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Geometric morphometric and heavy metals analysis of flathead grey mullet (*Mugil cephalus),* from Agusan River, Butuan city, Philippines

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Abstract

Geometric Morphometric used as a tool in quantifying phenotypic variations among species of the same population. The analysis of heavy metals in the fishes created to determine the extent of harmful elements found since it serves as a human food source. This study aims to determine the fluctuating asymmetry (FA) in the body shapes of *Mugil cephalus* and levels of cadmium (Cd), chromium (Cr), and lead (Pb) in the muscles. In FA, a total of 100 samples (50 males & 50 females) were randomly collected and subjected to Symmetry and Asymmetry Geometric Data software. Results of Procrustes ANOVA showed a highly significant difference ($P<0.0001$) between sexes. Females obtained the highest percentage of FA (77.93%) than males (77.43%). This indicates that females change in body shape was associated with sustaining homeostasis and metabolic responses during reproduction. For the analysis of Cd, Cr and Pb three stations were established with three replicates each. A total of nine fish of the same size and weight regardless of sexes were collected and subjected to analysis. The concentrations of heavy metal were found in the order of Pb>Cr>Cd. Pb had the highest concentrations (4.11 ± 0.11 ppm), Cr (0.5 ± 0 ppm) and Cd (BDL) Below Detection Limit. One-way ANOVA showed that the concentrations of heavy metals were significantly higher ($P<0.05$). Pb and Cr exceeded the recommended safe limit in foods set by US EPA, FAO and WHO (Pb ≤0.5 ppm, Cr ≤0.01 ppm). This suggests that frequent consumption may pose risk to human health.

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Introduction

Since fish inhabit high trophic levels, they are commonly used as a bioindicator of pollutants present in the aquatic ecosystem (Blasco *et al.*, 1998; Agah *et al.*, 2009). It performs as functional genetic models for the estimation of aquatic pollutants (Mitchell and Kennedy, 1992). Their existence works as a biomarker of effluents thus making them significant for bio-monitoring in this environment (Cabuga *et al.*, 2016). Provides appropriate representation for examining aquatic genotoxicity as they metabolize xenobiotics and accumulate pollutants (Grisolia and Cordeiro, 2000). Ecological deficiency contributes forces that may alter environmental capacity (Chau, 2008) thus causing impairment to the species (Duruibe *et al.*, 2007). The habitat variables and its imbalances would create developmental instability upon the resistance and the forces exerted of the organisms. Rapid change of the environment might alter genomic makeup of organisms; and may result in genetic alteration and diversity of the population (Trono *et al.*, 2015). The study shows, that adverse situation of the environment develops negative effects to its organisms affecting the morphology or to the extent of mortality (Jumawan *et al*, 2016). Increased deformities of organisms morphology would be reflected as the unfavorable response towards ecological settings. Evidently, organisms differ in morphology as a mechanism to compensate to the changing environment. Thus, morphology is a basis in which organisms may diverse among and within its population. The inconsistency of the environment suggests biological and morphological transformations due to the adaptation of the organism (Cazzaniga, 2002; Torres *et al.*, 2013).Therefore, morphology plays an essential factor in species differentiation. It is a primary component to quantify shape and shape variations (Cabuga *et al.*, 2016). Thus, in evolutionary biology, the importance of shape has been an ultimate requisite together with identifying shape variations is an argument in which biological forms diverged from any other (Richmeier *et al.*, 2002).

In determining morphological variations in the body shapes of *M. cephalus*, fluctuating asymmetry (FA) was employed. FA used as a tool to quantify variances among the left and right side of two-sided traits of an organism (Swaddle, 2003). It is known as an efficient mechanism to evaluate an environmental condition and organisms state of well-being (Lecera *et al.*, 2015). FA performs as morphometrics way of describing asymmetry; thus giving the overall dissimilarities of the bilateral symmetry of the species (Moller & Swaddle 1997; Palmer & Strobeck 2003). The FA widely recognized by many studies in investigating developmental instability and identifying subtle differences (Klingenberg and McIntyre 1998; Savriama *et al.*, 2012; Hermita *et al.*, 2013). Sommer (1996) concluded that stress is an unswerving factor that could develop fluctuation in organism morphology due to its resistivity in the environment. The increased levels of FA caused by various stresses could be lead to the undesirable development of individual organisms (Muallil *et al.*, 2014). Nonetheless, abiotic factor such as temperatures, nutrient deprivation, and pollutants are ecological perturbation causes developmental homeostasis (Mpho, 2000, Velichovic, 2004;).FA focus the likeness and dissimilarities of organism overall morphology (David Polly, 2012). FA, considerably a scientific means of detecting phenotypic modifications and evaluating ecological condition (Angtuaco and Leyesa, 2004). It is believed that it measures developmental instability of organisms due to genomic interference and ecological disturbances. Further, a great percentage of FA have been related to the exposure of organisms towards heavy metal pollution, industrial wastes, household runoffs and anthropogenic activities (Natividad *et al.*, 2015).

The study area was Agusan River, a part of the three sub-basins in Mindanao emptying Butuan Bay (CTI-Halcrow, 2008). Several studies have been conducted, including fluctuating asymmetry and heavy metal assessment. It was found out that the River known to have a mercury pollution from the study of (Roa, 2001) and associated with the mining activities happens in the upper sub-basin the Compostella Valley where Mt. Diwalwal located. Along with the study conducted by (Cabuga *et al*., 2016) lead (Pb), copper (Cu), mercury (Hg), and Cadmium (Cd) were positively present in the muscles of the fish *Mesopristes cancellatus* that is also found in the area. The local community depends on fish farming as livelihood source where the *M. cephalus* is part of their commodity. This species widely found in the area, consumed and available in the local market. This fish species ischaracterized as a freshwater, brackish and marine species and yet there were no comparable studies conducted. This study aims to determine the possible effects of pollutants into the fish morphology and determing the levels of cadmium, chromium and lead in the muscles. Thus, the results of the study would be an essential information both of the fish eating community as well as in the government, which has the authority for the management of the Agusan River.

Materials and methods

Description of the area

The study area was Agusan River located in Butuan City, Agusandel Norte Philippines (Fig. 1.).

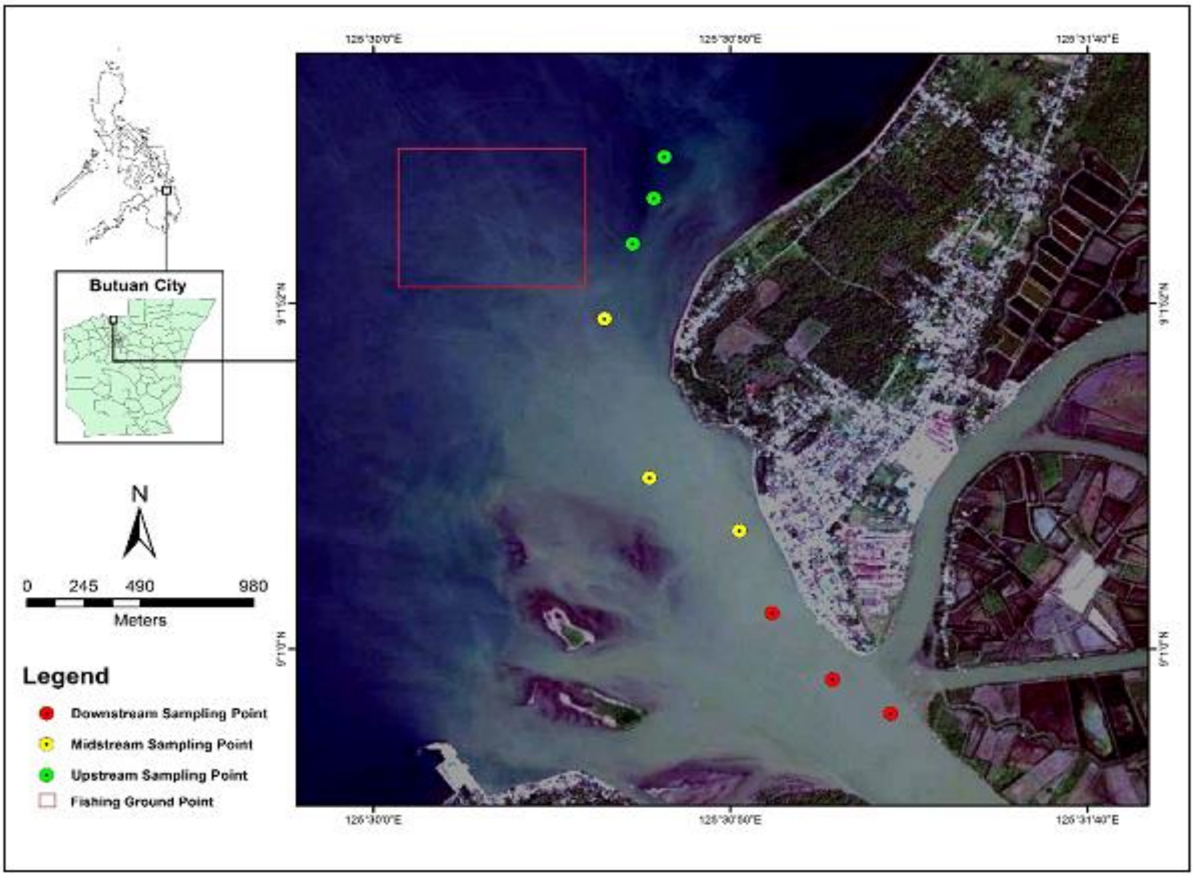

Fig. 1. Map of the study area: Agusan River, Butuan city, Agusandel norte, Philippines

It geographically lies between 9°01'58.36"N 125°30'27.41"E. The fish collection was done in the month of March 2017 through the aid of local fisherman utilizing motorized bancas and gillsnet as their catching gears. While the physicochemical water parameters was completed with the same date stated above.

Sample collection and processing

About 100 samples of *M. cephalus* (50 males and 50 females) were randomly collected in the study area. The collected samples were immediately transported to the laboratory and proper preservation was applied. After which, the individual fish were positioned in a Styrofoam for pinning of its fins.

To make it wider and visible to locate the point of origin for land marking methods; it was applied a 10% Formalin all over its fins to make it hardened using the small brush. The samples were then photographed using the digital camera (Lenovo-13 megapixels). To obtain the total length of the samples, the left and the right bilateral side were taken using a ruler (Natividad *et al.*, 2015).

Sex determination

The samples were then examined through looking its genitalia. Female samples were detected by the occurrence of eggs in the ovaries with yellow to orange granular textures while the male samples were observed by its testes where it is smooth and whitish with non-granular in texture (Requiron *et al.*, 2010).

Landmark selection and digitation

The acquired images were then sorted according to its sex and converted into TPS file format utilizing the tps Util. Digitation/land marking procedure of the samples done through tpsDig2 (version 2, Rohlf 2004). There were sixteen (16) anatomical landmark points (Table 1) were utilized to digitize the samples of *M. cephalus* (Fig. 2.).

Shape analysis

To lessen the measurement error, the samples were replicated three times. Its bilateral symmetry (left and right) was digitized using tpsDig2. The collected coordinates were then subjected to (SAGE) Symmetry and Asymmetry in Geometric Data (version 1.04, Marquez, 2007) software (Fig. 3). This data provides information about the acquired principal components of individual symmetry which indicates through a deformation grid (Natividad *et al.*, 2015).

The Procrustes ANOVA test was implemented to identify the significant difference in the symmetry of the three factors examined-individual, sides and interaction of individuals and side. The significant level was verified at $P<0.0001$. Along with, the variances of its side and the estimation of directional asymmetry also identifies.

The level of FA was stipulated through percentage (%) which were analyzed and compared between male and female samples (Natividad *et al.*, 2015).

Physico-chemical water parameters

By means of the standardized protocol, the test for physicochemical parameters was also determined in the study. Using the multi-parameter water quality meter (EUTECH PCD), the following were examined: Conductivity, Dissolve Oxygen (DO), pH, Salinity, Temperature and Total Dissolve Solids (TDS). In the study area, three sites (Upstream, Midstream and Downstream) were established accompanying with three replicates and randomly selected for the water measurements. Through Paleontological Statistics and Software (PAST), it was used to calculate the data and presented in the manner of mean ± standard error mean (SEM). One-way Analysis of Variance (ANOVA) was also employed for analyzing the significant difference of physicochemical parameters.

Fish preparation for heavy metals analyses

A total of nine (9) *M. cephalus* were utilized for analyses of levels of cadmium, chromium and lead. The total length of fish utilized ranges from 20-24 cm and its body weight ranges from 240-265 g. The collected samples has a mean weight 250 ± 4.968 SEM range (240-265 g) and its mean length 21.889 ± 1.202 SEM range (20-24 cm).

Digestion of fish muscles

Digestion process was adopted from the Analytical Methods for Atomic Absorption Spectroscopy (®PerkinElmer Incorporated). The samples of homogenized fish muscles were dried in the laboratory oven at 100°C for about 3 hours. The dried fish muscle samples were each ground with laboratory ceramic mortar and pestle to powder form. The 1g of homogenized dried fish muscles was weighed using a digital weighing scale. It was placed in a 50ml beaker covered with a watch glass and transferred to the hot plate under the fume hood. A concentrated 5 ml Nitric acid (HNO_3) and 5 ml Sulfuric acid (H_2SO_4) were added to the samples respectively.

The reaction of the chemicals was allowed to take place after which the temperature was set to 60°C for 30 minutes. A 10ml of concentrated Nitric acid (HNO_3) was added and heated to 120°C for 30 minutes.

The temperature was then increased to 150°C for 45 minutes. After the samples become brown, the temperature was then reduced to 30°C. A 5 ml concentrated Hydrogen Peroxide ($H2O^2$) was added continuously to the samples become clear or pale yellow. It was then allowed to cool for about 30 minutes. The resulting fish digested samples were then filtered using Wattman paper No.80 filter paper and diluted to 50ml volumetric flask added with distilled-deionized water. Sample containers were pre-treated with 90% distilled- deionized water and 10% Nitric Acid.

Determination of levels of Cadmium (Cd) Chromium (Cr) and Lead (Pb)

The analysis of levels of cadmium, chromium and lead was supervised by the Department of Agriculture-(Regional Soils Laboratory located at Brgy. Taguibo, Butuan City, Agusande lNorte, Phils.). The analysis was administered using the Agilent Technologies® MY14300001 Atomic Emission Spectrophotometer with a detection limit of 0.1 ppm in all the heavy metals.

Correlation between fluctuating asymmetry and physico-chemical parameters

The correlation between fluctuating asymmetry and physico-chemical parameters were also determined. The Pearson-correlation coefficient was used to analyze the relationship between FA and Physico-chemical parameters. All data were analyzed using Graph Pad Prism 5.

Correlation between fluctuating asymmetry and heavy metal concentrations.

The correlation between fluctuating asymmetry and heavy metal concentrations were also determined. The Pearson-correlation coefficient was used to analyze the relationship between FA and heavy metal concentrations. All data were analyzed using the Graph Pad Prism 5.

Results and discussion

To draw the extent of fluctuations in the body shapes of *M. cephalus* Procrustes ANOVA was applied (Table 1). The left and right sides of individual samples were compared and analyzed. There were factors considered to determine the asymmetry in both female and male populations. These were: individuals, sides, and the interaction of individuals by sides.

Table 1. Description of the landmark points adapted from Paña *et al.* (2015).

Coordinates	Locations/Nomenclature
1	Snout tip
2	Posterior end of nuchal spine
3	Anterior insertion of dorsal fin
4	Posterior insertion of dorsal fin
5	Dorsal insertion of caudal fin
6	Midpoint or lateral line
7	Ventral insertion of caudal fin
8	Posterior insertion of anal fin
9	Anterior insertion of anal fin
10	Dorsal base of pelvic fin
11	Ventral end of lower jaw articulation
12	Posterior end of the premaxilla
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit
15	Dorsal end of operculum
16	Dorsal base of pectoral fin

The result shows a high fluctuating asymmetry among the factors analyzed and within the sexes (P<0.0001). It was identified that the individual fish reveals a high degree of fluctuating asymmetry, while comparing it with other fish samples. The left and right sides also displayed the high significant difference of fluctuating asymmetry as populations. While the factors of individuals by sides also shown highly significant difference implying fluctuating symmetry. Further, the fluctuating asymmetry (FA) of all the factors analyzed and among the female and male populations was observed.

Table 2. Procrustes ANOVA on body shape of *M. cephalus* in terms of sexes from Agusan River, Butuan City, Philippines.

Factors	SS	DF	MS	F	P-Value
Female					
Individuals	0.1221	1372	0.0001	2.148	0.0001**
Sides	0.641	28	0.0023	55.2812	0.0001**
Individual x Sides	0.0568	1372	0	5.1623	0.0001**
Measurement Error	0.0449	600	0	--	--
Male					
Individuals	0.1279	1372	0.0001	2.6335	0.0001**
Sides	0.0754	28	0.0027	76.0367	0.0001**
Individual x Sides	0.0486	1372	0	4.7674	0.0001**
Measurement Error	0.0416	5600	0	--	--

** (P<0.0001) highly significant.

The data revealed that the collected samples of *M. cephalus* in the study area were undergone asymmetrical in the manner of comparing as an individual, the left and right sides, and its relation being individual versus the sides because asymmetry in the body shapes of the female and male fish samples was detected and it might be a marker that the fishes in the study area experienced ecological stress. Typically, symmetry in the morphology could be observed among and within the fish populations. However, the detected fluctuating asymmetry would be related to habitat condition affecting the fish morphology.

Table 3. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks from Agusan River, Butuan city, Philippines.

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected landmarks
Female				
PC1	32.3974%	100%	30.669%	1,3,5,8,9,10
PC2	16.6451%		24.624%	3,4,5,6,8,9,10,15,16
PC3	14.092%		11.7227%	2,3,4,10,15,16
PC4	10.0633%		5.3071%	1,2,3,6,7,8,9,10,11,12,15
PC5	6.9804%		5.6069%	1,2,4,5,6,7,8,9,10,11,12,15,16
	80.18%		77.93%	
Male				
PC1	34.5459%	100%	33.7862%	1,5,8,9,10,13
PC2	18.9434%		15.5722%	2,3,5,6,9,10,11,15
PC3	11.3087%		13.4809%	6,7,8,9,10,15
PC4	10.8425%		8.8115%	1,2,3,4,10,15,16
PC5	5.5586%		5.7749%	2,3,4,11,12,16
	81.20%		77.43%	

The long-time exposure of the fishes in the unfavorable environment would create asymmetry. Nonetheless, *M. cephalus* would happen to be asymmetrical when continued exposure to pollution in the study area. Thus, organisms exhibiting a high degree of FA correspond with the perturbation of the environment (Hermita *et al.*, 2013).

Table 4. Mean concentrations (ppm) of heavy metals in the muscles of *M. cephalus* collected at Agusan River, Butuan city, Agusan del Norte, Phils.

Heavy Metals	Standard US EPA, FAO & WHO	Station 1 Mean ± SEM	Station 2 Mean ± SEM	Station 3 Mean ± SEM	Mean Mean ± SEM
Cadmium	≤0.05	BDL	BDL	BDL	BDL
Chromium	≤0.01	0.05 ± 0**	0.05 ± 0**	0.05 ± 0**	0.5 ± 0**
Lead	≤0.5	4.33 ± 0.33**	4 ± 0.29**	4 ± 0**	4.11 ± 0.11**

Note: Detection limit of the analyzer is 0.1 ppm, ** highly significant ($P<0.05$), BDL-Below Detection Limit
US EPA-United States Environmental Protection Agency
FAO– Food Agriculture Organization
WHO-World Health Organization.

The obtained data shows FA of the collected fish samples that could be attributed to the disturbed environment primarily towards various pollutants. Ecological condition highly affects organism's morphology and pollution that serves as a direct component altering the growth and development (Bonada and Williams, 2002). Further, the manifestation of asymmetry might due to the incapacity of organisms to compensate in a changing environment (Van Valen, 1962).Comparable study shows that fishes found to have high levels of fluctuating asymmetry credited to heavily polluted water ecosystem as well as Chironomid larvae and shrimp in the area were exposed to agricultural fertilizer (Tomkins and Kotiaho, 2001).

Table 5. Mean values of physico-chemical parameters from Agusan River, Butuan city. Philippines.

Water Parameters	Standard DAO 90-34 & Water Watch Australia National Technical Manual (2002)	Upstream Mean ± SEM	Midstream Mean ± SEM	Downstream Mean ± SEM	Mean Mean ± SEM
Conductivity	100-2000 µS/cm	280.33 ± 0.67	258.67 ± 14.33	220.87 ± 1.07	253.29 ± 17.37^{ns}
DO	>5mg/L	5.57 ± 0.15	4.93 ± 0.03	4.87 ± 0.03	5.12 ± 0.22^{ns}
pH	6.5-8.5	7.61 ± 0.05	7.33 ± 0.06	7.14 ± 0.57	7.36 ± 0.14^{ns}
Salinity	<0.5ppt	0.14 ± 0.01	0.12 ± 0.01	0.1 ± 9.81	0.12 ± 0.01^{ns}
Temperature	3^{o}C rise[a]	28.5 ± 0.1	28.17 ± 0.09	28.1 ± 2.51	28.26 ± 0.12^{ns}
TDS	<1000mg/L	101.13 ± 0.52	128.47 ± 9.69	105.3 ± 0.60	111.63 ± 8.50^{ns}

Note: DAO – DENR Administrative Order, ns-non-significant.

The Principal Component Analysis (PCA) was used to determine the affected landmarks which commonly found in both sexes through symmetry and asymmetry scores. The result shown five principal components from the collected female and male samples. This establishes the fluctuating asymmetry in the morphology of the fishes (Table 3).

Further, the collected female samples generated five principal component scores accounting 80.18%, while it has 77.93% of Interaction or Fluctuating Asymmetry. The commonly affected landmarks among the five PC scores were 10 (Dorsal base of the pelvic fin). In male samples, it generated five component scores accounting for 81.20%, while it has

77.43% of Interaction or Fluctuating Asymmetry. The males do not show common affected landmarks among the five PC scores.

This suggests that species of the same population had different mechanism to buffer environmental condition and changes. It was observed that females have the highest percentage of Interaction or Fluctuating Asymmetry (77.93%) when compared to the male which is (77.43%).

Accordingly females were more acquired FA and this could be attributed with its adaptability in a changing environment while the change in body shape was associated with sustaining homeostasis and metabolic aspects of reproduction (Requiron *et al*., 2012).

Females were susceptible from pollution most especially throughout the gestation period (Cabuga *et al*., 2016).

Fig. 2. Landmark points of *M. cephalus* (top) female (bottom) male.

Thus, the indication of FA in the morphology of *M. cephalus* corresponds to the environmental condition where the fishes inhabit. On the other hand, the two highest principal component (PC) scores among the female and male population was obtained and presented in (Fig. 4). While the histogram provides essential information of the skewness along with it is the deformation grid to distinguish the affected landmarks of the fish morphology (Fig. 5 & 6).

The concentrations of Cd, Cr and Pb in the muscles of *M. cephalus* was presented in (Table 4). The result shows that the concentrations were in the order of Pb>Cr>Cd. Among the heavy metals analyzedPb had the highest mean concentrations (4.11 ± 0.11ppm) and has exceeded the recommended safe limits in foods set by USEPA (United States Environmental Protection Agency), FAO (Food Agriculture Organization) and WHO (World Health Organization). Followed by Cr with the mean concentrations (0.5 ± 0 ppm) which also exceeded the recommended safe limits in foods set by USEPA, FAO and WHO. However Cd recorded the BDL (Below Detection Limit).One-way ANOVA showed high significant difference ($P<0.05$) among the heavy metals analyzed and from the three sampling stations. Lead (Pb) had the highest mean concentrations (4.11 ±0.11ppm) which exceeded the recommended safe limits in foods ≤0.5 set by US EPA, FAO, and WHO.

This indicates that continuing consumption may pose risk to human health. High concentration of Pb in the body may result to adverse effect and could develop severe health problems (Conclu, 1986). Accordingly, a greater amount of Pb highly affects bones and cause neurotoxicity (Herreros *et al.*, 2008). The possible build-upof Pb in the muscles of *M. cephalus* due to the information that the element is naturally occurring in the environment as a result of anthropogenic activity (WHO, 1985).

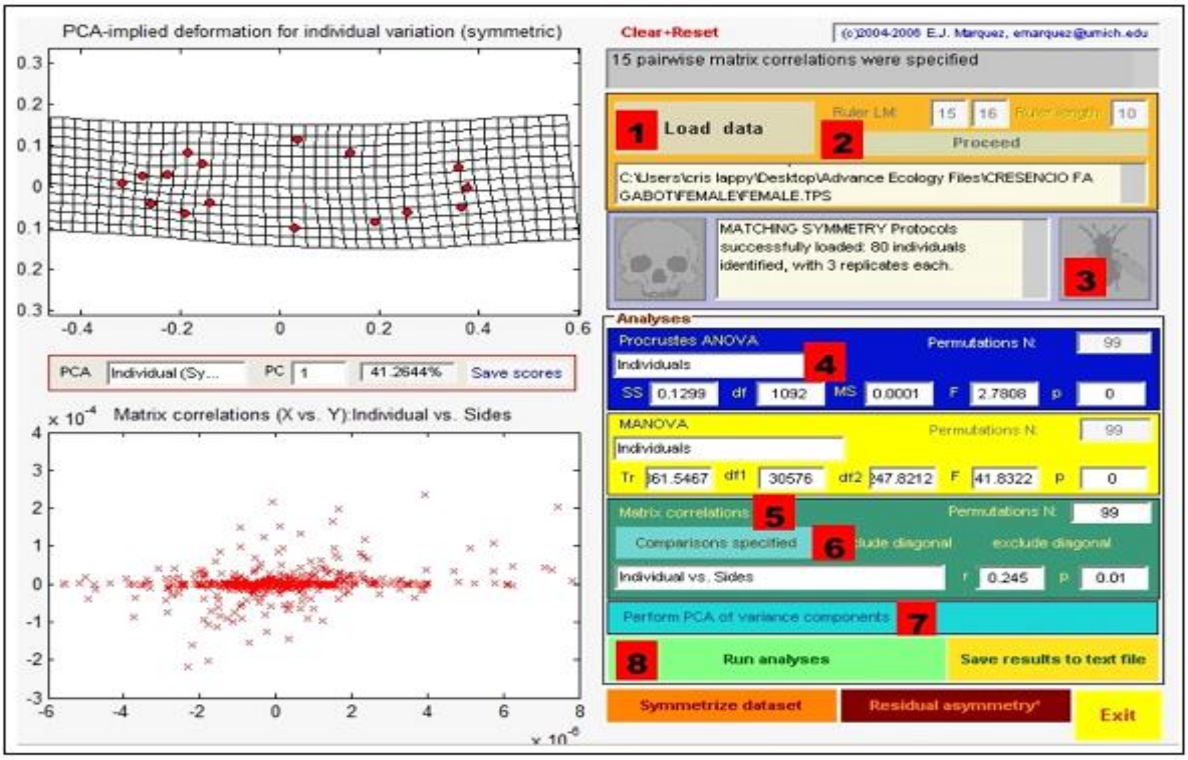

Fig. 3. Overview of the schematic flow of shape analysis using SAGE.

Its dissolution in the water bodies primarily because of natural sources yet its greater amount comes from household plumbing (Edward *et al.*, 2013).While point sources in the environment are from the burning of fossil fuel and mining (Muzyed, 2011). Further, Pb is discharged from mining and smelting activities as well as from the combustion of petroleum fuels emitted from automobile (Juberg, 2000). Relatively, from the study conducted by (Cabuga *et al.*, 2016) also found out a high concentration of lead (30.056 ± 0.0475 ppm) in the muscles of *M. cancellatus* a freshwater fish collected from Agusan River.

On the other hand, Cr had the mean concentrations (0.5 ± 0 ppm) which exceeded the recommended safe limits in foods ≤0.01 set by US EPA, FAO, and WHO. This implies that frequent consumption may result in health problems.

Lead (Pb) and Chromium (Cr) are heavy metals commonly found in the aquatic environment. Specifically, Cr is identified to be the toxic form of chemicals that is present in natural waters discharge from several sources including industrial runoffs. This could be harmful when ingested in a high concentration (Sireli *et al.*, 2006). Further, when happening that this element accumulated in the human body, it will result to an adverse health condition (Even and Ghaffari, 2011). The comparable study also shows the presence of chromium from the different organs in the marketed fish in Metro Manila (Solidum *et al.*, 2013). Further, the concentrations of heavy metals along the three stations were illustrated in Fig. 7.

Pearson-correlation showed that FA and the levels of cadmium, chromium, and lead were positively correlated in male samples (r=0.7326) while in female samples slightly positive (r=0.8321) (Fig. 8).

The results suggested that heavy metals were directly influenced the FA in the male samples. Fishes that found to have high levels of FA associated with heavily polluted water ecosystem, as well as Chironomid larvae and shrimp in the area, were exposed to agricultural fertilizer (Tomkins and Kotiaho, 2001).

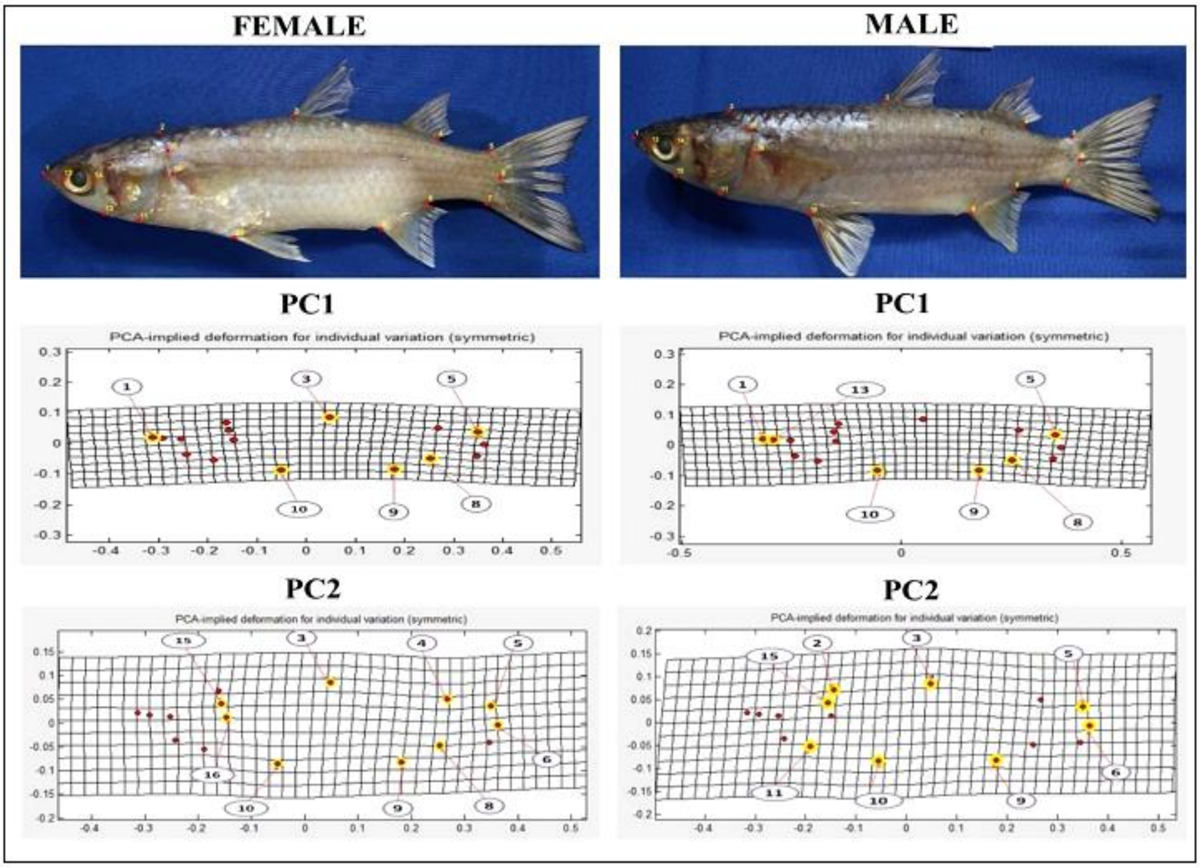

Fig. 4. Actualized picture of digitized female and male *M. Cephalus*collected at Agusan River with affected landmarks shown in PCA-deformation grid for PC1 and PC2.

Also, evaluating the concentrations of heavy metals in the different organs of the fish possibly due to its size, mass, and age (Cabuga *et al.*, 2016). Heavy metal is a form of pollutant that usually found in the water bodies. Nonetheless, freshwater environment frequently affected by various chemicals where most of the fishes life stages occur and this could affect their normal growth (Conclu, 1986).

However, female samples showed slightly positive and the occurrence of FA was not directly influenced by the heavy metal concentrations. It possibly due to another factor which is not determined in the study. The obtained data for physicochemical water parameters were presented in (Table 5).

One-way ANOVA shows that the mean values of the tested parameters have no significant difference. However, it was found out that the values were not exceeded from the suggested standards of DAO (DENR Administrative Order) & Water Watch Australia National Technical Manual (2002). The conductivity is the measurement of electric current of the water and influenced by the dissolved salts such as potassium chloride and sodium chloride (Jena *et al.*, 2013). It is also affected because of the dissolution of substances like chloride, nitrate, magnesium sulfate, and phosphate ions (USEPA, 1997). Dissolve Oxygen (DO) is the amount of oxygen dissolve in the water bodies and it is essential for the survival of aquatic organisms; thus indicating a low level of DO could be associated with pollution (Martinez and Galera, 2011).

Accordingly, DO represent the extent of pollution in the aquatic environment (Gopalkrushna, 2011). The pH affects different chemical and biological processes in the water and the largest variety of aquatic organisms prefer a range of 6.5-8.0 (USEPA, 1997).

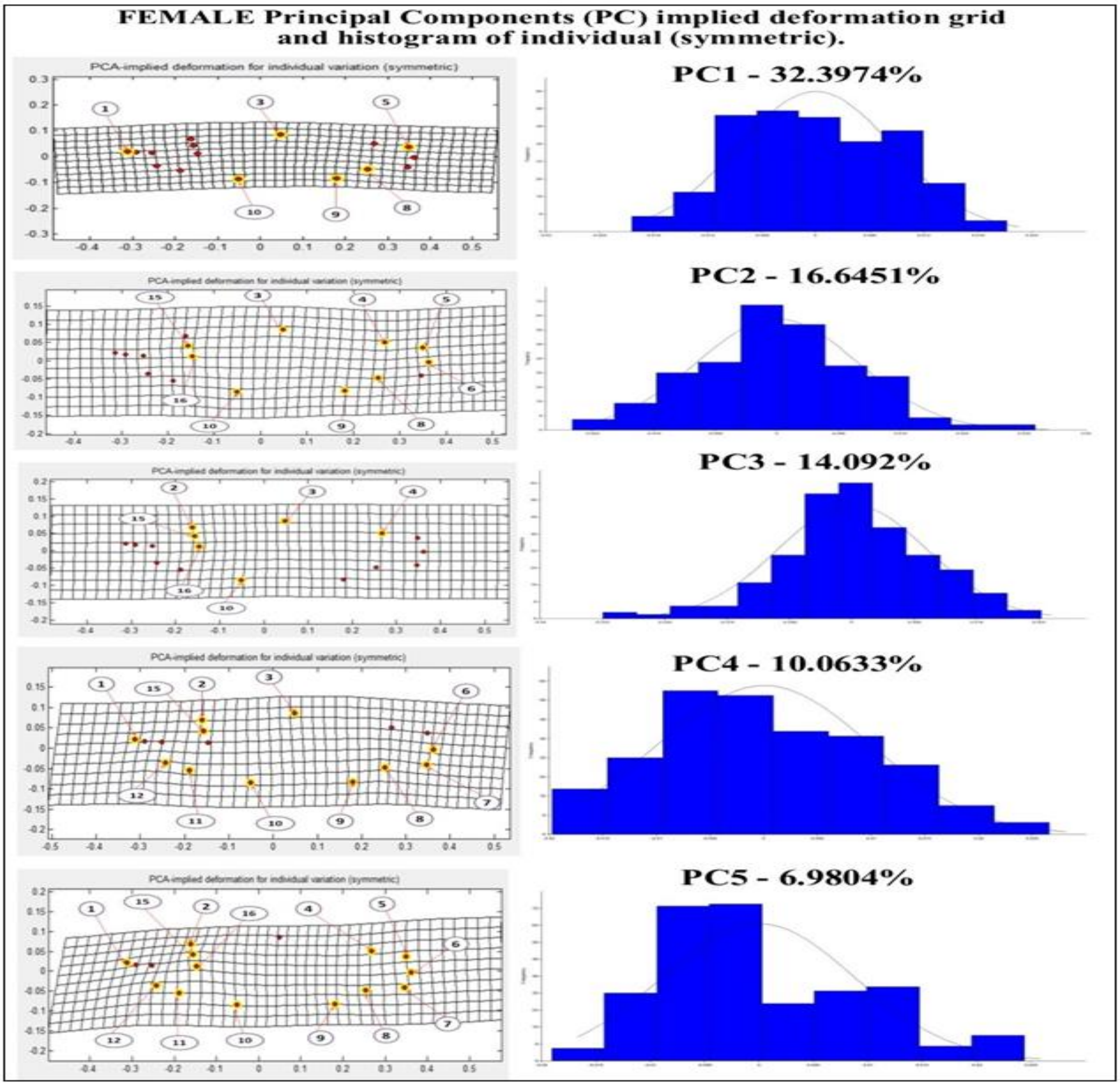

Fig. 5. Principal Component (PC) implied deformation grid and histogram of individual (symmetric) of female *M. cephalus*.

It indicates the basicity and the acidity of the water, thus the scale of pH level is highly important to the health of aquatic life (Martinez and Galera, 2011). The salinity is linked to the dissolve inorganic ions such as sodium chloride (salt). The suggested amount of salinity that freshwater systems should have is not the less than <0.5mg/L (USEPA, 1997). Further, the temperature has an influence in the presence of different chemicals found in the water system.

The tolerability of aquatic contaminants depends on the temperature while this affects the growth of microorganism that later causes the water condition (Ombaka and Gichumbi, 2012). Lastly, TDS or total dissolves solids is used to assess the drinking quality of the water since it represents the quantity of the ions, when TDS is high or low it can directly affect the growth of aquatic organisms (Sreeja *et al.*, 2012).

Accordingly, water quality highly affects the biodiversity of aquatic ecosystem (Cabuga *et al.*, 2017) and plays an important factor in the organism's growth and development (Venkatesharaju *et al.*, 2010). The influence of vegetated riparian zones greatly affects the condition of lakes, streams and rivers in decreasing the runoffs of chemicals from agricultural uplands (Baker *et al.*, 2000; Dosskey, 2001; Hefting *et al.*, 2005).

Nonetheless, determining the health status, sustainability and productivity of aquatic environment merely depend on the water parameters (Djukie *et al.*, 1994). Thus, the physical and chemical property of water bodies also constitutes in the richness and distribution of aquatic lives (Unanam and Akpan, 2006).

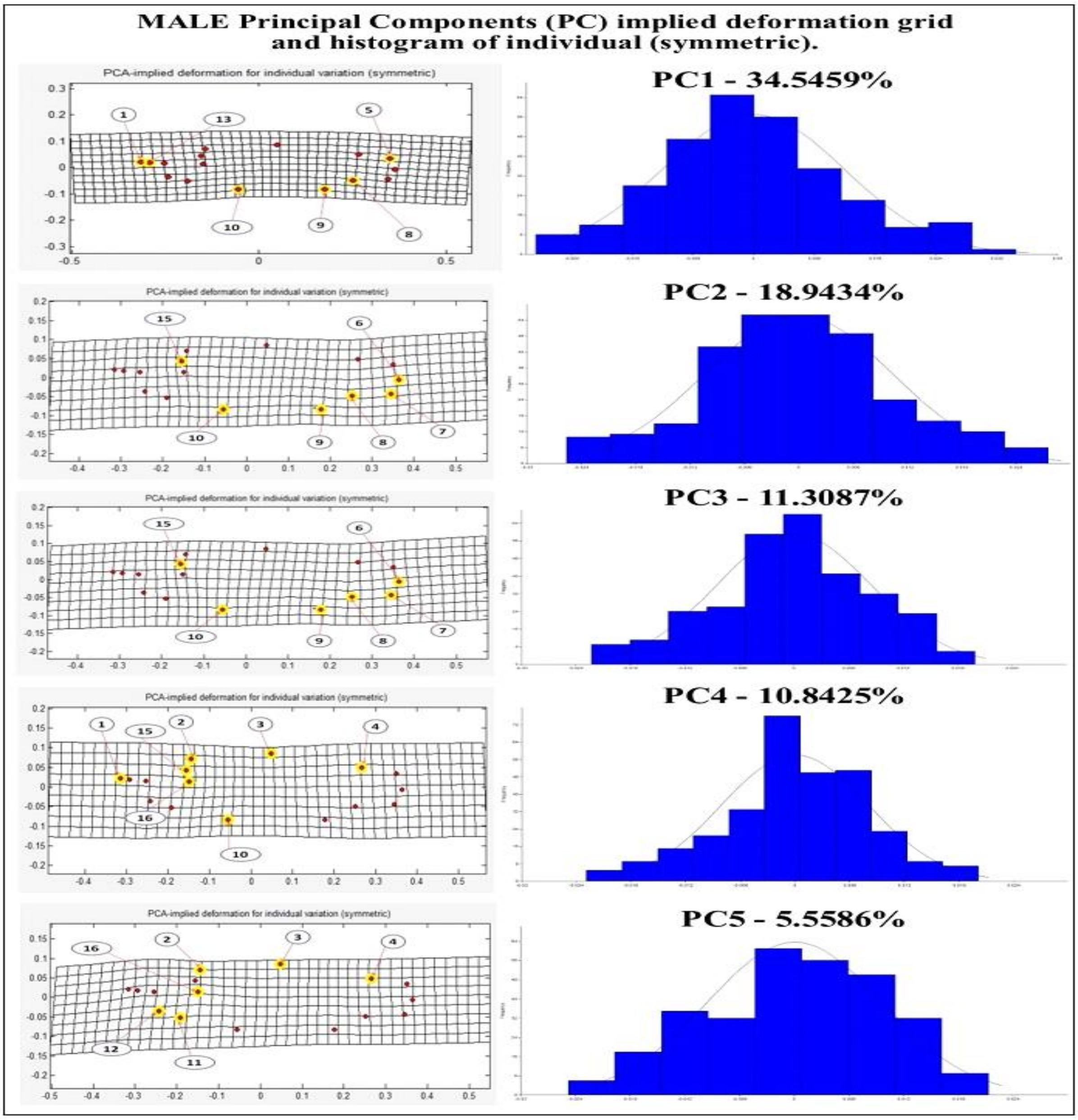

Fig. 6. Principal Component (PC) implied deformation grid and histogram of individual (symmetric) of male *M. cephalus*.

The Pearson-correlation between the physicochemical parameters and FA in the female fish samples shows a slightly positive (r=0.7402) (Fig. 9) and very low significant correlation. This implies that FA is not influenced by the water parameters and could be attributed to other factors that were not determined in the study. Accordingly, the level of FA in an organism is parallel to the stressed environment accompanying with genomic alterations that result to developmental variability (Ducos and Tabugo, 2015).

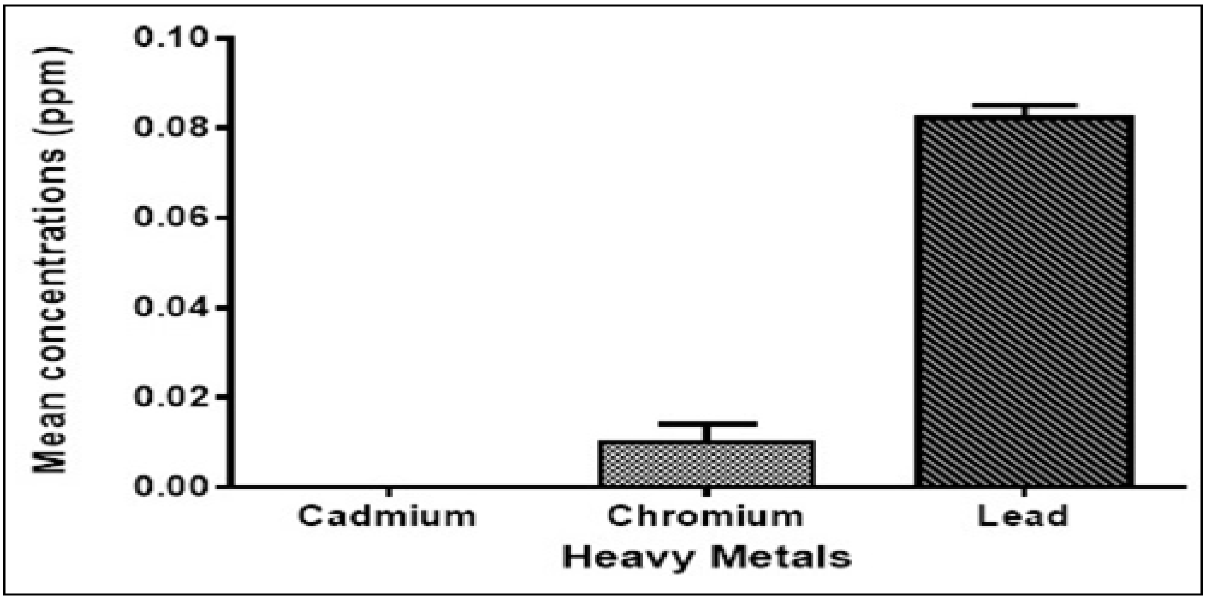

Fig. 7. Graphical presentation of heavy metals concentration along three stations.

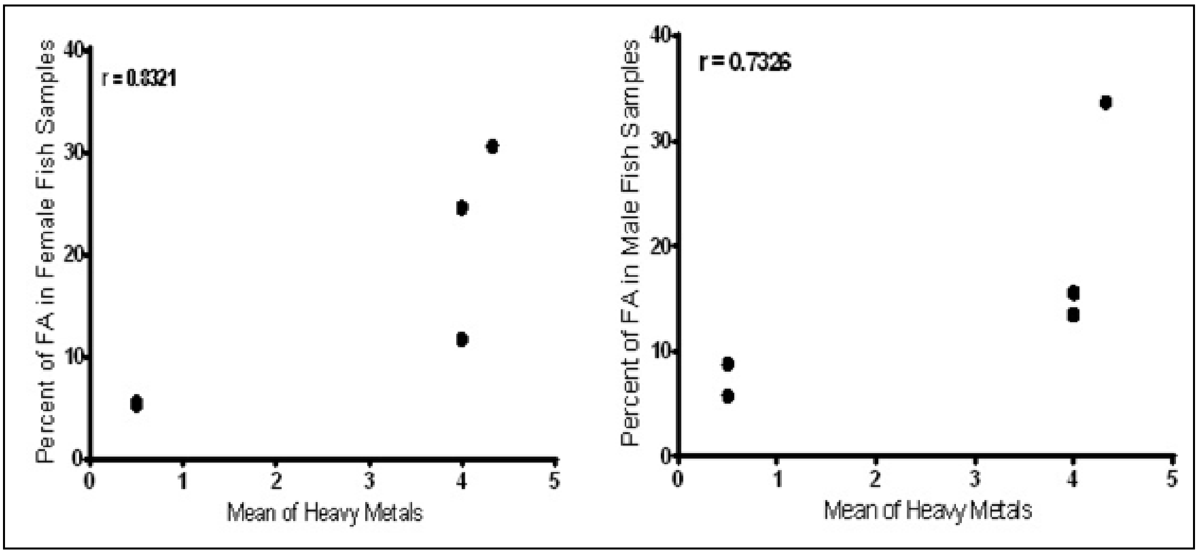

Fig. 8. Scatter plot showing correlation of FA versus heavy metal concentrations (Left) Female (Right) Male.

In comparison, fishes that found to have high levels of fluctuating asymmetry associated with heavily polluted water ecosystem, as well as Chironomid larvae and shrimp in the area, were exposed to agricultural fertilizer (Tomkins and Kotiaho, 2001).

While the physicochemical parameters versus FA in male samples show positively correlated (r=0. 9427) (Fig. 9). This suggests that the incidence of FA might directly be associated with the water components.

Because, water is significant abiotic components during the growth, development and metabolic responses of the fishes. Further, its physical properties that include, temperature and rate of suspended solids, chemical parameters like hardness, alkalinity, pH and metals are important for fish growth and production (Viadero, 2005). Thus, the observed FA in female and male samples suggest that each sex has the ability to buffer environmental alterations.

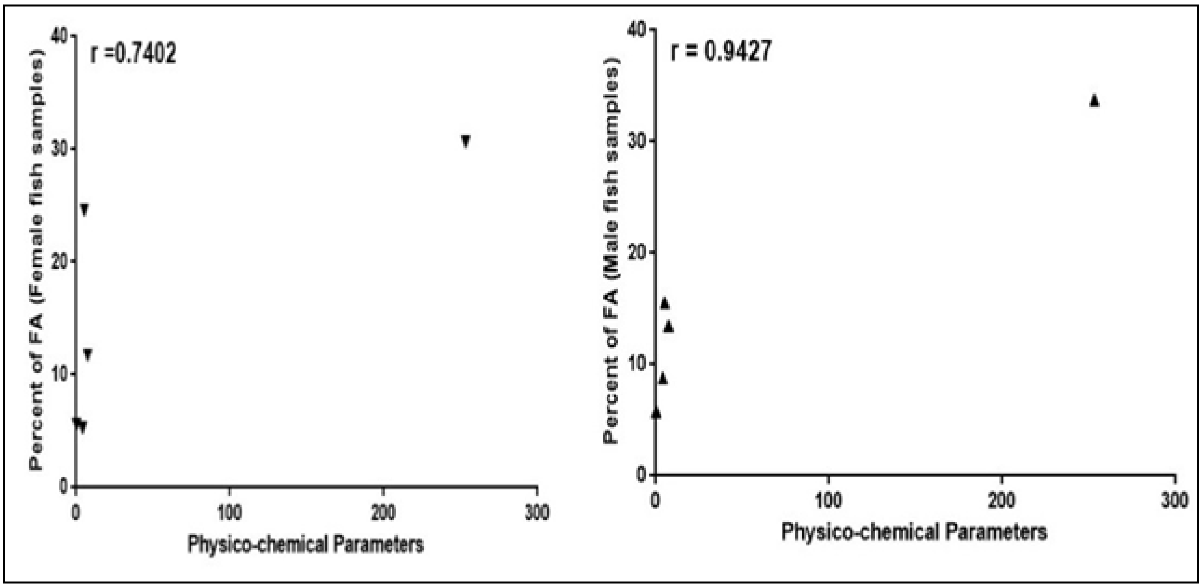

Fig. 9. Scatter plot showing correlation of FA versus physico-chemical water parameters (Left) Female (Right) Male

Conclusion

The importance of fluctuating asymmetry (FA) advances to quantify phenotypic variability among species of the same population and understands the ecological condition. The results of Procrustes ANOVA indicates high significant difference $P<0.0001$ in male and female populations. Females had the highest percentage of FA (77.93%) when compared to males (77.43%). This was associated with sustaining homeostasis and metabolic responses of the fishes during reproduction. Pearson-correlation between FA and physicochemical parameters in female samples shows slightly positive ($r=0.7402$) while male samples show positively correlated ($r=0.9427$). In heavy metals analysis, lead (Pb) had the highest mean concentrations (4.11 ± 0.11 ppm) and followed by chromium (Cr) (0.5 ± 0 ppm) both exceeded the recommended safe limits set by the authorized agencies. One-way ANOVA showed that the concentration of heavy metals in the muscles was highly significant ($P<0.05$). Pearson-correlation between FA and heavy metal concentrations in female samples shows slightly positive ($r=0.8321$) while in male samples shows positively correlated ($r=0.7326$). This implies that heavy metal concentrations were directly influenced the FA and could develop morphological differences. Thus, using these techniques identifies shape differences, heavy metal contents and ecological standing based on the result obtained.

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