Investigating the fluctuating asymmetry in the metric characteristics of tilapia *Oreochromis niloticus* sampled from Cabadbaran River, Cabadbaran City, Agusan del Norte, Philippines

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Abstract. This study was conducted to assess the status of *Oreochromis niloticus* in Cabadbaran River, Agusan del Norte by assessing the level of fluctuating asymmetry in the population of the species. Fluctuating asymmetry (FA) measurements are very useful to determine the status of *O. niloticus.* The mentioned species is a hermaphrodite that contains both sexes. A total of 76 individuals were collected. By means of thin-plate spline (TPS) series, landmark analyzes were acquired and subjected to symmetry and asymmetry in geometric data (SAGE) software. The symmetry and asymmetry scores were used in ordination of data through deformation grids and histogram. Outcomes in Procrustes ANOVA indicated that although individual symmetry shows a high significant difference ($P<0.0001$), sides (directional asymmetry) and interaction (fluctuating asymmetry) displayed no significant change. A total of 75.1259% of cumulative variation in the effective principal components (PC1-PC5) were considered in the analysis. Affected landmarks due to FA were largely from parts of the head and the ventral fins. The study validates the use of FA in determining the status of the fishes caught in Cabadbaran River equating to what the status of the environment is based on the results. The results of the study will be essential to the local government in the management of the river system.

Key Words: morphological asymmetry, geometric morphometry, anthropogenic activities, environmental stress, developmental flux.

Introduction. Numerous freshwater fishes serve as biological indicators and may function as a potential organism in describing environmental condition. The ability of the species to fend off in the environmental settings has an effect to their morphological array. Thus, determining the morphological asymmetry was the key interest of this study. The different species of fish in freshwater systems have evolved over millions of years and have modified to their favored environments over extensive periods of time (Wurts 1998). Since fishes play a vital role in the condition of aquatic ecosystems, it is equally important to determine their condition on the selected site. Fish development could be affected by direct or indirect exposure to aquatic pollution (Kime 1995). The effects of these pollutants would be detrimental in the fish physiological and morphological conditions. Over the years, scientific approach contributes explanation on how the species evolved over time. Considering the morphological features, it influences how species grown and developed relative to its environment. One of the accepted scientific approaches to correlate environmental condition to the species morphology is the use of geometric morphometrics (GM). It is the quantitative representation and analysis of morphological shape using geometric coordinates instead of measurements (David Polly 2012). The foremost goal of GM is to measure morphological similarity and difference (David Polly 2012). The relationship between the morphology, development, and phylogeny in organisms has been a long-standing biological interest. It acquired a definite quantitative approach as it was revived by the work of other scientists since its introduction by Von Baer (Gould 1977, 1981; Huxley 1932).

The phenotypic aspects of species generally vary in terms of morphology that includes size and shape. These two elements were biologically important in determining morphological array. Environmental conditions such as anthropogenic activities, agricultural runoff and disposing of large quantities of waste pose detrimental to environment specifically aquatic system (Natividad et al 2015). Over the years, aggregate pollution of aquatic environments has become a major concern (Dikshith et al 1990). These were direct factors affecting the morphological feature of some aquatic species. One aspect in geometric morphometry that can identify deviation in morphological features is the fluctuating asymmetry (FA). It refers to all deviations from a prior expectation of symmetric development in morphological traits (Ludwig 1932). These morphological asymmetries were assumed to result from imperfect development. Moreover, they were perceived to reflect the inability of the genome to buffer developmental processes against intrinsic, random noise (Ludwig 1932; Waddington 1957; Zakharov 1992). It identifies the causative influence of environmental stress and provides advantages over other manifestations of stress since FA is practical and easy to measure (Clarke 1993). Furthermore, FA has long been regarded as indicative tool in determining the quality and condition of organisms. Therefore a change in FA should be biologically relevant and an important application (Sommer 1996).

Freshwater systems in southern Philippines especially the Cabadbaran river was poorly studied. There are no studies yet that assess its water quality which would help us determine its present condition.

The commonly caught fish in the river is *Oreochromis niloticus* known as tilapia. The fish has economic significance in the area because it is consumed and sold in the marketplaces by the local communities. The fish tilapia was known to be capable of tolerating varying environmental conditions (Chervinski 1982). However, agricultural runoffs and other pollutants from the river can also add up to the stresses that freshwater organisms might experience (Velichović 2004; Mpho et al 2000).

It is therefore the interest of the study to investigate the FA in the metric characteristics of tilapia, *O. niloticus*. It is the first attempt to determine the manifestation of stress and developmental flux of the species exposed in Cabadbaran river conditions using FA. This study is important and relevant as rapid developments in the area may further aggravate the conditions of the river.

Material and Methods

Study area. Cabadbaran City is located at Agusan del Norte (Figure 1C). It geographically lies between 9°07’20.52”N and 125°32′02.20″E. Online mapping was used to obtain area mapping through Global Positioning System shown in Figure 1. Sampling was conducted from July to August, 2015.

Sample processing. There were 76 samples of *O. niloticus* collected in the sampling area. Digital imaging was done using Olympus digital camera (Canon, 10 megapixels). Both the left and right lateral side of each sample was photographed with a ruler parallel to the length of each individual. Blue background was used during imaging to show well-defined body morphology. Captured images were digitized using the tpsDig2 software (version 2.0, Rohlf 2004) and saved as a TPS file.

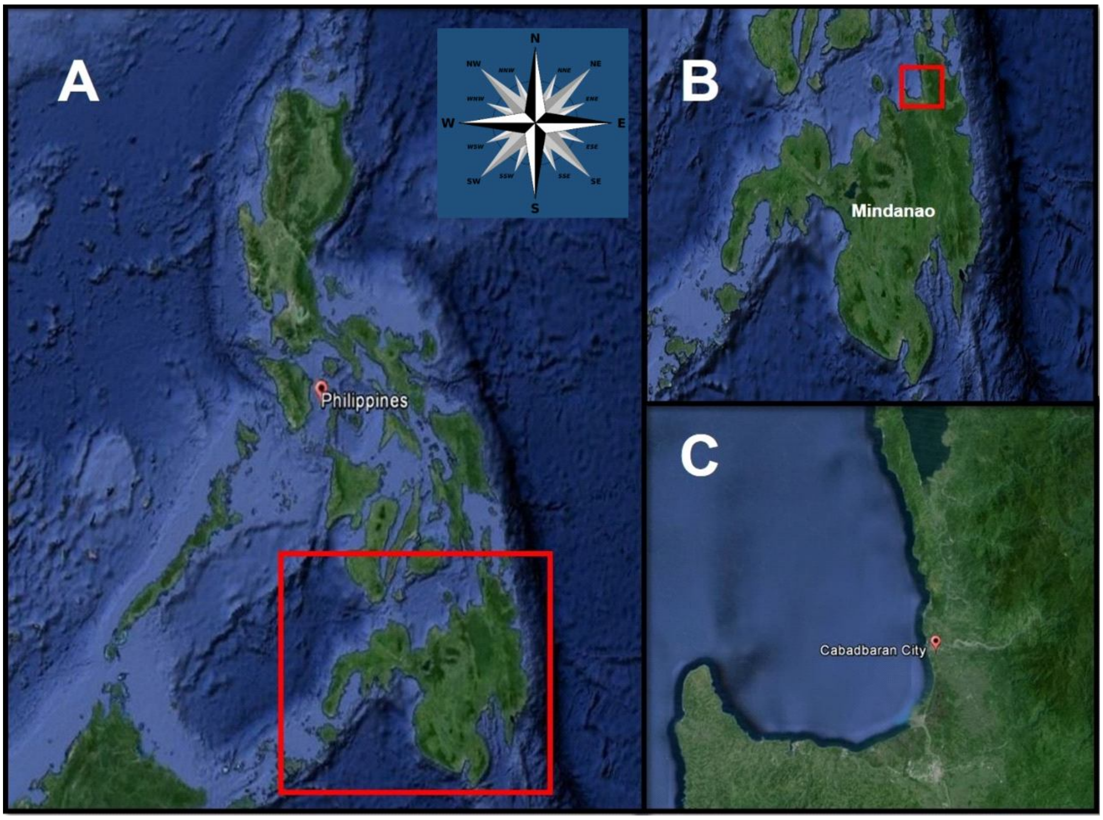

Figure 1. Location of the sampling area showing the map of the Philippines (A), Mindanao island (B), and Cabadbaran City in Agusan del Norte (C).

Landmark selection and digitization. Thin-Plate Spline (TPS) software was used for the analysis of landmarks and integrated the warped features of the images from both sides. Evolutionary and functional significance were attained using the standard forms of the digitized landmarks used in fish morphometric. Landmarks were designated to provide standardized outline of body morphology (Figure 2) using the tpsDig2 software.

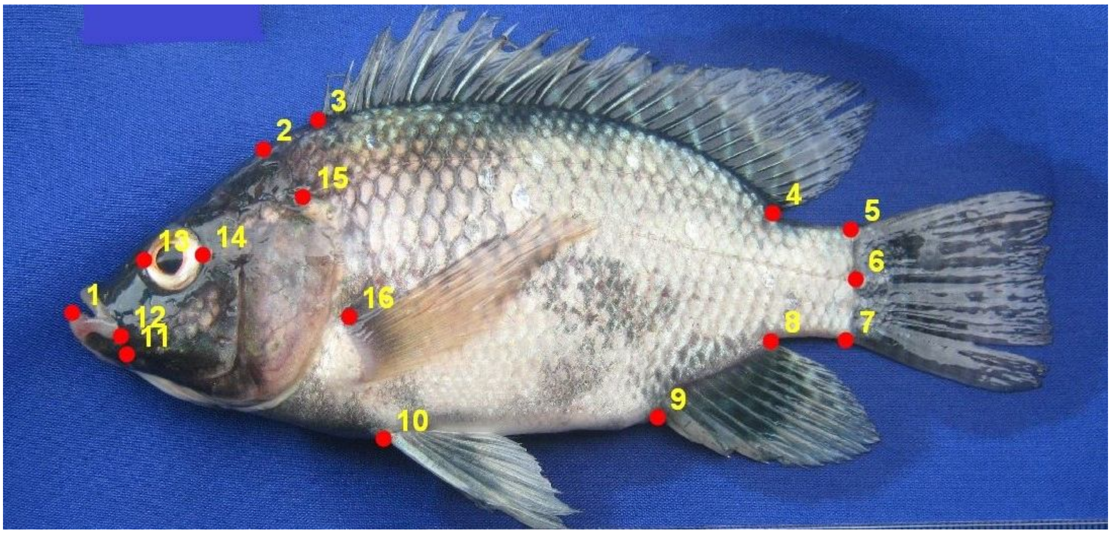

Figure 2. The designated landmarks of *Oreochromis niloticus.*

To signify the external shape of the body, 16 landmarks were identified (Table 1). Further analysis was applied on the X and Y coordinates of the landmarks. Fish samples were copied in triplicates upon digitization to lessen the inaccuracies in plotting landmark points.

Table 1

Description of the landmark (Paña et al 2015)

No.	Description
1	Snout tip
2	Posterior end of nuchal spine
3 & 4	Anterior and posterior insertion of the dorsal fin
5 & 7	Points of maximum curvature of the peduncle
6	Lateral line
8 & 9	Posterior and anterior insertion of the 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

Shape analysis. Standard data used in analyzing FA of freshwater fishes was obtained from the X and Y coordinates generated from tpsDig2 (Figure 3).

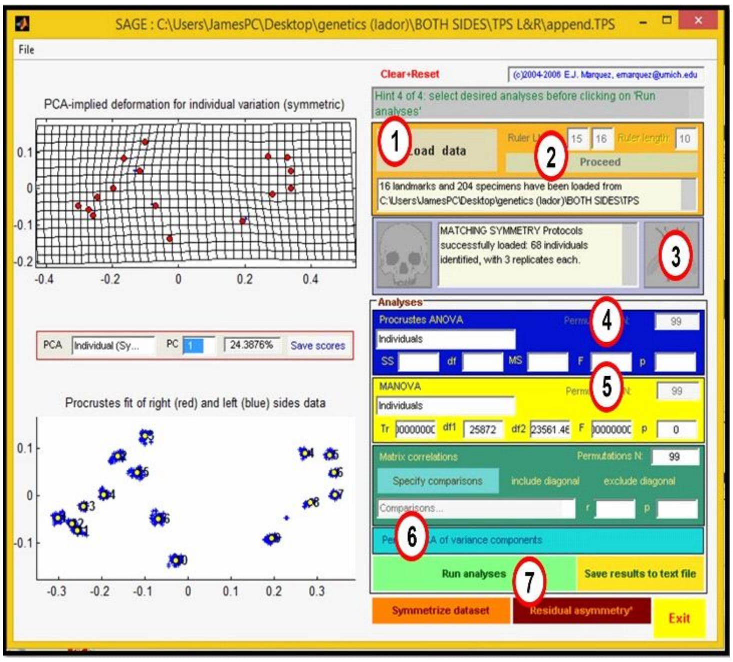

Figure 3. Overview and the schematic flow of shape analysis using SAGE software.

Digitized images from left and right sides of the TPS version was subjected to symmetry and asymmetry in geometric data (SAGE) software (version 1.04, Marquez 2007). Geometric data of objects with highlights on its asymmetry were identified. Symmetrized data sets, residuals from symmetric components, as well as estimated covariance matrices were also obtained from SAGE. Procrustes ANOVA was employed in triplicates with 99 permutations to analyze the residual asymmetry and percentage of FA. Results from the Procrustes ANOVA indicated the variations to the measured factors. It was considered as the measure of directional asymmetry.

Results and Discussion. 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 interaction of individuals and sides symmetry among the fish samples showed highly significant difference ($P<0.0001$). However, no significant differences were observed to individuals and sides. The results implied asymmetry on the interaction of individuals and sides on each sample. However, the individuals were symmetrical along with the left and right sides of each sample. The indicated FA among the interaction of individual and sides of *O. niloticus* was a possible effect of stressed environment (Barrett 2005). It eventually affects the fishes reproduction and development (Bonada & Williams 2002). The species inability to thrive and buffer environmental disturbances is the cause of these asymmetries (Van Valen 1962). The summary of Procrustes ANOVA is shown in Table 2.

Table 2

Procrustes ANOVA for Shape of *Oreochromis niloticus*

Effect	*SS*	*dF*	*MS*	*F*	*P-value*
Individuals	0.1962	2380	0.0001	0.7568	1
Sides	0.001	28	0	0.3419	1
Individual x Sides	0.2592	2380	0.0001	25.1742	0.0001**
Measurement error	0.0417	9632	0	-	-

** ($P<0.0001$) highly significant.

Principal component analysis was employed to determine the affected landmarks. A total of 79.2764% of cumulative variation was accounted from upper 5% effective principal components (PC1-PC5) of *O. niloticus* (Table 3).

Table 3

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

PCA	*Individual (Symmetry)*	*Sides (Directional asymmetry)*	*Interaction (Fluctuating asymmetry)*	*Affected landmarks*
PC1	23.4473%		26.3758%	1,3,9,10,11,12,14,15,16
PC2	21.7017%		20.9898%	1,2,3,4,5,6,7,9,10,11,12,14,16
PC3	13.4939%	100%	14.8479%	1,2,3,9,10,11,12,15,16
PC4	9.9071%		9.6784%	2,5,7,9,10
PC5	6.6214%		7.3845%	4,5,8

According to the results in PC 1, asymmetry can found greatest in the area covered by landmark: 1 (snout tip), 3, 9, 10, 11, 12, 14, 15, and 16. PC2 on 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 14 and 16. The affected landmarks were generally located in parts of the head region and ventral fins. The results specified FA in *O. niloticus*. Figure 4 showed the actual digitized image of the male and female fishes with the PCA deformation grid to visualize the actual affected landmarks.

The ordination of data in the deformation grid showed the distribution of asymmetrical shape of *O. niloticus* fishes. Symmetric individuals were depicted as bar graph originating. Blue marks represented differences indicating fluctuation on the affected landmarks. The resulting histogram revealed the spread, central location, and shape of the individual symmetry. Skewed histogram indicated evidence of asymmetry.

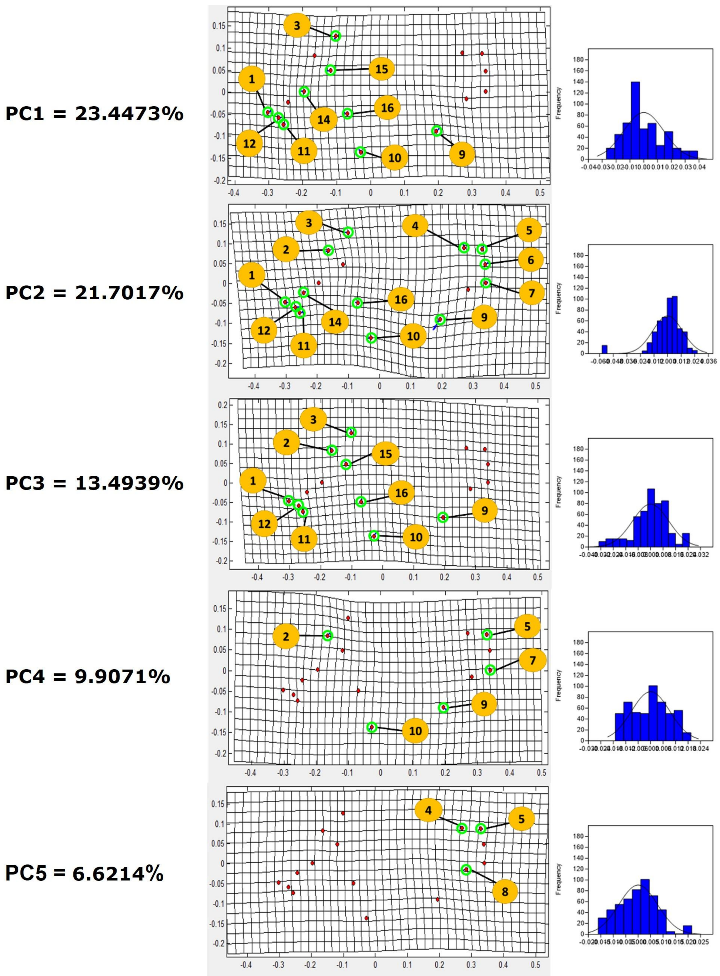

Figure 4. Percentage variation of accounted PC was used to depict the deformation grid and histogram.

The identified landmark points affected by FA were shown together with the actual photograph of the fish sample (Figure 5). The defined illustration was summarized using affected PC1 and PC2 landmarks because the two principal components were the highest accounted variation.

A recent study on *Glossogobius celebius* from Lake Sebu South Cotabato showed parallel results, which only means that the inconsistencies in the right and left sides of individuals can be attributed to stressful environments (Natividad et al 2015). Significant levels of FA in fish sample morphology could be the result of their adaptation to the stressful environment in order to survive. From the study of Chervinski (1982), *O. niloticus* is known to tolerate a wide range of environmental variation, which implies that it can survive in stressful environments. The widespread distribution of the species was only possible because of its highly adaptive mechanisms. Despite the efficiency of the species, FA was still detected under its condition in Cabadbaran River. The possible exposure to pollutants in the sampling area might be a factor to the asymmetry. But there are no studies yet that might provide adequate information as to what is the present condition of the Cabadbaran River. This study would encourage future studies on the water quality of the Cabadbaran River. Water quality largely influences the development of fishes (Schlosser 1991). An increase in FA reflected poorer developmental homeostasis in the molecular, chromosomal and epigenetic levels in impaired environmental conditions (Parsons 1990). The effect eventually manifested in the resulting FA and the asymmetry was largely affected the dorsal-cephalic region and the pectoral fin of the individual fish samples.

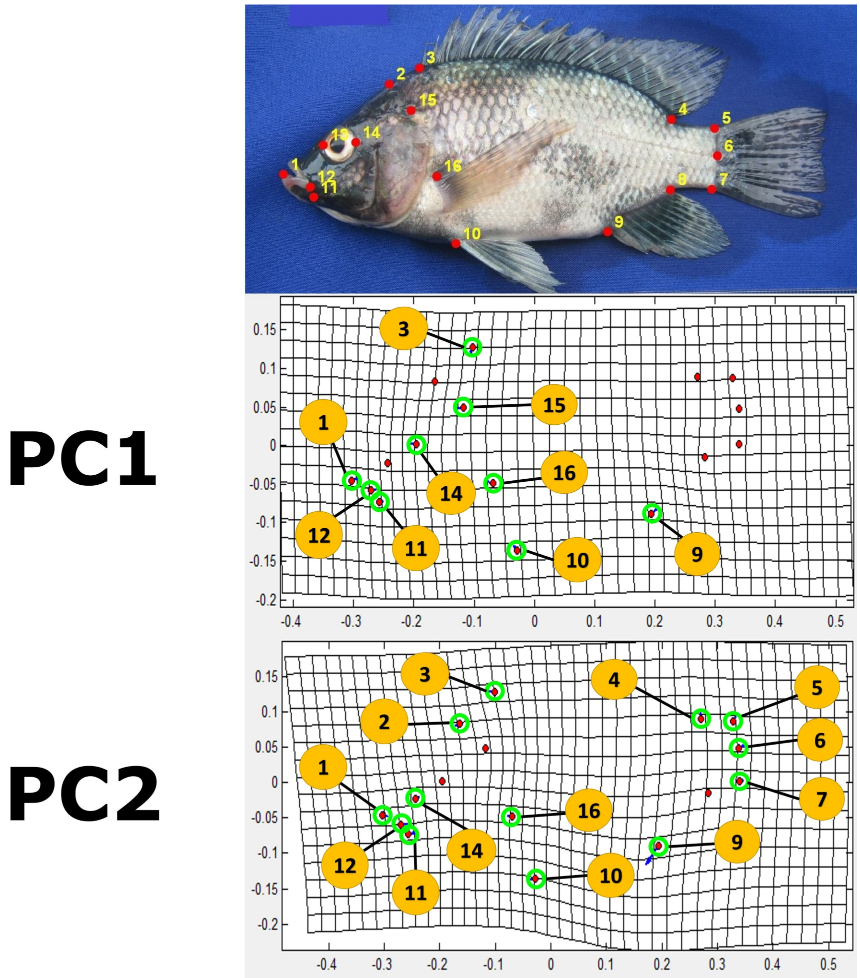

Figure 5. Data ordination of digitized fish with the affected landmarks shown in PCA-deformation grid for PC1 and PC2.

Conclusions. The results indicated evidence of FA among samples of *O. niloticus*. There was a highly significant difference in the interaction of individuals and sides of the species using Procrustes ANOVA (P<0.0001). Principal component analysis was employed in determining the affected landmarks. A total of 79.2764% of cumulative variation in the effective principal components (PC1-PC5) considered in the analysis. Affected landmarks due to FA were largely from parts of the head and the ventral fins. The symmetry and asymmetry scores were used in ordination of data through deformation grids and histogram. The resulting FA of *O. niloticus* sampled from Cabadbaran River could be due to instability of the water condition. The presence of pollutants in the river might have caused morphological asymmetry as the fish buffer the effects of pollution. Tilapia (*O. niloticus*) was known to thrive and tolerated wide range of aquatic conditions (Chervinski 1982). However, FA was determined in the results of analyzed data. The study demonstrated the use of FA as a tool in investigating the morphological asymmetry using metric traits in *O. niloticus* sampled from Cabadbaran River. The results of the study will be important to the local government in the management of the river system and for future studies in the area.

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