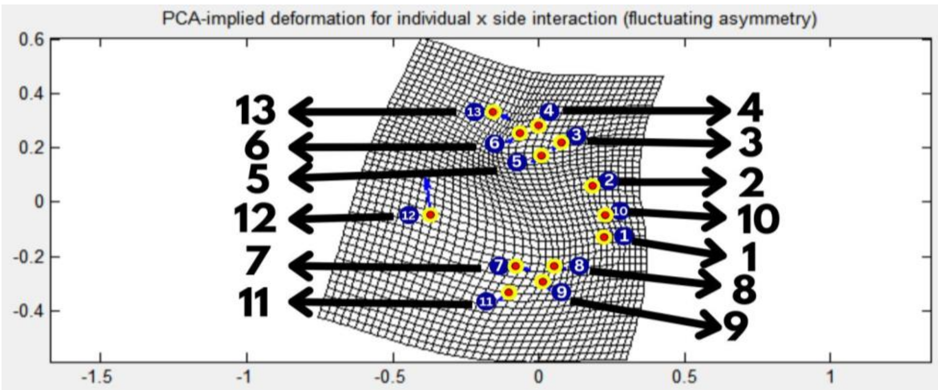
<table>
<tr><th>PCA</th><th>Individual (Symmetry)</th><th>Sides (Directional Symmetry)</th><th>Interaction (Fluctuating Asymmetry)</th><th>Affected Landmarks</th></tr>
<tr><td>PCA 1</td><td>35.24%</td><td rowspan="6">100%</td><td>30.81%</td><td>10, 12, 13</td></tr>
<tr><td>PCA 2</td><td>14.56%</td><td>24.59%</td><td>1,2,3, 4, 5, 6, 7, 8, 9, 10, 11, 12,13</td></tr>
<tr><td>PCA 3</td><td>10.91%</td><td>8.23%</td><td>1, 3, 4, 5, 10, 13</td></tr>
<tr><td>PCA 4</td><td>6.94%</td><td>6.27%</td><td>3, 6, 10,</td></tr>
<tr><td>PCA 5</td><td>5.49%</td><td>5.44%</td><td>3, 4, 6, 7, 10, 11, 13</td></tr>
<tr><td>Overall</td><td>73.14%</td><td>75.34%</td><td></td></tr>
</table>

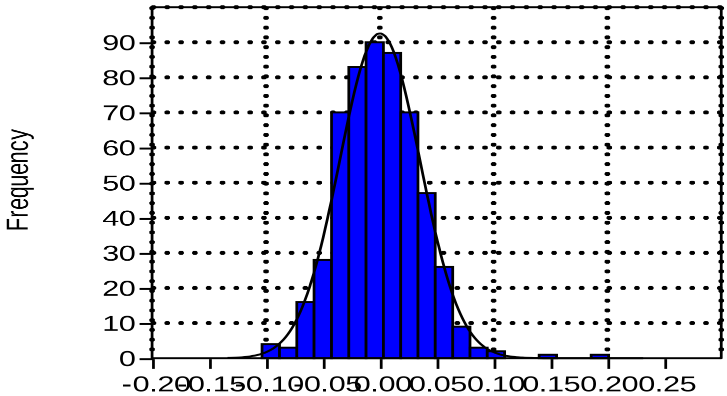
PC1=
30.81%



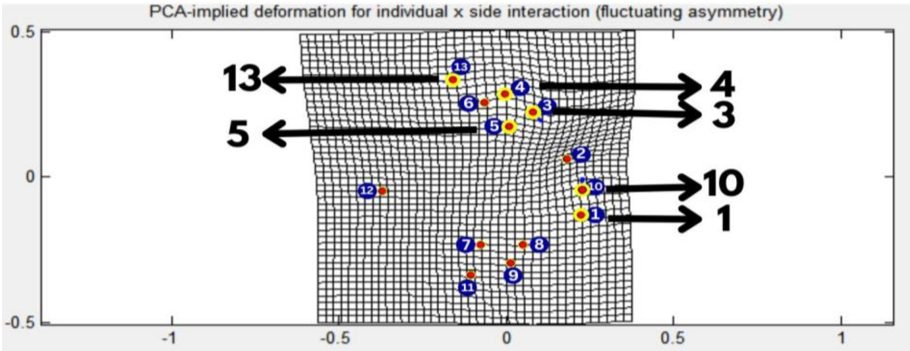


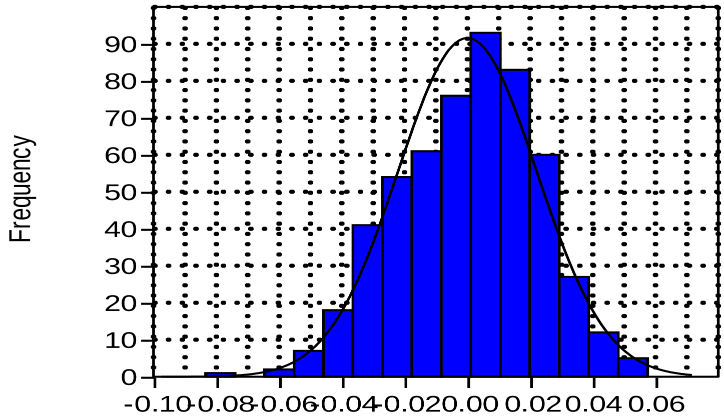
PC2=
24.59%



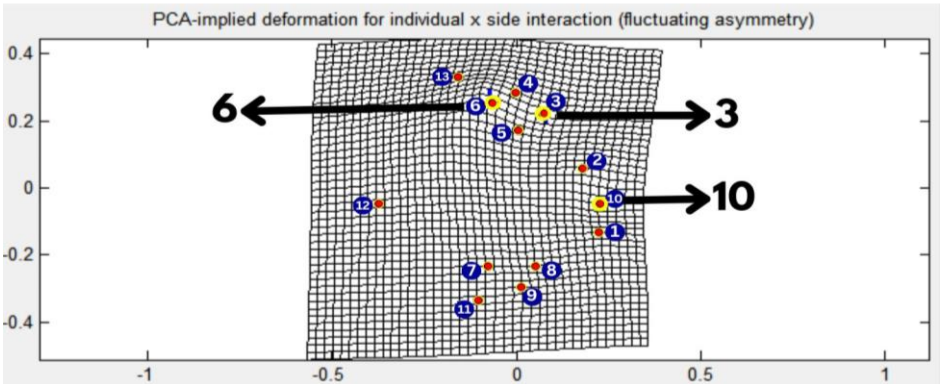


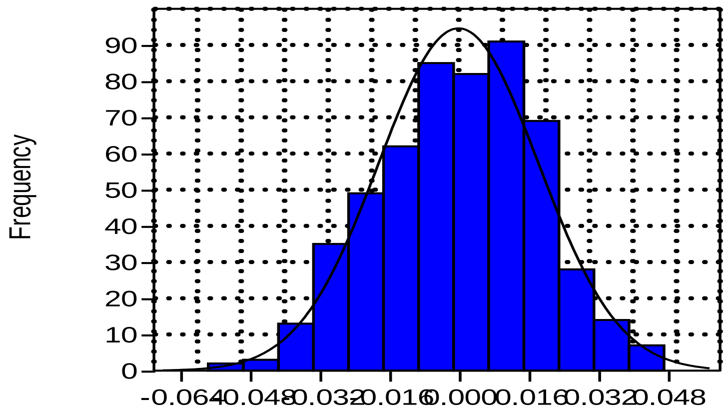
PC3= 8.23%



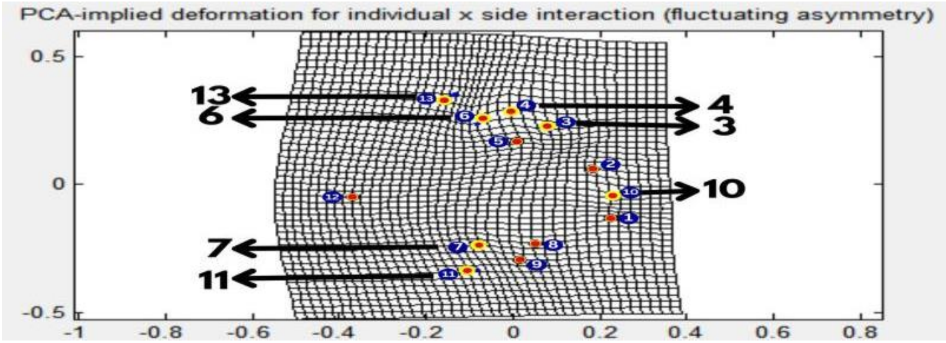


PC4= 6.27%





PC5=
5.44%



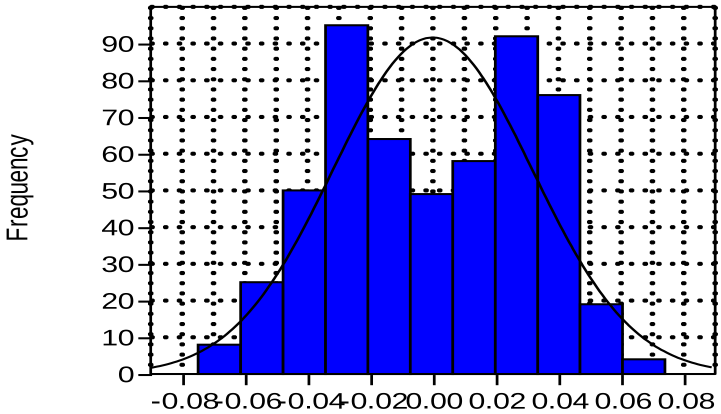


Figure 3. Internal Valve Deformation Grid and Symmetry Histogram for *P. erosa*.

Different environments may exert selective pressures that result in variations in these landmarks, enabling organisms to cope better with their surroundings (Bernales *et al.*, 2022).

The present study identifies fluctuating asymmetry pertaining to the internal valve of *P. erosa* that are experiencing environmental stress. According to Ducos (2015), species that has greater value of fluctuating asymmetry has underneath ecological stress. Acquiring different levels of FA indicating that species may buffer from developmental variability is due to environmental pressures (Graham *et al.*, 1993; Lens *et al.*, 2002). The unfavorable environmental condition exhibits morphological variations of an organism affecting its symmetry (Natividad *et al.*, 2015). The outcome of this study will be important information to assess the current standing of the river of La Union, Cabadbaran City, Agusan del Norte. These results could help identify effective waste management strategies for preventing water pollution and validate the existing control measures in pig farming. Thus, FA is a valuable effective mechanism to evaluate an environmental stress and developmental variability of an individual. Fluctuating asymmetry shown in mud clams may increase due to environmental and genetic stress in individual and population level, thus become a potential tool for biomonitoring and conservation programs (Tomkins and Kotiaho, 2001).

CONCLUSION

The analysis of ventral shape fluctuations in *P. erosa* (mud clam) revealed significant fluctuating asymmetry attributed to individual symmetry, sides, and the interaction between individuals and sides. The observed morphological changes, hypothesized to result from improper waste disposal and the proximity of a pig farm, suggest a potential link to environmental stressors, especially pollutants. The physico-chemical analysis of water quality parameters showed generally acceptable conditions for mud clam internal valve health, with only dissolved oxygen exceeding the standard. However, prolonged exposure to lower dissolved oxygen levels could pose risks. The alarming level of Nickel in sediments, particularly attributed to mining activities, necessitates local intervention to regulate such activities and mitigate environmental impacts. High concentrations of Nickel can potentially impact the integrity and growth of mud clam shells, making them more vulnerable to various stressors. The mud clam's feeding habit of filtering sediments makes them a valuable bio-indicator of marine ecosystems, highlighting the importance of monitoring their health.

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