Geometric morphometric analysis of three spot gourami, *Trichopodus trichopterus* in Masao River, Butuan City, Mindanao, Philippines

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Abstract. Fluctuating asymmetry (FA) determines the health status of the bodies of water by way of measuring the variations in body shapes of the fish. Species in this environment are said to normally buffer the genetic and environmental stresses during their ontogeny to maintain precise development. FA measures the pattern of morphometric differences of bilateral symmetry of an organism caused by these environmental disturbances. In this study *Trichopodus trichopterus* was used as bioindicator, to determine the FA in its body collected from Masao River, Agusan del Norte, Philippines. The river is situated and inhabited by congested nearby households. There were 60 species collected using a cast net and local traps. Geometric Morphometric (GM) and Analyses Procrustes ANOVA were used to calculate, show and obtain morphological intraspecific variations. A total of 20 landmarks from 60 samples of fish, 30 per sexual category, were used. These selected points of landmarks were digitized both in left and right side of the specimen images. Then, the 20 landmark coordinates from fish were subjected to Symmetry and Asymmetry Geometric Data (SAGE) software after being digitized using tpsDig2 and being saved as TPS file. The result in procrustes ANOVA showed a significant high level ($p < 0.0001$) of FA which indicates a high significant variation showing the percentage variation of shapes in deformation grid with Principal Component Analysis (PCA) scores of 70.8115% and 79.8394% in female and male, respectively. However, the female has a significant variation among individual genotypes as reflected on individual effect while the male does not. The study provides an evidence sufficient to indicate the environmental stress through FA that directly impact fish mobility that was concentrated mostly in the affected landmarks such as those in the dorsal cephalic region, in the pectoral fin, and some parts for fish locomotion as in anterior and posterior insertion of the dorsal fin. These signify that the river is not at good ecological condition due to the nutritional disturbances that affect the FA both in male and female, as well as other environmental and genetic stresses.

Key Words: fluctuating asymmetry, *Trichopodus trichopterus*, environmental stress, SAGE.

Introduction. Our environment is continuously experiencing contamination due to anthropogenic activities since the early technological discoveries that pollute especially the bodies of water. Organisms living in water are a good predictor of the condition of the environment. Stressful habitat develops morphological configuration on fishes. They are affected by this pollution or contamination of organic waste, nutritional load and other environmental impairment that lead to intraspecific variation in species morphology (Almeida et al 2008).

Morphometric measurement is one of the known and cost-effective methods identifying morphological differences. This is widely used to determine differences between population or intraspecific variations (Cheng et al 2005; Buj et al 2008; Torres et al 2010). It is, therefore, essential to observe the pattern of variation within a population (Beheregaray & Levy 2000) when studying morphological variation within species. During the development of fish, fluctuating asymmetry (FA) is associated with perturbation and stresses (Allenbach et al 2009). Environmental and genetic stress has shaped the morphological differences regarding elevated levels of FA as perturbed the natural development during ontogeny (Palmer & Strobeck 2003; Markow 1995). Hence, FA indicates subtle differences between the left and right lateral sides as a pattern of bilateral variations in a sample of fishes as an adaptation to environmental stress (Swaddle 2003).

Three spot gourami (*Trichopodus trichopterus*) belongs to an air-breathing group of fish native or indigenous to Africa and some parts of southern Asia. Consequently, they can be found in Laos, China, Cambodia, Thailand, Vietnam, Malaysia, Indonesia and Philippines (Dorado 2012). This group consists of three families, 19 genera and about 120 species of air-breathing fishes (Nelson 2006). In the Philippines, *T. trichopterus* is considered as one of the important fishes found in some parts of Mindanao such in Masao River in Butuan City. *T. trichopterus* is also a better specimen for study since it can thrive a complex range of environmental condition (Priest 2002) to be subjected to Landmark-based geometric morphometric analysis.

In this study, FA was used to assess the condition of Masao River in Butuan City, Agusan del Norte. Geometric Morphometric analytical method is the technique used to obtain FA to visualize the shape analysis of fish to describe the intraspecific variations within species (Rohlf 1990). *T. trichopterus* was used to show its asymmetry in residing to its body of water crowded with nearby houses along this river. Hence, this study is conducted to determine the levels of asymmetry.

Material and Method

Fish sampling and study area. Specimens of *T. trichopterus* were collected using a cast net and local traps in two sampling areas from Masao River, which lies between 9º0'0"N and 125º28'59.99" E located in Barangay Masao, Butuan City, Philippines. Global Positioning System (GPS) was obtained by the use of an online mapping shown in Figure 1. A total of 60 specimens (30 males and 30 females) from the habitat to be used for body measurement were collected on December 20-22, 2015. Samples were placed on the box of ice for transport to the laboratory maintaining 4^{o}C until actual processing of fish specimen. With the use of the camera (Kodak Easyshare C1450 5X), specimens' images were captured with three replicates. Later, sex identification was made by direct examination right after identifications its external morphological structures.

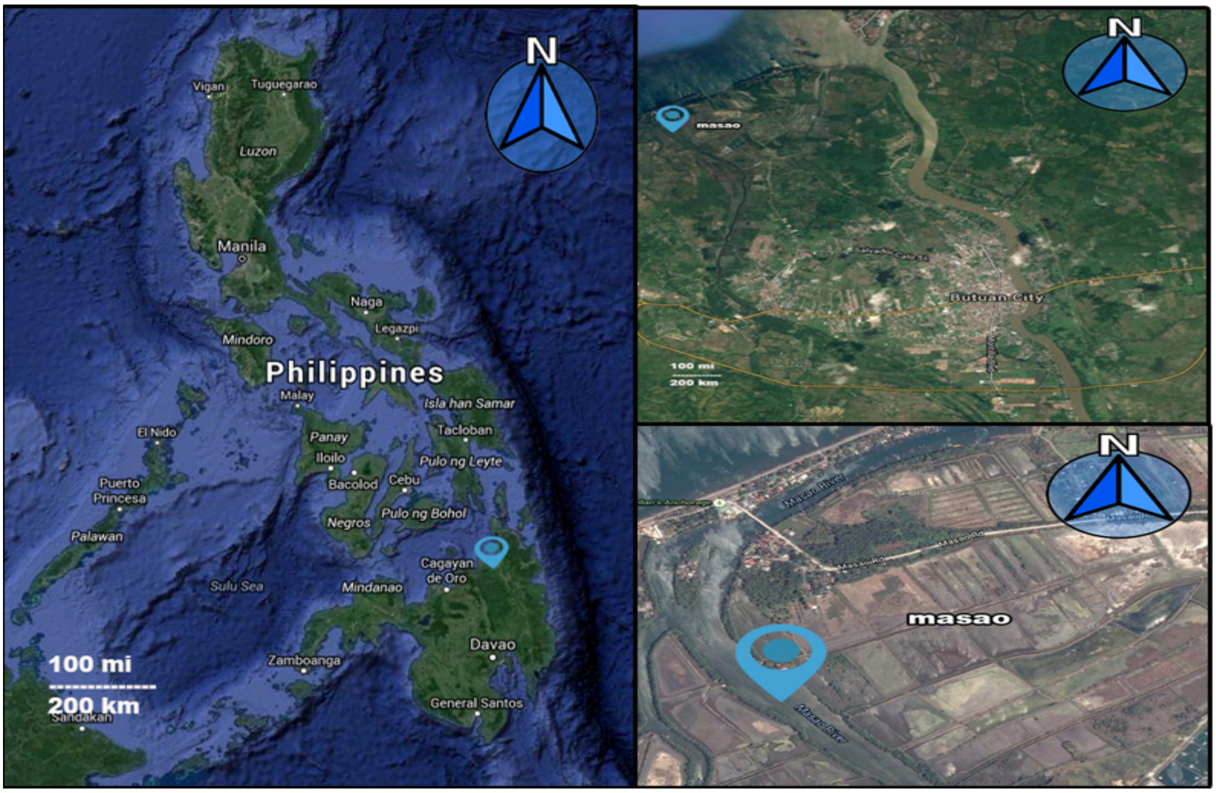

Figure 1. Showing the exact location of the study site in Masao River, Butuan City, Philippines (www.google.com.ph/maps).

Landmark digitization on specimen images. Using Thin-Plate Spline series (TPS), landmarks of 20 points were assigned to the morphological structure of fish (Figure 2). Twenty (20) landmarks equivalent to 20x and 20y Cartesian coordinates on images were selected both in left and right lateral sides to be used later for analysis (Table 1). These landmarks were digitized to each digital image of the specimen for TPS filing using tipsDig 2.10 version (Rohlf 2006). Digitization was done in tri-replicates for each fish sample to minimize errors in assigning landmarks.

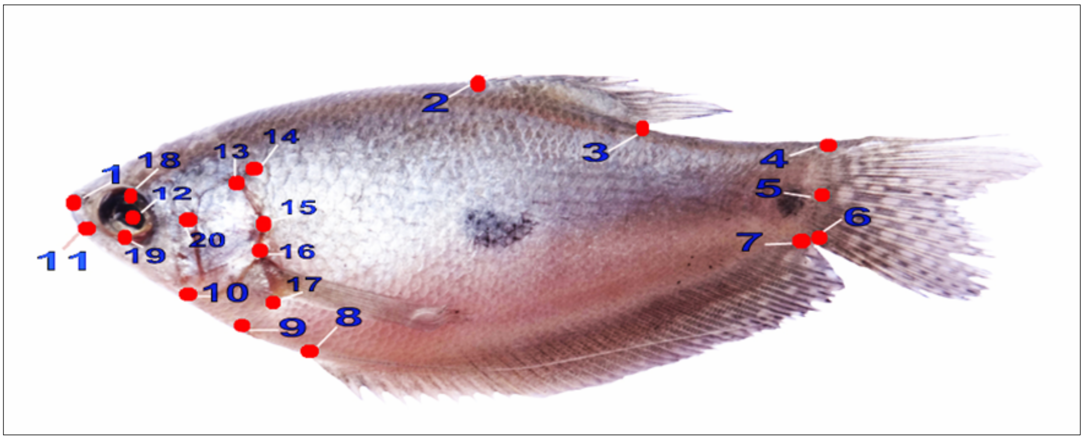

Figure 2. Location of the 20 landmark points on *T. trichopterus.*

Table 1

Description of the landmark points according to Dorado et al (2010)

Coordinates locations	*Descriptions*
1	Snout tip
2	Anterior insertion of dorsal fin
3	Posterior insertion of dorsal fin
4 & 6	Points of maximum curvature of the peduncle
5	Posterior body extremity
7	Posterior insertion of anal fin
8	Anterior insertion of anal fin
9	Insertion of the pelvic fin
10	Insertion of the operculum at the lateral profile
11	Posterior extremity of premaxillary
12	Center of the eye
13	Superior insertion of the operculum
14	Beginning of the lateral line
15	Point of maximum extension of operculum on the lateral profile
16	Superior insertion of pectoral fin
17	Inferior insertion of the pectoral fin
18	Superior margin of the eye
19	Inferior margin of the eye
20	Superior margin of the preoperculum

Shape variation analysis. The Symmetry and Asymmetry in Geometric Data (SAGE) software was used to determine FA levels on the generated x and y coordinates of landmarks in TPS format. Procrustes ANOVA was also used to calculate, quantify and obtain the overall FAs between sexes (Klingenberg et al 1998) for the purpose of detecting variances and deviations from the paired landmark coordinates of the left and right side images. Here, FA was generated between sexes as well as variations between sides of specimen performed by interpolation based on the TPS through Principal Component Analysis (PCA) where the shape changes as landmarks displaced in the deformation grid (Albarran-Lara et al 2010). Paleontological Statistics (PAST) software (Hammer et al 2001) was used to compare between variation and individual symmetry of male and female specimen. The generated comparison was in as histogram such the box plot.

Results and Discussion. Procrustes ANOVA showed the individual body shape fluctuation, as depicted in Table 1. Regarding genotypic variation among individuals, it has a significant level of symmetry in individuals of female but not significant in individuals of male *T. trichopterus.* In contrast to the study of Lecera et al (2015) that variations in female specimen were absent since female possess the mechanism that buffers developmental noise and being able to maintain homeostasis after experiencing lesser developmental perturbations and stresses, in our study the incidence of FA in females is at higher level as exposed to poor environmental condition. This means that the degree of FA indicates the inability in maintaining homeostasis when exposed to stress during development (Dongen 2006) as depicted in individual effect or variation among individual genotype. Moreover, it is found out that the unfavorable condition can cause the organism to have poor developmental homeostasis which means the inability to buffer environmental disturbances thus affecting developmental ability (Natividad et al 2015). Similar to the study of Almeida et al (2008), with high eutrophication level which fishes can feed on organic waste thrown by people into the water is considered as a nutritional disturbance. On the other hand, Nacua et al (2010) reveals sexual dimorphism between sexes is common among fishes. This means that male and female differ in their body shapes. Female fish possessed deeper body while the male is fusiform. It is in concordance with this study that male shape is fusiform suggesting to adapt for rapid acceleration since the environment is fast flowing like in the case that of Masao River. The male has naturally adapted to the fast flowing water and for courtship purposes (Walker & Bell 2000), hence, preferred as sexual selection (O'Donald 1967). As a result, male individuals likely to show not significant in individual body shape fluctuation as depicted in Table 1. However, variations in the side scores that is, the left and right sides of the sexes were identified to have a significant high level. This simple means that FA has occurred in the body shapes of the male and female specimen as a whole. According to Barrett (2005) as cited by Natividad et al (2015) stressors in the environment affect the species' ability to develop a desired path phenotypically.

Table 2

Procrustes ANOVA for shape of *T. trichopterus* between sexes

Effect	*Sum of square*	*Degrees of freedom*	*Mean of squares*	*F-value*	*P-value*
		Female			
Individuals	0.1742	1044	0.0002	1.3393	0.0001**
Sides	0.0129	36	0.0004	2.8725	0.0001**
Individual x sides	0.1301	1094	0.0001	6.4599	0.0001**
Measurement error	0.0833	4320	0	-	-
		Male			
Individuals	0.1217	972	0.0001	0.9444	0.8137
Sides	0.0388	36	0.0011	8.1344	0.0001**
Individual x sides	0.1289	972	0.0001	5.4846	0.0001**
Measurement error	0.0975	4032	0	-	-

**highly significant ($p < 0.005$).

The upper 5% effective interaction value of principal components from PC1 to PC5 is in Table 3 (female) and Table 4 (male) accompanied with the histogram to show the shape variation from landmarks of male (Figure 3) and female (Figure 4) samples. A total of 70.8115% FA interaction of *T. trichoterus* female were accounted to indicate variation in shapes and significantly varied in individual body symmetry (61.5922%). PC1 asymmetry can found greatest in the area covered by landmark 1, 2 and 3, 4 and 6, 5, 7 and 8, 9, 10, 11, 14, 15, and 16 and 17; landmarks for asymmetry in PC2 can be found in 1, 2 and 3, 4 and 6, 5, 11, 15, and 16 and 17, 18, and 20. Male's bilateral asymmetry has a total of 79.8394% that which depicts movement of landmarks to the other region such: PC 1 on 1, 2 and 3, 4, 5, 8, 9, 10, and 16 and 17. The data 76.0632% individual symmetry and 79.8394% did not vary significantly due to the slight percentage difference.

Furthermore, the PCA provides a sufficient evidence to indicate the environmental stress through FA that directly impact fish mobility. These were concentrated mostly on affected landmarks such as those in the dorsal cephalic region, in the pectoral fin (Natividad 2015), and some parts for fish mobility as in anterior and posterior insertion of dorsal fin.

Table 3

Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks (female)

PCA	*Individual (symmetry)*	*Sides (directional asymmetry)*	*Interaction (fluctuating asymmetry)*	*Affected landmarks*
PC1	28.3238%	100%	25.191%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 17
PC2	19.0163%		16.8535%	1, 2, 3, 4, 5, 6, 8, 9, 11, 13, 15, 16, 17, 18, 20
PC3	12.3085%		12.444%	2, 3, 8, 14, 16
PC4	7.8762%		8.7569%	3, 8
PC5	6.3759%		7.5696%	1, 3, 8, 11, 13, 14, 16, 17, 18
	61.5922%		70.8115%	

Table 4

Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks (male)

PCA	*Individual (symmetry)*	*Sides (directional asymmetry)*	*Interaction (fluctuating asymmetry)*	*Affected landmarks*
PC1	23.045%	100%	29.2687%	1, 2, 3, 4, 5, 8, 9, 10, 16, 17
PC2	16.9989%		22.0821%	2,8, 13
PC3	15.33%		13.0957%	1, 2, 4, 5, 8, 9, 11, 13, 14, 15, 16, 17
PC4	12.8209%		8.9131%	1, 2, 3, 10, 11, 13, 15, 16, 17, 20
PC5	7.8684%		6.4798%	1, 2, 3, 11, 14
	76.0632%		79.8394%	

Figures 3 and 4 are TPS deformation grids showing asymmetric distribution on the shapes of male and female *T. trichopterus,* respectively. These shapes represented the variations in black color markings indicating the displacement or movement of the affected landmarks which imply that asymmetry is greatest. Besides are histograms as bar graphs to show the common centroid, statistical shape and spread of individual symmetry.

This result reveals that FA has a direct relationship with developmental stability since FA was thought to reflect the ability of the organism to cope with both genetic and environmental perturbation. This has taken into consideration that developmental stability is the ability of the genotype to resist to developmental stress (Graham et al 2010) and consistent with the studies that stress such as genetic and environmental instability (Mpho et al 2000), such as parasitic load and nutritional stress can cause an increase in fluctuating asymmetry (Pojas et al 2015).

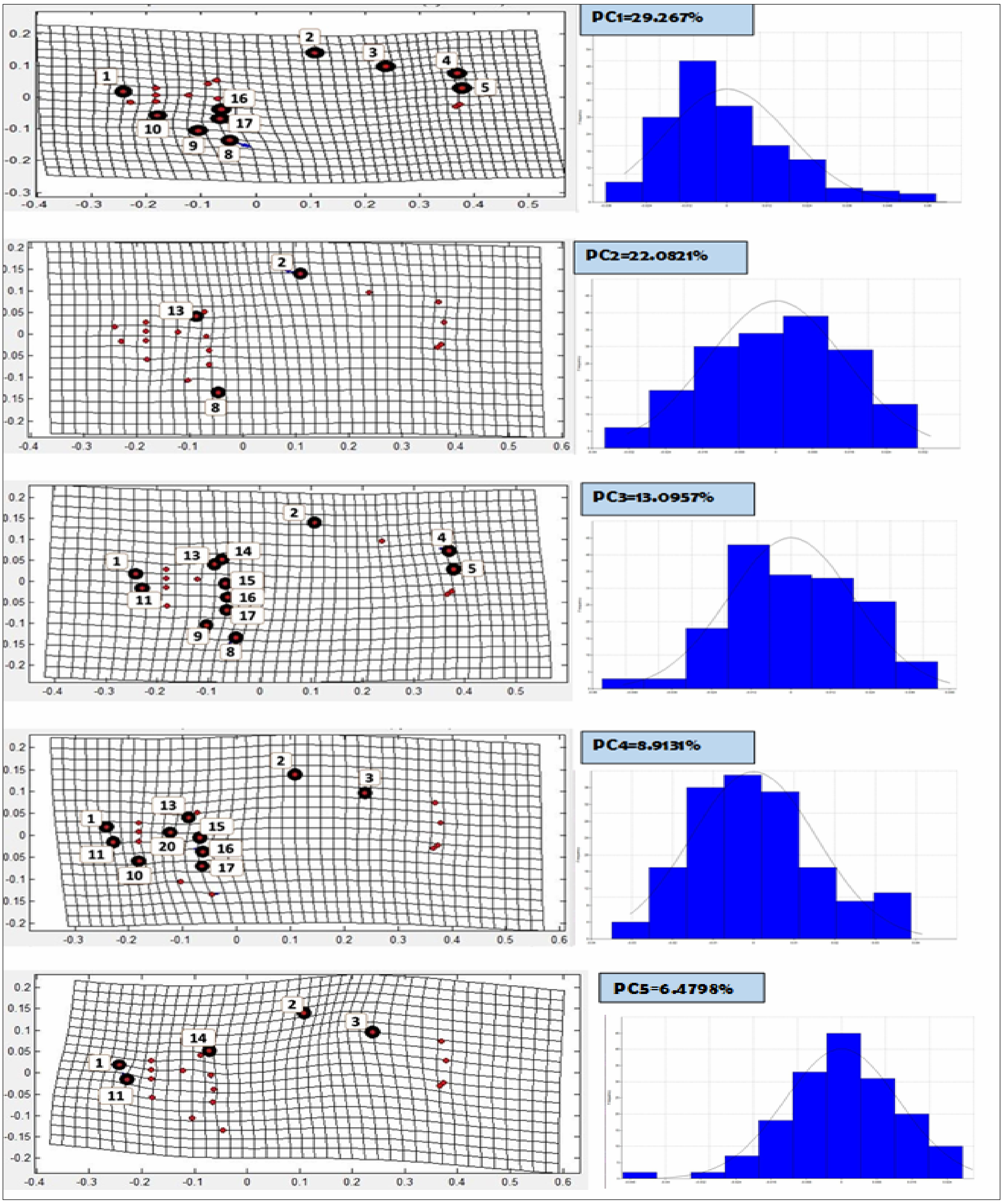

Figure 3. PCA indicates deformation of individual x side interaction (FA) with a histogram of male *T. trichopterus.* The percentages signify the proportions of variation for which the respective principal component account was showing the movement of the affected landmarks in the region.

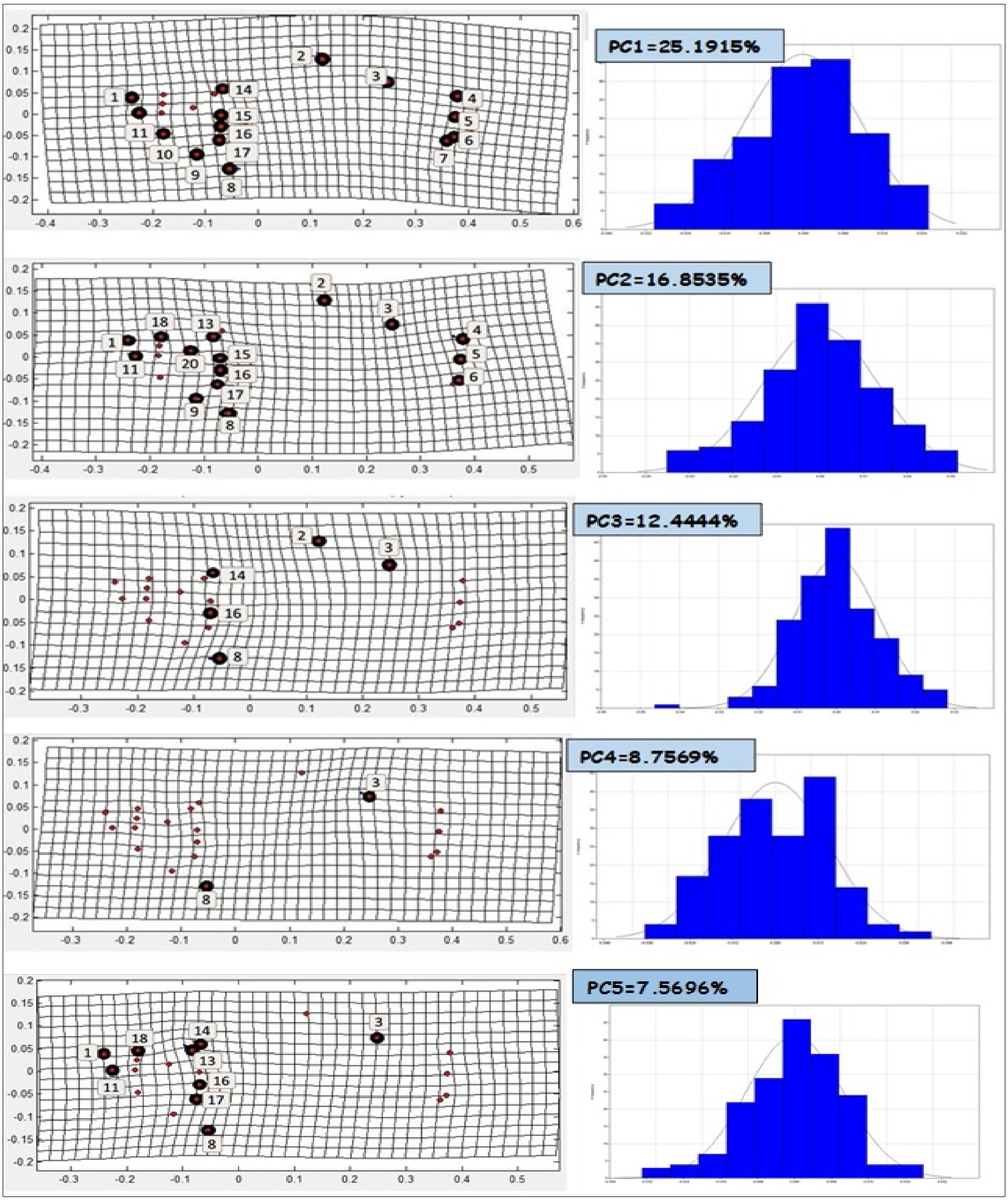

Figure 4. PCA indicates deformation of individual x side interaction (FA) with a histogram of female *T. trichopterus.* The percentages signify the proportions of variation for which the respective principal component account was showing the movement of the affected landmarks in the region.

Conclusions. Based on the study, the analysis showed the significant high level of variations ($p < 0.005$) both in male and female *T. trichopterus*. This FA in the shape both right and left sides has a percentage of 70.8115% in female and 79.8394% in male. These provide evidence on environmental stresses in Masao River as well as validate its health status due to crowded nearby households that contribute adverse effect to the body of water. The result reflects unfavorable environmental condition as it has a significant higher level of FA values of the specimen.

Acknowledgements. The authors would like to thank the faculty of Caraga State University (CSU)-Ampayon, Butuan City and DEpEd family for unending moral support.

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Received: 21 June 2016. Accepted: 29 August 2016. Published online: 26 September 2016.
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How to cite this article:
Eviota M. P., Jumawan J. C., Joseph C. C. D., Samson N. L., 2016 Geometric morphometric analysis of three spot gourami, *Trichopodus trichopterus* in Masao River, Butuan City, Mindanao, Philippines. AACL Bioflux 9(5):1011-1019.