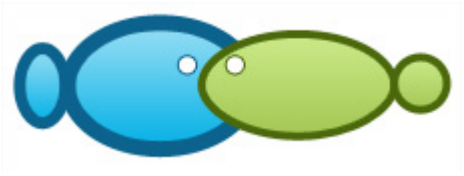

Assessment study using fluctuating asymmetry in the body shapes of *Trichopodus trichopterus* as bio-indicator of stress in Ubod-Ubod Creek, Baan, Butuan City, Agusan del Norte, Philippines

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Abstract. Fluctuating asymmetry was used to determine the ecological condition of Ubod-Ubod Creek, Baan, Butuan City. This method would measure the level of the stresses that affects *Trichopodus trichopterus* body symmetry and could also assess water quality of the said area which could affect it's morphological traits. In this field of study, thin-plate spline (TPS) series was utilized, landmark analyses were obtained and subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software. Results in Procrustes ANOVA showed that although individual symmetry depicts no significant difference, Sides (Directional Asymmetry) and Interaction (Fluctuating asymmetry) showed a highly significant difference ($p < 0.0005$). The results of Principal Component Scores display a high percentage fluctuating asymmetry of male (65.18%) and female (73.73%). In females, Principal Component PC 1 (31.42%) and PC 2 (18.62%) and interaction were found to have significant variations affecting *T. trichopterus* rostral tip of premaxillar, posterior insertion of dorsal fin, dorsal insertion of caudal fin, midpoint of caudal border of hypural plate, anterior insertion of anal fin, ventral end of lower jaw articulation, anterior margin through midline of orbit, posterior margin through midline of orbit, and dorsal base of pectoral fin. In males, PC 1 (26.45%) and PC 2 (22.29%) affected the rostral tip of premaxillar, anterior insertion of dorsal fin, posterior insertion of dorsal fin, posterior insertion of anal fin, anterior insertion of anal fin and dorsal base of pelvic fin. This study shows the importance of using fluctuating asymmetry in determining the status of the said area and also the current condition of the *T. trichopterus.*

Key Words: fluctuating asymmetry, environmental condition, Ubud-Ubud Creek, *Trichopodus trichopterus*.

Introduction. Fish serves as a bio-indicator of environmental conditions due to their tolerance to pollution in aquatic environment that may reflect their feeding habits and tolerance as a sensitive marker (Pascual & Abollo 2005). In determining the grade of pollutants in the morphology of the fish, fluctuating asymmetry (FA) was employed in order to determine the effects of environmental changes made by humans that may alter their population up to genetic level (Jones 1987). FA also measures the noise and the developmental instability of an individual species (Bergstrom & Reimchen 2002). This serves as a valuable detector of stressors of today's extreme environment (Tomkins & Kotiaho 2001). FA, deliberately an aspect for determining developmental stability because it reflects both genetic and environmental stress and this has been an important

theory in evolutionary biology for decades (Palmer 1994). FA performs to be taxon, feature and stress specific, indicating that environmental noise has a different impact on developing organs and organisms (David et al 1998; Roy & Stanton 1999; Vollestad et al 1999; Bjorksten et al 2001).

FA noticeably used in several studies such as an indication of organisms exposure to stressors, maintenance based on their growth and development in a random directional pattern (Palmer & Strobeck 1986). FA became a significant tool in morphometric studies because it could determine random deviations based on their morphological traits (Swaddle 2003). As well as, FA has been identified to be an efficient tool for measuring developmental stability of changing environment (Lecera et al 2015). The genetic level of an individual species could also reflect their response to the sudden change of their environment which could alter their genetic makeup and resulted to genetic variations and diversity in their population (Trono et al 2015). FA constitutes a wide range of variability in terms of measuring developmental stress due to environmental condition. Thus, using FA as an indicator of developmental stability and assessing the extent of environmental stress is established on the theory that a disturbed environment would effect in higher FA levels than those observed in optimum environments (Velickovic 2004; Parsons 1961, 1962, 1990, 1992; Van Valen 1962; Palmer & Strobeck 1986; Leong et al 2013).

In expressions of biological diversity, freshwater habitats are classified as one of the richest (Ward & Tockner 2001). As mentioned, freshwater ecosystems have been critical in sustaining life which directly affects the aquatic species in their environment (Combes 2003). Such, studies show that commonly threatened groups are freshwater fishes because of great susceptibility to aquatic modification (Laffaille et al 2005; Kang et al 2009; Sarkar et al 2008). In relation to the assessment of *Trichopodus trichopterus* as bio-indicator of stressors, Geometrics Morphometrics was applied in order to detect the variations in the body shapes of fishes in most freshwater species (Swaddle 2003). This tool became an efficient edge to evaluate the survival and adaptation of an organism (Jones 1987).

At present, the Ubod-Ubod Creek's aquatic environment was affected by human activities due to sewage disposals, household run-offs, and smoke from vehicles which could enter the atmosphere and then fall back to earth as rain which causes water pollution even at a little by little process. This study encourages the researcher to assess and validate that fishes serves as a bio indicator of pollutions which currently exist in their environment. This study also serves as a basis for the intervention and control measures for the management of water pollution.

This present study serves as a significant tool in water quality assessment in determining stressors, and environmental changes which could assess fishes sensitivity to stressors that enable them to become a good bio indicator of pollution of which sudden alterations would affect the bodies of water in Ubod-Ubod Creek. FA in *T. trichopterus*, easily reflects and indicates various deviations happen to their symmetries, at the same time assessing the ecological condition and the water quality found in Ubod-Ubod Creek of Butuan City, Agusan Del Norte.

Material and Method. The study area was part of Butuan City, Agusan del Norte. It was located in Brgy. Baan, Butuan City and geographically lying between $8^{o}57^{'}16.86^{"}$N $125^{o}34^{'}16.57^{"}$E (Figure 1). The sampling of fishes was conducted in the month of July 31, 2015.

Processing of fish samples. The collected fish samples were using fish identification manuals and verified based on www.fishbase.org. A total of 60 samples were collected, 19 were females and 41 were males. *T. trichopterus* 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. Sex was determined and identified based on

www.fishbase.com. Males and females were identified by direct examination of their gonads and dorsal fin: males have a longer and narrow fin, females have a shorter and rounded one. Male fish had whitish soft textured gonads while yellowish coarsely textured gonads with eggs for female specimens (Natividad et al 2015).

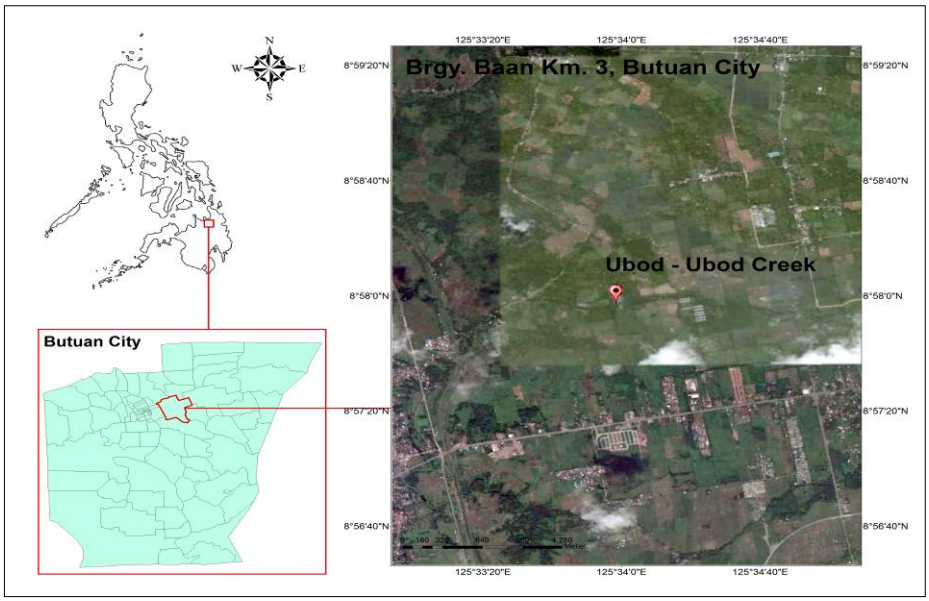

Figure 1. Map showing the Philippines (up-left); Map showing the location of Butuan City (down-left); Map showing the location of Ubod-Ubod Creek located in Brgy. Baan (right).

Landmark selection and digitization. Using thin-plate spline (TPS) series, landmark analyzes 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 was shown in Table 1. For the analysis, X and Y coordinates of landmarks on images were then obtained. Digitizations were copied in triplicates for each sample to reduce the discrepancies and errors in plotting the landmark points (Natividad et al 2015).

Figure 2. Landmark point of *T. trichopterus*: female (A) and male (B).

Table 1

Description of the landmark points in the body shape of *T. trichopterus* (adapted from Chakrabarty 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
12	Posterior end of 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

Shape analysis. Generated x and y coordinates had functioned as baseline data in analyzing fluctuating asymmetry of freshwater fishes. Symmetry and Asymmetry in Geometric Data (SAGE, Marquez 2007) were used for the left and right flatform landmark coordinates of the TPS.

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 see (Figure 3). Procrustes ANOVA was used to determine significant difference in the symmetry of the factors considered. These factors were individuals, sides and interaction of individuals and sides of *T. trichopterus* Level of significance was tested at $p < 0.0001$. The variation between the side and the measure of directional asymmetry also indicates. Percentage (%) FA were obtained and compared between the sexes (Natividad et al 2015).

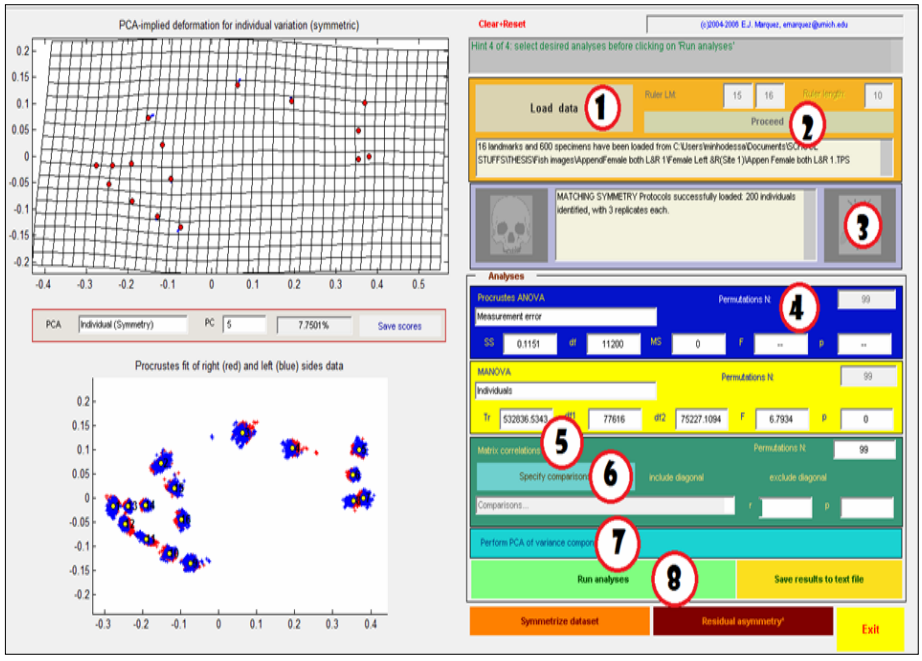

Figure 3. Overall schematic diagram of shape analysis using SAGE software.

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 (PAST) Paleontological Statistics Software (Hammer et al 2001).

Results and Discussion. Procrustes ANOVA was used to illustrate the individual body shape fluctuations. The analysis to compare individual symmetry of left-right size and shape was shown in Table 2. There were 3 factors considered in the analysis the individuals, sides, and the interaction of individuals and sides. The analysis was applied to both male and female of the fish species. The results showed FA for the 3 factors considered and also between male and female ($p < 0.0001$). There was a highly significant difference observed in the individual fish indicating FA when one individual was compared to the other individual samples. The sides also had highly significant difference suggesting FA in the left and right sides of the samples. Generally, the interaction between individuals and sides also showed highly significant difference which implied FA contributed among the interaction of individuals and sides. The FA of the three factors was observed in both male and female fishes.

Table 2

Procrustes ANOVA shape of *T. trichopterus* in terms of sexes

Factors	*SS*	*DF*	*MS*	*F*	*P-value*
		Female			
Individuals	0.1528	504	0.0003	3.1216	<0.0001**
Sides	0.0087	28	0.0003	3.8748	<0.0001**
Individual x Sides	0.0489	504	0.0001	6.0234	<0.0001**
Measurement Error	0.0192	2128	0	-	-
		Male			
Individuals	0.7044	1120	0.0006	5.3263	<0.0001**
Sides	0.0457	28	0.0016	13.82	<0.0001**
Individual x Sides	0.1322	1120	0.0001	2.5728	<0.0001**
Measurement Error	0.2107	4592	0	-	-

** ($p < 0.0001$) highly significant.

The data showed that *T. trichopterus* in Ubod-Ubod creek were already distorted in terms of comparison as an individual, its left and right side morphology, and the interaction of its individuals and sides. The asymmetry in the three considered factors were both similarly observed when samples were pooled as male and female. The asymmetry in the morphology of the three factors among males and females could be a sign that the species in the study area was under environmental stress. Symmetrical appearance on the fish species could be expected under normal conditions. However, the observed FA would suggest that the aquatic ecosystem might comprise pollutants affecting the morphology of the fish. The over exposure to unstable and polluted water condition eventually will lead to asymmetrical appearance of *T. trichopterus* in the sampling area.

The data showed the evidence for FA of the fishes that can be credited to a damaged condition in the environment possibly from contaminants. As a result, the unstable ecosystem will cause the species to change morphologically as these stressors act during their development (Bonada & Williams 2002). Thus, the asymmetry is the manifestation to inability of the species to thrive and buffer environmental disturbances (Van Valen 1962). The developmental instability of *T. trichopterus* eventually resulted to this FA.

Principal component analysis was applied to identify the affected landmarks using the symmetry and asymmetry scores. There were three principal components (PC) considered in male and female samples. The three 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 4 and 5).

In females variations based on the four principal components was 81.24%. The highest variation was accounted 31.41% in PC1. The common affected landmarks to the four PC score were 1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 14, 15, and 16 (Table 3). These were portion of the head (rostral tip and nuchal spine) and the fins (parts of caudal, anal, and pelvic fins). In male samples, the three PC constituted to 65.18% of the cumulative variation. PC 1 contributed the highest accounted variation with 26.45%. The commonly affected landmarks in male samples were landmarks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 (Table 3). These landmarks were largely of 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.

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	48.6735%	100%	31.4176%	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 14, 15
PC2	14.6032%		18.6234%	1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16
PC3	11.6601%		13.8861%	1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 14, 15, 16
PC4	6.7526%		9.808%	1, 2, 3, 4, 6, 7, 9,11, 12, 13,14, 15
	81.2431%		73.7351%	
			Male	
PC1	64.908%	100%	26.455%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
PC2	7.7689%		22.2923%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
PC3	6.7134%		16.4358%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16
	79.3903%		65.1831%	

It was noticed that both females and males, the heavily affected landmarks were portion of the head, fins and caudal fin. It was remarkable to note that affected landmarks were the same 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 4, 5, 6).

A great percentage of FA suggested a significant dissimilarities level of FA. Comparable study also shows that affected morphology of fish caused by environmental condition. According to Ducos & Tabugo (2014, 2015) with *Gafrarium tumidum* as a bioindicator had confirmed that the greater the FA value the higher the stress a species are undertaken due to the environmental situation. Similar studies also validated that in female *Leiopotherapon plumbeus* great significant level of FA specifies difficulty of sustaining specific development that results to adverse effects on the population through time (Markow 1995). Likewise, a high level of FA confirms that organism possess a poor developmental ability (Palmer 1994). The unstable condition of the environment will also cause instability during the development of an organism that also affects its asymmetry (Natividad et al 2015).The result of this study will be an significant information of the present condition of Ubod-Ubod Creek and certainly identifies that FA is said to be an effective indicator of environmental condition.

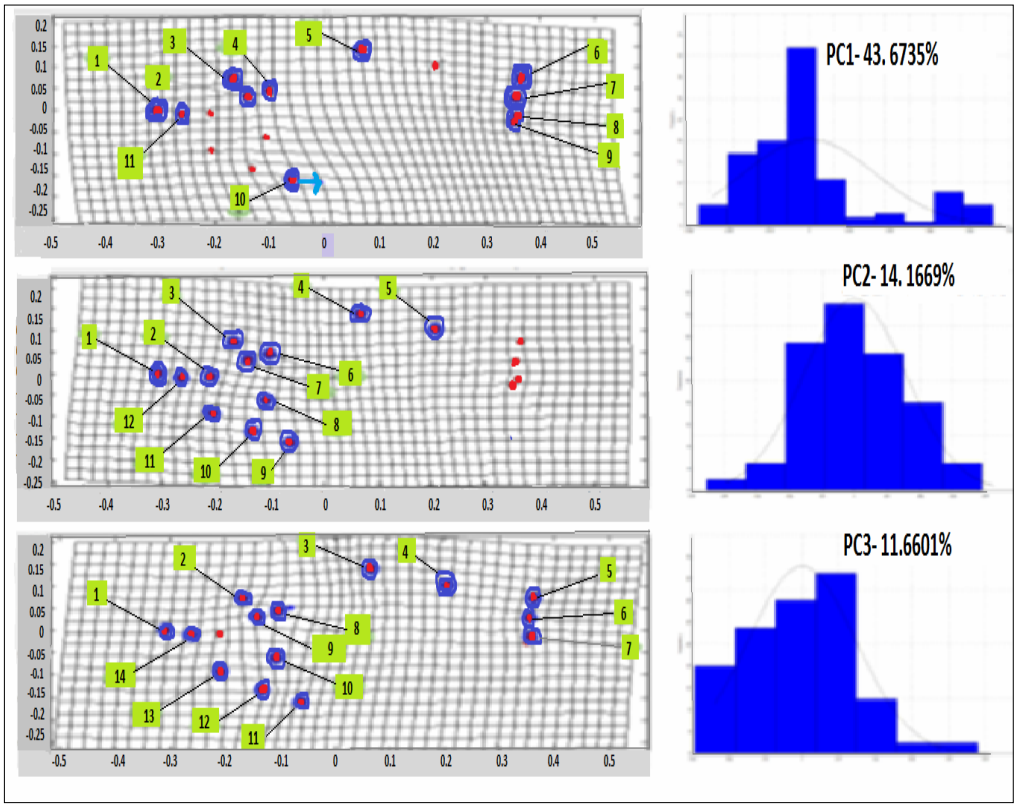

Figure 4. Principal components (PC) implied deformation grid and a histogram of individual (symmetric) in *T. trichopterus* female species.

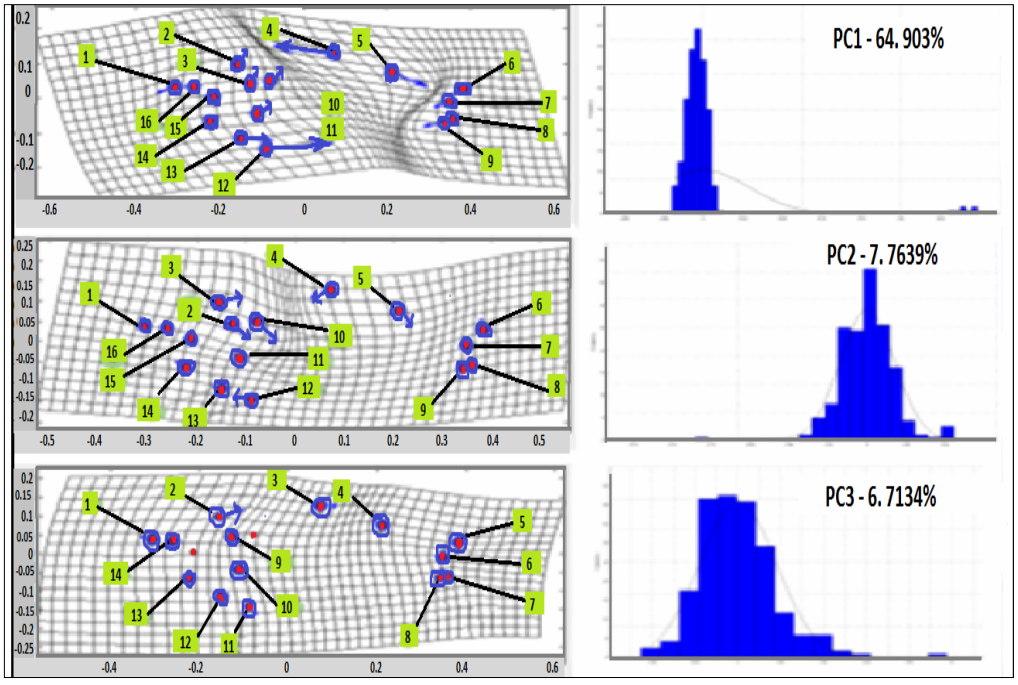

Figure 5. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *T. trichopterus* male species.

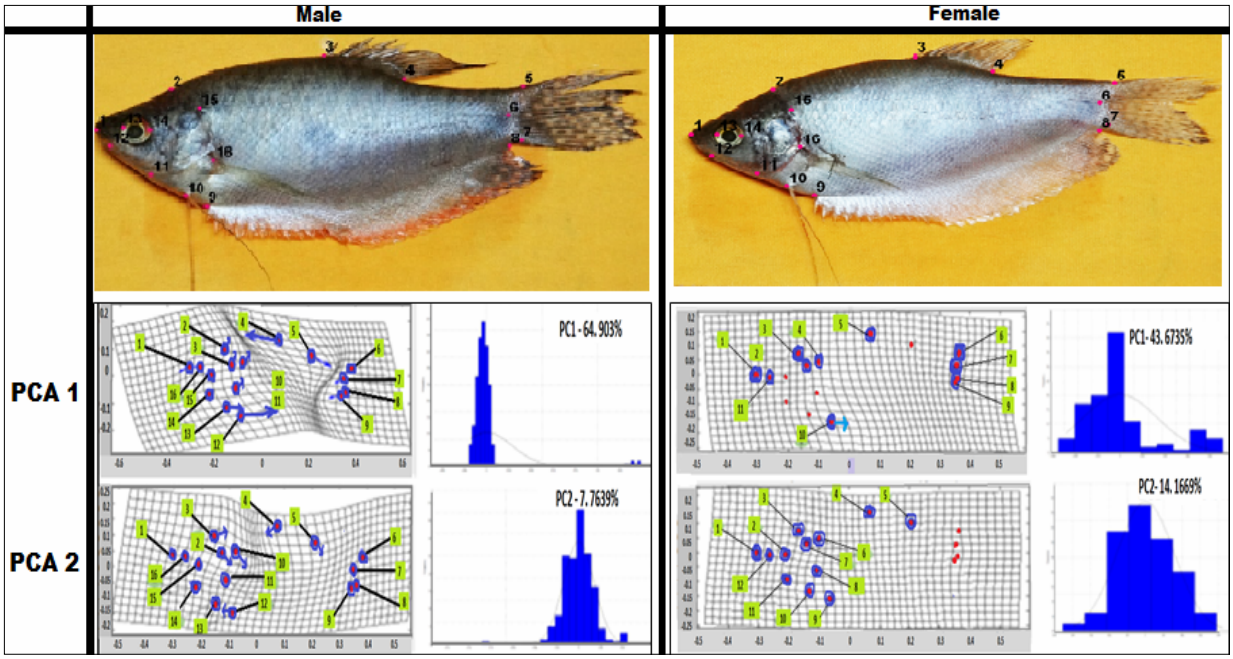

Figure 6. Actualized picture of digitized male (A) and female (B) fish with the affected landmarks shown in PCA- deformation grid for PC1 and PC2.

Conclusions. This study illustrates the significance of FA in assessing environmental stressors and the use of *T. trichopterus* as bio indicators based on the degree of pollution at Ubod-Ubod Creek. Disposal of sewage and household run-offs are the direct activities affecting the morphology of *T. trichopterus.* The result shows a high significance ($p < 0.0001$) of FA on both sexes (female = 81.24% and male = 65.18%) considering the three factors given. It manifested that the sampling area was polluted. The contamination of the Ubod-Ubod creek was a detrimental factor to be a reason to affects the other species that inhabits. Moreover, this would serve as a basis for the intervention and control measures of local government for the management of water pollution for future studies and for the preservation of the native species found at Ubod-Ubod Creek.

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