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Using Geometric Morphometrics to study the population structure of the silver perch, *Leiopotherapon plumbeus* from Lake Sebu, South Cotabato, Philippines

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Abstract. Fluctuating asymmetry refers to the particular form of organism biological asymmetry, characterized by small random deviations from perfect symmetry; thought to reflect an organisms ability to cope with genetic and environmental stress. It has been used as a measurement of developmental stability of individuals to withstand environmental and genetic perturbations to produce the phenotype. This study was conducted to determine the levels of fluctuating asymmetry (FA) in the bodies of the fish *Leiopotherapon plumbeus* collected from Lake Sebu in South Cotabato, Philippines. The method of Geometric Morphometrics (GM) was used in the study and analyses using Procrustes ANOVA was utilized in the test for FA. A total of 16 landmarks were used to extract biological shape information from 120 fish samples. Results showed immense intra-population diversity in the shapes of the fish bodies. More importantly, GM and the Procrustes ANOVA revealed significant FA in both sexes of the fishes especially in female *L. plumbeus*. The meaning of these asymmetry and how it pertains to the life history is discussed in this paper.

Key Words: fluctuating asymmetry, environmental stress, *Leiopotherapon plumbeus*, Lake Sebu.

Introduction. There are innumerable ways of determining the health of populations of organisms. Some of these methods include assessing population dynamics, genetic composition, energy acquisition, storage and use, presence of contaminants and diseases, and population structure (Pope et al 2010). These methods are useful especially in assessing fish stock health which is important in predicting problems requiring management action. For fishes, assessing the population structure involves collecting information on the mean body length, proportional size distribution and length at age using traditional morphometry. Aside from these size measure, data on mean age, year classes per sample, juvenile:adult ratio, sex ratio, age at maturity, and weight at maturity are also collected to give a more comprehensive picture of the population structure of the fishes (Pope et al 2010).

In this study however, asymmetries in three bilateral traits are used to determine the population structure of the fish *Leiopotherapon plumbeus* (Kner, 1864) from Lake Sebu, South Cotabato, Philippines. In collecting asymmetry data, the landmark-based method of geometric morphometrics (GM) was used instead of the traditional linear morphometric data. It is widely thought that GM can extract a more comprehensive picture of the anatomical typology of the fishes as it can collect not only information on size but the shape of the fishes as well.

A number of studies have established the utility of fluctuating asymmetry (FA) in determining the population structures of fishes. The rationale for using FA goes back to the presupposition that under "normal" conditions, an organism that is bilaterally

symmetrical will develop left and right sides that mirror each other. In other words, the left and right parts should be identical halves. This happens since the production of bilateral traits is genetically regulated, hence should develop identical structures (Klingenberg 2003; Clarke 1995). However, this symmetry is broken in the presence of stressors during the development of the organism. Studies showed that stress is a disturbing source that is capable to induce qualitative and quantitative changes in the symmetry of organs or bodies with bilateral structure (Daloso 2014). Consequently, deviation from symmetry has been proposed as a quantitative biomarker to predict the presence of individual and population stress produced by a number of factors. These stressors include physico-chemical contaminants (Pankakoski et al 1992; Gileva & Nokhrin 2001; Nunes et al 2001; Velickovic 2007; Sanchez-Chardi et al 2008), anthropogenic and natural disturbances (Badyaev et al 2000; Sanchez-Chardi et al 2008), such as availability of parasites (Pojas & Tabugo 2015) and other endogenous factors such as inbreeding depression that affect the health of the gene pool (Sheridan & Pomiankowski 1997; Waldmann 2001).

The use of GM in determining trait FA has also been established in a number of studies (Klingenberg et al 1998; Savriama et al 2012; Hermita et al 2013). This method analyzes shape differences of biological structures using Cartesian or landmark coordinates (representing the X and Y coordinates in a 2-dimensional system; and X,Y and z in a 3-dimensional system). Extracting shape information from these coordinates require processing of data into Procrustes residuals after subjecting the raw X and Y coordinates into a generalized least squares fitting analysis. The resulting Procrustes values are residuals after size and symmetry components of shape variation have been systematically removed from the original data coordinate data set.

There are actually a number of GM tools which can be used for FA studies. These include outline- and landmark-based analysis. In this study, the method of Procrustes-fitting and the landmark-based Relative Warp Analysis was used. Furthermore, data was subjected to symmetry analysis using an algorithm in the Symetry and Asymmetry in Geometric Morphometric Data (SAGE version 1.04, Marquez 2007) software.

This study therefore aimed at using the methods of landmark-based GM to assess the level of asymmetry in three bilateral traits in the fish *L. plumbeus* from Lake Sebu, South Cotabato. This fish, which is locally widespread in the area, is a candidate biological indicator species because of its sheer number. In doing so, it is hoped that the results of this study will find its utility in pro-active management actions towards the protecting Lake Sebu.

Material and Method

Study area. The study was conducted during last week of Januay 2015 and the fish samples were collected from Lake Sebu (613'0"N - 12442'0"E) which is situated in one of the municipalities of the province of South Cotabato, Philippines (Figure 1). At present, the local government of the municipality of Lake Sebu has adopted several measures to protect and conserve the lake which is known to be rich in biodiversity. However, the presence of fish ponds where feeds are dispersed everyday and waste water coming from the communities along the lake are generally believed to affect the health of the lake.

Collection and preparation of fish samples. Local fishermen from the area were made to collect the specimens used in this study. The collected fishes that were brought to the landing sites and were immediately processed for image capture and analysis. Digital image of the left and right lateral side of each sample were taken using an Olympus digital camera (SP-800uz, 14 megapixels) (Figure 2). These images were then converted to TPS format using tpsDig2 program (version 2.0, Rohlf 2004). After thorough evaluation of the fish samples and careful scrutiny of the quality of the images produced, only a total of 360 images from 60 females and 60 males were used in this study. These images were then tri-replicated for the purpose of determine digitizing error prior to asymmetry analysis.



Figure 1. A) Map of the Philippines showing Map of Mindanao, (B) Map of South Cotabato showing Map of Lake Sebu.





Figure 2. Left male (A1), right male (A2), left female (B1), right female (B2) images of Silver perch (*Leiopotherapon plumbeus*).

The sex of the specimens was also determined. The sexes were identified after careful examination of the specimen's genitalia. Females were readily identified by the presence of eggs and ovaries. The fishes ovaries appeared tubular in shape and pink, yellow or orange in color (Cailliet et al 1986). 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.

Manual assignment of landmarks and shape analyses. The coordinates that were used to analyze the body shapes of the fishes were extracted from a total of sixteen landmarks, the locations of which are shown in Figure 3. The coordinates were manually collected using the tpsDig2 software.



Figure 3. Landmarks used for digitizing image of freshwater fish species: (1) rostral tip of premaxilla, (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 opercle, (16) dorsal base of pectoral fin. Base figure from Chakraborty et al (2008).

The coordinates data taken from both sides of the bodies of the fishes were then subjected to two way mixed model ANOVA with side (left and right) as the fixed factor, and individual and repeated measurements as other factors. This model allows for measurement of FA while controlling for measurement error. Geometric configurations were obtained from the digitized landmarks generated by the image analysis and processing software. All asymmetry analysis was conducted using the software SAGE (version 1.04, Marquez 2007).

Results and Discussion. The results of the analysis on the patterns of asymmetry in the shapes of the bodies of the fish *L. plumbeus* from Lake Sebu are shown in Table 1. The values in the table reveal high inter-individual variation in body shapes among the specimens in both sexes. This clearly shows immense phenotypic variation among the individuals collected. Moreover, the table also showed high levels of FA. This asymmetry cannot be attributed to digitization, hence can only be attributed to the sheer difference between the shapes of the left and right sides of the bodies of the fishes.

According to Table 2, asymmetry are said to be localized to certain parts of the body. For example, nearly 40% of the observed asymmetries can be attributed to more than 9 landmarks, most important of which are located in the mouthparts of the fishes. Does this asymmetry in the feeding apparatuses of the fishes mean anything? Does it have any functional significance? This is yet to be explained through further analysis of the microhabitats characterizing Lake Sebu.

To further support the first analysis, based on the Table 2, PCA was performed to visualize shape variation in the bilateral characteristics and also used to investigate in patterns of covariation in the positions of landmarks (Dryden & Mardia 1998). PCA shows that about 14% of the asymmetrical variation in both sexes can be seen in the anterior region of the head part of the fishes. Although, there seems to be differences in the number of affected landmarks, asymmetry is at least commonly observed in a total of five landmarks. Moreover, more than 11% of the total asymmetrical variation can be seen round landmarks 1 (rostral tip of premaxilla) and 8 (posterior insertion of anal fin). In addition, the results revealed that there is a total of 80.98% FA interaction from the upper 5% of PCA for female while 78.68% in male *L. plumbeus*. A possible explanation

for these high levels of FA arises from the differences in genetic composition of the populations resulting in different tolerance to stress (Ducos & Tabugo 2014). The significantly increased levels of FA in a population especially in female *L. plumbeus* may indicate that individuals had high difficulty in maintaining precise development resulting in negative effects on the population over time (Markow 1995). On the otherhand, a high rate in FA in male *L. plumbeus* affects its sexual selection. Which is broadly represented by aggressive competition between males for access to females or their ova (male–male competition/sperm competition) and by choice by females among potential mates (Tomkins & Kotiaho 2001). This means that the male *L. plumbeus* reduced its fitness and vectorial capacity (Galbo & Tabugo 2014). They are also subjected to survival, because female are very choosy in terms of the quality of their mates, this may indicate that only symmetrical males can have sexual union with them. As a result, individuals with higher developmental instability had reduced survival (Hendrickx et al 2003; Lens et al 2002).

Table 1

Effect	Sum of	Degrees of	Mean of	F	<i>P-</i>			
	squares	freedom	squares	value	value			
Male								
Individual (shape/symmetry	0.1693	812	0.0002	2.369	< 0.8752 ^{ns}			
variation)								
Sides (directional asymmetry)	0.0345	28	0.0012	14.017	< 0.0001**			
Individual x sides (fluctuating	0.0714	812	0.0001	8.3914	< 0.0001**			
asymmetry)								
Measurement error	0.0352	3360	0					
Female								
Individual (shape/symmetry	0.01797	812	0.0002	2.9053	< 0.5383 ^{ns}			
variation)								
Sides (directional asymmetry)	0.0329	28	0.0012	15.4249	< 0.0001**			
Individual x sides (fluctuating	0.0618	812	0.0001	3.8301	< 0.0001**			
asymmetry)								
Measurement error	0.0668	3360	0					

Procustes ANOVA analysis of L. plumbeus

** significant levels of fluctuating asymmetry in both sexes of *L. plumbeus* (p < 0.005), ^{ns} not significant.

Table 2

Percentage contribution of the first five important principal components

	PCA	% Interaction	Affected landmarks		
Male					
	1	39.85	1**, 2**, 5, 6**, 8, 9**, 10**, 12**, 14**, 15**, 16**		
	2	14.74	1, 2**, 3**, 8**, 9**, 15, 16**		
	3	11.86	1**, 2, 3, 4, 8**, 10, 15		
	4	7.33	10, 15		
	5	4.9	3, 10, 11		
Total		78.68			
Female					
	1	38.22	1**, 2, 3, 4, 5, 6**, 7, 9**, 10**, 11, 12**, 14**, 15**, 16**		
	2	13.51	2**, 3**, 5, 8**, 9**, 11, 14, 16**		
	3	11.77	1**, 8**, 11		
	4	11.02	4, 5, 6, 7		
	5	6.4	2, 15, 16		
Total		80.92			

** affected landmarks that are shared between sexes of L. plumbeus; PCA - Principal Component Analysis.

The Thin-Plate Spline deformation grids that follows are graphical presentations of the asymmetries that were described in Tables 1 and 2 (Figures 4 and 5). The graphs show

the locations where asymmetry is greatest. Figure 6 shows that the black circles from some of the red landmarks are the highest affected landmarks in both sexes of the fish and observed that there were differences in the shape of both female and male *L. plumbeus* landmarks.



Figure 4. PCA implied deformation of individual x side interaction (FA) in male L. plumbeus.



Figure 5. PCA implied deformation of individual x side interaction (FA) in female L. plumbeus.





Figure 6. The highest affected landmarks in male and female *L. plumbeus.*

Conclusions. This study revealed enormous inter-individual variation in the shapes of the bodies of *L. plumbeus*. More importantly, this study also points to a high level of FA in this stock of fishes.

Analysis of the data also revealed a non-equitable distribution of asymmetry among the 16 landmarks. Some of the landmarks were shown to exhibit more FA than others. For example, most of the parts associated with the feeding apparatus in the head of the fishers were shown to manifest highest FA. The connection between trait FA in this part of the head and some factors such as availability of food and resources can thus be a good study in the future.

In general, this study showed the practical importance of GM in measuring between-sides variation in fishes. Thus, GM can be useful in determining fish stock population structure and helpful in identifying management actions in fisheries.

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