Fluctuating asymmetry in the body shapes of goby fish *Glossogobius giuris* from Agusan River, Butuan City, Agusan del Norte, Philippines

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Abstract. This study was led in determining the health of area in Agusan River, Butuan City, Agusan Del Norte by looking into the level of fluctuating asymmetry (FA) in populations of *Glossogobius giuris*. Numerous studies have shown that obtaining the FA can be used to evaluate the water quality including the health of the ecosystem. In this study, *G. giuris* was used due to its abundance in the area. Thin-plate spline (TPS) series was used for landmark analyses of each sample and were subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software. Results of the Procrustes ANOVA showed that individual symmetry of L-R sides presented were not significant. However, variations in both sides of the sexes were identified to be highly significant ($p < 0.0001$). The results of Principal Component (PC) scores present a high percentage FA of female (71.33%) and male (93.3611%), respectively. In females, all landmark points were affected in PC 1 (47.06%) and PC2 (17.32). In males, PC 1 (60.8437%) and PC 2 (18.913%) have all landmark points affected except for PC 2, where posterior end of the nuchal spine, ventral end of lower jaw articulation, posterior end of maxilla, anterior and posterior midline of orbit, dorsal end of operculum and dorsal base of pectoral fin were not affected. This study validates the use of FA in assessing the status of the ecological health of Agusan River.

Key Words: fluctuating asymmetry, environmental health, Agusan river, *Glossogobius giuris*.

Introduction. Today environmental condition are disturbed due to population growth, industrialization, and household run-offs. Freshwater bodies are the most affected ecosystems because of alteration by human activities. Studies showed that freshwater ecosystems comprise only 1% of the entire expanse of the earth (Helfrich et al 2009). Biological diversity can be found utmost in freshwater bodies (Ward & Tockner 2001). The record shows about 28,900 freshwater fishes have been identified (Butler 2006). Freshwater fishes are the most endangered because of aquatic modifications (Laffaille et al 2005; Kang et al 2009; Sarkar et al 2008). Contamination of the aquatic environment has become a global problem. Studies were conducted in order to measure the effect of pollution. Aquatic organisms such as fishes are the most suitable sample media (Jenner et al 1990). Moreover, fishes are an important source of food for humans (Kumar et al 2011). Also, they are commonly used as an indicator of environmental stress since they occupy higher trophic levels (Blasco et al 1998; Agah et al 2009).

Such, environmental disturbances that instigate the change of temperature, nutrient intake, and physicochemical characteristics may also operate to synchronize reproduction cycles and physiological changes of organisms specifically freshwater fishes. Environmental stress greatly affects fishes that limit its circulation in the water system

(Lecera et al 2015). According to Daloso (2014), environmental stress is a direct entity that can reduce the overall symmetry of organisms relating to its bilateral structure. The ability of organisms to buffer environmental stress prior to its ontogeny refers to developmental stability (Waddington 1942; Zakharov 1992; Clarke 1998). Pollutants are components that can directly affect organisms physiology and morphology. The state of a single organism within population directly embodies the ecological condition of the total population particularly, the homozygosity between affected species that occur (Natividad et al 2015).

Glossogobius giuris (Hamilton, 1882) or known to be called in the area as ”Pijanga” was used in this study. Goby fishes are widely used as an indicator of developmental instability and environmental stress because of its abundance and wide distribution in fresh water bodies and estuaries (Hoese & Allen 2009; Lekshmi et al 2010). The study area was Agusan river considered to be the third longest river in the Philippines that has a total length of 350 km which located at the north-eastern part of Mindanao. Currently, this river system was threatened by different stressors that have great impacts in biodiversity of the species. These are industrialization, chemical and household run-offs and heavy metal contamination (Natividad et al 2015). This different tensions in the freshwater ecosystems in the Lower Agusan river were caused by invasive species, siltation, and erosions (Aguilar 2012). The sampling area is undertaken heavy metal contamination due to mining activities located in nearby places that presently operates. In addition, deforestation, harmful discharge of pollutants from the household and factories situated along the river banks greatly affect the health of the riverine.

To understand the effect of these stressors in the morphology of *G. giuris* and to assess the health condition of Agusan river, fluctuating asymmetry (FA) was used as a tool to identify the symmetry and asymmetry of the fish sample. Using Geometric Morphometry (GM) in shaping trait FA has also been recognized in many studies (Klingenberg & McIntyre 1998; Savriama et al 2012; Hermita et al 2013). The importance of FA is to distinguised morphological difference and simililarity (David Polly 2012). FA measures developmental disturbance that reflects the average state of adaptation and coadaptation (Waddingon 1942; Graham et al 1993). It was noted that high FA corresponds significant developmental instability (Parsons 1990). FA is said to be the potential aggravation of homeostasis of an organism (Ducos & Tabugo 2015). FA is an important morphometrics tool in the aspects of lesser and unplanned nonconformity from the perfect morphology of species due to its ability to show a total variance both the left and the right side of a bilaterally symmetrical organism (Moller & Swaddle 1997; Palmer & Strobeck 2003). Additionally, FA shows essential evidence over many bioindicators of developmental instability because of its efficiency and low-cost aspect (Clarke 1993).

This study aims to determine the percentage of the FA in *G. giuris* found in Agusan river and to equate the asymmetrical differences in both sexes, as well as to characterized the environmental condition of the river system. This study will confirm if the species had undertaken ecological and genetic disturbances in the selected area.

Material and Method

Study area. The study area lies geographically between 08°57′25.28N and 125°32′36.63 E. Mapping was obtained through http://maps.google.com shown in Figure 1. Sampling was obtained out from July 31-August 2, 2015.

Sample collection and processing. A total of thirty (30) samples of *G. giuris* were collected. Twenty (20) were females, and ten (10) were males. Samples were prepared for photo processing. Each of the sample was flanked on a Styrofoam covered with a blue cloth with fins pinned to show its point of origin. In order to preserve the fish, 10% formalin was used and so as to make the fins hardened to obtain a decent image of the fish and its point of origin. Sony DSC-TX20 (16.2 Megapixels) was being used in digital imaging. Both the left and the right lateral side of each sample were photographed together with a ruler to determine the individuals' actual length. The captured image was then converted into thin-plate spline (TPS) file using tpsDig2 program version 2.0.

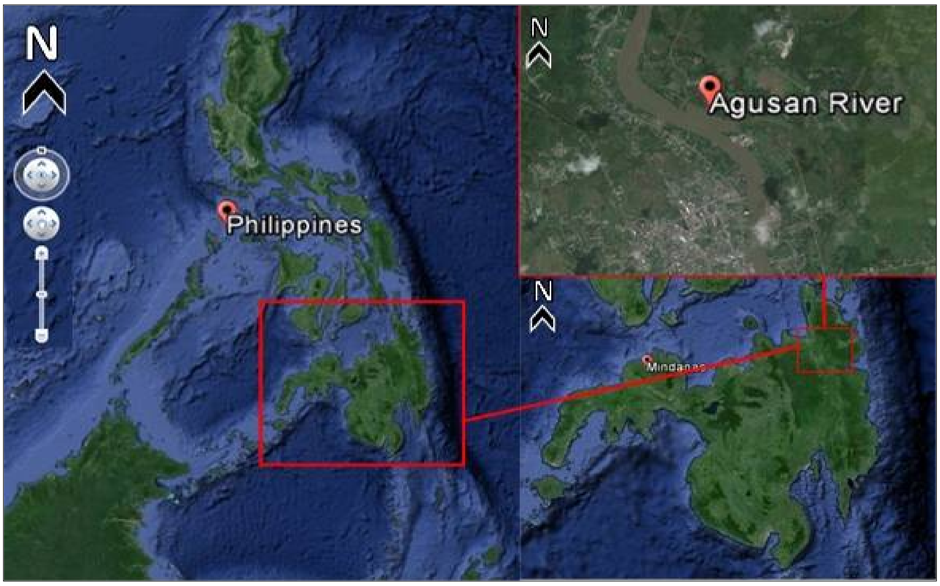

Figure 1. Map of the Philippines (left), map showing Mindanao (below right), map showing the location of Agusan River, Butuan City, Agusan Del Norte (upper left).

Sex determination. After capturing digital images, sex of *G. giuris* will then be determined. Males were identified by observing its testes; testes were typically smooth, whitish and non-granular in appearance. On the other hand, females were recognized by exploring its ovary, eggs are normally pink, yellow or orange.

Landmark selection and digitization. TPS series, landmark analyses were used to get its distorted features within the images. Landmarks were designated to have a homogenous outline of fish body shape (Figure 2) using software tpsDig2. A total of 16 landmarks (equivalent to 16 X and 16 Y Cartesian coordinates) were recognized to have the best representation of the external shape of the body. Landmark description was shown in Table 1. X and Y coordinates of the landmarks on the images were obtained for analysis. Digitization was obtained, three replicates for each fish sample to minimize the error in plotting the landmark points were used.

Table 1

Description of the landmark points of *Glossogobius giuris* was based on Paña et al (2015)

No.	*Description*
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 of caudal border of hypural plate
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 maxilla
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

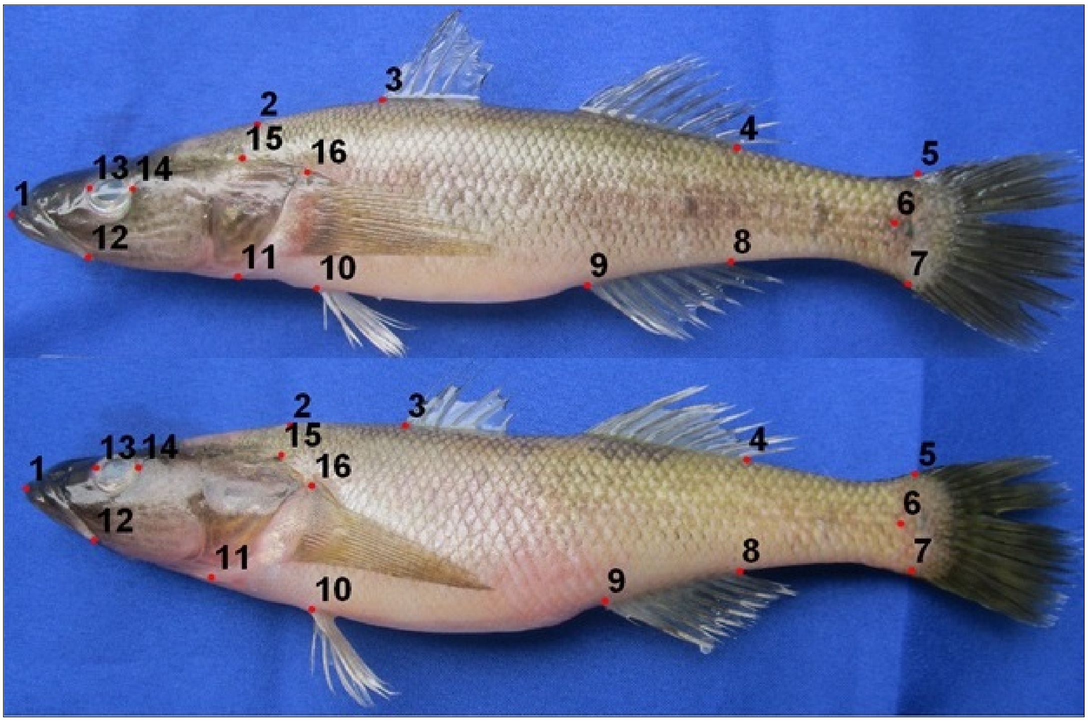

Figure 2. Landmark point of the female *G. giuris;* female in the upper part and male in the lower part.

Shape analysis. Procrustes ANOVA was employed in determining the individual symmetry and sides of L-R size and shape. X and Y coordinates serve as the starting point in examining or analyzing the FA of the fresh water fish like *G. giuris*. Left and right phase landmarks of TPS image was processed in Symmetry and Asymmetry in Geometric Data (SAGE) software (version 1.04, Marquez 2007). It was used to know the geometric data of the sample prior to its asymmetry (Figure 3). SAGE software is useful in shape conformation of individuals' variation (symmetric, asymmetric and error) including the probable covariance condition. The percentage (%) of FA was achieved and the differences between both sexes were also determined. Three factors were considered; individuals, sides and interaction of individuals and sides in measuring FA in *G. giuris*.

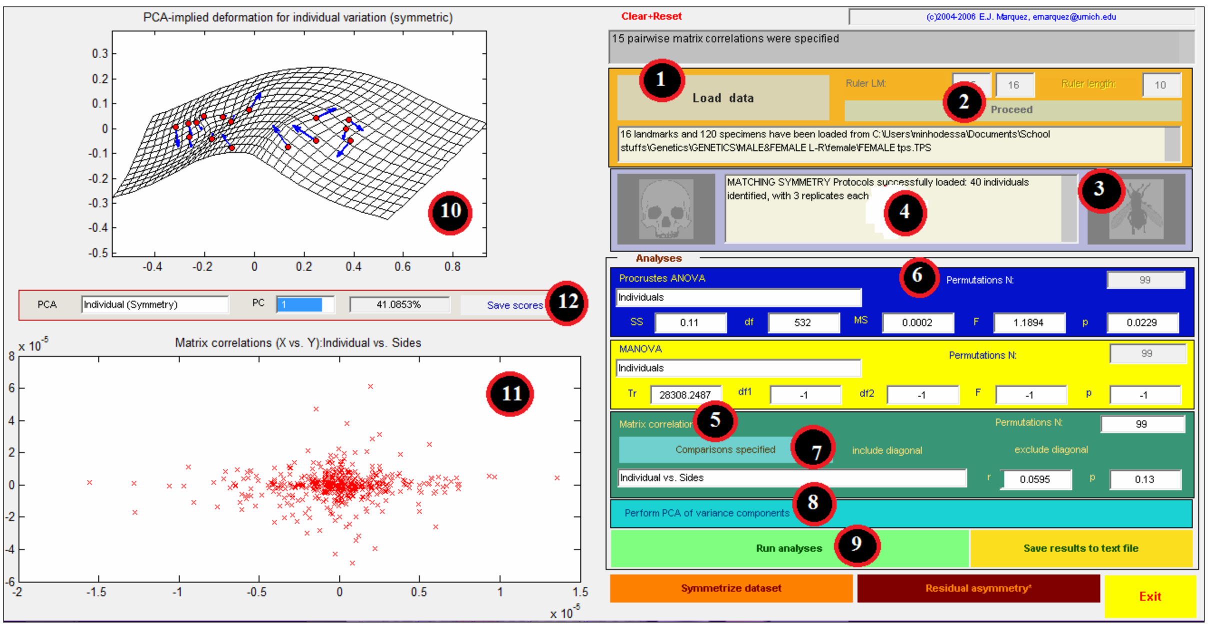

Figure 3. Overview of the schematic flow of shape analysis using SAGE software.

Intraspecific variation between sexes. The PAST (Paleontological Statistics) software (Hammer et al 2001), was being used to help and clearly compare the individuals symmetry between the sexes by generating appropriate statistical representations such as the histogram.

Results and Discussion. Results of the Procrustes ANOVA are shown in Table 2. The individual symmetry and sides of L-R size and shape in females were not statistically significant, while in males only the individual symmetry was not statistically significant. Out of the three factors considered, two shows high significance ($p < 0.0001$), the interaction of individuals and sides which is depicted on both sexes and the sides for males. It indicates the incidence of FA in *G. giuris* which was attributed to the effects of environmental stressors which can cause developmental instability to the species (Barrett 2005; Bonada & Williams 2002). To determine affected landmarks, principal component analysis (PCA) was employed on both sexes (Table 3). Male *G. giuris* shows a total of 93.77% of FA interaction from the upper 5% effective principal components from PC1–PC4. PC1 shows that all areas covered by these landmarks were found to have greater asymmetry.

Table 2

Procrustes ANOVA for the body shape of *G. giuris* fish in terms of sexes

EFFECT	*Sum of Squares*	*Degrees of Freedom*	*Mean Square*	*F value*	*P-VALUE*
		Female			
Individuals	0.11	532	0.0002	1.1894	0.022 ^{ns}
Sides	0.0091	28	0.0003	1.8655	0.005 ^{ns}
Individual x Sides	0.0925	532	0.0002	2.2438	0.0001**
Measurement Error	0.1736	2240	0	--	--
		Male			
Individuals	0.1463	420	0.0003	1.0009	0.496 ^{ns}
Sides	28	0.0127	0.0107	30.7039	0.0001**
Individual x Sides	0.1462	420	0.0003	36.2497	0.0001**
Measurement Error	0.0172	1792	0	--	--

**highly significant ($p < 0.0001$), ^{ns} not significant.

Table 3

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

PCA	*Individual (Symmetry)*	*Sides (Directional asymmetry)*	*Interaction (Fluctuating asymmetry)*	*Affected landmarks*
		Female		
PC1	41.09%		47.06%	1,2,3,4,5,6,7,8,9,10,11, 12,13,14,15,16
PC2	30.05%		17.32%	1,2,3,4,5,6,7,8,9,10,11, 12,13,14,15,16
PC3	8.48%	100%	9.6825&	1,5,7,8,10,11,12,15,16
PC4	5.68%		6.95%	1,5,8,10,15,16
	85.30%		71.33%	
		Male		
PC1	71.1123%		60.8437%	1,2,3,4,5,6,7,8,9,10,11, 12,13,14,15,16
PC2	13.6931%		18.913%	1,3,4,5,6,7,8,9,10
PC3	6.6798%	100%	10.6457%	2,7,4,11,12
PC4	2.2849%		2.9587%	1,2,4,8,10,16
	93.7701%		93.3611%	

On the other hand, female *G. giuris* shows a total FA interaction of 85.30% from PC1-PC4. Affected landmarks were the same as with males. This indicates the bilateral asymmetry of both male and female species. The male species experienced the higher level of environmental stress. The asymmetrical shape of *G. giuris* between male and female fish is shown in Figures 4 and 5.

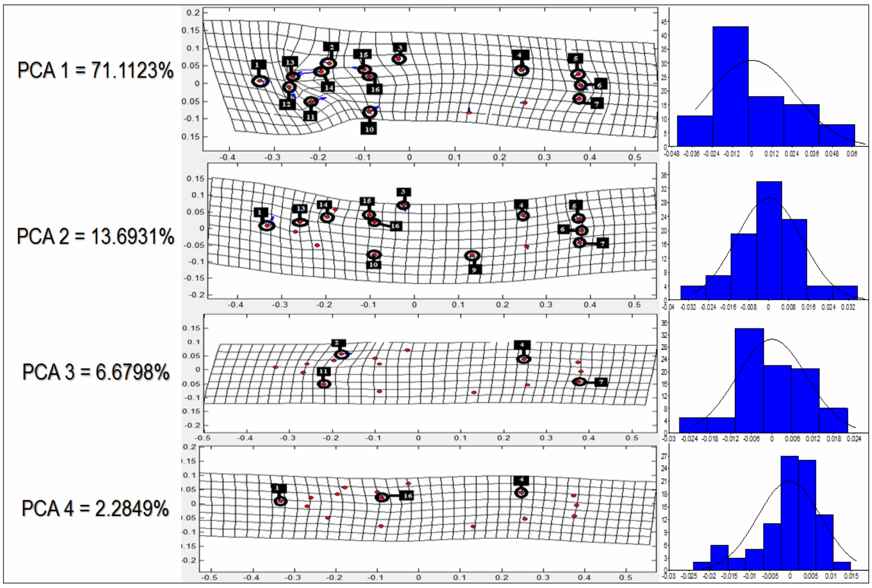

Figure 4. Principal components (PC) implied deformation grid and a histogram of individual (symmetric) in *G. giuris* male.

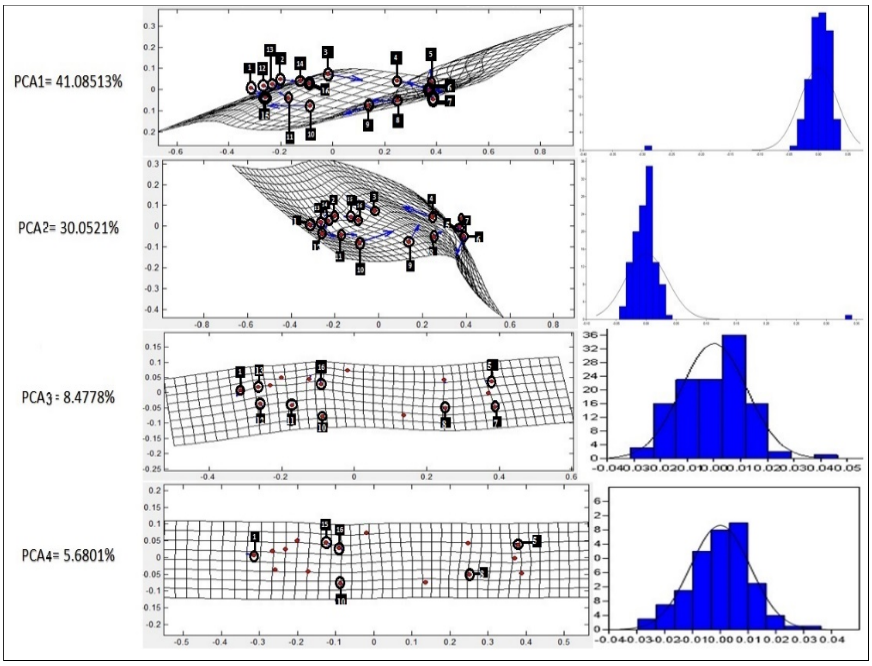

Figure 5. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *G. giuris* female.

Figure 6 shows the actual digitized image of the male and female fishes with the PCA deformation grid to show actual affected landmarks. The bar graph shows the number of symmetric individuals generating from point 0. Variations in different areas are represented by the blue marks that indicate the movement of the affected landmark areas. Skewness was observed on both sexes, indicating evidence of asymmetry.

Similar results were also observed on a recent study by Natividad et al (2015) in Lake Sebu, with *Glossogobius celebius* as bioindicator. It indicated high FA in female than in male samples. The adaptive mechanism of organisms to survive in a stressful environment is manifested on the significant levels of FA in the morphology of species. Fish mobility is one of the advantage mechanisms in determining the impact of stressors in the environment of fish specifically in the regions used for swimming. The increase in FA levels reflects a poor developmental stability. Thus, environmental stressors affect greatly in the developmental growth of fishes (Schlosser 1991).

Based on the results, all of the selected landmarks were affected, which means that *G. giuris* cannot tolerate stressed environments. The cephalic, pectoral and caudal regions were strongly affected since these regions are involved in fish mobility and foraging. Dumping of organic waste, fecal matter and erosion due to logging are major contributors to pollution in the area. Pollution in the Lower Agusan River poses a serious threat to the organisms living in it and public health in the communities in its banks (Aguilar 2012).

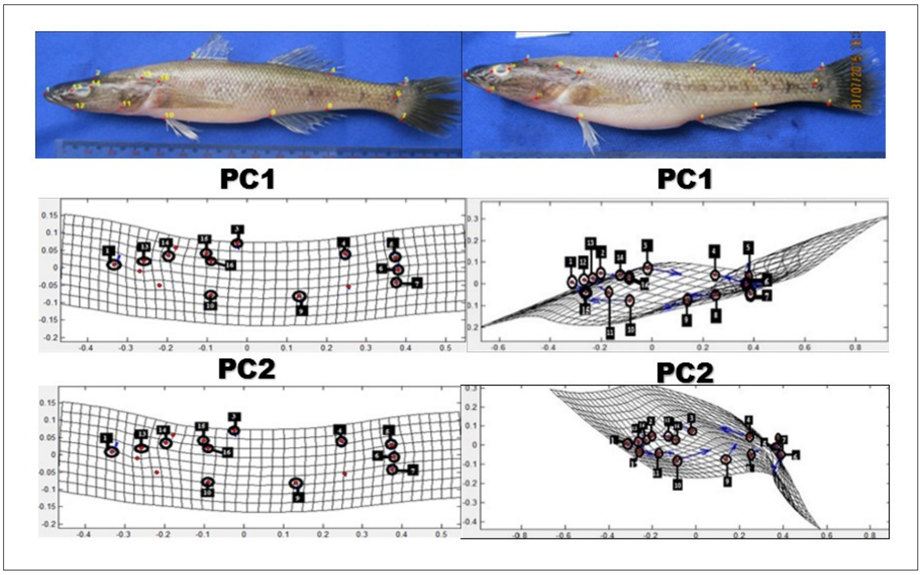

Figure 6. Actualized picture of digitized male and female fish with the affected landmarks shown in PCA- deformation grid for PC1 and PC2.

Conclusions. The statistical outcome shows high variations ($p < 0.0001$) on the left and right side of the bilateral having a percentage of 93.77% in male and 83.30% the female which represents high FA. Also, it shows that male G. *giuris* has higher symmetry compared to female. PCA also showed high skewness in the deformation grid which also signifies high FA. Affected landmarks were portions of the cephalic, pectoral and caudal regions which are involved in fish mobility. Elevated FA in *G. giuris* could be due to the poor condition of the Agusan River. It was known that the area was heavily polluted by several sources like household run-offs, mining discharges and industries wastes. These are the immediate factors affecting the morphological asymmetries of the *G. giuris*. This study contributes essential information about the current condition of lower Agusan River and confirms the use and importance of FA in determining the ecological condition.

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