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Fluctuating asymmetry as bioindicator of ecological condition in the body shapes of *Glossogobius celebius* from Lake Sebu, South Cotabato, Philippines

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Abstract. This study was conducted to assess the condition of Lake Sebu in South Cotabato by looking into the level of fluctuating asymmetry in populations of *Glossogobius celebius*. Several studies have shown that fluctuating asymmetry can be used to indirectly assess water quality of an ecosystem. In this study, *G. celebius* was used as a bio indicator because of its abundance in the lake. 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 although individual symmetry depicts no significant difference, Sides (Directional Asymmetry) and Interaction (Fluctuating asymmetry) showed a highly significant difference ($p < 0.0001$*). The results of Principal Component Scores display a high percentage fluctuating asymmetry of female (73.78%) and male (79.13%). In female, Principal Component (PC) 1 (26.53%) and PC 2 (20.67) and interaction were found to have significant variations affecting the *G. celebius* snout tip, insertion of the anterior dorsal fin, curvature of peduncle, anal fin (including posterior), operculum and pectoral fin. In male, PC 1 (29.18%) and PC 2 (23.29%) affected the snout tip, posterior body extremity and anal fin, beginning of the lateral line and superior and inferior insertion of pectoral fin and margin of the preoperculum. This study validated the use of fluctuating asymmetry in determining the populations of the *G. celebius* status of Lake Sebu was possible that it might indicate the condition of the aquatic ecosystem.
Key Words: geomorphometrics, Goby fishes, biomarker, environmental condition.

Introduction. Fluctuating asymmetry refers to these directionally random, subtle departures from perfect symmetry and is hypothesized to indicate the inability of an organism to maintain precise development (Palmer & Strobeck 1986). Fluctuating asymmetry is an essential morphometrics tool in terms to small, random deviation from the ideal morphology of organism due to its capability of giving absolute difference between the left and right hand side of a bilaterally symmetrical organism (Moller & Swaddle 1997; Palmer & Strobeck 2003a). To measure the difference of the right and left hand side of bilaterally symmetrical organism using fluctuating asymmetry, there are three methods for measuring the dispersion: classic linear measurement using variance, landmark methods, and continuous symmetry measure. The results in fluctuating asymmetry measurement may indicate the condition of an individual organism.

The condition of the individual organism within the population directly represents the ecological status of the entire population especially when homozygosity inbreeding between affected species occurs. Even unaffected species can be affected due to the disturbances in the environment by means of the pollutants especially during embryonic stage. This stage will express noticeable morphological changes if species is susceptible to high exposure to pollutants. The introduction of the pollutants to the individual organism shows direct relationship which represents the level of pollutants where the

organism lives. When there is high level of pollutants, the individual organism will portray skewed parts which is distinct from its symmetrical form. These skewed parts are the primary bases for the measurement of its fluctuating asymmetry in which in this study, G. celebius was used as the test organism served as the water body's bio indicator.

Gobies were used as indicators of water quality due to its diverse population across the country which carries about 16 species known to occur in the Philippines only (Hoese & Allen 2009; Lekshmi et al 2010). The species also are less studied, the last reference of the *G. celebius* study and its life cycle was conducted by Manacop (1953) along the Cagayan de Oro River. *G. celebius* is effectively used as an indicator of developmental stability and measure of ecological stress (Parsons, 1961, 1962, 1990, 1992; Van Valen 1962; Palmer & Strobeck 1986; Leary & Allendorf 1989; Markow 1995; Clarke & McKenzie 1992; Imasheva et al 1997; Roy & Stanton 1999; Kark et al 2001; Mpho et al 2002; Velickovic 2004). A review of Leung & Forbes (1996) states that fluctuating asymmetry variance of populations and an absolute fluctuating asymmetry value of individuals has been found to increase with stress. Support information also suggested that increased asymmetry reflects an increased inability to cope with stressful situations (Palmer & Strobeck 1986; Pomiankowsky 1997). Stress may also reduce the energy available to maintain developmental precision (Sommer 1996). Exposure to environmental contaminants is a global problem, and fish populations offer appropriate models for examining the effects of contamination (Jenner et al 1990) on the ecosystem. This amount of stressors affects the degree of fluctuations of the biological indicators used such as *G. celebius* which predefines the ecological health of the species habitat. In this study, Lake Sebu was the primary concern due to the massive fish kills which locals calls as "kamahong". This occurrence takes place when there is an extreme increase of water temperature leading to oxygen depletion in the waters of the lake due to overpopulated fish stock.

With Lake Sebu occupying 354 hectares which is fragmented by means of huge fish cages made by the fish cage operators, it is visible that the area is disturbed which has probably caused stress on the *G. celebius* species. To monitor the stress condition of the fishes, it is essential to use bio indicators for the assessment of the environment. In the case of polluted ecosystem, fishes were observed to have high fluctuating symmetry in residing to stressful environments considering they have to compensate stress by requiring energy. And this amount of fluctuating asymmetry was significantly linked to the ecological condition of the *G. celebius* habitat.

Fluctuating asymmetry was used in this study to assess the condition of Lake Sebu through the use of *G. celebius* as the bioindicator through assessing its level of asymmetry. This is to show how the exposure of *G. celebius* polluted the water body especially when the practice of overstocking and over feeding the fishes from the several fish cages are present in the area aside from municipal effluents causing degradation of the lake. Hence, the results of the fluctuating asymmetry of *G. celebius* can determine if the species has undergone environmental in Lake Sebu considering the lake system is one of the most productive ecosystems in South Cotabato as it covers good coverage of abundant fishing.

Material and Method

Study area. Lake Sebu is located at South Cotabato and it lies between 06°13′N and 124°42′E. Mapping was obtained through Global Positioning System (GPS) by the use of an online mapping (http://maps.google.com) shown in Figure 1. Sampling was carried out from November 28-30, 2014.

Figure 1. Map of the Philippines (left), map showing Mindanao area pointing South Cotabato (right above), map showing Lake Sebu in South Cotabato area (right below).

Fluctuating asymmetry of G. celebius in Lake Sebu

Sample processing. Thirty male and thirty female individuals of *G. celebius* were collected. *G. celebius* were preserved using 90% ethanol and were placed in a Styrofoam box. Digital imaging was done using Olympus digicam (SP-800uz, 14 megapixels). It was noted that both the left and right lateral side of each sample were taken with a ruler parallel to it for the length determinations of each *G. celebius* fishes. The captured images were digitized using tpsDig2 program (version 2.0, Rohlf 2004) and were saved as a TPS file. After taking the digital images of the fish samples, species name were identified based on www.fishbase.org by searching the common name and searching the similar description of the species morphology. Male and female sexes were identified based on their external morphology and later confirmed by direct examination of the gonads. A male fish has whitish soft textured gonads while female *G. celebius* fish has yellowish coarsely textured gonads with eggs (Requieron et al 2010).

Landmark selection and digitization. Using thin-plate spline (TPS) series, landmark analyses were obtained in order to incorporate curving features within the images. Both evolutionary and functional significance were obtained through the standard forms of the digitized landmarks used in fish morphometric. Landmarks were selected to provide homogenous outline of body shape as shown in Figure 2 using software tpsDig2. A total of 20 landmarks (equivalent to 20 X and 20 Y Cartesian coordinates) were identified in order to best represent the external shape of the body. Landmark description was shown in Table 1. X and Y coordinates of landmarks on images were then obtained for further analysis. To lessen the inconsistencies and/or errors in plotting the landmark points, digitization were copied in triplicates for each fish samples.

Table 1

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

No.	*Description*
1	snout tip
2 and 3	anterior and posterior insertion of the dorsal fin
4 and 6	points of maximum curvature of the peduncle
5	posterior body extremity
7 and 8	posterior and anterior insertion of the anal fin
9	insertion of the pelvic fin
10	insertion of the operculum at the lateral profile
11	posterior extremity of premaxillar
12	centre of the eye
13	superior insertion operculum
14	beginning of the lateral line
15	point of maximum extension of operculum on the lateral profile
16 and 17	superior and inferior insertion of the pectoral fin
18 and 19	superior and inferior margin of the eye
20	superior margin of the preoperculum

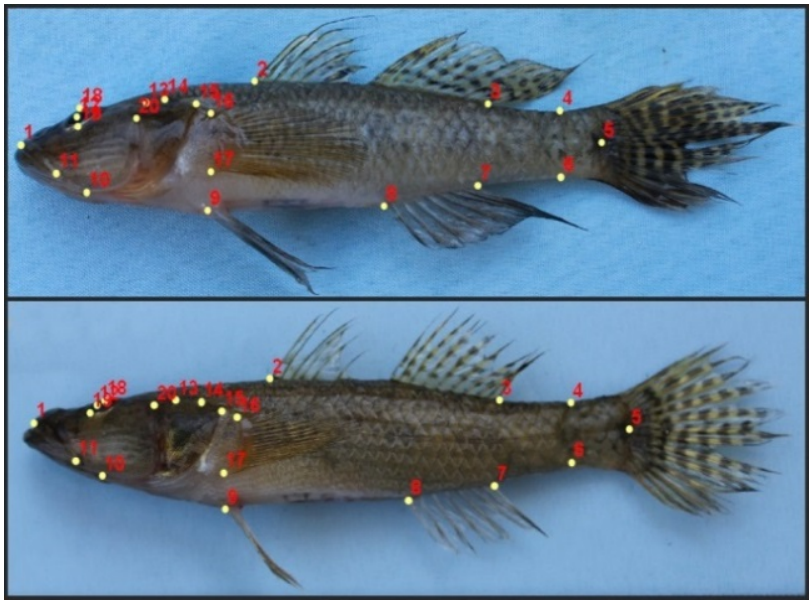

Figure 2. Landmark point of the female (upper) and male (lower) of *G. celebius.*

Shape analysis. Generated x and y coordinates had served as baseline data in analyzing fluctuating asymmetry of *G. celebius*. Left and right flat form landmark coordinates of the TPS version was subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software (version 1.04, Marquez 2007) to identify geometric data of object with emphasis on its asymmetry. Sage generates symmetrized data sets, residuals from symmetric components, in addition to shape configuration of each component of variation (symmetric, asymmetric, and error) as well as expected covariance matrices. Procrustes ANOVA were employed with triplicates and with 99 permutations to calculate and quantify the residual asymmetry. This indicates the variation between sides and is the measure of directional asymmetry. Percentage (%)fluctuating asymmetry were obtained and compared between the sexes.

Intraspecific variation between sexes. By using Paleontological Statistics (PAST) software (Hammer et al 2001), comparisons between sexes and individual symmetry were analyzed by generating relevant statistical representations such as histogram, box plot and scattered plot.

Results and Discussion. Individual body shape fluctuations through Procrustes ANOVA were shown. Individual symmetry of L-R size and shape in Table 2 were depicted to have no significance statistically within two sexes. However, variations in left and right sides of the sexes were depicted to have high significance (< 0.0001**) on the side scores indicating the occurrence of fluctuating asymmetry in the body shapes of the *G. celebius*. This indicates that the differences in the shapes between the left and right sides of the fishes can be attributed to perturbations in the environment resulting from poor water quality. Stressed environment according to Barrett (2005) translates to the inability of the species to develop phenotypically to a desired path as these stressors affects the individual species, in which this study used *G. celebius* as the test organism, during their development (Bonada & Williams 2002). In the study of Van Valen (1962), these asymmetries developed errors in the developmental processes because of the species' inability to thrive and buffer environmental disturbances. Developmental instability of the *G. celebius* correlates to the species' fluctuating asymmetries.

Table 2

Procrustes ANOVA for shape of *G. celebius* fish in terms of sexes

Effect	*Sum of squares*	*Degrees of freedom*	*Mean of squares*	*F value*	*P-value*
		Female			
Individuals	0.0813	1044	0.0001	0.9707	0.6843 ^{ns}
Sides	0.0691	36	0.0019	22.9425	< 0.0001**
Individual x sides	0.0837	1044	0.0001	9.6619	< 0.0001**
Measurement error	0.0358	4320	0	--	--
		Male			
Individuals	0.0996	1044	0.001	1.0363	0.2823 ^{ns}
Sides	0.0814	36	0.0023	24.5638	< 0.0001**
Individual x sides	0.962	1044	0.0001	8.9039	< 0.0001**
Measurement error	0.0447	4320	0	--	--

** highly significant ($p < 0.005$), ^{ns} not significant.

A total of 79.1259% of fluctuating asymmetry interaction from upper 5% effective principal components (PC1-PC5) of *G. celebius* in male is measured. Table 3 shows the Principal Components for the variation for male and female samples. According to the results in PC 1, asymmetry can found greatest in the area covered by landmark: 1 (snout tip), 2 (anterior and posterior insertion of the dorsal fin), 4 (points of maximum curvature of the peduncle), 7 and 8 (posterior and anterior insertion of the anal fin), 10 (insertion of the operculum at the lateral profile), 11 (posterior extremity of premaxillar) and 17 (superior and inferior insertion of the pectoral fin); PC 2 on the landmarks 1, 10, 11 and the lateral profile. This result best indicates the bilateral asymmetry of the males. Fluctuating asymmetry can also be seen among females with a total of 73.7794% fluctuating asymmetry interaction that shows movement of landmarks on the different regions depicted on the following landmarks: PC 1 on 1 (snout tip), 5 (posterior body extremity), 14, 15, 16 (lateral profile). These landmark areas cover a different set of landmarks than that of females. The values of fluctuating asymmetry and individual symmetry did not vary significantly because of the very small percentage difference.

Adaptive mechanism of the fish in order to compensate with the stressful environment probably result to significant levels of fluctuation in its morphology. Fish mobility is considered an advantage for determining direct impact of stressors of the fish specifically in the dorsal cephalic region and in the pectoral fin. According to Parsons (1990) an increase in fluctuating asymmetry reflects poorer developmental homeostasis in the molecular, chromosomal and epigenetic levels in impaired environmental conditions. Moreover it has been known that water quality affects or greatly influence developmental growth of fishes (Schlosser 1991).

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		
PC 1	26.0318%	100%	26.5261	1,2,4,7,8,10,11,15,17
PC 2	21.9442%		20.6678	1,10,11,14,15,20
PC 3	13.8197%		13.0208%	1,8,9,13,16
PC 4	7.5711%		8.3029%	8,15,16,17
PC 5	6.6842%		5.2618%	8,16
	76.051%		73.7794%	
		Male		
PC 1	33.6543%	100%	29.177%	1,5,14,15,16
PC 2	18.6333%		23.2935%	1,2,5,8,17
PC 3	11.0866%		14.3618'%	10,11,17
PC 4	8.546%		6.4093%	17
PC 5	7.8451%		5.8825%	1
	79.7653%		79.1259%	

Figure 3 and 4 shows the distribution of asymmetrical shape of male and female *G. celebius*. The number of symmetric individuals is shown as the bar graph emanating from point 0. Variations are represented by the blue marks signifying the movement of the affected landmarks. Adjacent is the histogram to visualize the central location, spread, and shape of the individual symmetry. PC 1 and PC 2 in male have an individual (symmetry) scores that were skewed to the left which signify effect on its asymmetry. Whereas in the female *G. celebius,* PC 1 is skewed to the right implying maximum individual symmetry and depicted minimal asymmetry and PC 2 shows to have no significant variations. Defined illustration in Figure 5 shows the affected PC 1 and PC 2 landmarks of both sexes showing the actual photograph of *G. celebius*.

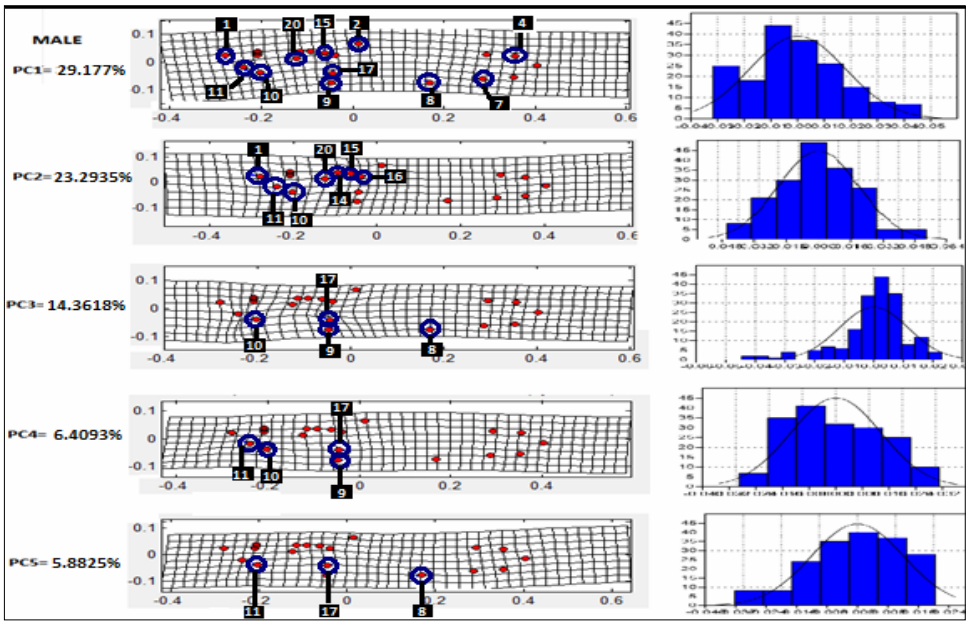

Figure 3. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *G. celebius* males showing distribution of assymetry. The percentages indicate the proportions of variation for which the respective principal components account.

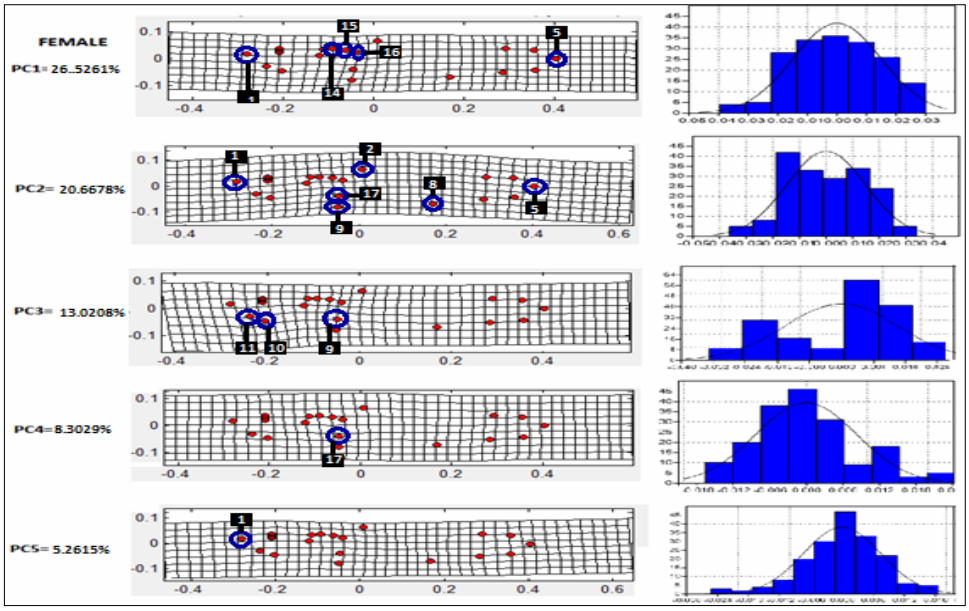

Figure 4. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *G. celebius* females.

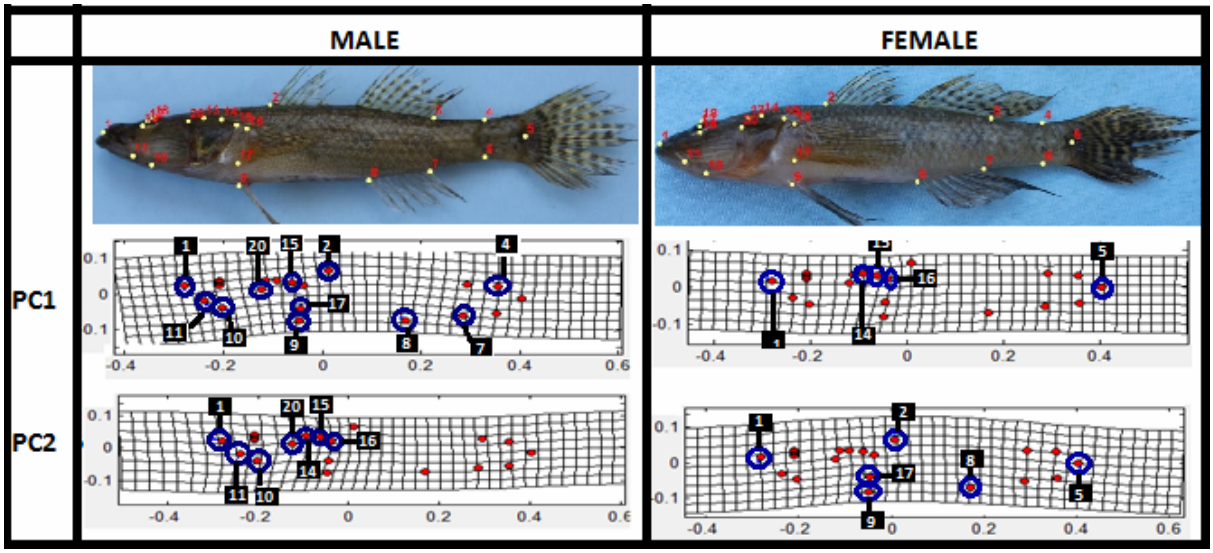

Figure 5. Actualized picture of digitized male and female fish with the affected landmarks shown in PCA- deformation grid for PC 1 and PC 2.

This high level of fluctuating asymmetry proposes a disturbed environment in the areas of Lake Sebu with the overcrowded fish stock is beyond the carrying capacity of the lake that has caused fish kill in the last three years. Similar study from Ducos (2015) with *Gafrarium tumidum* as a bioindicator had validated that the higher the F value the greater the stress an organism experiences because of the ecological stress. Other studies imply that presence of parasite affects fluctuating asymmetry in host species having implication on species fitness and adaptation (Pojas 2015). This developmental instability and asymmetry developed by an organism were carried out by the unfavourable condition of the organism's ecosystem. It causes the organisms to have poor developmental hemeostasis and have an inability to buffer its developmental pathways against any environmentally derived and genetic stressors as exposed to stressful environments thus, affecting developmental stability. This study provides information on the condition of Lake Sebu's aquatic condition and proved FA as an effective indicator of ecological stress and environmental instability.

Conclusions. The statistical results showed high variations ($p < 0.005$) on the left and right sides of the bilateral with a percentage of 79.1259% in male and 73.7794% in female indicating high fluctuating asymmetry. Results also showed higher asymmetry in female *G. celebius* than male. This study validates the use of fluctuating asymmetry in determining the status of the ecological health of Lake Sebu confirming that indeed results of high fluctuation equates for the incidence of fish kills in the area.

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