Shell morphometric variation of the freshwater snail, *Melanoides tuberculata* (Müller, 1774) across the seven lakes of San Pablo City, Laguna, Philippines

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ABSTRACT

The seven maar lakes of San Pablo City in Laguna Province constantly face natural and anthropogenic disturbances that affect the water quality and habitat condition of various aquatic species, including freshwater gastropods. Freshwater gastropods exposed to multiple biotic and abiotic stressors are known to alter their shells as a form of ecomorphological adaptation. However, there are no studies yet regarding shell variations across these lakes as subjected to perturbations in water quality. The shell morphometrics of the common native freshwater snail, *Melanoides tuberculata,* distributed across the seven lakes was examined to address this research gap. Various water parameters were measured prior to collection of snails along the littoral zones. One hundred ninety-seven *M. tuberculata* shells were subjected to linear and geometric morphometrics. The biggest shells were sampled in Lake Sampaloc while the smallest was in Lake Bunot. Centroid size also contributed significantly to the shell shape variation. Generalized linear mixed modeling results

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suggest water temperature had the most significant effect on shell shape change. Shells exposed to colder waters exhibited wider and shorter whorls with constricted apertures, whereas those collected in warmer waters have slender and slightly taller whorls with expanded apertures. The present study demonstrated the possible plasticity of *M. tuberculata* shells which could serve as important information in understanding the habitat conditions of the maar lakes of San Pablo City.

INTRODUCTION

Freshwater gastropods are considered good models for studying shell ecomorphological plasticity since they can manifest changes in their shell structure due to their sensitivity in nature (Samsi et al. 2017). Over time, these changes may result in the expression of new phenotypes to adapt to certain environmental conditions, i.e., response to predation and environmental cues which explains why extensive phenotypic variation is associated with specific habitats of freshwater gastropods (Negovetic and Jokela 2001), thus, allowing them to occupy several niches. An excellent example of this is the caenogastropod snail, *Melanoides tuberculata* (O.F. Müller, 1774). Although they are not as sensitive as other freshwater snails, they exhibit variations in shell morphology called morphs that are phenotypically distinct (Pointier et al. 1993). They are highly adaptable to harsh conditions as they can withstand desiccation (Dudgeon 1986), different degrees of eutrophication (Vogler et al. 2002), and high temperatures contribute to their successful invasion. As a result, they can thrive in almost every freshwater and brackish habitat, such as rivers, streams, springs, wetlands, pans, and coastal lakes (de Kock and Wolmarans 2009), making them a cosmopolitan species, ranging from East Africa to the Middle East and to Southeast Asia, including the Philippines (Brown 1994; Facon et al. 2003; Abdelhady et al. 2018).

In San Pablo City, Laguna Province, there are seven small maar lakes that are geographically situated proximally to each other: Bunot, Calibato, Mohicap, Palakpakin, Pandin, Sampaloc, and Yambo. These lakes have high human activities, such as commercial fish production, aquaculture, and ecotourism. Due to limited conservation and management plans, however, these small lakes face challenges compromising water quality. For instance, lakes Palakpakin, Bunot, and Calibato still suffer from eutrophication and high acidification (Mendoza et al. 2019). In addition, trace organic chemical pollutants such as pesticides (due to rice fields and fruit plantations), surfactants (caused by domestic activities), artificial sweeteners, and phosphate-based fire retardants (used for ecotourism activities) were also found in Lakes Palakpakin, Sampaloc, and Pandin (Dimzon et al. 2018).

Many studies have reported the modification of gastropod shells in areas with heightened human activities in marine and freshwater ecosystems (Harayashiki et al. 2020b; Primost et al. 2021). These shell changes include decreased morphological breadth (Abdelhady et al. 2018) and shifts from narrow to rounded shapes and *vice versa* (Primost et al. 2016) as responses to adapt to their habitats. These gastropod shells are quantified using morphometric analysis to measure the average sizes and shapes in a specific set of environmental conditions (Rohlf 1990). Two methods are applied in morphometric analyses: linear and geometric. Linear or traditional morphometrics measures the size of an organism's morphological characters (e.g., shell length and diameter). On the other hand, geometric morphometrics quantifies the shape by utilizing digitized landmarks as coordinate points in two or three-dimensional figures (Roth & Mercer, 2000). Both are equally important in quantifying morphometrics as they are significant in ecological and evolutionary studies. Thus, gastropod shells can provide information about the specific factors in the environment by studying their shell morphometric patterns induced by specific environmental conditions.

No study has yet explored the morphometric variation of freshwater snails across the seven lakes of San Pablo City, Laguna. The study examined the pattern in the shell morphometrics of the common native freshwater gastropod, *Melanoides tuberculata*, distributed across the seven lakes to fill this information gap. Specifically, it 1) determined the linear and geometric shape character changes of *M. tuberculata*; and 2) analyzed the relationship of the morphometrics of *M. tuberculata* shells with the water quality of the seven lakes. The study only focused on the shell morphometrics of *M. tuberculata* exposed to field conditions. It did not cover its visceral mass's morphological and developmental patterns and other tissues. Also, it did not examine the effect of predators as environmental selective pressures on shell plasticity. Lastly, no genetic testing was conducted to verify species lineages among snails from the lakes.

MATERIALS AND METHODS

Sampling Sites

The seven lakes of San Pablo City in Laguna Province were the areas for this study (Figure 1). The number of sampling sites was determined by dividing the total area of a lake by 10 hectares. Sampaloc (106 ha/10), Palakpakin (47.98 ha/ 5), Calibato (43 ha/ 4), Yambo (30.5 ha/ 5), Bunot (30.5/ 3), Pandin (24 ha/ 2), and Mohicap (22.89 ha/ 3)]. Lake Pandin (14.1153278 °N, 121.3686028 °E) and Lake Yambo (14.1181583 °N, 121.3675222 °E) are transboundary twin lakes that are separated by a narrow strip of land (Figures 1 and 2). Lake Palakpakin (14.1113° N, 121.3384° E) is the shallowest yet the second widest. On the other hand, Lake Calibato (14.1033° N, 121.3775° E) is the deepest and has the most significant volume of water. Lake Mohicap (14.1259° N, 121.332.2° E) is the smallest among the seven lakes. Lake Bunot (14.0822° N, 121.3442° E) mainly operates fish farming using floating cages. Lake Sampaloc (14.0791° N, 121.3299° E) is the city's prime tourist spot with the largest area.

Measurement of Environmental Variables

The environmental variables were collected from September to October 2019. Garmin 12 hand-held global positioning system was used to determine the geographic coordinates. Water physicochemical variables such as temperature, salinity, conductivity, pH, dissolved oxygen, and total dissolved solids (TDS) were measured *in situ* using YSI ProPlus Water Quality Meter (Xylem Analytics, USA) in all sampling sites.

Collection of *Melanoides tuberculata*

The *M. tuberculata* snails were collected, starting at least 1 m from the shoreline, using a direct hand search on habitats preferred by the snails, such as floating macrophytes and logs. Additional shell samples were obtained to minimize size-bias sampling by subjecting 1 kg sediments per sampling point to cascade sieving using metal mesh (3-, 2- and 1mm) in the Malacology Laboratory, UPLB. Random shells samples were pooled together since all sampling sites within each lake's littoral zones have negligible differences and comparable water quality values. All samples were stored in sealed plastic bags or in 50 ml conical tubes filled with absolute ethanol for preservation and morphometric analysis. The species identity of *M. tuberculata* was confirmed using published literature and museum specimens.

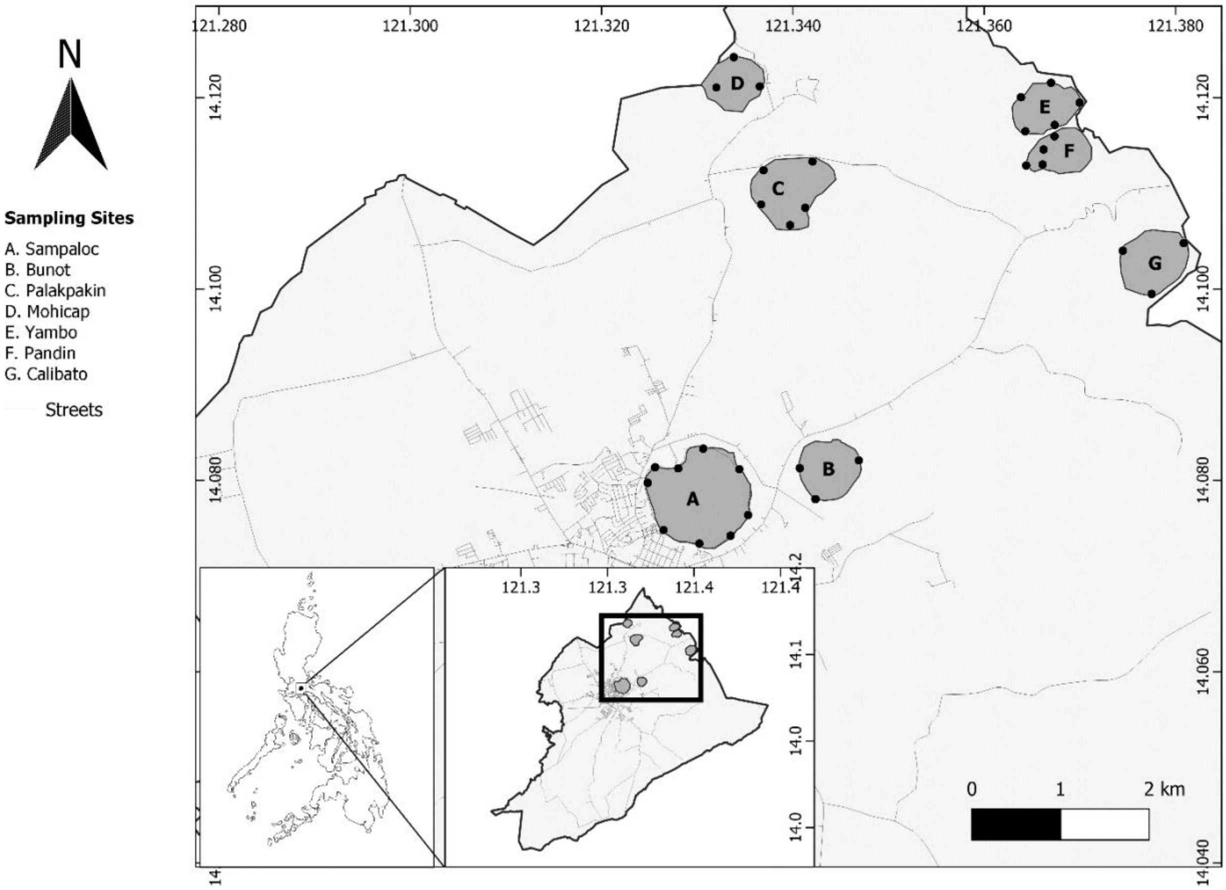

Figure 1: Sampling sites across the littoral zones of the seven lakes of San Pablo City, Laguna.

Linear Morphometrics

Digital photographs of the *M. tuberculata* shells oriented vertically with exposed aperture were taken using Canon 600D digital single-lens camera attached to a desk tripod. *ImageJ* software was used to calibrate and measure the photos of shell characters [shell height (SH), shell width (SW), body whorl height (BWH), aperture length (AL), and aperture width (AW)] following the method of Dillon and Jacquemin (2015) (Figure 2). All values were recorded and exported in MS Excel and *PAST 3* (Paleontological Statistics) software. Values were then $\log_{10}$ transformed to remove the size effect (Mosimann 1970). Principal component analysis (PCA) was run under the covariation option. Eigenvalues and percent variances were computed to examine the number of components. Character loadings of PCA axes were ranked based on their absolute value to determine the most dominant shell characters.

Geometric Morphometrics

The same shell photographs were used for geometric morphometrics. Files were exported in the *tps* package software that contained two programs: *tps*Util and *tps*Dig. Each image exported was built first using the former with a *tps* file type for it to be compatible with the latter program. Type I and Type II landmarks were then added to each image on *tps*Dig. Thirteen landmarks were assigned to *M. tuberculata* shells. Procrustes fit was employed using the *MorphoJ* program to align image data and remove the effect of size and positional bias on the landmarks. Classifiers were generated to group samples according to lakes. The shells' transformation grids and wireframe diagrams were constructed to form an outline by linking landmark points.

The centroid size, based from the landmarks, of the shells was computed and subjected to One-way ANOVA with Tukey post-hoc test using SPSS v.18. Canonical variate analysis (CVA) was

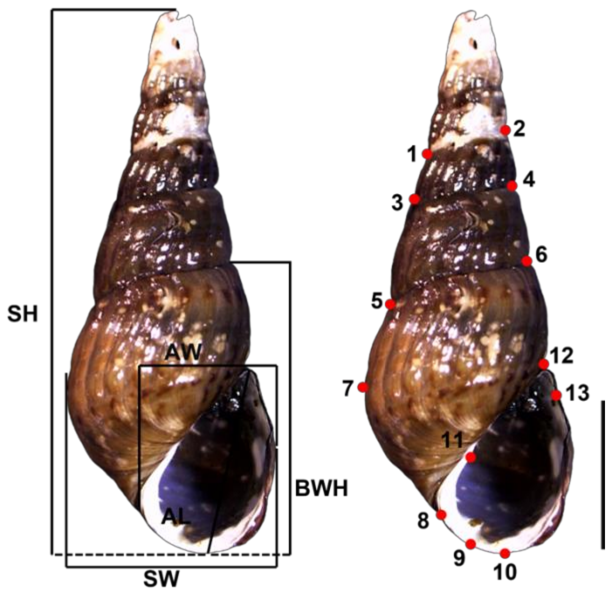

Figure 2: Shell linear characters (A) and landmarks (B) measured in *Melanoides tuberculata*. Shell linear characters: SH – shell height; SW – shell width; AW – aperture width; AL – aperture length; BWH – body whorl height. Landmarks (Albarran-melzer et al., 2019): (1-2) 3rd suture above 3rd whorl; (3-4) 2nd suture above 2nd whorl; (5-6) 1st suture above body whorl; (7) left most part of body whorl; (8) body whorl and aperture lip midpoint; (9) inner bottom lip; (10) outer bottom lip; (11) inner upper lip widest point; (12) lip and body whorl union; (13) inner lip and posterior canal union. Scale bar = 5 mm

then performed to find the optimal distance between identified groups. Ellipses with equal frequencies were added in the CVA plot to determine if overlaps within groups exist. Mahalanobis distances (MD) were generated to further explain clusters between and among groups. A huge overlap, according to the clusters grouped by confidence ellipses with equal frequencies, was observed across the lakes as sampling sites. Permutation tests were conducted for MD at 1000 permutation rounds to determine if each lake is significantly different from the other lakes. All lakes displayed significant MD difference ($p < 0.0001$).

Relative Warp as Shell Shape vs. Shell Size

The *tps* file built containing the shell landmarks for geometric morphometrics was exported to PAST and was subjected to principal component analysis (PCA) under the 2D geometry option. Eigenvalues and variances were then examined to choose which components displayed the highest effect on the landmarks of each shell. The same component was assigned as the relative warp to serve as the shape of each shell. Scores of the component with the highest eigenvalues and variance in the shells subjected to PCA in linear morphometrics served as the size for all shells. Values from relative warp and size were then aligned and exported in SigmaPlot 10.0.

Data Analysis

Correlation analysis was initially conducted to all measured water parameters. Salinity and TDS were highly correlated (0.99) with other variables and thus dropped. To quantify which among the water parameters measured in the seven lakes influences the shell size and shape of *M. tuberculata*, generalized linear mixed modeling (GLMM) was conducted (Bolker et al. 2009). It allows the generation of models to estimate the influence of each factor on the shell variation. The environmental parameters (conductivity, pH, dissolved oxygen, turbidity, and temperature) and shell shape was set as fixed and response variables, respectively. Before subjecting to GLMM and to remove the error of scale, data were *z*-transformed wherein the mean of each environmental variable was subtracted from the raw value and was then divided by the number of their standard deviations. Models were then generated with these variables ascribed with Gaussian error distribution and log link function (Burnham and Anderson 2004). The packages: lme4, *MuMIn*, and arm were used in RStudio (Barton 2009). Akaike values were then computed to assess which among the models affected the shell shape.

RESULTS AND DISCUSSION

Water Quality of the Seven Lakes of San Pablo City

Temperature ranged from 28.0 - 30.0 °C across the seven lakes (Table 1). Conductivity was 0.196 – 0.366 S/m, in which Lake Pandin had the lowest value while Lake Sampaloc the highest. All the lakes were found to be acidic since they were all below 7.0, ranging from 5.70 - 6.34. The DO had low average values in all lakes, particularly in Lakes Bunot (2.585 ± 0.48) and Sampaloc (2.74 ± 0.53). Among these environmental variables measured, TDS and turbidity values were the most variable.

Table 1: Water quality parameters (average ± SD) in the seven lakes of San Pablo City, Laguna. BUN – Bunot, CAL – Calibato, MOH – Mohicap, PAL – Palakpakin, PAN – Pandin, SAM – Sampaloc, YAM – Yambo. TEM – temperature, CON – conductivity, TDS – total dissolved solids, SAL – salinity, DO – dissolved oxygen, pH, TUR – Turbidity).

	TEM (°C)	**CON** (S/m]	**TDS** (mg/L)	**SAL** (ppt)	**DO** (mg/L)	**pH**	**TUR** (m)
BUN	30.14 ± 0.49	0.3009 ± 0.003	178.34 ± 1.30	0.13 ± 5.75E-17	2.585 ± 0.48	6.177 ± 0.04	1.042 ± 0.21
CAL	28.58 ± 0.28	0.272 ± 0.005	165.26 ± 2.69	0.12 ± 5.75E-17	4.20 ± 0.63	5.871 ± 0.15	0.83 ± 0.33
MOH	30.09 ± 0.15	0.38 ± 0.0008	227.11 ± 0.59	0.16 ± 0.004	4.17 ± 0.25	5.94 ± 0.21	2.15 ± 0.75
PAL	30.09 ± 0.55	0.303 ± 0.05	181.66 ± 22.75	0.15 ± 0.05	4.75 ± 0.87	6.01 ± 0.10	0.94 ± 0.20
PAN	29.49 ± 0.38	0.196 ± 0.003	120.84 ± 14.30	0.08 ± 0.002	5.56 ± 0.47	5.70 ± 0.11	2.31 ± 1.59
SAM	28.87 ± 0.33	0.366 ± 0.004	221.26 ± 1.22	0.16 ± 8.42E-17	2.74 ± 0.53	6.34 ± 0.10	1.57 ± 0.40
YAM	29.23 ± 0.20	0.188 ± 0.001	112.716 ± 0.79	0.08 ± 1.42E-17	5.28 ± 0.21	5.95 ± 0.37	3.44 ± 1.27

Linear Shell Characters

A total of 197 shells were sampled from all seven lakes. The sample sizes were Bunot = 22; Calibato = 30; Mohicap = 29; Palakpakin = 29; Pandin = 25; Sampaloc = 30; and Yambo = 32 (Table 2). Shells from Lake Bunot had the smallest average sizes [SW (mm)= 4.82 ± 1.53, BWH (mm) = 6.92 ± 2.29, AW (mm) = 2.94 ± 1.04, AL (mm) = 4.36 ± 1.52]. In contrast, the shells from Lake Sampaloc had the largest [SW (mm) = 7.07 ± 2.68, BWH (mm) = 10.69 ± 4.51, AW (mm) = 4.61 ± 1.88, AL (mm) = 6.61 ± 2.53] in all linear shell characters. As for the shells from the rest of the lakes, intermediate linear sizes were observed.

Table 2: Shell linear characters (average ± SD) of *Melanoides tuberculata* collected from the seven lakes of San Pablo City, Laguna. BUN – Bunot, CAL – Calibato, MOH – Mohicap, PAL – Palakpakin, PAN – Pandin, SAM – Sampaloc, YAM – Yambo. SW – shell width; BWH – body whorl height; AW – aperture width; AL – aperture length.

Lake	SW (mm)	BWH (mm)	AW (mm)	AL (mm)
BUN	4.82 ± 1.53	6.92 ± 2.29	2.94 ± 1.04	4.36 ± 1.52
CAL	6.58 ± 2.02	9.69 ± 3.34	4.08 ± 1.35	5.91 ± 2.12
MOH	6.28 ± 2.07	9.39 ± 3.78	4.07 ± 1.48	5.77 ± 2.21
PAL	6.05 ± 1.23	9.05 ± 2.20	3.96 ± 0.99	5.54 ± 1.29
PAN	6.27 ± 0.99	9.19 ± 1.44	3.84 ± 0.75	5.66 ± 0.93
SAM	7.07 ± 2.68	10.69 ± 4.51	4.61 ± 1.88	6.61 ± 2.53
YAM	6.54 ± 0.88	9.43 ± 1.49	4.10 ± 0.58	5.90 ± 1.04

Principal Component Analysis

The PC 1 accounted for most of the variance (98.46%) which is followed by PC 2 (0.91%) (Table 3). Character loadings was also generated using PC 1 and PC 2 (Figure 3). All characters had positive loadings. BWH (0.514) had the greatest loading, followed by AL (0.511) as the 2nd, AW (0.509) as the 3rd, and SW (0.466) as the last. PC 1 indicated that linear shell characters are all directly proportional to each other. As for PC 2 had positive loadings for both SW and AW, while BWH and AL had negative. The absolute values for each showed that AW (0.739) had the highest loading, followed by AL (0.656) as the 2nd, BWH (0.137) as the 3rd, and SW (0.064) as the last. Only AW and AL were used to explain the relationship among the linear characters since both BWH, and SW had negligible loading scores that did not significantly contribute to the linear characters of the shells. These shell linear character relationships based indicates that the shells from Lake Bunot had the smallest linear sizes with narrow and tall body whorls with wide apertures, whereas the shells from Lake Sampaloc had the largest linear sizes with short and wide body whorls with small apertures.

Table 3: Principal components of the linear shell characters of *Melanoides tuberculata* from the seven lakes of San Pablo City, Laguna.

Principal Component	Eigenvalues	Variance (%)
1	0.0666	98.461
2	0.0006	0.908
3	0.0002	0.342
4	0.0002	0.289

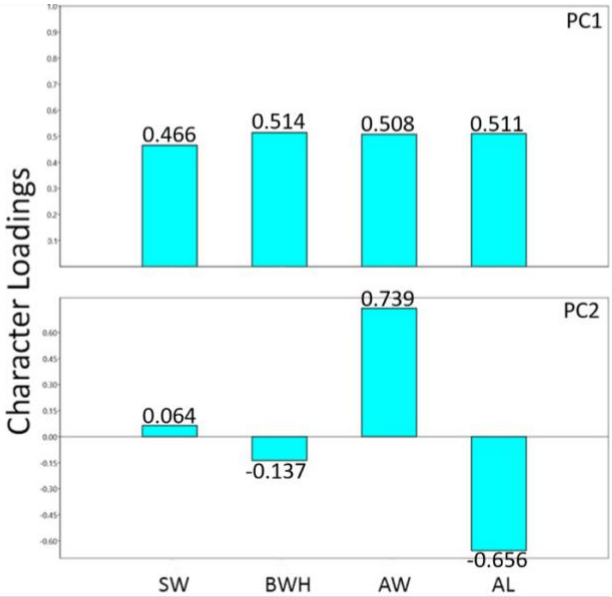

Figure 3: Loadings plot and scores of the four linear characters of *Melanoides tuberculata* in the Seven Lakes of San Pablo City, Laguna. SW – shell width; BWH – body whorl height; AW – aperture width; AL – aperture length. PC1 - Principal Component 1; PC2 - Principal Component 2

Centroid Size and Canonical Variate Analysis

The centroid size of *M. tuberculata* shells differed significantly (F=52.85, df= 196, $p < 0.0001$) across the seven lakes (Figure 4A). Shells sampled from Bunot had the biggest average centroid size (6.48 ± 0.08 mm, $p < 0.0001$), followed by those in Sampaloc (5.68 ± 0.07 mm), Yambo (5.68 ± 0.03 mm), Pandin (5.66 ± 0.04 mm) and Calibato (5.64 ± 0.6 mm). The smallest were shells from Mohicap (5.21 ± 0.06 mm) and Palakpakin (5.19 ± 0.5 mm). The CVA plot (Figure 4B) showed the shape patterns of *M. tuberculata* shells. CV 1 accounts for 48.80% total variability among the different shell groups. The CV 2 explained 21.59% of the total variance. Transformation grids and wireframe graphs (Figure 5) were generated using CV 1 and CV 2 to elucidate the specific shell shape patterns that the plots conform. The positive CV 1 values are associated to shells with wider but shorter body whorls and smaller apertures. On the other hand, positive CV 2 values are associated with a more slender and slightly taller body whorls and wider apertures.

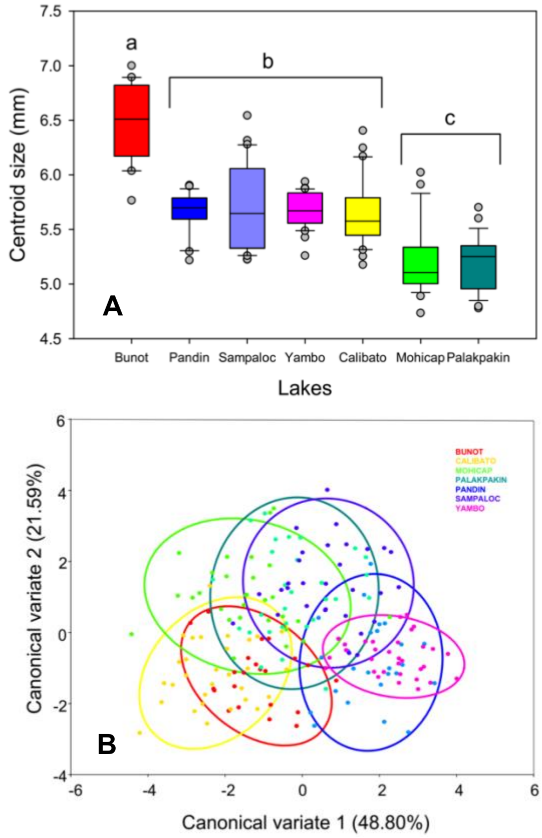

Figure 4: Boxplot of shell centroid sizes of *Melanoides tuberculata* (A) and the shell shape diagram of the first two canonical variates (B) across the seven lakes of San Pablo City. Different consecutive letter indicates significant difference at *p* < 0.0001. n=197

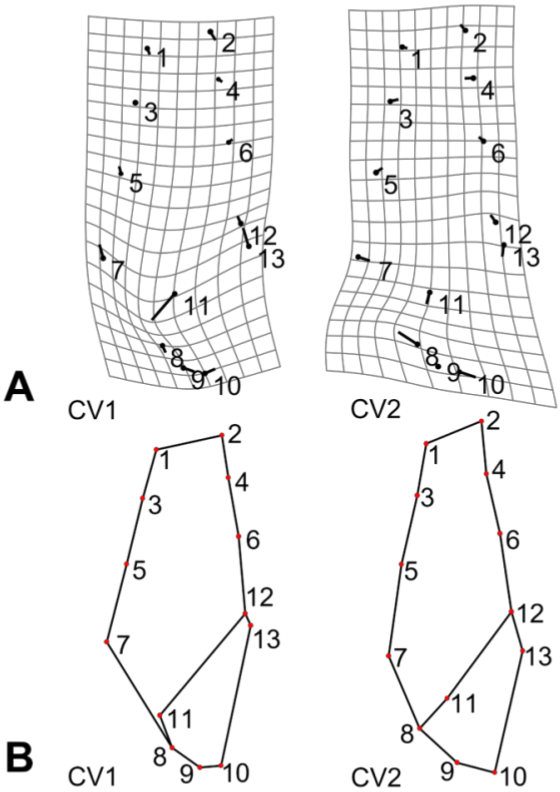

Figure 5: Transformation grids (A) and wireframe diagrams (B) of *Melanoides tuberculata* shells showing the two dominant canonical variates across the seven lakes of San Pablo City, Laguna (CV 1 and CV 2).

Size versus Shape and Environmental Variables of Lakes

Linear size was compared with the relative warp, a proxy for shell shape, to determine how the linear and geometric characters coincide (Figure 6). The PC 1 accounted for 90.08% of the total variance of the shell landmarks. A clear separation was observed among shells from various lakes. Samples from Lakes Sampaloc, Yambo, Calibato, and Pandin clustered in the uppermost part of the graph, whereas the shells from Lakes Bunot, Mohicap, and Palakpakin grouped below. However, several plots were still horizontally comparable despite their distinct separation in the RW 1 axis.

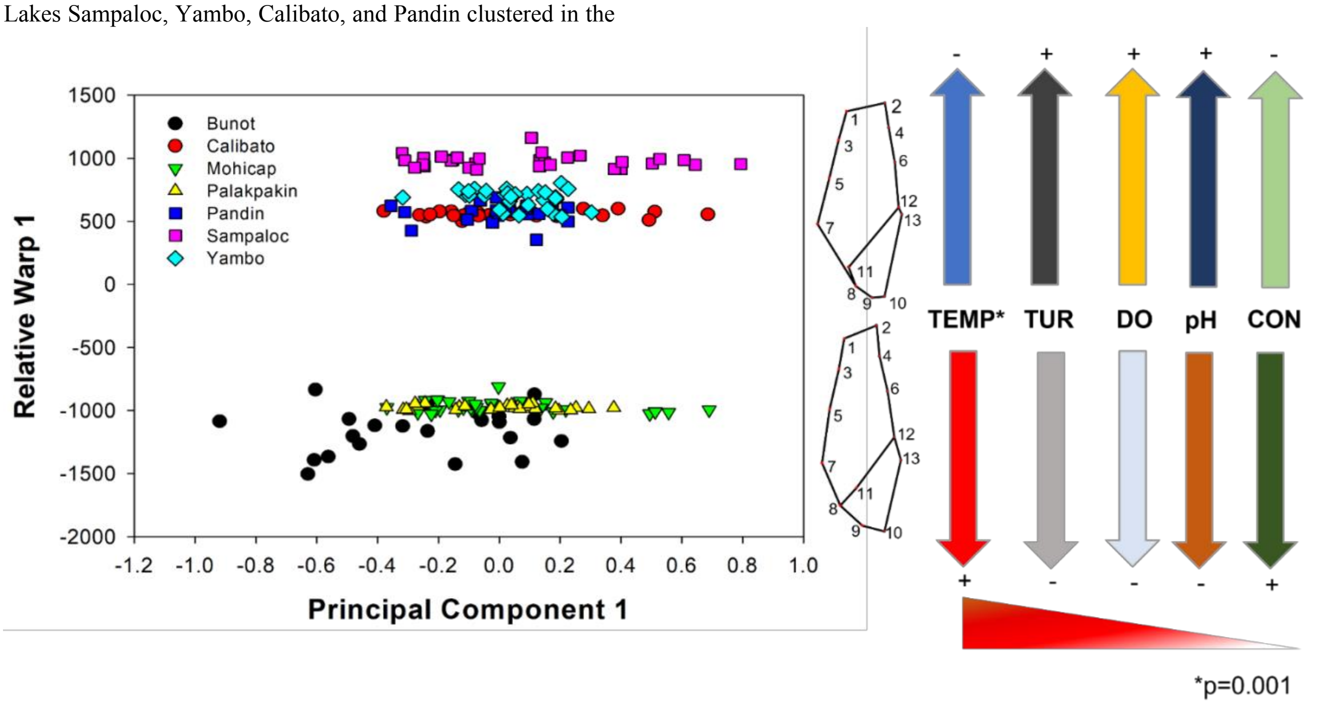

Figure 6: Principal component 1 vs relative warp 1 of *Melanoides tuberculata* shells in relation to water quality parameters of the seven lakes of San Pablo City, Laguna arranged from highest to lowest significant effects. TEMP-temperature; TUR- turbidity; DO- dissolved oxygen; CON-conductivity; n=197

Both salinity and TDS were shown to be highly correlated and serve as only proxies for the remaining models, thus, it was excluded. All variables were set as fixed factors in the shell size (PC1) and shape response (RW1) to associate the environmental variables (temperature, conductivity, dissolved oxygen, pH, and turbidity) with the scatter plot and establish a pattern of how these variables affect the shape of the shells across the lakes. Model-averaged coefficients (Table 4) pointed out that the lakes' temperature (E = -746.10, p = 0.001) had the highest significant effect on the shells that is inversely related to the PC1 vs RW1 plots followed by turbidity (E = 227.35, *p* = 1), DO (E = 130.59, *p* = 1) and pH (E = 163.72, *p* = 1) that all had a direct relationship with the scatter plot. Conductivity (E = -90.88, *p* = 1) had the least significant effect on all the variables that was inversely related to the graph.

Table 4: Model-averaged estimates of the environmental variables of the seven lakes of San Pablo City, Laguna with respect to the shells. CON – conductivity; DO – dissolved oxygen; TEM – temperature; TUR – turbidity

Parameter	Estimate	Standard Error	*p*-value
(Intercept)	11.92	239.18	ns
CON	-90.88	356.87	ns
DO	130.59	559.23	ns
pH	163.72	488.67	ns
TEM	-746.1	248.65	0.001
TUR	227.35	317.79	ns

***ns- not significant**

Based on the degree of significance of each environmental variable, shells found in Lakes Sampaloc, Yambo, Calibato, and Pandin were significantly affected by low temperatures and conductivity but high turbidity, DO, and pH, which resulted in shells that have wider body whorls and smaller apertures. On the contrary, the shells from Lakes Bunot, Palakpakin, and Yambo have shells were significantly affected by high temperatures and conductivity, but turbidity, DO, and pH that possess slender and slightly taller body whorls and expanded apertures.

The linear and geometric morphometrics of *M. turbeculata* shells showed variations in size and shape across the seven lakes. Both morphometric analyses revealed that shells from Lake Sampaloc had the largest linear sizes with shorter and broader body whorls and smaller apertures. In contrast, shells from Lake Bunot had narrower and taller body whorls and wider apertures. Shells found in other lakes all had moderate sizes. However, when it comes to shell shape, the shells from Lakes Calibato, Yambo, and Pandin resembled those observed from Lake Sampaloc also reflected in their centroid sizes.

Lake Bunot has the poorest water conditions as it has consistently posted the highest DO, BOD, ammonia, phosphate concentration, turbidity, and chlorophyll-*a* concentrations

(LLDA 2008; Brillo 2015a). Moreover, the study of Mendoza et al. (2019) showed that Lake Bunot still had a eutrophic status according to its chlorophyll-*a* concentrations. Thus, gastropod shells are expected to decrease in size, which was reflected in the shells linear morphometrics. Although Lake Sampaloc has comparable conditions (Brillo 2016e), it can be hypothesized that its huge surface area, compared to Bunot, could minimize the effect of the degrading water conditions on the snails that may even allow some individuals to grow into the largest shell sizes. Moreover, a similarity in terms of shell shape was observed to be clustered in lakes with the same trophic statuses as reflected by the shells sampled in Lakes Sampaloc, Yambo, and Pandin that are mesotrophic lakes except for Lake Calibato, a eutrophic lake which served as an outlier. Convsersely, this was also seen in the shells of Lakes Bunot, Palakpakin with eutrophic levels except for Lake Mohicap, an outlier as it has a mesotrophic status. Regardless of shape however, sizes tend to be greater in mesotrophic lakes. This disagrees with the study of Silva et al. (2010) as they have determined a high correlation between biomass (which increases in eutrophic waters) and the shell lengths and apertures of the same species. However, this can possibly be explained due to the corrosive effect of acidic waters brought about by eutrophic water conditions characterized by low DO and pH.

Although temperature was found to have the most significant effect on the shape of *M. tuberculata* shells, this cannot be considered the primary basis for shape variations displayed across the seven lakes since temperatures only had marginal average differences. Nevertheless, several studies have explored how temperature influences the morphometrics of aquatic gastropods. For instance, *Melanoides* shells in the hot springs of Azraq Oasis, Jordan has large sizes due to high food availability despite high temperatures (Elkarmi and Ismail 2007). Low food availability may be a possible reason for the stunted growth of the shells in Lake Bunot (Saunders et al. 2009). In addition, this could be mainly due to DO, and pH as affected by temperature. An increase in temperature in the lakes would indicate a decrease in dissolved oxygen (EMB 2014) and therefore, a decrease in pH that renders the lakes more acidic. This might affect the calcification of shell-forming mollusks due to the absorbance in CO_2 that reacts with the calcium carbonates in the lakes. Chatzinikolaou et al. (2016) have documented this in the two intertidal gastropods, *Nassarius nitidus,* and *Columbella rustica*, exposed to elevated temperatures and reduced pH, which ultimately led to reduced shell densities. Therefore, the size and shape of shells in Lakes Bunot, Mohicap, and Palakpakin could be attributed to corrosion due to acidic water leading to thinner and lighter shells. This effect was also documented in *Pomacea canaliculata* induced by growing temperatures (Tamburi et al. 2018).

Several abiotic agents could further explain the morphometric patterns of these shells across the lakes that were not covered in this study. A common factor could be due to heavy metal pollution, as observed in the gastropods, *Echinolittorina subnodoa* and *Planaxis sulcatus*, found on the Egyptian coast of the Red Sea (Abdelhady 2016). Shells exposed to heavy metal polluted areas decreased in their morphological characters and had smaller ranges. However, the shape pattern observed in this current study contradicts theirs since the shells in their studies from the polluted sites had wider body whorls. In contrast, those from the non-polluted areas had more slender shapes and wider or ovate apertures. Regardless, heavy metals could still result in small sizes due to growth retardation or thinning of the shell as seen in the developmental abnormalities in *Radix quadrasi* (de Chavez and de Lara 2003; Factor and de Chavez 2013). Unregulated amounts of organic compounds such as tributyltin in marine environments based on the study of Primost et al. (2016) were found to induce shape-shifts in the morphometries of the intertidal snail, *Buccinanops globulosus*. Domestic activities, ecotourism, and agricultural settlements, such as the presence of rice fields and fruit plantations, were sources of organic pollutants in Lakes Palakpakin, Sampaloc, and Pandin (Dimzon et al. 2018). However, the shape pattern of *M. tuberculata* still opposed the shapes of *B. globulosus* exposed to organic compounds that bear more globular body whorls. However, it still coincided with the shape pattern exhibited by *Odontocymbiola magellanica* bearing a more slender and narrower shape in polluted zones. In contrast, those from slightly polluted areas had moderate geometric patterns (Márquez et al. 2011).

The effect of biotic factors on shell morphometrics of *M. tuberculata* was not explored in this study but could also be a basis for why such patterns were formed in the lakes. Interspecific variation induced by predation could be a possible factor since the seven lakes have several fish species dominated by Nile tilapia (*Oreochromis niloticus*) and silver therapon (*Leiopotherapon plumbeus*) (Paller et al. 2017). These species could prey on *M. tuberculata* shells and possibly induce morphological plasticity over time. For instance, a study by Miranda et al. (2016) discovered that the thiarid snail, *Tarebia granifera*, exhibited thick rotund shells with a reduced SH/SW ratio to be more crush resistant against its freshwater crab predators. Similarly, *Radix balthica* snails develop more rounded shells when subjected to combined predator treatments to increase its shell crushing resistance (Lakowitz et al. 2008). The globular shapes of *M. tuberculata* shells in Lakes Sampaloc, Yambo, Pandin, and Calibato could then probably be linked to the presence of fish predators.

Another possible factor that may have affected the samples' shape variations could be due to the morphotype complexity of *M. tuberculata* as a species. In the study of Duggan and Knox (2022), they were able to establish that the origins of *M. tuberculata* in the aquarium trade of New Zealand came from two distinct genetic clades, which originated from both Asia and Africa. There was an overlap between invasive and native populations of *M. tuberculata* in both Asian and African lakes, as revealed by phylogenetic analyses (Van Bocxlaer et al. 2015). These suggest that shell morphometrics alone could not be enough to attribute the shape variations of the samples to biotic and abiotic factors. Phylogenetic analyses and genetic testing are needed to eliminate the effect of species differences and achieve species uniformity in the samples, given the character similarities and ecophenotypic overlaps of various *Melanoides* lineages.

Overall, the shell morphometrics of *M. tuberculata* with distinct sizes and shapes across the seven lakes were affected by several probable factors that could either be abiotic, biotic, or both. The responses exhibited by gastropods could be a key factor in determining the causal agents that affect the water parameters of the lakes and the various species assemblages residing within each lake. The plasticity and variation of *M. tuberculata* shells displayed in these lakes serve as additional evidence that thiarid snails could reflect the current conditions of lakes and other freshwater ecosystems as it represents trade-offs in its shell morphology. These trade-offs strengthen the utilization of shell morphometrics that could serve as evidence in prompting government agencies and other concerned offices, such as the City Government of San Pablo and Laguna Lake Development Authority (LLDA), respectively, in sustaining healthy water quality.

CONCLUSION AND RECOMMENDATIONS

Although the shape patterns in this study disagreed with most studies that have explored the effect of detrimental water conditions on shells, the shape patterns may be attributed to the corrosive effect of the lake's water quality due to low DO and pH levels, exposing an acidic environment to the shells. The possible effect of climate change, urbanization, and aquaculture practices in the lakes might also contribute to the eutrophication of the lakes that, affect development and shell calcification. It is highly suggested that future studies explore how each water quality lake parameter in this study at varying degrees would affect the shell morphometrics of snails that are reared in the laboratory. In addition, simulating lake conditions in a laboratory setting can be incorporated to represent the shell morphometric differences of snails in the wild. It is also recommended to investigate the effects of these parameters on the embryological development and visceral mass tissues of snails to examine how those that lack a distinct operculum, such as lymnaeids, respond to such changes in freshwater ecosystems. The number of lineages that *Melanoides* species has may have also contributed to shell shape variations of the samples collected since there was no verification done to prove that all the samples belonged to a single species. Therefore, it is crucial to conduct genetic testing before morphometric analyses to conclusively prove that the possible shell shape variations exhibited are caused by biotic or abiotic parameters alone.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Giancarl Wincer A. Catalan is the corresponding author conducting morphometric measurements of the collected *M. tuberculata* shells. Emmanuel Ryan C. de Chavez is the research adviser who helped formulate the study's methodology, collection of shell samples, data analysis, and editing of the manuscript.

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