Journal of Environmental Science and Management SI-1: 40-57 (2019) ISSN 0119-1144

Morphological Changes in *Corbicula fluminea* (Muller 1774) Shells from Laguna de Bay, Philippines Due to Elevated Nitrate and Hexavalent Chromium

Marlon V. Elvira[1,2*]
Emmanuel Ryan de Chavez[3]
Decibel V. Faustino-Eslava[2]
Maria Victoria O. Espaldon[2]
Loucel E. Cui[2]

[1] College of Forestry and Environmental Science, Caraga State University, Ampayon Butuan City, Philippines 8600
[2] School of Environmental Science and Management, University of the Philippines Los Baños (UPLB), College, Laguna, Philippines 4031
[3] Institute of Biological Science, UPLB, College, Laguna, Philippines 4030

*Corresponding author: mvelvira@up.edu.ph

ABSTRACT

Developmental instability using fluctuating asymmetry is a tool for morphological assessment to reflect the state of species adaptation and individual fitness. This research focused on the conchological analysis of Corbicula fluminea (Muller, 1774) shells relative to key water parameters in Laguna de Bay, Philippines. Results from the western and eastern regions of the lake, characterized by marked differences in water qualities, indicate that linear shell characteristics are not significantly affected by water quality, in contrast to other geometric parameters. Geometric morphometrics of Malahanobis and Procrustes distances exhibit horizontal elongation in shells from the west, and vertical elongation in those from the east. Generalized linear mixed modelling of shell morphometry with water quality revealed an inverse relationship between NO_{3-} *and shell size, and a direct association between shell shape changes and elevated Cr (VI). The combination of* NO_{3-}*, water pH and total suspended solids were the factors identified to have the most effect on shell size, with Cr (VI),* NH_{4+}*, and water pressure playing significant roles in affecting shape. Results of this work demonstrate the utility of C. fluminea's fluctuating asymmetry as influenced by water quality to constitute important baselines for the management of a lake and its biological resources.*

Key words: *Corbicula fluminea, Asiatic clam, Laguna de Bay, conchometrics, hexavalent chromium, nitrate*

INTRODUCTION

The variance of morphometric differences between the right and left sides in bilaterally symmetrical organisms is generally known as fluctuating asymmetry. It measures how an organism can buffer its growth and development as an adaptive mechanism against environmental stress during ontogeny (*Palmer 1994*). Moreover, it refers to the developmental stability of organisms undergoing ontogenetic processes, notwithstanding both genetic and environmental perturbations (*Clarke 1998*). This ability is thought to be influenced by genotype during environmental interactions (*Dongen and Lens 2000*).

The fluctuating asymmetry level of individual organism and its population are found to be significantly influenced by environmental stresses (*Leung and Forbes 1996*). The use of fluctuating asymmetry in assessing developmental stability and ecological stress is based on the assumption that a stressful environment would result in higher fluctuating asymmetry levels than those observed in optimum environments (*Leong et al. 2013; Velickovic 2004*). The energy requirement of an organism as compensation for stress is indicative of the interaction between fluctuating asymmetry and environmental stress. Reproduction and growth are commonly affected due to the reduction in energy which may eventually affect the population (*Koehn and Bayne 1989*).

Previous studies measured developmental instability and assessed the morphology of an organism and its population using fluctuating asymmetry. This approach can reflect a population's state of adaptation, co-adaptation, fitness, and individual quality. It also evaluates the quality of the preferred habitat of species, the environmental and genetic perturbations to which they are subjected (*Moller and Swaddle 1997*), and the intra- and inter-species relationships using morphometric shape variables (*Rohlf 2002; Toro et al. 2010*).

Bivalves are frequently used in biological monitoring studies because of their widespread distribution, sedentary habits, hardiness, and ability to bioaccumulate pollutants without excessive mortality (*Farrington 1983; Elder and Collins 1991*). The use of bivalves as sentinel organisms is instrumental in assessing the bioavailability of metal contaminants (*Farrington 1983; O'Conner and Beliaeff 1995*). Many in situ studies on bivalves have found significant correlation between tissue metal concentrations and adverse effects including inhibition of growth and impaired reproduction (*Widdows et al. 1981; Widdows 1985; Salazar and Salazar 1991*).

Furthermore, various researches used shell allometry and growth models in understanding the population structure, growth behavior, and shell morphometrics with respect to environmental conditions (*Elkarmi and Ismail 2007*). However, there are some criticisms arguing that shell characters are useless for the analysis of morphology since they easily respond to environmental factors (*Smith and Hendricks 2013*). Although allometric shell pattern is more prone to homoplastic evolution compared to anatomical and molecular evolution, this approach is still important for phylogenetic hypotheses (*Hermsen and Hendricks 2008*).

Asiatic clam *Corbicula fluminea* (*Muller 1774*) is a good bioindicator species due to its sedentary nature, widespread distribution, long lifespan, capacity to accumulate chemicals as filter feeder, genetic variability and phenotypic plasticity, physiological tolerance to abiotic changes, opportunistic behavior (r-strategist), and self-fertilizing nature (*Doherty 1990; Graney et al. 1984; Sousa et al. 2008a; Kraemer and Lott 1977; Doherty et al. 1987a*). The combination of all these traits and its ability to bioaccumulate and amplify several contaminants make Asiatic clam a very convenient object in ecotoxicology (*Way et al. 1990; Bassack et al. 1997; Baudrimont et al. 1997a; Inza et al. 1997; Narbonne et al. 1999; Tran et al. 2001; Cataldo et al. 2001a; Cataldo et al. 2001b; Achard et al. 2004*). The clam can feed from both water and sediments, and its tissue can reflect ambient metal concentrations over time (*Peltier et al. 2008*). Comprehensive investigations have shown that this freshwater clam is capable of surviving exposure to polymetallic polluted environments (*Estefania et al. 2017*).

Due to continuous waste disposal, Laguna de Bay's ecosystem structure is increasingly altered and degraded. Bioaccumulation is vital to various filter feeder organisms in the lake including Asiatic clam. The extent of biomagnification had been amplified across higher trophic levels. Currently, there are only few studies on the bioaccumulation and biomagnification of different pollutants in the lake ecosystem. Moreover, there are no existing studies in Laguna de Bay on how the allometric structure of Asiatic clam had been altered due to waste disposals. This study aimed to determine the linear and geometric morphometric characters of Asiatic Clam from two regions of Laguna de Bay as affected by selected physicochemical properties and heavy metals in the water. The results of this study sheds light on the heavy metal contamination in the Asiatic clam and the ecosystems of the lake in general.

MATERIALS AND METHODS

Study sites

A three-fingered lake forms the central lobe called Laguna de Bay, or Laguna de Bay Caldera is a 200 km² depression. Reported approximate age of 27 to about 50 ka and has been erupted pyroclastic materials (*Catane and Arpa 1998; Arpa et al. 1999a; Catane et al. 2004*). Although the volume of erupted products has not been determined, the caldera is 15 x 30 km² (*Arpa et al. 1999b*), and pyroclastic units from this caldera have been observed in Metro Manila, 40 km to the northwest. Abundant fresh outcrops are observed in quarries near the caldera, but outcrops are poor, due to weathering, where quarries are absent.

Study of *Vogel et al.* (*2006*) revealed that Laguna de Bay Caldera contains lower concentrations of MgO and higher concentrations of Fe_2O_2(t) than the samples from domes and lavas. It was revealed further that the bay has more enrichment in incompatible trace elements. The silicic rocks from the domes, Makiling Volcano and Laguna de Bay Caldera all contain high alkalis and high KO/NaO ratios (*Vogel et al. 2006*).

Anthropogenic operations in the East region of Laguna de Bay particularly in the Municipality of Santa Cruz, Pila, and Victoria involved lithographic, construction, plastic, garment, livestock and agribusiness operations. The lithographic services involved in printing and fabrication of poster products. The manufacturer of plastic products and garment operation involved in woven fabrics are also present. The production of construction materials such as tiles and many other construction supplies are also operational within the vicinity of the lake. Furthermore, the most significant activities within the bay involved agribusinesses like manufacturing of feeds specialized in shrimps and fish feeds. Livestock operations, such as duck and goat farming are also present with vegetable, fruits, and main crop activities. These operations have an approximate distance of

0.849 to 5.91 km from the shoreline of Laguna de Bay. In the West Bay of Laguna de Bay, the major anthropogenic operations include manufacturing of automotive, chemical, metallurgical, construction, food, garment, paper and plastic, and agribusiness products. The nature of factories involved in automotive products include the production of molded rubber and equipment, machining, spare parts for machinery and equipment, fabricate carbon graphite products for mechanical and electrical applications, welding and assembly of machine tools, air-conditioner and refrigerator, heating equipment and other electrical appliances. The chemical production is also present commonly involved in the manufacturing of diesel engine, chemical for machinery, epoxy adhesive, marine paints, concrete admixture, industrial coating, and waterproofing solution. The metallurgical industries along the lakeshore are involved in metal cutting, gaskets, packing materials, hydraulic equipment, shipbuilding and hardware production such as bearing. Industries engaged in the productions of plastic crates, food container, trolleys and pallets, glass processing, fabricate metal, plastic, and rubber are also present. The operation for a manufacturer of construction supplies, such as waterproofing membranes, tiles, PUP sealants, tile adhesive, and other high-quality building and construction materials are also present. The manufacturing industries of food products mainly for food ingredient from seaweeds and other goods are also operational within the proximity of the lake. The garment operations involved in the manufacturing of heavy-duty tactic gear costume for military, button production, women and issues blouses and skirts are also present. Some industrial activities involved in the manufacturing of household cleaning and pharmaceutical products such as medication and vitamins, body and dishwashing soaps and laundry detergents are also present within the proximity of the lake. The agribusiness operations focused mainly on the manufacturing and marketing of interior and IT solution products and paper mill industry are also present. These industrial operations have an approximate distance of 0.206 to 6.45 km from the shoreline of Laguna de Bay.

The study was conducted in October 2017 along the East (Sub-urban/Agricultural Region) and West (Highly Urbanized Region) regions of Laguna de Bay, Philippines. Sampling stations in the East Bay is composed of Municipality of Santa Cruz, Pila, and Victoria. Generally, East Bay is a grassland covering 70% and cropland mixed with coconut plantation. Poultry and other livestock operations are also present in the area. Although, there are few industrial activities and settlements within this area, it is classified as sub-urban area. Stations in the West Bay are composed of San Pablo, Muntinlupa, and Taguig City. This bay has large build up areas where industrial operation and settlements are situated. This portion of Laguna de Bay is classified as an urbanized area. Three sampling replicates (Barangay) were established per station (Municipality). A total of 18 sampling replicates were determined across 6 sampling stations (**Figure 1**).

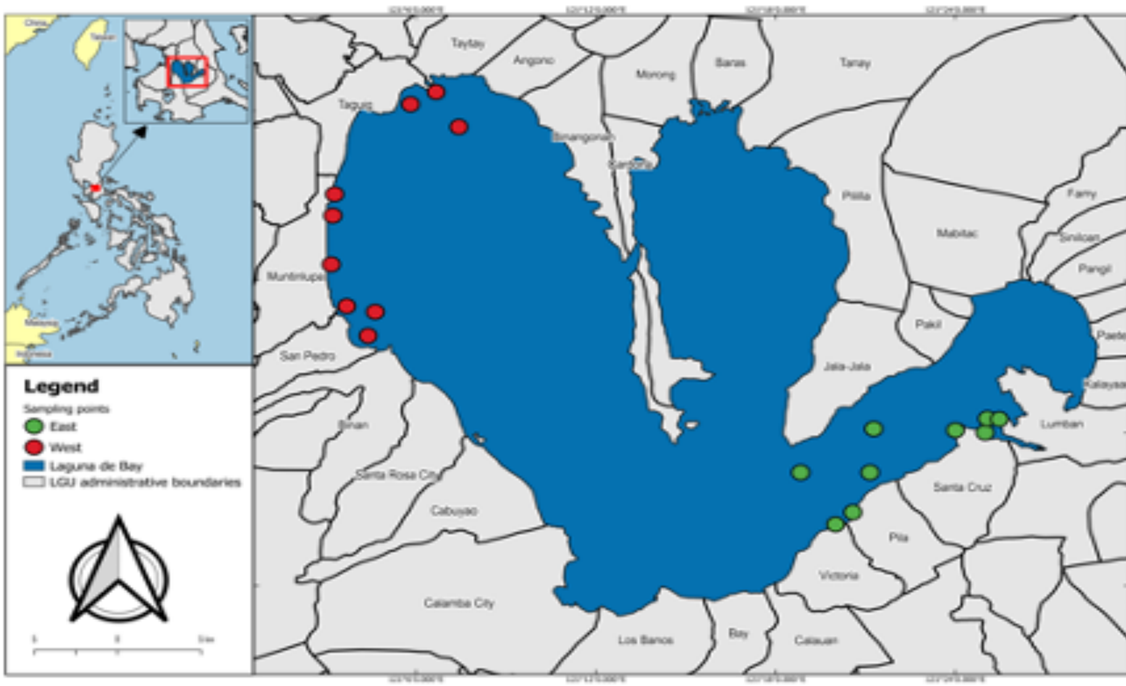

Figure 1. Map of Laguna de Bay showing the location of sampling sites. The sampling sites in the western region (Taguig, Muntinlupa and San Pedro) are marked by red circles while those in eastern region (Santa Cruz, Pila and Victoria) are marked by green circles.

Sampling of *Corbicula fluminea*

A total of 90 individuals of Asiatic clam were randomly collected from each sampling station in Laguna de Bay. In each area, three sampling replicates were established where 30 individuals were collected. The collected samples were kept in a plastic container with lake water during transport to the laboratory. The samples were sorted and placed in a laboratory set-up closer to natural conditions upon arrival at the Malacology Laboratory at the Institute of Biological Science, University of the Philippines Los Baños (UPLB). Each container was provided with an aerator to maintain the available dissolved oxygen for the collected clams. Chlorella sp. was used as food for the Asiatic clam throughout the set-up duration before sample analysis.

Environmental Variables

Physicochemical characteristics of water, such as pH, temperature, conductivity, turbidity, dissolved oxygen (DO), ammonium, nitrate, pressure, depth, transparency, and salinity were analyzed in-situ using Troll 900 multi-parameter portable water quality analyzer per sampling station. The total suspended solids (TSS) was analyzed ex-situ using the gravimetric method. The heavy metal in the water such as mercury (Hg), lead (Pb), cadmium (Cd), hexavalent chromium (Cr VI), nickel (Ni) and arsenic (As) were determined and analyzed ex-situ through the Trace20 Metalyser at the School of Environmental Science and Management Laboratory, UPLB. Results from the analyses of physicochemical parameters in the water were compared with the standard set by the Department of Environment and Natural Resources (DENR) under Administrative Order 08, series of 2016.

Linear Morphometrics

Niku-nuki method was employed to Asiatic clam samples to separate their shells and muscles (*Fukuda et al. 2008*). Shell samples were then used for the morphometric analysis. The soft tissues of the clams were removed and placed in a 70% formalin solution and stored in a refrigerator for future analysis. The shell length (SL) (*Scheltema 1983; Idris et al. 2008*), shell height (SH), and shell width (SW) (*Mass et al. 1999*) of thirty representative samples of Asiatic clam per sampling station were measured using a digital caliper DT-150 (in mm). The umbo length (UL), anterior length (AL) (*Mass et al. 1999*), posterior length (PL), cardinal tooth length (LCT), anterior adductor muscle scar width (PVM), length from anterior adductor muscle scar to anterior margin (AAAM), length from posterior adductor muscle scar to posterior margin (PAPM) and length from ventral margin to pallial line (PVM) (*Hamli 2015*) were also measured using ImageJ 1.51k (*Schneider et al. 2012*) for multidimensional image processing and analysis (**Figure 2**). Specimens were photographed using Canon EOS 600D digital camera.

Geometric Morphometrics

One hundred eighty individuals of Asiatic clam were used for the geometric morphometric analysis. All samples were cleaned of soft tissues, and the inner side of the right valves were used. The pictures were digitalized with the tps Utility Program version 1.74 and were utilized for the establishment of anatomical landmarks using tpsDig2 version 2.30 (*Rohlf 2017*). The forms of individuals were captured by the Cartesian coordinates of the two-dimensional configuration of 15 Type I and 16

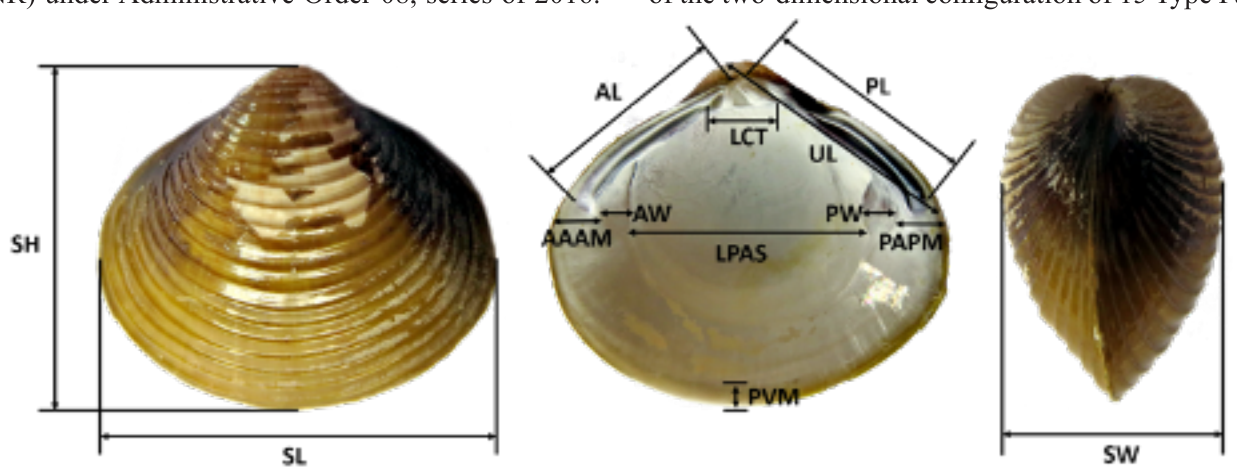

Figure 2. Diagram of linear morphometric character of Asiatic clam shell. Shell Length (SL), Shell Height (SH), Shell Width (SW), Umbo Length (UL), Anterior Length (AL), Posterior Length (PL), Cardinal Tooth Length (LCT), anterior adductor muscle scar width (PVM), length from anterior adductor muscle scar to anterior margin (AAAM), length from posterior adductor muscle scar to posterior margin (PAPM) and length from ventral margin to pallial line (PVM).

Type II anatomical landmarks. The Type I landmarks were placed in the adductor muscle scars and hinge teeth (*Sousa et al. 2007*). On the other hand, Type II landmarks were placed along the shell margin (**Figure 3**). Landmark configurations were superimposed by generalized Procrustes analysis (*Rohlf and Slice 1990; Slice et al. 1996*). This procedure translates and rotates the landmark configurations to a common origin and scales them to unit centroid size.

Data Analysis

For linear shell analysis, Pearson correlation was applied for measured shell characters to discriminate characters with high coefficients (r=0.90). After removing the highly correlated characters, principal component analysis (PCA) using variance-covariance matrix was used to extract the major components contributing to site variation.

The Procrustes transformation was used for geometric morphometric analysis. The variance-covariance matrix was generated before PCA to display the major features of length variation. Canonical variance (CV) analysis was performed to get the major features of shape variation in the first two CVs. Lollipop diagram and transformation grid were used to represent the values of CV 1 and CV 2. A wireframe diagram was also utilized to represent the extreme shell shape change over the average shape of Asiatic clam. Moreover, centroid shape, Mahalanobis and Procrustes distances were used to explain the possible significant difference (≤ p =0.05) between sampling areas and across sampling stations. Additionally, shell shape (RW1) and size (PC1) were computed and compared

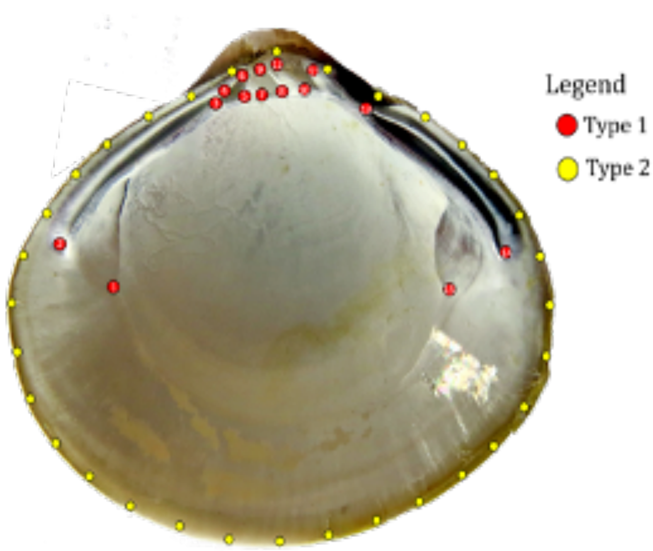

Figure 3. Locations of type 1 (red circle) (n=15) and type 2 (yellow circle) (n=32) anatomical landmarks on the Asiatic clam, *Corbicula fluminea*, shell.

using XY graph plot analyze the differences across sampling stations.

Environmental variables were calculated using correlation analysis to discriminate highly corelated variables at r=0.90. To determine the relationship of response variables (shell shape and size) against fixed factors (environmental variables), generalized linear mixed model (GLMM) using RStudio version 1.1.383 was employed. The first run for global model categorizing fixed and random variables was performed to determine the top five most significant variables that possibly affects the morphometric characters. The final run for global model categorizing fixed and random variables was performed to determine the top three most significant variables that affects the morphometric characters. Akaike's information criterion corrected for small sample size (AICc) was used to assess the most parsimonious model support (*Burnham and Anderson 2002*).

RESULTS AND DISCUSSION

Environmental Variables

The physicochemical analysis of water revealed only ammonium exceeded the standard at 0.05 mg L^{-1} set by the DENR for Class C freshwater with the highest recorded value of 0.21 mg L^{-1} (**Table 1**). Across sampling stations, parameters were tested with several replications revealed the maximum value of 8.68 for pH, 29.87 ^{o}C for temperature, 628.40 S cm^{-1} for conductivity, 57.00 NTU for turbidity, 20.34 mg L^{-1} for DO, 0.21 mg L^{-1} for ammonium, 0.94 mg L^{-1} for nitrate, 1.67 psi for pressure, 2.75 m for depth, 10.5.83 cm for transparency, 0.28 ppt for salinity, and 23.22 mg L^{-1} for TSS, respectively. In most sampling points tested across stations, the parameters such as pH, temperature, DO, Nitrate, and TSS were mostly at a tolerable level. Furthermore, temperature conductivity, DO, ammonium, nitrate, and salinity showed significance across sampling regions.

The analysis of heavy metals in the water revealed that Cr (VI) and Ni have values above the detectable limit (>0.005 mg L^{-1}) among other heavy metals such as Hg and Pb (<0.005 mg L^{-1}) and Cd and As (<0.003 mg L^{-1}) (**Table 2**). Cr (VI) and Ni with several replications tested among others revealed the maximum value of 0.11 mg L^{-1} and 1.90 mg L^{-1} across the sampling station. Although Cr (VI) and Ni were not significantly different (p = 0.7775 and 0.2919) across sampling regions, they exceeded the standard at 0.01 mg L^{-1} and 0.2 mg L^{-1} set by the DENR for Class C freshwater.

Table 1. Water physico-chemical parameters measured across the eastern and western regions of Laguna de Bay (n=18) compared with the Department of Environment and Natural Resources (DENR) water quality standards.

Parameter (unit)	Mean ± SD		*p*-Value	DENR Class C Standard
	East Bay	West Bay		
pH	7.87 ± 0.32	7.86 ± 0.41	0.9741ns	6.5 – 9.0
Temperature (°C)	27.57 ± 0.65	29.37 ± 0.35	<0.0001****	25-31
Conductivity (S cm^{-1})	198.95 ± 33.12	597.21 ± 29.32	<0.0001****	-
Turbidity (NTU)	34.89 ± 8.51	35.79 ± 13.33	0.8671ns	-
Dissolved Oxygen (mg L^{-1})	7.08 ± 1.28	11.99 ± 4.69	0.0079***	5.0
Ammonium (mg L^{-1})	0.06 ± 0.02	0.15 ± 0.04	<0.0001****	0.05
Nitrate (mg L^{-1})	0.49 ± 0.29	0.17 ± 0.24	0.0204*	7.0
Pressure (psi)	1.31 ± 0.22	1.33 ± 0.19	0.8420ns	-
Depth (m)	2.16 ± 0.44	2.07 ± 0.46	0.6703ns	-
Transparency (cm)	34.57 ± 27.55	37.56 ± 21.44	0.8010ns	-
Salinity (ppt)	0.09 ± 0.01	0.27 ± 0.01	<0.0001****	-
Total Suspended Solids (mg L^{-1})	10.65 ± 7.22	7.42 ± 6.97	0.3478ns	80.0

ns- p > 0.05, * p ≤ 0.05, **p ≤ 0.01, *** p≤ 0.001, **** p ≤ 0.0001.

Table 2. Heavy metals detected in the water across sampling areas in the eastern and western region of Laguna de Bay (n=18) compared with the DENR allowable heavy metals in water standard.

Parameter (unit)	Mean ± SD		*p*-Value	DENR Class C Standard
	East Bay	West Bay		
Mercury (mg L^{-1})	<0.005	<0.005	-	0.002
Lead (mg L^{-1})	<0.005	<0.005	-	0.05
Cadmium (mg L^{-1})	<0.003	<0.003	-	0.005
Hexavalent Chromium (mg L^{-1})	0.044 ± 0.033	0.040 ± 0.011	0.7775ns	0.01
Nickel (mg L^{-1})	0.556 ± 0.534	0.356 ± 0.133	0.2919ns	0.2
Arsenic (mg L^{-1})	<0.003	<,.0.003	-	0.02

Readings from the Trace20 Metalyzer with values less than 0.005 and 0.003 mg L^{-1} indicates below detectable limits (BDL).

Correlation analysis revealed that SL, LCT, AW, PW, AAAM, PAPM and PVM were not highly correlated (≤ r=0.90). The first two axes of the PCA in all seven variables accounted for 95.46% and 1.77% of the total variation (**Figure 4**). The character loading showed the contribution from each principal component and is quantified by the eigenvalue with significant contribution from the first two components. The plot assigned to the six municipalities revealed no distinct groupings, however, shell length contributed so much to the value of the first PC (0.98) (**Table 3**). Thus, Asiatic clam from West Bay (Cities of Taguig, Muntinlupa, San Pedro) and East Bay (Municipalities of Santa Cruz, Pila and Victoria) showed no differences in terms of its linear morphology.

Table 3. Shell character loadings of Asiatic clam, *Corbicula fluminea*, in the top principal components (PC) (n=180).

Character	PC 1	PC 2
SL*	0.97723	-0.16029
LTC	0.13635	0.93084
AW	0.056462	0.047742
PW	0.10979	-0.056751
AAAM	0.069191	0.24516
PAPM	0.07658	0.18786
PVM	0.02295	0.083367

*see text for description

Geometric Morphometrics

Lollipop diagram and transformation grid showed the shape changes and variation associated with CV 1 and CV 2 for Asiatic clam (**Figure 5**). The landmarks from distinct parts of the inner valve showed a significant transformation from the average shape to extreme shell shape change (**Figure 6**). It expressed the transformation of anterior and posterior length, anterior and posterior muscle scars length and the cardinal teeth length from the reference landmarks. The shell shape from CV 1 showed horizontal elongation pattern while CV 2 showed vertical elongation pattern (**Figure 6**).

The centroid size of Asiatic clam was computed to determine the shape differences across sampling stations.

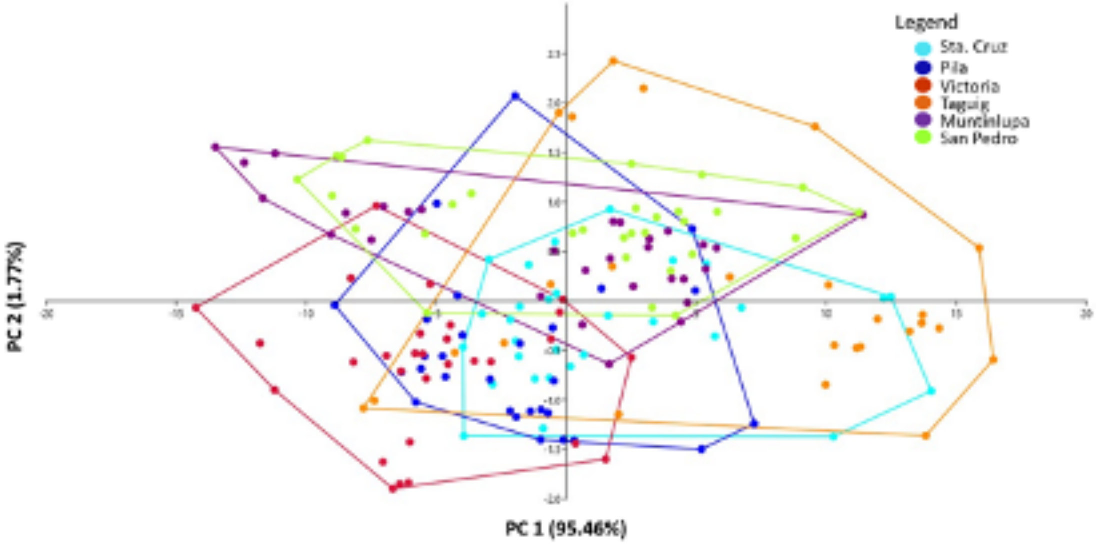

Figure 4. Scatterplot of seven shell characters of the Asiatic clam, *Corbicula fluminea*, across six sampling stations of Laguna de Bay. Replicates per site are bounded by a convex hull.

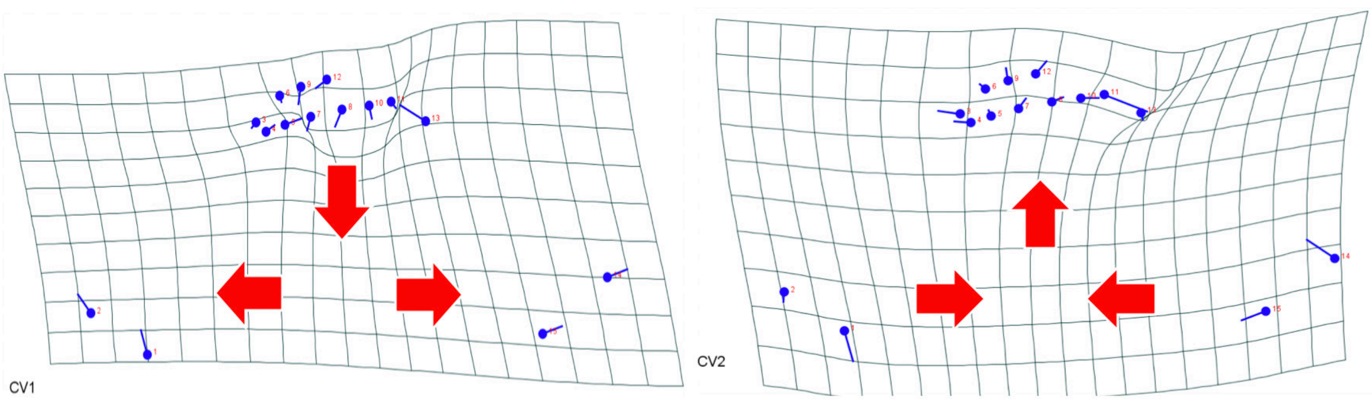

Figure 5. Lollipop diagram and transformation grid of Asiatic clam represented by canonical variate axis 1 (CV 1) and canonical variate axis 2 (CV 2). Red arrow represents the direction of shell shape change; blue head dot represents the original position while the tail is the final position.

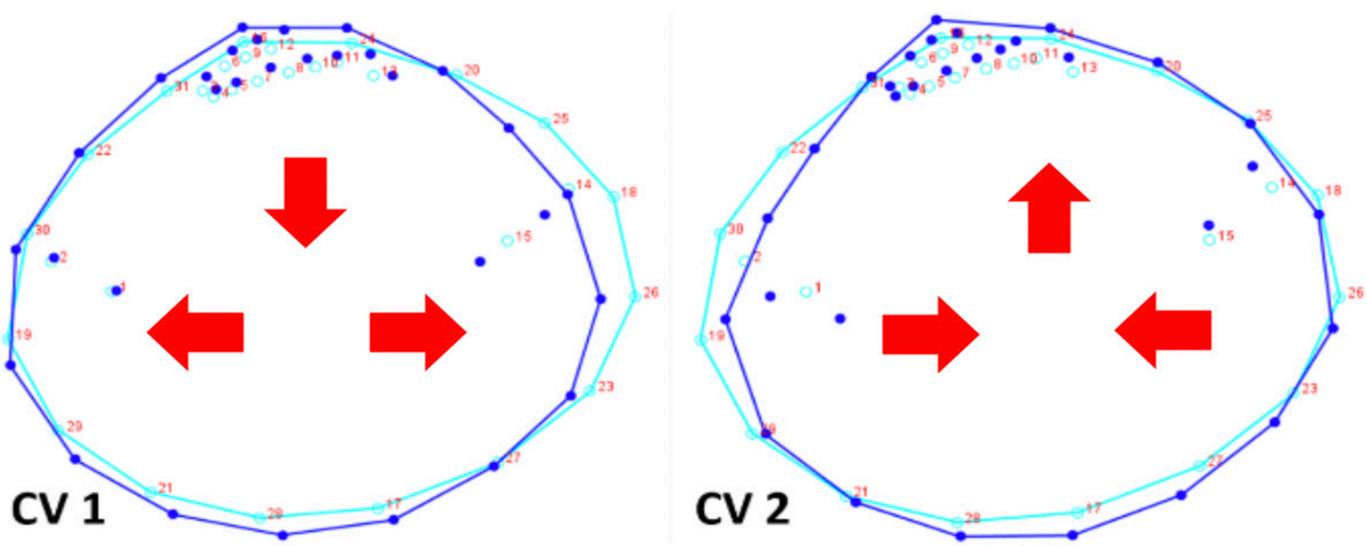

Figure 6. Wireframe diagram of Asiatic clam represented by canonical variate axis 1 (CV 1) and canonical variate axis 2 (CV 2). The red arrow represents the direction of shell shape change. The dark blue outline is the average shape while the light blue is the most extreme shape change.

It revealed that the shape of the clam samples from Santa Cruz was significantly different from those in Pila (p =0.0048) and Victoria (p =0.0027). Moreover, the shape of the clam samples from Taguig was significantly different from those in Muntinlupa (p =0.0004) and San Pedro (p =0.0269). Based on the differences between sites, the shape of clams from East Bay was significantly different from West Bay (p =0.012) (**Figure 7**).

Percentage of the total canonical variance were calculated (**Figure 8**). CV 1 accounted for 10.54% of the total variance among the 180 samples across six sampling stations while CV 2 accounted for 4.98%. Despite thislow percentage, CV 1 and CV 2 represents the most variance of the two CVs and contained the best data for CV score comparisons.

A wide distribution and considerable overlap were observed across sampling stations. However, p-values from the permutation tests for Mahalanobis distances on the geometric morphometric characters of the shell showed significant difference (p<0.0001) in Muntinlupa versus Pila, San Pedro and Victoria. Pila was significantly different (p<0.0001) from San Pedro, Santa Cruz and Taguig. San Pedro was also found to be significantly different (p<0.0001) form Santa Cruz and Victoria. Santa Cruz has a significant difference (p<0.0001) to Taguig and Victoria. Taguig and Victoria were also significantly different. Moreover, Muntinlupa was significantly different (p=0.023) from Taguig. San Pedro was also significantly different from Taguig (p=0.0086).

Another permutation test using Procrustes distance among stations was used. It revealed that Muntinlupa was significantly different (p<0.0001) from Pila, Victoria, Santa Cruz (p=0.0021) and Taguig (p=0.0447). Pila was significantly different from San Pedro (p= 0.002) and Santa Cruz (p=0.0016). Moreover, San Pedro was significantly different from Santa Cruz (p=0.0089), Taguig (p= 0.0195) and Victoria (p=0.0021). Santa Cruz was significantly different from Taguig (p=0.0003) and Victoria (p=0.0001). Taguig was significantly different from Victoria (p=0.0036). For the differences between West and East Bay, Mahalanobis and Procrustes distance showed a significant difference at p=0.0001 and p=0.018, respectively (**Figure 9**).

Shell shape (RW1) and size (PC1) were compared across sampling stations. Maximum and minimum shell shape measurement was observed in San Pedro City while maximum shell length measurement was observed in Muntinlupa City. Minimum shell length measurement of Asiatic clam was observed in Santa Cruz (**Figure 10**).

Environmental variables such as mercury (Hg), lead (Pb), cadmium (Cd), hexavalent chromium (Cr (VI)), nickel (Ni), arsenic (As), pH, temperature, conductivity, turbidity, dissolved oxygen (DO), ammonium (NH_4^+), nitrate (NO_3^-), pressure, depth, transparency, salinity and total suspended solids (TSS) were considered as fixed factors in the shell size and shape response of Asiatic clam. Correlation analysis was computed before the GLMM analysis. Only Cr (VI), Ni, pH, DO, NH_4^+, NO_3^-, pressure, and TSS were not highly correlated below r=0.70.

To model the shell size and shape of Asiatic clam across sampling sites, a generalized linear mixed model that considered the identified environmental factors that presumably had the strongest effects on the shell size and

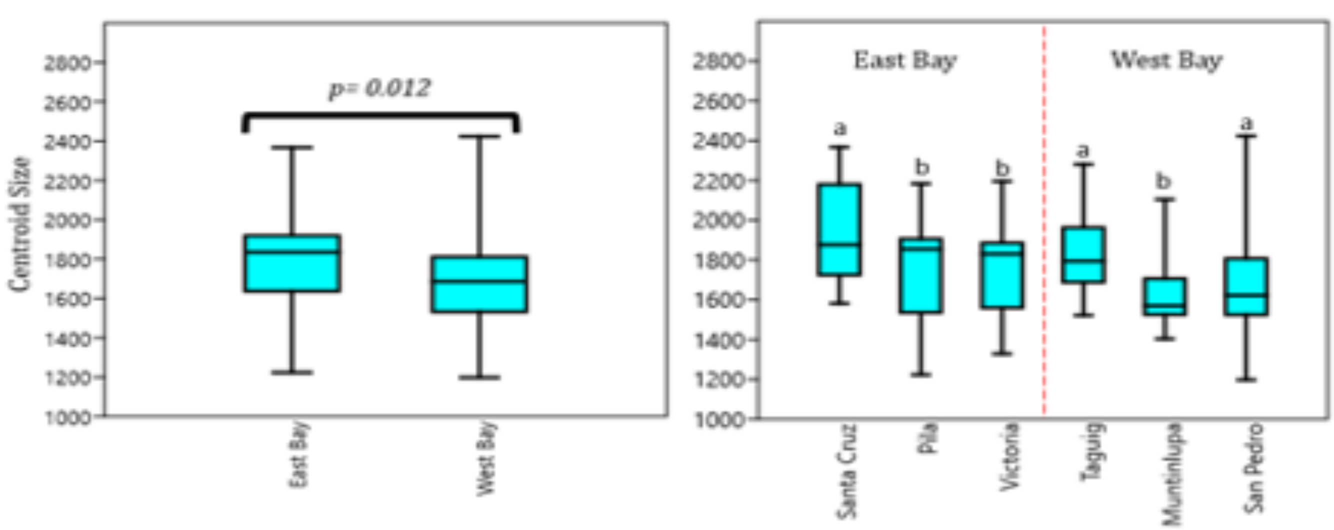

Figure 7. Centroid sizes of the Asiatic clam, *Corbicula fluminea*, shell across sampling stations and within sampling areas of Laguna de Bay. Consecutive different letters indicate significant difference at p ≤ 0.05.

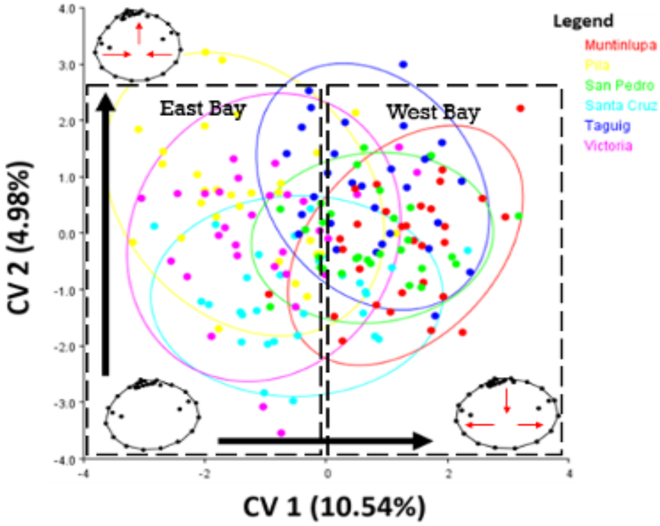

Figure 8. Canonical variance of Asiatic clam, *Corbicula fluminea*, represented by canonical variate (CV) 1 and CV 2 across six municipalities of Laguna de Bay.

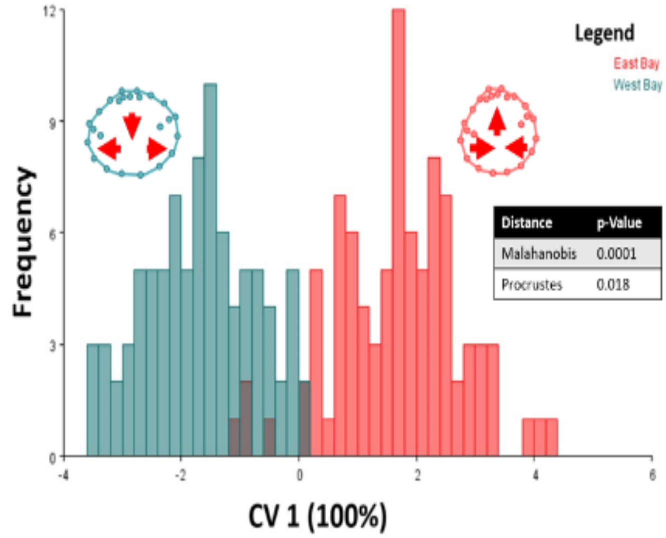

Figure 9. Discriminant analysis of Asiatic clam, *Corbicula fluminea*, represented by canonical variate (CV) 1 across east and west regions of Laguna de Bay.

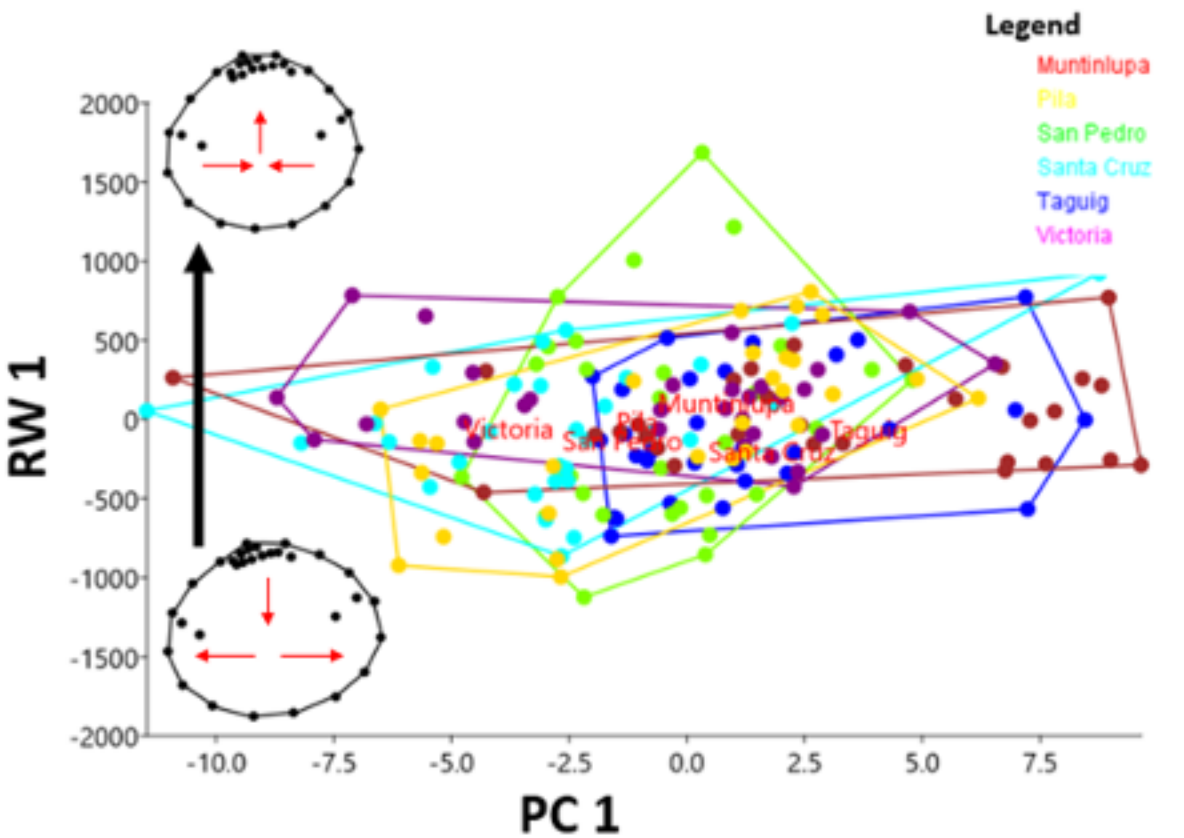

Figure 10. *Corbicula fluminea* shell shape (RW1) and size (PC1) variation among the six municipalities across Laguna de Bay. Arrow in the y-axis depicted a shift of shell shape from horizontal to vertical elongation while x-axis explained the accounted individuals with different shell sizes across sampling stations.

shape variation was constructed. NO_{3-} ($p<0.0001$) has a significant influence on the shell size change of Asiatic clam (**Table 4**). This environmental variable was among those with the highest relative importance value (RI=1.00). Furthermore, Cr (VI) influenced the vertical elongations of Asiatic clam but it was not statistically significant ($p = 0.924$). The combination of component model NO_{3-}+pH+TSS revealed positive influence on the shell size change (ΔAICc=0.00 and wAICc=0.59) while component model Cr(VI)+NH4++PRESS showed a positive influence on the shell shape change of Asiatic clam (ΔAICc=0.00 and wAICc=0.979) (**Table 5**).

Most of the water physicochemical parameters were significantly different across sampling sites which may be due to the variation in anthropogenic activities (*Verma et al. 2012*). Based on the reconnaissance activity, East Bay was categorized as suburban and agricultural while West Bay was categorized as highly urbanized. However, heavy metals were not significantly different across sampling sites. This can be attributed to the geogenic nature of Laguna de Bay; it is a large volcano-tectonic depression within Macolod Corridor while its neighboring Mount Makiling is one of the stratovolcanoes (*Forster et al. 1990*). The three-fingered lake forms the central lobe called the Laguna de Bay Caldera has an an approximate age of 27 to 50 thousand years and erupted with pyroclastic material (*Catane and Arpa 1998; Arpa et al. 1999a; Catane et al. 2004*). Abundant fresh outcrops were observed in quarries near the caldera, but outcrops are poor due to weathering, where quarries are absent. Based on the chemical analyses of pumice fragments, the pyroclastic deposits range in composition from 53 to 69 wt% SiO_2 (*Vogel et al. 2006*). Furthermore, even East Bay was considered as sub-urban area, however, industries involved in lithographic, construction, plastic and garment productions are also present which is similar to the West Bay where settlements and industrial operation such as manufacturing of automotive, chemical, metallurgical, construction, food, garment, paper and plastic, and agribusiness products were present. According to *El-Sayed and Wl-Sayed* (*2014*), the prevalent heavy metal pollution from the garment, plastic and lithographic industries are Pb, Hg, Cd, Cr, Co and As while operations from automotive, chemical, metallurgical, construction, foods and paper are Pb, Cr, and Cd, Cu, Zn, Ni, Co, Hg, and As (*Mendez-Garcia et al. 1996; Villalobos et al. 2009; Valdez-Vega et al. 2011; Sallaku et al. 2009; Nadal et al. 2005; El-Sayed and Wl-Sayed 2014*). Additionally, wind in Laguna de Bay is dominated by northeast wind direction. The wind direction is the major contributor for the direction of the flow of water in Laguna de Bay (*Cunanan and Salvacion 2014*). This means that the flow of the water from the West Bay and Central Bay move towards South Bay and eventually directed to East Bay. The flow of the water and the anthropogenic activities within Laguna de Bay could be the reasons why heavy metal

Table 4. Generalized linear mixed models testing each environmental variable on the shell size and shape of Asiatic clam, *Corbicula fluminea*, across sampling areas in Laguna de Bay (n=180).

Environmental Variables	Estimate	SE	p-Value	Relative Importance
Shell Size				
INTERCEPT	0.0131	1.4071	0.9926	
Nitrate	-3.5126	0.6099	<0.0001***	1
pH	-0.6954	0.7304	0.3428	0.93
Total Suspended Solids	-1.3697	0.6859	0.0468*	0.67
Shell Shape	-4.04052	0.64853	< 0.0001 ***	.82
INTERCEPT	301.1	412.7	0.730	-
Chromium	1757.2	1901.4	0.924	-
Ammonium	702.6	547.8	1.282	-
Pressure	-344.6	270.5	-1.274	-

Table 5. Summary statistics of model averaging for shell size and shape of *Corbicula fluminea* across sampling areas in Laguna de Bay (n=180). Models are ranked on Akaike's information criterion corrected for small sample size (AICC) where AICC weights (wAICC) >0.50 are considered. Predictor variables: NIT (Nitrate), PH (Potential of Hydrogen), TSS (Total Suspended Solids), AMM (Ammonium), CR (Chromium) and PRE (Pressure).

Component Model	k*	AICC	ΔAICC	wAICC
Shell Size				
NIT+PH+TSS	7	945.23	0.00	0.59
NIT+TSS	6	946.38	1.15	0.33
NIT+PH	6	949.38	4.15	0.07
Shell Shape				
AMM+CR+PRE	7	2647.6	0.00	0.979
CR+PRE	6	2657.1	9.51	0.008
AMM+PRE	6	2657.7	10.01	0.007

*k- number of parameters

concentrations across sampling sites were not significantly different.

The linear characters of Asiatic clam across sampling stations have no significant differences. However, the Asiatic clam from West Bay was observed to have horizontal elongations where the length from the cardinal teeth to pallial line was narrow while the anterior to posterior distance was wide. Samples from the East Bay have observable vertical elongations where length from the cardinal teeth to pallial line was wider while the anterior to posterior distance was narrow. These findings may support further the initial hypothesis of the researcher that shell shape of Asiatic clam from East Bay was different from West Bay. According to *Monteiro et al.* (*2000*), the phenotypes revealed in the final shape of structures, organs and organisms arise from the interfacing and complex combination between morphogenetic rules, ecological conditions, and deterministic and stochastic evolutionary forces. It is generally accepted that bivalve shells have great plasticity to adapt to the different environmental and ecological conditions which is very common in freshwater bivalve species (*Baker et al. 2003*). Comparative studies have shown that bivalve species exhibit several distinct morphological characteristics that allow them to adapt to epifaunal existence, to avoid predation and parasitism, to maintain adequate current flow under crowded conditions and adapt to wave exposure, type of substratum, salinity and calcium availability (*Stanley 1983; Gardner and Skibinski 1991; Willis and Skibinski 1992; Norberg and Tendengren 1995; Baker et al. 2003*).

NO_{3-} is a product of surface run-off from agricultural activities, particularly excessive fertilizer uses. This chemical is the primary cause of cultural eutrophication. Under eutrophic environment, excessive plant growth and decomposition is common in which favoring those simple algae and plankton over other more complicated plants. When algae die, they decompose, and the nutrients contained in that organic matter are converted into inorganic form by microorganisms. The decomposition process consumes oxygen, which reduces the concentration of dissolved oxygen. This phenomenon turns the aquatic ecosystem to become hypoxic and causes severe reduction in water quality. The Asiatic clam is a filter feeder that consumes zooplankton and phytoplankton for its regular diet. Asiatic clam grows rapidly, partly because it has a high filtration and assimilation capacity (*McMahon 2002*). The major part of its energy is allocated to growth and reproduction and only a small portion is devoted to respiration (*McMahon 2002*). This behavior is highly dependent on the availability of food resources (*Cataldo and Boltovskoy 1999; Mouthon 2001a and b*). Because of the scarcity of food available, the feeding habits of Asiatic clam are altered. This is a probable cause of why nitrate concentration is inversely proportional to the shell size of Asiatic clam (**Figure 11**).

A Class C water in the Philippines must have pH ranging from 6.5 to 8.5 level. In an enclosed water bodies, pH level may increase to values above 9 and become as high as 9.25 and 9.75 (*Macedo et al. 2001; Hansen 2002*). An increased pH greater than the range can be an indicator of photosynthesis by large quantities of algae. Algae can be considered r-strategist since they take optimal advantage of environmental resources (*Klaus 1990*). Algal blooms have dramatic effects on water chemistry, most notably pH and dissolved oxygen. High pH levels in freshwaters have indeed been measured during blooms of cyanobacteria (*An and Jones 2000; López-Archilla et al. 2004*) which means an elevated pH level is important for phytoplankton growth and succession (*Hinga 1992, 2002; Hansen 2002; Pedersen and Hansen 2003; Lundholm et al. 2004, 2005*). Furthermore, similar shifts in the dominance of phytoplankton communities from green algae to cyanobacteria believed to be a response to elevated pH levels (*Shapiro 1990*). During periods of rapid growth, which generate algal blooms, changes in the acidity of the water can not only influence the growth of phytoplankton populations, but also those of the animals feeding on them. This may, in turn, limit the source of food for Asiatic clam and prompts them to adapt in order to survive, thereby triggering further morphometric changes. If the water is ideal for survival, the survival rate of Asiatic clam is higher. Overcrowding and competition of spaces per unit area may ensue and may generate adaptation response from individual species. This is also a probable explanation why the increase of pH could lead to decreases in the shell size of Asiatic clam (**Figure 11**).

The increased level of TSS could also lead to a decrease in shell sizes of Asiatic clam. Urbanization is causally related to the increased concentrations of TSS in the water (*Mallin et al. 2009*). It is an important pollutant in water run-off as it degrades the quality of the receiving environment resulting in more turbid water and impeding the growth and diversity of aquatic flora and fauna. Sediment that settle at the bottom of the lake alter the habitats of benthic organisms (*Ferguson 1998*). TSS is a significant factor in observing water clarity (*Langland and Cronin 2003*) and can be associated with water turbidity. Water Turbidity can inhibit photosynthesis by trapping the sunlight. As a result, DO production will

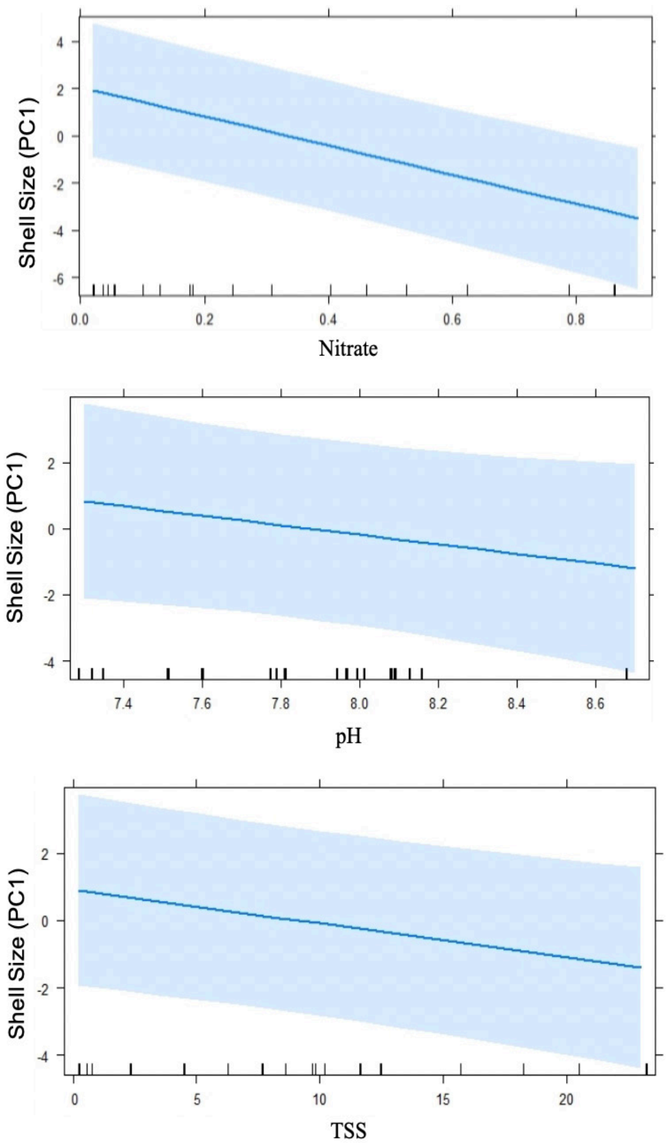

Figure 11. Environmental variables (nitrate, water pH and total suspended solids (TSS)) that have significant influence on the shell size change of Asiatic clam. The blue shade represents 95% confidence levels.

decrease resulting in lower survival of aquatic flora and fauna (*EPA 2012*). The subsequent decomposition of dead plants and animals can lower the level of DO even further. Due to less production of DO, Asiatic clam may develop adaptation strategies in response to a hypoxic environment and may struggle to find available resources. A deprived clam may exhibit lower growth rate.

Asiatic clam is comprised with different reproductive modes (*Korniushin and Glaubrecht 2003*) such as polyploidy, unreductional biflagellate sperm and androgenesis clonality (*Qiu et al. 2001*). Generally, Asiatic clam is considered as hermaphrodite. It has the ability to fertilize inside the paleal cavity in which the incubation of larvae happens in the branchial water tubes (*Sousa et al. 2008*). Moreover, this species deals with the embryonic nutrition of brooding individuals (*Rajagopal et al. 2000*), in which the eggs are rich in nutrients that are essential for the developing embryos (*Byrne et al. 2000*). After this protective period, larvae are released into the water, settle, and bury into the substratum (*Cataldo and Boltovskoy 1999; McMahon 2000*). When juvenile individuals are released, it anchors to sediments, vegetation or hard surfaces due to the presence of a mucilaginous byssal thread and can be re-suspended by turbulent flows and dispersed for long distances (*McMahon 2000*). The maturation period occurs within the first 3 to 6 months when the shell length reaches 6 to10 mm. The life span of this species is extremely variable, ranging from 1 to 5 years, with usual bivoltine juvenile release pattern (*McMahon 2000*). However, the number of annual reproductive periods changes from ecosystem to ecosystem.

Cr is a transition metal that exists in many different oxidation states in the environment, with Cr (VI) and Cr (III) as the most stable form (*Ciacci et al. 2012*). An oxidized form of Cr (VI) is considered a serious health hazard due to its carcinogenic effect, and it is a dominant environmental contaminant released from both domestic and industrial effluents. It represents the predominant levels in natural water in the 0.08-0.15 µg L-1 range (*Maanan 2007*). In aquatic organisms, including bivalve mollusks, the gills are the main site of uptake of metals present in soluble forms, and the possible first target for metal toxicity. Cr (VI) plays a role in the increased incidence of diseases seen in clams (*The et al. 2000*). Asiatic clam has the highest net production efficiencies recorded for any freshwater bivalve, reflected by short turnover times of only 73-91 days. Like other freshwater bivalve species, Asiatic clam transferred only a small percentage of assimilated energy to reproduction. Nevertheless, its elevated assimilation rates allow a high absolute energy transfer to reproduction when compared with other freshwater bivalves. Asiatic clam has a high fecundity but a low juvenile survivorship and a high mortality rate throughout the life span. This low adult survivorship leads to populations dominated by high proportions of juveniles (*McMahon 2000, 2002*). However, in some ecosystems, this population domination by immature juveniles is not so effective and the presence of adults in high abundance and having large sizes has been reported (*Sousa et al. 2008; Cooper et al. 2007*). Juvenile Asiatic clams are more vulnerable to pollutants than those adult individuals. This was observed in Laguna de Bay during sample collection as abundance of empty juvenile shells where noted. Thus, those mature individuals that survive within a polluted environment are those successful individuals throughout their juvenile stage. Since few survive, the remaining individual *C. fluminea* that mature into adults have enough area to occupy throughout their lifespan. They tend to increase their shape without resulting to a more elongated form. This is a probable reason even Cr (VI) is fatal to Asiatic clam when there is an increased concentration; the shape becomes more elongated (**Figure 12**).

NH_4+ is the waste product of the metabolism of animals (*Campbell and Reece 2002*). Biological oxidation of NH_4+ to nitrite, and eventually nitrate is a key part of nitrogen cycle (*Leoni et al. 2018*). Under anoxic conditions in a freshwater ecosystem, anaerobic oxidation of ammonium can occur known as anammox reaction. Meanwhile, nitrification in aerobic conditions involve different taxa of organisms namely archaeal and bacterial ammonia oxidizers (*Hayden et al. 2014; Mukherjee et al. 2016*). This process serves as a fundamental process in aquatic environment that influence the stability and functionality of an ecosystem (*Bernhard et al. 2007; Wang et al. 2014*). Moreover, ammonium is essential for cyanobacteria to proliferate in the lake ecosystems due to its high kinetic uptake (*Collos and Harrison 2014*). In similar way, when NH_4+ concentrations are sufficiently high, ammonium may suppress the overall growth of phytoplanktonic organisms (*Collos and Harrison 2014; Glibert 2016*). If planktons are abundant in the aquatic environment, organisms at higher trophic levels such as Asiatic clam will thrive since more food are available. With the abundance of resources, allocation of energy for the growth maintenance of Asiatic clam can be secured resulting in a more horizontal shell elongation (**Figure 12**). This could be a possible reason why ammonium is directly proportional to the shell shape of Asiatic clam.

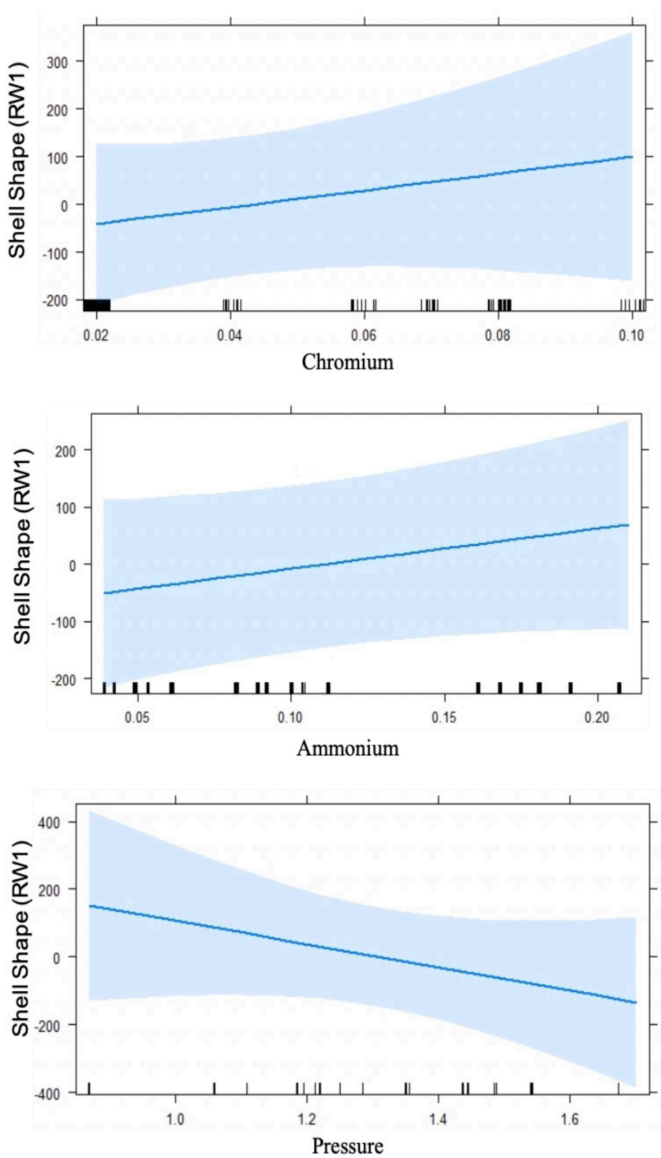

Figure 12. Environmental variables (chromium, ammonium and water pressure) that have significant influence on the shell shape change of Asiatic clam. The blue shade represents 95% confidence levels.

Water pressure showed a negative influence on the shell shape of Asiatic clam. A more vertical elongation in the shell where the distance from the pallial region to umbo has widened and where the posterior and anterior regions has narrowed was observed. The change in the shape of Asiatic clam as influenced by water pressure is a manifestation of self-adaptation for survival (**Figure 12**).

CONCLUSIONS AND RECOMMENDATIONS

The analysis of linear and geometric morphometric characters of Asiatic clam shells collected from the east and west regions of Laguna de Bay as affected by water physicochemical properties and heavy metals was determined. The results revealed that Asiatic clam had similar shell linear morphometric measurements across sampling stations. However, the shells from the industrial regions (West Bay) of Laguna de Bay have horizontal elongation while the shells from the agricultural regions (East Bay) tended to have vertical elongations. NO_3- had a negative influence on the shell size while Cr (VI) had a positive influence on the shell shape of Asiatic clam. This finding supports the cause-effect relationship between contaminant exposure and morphological changes reflected in the shell sizes and shapes of Asiatic clam. These chemicals that are continuously draining into Laguna de Bay could magnify their impacts not only on the Asiatic clam but also to organisms in a higher tropic level within the lake.

The study was limited to one sampling schedule for the collection of Asiatic clams and the physicochemical parameters of the water due to time and financial constraints. For future perspective of this research, it is important to include temporal data analysis to compare time scale variability with regards to the rate of heavy metal exposure via Asiatic clam's metabolism. Laboratory experiments on Asiatic clam exposed for a long period to different concentrations of NO_3- and Cr (VI) must be conducted to further support and validate the initial results from this study. It is also important to include the analysis of heavy metals on sediments and to examine the effect of heavy metals on the species not only on the morphological level but also at the physiological level.

Asiatic clam is abundant in Laguna de Bay and is one of the most domineering macro-invertebrate species in the area. It is significant to the lakeshore communities who derive benefits from it as food, as source of income, and as alternative feeds for ducks. The results can be used for human health risk assessment as they can provide a link between bioaccumulation and biomagnification of pollutants in Laguna de Bay. It is an essential result to assess the overall health and functioning of aquatic ecosystems. Also, it sheds light on the bioaccumulation of heavy metals in the clam from contaminated water.

The results could provide a good basis for policymakers to craft local laws, policies, and management strategies within the Laguna de Bay watershed, especially those in line with the control and regulation of point and non-point sources of pollution. A multi-dimensional, integrated, and holistic management is necessary to address the various concerns which contribute to the increasing degradation of Laguna de Bay. The complexity and interdependence of both the social and ecological dimensions of the problems confronting the lake ecosystem must be recognized. Collaborative engagements of various stakeholders, communities and institutions alike, are indispensable in achieving cohesive decisions and actions towards the protection of the lake and the communities that depend on it.

REFERENCES

Achard, M., Baudrimont, M., Boudou, A., and Bourdineaud, J.P. 2004. "Induction of a multixenobiotic resistance protein (MXR) in the Asiatic clam *Corbicula fluminea* after heavy metal exposure". *Aquatic Toxicology* 67: 347-357.

An, K.G., Jones, J.R. 2000. "Factors regulating bluegreen dominance in a reservoir directly influenced by the Asian monsoon". *Hydrobiologia* 432:37-48.

Arpa, M.C.B., Catane, S.G. and Mirabueno, M.H.H. 1999a. Identification of large-scale eruptions of Laguna caldera from Geologic mapping of volcanic deposits found in Rizal province, Metro Manila, and adjacent areas. Philippine Institute of Volcanology and Seismology, Diliman.

Arpa, M.C.B., Catane, S.G., Mirabueno, M.H.H., and Bornas, M.A.V. 1999b. Identification of large-scale eruptions of Laguna caldera from geologic mapping of volcanic deposits found in Rizal province, Metro Manila and adjacent areas. Philippine Institute of Volcanology and Seismology, Diliman.

Baker, A.M., Bartlett, C., Bunn, S.E., Goudkamp, K., Sheldon, F. and Hughes, J.M. 2003. "Cryptic species and morphological plasticity in long-lived bivalves (Unionoida: Hyriidae) from inland Australia". *Molecular Ecology* 12: 2707-2717.

Bassack, S.B., Oneto, M.L., Verrengia Guerrero, N.R., and Kesten, E.M. 1997. "Accumulation and elimination of pentachlorophenol in the freshwater bivalve *Corbicula fluminea*". *Environmental Contamination and Toxicology* 58: 497-503.

Baudrimont, M., Métivaud, J., Maury-Brachet, R., Ribeyre, F., and Boudou A. 1997a. "Bioaccumulation and metallothionein response in the Asiatic clam (Corbicula fluminea) after experimental exposure to cadmium and inorganic mercury". *Environmental Toxicology and Chemistry* 16: 2096-2105.

Bernhard, A.E., Tucker, J., Giblin, A.E., Stahl, D.A. 2007. "Functionally distinct communities of ammonia-oxidizing bacteria along an estuarine salinity gradient". *Environmental Microbiology* 9: 1439-1447.

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multi-model inference: a practical information-theoretic approach. 2nd edition. Springer-Verlag, New York, NY.

Byrne, M., Phelps, H., Church, T., Adair, V., Selvakumaraswamy, P., Potts, J. 2000. "Reproduction and development of the freshwater clam *Corbicula australis* in southeast Australia". *Hydrobiologia* 418: 185-197.

Campbell, N.A. and Reece, J.B. 2002. Biology, 6th edition. San Francisco: Pearson Education, Incorporated. 937–938 pp.

Cataldo, D., Boltovskoy, D., Stripeikis, J., and Pose, M. 2001a. "Condition index and growth rates of field caged Corbicula fluminea (Bivalvia) as biomarkers of pollution gradients in the Paraná river delta (Argentina)". *Aquatic Ecosystem Health Manage* 4: 187-201.

Cataldo, D., Colombo, J.C., Boltovskoy, D., Bilos, C., and Landoni, P. 2001b. "Environmental toxicity assessment in the Paraná river delta (Argentina): simultaneous evaluation of selected pollutants and mortality rates of *Corbicula fluminea* (Bivalvia) early juveniles". *Environmental Pollution* 112: 379-389.

Cataldo, D., Boltovskoy, D. 1999. "Population dynamics of Corbicula fluminea (Bivalvia) in the Paraná river delta (Argentina)". *Hydrobiologia* 380: 153-163.

Catane, S.G., Ui, T., Arpa, M.C.B. Cabria, H.B. and Taniguchi, H. 2004. "Potential hazards from the youngest explosive eruptions of Laguna Caldera to metropolitan Manila, Philippines". *Western Pacific Geophysics Supplemen*t 85: 33-89.

Catane, S.G. and Arpa, M.C.B. 1998. Large-scale eruptions of Laguna caldera: contributions to the accretion and other geomorphic developments of Metro Manila and adjacent provinces. Philippine Institute of Volcanology and Seismology, Diliman.

Ciacci, C., Barmo, C., Gallo, G., Maisano, M., Cappelo, T., D'agata, A., Leonzio, C., Mauceri, A., Fasulo, S. and Canesi, L. 2012. "Effects of sublethal, environmentally relevant concentrations hexavalent chromium in the gills of *Mytilus galloprovincialis*". *Aquatic Toxicology* 120(121): 109-118.

Clarke, G.M. 1998." The genetic basis of developmental stability: IV. Individual and population asymmetry parameters". *Heredity* 80: 55-561.

Collos, Y., and Harrison, P.J. 2014. "Acclimation and toxicity of high ammonium concentrations to unicellular algae". *Marine Pollution Bulletin* 80: 8-23.

Cooper, J.E. 2007. "*Corbicula fluminea* (Asian clam) in the Roanoke River, North Carolina: a stressed population?". *Southeastern Naturalist* 6: 413-434.

Cunanan, A.M., and Salvacion, J.W.L. 2014. "Analysis of Water Temperature of Laguna Lake Using EFDC Model". International *Journal of Scientific and Technology Research* Volume 3: 8.

Department of Environment and Natural Resources Administrative Order No. 2016-08 (DENR-DAO No. 2016-08). 2016. Water Quality Guidelines and General Effluent Standards of 2016. Department of Environment and Natural Resources Visayas Avenue, Diliman, Quezon City.

Doherty, F.G. 1990. "The Asiatic clam, Corbicula spp. as a biological monitor in freshwater environments". *Environmental Monitoring Assessment* 15: 143-181.

Doherty, F.G., Cherry, D.S., and Cairns J.J. 1987a. "Spawning periodicity of the Asiatic clam Corbicula fluminea in the New River, Virginia". *The American Midland Naturalist Journal* 117: 71-82.

Dongen, V., Lens, L. 2000. "The evolutionary potential of developmental stability." *Journal of Evolutionary Biology* 13(2): 326-335.

Elder, J. F. and Collins, J.J. 1991. "Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems". Reviews in *Environmental Contamination and Toxicology* 192: 37-79.

Elkarmi, A.Z., and Ismail, N.S. 2007. "Growth models and shell morphometrics of two populations of *Melanoides tuberculata* (Thiaridae) living in hot springs and freshwater pools." *Journal of Limnology* 66(2): 90-96.

El-Sayed, H.E.M., and El-Sayed, M.M.H. 2014. "Assessment of Food Processing and Pharmaceutical Industrial Wastes as Potential Biosorbents: A Review". *BioMed Research International* 146769: 24.

EPA. 2012. Channel Processes: Suspended Sediment Transport. In Water: Science & Technology. Retrieved from http://water.epa.gov/scitech/datait/tools/warsss/suspend.cfm.

Estefania, B., Sarmiento, A.M., Nieto, J.M., and Del Valls, A.T. 2017. "Does *Corbicula fluminea* Respond to Dissolved Metal(loid)s in Laboratory Exposure Bioassays". *Journal of Aquatic Pollution and Toxicology* 1: 11.

Farrington, J. W. 1983. "Bivalves as sentinels of coastal chemical pollution: the mussel (and oyster) watch". *Oceanus* 26(2): 18-29.

Ferguson, B.K. 1998. Introduction to Stormwater: Concept, Purpose, Design. John Wiley and Sons.

Forster, H., Oles, D., Knittel, U., Defant, MJ. and Torres, R.C. 1990. "The Macolod Corridor-a rift crossing the Philippine Island-Arc". *Tectonophysics* 183: 265-271.

Fukuda, H., Haga, T. and Tatara, T. 2008. "Niku-nuki: a useful method for anatomical and DNA studies on shell-bearing". *Zoosymposia* 1: 1-5.

Gardner, J.P.A. and Skibinski, D.O.F. 1991. "Biological and physical factors influencing genotype-dependent mortality in hybrid mussel populations". *Marine Ecology Progress Series* 71: 235-243.

Graney, R.L Jr., Cherry, D.S. and Cairns, J. Jr. 1984. "The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams". *Water Resources* 18: 833-842.

Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., and Kana, T.M. 2016. "Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions". *Limnology and Oceanography* 61: 165-197.

Hamli, H., Rahim, A.Z., Idris, M.H., Kamal, A.H.M. and King, W.S. 2015. "Morphometric variation among three local mangrove clam species of Corbiculidae". Songklanakarin *Journal of Science and Technology* 37: 15-20.

Hansen, P.J. 2002. "Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession". *Aquatic Microbial Ecology* 28:279-288.

Hayden, C.J., and Beman, J.M. 2014. High abundances of potentially active ammonia-oxidizing bacteria and archaea in oligotrophic, high-altitude lakes of the Sierra Nevada, California, USA. PLoS ONE 9: e111560.

Hermsen, E.J. and Hendricks, J.R. 2008. "Whither fossils? Studying morphological character evolution in the age of molecular sequences 1". *Annual Molecular Botanical Garden* 95: 72-100.

Hinga, K.R. 2002. "Effects of pH on coastal marine phytoplankton". *Marine Ecology Progress Series* 238:281-300.

Hinga, K.R. 1992. "Cooccurrence of dinoflagellate blooms and high pH in marine enclosures". *Marine Ecology Progress Series* 86: 181-187.

Idris, M.H., Arshad, A., Japar Sidik, B., Mazlan, A.G. and Daud, S.K. 2008. "Morphometric analysis as an application tool to differentiate three local pen shell species". Pertanika *Journal of Tropical Agriculture Science* 31: 387-398.

Inza, B., Ribeyre, F., Maury-Brachet, R., and Boudou A. 1997. "Tissue distribution of inorganic mercury, methilmercury and cadmium in the Asiatic clam (*Corbicula fluminea*) in relation to the contamination levels of the water column and sediment". *Chemosphere* 35: 2817-2836.

Klaus, L. 1990. Seaweeds: Their Environment, Biogeography, and Ecophysiology. A Wiley-Interscience Publication: John Wiley & Sons, Incorporated p. 358.

Koehn, R.K. and Bayne, B.I. 1989. "Towards a physiological and genetical understanding of the energetics of the stress response". *Biological Journal of Linnean Society* 37: 157-171.

Korniushin, A.V., and Glaubrecht, M. 2003. "Novel reproductive modes in freshwater clams: brooding and larval morphology in Southeast Asian taxa of *Corbicula* (Mollusca, Bivalvia, Corbiculidae)". *Acta Zoologica* 84: 293-315.

Kraemer, L.R., and Lott, S. 1977. "Microscopic anatomy of the visceral mass of Corbicula (Bivalvia: Sphaeriacea)". *Bulletin of American Malacological Union* 48-56 pp.

Langland, M. and Cronin, T. 2003. A Summary Report of Sediment Processes in Chesapeake Bay and Watershed. In Water-Resources Investigations Report 03-4123. New Cumberland, PA: U S Geological Survey. Retrieved from http://pa.water.usgs.gov/reports/wrir03-4123.pdf

Leong, R., de la Seña, C.A., Torres, M.A.J., and Demayo, C.G. 2013. "Describing morphological and enzyme polymorphism in the Ribbed Venus Clam Gafrarium tumidum from five marine coastal locations in Mindanao, Philippines". *AACL Bioflux* 6(3): 1-13.

Leoni, B., Patelli, M., Soler, V., and Nava, V. 2018. "Ammonium Transformation in 14 Lakes along a Trophic Gradient". *Water* 10: 265.

Leung, B. and Forbes, M.R. 1996. "Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis". *Ecoscience* 3: 400-413.

López-Archilla, A.I., Moreira, D., Lopez-Garcia, P., and Guerrero, C. 2004. "Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH". *Extremophiles* 8:109-115.

Lundholm, N., Hansen, P.J., and Kotaki, Y. 2005. "Lack of allelopathic effects of the domoic acid-producing marine diatom Pseudo-nitzschia multiseries". *Marine Ecology Progress Series* 288: 21-33.

Lundholm, N., Hansen, P.J., and Kotaki, Y. 2004. "Effect of pH on growth and domoic acid production by potentially toxic diatoms of the genera Pseudo-nitzschia and Nitzschia". *Marine Ecology Progress Series* 273:1-15.

Maanan, M. 2007. "Biomonitoring of heavy metals using Mytilus galloprovincialis in Safi coastal waters, Morocco". *Environmental Toxicology* 22: 525-531.

Macedo, M.F., Duarte, P., Mendes, P., and Ferreira, J.G. 2001. "Annual variation of environmental variables, phytoplankton species composition and photosynthetic parameters in a coastal lagoon". *Journal of Plankton Research* 23:719-732.

Mallin, M.A., Johnson, V.L. and Ensign, S.H. 2009. "Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream". *Environmental Monitoring Assessment* 159 (1-4): 475-491.

Mass, P.A.Y., O'mullan, G.D., Lutz, R.A and Vrijenhoek, R.C. 1999. "Genetic and morphometric characterization of mussels (Bivalvia: Mytilidae) from Mid-Atlantic hydrothermal vents". *Biological Bulletin* 196: 265-272.

McMahon, R.F. 2000. Invasive characteristics of the freshwater bivalve *Corbicula fluminea*. pp. 315-343 In: Nonindigenous Freshwater Organisms: Vectors, Biology and Impacts. (eds: R. Claudi and J. Leach). Lewis Publishers, Boca Raton.

Mendez-Garcia, T., Rodriguez-Dominguez, L., and Palacios-Mayorga, S. 1996. "Contamination levels of water of the Atoyac River in the state of Puebla by heavy metals, oils, fats and bore". *Terra* 14: 137-49.

Moller, A.P. and Swaddle, J.P. 1997. Asymmetry, developmental stability, and evolution. Oxford University Press, Oxford.

Monteiro, L.R., Bordin, B. and Reis, S.F. 2000. "Shape distances, shape spaces and the comparison of morphometric methods". *Trends in Ecology and Evolution* 15: 217-220.

Mouthon, J. 2001a. "Life cycle and population dynamics of the Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) in the Rhone River at CreysMalville (France)". *Archiv Fur Hydrobiologie* 151: 571-589.

Mouthon, J. 2001b. "Life cycle and population dynamics of the Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) in the Saone River at Lyon (France)". *Hydrobiologia* 452: 109-119.

Mukherjee, M., Ray, A., Post, A.F., McKay, R.M., Bullerjahn, G.S. 2016. "Identification, enumeration and diversity of nitrifying planktonic archaea and bacteria in trophic end members of the Laurentian Great Lakes". *Journal of Great Lakes Research* 1: 39-49.

Müller, O. F. 1774. Vermium terrestrium etfluviatilium, sen animalium infusoriorum, helminthicorum, et testaceorum, non marinorum, succincta historia, Vol. 2, Testacea. Havnie et Lipsiae. 214 pp.

Nadal, M., Bocio, A., Schuhmacher, M., Domingo, J. 2005. "Trends in the levels of metals in soils and vegetation samples collected near a hazardous waste incinerator". *Archives of Environmental Contamination and Toxicology* 49: 290298.

Narbonne, J.F., Djomo, J.E., Ribera, D., Ferrier, V., and Garrigues, P. 1999. "Accumulation kinetics of polycyclic aromatic hydrocarbon adsorbed to sediment by the mollusc *Corbicula fluminea*". *Ecotoxicology and Environmental* Safety 42: 1-8.

Norberg, J. and Tendengren, M. 1995. "Attack behavior and predatory success of *Asterias rubens* L. related to differences in size and morphology of the prey mussel Mytilus edulis". *Journal of Experimental Marine Biology and Ecology* 186: 207-220.

O'Conner, T. P. and Beliaeff, B. 1995. Recent trends in coastal environmental quality: results from the Mussel Watch Project 1986 to 1993. NOAA Technical Memorandum NOAA N/ORCA21. National Status and Trends Program, Silver Springs, MD, 40p.

Palmer, A.R. 1994. Fluctuating asymmetry analysis: a primer. In: Developmental instability: its origins and evolutionary implications. Markow TA. edition, London: Kluwer Academic 335-364 pp.

Pedersen, M.F., and Hansen, P.J. 2003. "Effects of high pH on a natural marine planktonic community". *Marine Ecology Progress Series* 260:19-31.

Peltier, G.L., Meyer, J.L., Jagoe, C.H., and Hopkins, W.A. 2008. "Using trace element concentrations in *Corbicula fluminea* to identify potential sources of contamination in an urban river". *Environmental Pollution* 154: 283-290.

Qiu, A., Shi, A., and Komaru, A. 2001. "Yellow and brown shell colour morphs of Corbicula fluminea (Bivalvia: Corbiculidae) from Sichuan Province, China, are triploids and tetraploids". *Journal of Shellfish Research* 20: 323-328.

Rajagopal, S., van der Velde, G., and bij de Vaate, A. 2000. "Reproductive biology of the Asiatic clams *Corbicula fluminalis* and *Corbicula fluminea* in the river Rhine". *Arch. Hydrobiologia* 149: 403-420.

Rohlf, F.J. 2017. Ecology and Evolution and Anthropology, Stony Brook University. Originally supported in part by grant from the National Science Foundation.

Rohlf, F.J. 2002. Geometric morphometrics and phylogeny. In: Macleod N., Forey P. (eds). Morphology, shape and phylogeny. Taylor & Francis, London. 175-193 pp.

Rohlf, F.J. and Slice, D.E. 1990. "Extensions of the Procrustes method for the optimal superimposition of landmarks". Systematic Zoology 39: 40-59.

Sallaku, F., Fortuzi, S., Tota, O., Huqi, B., Chachalis, D., and Darawsheh, M. 2009. "Heavy metal soil contamination around the metallurgical plant of Elbasani in Albania". *Journal of Food, Agriculture and Environment* 7: 2.

Salazar, M.H., and Salazar, S.M. 1991. "Assessing site-specific effects of TBT contamination with mussel growth rates". *Marine Environmental Research* 32: 131-150.

Scheltema, A. 1983. "Pinna deltodes, Menke newly described and differentiated from *P. Bicolor* Gmelin (Bivalvia, Ptreioida)". *Journal of the Malacolgica Society of Austalia* 6: 37-52.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. 2012. "NIH Image to ImageJ: 25 years of image analysis". *Nature Methods* 9: 671-675.

Shapiro, J. 1990. "Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH". *Verhandlungen des Internationalen Verein Limnologie* 24: 38-54.

Slice, D.E., Bookstein F.L., Marcus L.F., and Rohlf, F.J. 1996. A glossary for geometric morphometrics. pp 531–551 In: Advances in morphometrics (eds: L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice) Plenum, New York.

Smith, U.E. and Hendricks, J.R. 2013. "Geometric morphometric character suites as phylogenetic data: extracting phylogenetic signal from gastropods shells". *Systematic Biology* 62: 366-385.

Sousa, R., Antunes, C., and Guilhermino, L. 2008. "Ecology of the invasive Asiatic clam *Corbicula fluminea* (Muller, 1774) in aquatic ecosystes: an overview". *International Journal of Limnology* 44: 85-94.

Sousa, R., Freire, R., Rufino, M., Me´Ndez, J., Gaspar, M., Antunes, C. and Guilhermino, L. 2007. "Genetic and shell morphological variability of the invasive bivalve *Corbicula fluminea* (Mu¨ller, 1774) in two Portuguese estuaries". *Estuarine, Coastal and Shelf Science* 74: 166-174.

Stanley, S.M. 1983. Adaptative morphology of the shell in bivalves and gastropods E.R. Trueman, M.R. Clarke (Eds.), The Mollusca, vol. II, Academic Press, New York. 105-141 pp.

The, S.J., Werner, I. D. and Hinton, E. 2000. "Sublethal effects of chromium-VI in the Asian clam (*Potamocorbula amurensis*)". *Marine Environment Research* 50: 295-300.

Toro, M.V., Manriquez, G. and Suazo, I. 2010. "Morfometria geometrica y el studio de las formas biologicas: De la morfologia descriptive a la morfologia cuantitativa". *International Journal of Morphology* 28: 977-990.

Tran, D., Boudou, A., and Masabuau, J.C. 2001. "How oxygenation level influences cadmium accumulation pattern in the Asiatic clam *Corbicula fluminea*: a laboratory and a field study". *Environmental Toxicology and Chemistry* 20: 2073-2080.

Valadez-Vega, C., Zúñiga-Pérez, C., Quintanar-Gómez, S., Moralesgonzález, J.A., Madrigal-Santillán, E., and Villagómez-Ibarra, J.R. 2011. "Lead, cadmium and cobalt (Pb, Cd, and Co) leaching of glass-clay containers by pH effect of food". *International Journal of Molecular Science* 12: 2336-50.

Velickovic, M. 2004. "Chromosomal aberrancy and the level of fluctuating asymmetry in black-striped mouse (*Apodemus agrarius*): effects of disturbed environment". *Hereditas* 140:112-122.

Verma, P.U., Purohit, A.R. and Patel, N.J. 2012. "Pollution status of Chandlodia lake located in Ahmedabad Gujarat". International *Journal of Engineering Research and Applications* 2: 1600-1610.

Villalobos, M., Merino-Sánchez, C., Hall, C., Grieshop, J., Gutiérrezruiz, M.E., and Handley, M.A. 2009. "Lead (II) detection and contamination routes in environmental sources, cookware, and home-prepared foods from Zimatlán, Oaxaca, Mexico". *Science of Total Environment* 407: 2836-44.

Vogel, T.A., Flood, T.P., Patino, L.C., Wilmot, M.S., Maximo, R.P.R., Arpa, C.B., Arcilla, C.A. and Stimac, J.A. 2006. "Geochemistry of silicic magmas in the Macolod Corridor, SW Luzon, Philippines: evidence of distinct, mantle-derived, crustal sources for silicic magmas". *Contributions to Mineralogy and Petrology* 151, 267-281.

Wang, C., Liu, J., Wang, Z., and Pei, Y. 2014. "Nitrification in lake sediment with addition of drinking water treatment residuals". *Water Research* 56:234-245.

Way, C.M., and Hornbach, D.J. 1990. "Dynamics of filter feeding in *Corbicula fluminea*". *Canadian Journal of Zoology* 68: 115-120.

Widdows, J. 1985. "Physiological responses to pollution". *Marine Pollution Bulletin* 16(4): 129-134.

Widdows, J., Phelps, and Galloway, W. 1981. "Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay". *Marine Environmental Research* 4: 181-194.

Willis, G.I. and Skibinski, D.O.F. 1992. Variation in strength of attachment to the substrate explains differential mortality in hybrid mussel (*Mytilus galloprovincialis* and *M. edulis*) populations". *Marine Biology* 112: 403-408.

ACKNOWLEDGMENT

This research is part of M. Elvira's dissertation, and was funded by the University of the Philippines' Emerging Interdisciplinary Research Program and the Department of Science and Technology - Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) under the program "Ten Years After the Millennium Ecosystem Assessment of Laguna de Bay: Towards a Sustainable Future", Project 2: Exploring Pollution Monitoring Proxies for Characterizing Urban Lake Environments.