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Evidence of Shell Shape Variation in the Population of Golden Apple Snail (*Pomacea canaliculata* Lamarck, 1822) in the Second Largest Lake (Mainit) in Mindanao, Philippines

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ABSTRACT

Morphometric techniques have been increasingly employed to detect body shifts and variations within biological communities as indicators of environmental stress. This study investigates shell shape variation of the Golden Apple Snail (*Pomacea canaliculata*) using geometric morphometric (GM) analysis to assess the presence, spatial distribution, and sex-based differences in shape variation in the second largest lake in (Mainit) in Mindanao, Philippines. One hundred eighty adult shells of uniform size were collected from three selected areas within the lake. Sampling was stratified by site, with 60 individuals collected from each location, ensuring an equal representation of males and females (n = 30 per sex per site). Using Relative Warp Analysis (RWA) to discriminate the shell shapes, the anatomical landmark points were employed in the Multivariate Analysis of Variance, Principal Component Analysis, and Canonical Variate Analysis. The highest levels of fluctuating asymmetry were observed in the northern portion of the lake, particularly in the abapertural (55.76%) and apertural (56.89%) shell regions, followed by the southern portion and the middle part, respectively. MANOVA and RWA confirmed significant morphological variation across sites and sexes, suggesting that environmental factors strongly influence shell development in different lake regions. Findings revealed a significant difference ($p < 0.05$) in shell shape across the sampling sites and between the sexes. Additionally, the Pearson correlation coefficient (r = −0.3156) and found that the water did not affect on the shell shape of the gastropods. Thus, the results showed that *P. canaliculata* shell shape varies in size and shape and indicates distinguishable morphology across varying ecological locations..

Key words: gastropods, landmarks, morphometrics, morphology, shell shape

INTRODUCTION

The advancement of modern technology and development of specialized software have significantly improved the ability of the researchers to collect, analyze, and quantify data in the fields of ecology and biology. Among these tools, Geometric Morphometric (GM) analysis has emerged as a powerful and precise method in detecting subtle changes in the body shape of organisms (Adams et al., 2004; Bookstein, 1991; Roth & Mercer, 2000). By quantifying morphological variations using landmark-based approaches, it enables researchers to understand how environmental stressors or genetic factors may influence organismal development. (Adams et al., 2004; Debiais-Thibaut et al., 2014; Klingenberg & McIntyre, 1998; Roth & Mercer, 2000). This method has been widely applied in ecological and evolutionary studies, especially in aquatic ecosystems where fluctuations in environmental conditions often lead to morphological plasticity.

Lake Mainit is the second-largest lake in Mindanao and a vital freshwater resource in the Caraga region that spans several municipalities (Alegria, Jabonga, Kitcharo, Mainit). Because the lake is shared by multiple municipalities, it is highly susceptible to pollution and ecological disturbances resulting from various anthropogenic activities of each municipality. Previous studies have already reported evidence of fluctuating asymmetry in the lake's biota, suggesting environmental stress. For instance, Saura et al. (2021), Ratunil et al.

(2019), Libay et al. (2019), and a forthcoming study on *Giuris laglaizei* (likely 2024) used fish species to explore these stress indicators. These investigations revealed varying degrees of sexual dimorphism in Bugwan fish (*Hypseleotris agilis*), examined shape variations in Tank Goby fish (*Glossogobius giuris*) and among color morphs of Snakehead Gudgeon fish (*Giuris laglaizei*), and reported high levels of fluctuating asymmetry in Mudfish (*Channa striata*)—all potentially linked to environmental conditions. Furthermore, Cabuga et al. (2018) used water hyacinth (*Eichhornia crassipes*) as a model organism and found significant leaf shape variation and high fluctuating asymmetry within the plant community, suggesting that both environmental and genetic factors may influence its morphology.

While fishes are commonly used in such studies, snails particularly the Golden Apple Snail (*Pomacea canaliculata* Lamarck, 1822) also serve as important bioindicator species in freshwater ecosystems. As one of the dominant invertebrates in many lakes, *P. canaliculata* possesses characteristics that make it ideal for morphometric studies: it is invasive, highly adaptable, and exhibits rapid reproduction (Cowie & Hayes, 2012; Joshi, Martin & Sebastian, 2005). In the Philippines, several published studies have demonstrated the usefulness of GM in analyzing shell shape variation in freshwater gastropods, including the *P. canaliculata*, as indicators of environmental stress. For instance, Galliguez et al. (2009) applied landmark-based geometric morphometric analysis on *P. canaliculata* and found significant differences in shell shape between sexes, suggesting that factors such as geographic isolation, predation pressure, and nutrient availability may influence shell morphology. Similarly, outline-based GM approaches have been used on *Achatina fulica*, revealing shell shape differences across geographically isolated populations, indicating phenotypic plasticity. Catalan and de Chavez (2023) also confirmed that environmental variables, particularly water temperature, significantly affected shell morphology in *Melanoides tuberculata* across the seven lakes of San Pablo City. Collectively, these studies emphasize that freshwater snails, including the invasive *P. canaliculata*, can serve as reliable bioindicators, with shell asymmetry and variation reflecting the ecological conditions and stressors present in aquatic ecosystems.

This study aims to build upon previous research in the lake by investigating shell shape variation in the freshwater gastropod *P. canaliculata* using GM analysis. Specifically, it seeks to: (1) determine the presence and extent of fluctuating asymmetry across the population throughout the lake; (2) identify which portions of the lake exhibit the highest levels of fluctuating asymmetry; and (3) evaluate whether there are significant differences in fluctuating asymmetry between male and female individuals. By addressing these objectives, the study aims to provide further evidence of environmental stress within the lake and contribute to a broader understanding of morphological responses to ecological disturbances among freshwater organisms.

MATERIALS AND METHODS

Sampling Area

Lake Mainit is located in the provinces of Agusan del Norte and Surigao del Norte in northeastern Mindanao, Philippines (Figure 1). The lake covers an area of approximately 149.87 km^2 and holds an estimated water volume of 18.36 km^3 (Tumanda et al., 2003). Its maximum depth reaches around 219.35 m, with an average depth of about 122.48 m. Although there is no thorough or comprehensive report currently available on the land use around the lake, existing literature indicates that the surrounding area is characterized by a mix of agricultural land primarily rice paddies and coconut plantations and patches of natural vegetation (Padilla et al., 2015). Given the lake's vast surface area and ecological complexity, three sampling stations were strategically selected to represent distinct portions of the lake. Each station was chosen based on spatial distribution and environmental variability to capture potential differences in microhabitats, such as variations in water depth, vegetation cover, substrate type, and anthropogenic influence. This stratified sampling approach aims to ensure a more comprehensive assessment of habitat-specific biological responses within the lake ecosystem.

Data Collection and Processing

The collection of the samples was done through hand picking. A total of 180 shells of adult specimens *P. canaliculata* were randomly collected in uniform size across the sampling sites in the lake. In each study sites, there are 30 males and 30 females. The shell underwent preparatory procedures, including boiling in water and being washed under running water while the flesh was removed using forceps.

Subsequently, corroded, and cracked shells were not included (Mahilum and Demayo, 2014). Male and female samples were determined through its genitalia. Afterwards, the digital camera was used to take pictures of the dorsal and ventral view of the shell. To highlight the aperture view or make the apex visible, the shell was positioned in the columella at a 90-degree angle (Moneva et al., 2012). The samples were tri-replicated and taken at the same location to minimize measurement error.

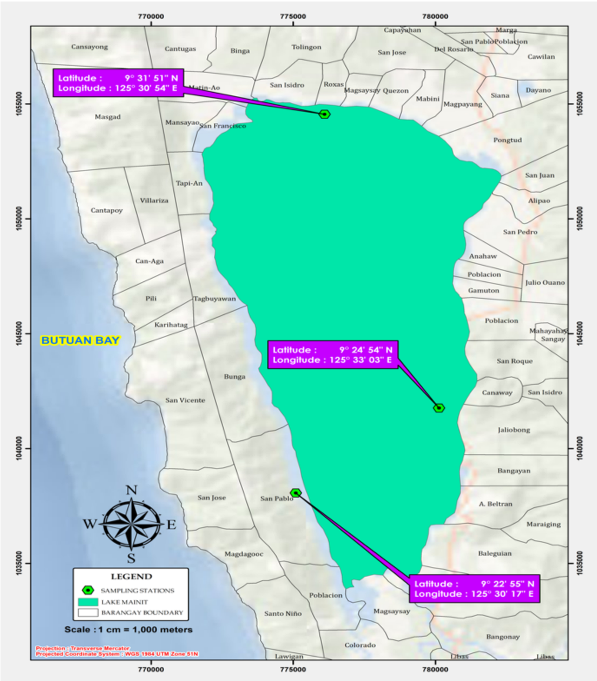

Fig. 1. Map of Lake Mainit, Caraga Region, Philippines showing the sampling stations: northern portion (Brgy. Magsaysay), middle portion (San Roque) and the southern portion (Brgy. San Pablo).

Physicochemical Parameters of the Water

To characterize the microhabitat conditions at each sampling site, a water quality assessment was conducted to describe the physico-chemical properties of the aquatic environment during gastropod collection. The parameters measured included temperature, salinity, conductivity, pH, total dissolved solids (TDS), and dissolved oxygen (DO). Sampling was carried out across three stations within the study area, with each station comprising three replicates and three randomized measurement trials. A multi-parameter water quality meter (EUTECH PCD) was used for in situ data collection. The collected data were analyzed using Paleontological Statistics Software (PAST), and the results were presented as mean ± standard error of the mean (SEM).

Landmark Selection and Digitization

The acquired photos were sorted by sex and then converted by using the tps file format. The samples were digitalized using the tpsDig version 2 for landmarking process (Rohlf, 2015). In this study, an adopted anatomical landmark locations in the ventral and dorsal aperture were presented in Tables 1 and 2. (Torres et al., 2011).

Shape Analysis

To determine the relative warp analysis (RWA), the converted tps files containing the anatomical landmarks were employed to tpsRelw. Histograms were used to illustrate and process the variations between the sexes in patterns of sexual dimorphism and shell form. Data analysis, such Multivariate Analysis of Variance (MANOVA), Principal Component Analysis (PCA), Canonical Variance Analysis (CVA), and Paleontological Statistics (PAST, Version 4.03) Software were used to quantify and draw shape differences (Figure 2). Additionally, CVA was employed to represent in a scatter plot for comparison patterns of variation in a population level.

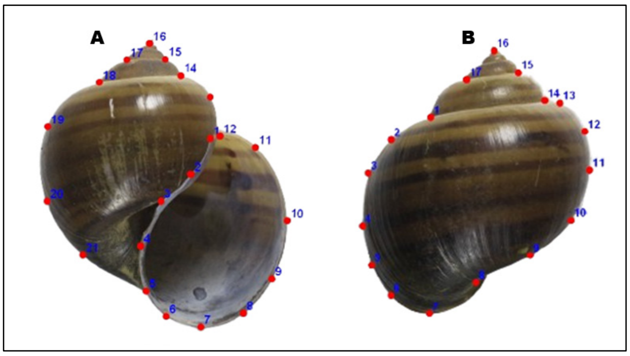

Fig. 2. Anatomical landmark points of the abapertural (**A**) and apertural view (**B**) of *P. canaliculate*.

Table 1. Anatomical landmark points of *P. canalicuta* apertural shell.

Coordinates	Landmark anatomical location on the gas apertural view type
1	Right border of the profile of the shell at the end of the upper suture of the last whorl
2	Right border of the profile of the shell at the end of the upper suture of the second to the last whorl
3	Apex of the shell
4	Left border of the profile of the shell at the end of the upper suture of the second to the last whorl
5	Right border of the profile of the shell at the end of the upper suture of the last whorl
6	Most external point below last whorl at the left profile of the shell
7	With a line perpendicular to the LM 11 touching the margin
8	LM in the aperture margin between LM 7 and LM 9
9	LM in the aperture margin between LM 8 and LM 10
10	With the line from LM 6, touching the lower aperture margin
11	LM in the aperture margin between LM 10 and LM 12
12	Umbilicus of the shell
13	With a line perpendicular to the LM 3 touching the margin
14	With a line perpendicular to the LM 2 touching the margin
15	Align to the LM 8
16	Align to the LM 7
17	Align to the LM 6

Table 2. Anatomical landmarks point of *P. canalicuta* abapertural shell.

Coordinates	Landmark anatomical location on the gas dorsal view type
1	Right border of the profile of the shell at the end of the upper suture of the last whorl
2	Right border of the profile of the shell at the end of the upper suture of the second to the last whorl
3	Apex of the shell
4	Left border of the profile of the shell at the end of the upper suture of the second to the last whorl
5	Right border of the profile of the shell at the end of the upper suture of the last whorl
6	LM near LM 5 which is perpendicular to the LM 10 below
7	Most external point below last whorl at the left profile of the shell on a perpendicular line to the axis of LM 13
8	Umbilicus of the Shell
9	LM in the aperture margin between LM 9 and LM 10
10	With the line from LM 3, touching the lower aperture margin
11	With a line perpendicular from LM 15
12	With a line it is align to LM 8
13	With a line it is align to LM 16
14	With a line perpendicular to the LM 11 touching the margin
15	Last whorl of the shell
16	With a line below LM 1 was the left most profile of the lip
17	Just above LM8, placed on the profile of the lip
18	Align to LM 14
19	Align to LM 13
20	Align to LM 3
21	Align to LM 4

RESULTS AND DISCUSSIONS

Shell Shape Variation of Pooled Sample Population Across the Lake

To further illustrate, the shell shape variation of the pooled samples of *P. canaliculata* across the three sites (Figures 3–5) was presented. Each panel represents the Relative Warp (RW) analyses derived from geometric morphometric methods using landmarks in the snail shells. These RWs represent axes of shape variation extracted via Principal Component Analysis (PCA) of Procrustes aligned landmark data (Rohlf & Slice, 1990). Thin-plate spline grids (left and right) show the variation in shape at each extreme of the relative warp axis. The histogram represents the distribution of shape variation along each relative warp axis, indicating how individual specimens vary around the mean shape. In the northern area (male-apertural), the first RW1 accounted for (47.57%), RW2 (15.88%), and RW3 (11.22%), respectively, with a majority of shape variation of 74.67%. For the (male-apertural) the first RW1 accounted for (36.90%), RW2 (17.67%), RW3 (10.94%), and RW4 (7.36%), respectively, with a majority of shape variation of (72.87%). In female-apertural, the first RW1 accounted for (44.23%), RW2 (19.71%), and RW3 (11.16%), with a majority of shape variation of (75.10%) respectively. For the female-abapertural, the first RW1 accounted for (33.37%), RW2 (17.22%), RW3 (12.23%), RW4 (9.90%), and RW5 (5.79%) with a majority of shape variation of (78.51%).

Meanwhile, in the middle area (male-apertural), the first RW1 accounted for (35.76%), RW2 (18.73%), RW3 (12.03%), and RW4 (9.36%) respectively, with a majority of shape variation of (75.88%). In male-abapertural, the first RW accounted for (38.12%), RW2 (19.23%), RW3 (11.32%), and RW4 (8.86%) respectively with a majority of shape variation of (77.53%). In female apertural, the first RW1 accounted for (33.01%), RW2 (19.49%), RW3 (13.03%), RW4 (7.59%), and RW5 (5.75%), respectively, with a majority of shape variation of (78.87%). In female-abapertural the first RW1 accounted for (31.17%), RW2 (23.30%), RW3 (10.24%), RW4 (8.86%), and RW5 (6.33%), respectively, with a majority of shape variation of (79.90%).

On the other hand, in the southern area, male-apertural, the first RW1 accounted for (44.72%), RW2 (17.24%), RW3 (11.36%), and RW4 (6.95%), respectively, with a majority of shape variation of (80.27%). In male-apertural, the first RW1 accounted for (45.24%), RW2 (19.86%), RW3 (8.96%), and RW4 (5.84%) respectively, with a majority of shape variation of (79.90%). In female-apertural, the first RW1 accounted for (36.50%), RW2 (15.47%), RW3 (13.38%), and RW3 (8.09%), respectively, with a majority of shape variation of (73.44). In female abapertural, the first RW1 accounted to (36.83), RW2 (20.38%), RW3 (9.48%), RW4 (8.55%) and RW5 (6.53) respectively with a majority of shape variation of (81.77%).

Meanwhile, asymmetrical shape variation can be observed between the left and right sides of the shell as inferred from apertural and abapertural views. The variance values are consistently high in RW1 across all sexes and sites. This suggests that the dominant shape variation is consistent and significant but likely not directional, a hallmark of fluctuating asymmetry (Klinberg, 2016). The figure supports the hypothesis of fluctuating asymmetry (FA) in the shell morphology of *P. canaliculata*, particularly due to non-directional shape deviations in symmetrical structures. Similarly, the symmetric placement of deformation grids indicates the presence of fluctuating asymmetry (FA), a slight random deviation from perfect symmetry, which is often interpreted as an indicator of developmental instability due to environmental or genetic stress (Palmer & Strobeck, 1986; Klingenberg, 2015). Both sexes show higher apertural variation in RW1, suggesting that this shell portion may be more susceptible to environmental pressure or adaptive shaping. The abapertural views show greater cumulative variation across axes, particularly in females which might relate to ecological or reproductive demands (Lajus et al., 2019). This observed FA in shell shape could be attributed to ecological pressures in Lake Mainit, such as pollution, habitat heterogeneity, or predation stress, which have been known to affect developmental stability in gastropods (Benitez et al., 2014; Dorgham et al., 2021). As Lake Mainit is undergoing increasing anthropogenic impact, FA is a useful bioindicator of environmental stress in its molluscan fauna. Moreover, differences in shape variation between sexes could reflect sex-specific history strategies and responses to ecological conditions which are common in gastropod morphology studies (Viscosi, 2015).

Shell Shape Variation per Pooled Sample Population Sampling Site

MANOVA test findings for combined male and female *P. canaliculata* in the apertural and abapertural regions (Table 3). The data indicate a highly significant value of ($p < 0.05$) among the female and male samples. Abapertural and apertural traits show significant differences across sites. While, middle area (Barangay San Roque) generally has the strongest differences in the abapertural region when compared to other areas. At the same time, the middle area (Barangay San Roque) ($p = 5.054 \times 10^{-7}$) and lower area (Barangay San Pablo) ($p = 3.649 \times 10^{-11}$) both show highly significant differences in ventral traits across samples. This implied that collected gastropods across the areas exhibited a unique shell shape based on the geographical locations. Accordingly, the height of the shell and its opening shape and size associated with morphological variations (Moneva et al., 2012). Differences in aspire height and length narrow to wide aperture, and/or narrow to wide body was observed (Mahilum and Demayo, 2014).

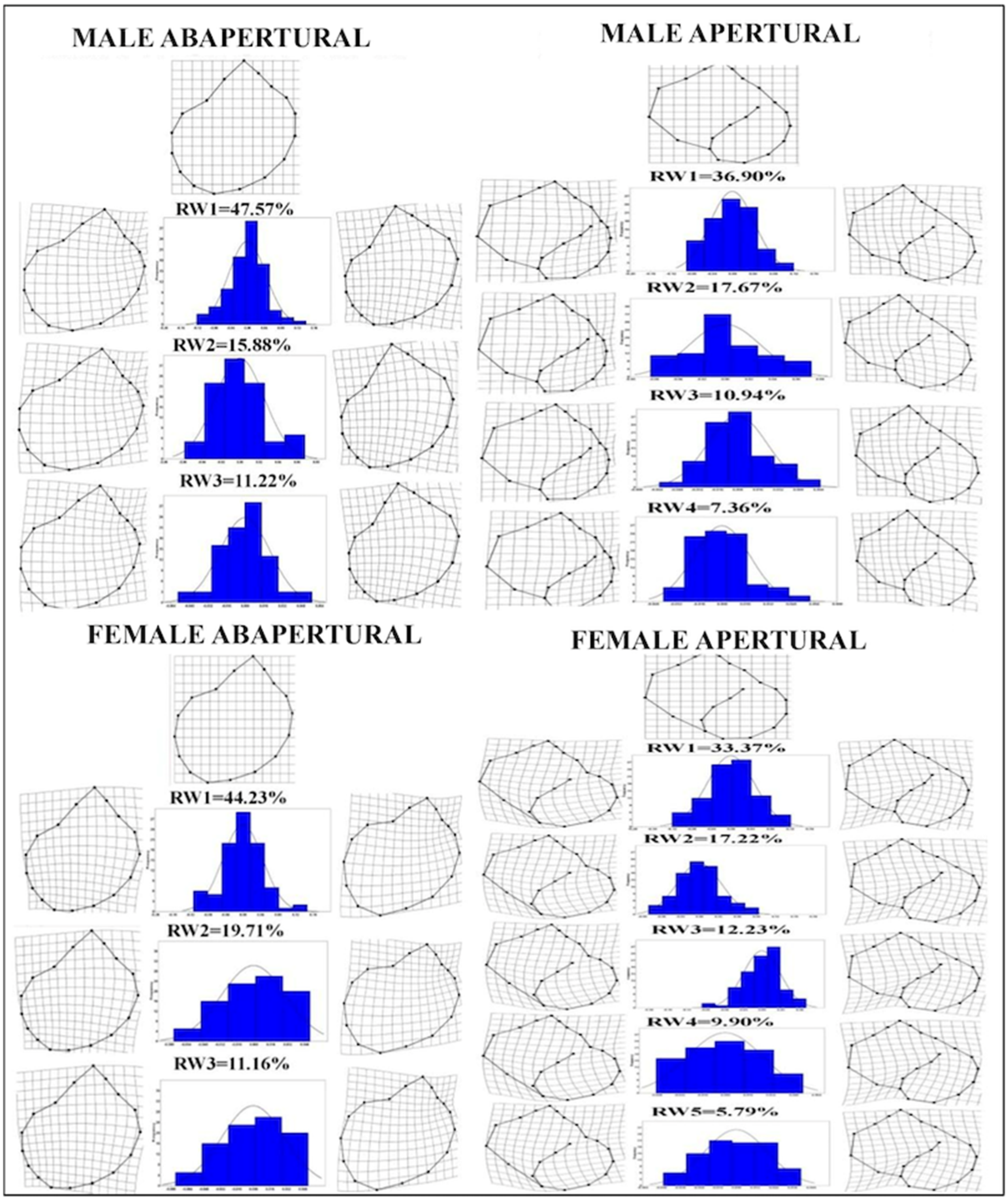

Fig. 3. Visualization of shell shape in *P. canaliculata* between sexes in Northen Area in Lake Mainit, Surigao Del Norte.

Table 3. MANOVA results for pooled female and male *P. canaliculata* abapertural & apertural region across the three sampling sites in Lake Mainit, Surigao Del Norte.

Sampling sites	Wilk's Lambda	df1	df2	F	*p* (same)
		Abapertural			
Northern Area	0.8605	4	174	7.081	2.663×10^{-5} **
Middle Area	0.6579	4	174	17.95	2.446×10^{-14} **
Southern Area	0.8156	5	175	9.385	3.326×10^{-7} **
		Apertural			
Northern Area	0.3330	4	175	87.63	9.694×10^{-41} **
Middle Area	0.8062	5	174	8.26	5.054×10^{-7} **
Southern Area	0.7189	5	174	13.56	3.649×10^{-11} **

** (p < 0.05) highly significant.

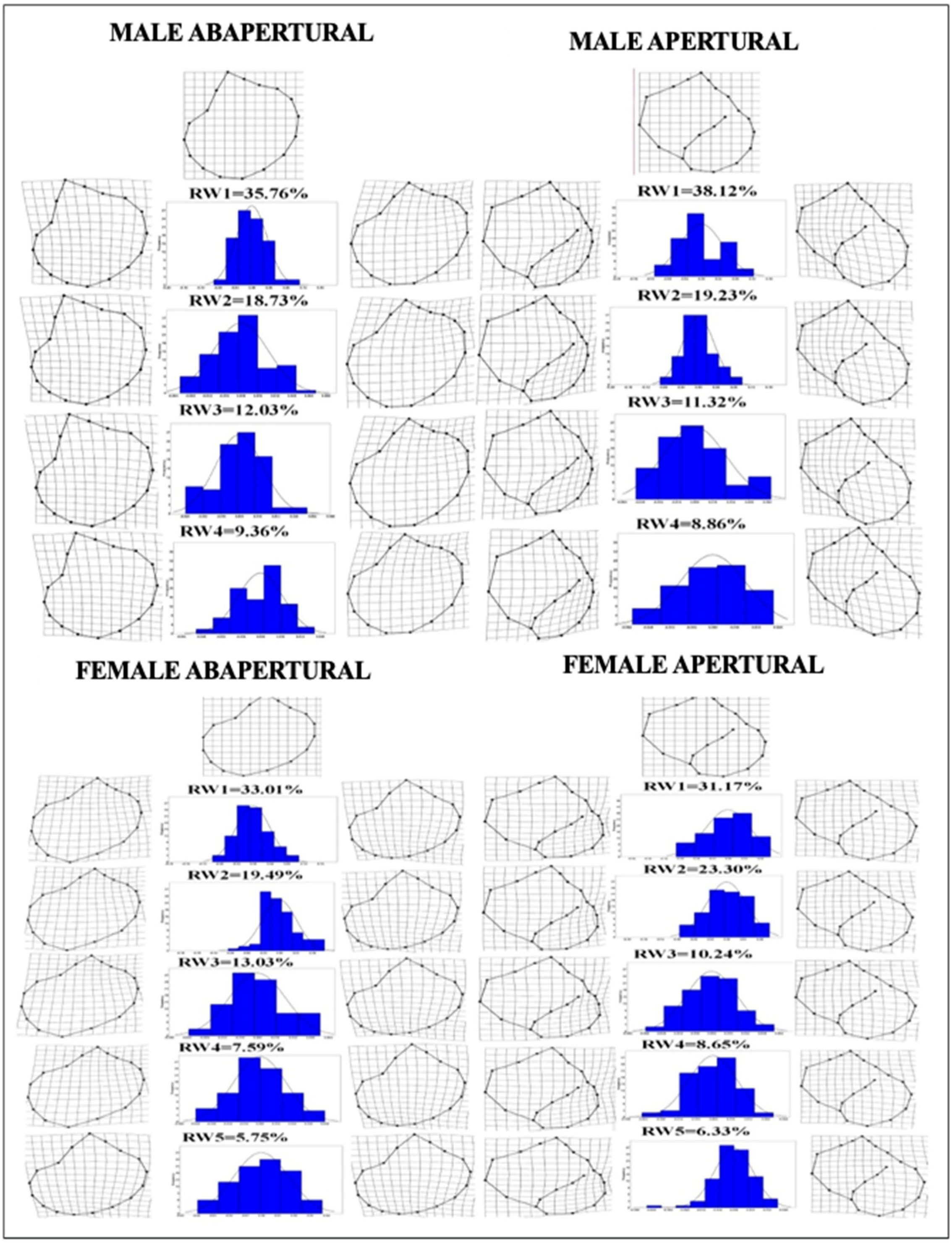

Fig. 4. Visualization of shell shape in *P. canaliculata* between sexes in Middle Area in Lake Mainit, Surigao Del Norte.

Additionally, changes in the shell opening's shape could be connected to its ability to defend against decapods, the main freshwater predators. (DeWitt, 2000). Moreover, as a physical defense and survival mechanism, the spire's height is also rather significant (Borra, 2006). In the freshwater environment, narrowed aperture may serve as a buffer against changes in the surrounding environment. Conversely, a wider aperture suggests that the subject is more vulnerable to predators (Williams et al., 2010). Natural organisms known as predators have the potential to damage shell-shaped apertures or openings (Mahilum and Demayo, 2014). Physical traits have been connected to the influence of lake temperature, and it is thought that temperature directly affects shell expansion. (Mahilum & Demayo, 2014).

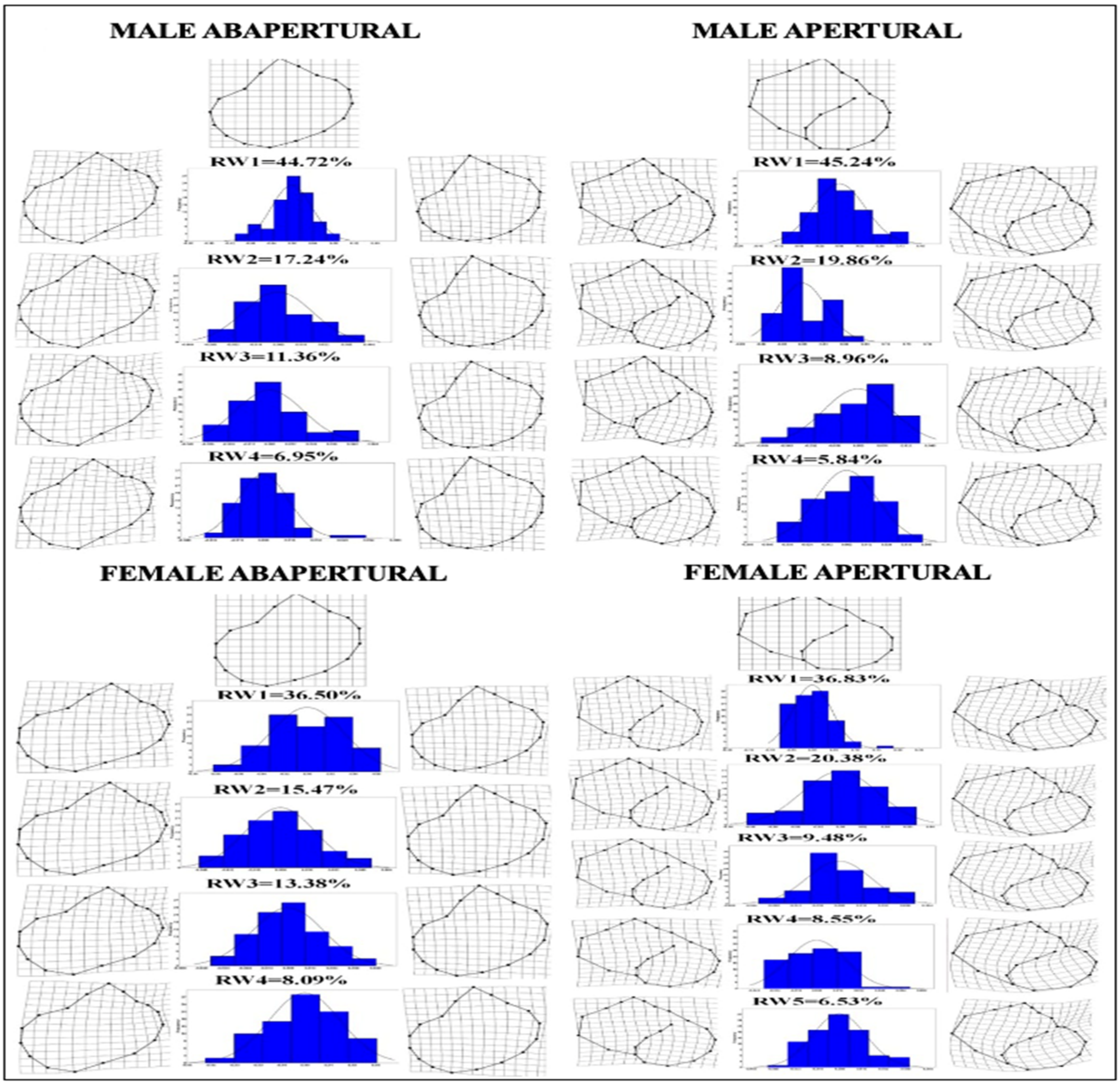

Fig. 5. Visualization of shell shape in *P. canaliculata* between sexes in Southern Area in Lake Mainit, Surigao Del Norte.

Furthermore, the shape of the shell is essentially a record of the organism's ontogeny since shell structural change is accretionary, meaning that points on surfaces accumulate. (Stone, 1998). Because of this, variations in shell shape among closely associated species could be a sign of developmental instability (Cabuga et al., 2023). Additionally, quantification makes it possible to identify intermediate forms, and evaluate degrees of similarity (Roth & Mercer, 2000). Even though there are many classical morphometric studies on gastropod shells in the literature and these techniques are easily applicable, however the use of GM in this field has not been done frequently (Carvajal-Rodríguez et al., 2005). Among the gastropod species, variations in apertural and spire height have a similar form. Consuming prey from beneath the substrate is one of the prevalent foraging behaviors among gastropods species and possibly corresponds to the shell opening (Kohn, 1956; Tursch & Greifeneder, 2001; Olivera et al., 1990; Stewart & Gilly, 2005). Nonetheless, these findings imply that gastropods, particularly the spire, and the shells are rather differentiated. Shells from gastropods are probably quite flexible but likely need to be isolated from spreading planktonic larvae to diversify (Catalan & de Chavez, 2023). On the other hand, the differences between sexes and geographical regions are based on the results of frequency distribution histogram (Figure 6). The samples collected in northern area (Barangay Magsaysay) exhibited the greatest variation in shell shape, abapertural (55.76%) & ventral (56.89%) in PC1 accounting for 4 PCA's. This was followed by southern area

(Barangay San Pablo), abapertural (51.92) ventral (47.25%) in PC1 with 5 PCA and middle area (Barangay San Roque), abapertural (41.79%) ventral (40.75%) with 5 PCA, respectively. The observed differences across geographical locations showed significant details on how the gastropods functions on its habitat.

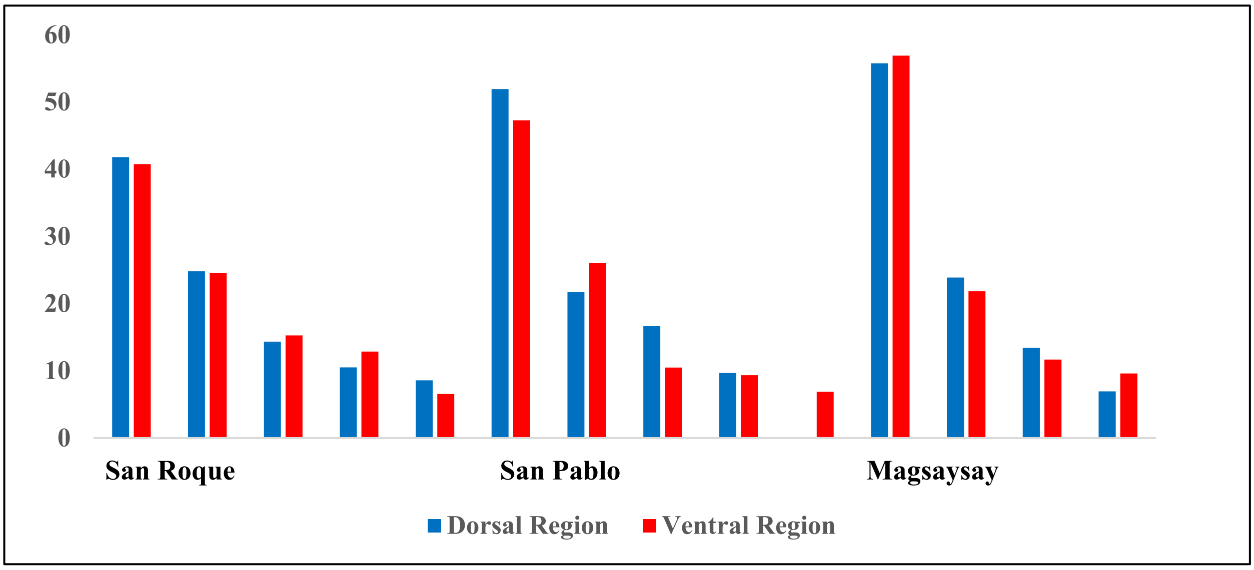

Fig. 6. Frequency distribution showing the variation in the appended abapertural/apertural region between sexes of *P. canaliculata* across three sampling sites in Lake Mainit, Surigao Del Norte.

The centroid/mean morphology and variations in the abapertural and apertural shell shape pattern of *P. canaliculata* from the three sampling sites as a result of the relative warps (RW) are summarized in the geometric morphometric analysis presented in Table 4. In Brgy. San Roque female snails (78.87%, 79.69%) exhibit slightly higher shape variation than males (76.18%, 77.53%) in both abapertural and apertural views. While in Brgy. San Pablo males have a higher RW value in the abapertural view (80.27%) compared to females (73.44%), but females show higher variation in the ventral view (81.77% vs. 79.90%). Also, in Brgy. Magsaysay the RW values are closer between sexes, but females still exhibit slightly higher variation in both views. In terms of percent of variances, it was seen that differences occurs from both abapertural and apertural regions between sexes of the samples. More so, the highest variations were found in the female ventral region when compared to male abapertural region across the sampling areas. Differences in RW values suggest that males and females of *P. canaliculata* exhibit shape variations, which might be associated to reproductive roles, habitat use, or environmental adaptation (Huo et al., 2024). Further, differences between geographic locations could indicate that local environmental conditions influence shell shape (Zhao et al., 2019). Additionally, the higher RW values may reflect adaptations for survival, movement, or reproduction among the species (Miner et al., 2005).

Characteristically, shell variation was seen mostly in its opening or apertural region. The detected differences could be associated to phenotypic trait, shell form results from the interplay between an individual's genetic makeup and their surroundings. While it was inferred that variations in shell dependent on environmental factors (Torres et al., 2011). Shape takes precedence over genetic variation, which will originate from genetic drift or isolation leading to a large ecophenotypic variant that overlap and exhibit nearly constant fluctuation (Estenebet, Martin and Burela, 2006).

Table 4. Summary of Relative Warp between sexes of *P. canaliculata* in 3 sampling sites

Sampling sites	Sexes	RW abapertural view	RW apertural view
Northern Area	Male	74.67%	72.87%
	Female	75.10%	78.51%
Middle Area	Male	76.18%	77.53%
	Female	78.87%	79.69%
Southern Area	Male	80.27%	79.90%
	Female	73.44%	81.77%

Furthermore, genetic, and environmental factors combine to explain the variance in shell shape found in different snail species. Study has shown that phenotypic plasticity, genetic structure, and environmental gradients all affect shell morphology in a variety of gastropod species, including *Tanousia subovata*, *Monodonta labio*, *Bostryx torallyi*, and Lanistes species. By measuring and comparing morphological variability across populations, geometric morphometric techniques have proven invaluable in identifying patterns driven more by historical or ecological causes than by clear species separation (Siquera, Piantoni, Marquez, 2022). The evolution of shell morphology has been influenced by natural selection and local adaptation, as demonstrated by links between shell shape variations and local environmental circumstances found in environmental modeling and molecular phylogeographic investigations (Zhao et al., 2019). Moreover, comparable study conducted on the Lanistes species has demonstrated genetic diversity in shell morphology (Van Bocxlaer et al., 2020; Miranda, 2020). Shell shape variation in gastropod species primarily occurs due to a combination of genetic divergence, environmental influences, and adaptive responses (Johnson, Fogel & Lambert, 2019). Studies on freshwater mollusks have shown that differences in shell morphology among species occupying different habitats have adaptive value, indicating a genetic basis for shell shape variations (Van Bocxlaer et al., 2020). Additionally, research on *Monodonta labio* suggests that shell shape variations are influenced by both local adaptation and phenotypic plasticity, driven by environmental factors like temperature gradients (Zhao et al., 2019). Furthermore, investigations on *Melarhaphe neritoides* demonstrate the presence of distinct ecomorphs in response to environmental adaptation, highlighting the role of adaptive polymorphisms in shaping shell characteristics (Cuña et al., 2011).

Phenotypic trait differences within organisms could have been shaped by a variety of ecological causes and amongst this invasive species' populations. The circumstances of the habitat are varied and have numerous factors, including the presence of predators, the simplicity of defense, the possibility of distance from human populations, climatic variations, offspring survival, and numerous other factors (Holt, 1987; Jones, 2001). The observed phenotypic changes can be explained by the snail's ability to change its development form or produce a different phenotype to fit the current ecological settings, even though genetic variability was not investigated in this work (Goodfriend, 1986; Miner et al., 2005; Wagele, 2004). This demonstrates their adaptive approach to reduce fitness loss in a more challenging setting or to increase fitness in a more advantageous environment (Madjos et al., 2015).

Nevertheless, shell shape variations in *P. canaliculata* across different geographical locations is evident in the present study. Studies in China and the Philippines reveal significant morphological differences among populations, indicating ongoing adaptation (Luo et al., 2022; Huo et al., 2024). In China, the species exhibits faster shell growth and stronger reproductive characteristics compared to its native area in Argentina, likely due to contemporary evolution under selective pressures and favorable climates (Madios et al., 2015). Geometric morphometric analyses in the Philippines show variations in shell shape among populations, influenced by ecological factors like substrates and water flow (Torres et al., 2011). Nonetheless, the use of CVA & PCA was intended to map in a scatter plot in order to draw patterns of sexual dimorphism and variation at the population level. The purpose was to compare the sexual dimorphism among the samples from the three sampling sites. Based on the data presented in the study, it was observed that sexual dimorphism or shared characteristics between the female and male gastropods was evident (blue and red lines). The collected samples of snails exhibited a common set of phenotypic traits that could be observed between the sexes.

Relative Warps in Male and Female Population

Conversely, sexual dimorphism in shell form is noticeable in certain apple snail species, such as *P. canaliculata*, where males typically exhibit characteristics like larger apertures. These findings collectively highlight the complex interplay of genetics, environment, and evolutionary pressures shaping the shell morphology of the gastropods across different regions (Tamburi et al., 2019). In northern area, the abapertural and apertural regions (A–B) of both males and females exhibited a high degree of similarity, as evidenced by their clustering on the X and Y axes in the scatterplot. This pattern was also observed in middle area (C–D). However, in lower area the abapertural region displayed a high degree of similarity, while the apertural region exhibited minimal traits, as indicated by the lines that transcended between the X and Y axes (Figure 7). The overlapping mechanism of traits provides evidence that sexual dimorphism was a widespread phenomenon among gastropod populations. Likewise, study showed that significant intersexual differences in shell shape were found in some populations, like in China and Argentina (Luo et al., 2022; Tamburi et al., 2019).

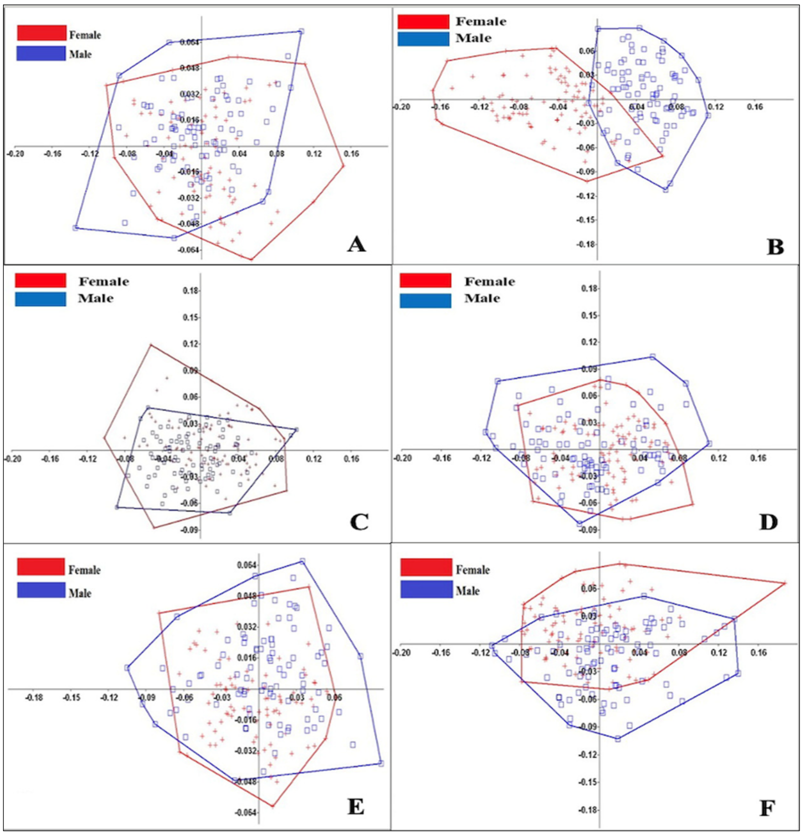

Fig. 7. Canonical Variance Analysis (CVA) scatterplot showing the pooled female and male abapertural and apertural regions (**A**,**B**) Northern Area (**C**,**D**) Middle Area (**E**,**F**) Lower Area, in Lake Mainit, Surigao Del Norte.

However, other studies suggest that sexual dimorphism may not be detectable in certain apple snail species, highlighting interspecific variation within the Ampullariidae family (Dong et al., 2011). Genetic analyses in China revealed some differentiation among populations, indicating wide adaptability (Torres et al., 2011). Morphological variation within and among populations of *P. canaliculata* has been observed, with natural selection and geographical isolation playing roles in evolution Geometric morphometric measurements have been crucial in detecting sexual differences in size, spire height, and shell opening, potentially linked to ecological responses (Tripoli et al., 2015). Because of their numerous adaptations, this species can survive in a variety of environmental situations. Generally, in regions that are tropical or subtropical, where they live in marshes, ditches, ponds, lakes, and rivers (Collier et al., 2011). To illustrate the potential for variation in gastropods, the *A. fulica* species is presented as an example. The observed phenotypic plasticity, in conjunction with the observed differences between individuals, may account for population differences (Albuquerque et al., 2008). In numerous gastropod species, differences between sexes related to shell shape have been associated with brooding; this usually entails a larger body whorl, aperture, umbilicus, or apertural callus to accommodate embryos, eggs, or egg capsules (Lindberg & Dobberteen 1981, Minton & Wang 2011).

The conducted water analysis across the three sampling sites in Lake Mainit, Surigao del Norte were presented in (Table 5). As indicated, the levels of dissolved oxygen (DO), pH, salinity, temperature, and total dissolved solids (TDS) within the permissible limits set by the Department of Environment and Natural Resources (DENR) in freshwater system and were within the acceptable range. Water analysis ensures the suitability of water for different purposes, detecting contaminants accurately and identifying different types of water sources. Proper water quality analysis is vital not only for environmental health but also to prevent hazards. (Roy, 2019). Conducting water quality analysis is essential to ensure safe and sustainable water use in various sectors, most specifically in aquatic environments (Richardson & Ternes, 2018).

Table 5. Physico-chemical Parameters across the three sampling areas in Lake Mainit, Surigao Del Norte.

Water parameters	Barangay San Roque Mean ± SEM	Barangay San Pablo Mean ± SEM	Barangay magsaysay Mean ± SEM	Standard DAO 90-34	Remarks
Conductivity	168.53 ± 0.54	174.94 ± 2.03	159.91 ± 78.02	100–2000 uS/cm	Passed
DO	8.03 ± 0.25	6.65 ± 0.25	6.36 ± 0.15	>5mg/L	Passed
pH	8.06 ± 0.08	7.71 ± 0.11	7.63 ± 0.04	6.5–8.5	Passed
Salinity	0.08 ± 0	0.08 ± 0	0.08 ± 0	<0.5ppt	Passed
Temperature	29.58 ± 0.12	28.47 ± 0.17	29.18 ± 0.37	3 °C rise [a]	Passed
TDS	80.00 ± 0.25	82.97 ± 1.00	171.78 ± 2.37	<1000 mg/L	Passed

Note: DAO—DENR Administrative Order., (a)- Sample was taken from 9:00am to 4:00pm

Understanding parameters like pH, temperature, conductivity, and nutrient levels aids in assessing soil fertility, water potability, effluent pollution levels, and ecological impacts. By analyzing physico-chemical properties, such as BOD, COD, TDS, and hardness, one can evaluate contamination levels, predict environmental impacts, and implement remedial measures (Momin, & Mohammed, 2020). This comprehensive analysis helps in safeguarding human health, preserving aquatic life, and maintaining ecological balance (Rane et al., 2017). The aim of the study was to assess the water quality of Lake Mainit for the survival and growth of aquatic life. Consequently, it provides insight into the conditions of the lake. Water is a fundamental abiotic factor that contributes to the holistic development of organisms. In addition, Pearson correlation analysis was conducted to ascertain whether the water had any effect on the shell shape of the gastropod sample. However, the results demonstrated no correlation ($r = -0.3156$) indicating that the water had no impact on the phenotypic variation observed in the *P. canaliculata* collected across the three sampling sites in Lake Mainit.

In general, the importance of evaluating additional environmental factors such as heavy metal levels in soil and water implies that these may have an impact on the variation in shell shape of the gastropod samples, which were not addressed in the present study. This can be done in the future to determine if their presence may have an adverse effect through correlation analysis. Hence, the interplay between genetic divergence, environmental pressures, and adaptive responses contributes significantly to the shell shape variation observed in gastropod species. Nevertheless, the occurrence of variations among the populations may have been acquired through other environmental responses that were not addressed in the present study. Lastly, the present study identified shell shape variation amongst the *P. canaliculata* population across geographical locations. Moreso, the gastropod's ecological reactions may be the cause of these morphological variations. The findings clearly show that geometric morphometric techniques may effectively identify variations between the sexes.

CONCLUSIONS

Shell shape variation of the invasive Golden Apple Snail in Lake Mainit was conducted. The results of the Multivariate Analysis of Variance (MANOVA) indicated that there were statistically significant ($p < 0.05$) differences in the abapertural and apertural regions among the female and male samples across the three sampling sites in Lake Mainit, Surigao del Norte. This implies that the gastropod populations exhibit significant variations in shell shape, which may be associated with geographical location and environmental responses. Moreover, shell size and shape were observed through the scatter plot, which represented the overlapping mechanisms of the coordinates. This suggests that the female and male gastropods exhibit specific characteristics. Nevertheless, the significance of employing morphometric analyses could result in the discernment of variations in shell shape and sexual dimorphism within intraspecific populations.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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