Fluctuating asymmetry employed in analyzing development instability of *Cheilopogon pinnatibarbatus* (Bangsi) from Cabadbaran City, Agusan del Norte, Philippines

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Abstract. The study was aimed to analyze the developmental instability of *Cheilopogon pinnatibarbatus* collected from the coastal areas of Cabadbaran City, Agusan del Norte, Philippines. Reports from the fisherfolks in the area suggested decreasing fish catch of the species. The prevailing samples were essential in analyzing developmental instability. Landmark analyses were obtained using thin-plate spline (TPS) series and loaded into Symmetry and Asymmetry in Geometric Data (SAGE) software. Procrustes ANOVA showed a highly significant difference ($p < 0.0001$) on the three measured factors for fluctuating asymmetry (FA). These factors were individuals, sides, and interaction of individuals and sides which were noted to be asymmetrical. The results of principal components scores indicated high fluctuating asymmetry of female (68.0391%) and male (90.2138%) cumulative values. In females, affected landmarks largely came from posterior insertion of dorsal spine, dorsal and ventral insertion of caudal fin as well as midpoint of caudal border of hypural plate, and the anterior insertion of anal fin. In males, the commonly affected landmarks were the snout tip, posterior end of nuchal spine, anterior and posterior insertion of dorsal spine, anterior and posterior insertion of anal fin, and dorsal base of pelvic fin. High FA values provided evidence of a stressful adaptation of the fish species in an unstable environment during its development. The study presented the use of FA tool in evaluating the developmental instability of the species.

Key Words: aquatic ecosystem, developmental instability, Geometric Morphometrics, ecology.

Introduction. *Cheilopogon pinnatibarbatus* locally known in the Philippines as "bangsi" belongs to family Exocoetidae, is widely distributed in the tropical and subtropical areas around the world (Oxenford et al 1995; Monteiro et al 1998). It is one commercially and economically important species, which is also kept in aquaria as a ornamental fish (Oliveira et al 2015; Gomes et al 1998; Parin 2002). *C. pinnatibarbatus* is distinguished by its elongated body, with a long pectoral fin and silver color. They usually feed on planktonic invertebrates at night (Oxenford et al 1995; Monteiro et al 1998). They inhabit marine waters which many stressors and disturbances from polluted environment contribute to the alteration of physiological system of the organism.

Fluctuating Asymmetry (FA) is one of the advanced methods that is suitable in studying variations of an organism (Hermita et al 2013). FA is used to measure the ability of an organism to maintain homeostasis and provides evidence of stress in fish populations (Van Valen 1962; Palmer 1994). *C. pinnatibarbatus*. Variations of genetically identical organisms that experience same stresses in the environment can be measured using FA. The measured morphological variations between fishes indicate the fitness of populations and the quality of fish stock (Parsons 1961, 1962, 1990, 1992; Van Valen 1962; Palmer & Strobeck 1986; Leary & Allendorf 1989). High trait FA is associated with the stresses and an alteration of the function of a biological system during its development. Moreover, a high value of FA showed lower survival rates acted upon by a stressful and subsidiary environment (Hermita et al 2013). The resulting FA indicates developmental instability as the fish buffers the effect of polluted environment.

Cabadbaran City is situated in the province of Agusan del Norte, northeastern part of Mindanao island. It is regarded as the most populated area in the province. The rich mineral deposits in the mountain areas gave rise to mining activities. Along with economic developments and industrialization, coastal areas were reported to be increasing trend in pollution (CTI Engineering International Co. Ltd; Halcrow; and Woodfiled Consultants, Inc. 2008). The contaminants cause growth reduction and prejudice on reproduction depending level of disturbance in the environment. Exposure to some of these stressors may lead to changes and alterations to some morphological aspects of organisms in response to the severity of the stressors contact (Jenkins 2004).

This study was aimed to determine the FA of *C. pinnatibarbatus* (bangsi) which were previously abundant in coastal areas of Cabadbaran City with accordance by the fish catch of the fishermen in the area. The decreasing catch of this fish was a concern from interviewed fishermen in the area. Presently, there were no reports on the status of *C. pinnatibarbatus* in the area, since we cannot find an article that can provide information about the status of the species in the area. There were limited articles in the condition of coastal areas in Cabadbaran City. Hence, this study is very important in updating reports on the use of FA in analyzing developmental instability of *C. pinnatibarbatus*.

Material and Method

Study area. The study area in Cabadbaran City was situated in Agusan del Norte, Philippines. It was geographically located between 9°7'25" North, 125°32'4" East (Figure 1). The fish samples were collected in the month of July, 2015.

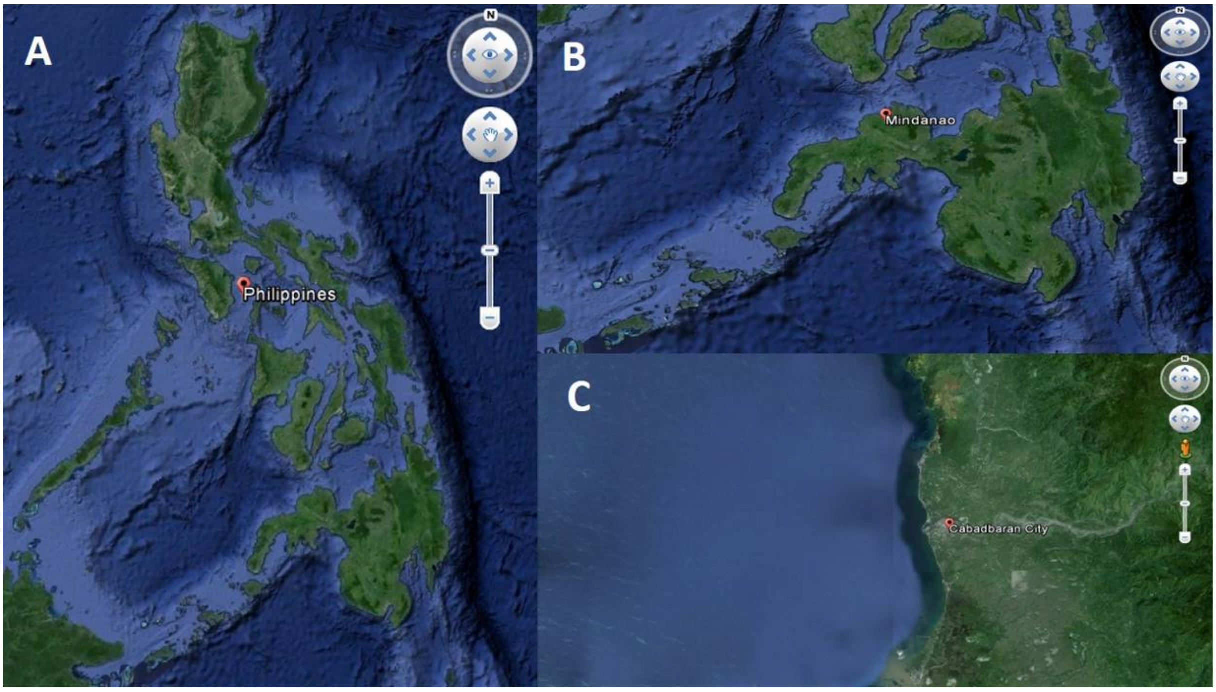

Figure 1. Map of the sampling area showing the Philippines(A), Mindanao island(B), and Cabadbaran City(C).

Processing of fish samples. Fifteen male and fifteen female of *C. pinnatibarbatus* were collected and analyzed. Samples were preserved using 10% formalin and placed in a styrofoam box. Digital imaging was done using Nikon DSLR D5300 with 24.9 megapixels. Left and right lateral side of each individual were photographed with a ruler parallel to its length. The sides were tri-replicated for the purpose in determining digitizing error prior to asymmetry analysis. Captured images were converted to TPS using tpsDig2 program. Sex of specimens was identified by dissection and gonad description after capturing images of the fish that is numbered to determine which fish was dissected. Female fish has yellowish and granular texture gonads while male fish has whitish and non-granular gonads (Requieron et al 2010).

Landmark selection and digitization. Landmark analysis using thin-plate spline (TPS) series was obtained to incorporate the curving part of the specimen in the images. Standard forms of the digitized landmarks in fish morphometrics were applied through tpsDig2. To get homogenous outline on the body shape of specimen standard form of digitized landmark used with a total of 16 landmarks (equivalent to 16 X and 16 Y Cartesian coordinates) were identified to represent best the exterior figure of the fish body as shown in Figure 2 and described in Table 1 (Natividad et al 2015).

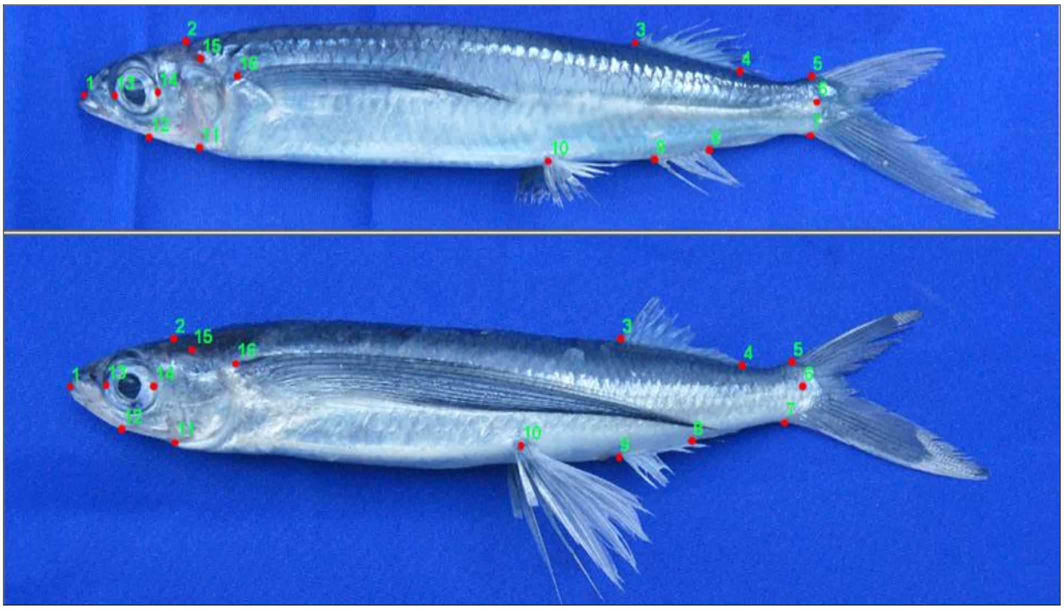

Figure 2. Landmark point of the female (upper) and male (lower) of *C. pinnatibarbatus*.

Table 1

Description of the landmark points in the body shape of *C. pinnatibarbatus* according to Paña et al (2015)

No.	*Description*
1	Snout tip
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

Shape analysis. Generated x and y coordinates served as a baseline data in evaluating FA of *C. pinnatibarbatus*. Symmetry and Asymmetry in Geometric Data (SAGE) were used from the left and right platform landmark coordinates of the TPS (Marquez 2007).

The symmetrized data sets and residuals from symmetric components were generated by SAGE. This was used for the identification of geometric data of object with essential on its asymmetry. Procrustes ANOVA was used to determine significant differences in the symmetry of the factors considered. These factors were individuals, sides and interaction of individuals and sides of *C. pinnatibarbatus*. Level of significance was tested at $p < 0.0001$. The variations of Sides and Interaction of individual and sides were also indicated. Percentage (%) FA were obtained and compared between the sexes (Natividad et al 2015).

Intraspecific variation between sexes. Comparisons between sexes and individual asymmetry analysis obtained using principal component analysis that is illustrated using relevant statistical representation such as histogram, box plot and scattered plot with the use of Paleontological Statistics (PAST) software (Hammer et al 2001).

Results and Discussion. Fluctuation on the body shape of the samples was evaluated through Procrustes Anova (Table 2). There were three factors considered in the analysis and these were: individuals, sides, and interaction between individuals and sides. The analysis was applied to both female and male samples. The results on Procustes Anova revealed a highly significant differences on the three factors considered ($p < 0.0001$). The individual fish samples were asymmetrical with other individuals. The left and right side of the samples were also asymmetrical. Also, the interaction of individuals and sides also contributed to accounted assymetry of the samples. The analysis revealed evidences of FA on the *C. pinnatibarbatus* samples collected from Cabadbaran City. Findings were observed to both female and male samples.

Data showed that samples of *C. pinnatibarbatus* in Cabadbaran City were already asymmetrical in terms of individual symmetry, bilateral symmetry and the interaction between individuals and sides. The observed FA could be an indication that the species sampled were under environmental stress in the area. Symmetrical appearance on the fish species were expected under normal conditions. However, poor water quality of the disturbed environment affected the morphological characteristics during the development of the fish species. The increase exposure in disturbed and polluted water conditions eventually lead to asymmetrical appearance and alter the growth and developmentof *C. pinnatibarbatus*in the sampling area. Thus, this developed FA occurred by buffering the environmental disturbances which eventually altered the developmental hemeostasis of an organism and developed variable phenotypic cherecteristic (Parsons 1990).

Table 2

Procrustes ANOVA shape of *C. pinnatibarbatus* (Bangsi) in terms of sexes

Factors	*SS*	*DF*	*MS*	*F*	*P-value*
		Female			
Individuals	0.1879	392	0.0005	15.4577	<0.0001**
Sides	0.006	28	0.0002	6.9442	<0.0001**
Individual x sides	0.0122	392	0	6.5451	<0.0001**
Measurement error	0.008	1680	0	--------	----------
		Male			
Individuals	0.0934	392	0.0002	4.0124	<0.0001**
Sides	0.0093	28	0.0003	5.5674	<0.0001**
Individual x sides	0.0233	392	0.0001	10.8613	<0.0001**
Measurement error	0.0092	1680	0	------	------

** highly significant.

Principal component analysis was applied to determine the affected landmarks using the symmetry and asymmetry scores. There were four principal components (PC) considered in female samples (PC1-PC4) and five PC considered in male samples (PC1-PC5). The highest PC scores determined landmarks which were commonly affected in FA of the samples (Table 3). Skewness of the histogram was reflected in every PC score along with the deformation grid to determine affected landmarks (Figures 3 and 4).

In female samples, the four principal components (PC) represented 91% of the cumulative variation. PC1 has the highest variation accounted 63.70%. These were portion of the head (rostral tip and nuchal spine) and the fins (parts of caudal, anal, and pelvic fins). The commonly affected landmarks in the considered four PC scores were 4, 5, 6, 7, and 9. These were some portions of the fins (parts of caudal, anal, and pelvic fins). In male samples, the five PC also constituted 91% of the cumulative variations. PC1 contributed the highest accounted variation with 37.30%. The commonly affected landmarks in male samples were landmarks 1, 2, 3, 4, 8, 9, and 10 (Table 3). These landmarks were largely of the head (rostral tip and nuchal spine), the fins (anterior and posterior insertion of dorsal and anal fin and dorsal base of pelvic fin).

Table 3

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

PCA	*Individual (symmetry)*	*Sides (directional symmetry)*	*Interaction (fluctuating asymmetry)*	*Affected landmarks*
			Female	
PC1	63.699%	100%	27.993%	1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16
PC2	10.864%		15.764%	1, 4, 5, 6, 8, 9, 10, 11, 13, 14, 16
PC3	9.706%		13.519%	4, 5, 7, 8, 9, 10
PC4	6.949%		10.763%	3, 4, 5, 6, 7, 8
	91.218%		68.039%	
			Male	
PC1	37.304%	100%	42.831%	1, 2, 3, 4, 5, 8, 9, 10, 15
PC2	21.529%		17.998%	1, 3, 4, 6, 7, 8, 9, 10, 11, 16
PC3	15.092%		14.656%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
PC4	10.638%		9.220%	2, 4, 5, 6, 8, 9, 10, 15
PC5	6.671%		5.507%	1, 2, 3, 9, 11, 14, 15, 16
	91.233%		90.214%	

It was observed that in females, the heavily affected landmarks were portion of the fins while in males were largely the head and in fins. It was interesting to note that affected landmarks were different from female and male samples. These affected landmarks were further shown in deformation grid and histogram of the values revealed skewness suggesting asymmetry in body form (Figures 3, 4 and 5).

Thus, the results indicates that the *C. pinnatibabatus* species of Cabadbaran City was already affected by the contaminants and pollutants in the area. Since, there were reports that the areas encouters many pollutants along with the economic development that may contribute to the perbutations and fluctuating asymmetry of a species (CTI Engineering International Co. Ltd; Halcrow; and Woodfiled Consultants, Inc. 2008).

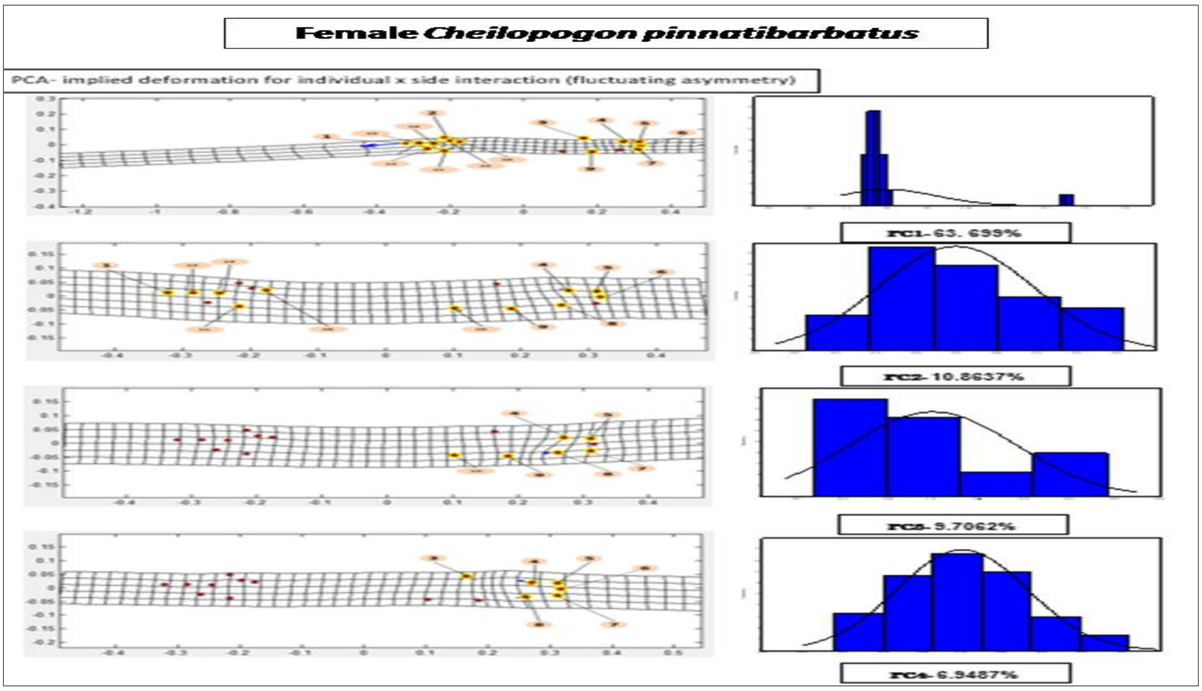

Figure 3. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *C. pinnatibarbatus* female.

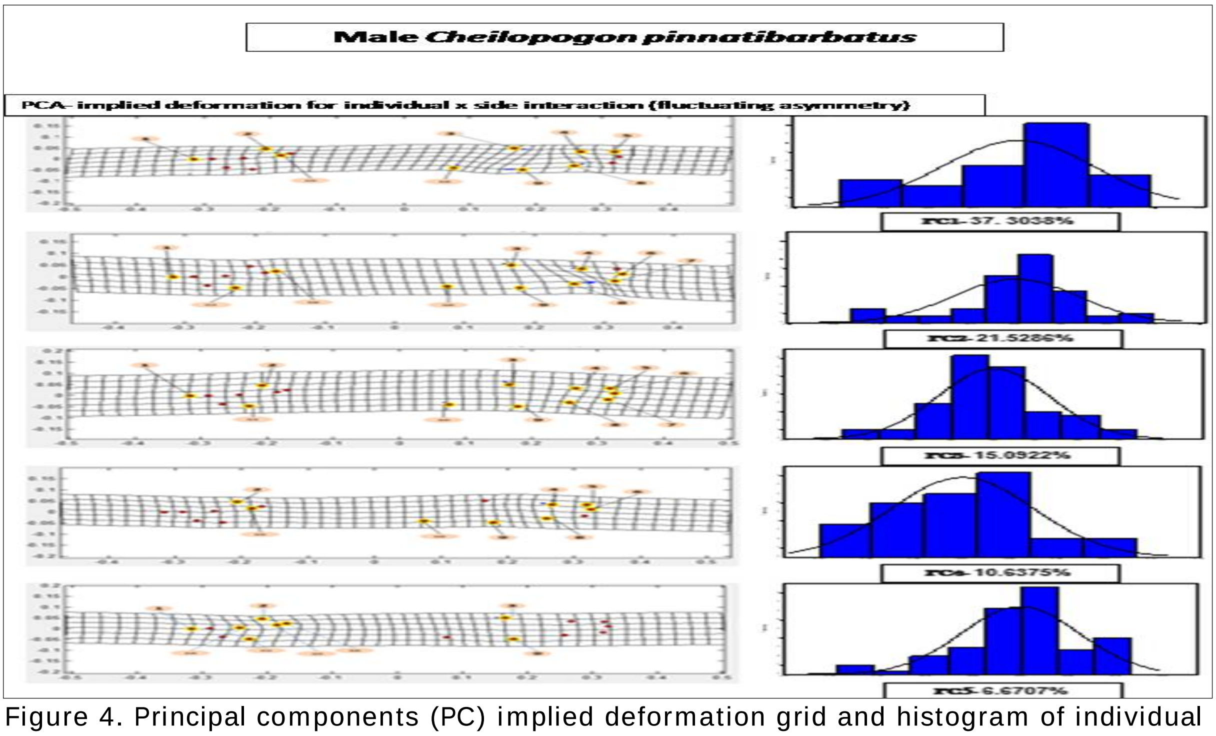

Figure 4. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *C. pinnatibarbatus* male.

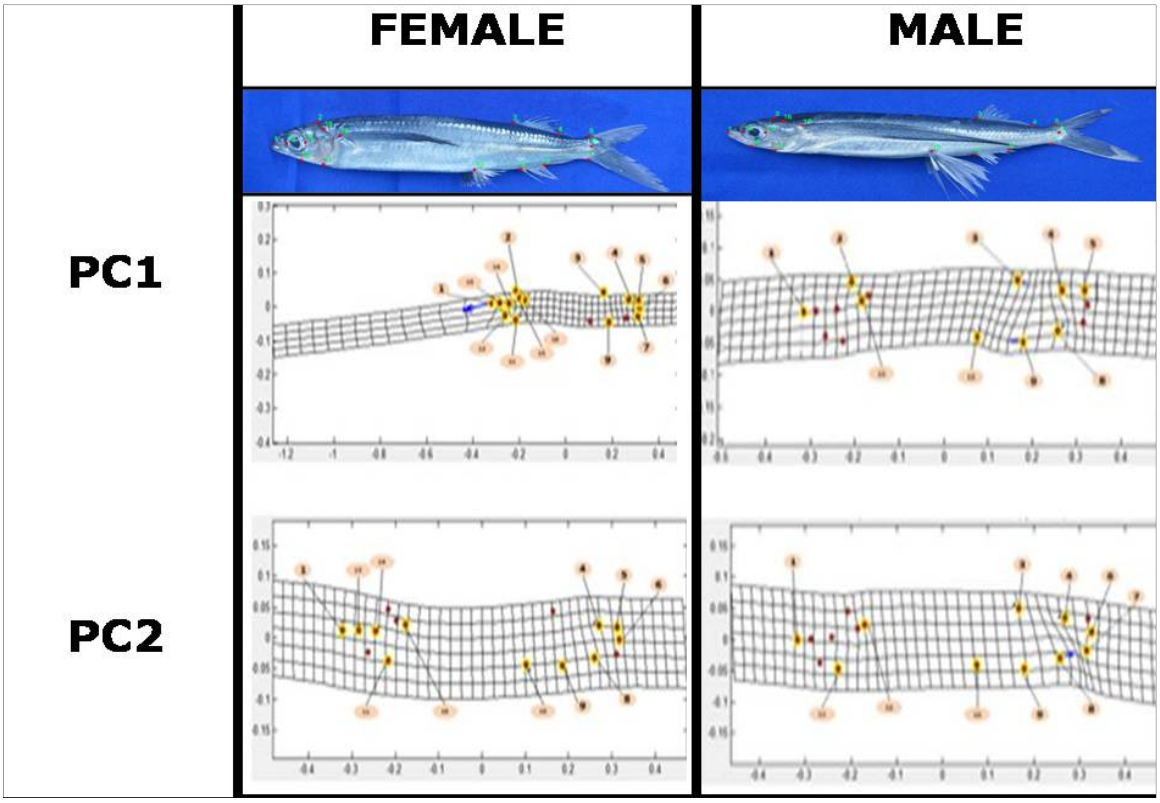

Figure 5. Actualized picture of digitized male and female fish with the affected landmarks shown in PCA-deformation grid for PC1 and PC2.

Conclusions. The study verified the use of FA as a tool in examining the direct contact of stressors in the environment of *C. pinnatibarbatus* samples in Cabadbaran City marine ecosystem. It is been reported that mineral resources attracted mining activities and the leaching into aquatic environments provided the sources of environmental stress. Yet, there were no studies conducted in assessing the condition of *C. pinnatibarbatus* that may be exposed to possible pollutants from Cabadbaran marine ecosystem. The statistical results showed high variations ($p < 0.0001$) on the left and right sides of the bilateral specimen that has 90.2138% in male and 68.0391% in female indicated a high FA. The study demonstrated the use of FA in determining the possible condition of aquatic ecosystem in Cababadbaran marine water. The results also provided information that may used in the fishery of *C. pinnatibarbatus* in the area.

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