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Fluctuating asymmetry of guppy, *Poecilia reticulata* (Peters, 1860) as a stress indicator in Lake Sebu, South Cotabato, Philippines

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Abstract. Guppies (*Poecilia reticulata*) were reported as indicators of waters in poor conditions, representing one of the most significant models in evolutionary ecology due to the species' quick response to natural selection. The inability of these species to develop perfect symmetry can be associated to the disturbances in their environment. Determining the fluctuating asymmetry (FA) as a measure of the species developmental instability of *P. reticulata* found in Lake Sebu, South Cotabato, Philippines is an effective tool in finding out the ecological health and to fully understand the shape variability of guppies found in the area. Landmark-based geometric morphometrics was used in the study to analyze the biological shape information of the samples. Procrustes ANOVA was employed for the assessment of FA. Statistical analyses showed that there is a significant FA on both sexes of the fishes ($p<0.05$) while individual symmetry showed otherwise. Results revealed that female guppy showed higher value in FA (83.69%) compared to male's (81.63%). The results taken reflect a high FA in the body shapes of *P. reticulata*, thus, imply a high level of stress in the water systems in Lake Sebu. This study proves the effectiveness of FA studies to indicate perturbations in the water environment using guppies as stress indicators.
Key Words: symmetry, ecological health, Poeciliid, landmark-based geometric morphometrics.

Introduction. In the studies made in relation with fish population distribution in deteriorated water systems, guppies *Poecilia reticulate* (Peters, 1860) were reported as indicators of waters in poor conditions (Araújo et al 2009). That is why they are considered to be an interest of study, representing one of the most significant model in evolutionary ecology due to the species' quick response to natural selection (Reznick & Bryga 1987; Reznick et al 1990; Reznick et al 1997; Reznick et al 2001; Magurran 2005). Taking also into consideration that guppies are found incredibly in wide array of habitats, they also encompass an extensive range of groupings to adapt comparative level of predation (Haskins et al 1961).

Fishes are good indicators of long-term effects and broad habitat conditions since they are relatively long-lived and mobile such that it is an advantage to use them over other organisms present in rivers and lakes (Karr et al 1986). Moreover, fishes are relatively easy to collect and identify at the species level. Locally, fishes in Lake Sebu, South Cotabato, Philippines presents a good study since the species are exposed to pollution-stressed environments due to the influence of overstocking and overfeeding of fishes from the several fish cages present within area (Sorenson et al 2013). Determining the condition of the waters in Lake Sebu can be done via calculation of the *P. reticulata* fluctuating asymmetry (FA).

FA is an organism's deviation from the bilateral symmetry. It is the degree with which the average individual species departs from its perfect symmetry (Astauroff 1930). FA results from the incapability of species to undergo identical development from its

bilateral trait between both sides of the body represented by the difference between the right and the left side measurements (Van Valen 1962). Furthermore, individuals who have the inability to maintain homeostasis throughout their development, when exposed to either genetic or environmental stress, would directly reflect upon their FA (Møller & Swaddle 1997).

Knowingly, fishes, which are bilaterally symmetrical, are supposed to develop sides that mirror each other. Since the development of bilateral symmetry is genetically controlled, fishes should form alike structures in both left and right sides. However, this symmetry is interrupted due to many stressors present in the environment of the species during its development stages. Subsequently, aberrations in the symmetry of the body shapes of fishes are propositioned as bioindicator to foresee the presence of individual and population stress and disturbances by several factors. Common factors such as anthropogenic and natural disturbances (Badyaev et al 2000), acidification, toxic chemicals or heavy metals are common stressors which produce elevated levels of FA (Allenbach et al 1999; Franco et al 2002; Estes et al 2006).

Literature on FA especially on fish species is very diverse. However, very few published articles about guppies particularly on morphology-related topics are available for reference. Due to the lack of valid sources, it would result to non-uniform measurements and methods which can result to the formulation of inconsistent and uncertain data.

The study is highly significant in order to fully understand the shape variability of guppies found in Lake Sebu, South Cotabato, Philippines focusing on FA as a measure of species developmental instability due to genetic or environmental stress. Asymmetry-related studies especially on fish species serves as a biologically relevant indicator to determine epigenetic measure between stressed and unstressed populations. Furthermore, efforts in utilizing fluctuating asymmetry of fish populations would serve as an inexpensive yet accurate tool in measuring the ecological health of the species' habitat with which proves to be vital in the estimation and management of both the ecosystem and human health. This will serve as baseline data for local management and legislators to do drastic measures to save and protect river ecosystems in the area before stress irreversibly damages populations (Clarke 1995; Bunn 1995).

The province was chosen to be the sampling site for the conduct of research since the area is surrounded with vast bodies of water having pollution stress environments exposed to human activity, thereby providing excellent data for comparative assessments. This study aims to investigate the FA of *P. reticulata* thereby providing beneficial data for early-warning mechanisms for population and environmental monitoring that would help in the conservation of both the species and the overall health of its habitat in Lake Sebu, South Cotabato.

Material and Method

Study area. The municipality of Lake Sebu, which is situated (6°13′0″N - 124°42′0″E), is a part of the South Cotabato Province located in its southwestern part (Figure 1). The health of the lake is believed to be affected by the presence of fish cages and fish ponds where overfeeding and overstocking of the fishes is employed by the local fish breeders. The wastes coming from the communities along the lake also contribute to the declining water quality of the lake. Lake Sebu is considered as threatened in terms of its water quality since overfishing stress and moderate pollution is occurring in the vicinity.

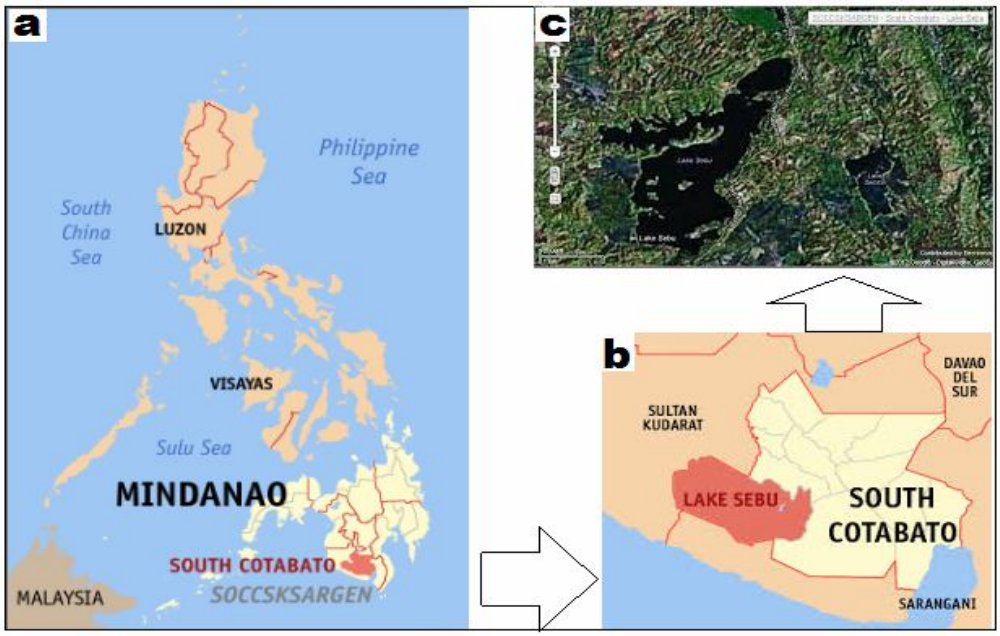

Figure 1. Map of the Philippines (a) showing South Cotabato located in Mindanao; (b) enhanced map showing Lake Sebu in South Cotabato; (c) aerial view of Lake Sebu (www.google.com).

Fluctuating Asymmetry of the guppy

Digitization of the fish specimen. Digital images of both the left and right lateral side of each sample was taken using Olympus digicam (SP-800uz, 14 megapixels). Digital images captured will then be converted to TPS format files using tpsDig2 program (version 2.0, Rohlf 2004). Samples was then preserved in 90% ethanol.

Identification and separation by sex. After capturing digital images of freshwater fishes, sexes were determined. Fishes was identified based on www.fishbase.com. Males and females were identified based on external morphology and later confirmed by direct examination of the gonads. Male fishes had whitish soft textured gonads while yellowish coarsely textured gonads with eggs for female specimens (Requieron et al 2010).

Landmarking. Landmark analyses were obtained using Thin-Plate Spline (TPS) series in order to incorporate curving features within the images. Landmarks were selected to provide homogenous outline of body shape using software tpsDig2. A total of 14 landmarks were identified in order to best represent the external shape of the body. X and Y coordinates of landmarks on images was obtained for further analysis (Figure 2).

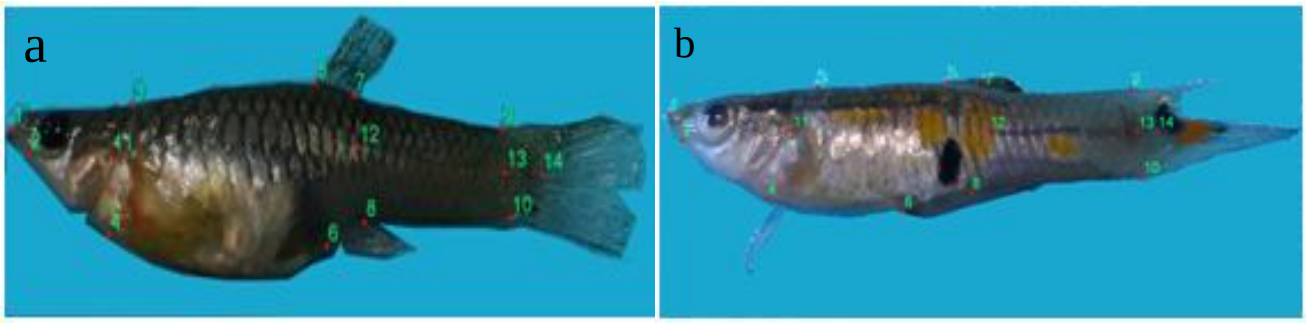

Figure 2. Landmarks' description of the guppy, *Poecilia reticulata* (a. female, b. male): (1) snout tip; (2) posterior extremity of premaxillar; (3) dorsal occiput; (4) insertion of operculum on the lateral profile; (5) and (7) posterior and anterior insertion of the dorsal fin; (6) and (8) anterior and posterior insertion of the anal fin; (9) and (10) dorsal and ventral insertion of the caudal fin; (11) point of maximum extension of operculum on the lateral profile; (12) and (13) anterior and posterior region of the lateral line from the middle part of the body to the caudal peduncle; (14) posterior most body extremity.

Data analyses

Fluctuating Asymmetry analysis. Generated x and y coordinates serve as baseline data in analyzing FA of freshwater fishes. Left and right flat form landmark coordinates is loaded to software Sage (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.

Statistical tools have been developed that effectively allow the separation of measurement error from the analysis of left-right asymmetry. One procedure uses two-way mixed analysis of variance (ANOVA) models, which determine whether fluctuating asymmetry is significantly different from measurement error, and whether the asymmetry distribution has a mean of zero, with "individual" scores as the fixed factor, and "side" and the "individual x side" interaction scores as the random factors (Palmer & Strobeck 1986; Palmer 1994; Swaddle et al 1994; Merilla & Bjorklund 1995) is used in determining the differences in developmental instability of *P. reticulata.*

Procustes ANOVA is employed and calculated. Percentage (%) FA was obtained and compared between the two sexes.

Intraspecific and interspecific variation. Comparison of FA variability between sexes, and individual bilateral symmetries among individuals was analyzed using paleontological statistics (PAST) software (Hammer et al 2001).

Results and Discussion

Using Procrutes ANOVA, individual body shape fluctuations are calculated and shown (Table 1). Statistical results clearly reveal a high phenotypic variation among the specimens collected. It is observed that the individual symmetry of shape of left-right orientations of both sexes is statistically insignificant. However, variations on the left and right sides of both sexes showed a highly significant value (0.000**) on the sides and individual X sides scores. This is an evidence for the occurrence of FA in the body shapes of guppies in Lake Sebu. This means that there is an immense variability in the left from the right sides of the bodies of *P. reticulata.*

Table 1

Procrustes ANOVA for shape of *Poecilia reticulata* in terms of sexes

Effects	*SS*	*dF*	*MS*	*F*	*p-Value*
Female					
Individuals	0.1048	696	0.0002	0.9365	0.8066^{ns}
Sides	0.0124	24	0.0005	3.2189	0.0000**
Individuals X Sides	0.1119	696	0.0002	13.7351	0.0000**
Measurement error	0.0337	2880	0.0000	-	-
Male					
Individuals	0.1749	696	0.0003	1.1757	0.0164^{ns}
Sides	0.0163	24	0.0007	3.1752	0.0000**
Individuals X Sides	0.1487	696	0.0002	7.6112	0.0000**
Measurement error	0.0809	2880	0.0000	-	-

** highly significant (P<0.005) , ns - not significant.

Results obtained from the Procrustes ANOVA for shape of *P. reticulata* in both male and female significantly indicate the stress in the environment in Lake Sebu due to its threatened water quality (Guerero III 1999). In water ecosystems, greater levels of FA have been found in fish from areas that have been heavily polluted (Tomkins & Kotiaho 2001). According to Clarke (1995) and Møller & Swaddle (1997), environmental perturbations can give rise to decreased developmental stability of individuals, which may result in reduced performance of fitness components. In addition, in the studies of Clarke & McKenzie (1992) and Imasheva et al (1997), the results in FA is indicative of random errors in the phenotypic development of an organism in response to environmental disturbances and stressors such as poor water quality and heavy metal and toxic chemical contaminations (Allenbach et al 1999; Franco et al 2002; Estes et al 2006). The

values obtained from the calculations of FAs of *P. reticulata* associate to the inability of the species to develop perfect bilateral asymmetry.

To support the analysis using Procrustes ANOVA to indicate significant differences in the FA of *P. reticulata,* the upper 5% interaction value of principal components of the sample is measured (Table 2).

Table 2

Principal component (PC) scores showing the values of symmetry and asymmetry scores and the affected landmarks

PCA	*Individual (Symmetry)*	*Sides (Directional asymmetry)*	*Interaction (Fluctuating asymmetry)*	*Affected landmarks*
		Female		
PCA1	34.8313%	100%	39.3045%	1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 14
PCA2	21.2448%		23.0041%	1, 2, 3, 4, 5, 6, 7, 8, 11, 13, 14
PCA3	19.2422%		11.0913%	1, 2, 5, 6, 7, 10, 11, 13
PCA4	7.0954%		10.2894%	3, 6, 8
Total	82.41%		83.69%	-
		Male		
PCA1	34.6407%	100%	30.8285%	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13
PCA2	24.565%		24.2997%	1, 2, 3, 6, 8, 9, 11, 12, 13, 14
PCA3	10.8053%		13.2422%	1, 2, 4, 5, 6, 7, 8, 10, 13
PCA4	8.271%		8.6661%	1, 2, 3, 5, 6, 7, 8, 10, 11
PCA5	5.7333%		4.59%	2, 5, 6, 7, 8, 9, 10, 11
Total	84.02%		81.63%	-

Out of the values obtained and were shown in Table 2, the female's FA showed higher value, with 83.69% interaction, than in male with 81.63% from PCA1 – PCA5. Results in PCA1 in female showed that differences in asymmetry can be located extensive in the affected landmarks: 1 (snout tip), 2 (posterior extremity of premaxillar), 4 (insertion of operculum on the lateral profile), 5 (anterior insertion of the dorsal fin), 6 (anterior insertion of the anal fin), 7 (posterior insertion of the dorsal fin, 8 (posterior insertion of the anal fin), 9 (dorsal insertion of the caudal fin), 11 (point of maximum extension of operculum on the lateral profile), 12 anterior region of the lateral line from the middle part of the body to the caudal peduncle, and 14 (posterior most body extremity). PCA2 also showed numerous points in body of guppies where FA can be found.

In male *P. reticulata,* affected landmarks in PCA1 were in: 1 (snout tip), 2 (posterior extremity of premaxillar), 3 (dorsal occiput), 4 (insertion of operculum on the lateral profile), 5 (anterior insertion of the dorsal fin), 6 (anterior insertion of the anal fin), 7 (posterior insertion of the dorsal fin, 8 (posterior insertion of the anal fin), 9 (dorsal insertion of the caudal fin), 10 (ventral insertion of the caudal fin), 11 (point of maximum extension of operculum on the lateral profile), and 13 (posterior region of the lateral line from the middle part of the body to the caudal peduncle). PCA deformation grid was obtained to visualize the actual affected landmarks (Figure 3 & 4). Actual landmarks of the *P. reticulata* were also shown together with the PCA1 and PCA2 deformation grids for comparison.

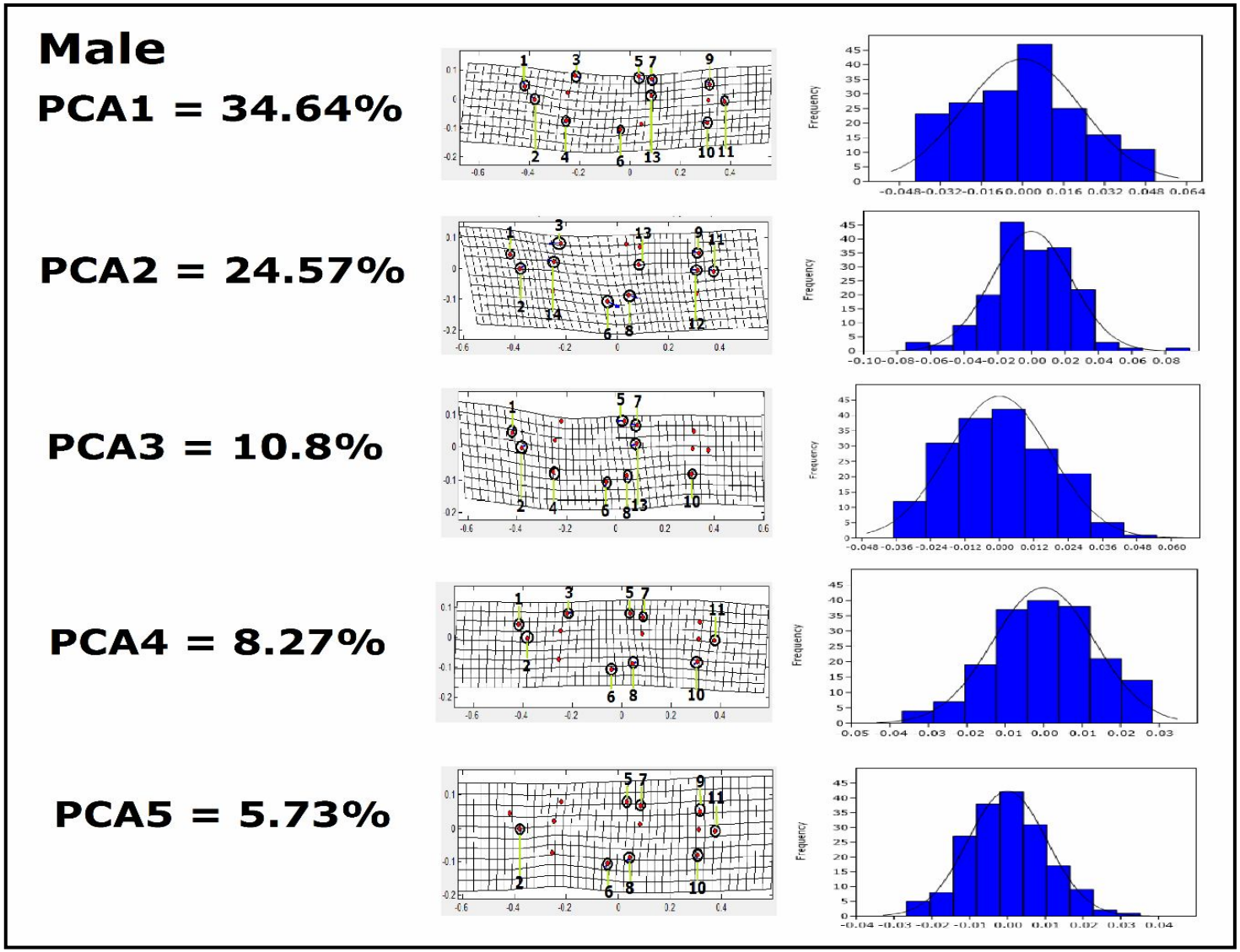

Figure 3. Principal components (PC) implied deformation grid and histogram of individual (symmetry) in *Poecilia reticulata* male specimens.

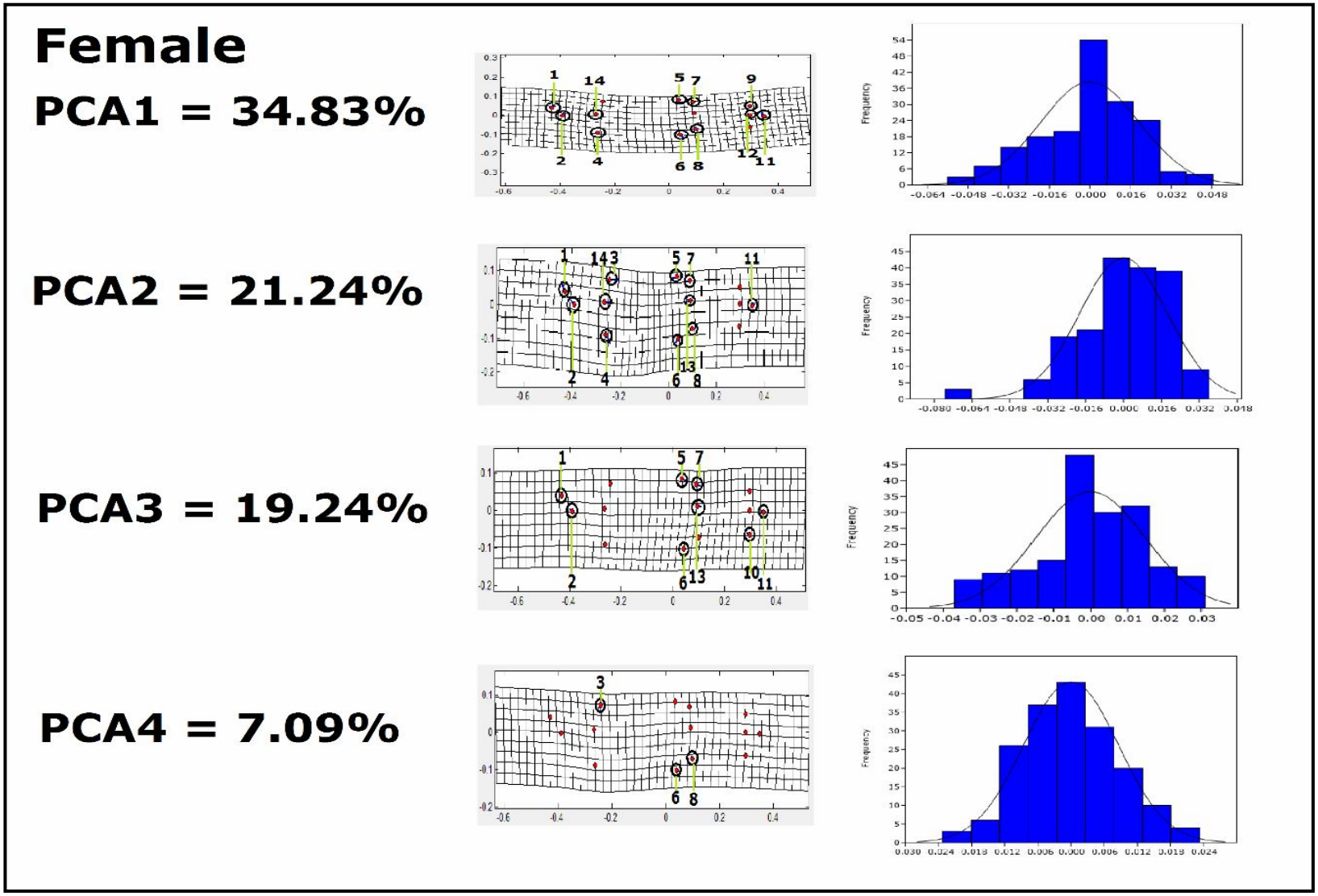

Figure 4. Principal components (PC) implied deformation grid and histogram of individual (symmetry) in *Poecilia reticulata* female specimens.

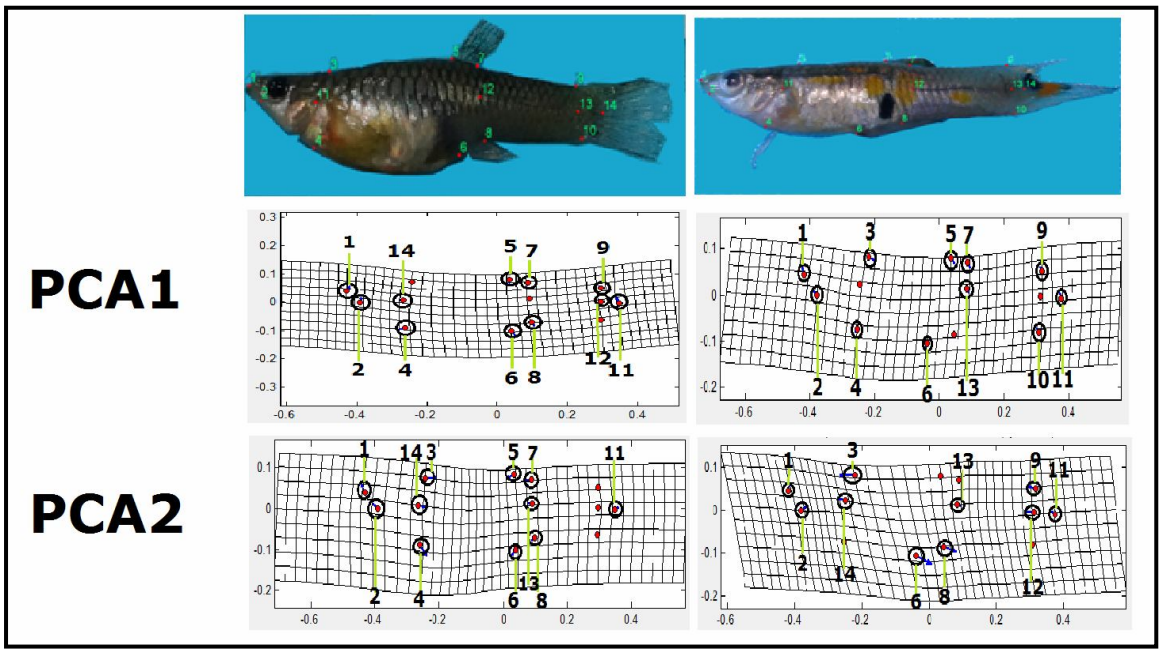

Figure 5. Actual digitized picture of the male and female guppy *Poecilia reticulata* and the affected landmarks shown in PCA1 and PCA2.

Figure 3 & 4 shows the distributions of asymmetry shapes through principal components implied deformation grid of both sexes and histogram of individual (symmetry) that is obtained originating from point 0. The affected landmarks are encircled to show the movement of disturbances in the body shape of the samples on both sides and on both sexes. The scores that are shown through bar graph show inclinations to the left and right that signify minimal and maximum individual asymmetry of *P. reticulata* in PCA1 and PCA2 in the male species. In female, PCA1 and PCA2 scores are tilted to the left that show result to its asymmetry.

Developmental instability relates to the inability of organisms in compensating for disturbances during development (Zakharov & Graham 1992), and would furthermore be affected by stress-related genetic and environmental conditions.

Taking into consideration environmental stress causes developmental instabilities, high fluctuating asymmetry could indicate the lower condition of guppies that were experiencing unfavorable environments.

These findings agree with results taken from several FA studies conducted prior to present research, where Almeida et al (2008), pointed out that FA appears to be a useful indicator in stress-induced environment of goldfishes and carp. Øxnevad et al (2002) found an increase in FA for gill rakers of Eurasian perch *Perca fluviatilis L.* with acidification of the lake. Likewise, Al-Mamry et al (2011) also predicted that the changes in the morphology of Indian mackerel *Rastrelliger kanagurta* in Oman Sea are correlated to the environmental pollution.

Conclusions. Out of the findings obtained in the study, the female *P. reticulata* showed higher FA value (83.69%) compared to the male species (81.63%). These results indicate a high FA, thus, specifies high level of perturbations in the environment in the lakes in Lake Sebu. This is validated by getting the ANOVA and the results showed a highly significant value ($p<0.05$). It can also be inferred that that the female species, having greater value in the interaction (FA), compensated more to the poor quality of water in the area evident to its deformities. It is suggested that efforts in connection to cleaner and improved water qualities will be done in the sampling area. These conclusions will greatly support to the effectiveness of the FA studies to be one of the bio-indicators of environmental stress.

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