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# Fluctuating asymmetry in the body shapes of Threadfin Bream Fish, *Nemipterus japonicus* as a stress indicator in Surigao River, Surigao del Norte, Philippines

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**Abstract**

Fluctuating asymmetry study on the body shapes of *Nemipterus japonicus* in Surigao River, Surigao del Norte was conducted to indicate the environmental and aquatic condition in the area. Thirty four samples of *N. japonicus* were gathered, upon sexing there were 19 females and 15 males were sampled and collected. Threadfin Bream Fish was used in this study as a bio-marker due to their tolerance on pollution serving as sensitive markers of disturbances in the area based on their fluctuating asymmetry. In the study, thin-plate spline (TPS) series were utilized, landmark analyses were obtained and subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software. Procrustes ANOVA showed that although individual symmetry depicts no significant difference, Sides (Directional Asymmetry) and Interaction (Fluctuating asymmetry) showed a highly significant difference ($p < 0.0005$*). Principal Component Scores display a high percentage of fluctuating asymmetry in male (81.34%) and female (65.62%) samples. The body shape and directional asymmetry of *N. japonicus* indicates their response to the changing environment. The morphological changes during the ontogeny of this species are associated to the snout length, trunk length and body depth that may be related as a change in feeding habit and adaptation to their environmental conditions. This suggests that FA positively correlated with the results and the fish' FA was useful as a bio-marker of stress, concluding that Surigao River has a poor ecological status.

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**Introduction**

Fluctuating asymmetry (FA) is a commonly used tool in determining the ecological status of a given bodies of water due to its relevant results reflected in the body shapes and symmetries of certain organisms such as fish, snails, and mollusks. It is a great help in assessing the water quality which serves as an indicator prior to the development of a species in an asymmetrical pattern seen in its morphological trait (Ludwig, 1932). In the living world, the normal distribution of an individual can be determined across a population, where asymmetries that are signed (differences in left and right sides) should result to a zero mean (Weyl, 1952). However, there should be an approximate approach to be found in a perfectly symmetrical individuals and this can be demonstrated using fluctuating asymmetry (Astaurov, 1930; Ludwig, 1932). With this, FA can be utilized in measuring the noise and the developmental instability of an individual (Mather, 1953; Soulé, 1967; Van Valen, 1962).

Freshwater ecosystems serve as a sustainable habitat for most plants and animal species which were dictated by environmental conditions that somehow leads to their extinction (Winter, 2003). This resulted in developing tools helpful in determining the condition of a certain water body as well as the organisms' condition in the environment they are exposed in; and this is were fluctuating asymmetry is used (Zakharov and Graham, 1992; Markow, 1994; Møller and Swaddle, 1997; Polak, 2003). The organisms found in the hydrosystems could serve as a bio-indicator because their taxon's specific trait response to stressors which affect their symmetry (Leung and Forbes, 1996). These asymmetries represent the deviations in the levels of genetic variations and environmental stresses being experienced by the individuals and populations during development (Van Valen, 1962; Palmer, 2000; Gangestad and Thornhill, 1999).

In pursuit of studying the fluctuating asymmetry of *Nemipterus japonicus* as a bio-indicator of environmental stresses, Geometrics Morphometrics (GM) was utilized in defining variations in its body shape which can be applicable in many fields especially in freshwater biology (Zakharov and Graham, 1992; Markow, 1994; Møller and Swaddle, 1997; Polak, 2003). Fish serves as bio-markers due to its morphological traits that are clearly seen in their feeding habits and adaptation to environmental conditions as they continue to possessed high intolerability on pollution and relatively sensitive markers (Pascual and Abollo 2005). It is a valuable tool in assessing the population state and adaptation of an organism up to genetic level (Jones, 1987). This also serves as a great deal of attention because nature is commonly extreme (Van Valen, 1962; Palmer, 1994; Gangestad and Thornhill, 1999).

Recently, Surigao River was exposed to small gold mining activities that were utilizing mercury and an estimated 20 tons of it were discharged into the river bodies of Mindanao particularly in Surigao River. This study will serve as a significant tool in determining the existing chemical speculation of mercury in the environment and especially to humans, which directly affects the health and status of the environment. Results of this study will be used as a basis in making an appropriate intervention and control measures for the treatment and management of mercury pollution in the area as well as for future studies (Narvaez, 2002). It will also validate the importance of assessing the alterations and changes in the aquatic environment and its effects on the population of *Nemipterus japonicus* commonly known as Threadfin Bream Fish particularly found in Surigao River. This pioneering study in the body shapes of Threadfin Bream Fish in Surigao River using FA will survey the vulnerability of *Nemipterus japonicus* in response to environmental stress and as a pollution indicator in the hydrological ecosystem.

**Materials and methods**

*Description of the Area*

The sampling was conducted in Surigao River, Surigao del Norte, Philippines on July 31, 2015. Its geographical coordinates lies between 9°544’11.19” N 125°32’30.40” E. Habitat description was based on the assessment from FAO/WHO Global Forum of Food Safety Regulators (Narvaez, 2002). The coordinates of the sampling sites selected were each recorded using a Global Positioning System (GPS) device.

*Processing of fish samples*

Thirty four samples of *N. japonicus* were gathered; upon sexing there were 19 females and 15 males collected. Threadfin Bream fish individuals were preserved using 1% formalin solution and were placed in a Styrofoam box. Digital imaging was done using DSLR (SP-800uz, 14 megapixels). It was noted that both the left and right lateral side of each sample were taken with a ruler parallel to it for the length determinations of each individuals. The captured images were digitized using tpsDig2 program (version 2.0, Rohlf, 2004) and were saved as a TPS file. After having photographed, the threadfin bream fish’ sex was determined and identified based on (www.fishbase.com). Males and females were identified based on its 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 (Natividad *et al*., 2015).


**Fig. 1.** Map of the sampling area showing the Philippines (A), Surigao City (B) , Surigao River (C).

*Landmark Selection and Digitization*

Using thin-plate spline (TPS) series, landmark analysis were obtained to incorporate curving features within the images. Standard forms of the digitized landmarks in fish morphometrics were applied. Landmarks were selected to provide a homogenous outline of body shape using software tpsDig2 (Figure 2). A total of 16 markers (equivalent to 16 X and 16 Y Cartesian coordinates) were identified to represent best the exterior figure of the fish’ body. Description of the landmark is shown in Table 1. For the analysis, X and Y coordinates of the images’ landmarks were then obtained. Digitization was saved in triplicates for each sample to reduce the discrepancies and errors in plotting the landmark points (Natividad *et al*., 2015).

*Shape Analysis*

Generated x and y coordinates function as a baseline data in analyzing the fluctuating asymmetry of freshwater fishes. Symmetry and Asymmetry in Geometric Data (SAGE) (Marquez, 2007) was used for the left and right flat form landmark coordinates of the TPS which generates the symmetrised data sets and residuals from the symmetric components. This was used for the identification of geometric data of object with essential on its asymmetry see (Figure 3). Procrustes ANOVA was used to determine significant difference in the symmetry of the factors considered. These factors were the individuals, sides and interaction of individuals and sides of N. japonicus. Level of significance was tested at P<0.0001. The variation between the side and the measure of directional asymmetry is also indicated. Percentage (%) of FA`s were obtained and compared among the sexes (Natividad *et al.*, 2015).

*Intraspecific Variation between sexes*

The comparisons among sexes and individual symmetry were analyzed using Principal Component Analysis. The resulting data was used in creating significant statistical illustrations like histogram, box plot and scattered plot through Paleontological Statistics Software (PAST) (Hammer *et al.*, 2001).

## Results and discussions

In the illustration of the body shape fluctuations of *N. japonicus*, Procrustes ANOVA showed a significant level of asymmetry in individuals of both male and female with the left-right size and shape (Table 2 and 3). In the analysis of each individual, three factors were used as a basis that includes each sample, their sides, and the interaction of the individuals and its sides.

**Table 1.** Description of the landmark points adapted from Chakraborty *et al.*, 2008.

| No. | Description |
|---|---|
| 1 | Rostral tip of premaxillae |
| 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 Posterior end of |
| 12 | maxilla |
| 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 |

**Table 2.** Procrustes ANOVA for shape of female *Nemipterus japonicas*.

| *Effect* | *SS* | F EMALE *dF* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| Individuals | 0.1328 | 504 | 0.0003 | 3.5349 | 0.0001** |
| Sides | 0.0081 | 28 | 0.0003 | 3.8748 | 0.0001** |
| Individual x Sides | 0.0376 | 504 | 0.0001 | 6.0234 | 0.0001** |
| Measurement error | 0.0263 | 2128 | 0 | --- | --- |

** Highly significant (P<0.001)- $^{ns}$ not significant.

The analysis was done in both sexes considering the (P<0.0001) which showed the fluctuating asymmetry. The results taken was highly significant based on the three factors found in the different individual sides of both male and female *N. japonicus* and also observed in their fluctuating asymmetry.This clearly shows an immense phenotypic variation among the individual species.

**Table 3.** Procrustes ANOVA for shape of male *Nemipterus japonicas*.

| Effect | SS | *M ALE* dF | MS | F | P |
|---|---|---|---|---|---|
| Individuals | 0.0901 | 392 | 0.0002 | 3.7886 | 0.0001** |
| Sides | 0.0233 | 28 | 0.0008 | 13.7436 | 0.0001** |
| Individual x sides | 0.0238 | 392 | 0.0001 | 5.597 | 0.0001** |
| | 0.0182 | 1680 | 0 | --- | --- |
| Measurement error | | | | | |

** Highly significant (P<0.001)- $^{ns}$ not significant.

**Table 4.** Principal component scores showing the value of symmetry and asymmetry scores with the summary of the affected landmarks.

**FEMALE**

| PCA | Individual (Symmetry) | Sides (Directional Asymmetry) | Interaction (Fluctuating Asymmetry) | Affected landmarks |
|---|---|---|---|---|
| PC1 | 45.0597% | 100% | 23.5392% | 1,4,5,6,10,11,12,14,16 |
| PC2 | 14.6032% | | 19.8652% | 1,2,4,8,9,10,12,15 |
| PC3 | 10.3982% | | 15.1187% | 2,3,4,10,11,14,15 |
| PC4 | 7.3417% | | 11.281% | 2,4,8,9,10,11,12,14,16 |
| PC5 | 6.0574% | | 7.0998% | 1,2,4,6,7,9,11,15,16 |
| | 83.4602% | | 65. 6229% | |

To validate the results, the morphology of *N. japonicus* was also studied to compare their individual sides interaction. It showed that *N. japonicus* was already asymmetrical, the same with those seen on their pooled samples for both sexes. This only suggests that an individual's species encounters an increase of difficulty in the maintenance for a precise development resulting to a negative effect to the population over time (Markow, 1995). With this, *N. japonicus* can be considered as a good bio-indicator of stresses to determine the current status of an aquatic environment in a natural way that could alter their physique traits and reflects their exposure to stressors, and the presence of pollutants in their habitat.

This would enable humans to be aware and prepare of such environmental changes. In general, the higher the FA, the lower the homeostatic development on the genetic level of an organism and could indicate a disturbed environment (Parsons, 1990).

On the other hand, depicting the environmental conditions affected by the current pollutants and stressors were shown in their morphological structures (Tables 4 and 5) during their growth and development based on the analysed data revealing the fluctuating asymmetry (Bonada and Williams, 2002).

This implies that the species has the ability to tolerate such environmental stresses based on the changes in their symmetry (Van Valen, 1962). The results suggest that the physique trait reflects the fluctuations on their symmetry leading to the individuals' developmental instability.

**Table 5.** Principal component scores showing the value of symmetry and asymmetry scores with the summary of the affected landmarks.

**M A L E**

| PCA | Individual (Symmetry) | Sides (Directional Asymmetr) | Interaction (Fluctuating Asymmetry) | *Affected* landmarks |
|---|---|---|---|---|
| PC1 | 34.5862% | 100% | 36.0722% | 1,2,5,6,7,8,11,12,13,14,15 |
| PC2 | 27.4588% | | 21.4872% | 1,3,4,5,6,7,8,9,10,11,14,15,16 |
| PC3 | 16.8234% | | 12.9116% | 1,2,3,5,6,8,9,10,12,16, |
| PC4 | 6.1493% | | 10. 8676% | 1,2,10,11,12,14,15,16 |
| | 85. 0177% | | 81.3386% | |

The fluctuating symmetry and asymmetry were scored in order to determine the affected landmarks through the use of Principal component analysis. Landmarks and histograms were used to reflect the score in every PC samples which could point out the affected landmarks (Fig. 4 and 5).

The three principal components (PC) in females have a cumulative variation of 83. 4602%. The highest variation was accounted with 45. 0597% in PC1. The commonly affected landmarks were 1, 4, 5,6,10,11,12,14, and 16 (Table 4). These were in portion of the head (rostral tip and nuchal spine) and the fins (parts of caudal, anal, and pelvic fins). For the male samples, the three PC constituted an 85.0177% for the cumulative variation. PC 1 was accounted as the highest in terms of variations with 34.5862%.


**Fig. 2.** Landmark points of male (A) and female (B) *N. japonicus*.

The commonly affected landmarks in male samples were landmarks 1, 2,5,6,7,8,11,12,13,14, and 15 (Table 5). These landmarks were largely on the head (rostral tip, nuchal spine, posterior end of maxilla, anterior margin through midline of orbit, and dorsal end of operculum); and dorsal insertion of caudal fin.

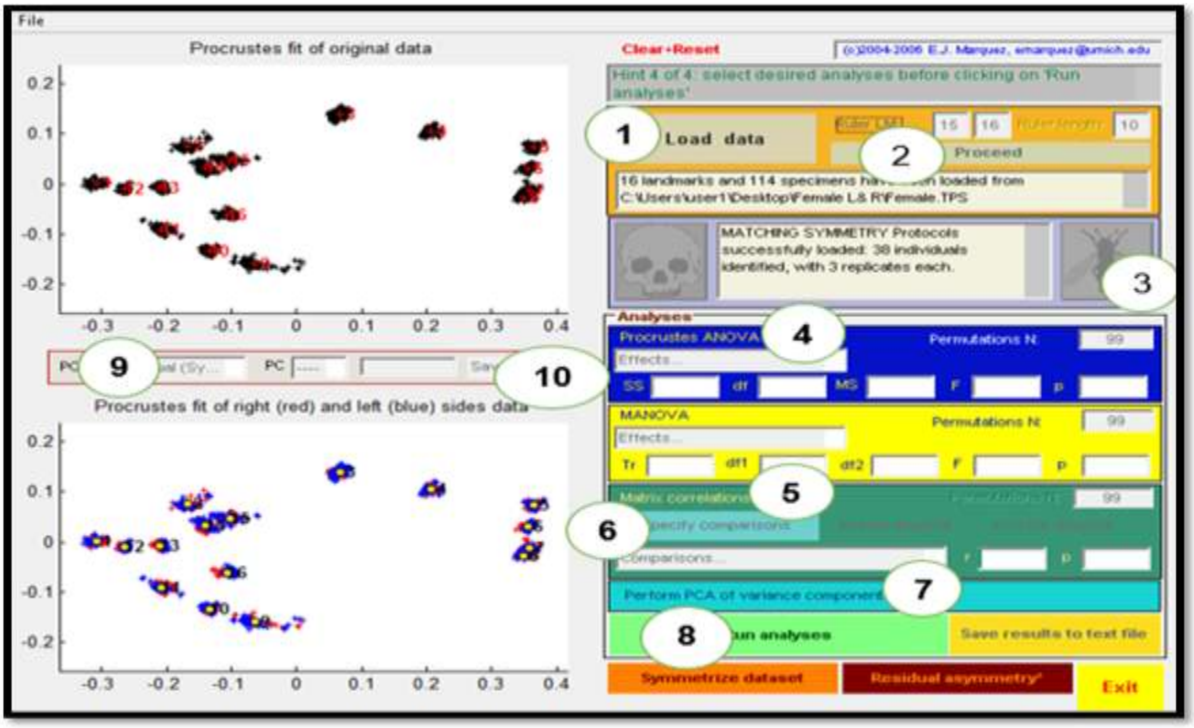


**Fig. 3.** Overall schematic diagram of shape analysis using SAGE software.

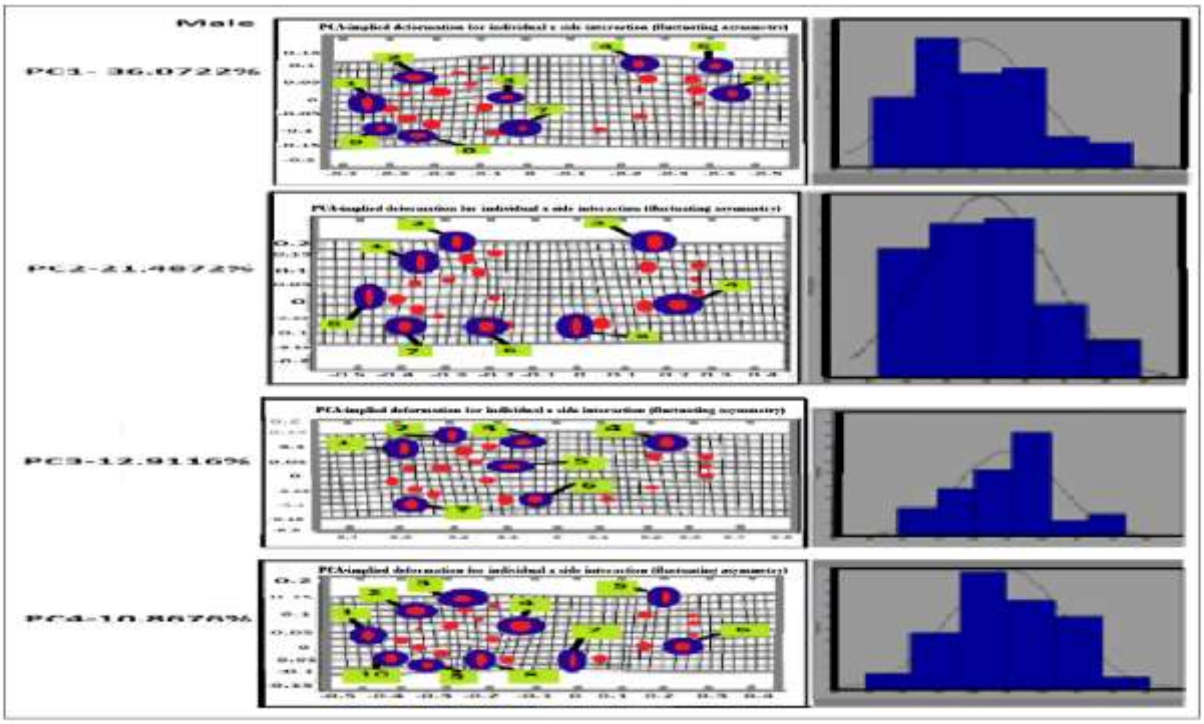

**Fig. 4.** Principal components (PC) implied deformation grid and a histogram of individual (symmetric) in *N. japonicus* male species.

The analysis of landmarks implied that the head and fins were heavily affected in female while head and caudal fin in males *N. japonicus*. These landmarks varies in both the sampled sexes. The deformation grid and histiogram were utilized to reveal the affected landmarks in the asymmetrical body form of fishes (Fig. 4, 5 and 6).

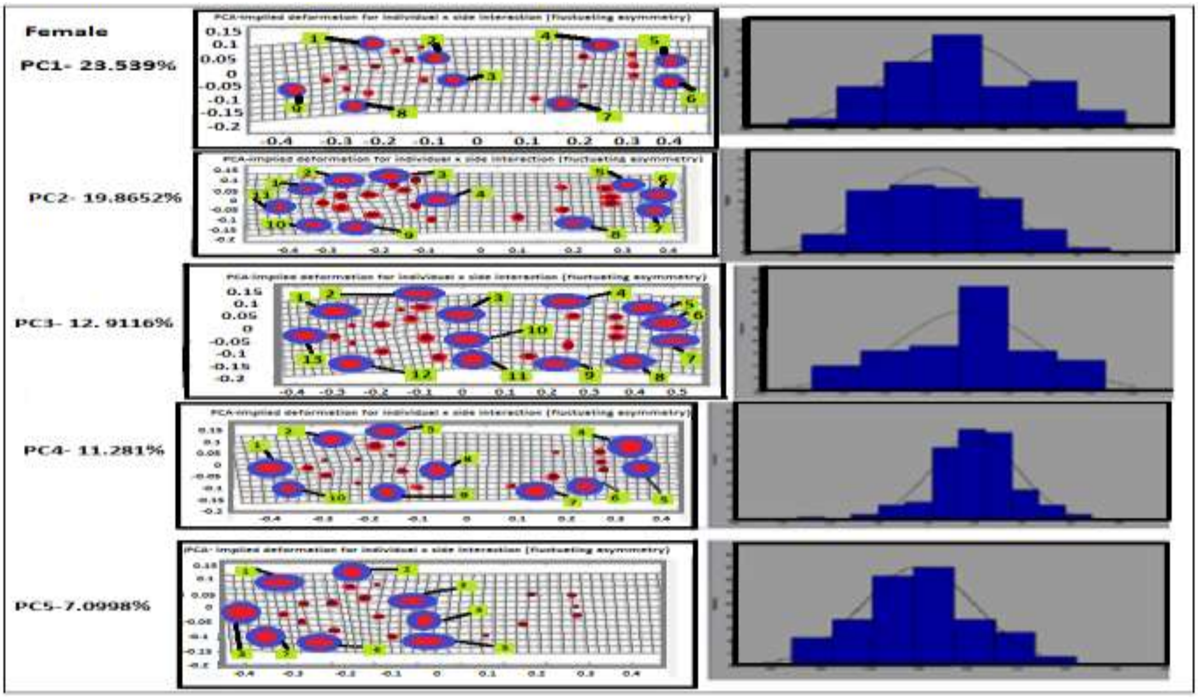


**Fig. 5.** Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *N. japonicus* female species.

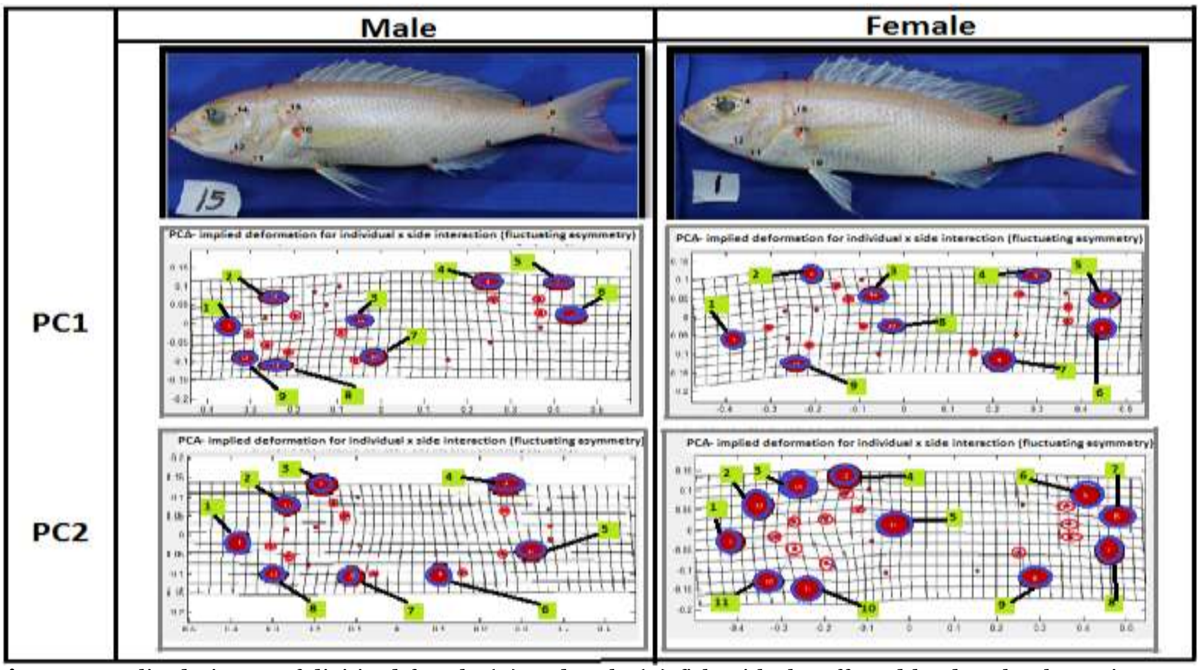


**Fig. 6.** Actualized picture of digitized female (A) and male (B) fish with the affected landmarks shown in PCA-deformation grid for PC1 and PC2.

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**Conclusion**

After undergoing several statistical analysis, results showed high deviations (P<0.001) on both sides of the male (81.3386%) and female (65. 6229%), revealing a very high fluctuating asymmetry for the both species. Male species is somehow more affected than the female species since they have higher values in the Principal Component Analysis (PCA). All in all, we can determine now the ecological status of the hydrosystem being studied. Therefore, FA observed on the fishes confirms that Surigao River has a poor water quality sustained with the presence of small gold mining activities that utilizes mercury. As for the appropriate intervention and control measures for the treatment and management of mercury pollution in the area, further study should be done focusing only on mercury and its accumulation on the river as well as on the aquatic organisms thriving in the area.

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