Article

Evaluating the developmental instability of *Scatophagus argus* (Linnaeus, 1766) from Lower Agusan River Basin, Butuan City, Philippines using fluctuating asymmetry

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Abstract

Fluctuating Asymmetry (FA) is the most frequently used tool for measuring developmental instability. It is used as a measure of ecological stress and a sign of developmental stability of organisms. The study evaluated the use of FA in assessing the condition of *Scatophagus argus* in lower Agusan river basin. High FA values would indicate exposure to polluted aquatic environment. The study selected *S. argus* because of its observed susceptibility to aquatic pollution in the area. There were a total of 60 samples collected (30 males and 30 females). Using Thin-Plate Spline (TPS) series, landmark analyzes were obtained and subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software. Procrustes ANOVA showed high significant differences (P<0.0001) to the three measured factors (individuals, sides and interaction of individuals and sides). Similar findings were observed to both male and females samples suggesting high FA values. The principal component analysis was implemented to determine the affected landmarks. More landmarks were affected in males (11 landmarks) than in females (5 landmarks). Deformation grids and histograms were used to display the ordination of affected landmarks. The data would be important to environmental planners in the management of lower Agusan river basin. Scores display a high percentage FA of female (70.94%) and male (78.67%). In the female samples, PC 1 (33.26%) and PC2 (13.53%) were found to have significant variations affecting the rostral tip of premaxilla, posterior extremities, and the lateral profile. In the male samples, PC 1 (35.63%) and PC 2 (14.24%) have the same affected landmarks as in females but have greater variations. Significant levels of FA in the fish morphology are the result of its adaptive mechanism to cope up with the stressed environment. The dorsal cephalic region and the pectoral fin were the most affected landmarks and may be used to determine the effect of stressors to the fish since these areas were involved in fish mobility. Thus, the high FA in the body shapes of spotted scat suggests that ecological health in the area is in not good standing.

Keywords fluctuating asymmetry; developmental instability; *Scatophagus argus*; lower Agusan river basin.

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1 Introduction

Fluctuating asymmetry (FA) is the inconsistency in subtle alterations between the left and the right sides or some parts in bilaterally symmetrical organisms. It is assumed to point out the inability of an organism to maintain their optimum level of development (Palmer and Strobeck, 1986). FA is the most frequently used tool for measuring developmental instability because they have a direct relationship. An FA analysis hypothesizes that the development of the left and right sides of a bilaterally symmetrical organism is influenced by the same genes. Therefore, the non-directional differences between both sides are not caused genetically but rather are environmental in origin. FA reveals a population's state of adaptation, coadaptation, fitness and individual quality (Ducos and Tabugo, 2015). The results in FA measurement will determine the condition of an individual organism (Natividad et al., 2015).

Studies show that both genomic and environmental changes can increase FA, which represents a possible deterioration in developmental homeostasis of an organism. This is apparent in the organisms' adult morphology. Such genetic perturbations included intense directional selection and linked to specific genes (Vollestad et al., 1999). Meanwhile, extreme temperatures, audiogenic stress, protein deprivation, and pollutants are some of the environmental perturbations (Velichovic, 2004; Mpho, 2000). FA was used as a measure of ecological stress and a sign of developmental stability of organisms. It is based on the theory that FA levels among organisms that inhabit stressful environments are higher than to those organisms that inhabit optimum environments (Parsons, 1961, 1962, 1990, 1992; Van Valen, 1962; Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Ducos and Tabugo, 2015). FA was also used as direct correlation to water quality since presence of pollutants directly affects the symmetry of fishes in the aquatic environment. Bioindicators such as fishes are the primary inhabitants in aquatic areas and are used in the assessment. If FA is applied, high FA value would mean that the water is highly likely polluted. This will be manifested as stressed condition of the fishes. On the other hand, a low FA value would indicate good condition. For this study spotted scats were used in the assessment because of its observed susceptibility to the water quality in the sampling area .

The spotted scat, *Scatophagus argus* (Perciformes: Scatophagidae) is a euryhaline teleost found in freshwater, brackishwater and marine habitats (Gandhi et al., 2014). They are widely distributed in mudflats, mangrove swamps, harbors, upstream swamps, estuaries and marine habitats of Indo-Pacific, the Malay Archipelago, the Philippines, Australia and South and Southeast Asia. Areas inhabited by scats are characterized by fluctuations in salinity, temperature, dissolved oxygen, tidal movements, turbidity and turbulence. These adaptations to living in such ever changing environments endow them with many biological attributes highly desired in cultured finfish (Barry and Fast, 1988). Spotted scats are an important food source in Southeast Asian countries (Musikasung et al., 2006) and also consumed by the local community in the sampling area. Because of their favorable biological characteristics and economic importance, considerable interest exists in developing propagation and culture techniques for the spotted scat (Sivan and Radhakrishnan, 2011). The *S. argus* was selected because it was observed to be susceptible to aquatic pollution in the study area.

The lower Agusan river basin eventually drains to Butuan Bay. Fish propagation is common in the area, with various fish ponds for the people's livelihood and consumption. Some efforts were made in the past to conduct water quality assessments and conditions of biological organisms. However, there were no reports in the application of FA and the condition of *S. argus* in the area. The Agusan river basin, as a whole, experienced ecological pressure from industrialization, sewage, and mining activities. Bacterial and heavy metal contamination has been the relevant issues in the past years. The rapid economic developments were poised to threaten this fragile aquatic system. Yet, there were limited reports and studies conducted to assess the condition of fishes and specifically *S. argus*. Thus, this study is highly relevant because it will evaluate the developmental instability and ecological stress using FA in the body shapes of *S. argus*. The generated data will be of immense importance to environmental planners in the management of this aquatic system.

2 Materials and Methods

2.1 Study Area

Fig. 1 showed the location of the Lower Agusan River Basin in Butuan City. It geographically lies between 08°53′50.06’’N and 125°48′31.63’’E. Sampling locations were attained using Global Positioning System (GPS) and displayed through Google Earth and map photos on lower Agusan river basin (CTI-Halcrow, 2008). Sampling was conducted in the month of August, 2015.

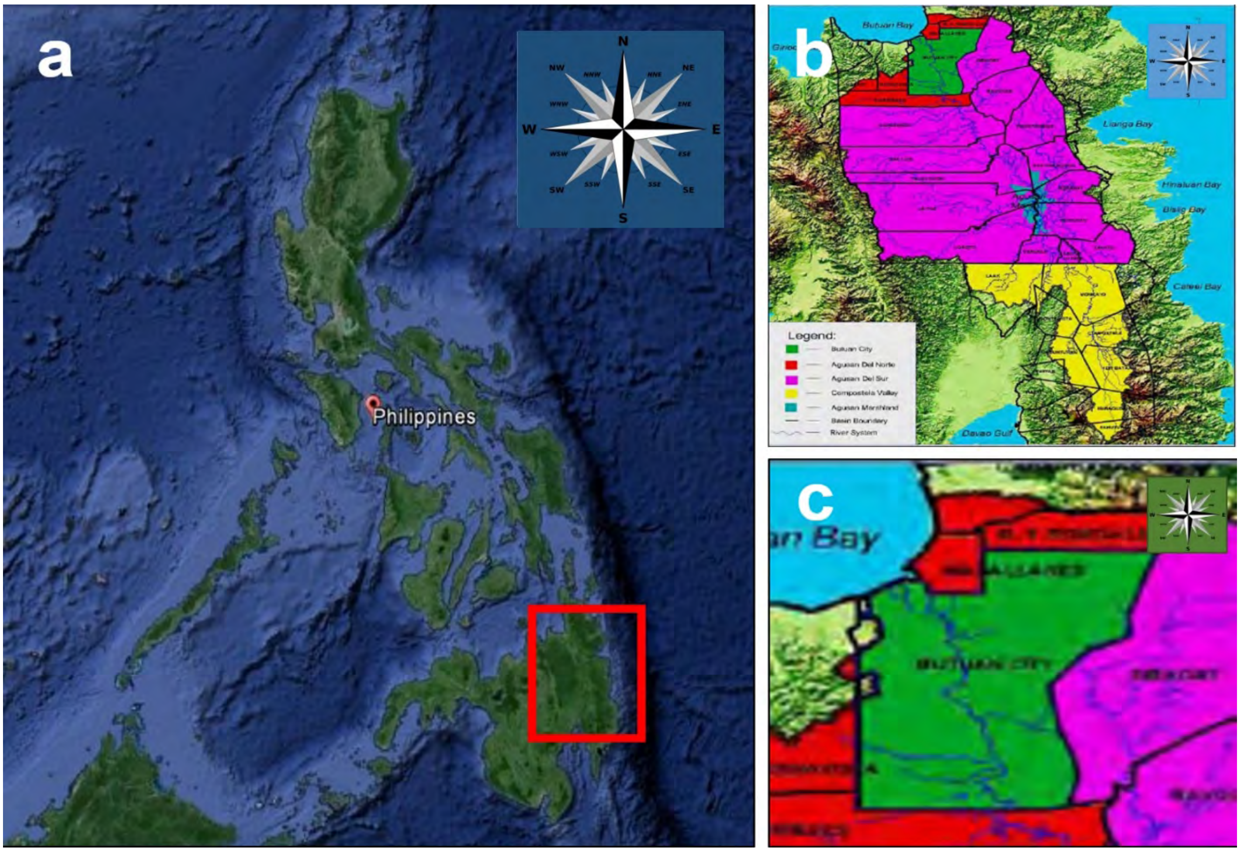

Fig. 1 Map of the Philippines, (b) Agusan River Basin (c) Butuan City in Lower Agusan River Basin

2.2 Fluctuating asymmetry of spotted scat *Scatophagus argus* in Agusan River

2.2.1 Sample processing

Samples of *S. argus* were collected with thirty specimens per sex (male and female). Imaging was done using Sony C6603 (15 megapixels) in a blue background with a ruler placed parallel to each sample for length determination of individuals, both for the left and right lateral sides. After imaging all samples, sex was then determined by dissecting each samples and identify the gonads. The testes are whitish soft textured while the ovary is yellowish coarsely textured with eggs (Requieron et al., 2010). Images were assorted according to sex and digitized using the tpsDig2 program (version 2.0, Rohlf, 2004) and were saved as TPS file.

2.2.2 Landmark selection and digitization

To incorporate curving features within the images, landmark analyses were performed using the Thin-Plate

Spline (TPS) series. Using the standard forms of digitized landmarks in fish morphometric, both evolutionary and the functional significance were obtained. These landmarks were selected to give a homogenous outline of the body shape of individual species (Fig. 2) using tpsDig2 software. There were 16 landmarks identified to represent best shape of the body, which is equivalent to 16 X and 16 Y Cartesian Coordinates. The Description of each landmark was shown in Table 1. Digitization was copied in triplicates for each sample to lessen inconsistencies and errors in plotting the landmark points. The X and Y coordinates of landmarks on the images were used for further analysis. Generated x and y coordinates served as baseline data in analyzing fluctuating asymmetry of freshwater fishes (Natividad et al., 2015).

Table 1 Landmark point descriptions (Dorado et al., 2012.)

No.	Description
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 or lateral line
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 the premaxilla
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

2.2.3 Shape analysis

The software Symmetry and Asymmetry in Geometric Data (SAGE) (version 1.04, Marquez, 2007), was used to analyze left and right landmark coordinates of the TPS software. Geometric data of the object with emphasis on its asymmetry was identified following the schematic flow (Fig. 3). SAGE software generated symmetrized data sets, residuals from symmetric components, in addition to shape configuration of each component of variation (Symmetric, Asymmetric, and error) as well as the probable covariance matrices. Procrustes ANOVA was employed on individuals with triplicates with permutations of 99 to compute and measure the residual asymmetry. It indicated variations between left and right sides and the measure of directional asymmetry. Percentage (%) FA was obtained and compared between both sexes.

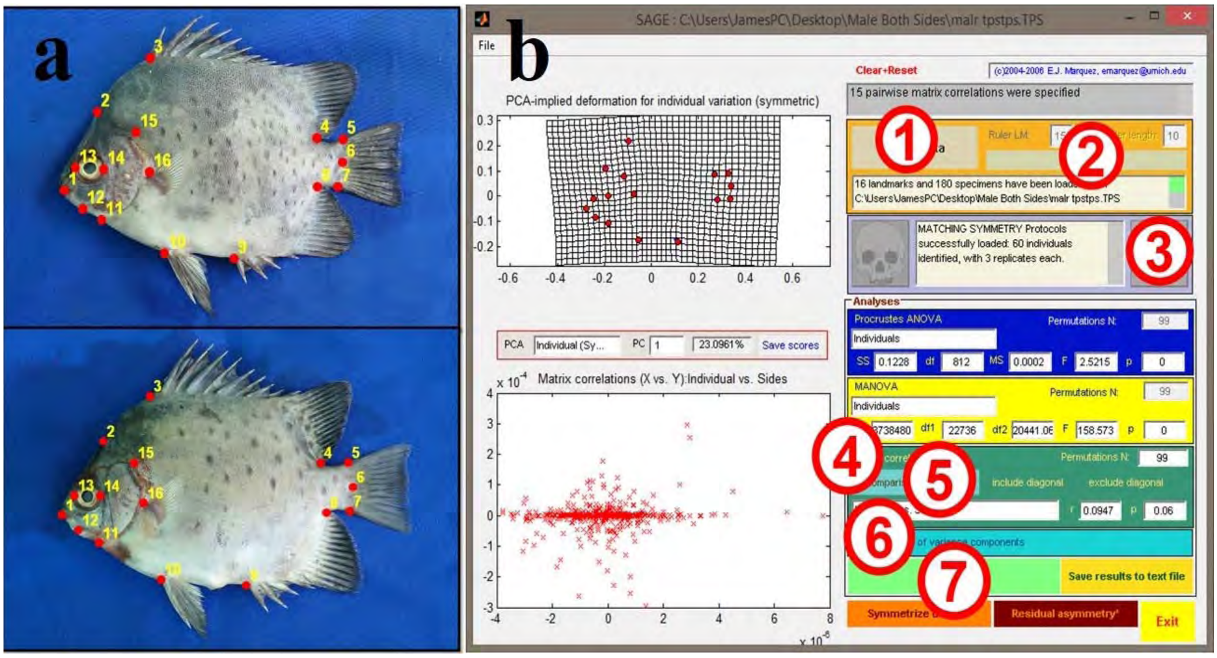

Fig. 2 (a) Landmark points of *S. argus* samples and **(b)** overview of the schematic flow of shape analysis using SAGE.

2.2.4 Intraspecific Variation between sexes

The comparisons between male and female sexes and individual symmetry were examined using the Paleontological Statistics (PAST) software (Hammer et al., 2001). Significant statistical representations such as box plots, histograms, and scattered plots were generated.

3 Results and Discussion

Procrustes Analysis of Variance (ANOVA) of the SAGE software was used to show the individual body shape fluctuations. Three factors were considered in the analysis; individuals, sides, and interaction of individuals and sides. The results indicated highly significant differences on the three factors indicating high FA values ($P<0.0001$). This observation was similar to both male and female samples (Table 2). Variations were observed on the left and right sides of fish samples generating asymmetry in morphology. The indicated FA in the body metrics of *S. argus* may imply poor water quality in the sampling area. Extended exposure to polluted water conditions will manifest into asymmetrical body shapes in the long run.A recent study by Ducos and Tabugo, 2015 shows that high FA values indicate greater exposure to environmental stress.Stressed environment renders to the failure of the species to develop phenotypically to the desired path as these stressors act upon the development of individual species (Barrett, 2005, Bonada and Williams, 2002). Therefore the observed FA in *S. argus* in the sampling area could likely be due to extended exposure in disturbed and polluted aquatic system.

Principal component analysis (PCA) was implemented using covariance shape change in principal components (PC) to get the general direction and magnitude of the fluctuation for each landmark (Table 3). In females, a total of 78.44% of cumulative variation were accounted (PC1-PC5). The frequently affected landmarks were landmarks 1, 2, 3, 9, and 10. These parts were rostral tip of premaxilla, posterior end of nuchal spine, anterior insertion of dorsal fin, anterior insertion of anal fin, and dorsal base of pelvic fin. In males, a total of 77.02% of cumulative variation were accounted (PC1-PC6). The frequently affected landmarks were 2, 3, 6, 7, 9, 11, 12, 13, 14, 15, and 16. These were posterior end of nuchal spine, anterior insertion of dorsal fin, midpoint or lateral line, ventral insertion of caudal fin, ventral end of lower jaw articulation, posterior end of the premaxilla, anterior margin through midline of orbit, posterior margin through midline of orbit, dorsal end of operculum, and dorsal base of pectoral fin.

Table 2 Procrustes ANOVA for shape of *S. argus* fish in terms of sexes.

Effect	SS	dF	MS	F	P-Value
		Female			
Individuals	0.1095	812	0.0001	3.9586	<0.0001**
Sides	0.0059	28	0.0002	6.0281	<0.0001**
Individual x Sides	0.0277	812	0	7.3523	<0.0001**
Measurement Error	0.0156	3360	0	----	----
		Male			
Individuals	0.1228	812	0.0002	2.5215	<0.0001**
Sides	0.0463	28	0.0017	27.5861	<0.0001**
Individuals x Sides	0.0487	812	0.0001	6.0776	<0.0001**
Measurement Error	0.0332	3360	0	----	----

**highly significant (P<0.0001)

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		
PC1	26.0572%	100%	33.2568%	1,2,3,9,10,11
PC2	22.2168%		13.5305%	2,3,9,10
PC3	14.1312%		9.6878%	1,10,12
PC4	8.8037%		7.9477%	5
PC5	7.2343%		6.521%	9
		Male		
PC1	23.0961%	100%	35.6315%	1,2,11,12,14,15,16
PC2	20.558%		14.2449%	3,6,7,9,11,13,14,15
PC3	12.1339%		10.4849%	2,3,6,8,9,14,15,16
PC4	7.6792%		6.4294%	3,4,6,7,15
PC5	7.2324%		6.3735%	2,6,7,12,13,16
PC6	6.323%		5.503%	2,5,6,7,14,15,16

It was observed that there were more affected landmarks in males than in females (Figs 4 and 5). There were 5 major landmarks that were affected in females which are largely located in dorsal cephalic region (3 landmarks) and ventral fins (pelvic and anal fins). However, there were 11 affected landmarks in males which largely constituted the head region (landmarks 2, 11, 12, 13, 14, 15 and 16), caudal parts (landmarks 6 and 7), and some fins (landmarks 3 and 9). The results indicated that males were prone to asymmetry than females.

An increase in FA would reflect poorer developmental homeostasis in the molecular, chromosomal and epigenetic levels in impaired environmental conditions (Parsons, 1990). The effect was more pronounced in males in comparison to females.

The affected landmarks were shown in deformation grids to visualize the asymmetrical points in *S. argus* (Figs 4, 5, and 6). The histogram displayed the symmetry and asymmetry scores indicating skewed distribution suggesting evidences of FA. These major affected landmark points in males and females were summarized into two principal components (PC1 and PC2) shown in Fig. 6.

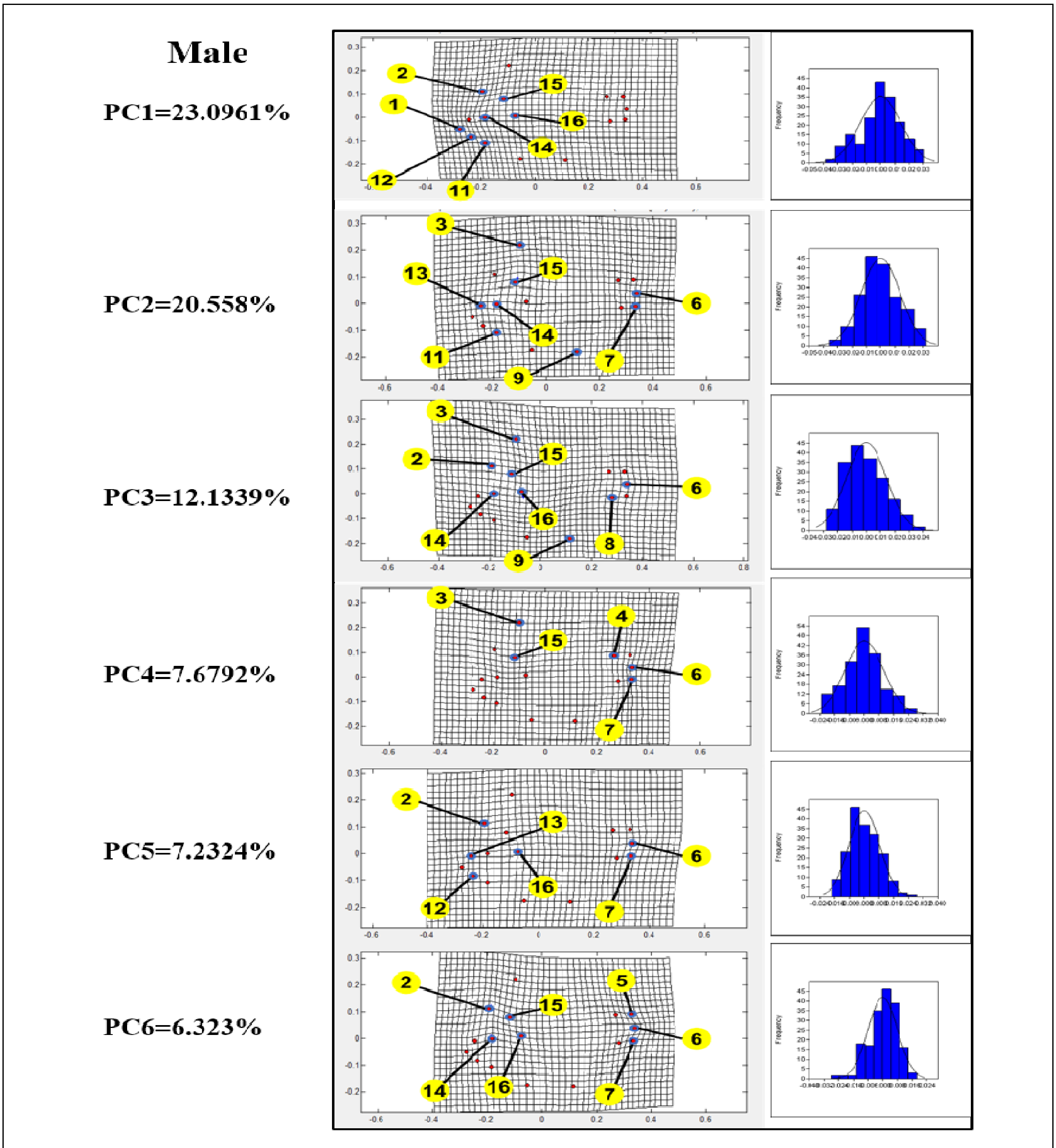

Fig. 3 Principal components (PC) inferred deformation grid and histogram of symmetric individuals in *S. argus* (Male) displaying distribution of asymmetry. Percentages on the sides indicate quantities of variation for which each PC account.

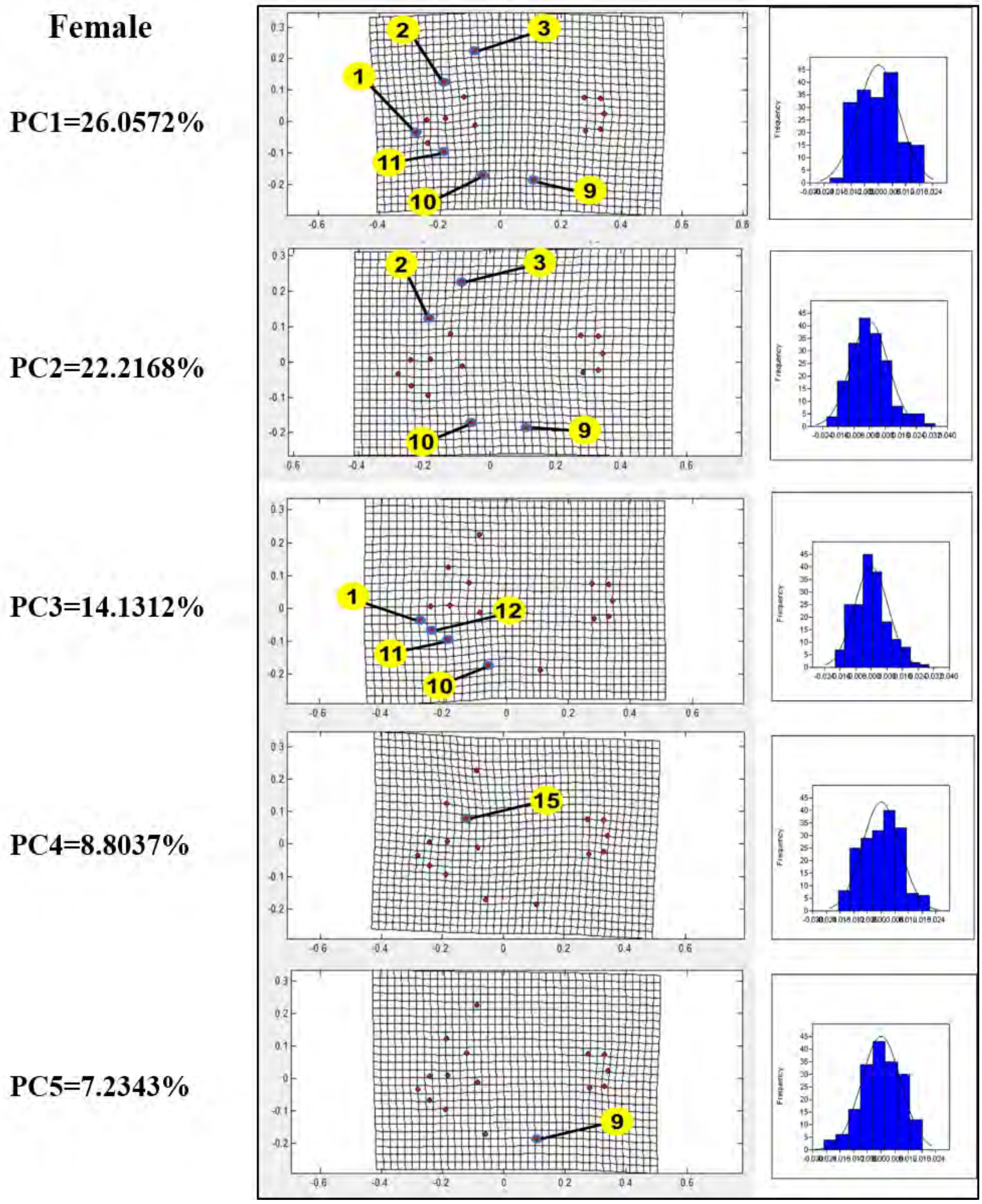

Fig. 4 Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *S. argus* female species.

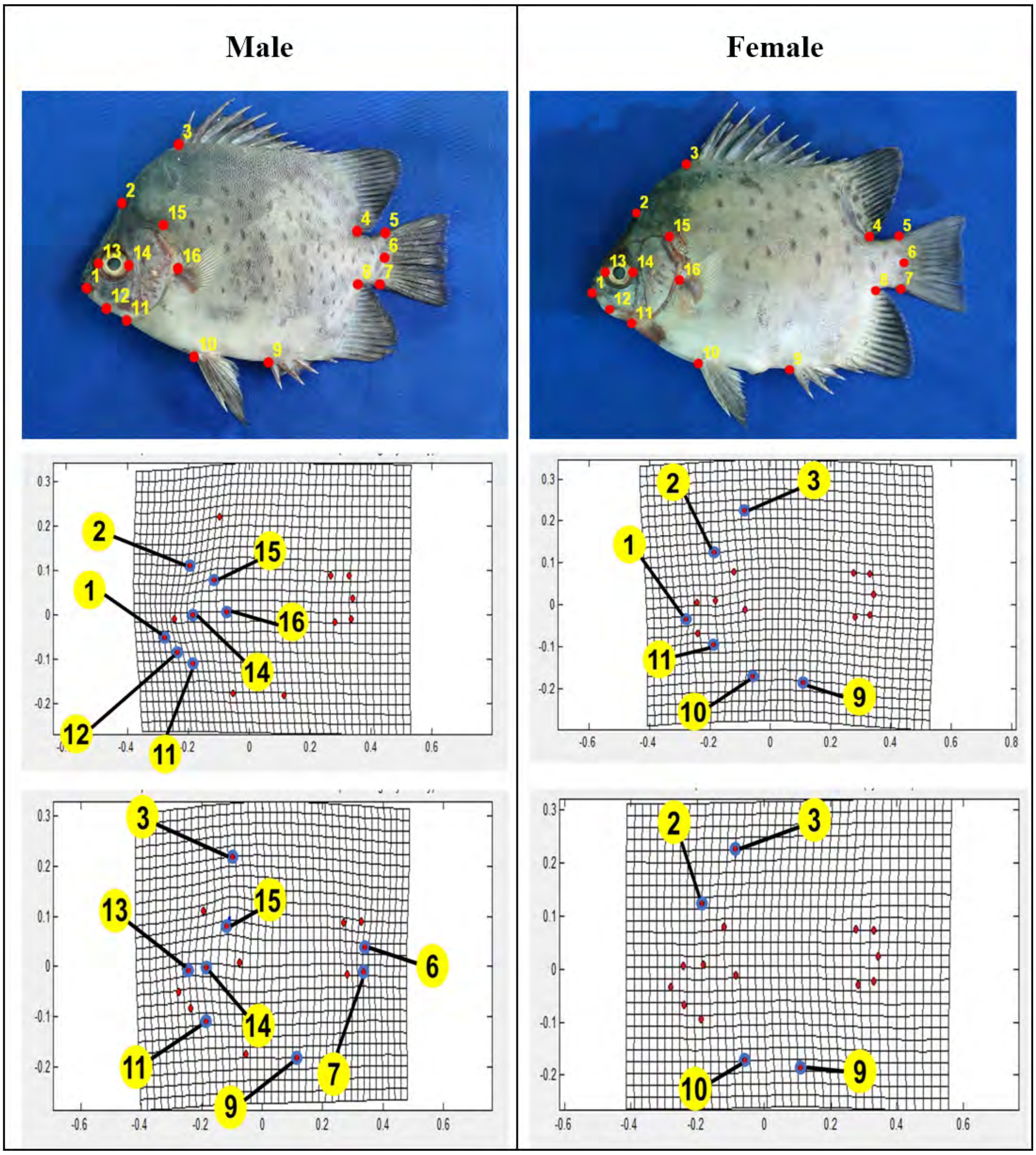

Fig. 5 Actualized picture of digitized male and female fish with the affected landmarks shown in PCA- deformation grid for PC1 and PC2.

4 Conclusion

The fish Spotted Scat (*Scatophagus argus*) was used to evaluate developmental instability and ecological stress manifestations using fluctuating asymmetry (FA). The metric traits of the fish were used to locate standard landmarks. The results on Procrustes ANOVA revealed highly significant differences in the three factors considered ($P<0.0001$). These factors were individuals, sides, and interaction of individuals and sides. Similar FA results were observed to both male and female samples. The affected landmarks were further analyzed using principal component analysis (PCA). PCA results showed more affected landmarks in males (11 landmarks) than in females (5 landmarks). The affected landmarks were displayed through deformation grids and histogram. The results of the study revealed evidences for FA in *S. argus*. The affected fish landmarks were also determined. The occurrence of FA is a manifestation of developmental instability and environmental stress undergone by the fish. Pollution and unstable water characteristics will have direct effects on the fish. Extended exposure to polluted waters will lead to asymmetrical appearance as the fish buffer the effects of unstable water conditions. The results of the study will contribute to environmental managers in lower Agusan river basin.

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References

Amarasinghe US, Amarasinghe, MD, Nissanka C. 2002. Investigation of the Negombo Estuary (Sri Lanka) brush park fishery, with an emphasis on community-based management. Fisheries Management and Ecology, 9: 41-56

Barrett C. 2005. Fluctuating dental asymmetry as an indicator of stress in Prehistoric Native Americans of the Ohio River valley. Dissertation. 44-45

Barry TP, Fast AW. 1988. Natural history of the spotted scat (*Scatophagus argus*) In: Spawning induction and Pond Culture of The Spotted Scat (Scatophagus argus) in Philippines (Fast AW, ed). 4-30, Tech. Rep. Hawaii Institute of Marine Biology, USA

Bonada N, Williams DD. 2002. Exploration of utility of fluctuating asymmetry as an indicator of river condition using larvae of caddisfly *Hydropsychemorosa* (Trichoptera: Hydropsychidae). Hydrobiologia, 481: 147-156

CTI-Halcrow. 2008. Philippines: Master Plan for the Agusan River Basin. Final Report. Chap. 2, p.1. Philippines

Ducos MB, Tabugo SRM. 2015 Fluctuating asymmetry as bioindicator of stress and developmental instability in Gafrarium tumidum (ribbed venus clam) from coastal areas of Iligan Bay, Mindanao, Philippines. AACL Bioflux, 8(3): 292-300

Gandhi V, Venkatesan VN, Ramamoorthy. 2014. Reproductive biology of the spotted scat *Scatophagus argus* (Linnaeus, 1766) from Mandapam waters, south-east coast of India. Indian Journal of Fisheries, 61(4) : 54-58

Hammer O, Harper DAT, Ryan PD. 2001. Past: Paleontological Statistics software package for education and data analysis. Palaeotological Electronica, 4(1): 9

Leary RF, Allendorf FW. 1989: Fluctuating asymmetry as an indicator of stress: implications for conservation biology. Trends in Ecology and Evolution, 4: 214-217

Marquez Eladio. 2007. Sage: Symmetry and Asymmetry in Geometric Data Version 1.05 (compiled09/17/08).http://www.personal.umich.edu/~emarquez/morph/.

Mpho M, Holloway GJ, Callaghan A. 2000. The effect of larval density on life history and wing asymmetry in the mosquito Culex pipiens. Bulletin of Entomological Research, 90: 279-283

Musikasung W, Danayadol Y, Songsangjinda P. 2006. Stomach content and ecological feature of *Scatophagus argus* (Linnaeus) in Songkhla Lake. Technical Paper No. 47. Coastal Aquaculture Research Institute, Coastal Fisheries Research and Development Bureau, Philippines

Natividad EMC, Dalundong ARO, Ecot J, Jumawan JH, Torres MAJ, Requieron EA. 2015. Fluctuating asymmetry in the body shapes of Gobies *Glossogobius celebius* (Valenciennes, 1837) from Lake Sebu,

South Cotabato, Philippines
Palmer AR, Strobeck C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics, 17: 391-421
Parsons PA. 1961. Fly size, emergence time and sternopleural chaeta number in Drosophila. Heredity, 16: 455-473
Parsons PA. 1962. Maternal age and developmental variability. Journal of Experimental Biology, 39: 251-260
Parsons PA. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biological Reviews, 65: 131-145
Parsons PA. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. Heredity, 68: 361–364
Requieron E, Torres MAJ, Manting MME, Demayo CG. 2010. Relative warp analysis of body shape variation in three congeneric species of ponyfishes (Teleostei: Perciformes: Leiognathidae). ICSCT, 2: 301-305
Sivan G, Radhakrishnan CK. 2011. Food, Feeding Habits and Biochemical Composition of *Scatophagus argus*. Turkish Journal of Fisheries and Aquatic Sciences, 11: 603-608
Van Valen L. 1962. A study of fluctuating asymmetry. Evolution, 16: 125-142
Velichovic. 2004: Chromosomal aberrancy and the level of fluctuating asymmetry in black-striped mouse (*Apodemus agrarius*): effects of disturbed environment. Hereditas, 140: 112-122
Vollestad L, Hindark A, Moller AP. 1999. A meta-analysis of fluctuating asymmetry in relation to heterozygosity. Heredity, 83: 206-218