Article

Fluctuating asymmetry in evaluating the developmental instability of *Glossogobius giuris* (Hamilton, 1822) from Lake Mainit, Surigao del Norte, Philippines

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Abstract
This study was conducted to assess the status of Lake Mainit by using fluctuating asymmetry in the populations of *Glosogobius giuris*. A total of 200 *G. giuris* was collected with 100 per sex. All the samples were placed in a flat styrofoam for the pinning of its fins to make it wider and to clearly see the samples point of origin for the land-marking process. 10% Formalin was applied in all the fins of the fish samples to make it hardened using a small brush. Twenty (20) landmarks were used to analyze the body shape of the fish. Several studies proved that FA can be used to directly assess water quality and the overall status of the ecosystem. Using thin-plate spline (TPS) series, landmark analyses were obtained and subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software. Results in Procrustes ANOVA showed that individual symmetry showed a highly significant difference (P = 0.00) as well as Sides (Directional Asymmetry) and Interaction (Fluctuating Asymmetry) in the study area. All the samples showed FA in both sexes. The results of Principal Component Scores displayed a higher percentage in female (69.2797%) than male (63.9214%) from Lake Mainit. Variations are almost found in all the body part of the goby in female and male gobies except for anterior insertion of second dorsal fin and superior margin of the preoperculum. Females have a higher fluctuating asymmetry than in males. Females spawn and males protect the eggs from any predators. In this case, females are more susceptible to stress because they have to forage in order to compensate the energy used up during spawning. Males are less susceptible because they usually stay on the nesting ground, and don't have to cover far distances in finding food. This study validates the use of FA in determining the status of the fishes caught connecting to what the status of the environment is based on the result.

Keywords environmental condition; bio indicators; morphological variations.

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1 Introduction

The environment has been frequently disturbed due to anthropogenic activities caused by humans. Some of these factors include climate change which subjects the environment to extreme temperatures and there is also pollution. Many studies have concluded that pollution eventually weakens or destroys the normal ecosystem which supports human health, production of food and biodiversity (Tare and Bhojwani, 2012).

Fluctuating asymmetry refers to small abnormalities from a prior expectation of symmetric development in morphological traits (Swaddle, 2003) which will be measuring the difference between traits on the left- and right-hand side of a bilaterally symmetrical organism (Moller and Swaddle, 1997). It is a quantitative biomarker for it detect individual and population stress produced by physicochemical contaminants during the developmental process (Pankakoski et al., 1992) as well as natural disturbances that can lower the number of population of distinct fish (Badyaev et al., 2000). In the case of a polluted ecosystem, fishes were observed to have high fluctuating asymmetry that lives in stressful environments considering they have to compensate stress by requiring energy (Natividad et al., 2015).

Fish is one of widely used bio indicators known, they are valuable bio indicators because they are relatively easy to determine in their numbers, biological diversity and behaviors. Also, they are less sensitive than lower organisms to natural micro-environmental changes, which makes them suitable for the evaluation of macro-environmental changes (Gadzała-Kopciuch et al., 2004). Fish growth is considered as a biomarker for riverine pollution because it integrates all effects within the fish (Shakir and Qazi, 2013). Declining of the production of fish and dominance of physical deformities are the major signs of deteriorating genetic make-up (Natividad et al., 2015). *Glossogobius giuris* or the Tank goby is the main focus of this study as a bioindicator in the two areas. Since *G. giuris*is good sensitive indicator (Venkataraman et al., 2007), any morphological variation of its body will indicate a stress (Natividad et al., 2015). Tank goby is common and abundant in Lake Mainit (Tumandam et al., 2005).

Lake Mainit is the fourth largest lake in the Philippines which is geographically located in the Province of Surigao del Norte and Agusan del Norte on the Island of Mindanao. According to Mosende and Mozol (2011), Lake Mainit has been identified to have a rich biodiversity, particularly on aquatic resources. Threats and disturbances are also present in the lake. The mouth of the lake outlet is becoming shallow which prevents rapid exit of the water causing it to flood in the lower portions of the lakeshore. Another disturbance is the erosion from denuded mountainside where timber and mining companies operate. Mining waste, domestic sewage, fertilizer, and pesticides are also serious pollutants in the lake. Gobies found in lake Mainit had a size range of 57-242 mm, which is generally smaller now than in 1990's (de Guzman, 2008)

This paper aims to determine the morphological variations in the body shape between sexes of *Glossogobius giuris* found in Lake Mainit using fluctuating asymmetry. According to Unito-Ceniza (2012), it is important to study the morphological variations in the body shapes of fishes using fluctuating asymmetry to provide a better understanding of the genetic structure of fish species, it also be an awareness of the status of the environment, and provide scientific basis for formulating a comprehensive management plan for fisheries.

2 Materials and Methods

2.1 Study area

This study was conducted in Lake Mainit which lies between 9°27'07.45" N 125°30'39.79" in Surigao del Norte, Philippines. Fish collection was done using nets with small holes in the month of September, 2015.

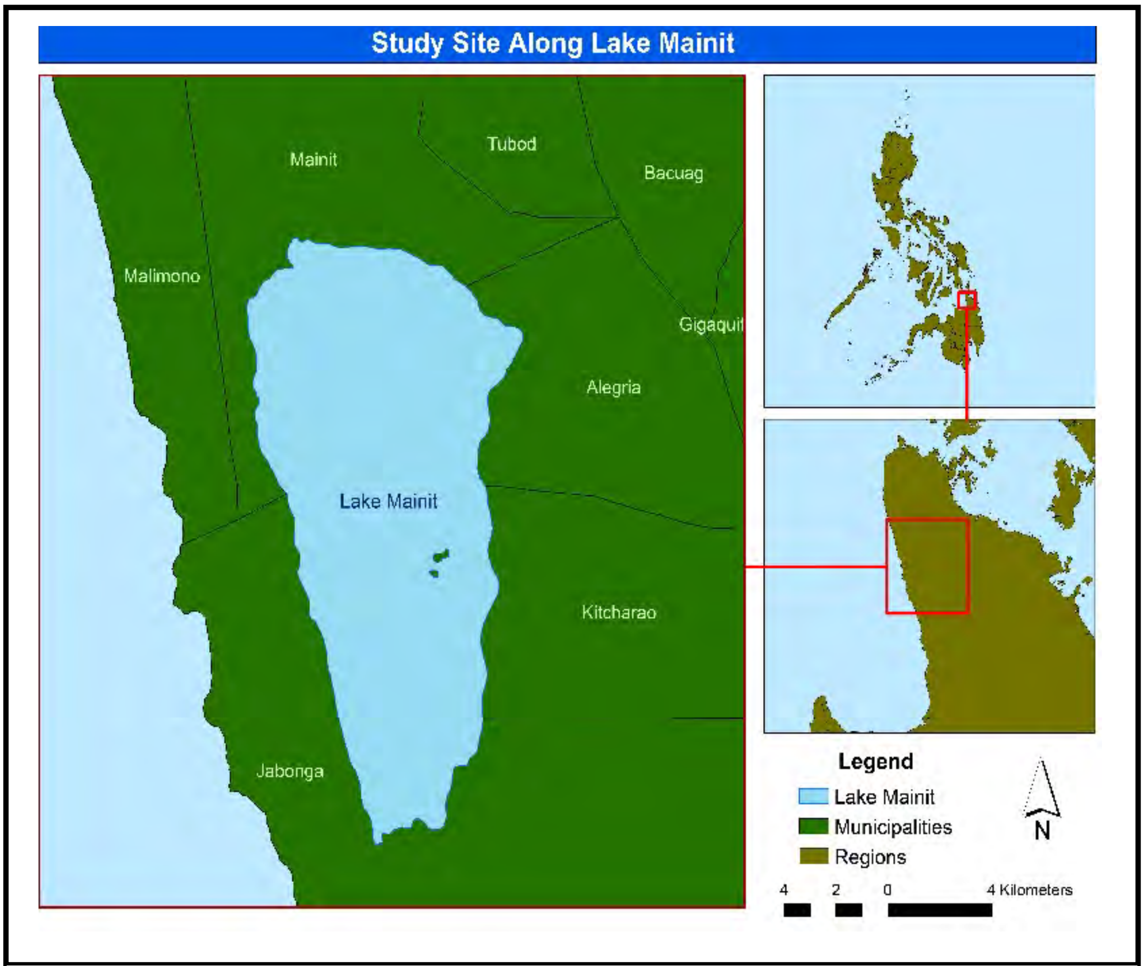

Fig. 1 Map showing Lake Mainit, Surigao del Norte, Philippines.

2.2 Fluctuating asymmetry of Tank Goby in Lake Mainit

2.2.1 Sample processing

Fish samples that used in this study were purchased from the local fisher folk in the area, prior to its species with the total of 200 individuals (100 male & 100 female). Samples were placed in styro box with ice water to maintain its freshness. The collected samples will then be process for image capturing and analysis.

The sample fish was placed in a flat styrofoam for the pinning of its fins to make it wider and to clearly see the samples point of origin for the land-marking process. 10% Formalin was applied in all the fins of the fish samples to make it hardened using a small brush. Digital image of the left and right lateral side of each sample was taken using Samsung camera (14 mega pixels).

Sex of the specimens were also determined. The sex was identified after careful examination of the specimen's genitalia. Females were easily identified by the presence of its eggs and ovaries, which is in general yellow or orange and granular in appearance. The males on the other hand, were determined based on the presence of their testes which were typically smooth, whitish and non-granular in appearance eggs (Requieron et al., 2010).Images were assorted according to sex and digitized using the tpsDig2 program (version 2.0, Rohlf, 2004) and were saved as TPS file.

2.2.2 Landmark selection and digitization

These images were tri-replicated for the purpose of determine digitizing error prior to asymmetry analysis. These images were then converted to TPS format using tpsUtil program (Fig. 2) and subjected to tpsDig2 program (version 2.0, Rohlf 2004) for the assignment of landmarks. The coordinates that were used to analyze the body shapes of the fishes using tpsdig2w32 were extracted from a total of twenty landmarks, the locations of which are shown in Table 1 and Fig. 3.

Table 1 Description of the landmark points according to Dorado et al. (2012).

COORDINATES	LOCATIONS
1	Snout tip
2	Posterior end of nuchal spine
3 & 4	Posterior & anterior insertion of 1st dorsal fin
5 & 6	Posterior & anterior insertion of 2nd dorsal fin
7 & 9	Dorsal and ventral insertion of caudal fin
8	Lateral line
10 & 11	Posterior & anterior insertion of anal fin
12	Insertion of the pelvic fin
13	Insertion of the operculum at the lateral profile
14	Posterior extremity of premaxillar
15	Anterior margin through midline of orbit
16	Posterior margin through midline of orbit
17	Superior margin of the preoperculum
18	Point of maximum extension of operculum on the lateral profile
19 & 20	Superior and inferior insertion of the pectoral fin

2.2.3 Shape analysis

The coordinates data taken from both sides of the bodies of the fishes was then subjected to Symmetry and Asymmetry in Geometric Data (SAGE) (version 1.04, Marquez, 2007) software to get the principal components which implies the deformation grid of the individual asymmetry (Natividad et al., 2015) and also the data of procrustes ANOVA was also be obtain for further data analysis. Procrustes ANOVA tests are performed to assess the significance of symmetry (= individual), directional asymmetry, and fluctuating asymmetry of shape and size, given samples with three replicates per specimens. Both object (bilaterally symmetric structures) and matching (bilaterally symmetric parts) types of symmetry are handled. Covariance matrix correlations are also computed between symmetric and asymmetric components of variation. SAGE allows saving symmetrized datasets.

2.2.4 Intraspecific variation between sexes

The comparisons between male and female sexes and individual symmetry were examined using the Paleontological Statistics (PAST) software (Hammer et al., 2001). Significant statistical representations such as box plots, histograms, and scattered plots were generated.

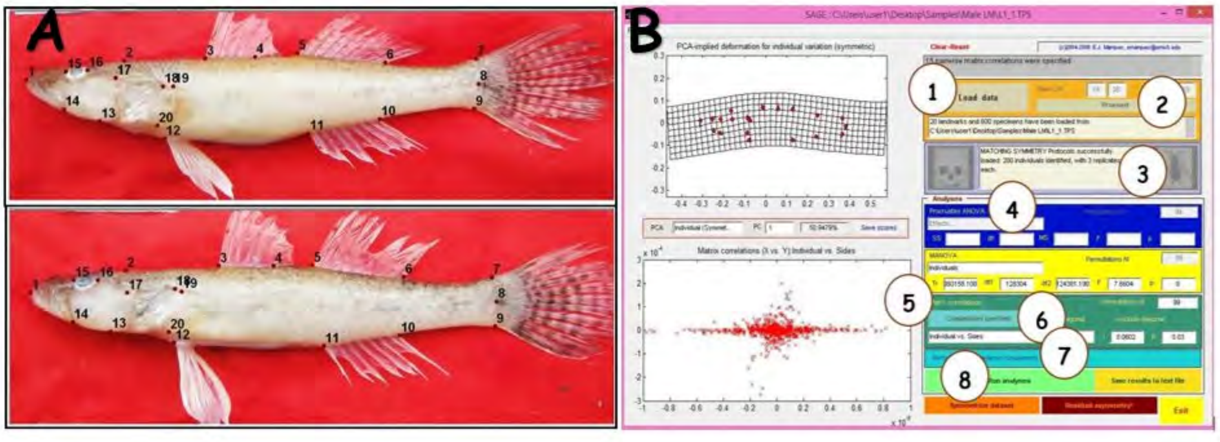

Fig. 2 (A) Landmark points of *G. giuris* samples and **(B)** overview of the schematic flow of shape analysis using SAGE Software.

3 Results and Discussion

Table 2shows the result obtained from Lake Mainit. The Procrustes ANOVA suggested an evidence for FA in one of the factors considered. Three factors were analyzed for FA and these were individuals, sides, and interaction of individuals and sides. The individual symmetry among the fish samples showed highly significant difference (P<0.0001). It was also observed to have a highly significant difference (P<0.0001) between sides and interaction of individuals and sides. The results implied that asymmetry is found in individuals and in sides (Left and right). All factors were observed to be asymmetric showing morphological changes that might be caused by high exposure of pollutants (Natividad et al., 2015). Reducing the survival rate over the population (Schwindt et al., 2014), and might affect the fishes' reproduction and their development (Bonada and Williams, 2002). The summary of Procustes ANOVA was shown in Table 2.

Table 2 Procrustes ANOVA for shape of *G. giuris* in terms of sexes in Lake Mainit.

EFFECT	SS	dF	MS	F	P-VALUE
			Female		
Individuals	0.5639	3564	0.0002	3.2735	0.0001**
Sides	0.0463	36	0.0013	26.6256	0.0001**
Individual x Sides	0.1722	3564	0	9.8775	0.0001**
Measurement Error	0.0705	14400	0	--	--
			Male		
Individuals	0.5954	3564	0.0002	3.3451	0.0001**
Sides	0.0518	36	0.0014	28.8028	0.0001**
Individual x Sides	0.178	3564	0	14.0884	0.0001**
Measurement Error	0.051	14400	0	--	--

**highly significant (P<0.0001)

A total of 69.2797%of FA interaction from upper 5% effective principal components (PC1-PC4) of *G. giuris*in female is measured. Table 3 shows the Principal components for the variation for male and female samples. According to the results in PC1, asymmetry can found greatest in the area covered by landmark: 1 (snout tip), 2 (posterior end of nuchal spine), 3 & 4 (posterior and anterior insertion of 1st dorsal fin), 5 (posterior insertion of 2nd dorsal fin), 7 & 9 (dorsal and anterior insertion of caudal fin), 8 (lateral line), 10 & 11 (posterior and anterior insertion of anal fin), 12 (insertion of pelvic fin), 13 (insertion of the operculum at the lateral profile), 14 (posterior extremity of premaxillar), 15 & 16 (posterior & anterior margin through midline of orbit), 18 (point of maximum extension of operculum on the lateral profile), 19&20 (superior and anterior insertion of pectoral fin). The only unaffected are 6 (anterior insertion of 2nd dorsal fin) & 17 (superior margin of preoperculum).

FA is also seen among males with the total of 63.9214% FA interaction which is which is lower than female, almost of the region are affected except 2 (posterior end of nuchal spine), 6 (anterior insertion of 2nd dorsal fin) and 17 which is the superior margin of the preoperculum which are not affected by any stress in the environment.

High level of FA in fish sample morphology could be a result of their adaptation to their stressful environment in order to survive. Since *G. giuris*is good sensitive indicator (Venkataraman et al., 2007), any morphological variation of its body will indicate a stress (Natividad et al., 2015).

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	52.7498%	100%	25.4076%	1,2,3,4,5,7,8,9,10,11,12,13,14,15,16,18,19,20
PC2	10.1919%		18.4708%	1,4,5,6,7,8,9,10,11,17,18,19,20
PC3	8.008%		14.2151%	1,2,3,4,5,7,8,9,12,14,15,16,20
PC4	5.967%		11.1862%	1,5
	76.9167%		69.2797%	
		Male		
PC1	50.9479%	100%	20.4974%	1,3,4,5,7,8,9,11,12,13,14,15,16,18,19,20
PC2	10.4175%		17.6469%	1,4,5,6,7,8,9,10,11,17,18,19,20
PC3	9.4775%		13.6001%	1,2,3,4,5,6,7,8,9,11,12,14,15,16,17,18,19,20
PC4	5.0209%		12.177%	1,2,3,4,7,8,9,11,12,18,19
	75.8638%		63.9214%	

Females have a higher fluctuating asymmetry than in males. Study shows that morphological variations on body shapes of fishes do adapt to its habit and habitat. Since Lake Mainit is an enclosed ecosystem, fish are isolated geographically that tend to have morphological variations to adapt by changing necessary morphometric. Female and male goby have different habits conforming to their positive adaptation (Unito-Ceniza, 2012). Females spawn and males protect the eggs from any predators. In this case, females are more susceptible to stress because they have to forage in order to compensate the energy used up during spawning. Males are less susceptible because they usually stay on the nesting ground, and don't have to cover far distances in finding food.

As observed, dorsal fins, caudal fin, anal fin, pelvic fin (pectoral fin), and parts of the head are affected. It might be affected because fins are used for swimming or locomotion, propelling, stability and protection from predators (Crenshaw, 2009). The mobility of the fish is considered as a factor in determining direct effect of stressor of the fish especially in the dorsal, caudal, anal and pelvic fin or pectoral fin (Natividad et al., 2015).

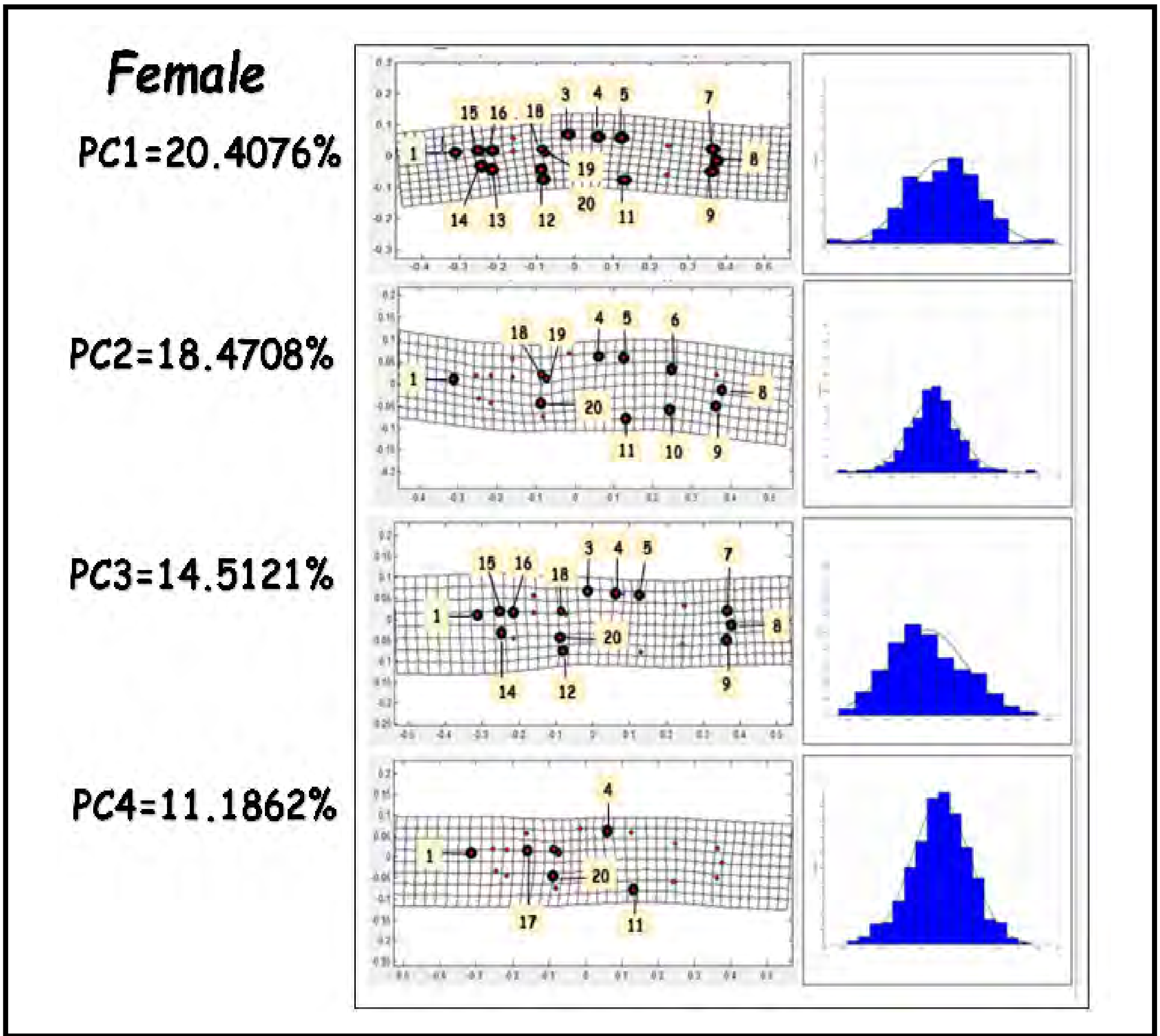

Fig. 3 Principal components (PC) inferred deformation grid and histogram of symmetric individuals in *G. giuris*(Female) displaying distribution of asymmetry. Percentages on the sides indicate quantities of variation for which each PC account.

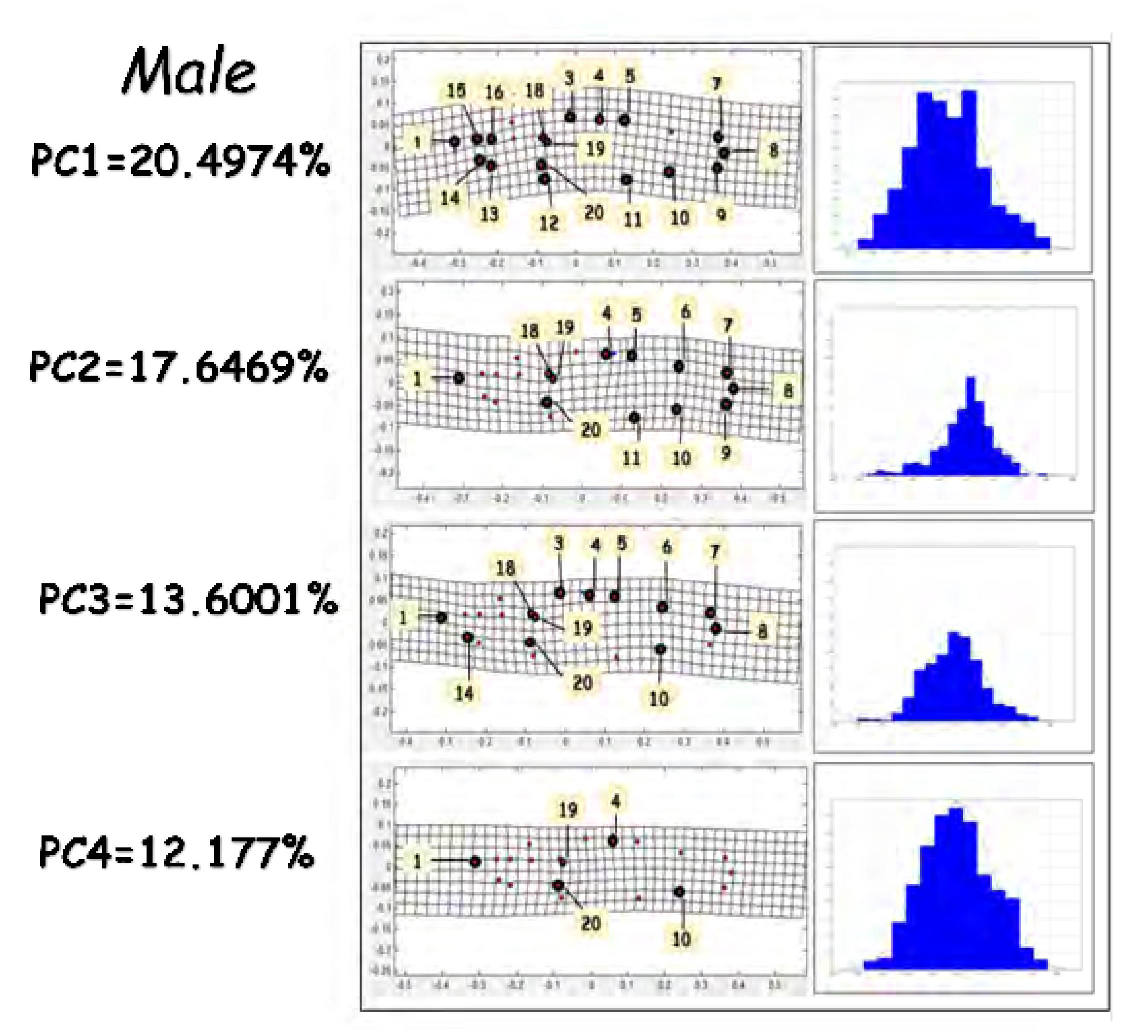

Fig. 4 Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *G. giuris* male species.

To visualize the actual affected landmarks, Fig. 3 and 4 shows the actual digitized image of the male and female fishes with the PCA deformation grid which displayed the asymmetrical shape of *G. giuris* species. Blue marks represented differences indicating fluctuation on the affected landmarks suggesting the evidence of FA. The identified landmark points affected by FA were shown together with the actual photograph of the fish sample (Fig. 5). The defined illustration was summarized using affected PC1 and PC2 landmarks because it was the two principal components to have the highest accounted variation.

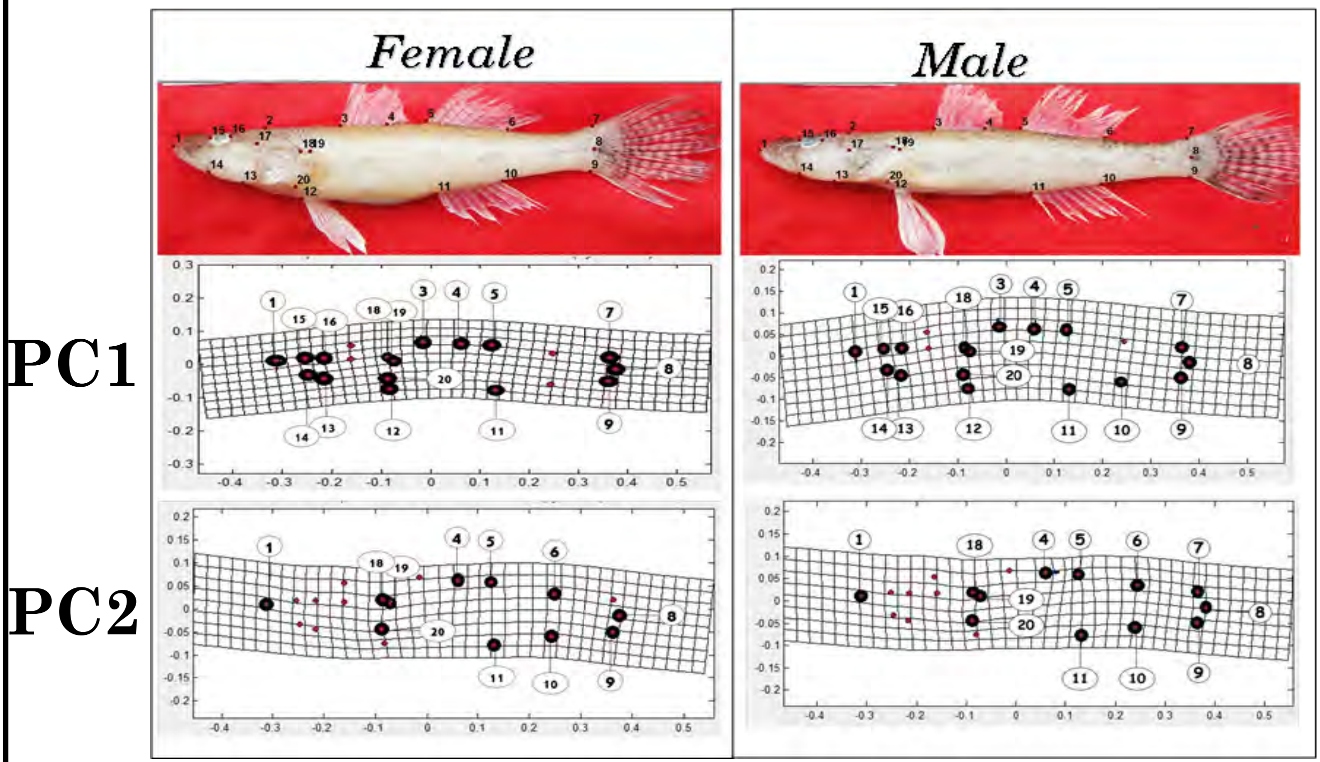

Fig. 5 Actualized picture of digitized female and male fish with the affected landmarks shown in PCA- deformation grid for PC1 and PC2

4 Conclusion

Fluctuating Asymmetry was used in this study to assess Lake Mainit, white goby or *Glossogobius giuris* was used as the sample medium because of its abundance in the said lake of. A total of 200 specimens was collected, 100 of female *G. giuris* and 100 of male. The results indicated evidence of FA among samples of *G. giuris* from Lake Mainit of both sex, Procrustes ANOVA showed a highly significant differences in the three factors (P<0.0001) which were individuals, sides, and interaction of individuals and sides. Principal components analysis was employed in determining the affected landmarks. Female has a higher affected landmarks with 69.2797%while male has 63.9214%. Affected landmarks due to FA were seen almost of its entire body. Since Lake Mainit is an enclosed ecosystem, introduced pollution can't be drained outside. The study demonstrated the use of FA as a tool in investigating the morphological asymmetry using metric traits of *G. giuris* samples from Lake Mainit. The results of the study will be important to the local government in the management of the said Lake.

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