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Fluctuating asymmetry analysis of trimac *Amphilophus trimaculatus* as indicator of the current ecological health condition of Lake Sebu, South Cotabato, Philippines

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Abstract. Lake Sebu is one of the important watersheds in the country supplying water to the lowland areas and contributed to the economic aspects of the place wherefore, the environmental status and condition of which is highly significant. Catastrophes such as fish kills recently happened in the lake, thus, the researchers aimed to monitor the current environmental status of the lake by evaluating the living organisms inhabiting the lake. Trimac *Amphilophus trimaculatus* is one of the major organisms found in the lake and it may serves as bio indicator of the environment. An evaluation among its trait asymmetry was used and it is believed to be an indicator of developmental noise and predict the lake status. In this study, 180 individuals, 90 species per sexual category were collected by net fishing techniques. Variations in the patterns of fluctuating asymmetry (FA) in a total of sixteen bilateral traits of the *A. trimacultaus* were determined using the method of landmark-based geometric morphometric. The paired landmark coordinates were subjected to the procustes ANOVA using the SAGE software version 1.0. The overall result of the study showed only minimal stress effects through the body shape analysis of *A. trimaculatus* is experienced, signifying that the lake is currently in good condition after the latest fish kill phenomenon. However, individual asymmetry in male proved, otherwise individual symmetry, and in female showed no significant differences because of the mechanisms that buffer developmental noise that made it capable of maintaining homeostasis.
Key Words: Geometric morphometrics, fish morphology, fish stock health, SAGE.

Introduction. Our environment is constantly bombarded with anthropogenic pollutants as a result of high technological output. This has been an issue since the new era of advancement begun. Those that are directly affected are the organisms dwelling in areas where they come in contact to sewage leading to bodies of water or even the atmosphere. One good source of indicator would be the living organisms in bodies of water such as Lake Sebu.

In Lake Sebu, Trimac *Amphilohus trimaculatus* or locally known as “flowerhorn” is identified by some natives as an edible fish that can be best serve as stew in vinegar. Thus, it contributed to the economic impact particularly in tourism. Lake Sebu is one the best tourist spots in the Philippines, the amazing view and seeming nature recognized the place. In addition, the variety of native recipes with the main dish of flowerhorn also signatures the spot wherein many people fascinated. As for now, its economy is based on aquaculture of flowerhorn grown in large fish cages and tourism, however, catastrophes such as fish kill happened in the lake. The latest massive fish kill on the first week of August, 2012, downed 8,000 kg of tilapia in a single week (Magbanua & Maitem 2011). This was considered a disaster by the local fish pen owners. The Local Government Units, Bureau of Fisheries and Aquatic Resources, even media reports paint a grim event in the fishery industry of the municipality, a view solely founded on its economic value (Beniga 2013). Monitoring the current environmental status of the lake after the disaster is very necessary and much related for the habitation of the living organism. To assess the quality of the environment and its condition, bio indicators *e.g*. fishes are considered (Prokosch et al 2005).

Fishes are greatly affected by environmental attributes that restrict the distribution of certain species in water system. This may be applied to *A. trimaculatus* which indicates the recent environmental quality of the lake. Fluctuating asymmetry (FA) appears to be a good bioindicator of the state of environmental quality. This is an easy way to do and inexpensive way of determining if the environment is capable of sustainable development (Angtuaco & Leyesa 2004). FA, defined as a subtle differences between the left and right sides of bilateral traits (Swaddle 2003), is believed to be an indicator of the fitness of populations and environmental status. Generally, high trait FA is associated with perturbations and stresses during the development of an individual fish (Hermita et al 2013). Stressful and marginal environment manifested high trait FA. Elevated FA is a reflection of poor developmental homoeostasis at the molecular, chromosomal and epigenetic levels (Pursons 1990). The relationships between homeostasis and FA can be further described as follows: high FA results poor developmental homeostasis; and poorer developmental homeostasis results from high developmental instability (Hermita et al 2013). Developmental instability, in this case, is defined as a suite of processes that tend to disrupt the precise developmental of bilateral traits in organism (Palmer 1990) and is believed to stem from various exogenous and endogenous stresses collectively referred to as developmental noise or perturbations (Reimchem & Nosil 2001).

There are several means of determining and presenting FA data and this is summarized by Palmer (1990). Whilst there are many available methods to determine trait FA using traditional linear distance measures, advances in digital imaging (Bookstein 1993) has made it possible to describe developmental instability using the landmark-based method of geometric morphometric (GM) (Demayo et al 2013). Thus, this tool was used in this study to quantify deviations from bilateral asymmetry and determine levels of FA in a total of 16 traits in *A. trimaculatus* inhabiting in Lake Sebu, South Cotabato, Philippines.

Material and Method

Study area*.* The study was conducted from the last week of December (2014) to January (2015) in Lake Sebu, South Cotabato, which is located at south-central Mindanao that geographically lies between 6° 10.45' N and 124° 43.95' E (Figure 1).

Species accumulations. The specimens of *A. trimaculatus* were collected using net fishing techniques in Lake Sebu, South Cotabato. A total of 180 individuals 90 per sexual category and were analyzed for body shape differences.

In determining the sexual category of each specimen, females (Figure 2a) and males (Figure 2b) were identified based on external morphology and later confirmed by direct examination of the gonads (Love & Chase 2009). Males had whitish soft textured gonads whereas females had yellowish coarsely textured gonads with eggs (Requieron et al 2010). Digital images were taken from the left side of the specimen using Olympus Digital Camera (14.1 megapixels) (Figure 2).

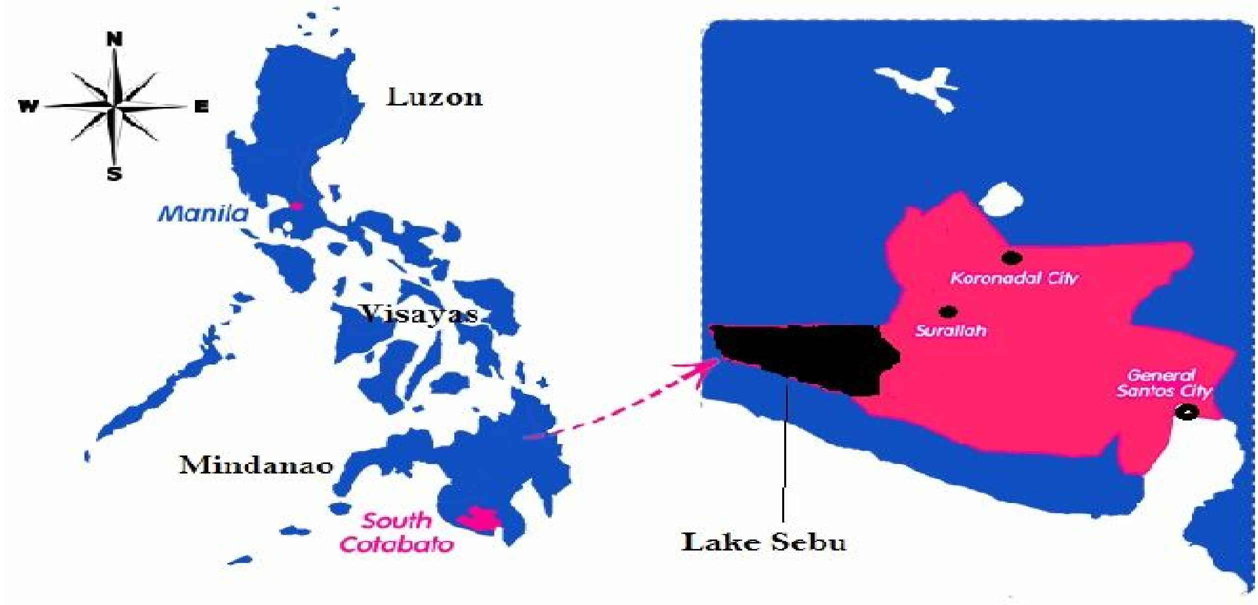

Figure 1. Map of the Philippines (Left) showing South Cotabato located in Mindanao; (right) enhanced map showing Lake Sebu in South Cotabato.

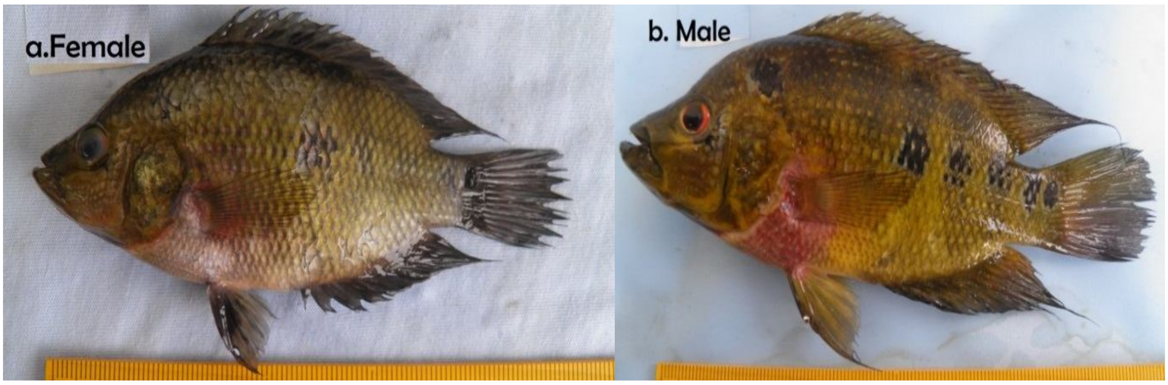

Figure 2. Trimac *Amphilophus trimaculatus*. A – Female, B - Male (Image obtained by Olympus Digicam 14.1 megapixels).

Landmarking. A total of 16 landmarks were digitized using the tpsDig version 2.12 (Adams et al 2004). The location of the landmarks and the anatomical descriptions of each are presented in Figure 3.

Measuring fluctuating asymmetry. FA were determined by subjecting the paired landmark coordinates to procustes ANOVA (Hermita et al 2013) following the method of Klingenberg (1992) and using the SAGE software version 1.0 (Marquez 2006).

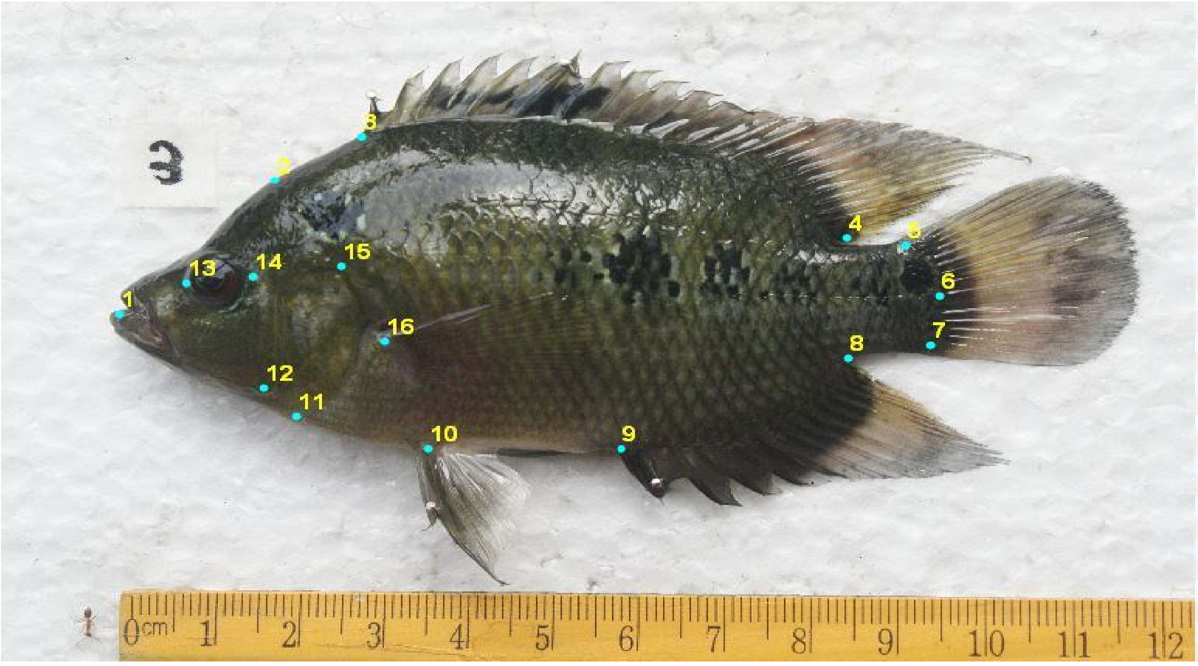

Figure 3. Landmarks used for digitizing the image of the subject. 1 - Rostral tip of premaxilla; 2 - Posterior end of nuchal spine; 3 - Anterior insertion of dorsal fin; 4 - Posterior insertion of dorsal fin; 5 - Dorsal insertion of caudal fin; 6 - Midpoint of caudal border of hypural plate; 7 - Ventral 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 Sparks & Chakrabarty (2007).

Results and Discussion. Procrustes ANOVA showed a significant level of symmetry in individuals of male *A. trimaculatus* (Table 1) and not significant level in female specimens (Table 2). The measurement error recorded a low mean square value in sides compared to data recorded in individual x side interactions and how the asymmetry in the landmarked subject is FA (Penaredondo & Demayo 2014). To further support the analysis, the upper five percent (5%) interaction value of PCA was performed (Table 3) to visualized shape variation in male (Figure 4) and female (Figure 5) landmarked subject together with the histogram, and also used to investigate patterns of covariation in the positions of landmarks (Dryden & Mardia 1998).

Table 1

Procrustes ANOVA analysis for male *Amphilohus trimaculatus*

Source	*SS*	*dF*	*MS*	*F*	*Remarks*
Individual	0.1994	812	0.0002	0.9982	significant
Directional asymmetry	0.1617	28	0.0058	23.4753	Not significant
Fluctuating asymmetry	0.1998	812	0.0002	4.1672	Not significant
Error	0.1984	3360	0.001	-	-

The factor in each test was sample locations. dF - degrees of freedom; SS - sum of squares; MS - mean of squares; F - F-ratio.

Table 2

Procrustes ANOVA analysis for female *Amphilohus trimaculatus*

Source	*SS*	*dF*	*MS*	*F*	*Remarks*
Individual	0.1907	812	0.0002	1.7491	Not significant
Directional asymmetry	0.0486	28	0.0017	12.925	Not significant
Fluctuating asymmetry	0.109	812	0.0001	2.6197	Not significant
Error	0.1722	3360	0.001	-	-

The factor in each test was sample locations. dF - degrees of freedom; SS - sum of squares; MS - mean of squares; F - F-ratio.

Table 3

Upper 5% principal components analysis (PCA) in *Amphilohus trimaculatus*

PCA	*% Interaction (FA for females)*	*% Interaction (FA for males)*
PC1	23.1995	33.189
PC2	16.2061	14.3297
PC3	12.6127	12.166
PC4	11.0276	7.7665
PC5	6.2247	6.2946
Overall percentage	69.2706	73.7458

PC - Principal component.

Looking at the overall body shapes of the fishes, the results of the analyses manifested that all species exhibited a not significant levels of FA (Table 1 & 2). However, when all sixteen bilateral traits were examined separately, the results identified that male trimac *A. trimaculatus* exhibit the trait FA with 12 out of 16 characters with significant levels whilst female trimac *A. trimaculatus* exhibit 8 out of 16 characters with significant levels (Table 4).

Table 4

Presence of trait asymmetry of the trimac *Amphilohus trimaculatus*

Character	*Male*	*Female*
Rostral tip of premaxilla	*	*
Posterior end of nuchal spine	*	*
Anterior insertion of dorsal fin	*	*
Posterior insertion of dorsal fin	-	-
Dorsal insertion of caudal fin	-	*
Midpoint of caudal border of hypural plate	*	-
Ventral insertion of caudal fin	-	-
Posterior insertion of anal fin	-	-
Anterior insertion of anal fin	*	*
Dorsal base of pelvic fin	*	*
Ventral end of lower jaw articulation	**	-
Posterior end of maxilla	**	*
Anterior margin through midline of orbit	**	-
Posterior margin through midline of orbit	**	-
Dorsal end of opercle	**	*
Dorsal base of pectoral fin	**	-
Total	12	8

First two PCA test for the presence of traits asymmetry: *(present at least in a PCA), ** (present in first 2 PCA).

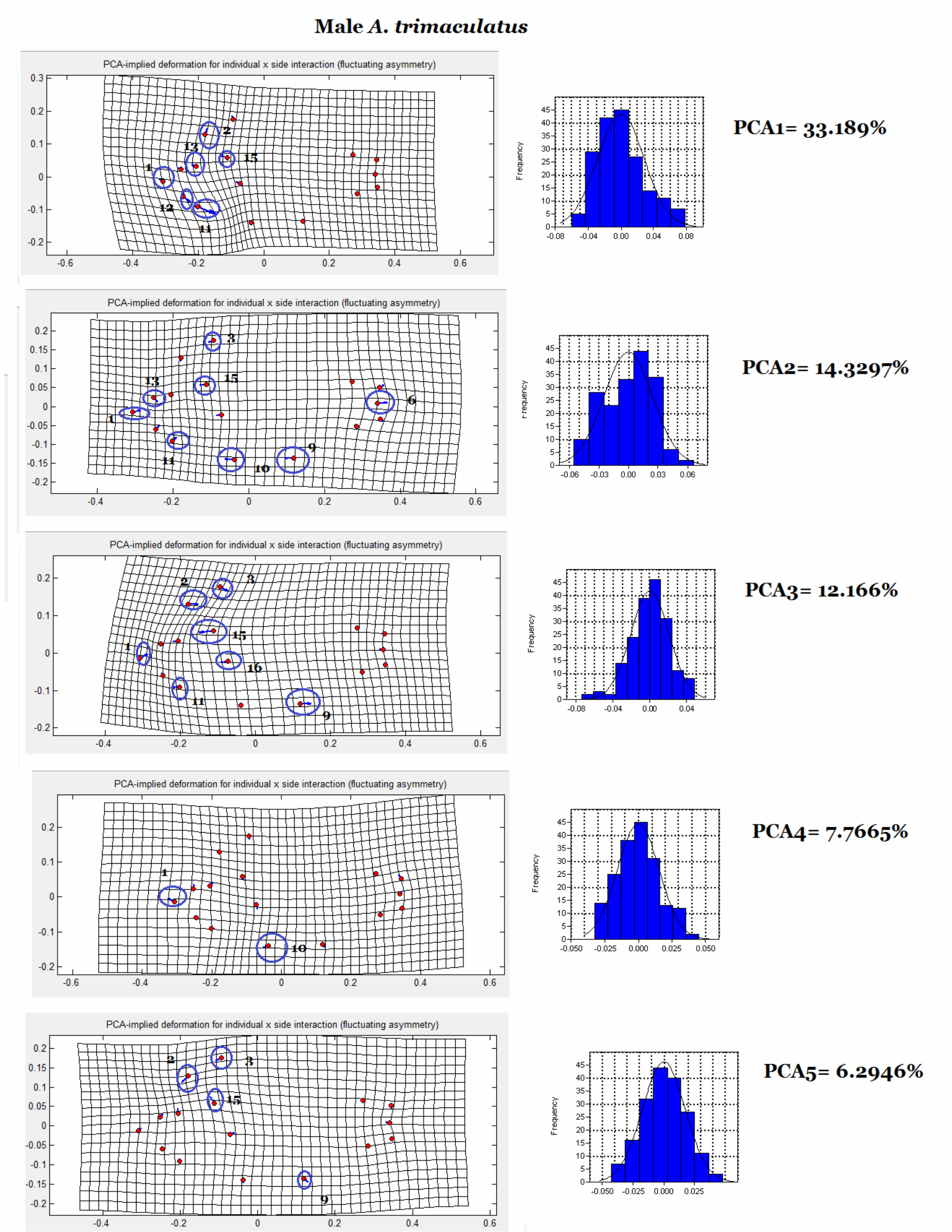

Figure 4. PCA implied deformation of individual x side interaction (FA) with histogram of male *Amphilohus trimaculatus.* The percentages indicate the proportions of variation for which the respective principal components account. (Circled dots signify the movement of the affected landmarks and the affected regions).

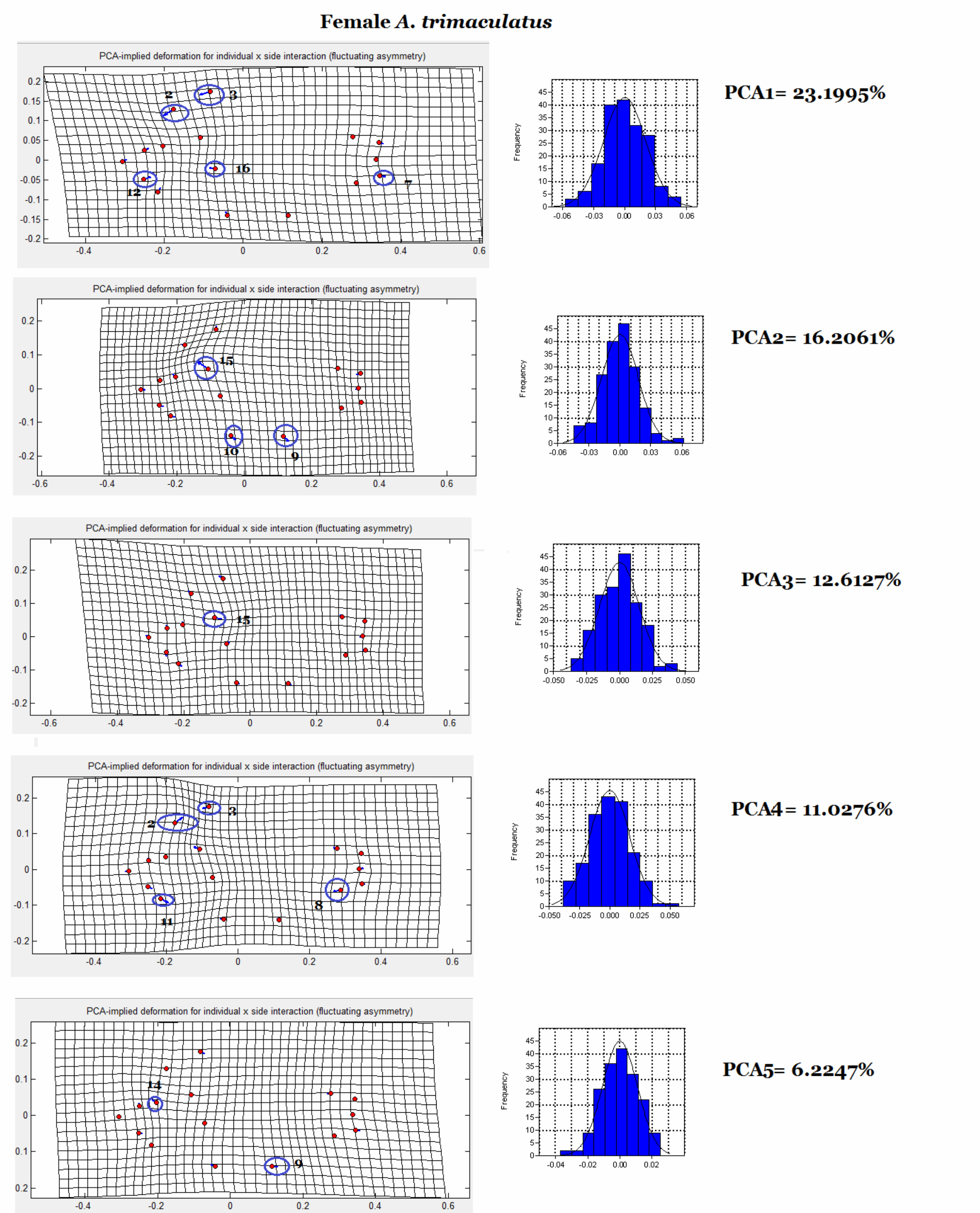

Figure 5. PCA implied deformation of individual x side interaction (FA) with histogram of female *Amphilohus trimaculatus.* The percentages indicate the proportions of variation for which the respective principal components account. (Circled dots signifies the movement of the affected landmarks and the affected regions).

Differences between sexes were evident in the subjected fishes examined. For example, only the female showed significant differences in dorsal insertion of caudal fin, and only males were found with significant differences in midpoint of caudal border of hypural plate. The observed differences in the patterns of FA between sexes might mean that there might exist differences in the levels of developmental homoestasis between males and females (Demayo et al 2013). The results of this study is similar to the findings of other studies in that not all characters examined in the fish exhibited FA (Swaddle 2003; Costa & Cataudella 2007; Graham et al 1993). The difference in the levels of FA among characters in that the trait differs in their ability to buffer developmental noise and achieve homeostasis (Graham et al 1993; Lens et al 2002).

Conclusion. Based on the overall results of the study, analysis showed *A. trimaculatus* in Lake Sebu, South Cotabato, exhibited not significant level of FA, thus might mean that Lake Sebu is in good environmental status and condition after the latest disaster *e.g.* fish kill. These results may also imply that the fishes in Lake Sebu experienced lesser developmental perturbations and stresses (Almeida et al 2008). Withal, if bilateral traits were examined separately the male *A. trimaculatus* gained more significant levels than the female, hence, the female possess mechanisms that buffer developmental noise that made it capable of maintaining homoeostasis.

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