



Assessment of fluctuating asymmetry in the body shapes of Nile tilapia (*Oreochromis niloticus*) from Masao river, Butuan city, Philippines

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Abstract. Nile tilapia (*Oreochromis niloticus*) is one of the major organisms found in the river and is used as the indicator of developmental noise and predict the status of the river. Thus, this study was conducted to assess the current ecological condition of Masao river, Butuan City using the fluctuating asymmetry in the body shapes of *O. niloticus*. A total of 100 individuals were collected by net fishing techniques. The landmark-based geometric morphometric method was used in determining the variations in the patterns of fluctuating asymmetry (FA) of *O. niloticus* with a total of sixteen bilateral traits. FA were analyzed using symmetry and asymmetry geometric data (SAGE) program version 1.0. Results in Procrustes ANOVA showed highly significant levels of FA ($p < 0.0001^*$) and principal component scores showed a high percentage FA (71.4051%), possibly mean that Masao river is in poor environmental status and fishes in the area experienced greater developmental perturbations and stresses. Principal component (PC) 1 has the highest percentile (44.3596%) of FA. The most common affected landmarks were found in landmarks 1, 3, 5, 9, 10 and 16. These are the rostral tip of premaxilla, anterior insertion of dorsal fin, dorsal insertion of caudal fin, anterior insertion of anal fin, dorsal base of pelvic fin, and dorsal base of pectoral fin because these parts were used mostly for fish mobility. Findings of this study validated the use of FA to be a useful tool in determining the current ecological status of Masao river.

Key Words: morphometry, river status, ecological condition, environmental stress, bioindicator.

Abstrait. Tilapia du Nil (*Oreochromis niloticus*) est l'un des principaux organismes trouvés dans la rivière et utilisé comme indicateur des niveaux de bruits acceptables ou pour prédire l'état de la rivière. Ainsi, cette étude a été menée pour évaluer l'état écologique actuel de la rivière du Masao, de la ville de Butuan selon l'asymétrie fluctuante de la forme du corps de *O. niloticus*. 100 individus ont été recueillies au moyen de la technique de la pêche aux filets. La morphométrie géométrique basée sur les landmarks a été utilisée pour déterminer les variations du niveaux de l'asymétrie fluctuante (FA) chez *O. niloticus* pour seize caractères bilatéraux. L'asymétrie fluctuante a été analysée au moyen de la symétrie et l'asymétrie géométrique des données (SAGE) version du programme 1.0. Les résultats à travers Procuste ANOVA indique des niveaux très significatifs de FA ($p < 0,0001^*$) et les scores des composantes principales indique un pourcentage élevé d'asymétrie fluctuant FA (71,4051%), il semble que la rivière du Masao est en état de l'environnement pauvre et les poissons dans la région ont fait face aux élevés perturbations et contraintes du développement. Le composant principal (PC) 1 a le plus haut pourcentage (44,3596%) d'asymétrie fluctuante. Les landmarks touchés les plus courants ont été trouvés dans les sites d'intérêt 1, 3, 5, 9, 10 et 16 (la pointe Rostal du prémaxillaire, l'insertion antérieure de la nageoire dorsale, l'insertion dorsale de la nageoire caudale, l'insertion antérieure de la nageoire anale, la base dorsale de la nageoire pelvienne, et la base dorsale de la nageoire pectorale respectivement) parceque ces parties ont été principalement utilisés pour la mobilité des poissons. Les résultats de cette étude ont validé l'utilisation de l'asymétrie fluctuante (FA) comme un moyen utile pour déterminer l'état écologique actuel de la rivière du Masao.

Mots-clés: asymétrie fluctuante, la rivière du Masao, le tilapia, le stress environnemental, bioindicateur.

Introduction. Fish is considered as valuable source of animal protein and other nutrients hence, the most important component in the diet of the Filipinos. About 36 kg/year is the estimated per capita consumption of fish and fish products. Tilapia is dominant among freshwater fish, and its per capita consumption increased from an average of 0.66 kg/year (1979–1988) to an average of 1.61 kg/year (1989–1997), an increase of 144.5% (Food and Nutrition Research Institute of the Philippines 1993).

The Nile tilapias, *Oreochromis niloticus* (Linnaeus, 1758) are native to central and North Africa and the Middle East (Boyd 2004). This species is usually found in a shallow, still waters of lakes and rivers with abundant vegetation. Furthermore, *O. niloticus* is considered as estuarine and tropical freshwater species (Picker & Griffiths 2011). It can grow to a maximum length of 62 cm and weighs 3.65 kg (at an estimated 9 years of age) (FAO 2012) and has an average size (total length) of 20 cm (Bwanika et al 2004). The growth of *O. niloticus* will be affected through the availability of food and water temperature (Kapetsky & Nath 1997).

Masao River is a stream located in the western part of Butuan City. It served as the direct source of freshwater for the estuary and it is poorly studied freshwater system. Nowadays, settlements existed along the river making it slowly changed into a swampy and marshy environment. Time and floods created a widespread blanket of sand, silt, and clay forming an extensive coastal plain in the entire Butuan area. The present flood behavior of Agusan and Masao Rivers indicates that they still reach the height sufficient enough to flood Butuan City (<http://hnrictbn.tripod.com/Bgeninfo.html>).

The developmental stability is the ability of an organism to buffer its development against genetic or environmental disturbances encountered during development to produce the genetically predetermined phenotype (Waddington 1942; Pojas & Tabugo 2015) and a basic characteristic of development (Waddington 1942; Møller & Swaddle 1997; Pojas & Tabugo 2015). Fluctuating asymmetry (FA) is the most frequently used estimate of developmental stability (Waddington 1942; Møller & Swaddle 1997; Pojas & Tabugo 2015). Hence, reduced performance of fitness components is the result of decreased developmental stability of individuals brought by environmental stress (Clarke 1995; Møller & Swaddle 1997; Almieda et al 2008).

Moreover, the level of fluctuating asymmetry (FA), which occurs when otherwise bilateral traits show small random variations in the size of the two sides of the character around a bilateral symmetry axis is often used to estimate the developmental instability of individuals and populations (Palmer & Strobeck 1986; Almieda et al 2008). The inability of individuals to maintain homeostasis during development when faced with stress: either genetic or environmental is reflected through the degree of FA (Møller & Swaddle 1997; Dongen 2006; Almieda et al 2008). Thus, poor developmental homeostasis results to high FA; and high developmental instability results to poorer developmental homeostasis (Palmer 1994; Hermita et al 2013).

FA is usually calculated using the difference in the measurements of bilateral traits (Reimchen & Nosil 2001; Carter et al 2009; Hermita et al 2013). The library of tools that can be used to separate the three components of body form, which are size, shape and symmetry, is called Geometric Morphometrics or GM (Adam et al 2005; Hermita et al 2013). Hence, in this study, fluctuating asymmetry was used to assess the level of asymmetry in the three bilateral traits in the body of *O. niloticus* as bioindicator of the current ecological condition of Masao River, Philippines.

Material and Method

Study area. The study was conducted during the last week of December 2015 in Masao river (9°0'0" N and 125°28'59.99" E), located in Barangay Masao, Butuan city, Philippines. Figure 1 shows the map of the study area (google map).

Collection and preparation of fish samples. A total of 100 Nile tilapia (*O. niloticus*) individuals were used in this study. The individuals were transported in a styrofoam box with crushed ice and water to maintain its freshness and were then processed immediately in the laboratory for image capture and analysis. Ten percent (10%) formalin was used to harden the fins of the samples.



Figure 1. Map of the Philippines (left above) showing Mindanao area pointing Butuan city (left below), showing Masao river (right) (source: google map).

Landmark selection and digitization. There were 16 landmarks used to characterize the peripheral outline of the body (Figure 2). Table 1 shows the description of landmarks (Chakraborty et al 2008). Digital images of the left and right lateral sides of each fish were generated using a Kodak Easyshare C1450 5X and then converted to thin-plate spline (TPS) format using tpsDig2 software (version 2.0, Rohlf 2004). These images were then tri-replicated to lessen the error before asymmetry analysis.

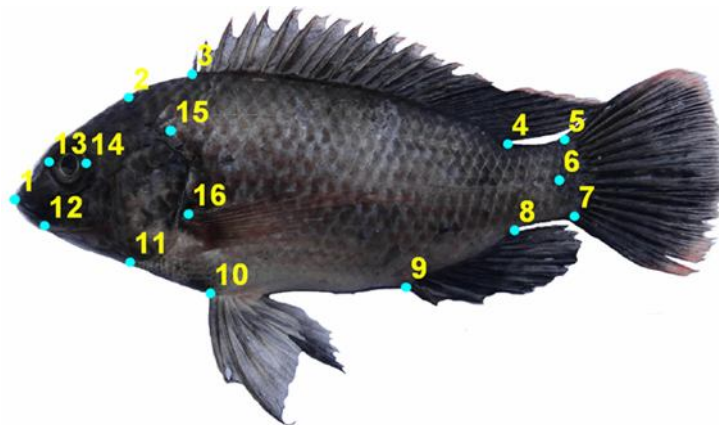


Figure 2. Location of the 16 landmark points on *Oreochromis niloticus* (original).

Measuring fluctuating asymmetry. The paired landmark coordinates were subjected to procrustes ANOVA to determine the FA (Hermita et al 2013; Lecera et al 2015) following the method of Klingenberg (1992) and using the symmetry and asymmetry in geometric data (SAGE) software version 1.0 (Marquez 2006).

Table 1

Description of the landmark points according to Chakraborty et al (2008)

| <i>Landmark No.</i> | <i>Locations</i> |
|---------------------|---|
| 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 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 opercle |
| 16 | Dorsal base of pectoral fin |

Results and Discussion. Table 2 shows that the Procrustes ANOVA were used to demonstrate the individual body shape fluctuations of *O. niloticus*. Left and right sides were analyzed to equate its fluctuating asymmetry. There were 3 factors used in the analysis; individual, sides and the interaction of individuals by sides. It showed that when individuals were compared in other individuals to be not significant (not $P < 0.0001$) which implies to be symmetrical with each other. However, the interaction of individuals by sides and interaction of left and right sides showed to be highly significant ($P < 0.0001$) indicating to have fluctuating asymmetry of the samples. It showed that comparing the individual shape and size attributed to perturbations in the environment resulting from poor water quality (Natividad et al 2015). It further suggests that the observed increased asymmetry reveals that the organism fails to buffer environmental disturbances (Van Valen 1962). These instabilities include genetic, environmental or product of genotype-environment interaction (Trono et al 2015) and fails to cope with stressful conditions (Palmer & Strobeck 1986; Pomiankowsky 1997; Natividad et al 2015). These stressors include physicochemical contaminants (Pankakoski et al 1992; Gileva & Nokhrin 2001; Nunes et al 2001; Velickovic 2007; Sanchez-Chardi et al 2008; Paña et al 2015), anthropogenic and natural disturbances (Badyaev et al 2000; Sanchez-Chardi et al 2008; Paña et al 2105) such as availability of parasites (Pojas & Tabugo 2015; Paña et al 2105). Other factor is endogeneous such as inbreeding depression that affect the health of the gene pool (Sheridan & Pomiankowski 1997; Waldmann 2001; Paña et al 2105), habitat defragmentation, that often leads to harmful consequence (Trono et al 2015) and affects its development (Bonada & Williams 2002).

Table 2

Results of the Procrustes ANOVA for the mean shape of tilapia (*Oreochromis niloticus*) in Masao river

| Factors | SS | dF | MS | F | P-VALUE |
|--------------------|--------|-------|--------|---------|-----------|
| Individuals | 0.5357 | 3108 | 0.0002 | 0.8563 | 1 |
| Sides | 0.0539 | 28 | 0.0019 | 9.5724 | <0.0001** |
| Individual x Sides | 0.6256 | 3108 | 0.0002 | 15.2329 | <0.0001** |
| Measurement error | 0.1658 | 12544 | 0 | - | - |

** (P<0.0001) highly significant.

Table 3

Principal component showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of tilapia (*Oreochromis niloticus*) in Masao river

| PCA | Individual (symmetry) | Sides (directional asymmetry) | Interaction (fluctuating asymmetry) | Affected landmarks |
|-------|-----------------------|-------------------------------|-------------------------------------|--------------------------------------|
| PC1 | 29.7626% | | 44.3596% | 1,2,3,5,6,7,8,9,10,11,12,13,14,15,16 |
| PC2 | 16.0883% | | 9.4427% | 1,4,9,10,11,12,13,14,16 |
| PC3 | 9.005% | 100% | 7.6518% | 1,2,3,5,7,10,11,12,15,16 |
| PC4 | 6.7236% | | 5.044% | 1,3,4,5,8,9,13,14 |
| PC5 | 5.2287% | | 4.907% | 3,4,5,6,7,8,9,10,16 |
| Total | 66.8082% | | 71.4051% | - |

PCA was performed to visualize the variation in landmarks (Dryden & Mardia 1998; Paña et al 2015). The PCA percentage values signify the total variation in fluctuating asymmetry with the summary of the affected landmarks of *O. niloticus* (Table 3). Results revealed that there is a total of 66.8082% of the individual symmetry and 71.4051% fluctuating asymmetry interaction from the upper 5% of PCA of *O. niloticus*. This indicates to have a high fluctuating asymmetry in the body shapes of *O. niloticus*. It illustrates that in PC1 which is the highest percentile constituting 44.3596% of fluctuating asymmetry. The affected landmarks are the following; 1 (Rostral tip of premaxilla), 2 (posterior end of nuchal spine), 3 (anterior insertion of dorsal fin), 5 (dorsal insertion of caudal fin), 6 (midpoint/lateral line), 7 (ventral insertion of caudal fin), 9 (anterior insertion of anal fin), 10 (dorsal base of pelvic fin), 11 (ventral end of lower jaw articulation), 12 (posterior end of premaxilla), 15 (dorsal end of opercle), and 16 (dorsal base of pectoral fin).

The most common affected landmarks were found in landmarks 1, 3, 5, 9, 10, and 16. These landmarks were located mostly in the head part, a rostral tip of the premaxilla, anterior insertion of dorsal fin, a dorsal insertion of the caudal fin, anterior insertion of the anal fin, a dorsal base of the pelvic fin, and a dorsal base of pectoral fin. These parts were used mostly for mobility especially the dorsal cephalic region and pectoral fin which were considered an advantage in determining direct impact of stressors in the fish (Natividad et al 2015). Poorer developmental homeostasis in the molecular, chromosomal and epigenetic levels in impaired environmental conditions caused an increased in FA (Parsons 1990). Furthermore, a developmental growth of fishes is greatly affected by the water quality (Schlosser 1991).

Figure 3 shows the distribution of asymmetrical shape of *O. niloticus* and the locations of the greatest asymmetry. In this study, the morphological landmark was represented by the red dots while the direction and the magnitude of the fluctuation were denoted by the blue arrow.

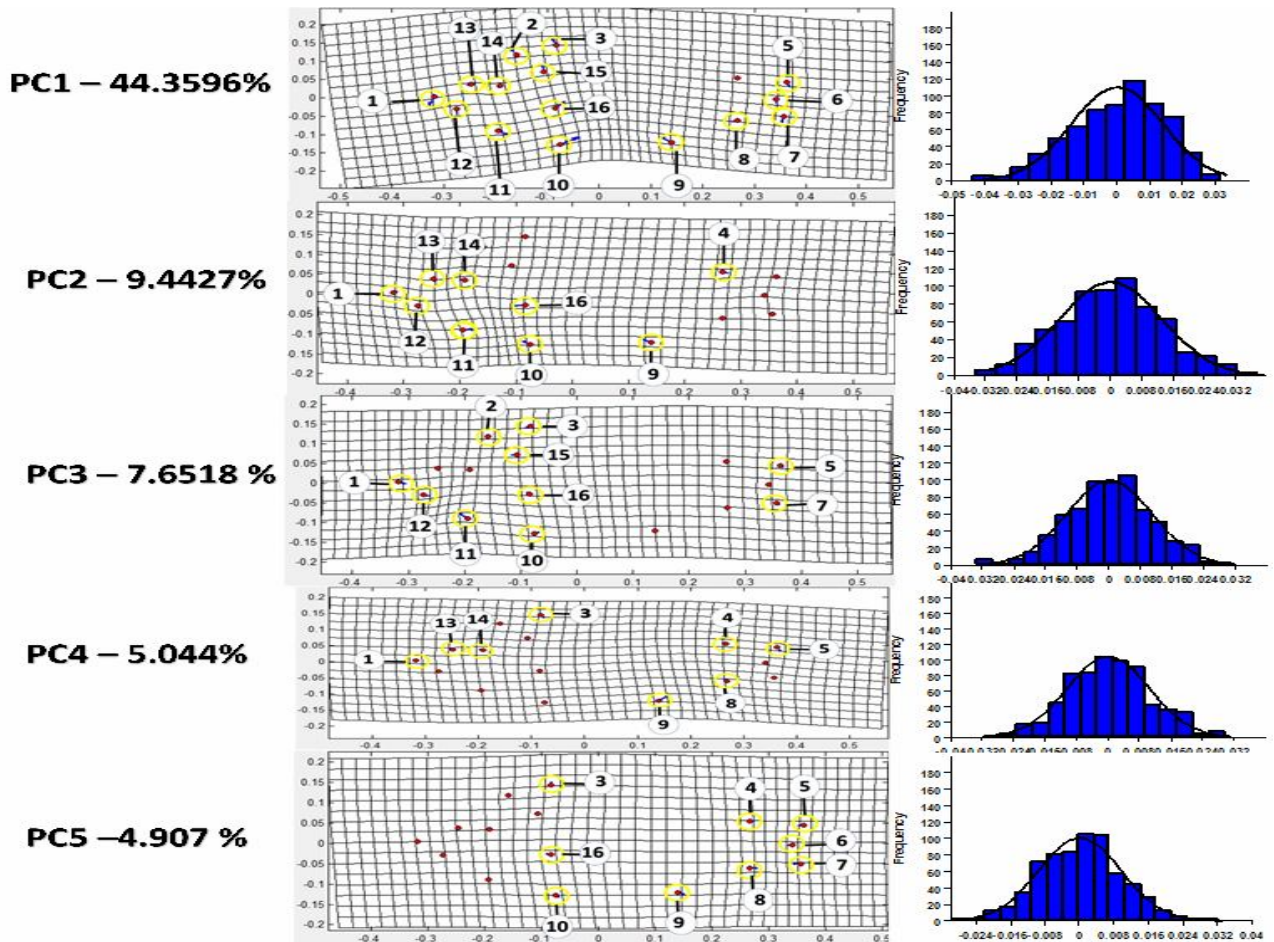


Figure 3. Principal component (PC) implied deformation grid and a histogram of individual (symmetric) of (*Oreochromis niloticus*), Tilapia in Masao river.

Figure 4 shows the identified landmark points affected by FA with the actual photograph of the fish sample. The encircled red dots indicated the movement of the highest affected landmarks and regions emphasized that there were differences in the shape of *O. niloticus*.

The level of variability in the data was represented by the percentage values of PCA (Marquez 2006). This study revealed the same findings with other studies in such a way that not all characters examined in the fishes exhibited FA (Graham et al 1993; Swaddle 2003; Costa & Cataudella 2007; Lecera et al 2015). The level of FA among characters may differ because they also have different ability to buffer developmental noise and achieve homeostasis (Graham et al 1993; Lens et al 2002; Lecera et al 2015). Moreover, *O. niloticus* has been found to survive in a stressful environment thus; it can tolerate in a wide range of environmental variation (Chervinski 1982). Even though, it has an extremely high adaptive mechanism; FA was still noticed under its situation (Jumawan et al 2016) in Masao river and this might be due to the high level of pollutants in the area.

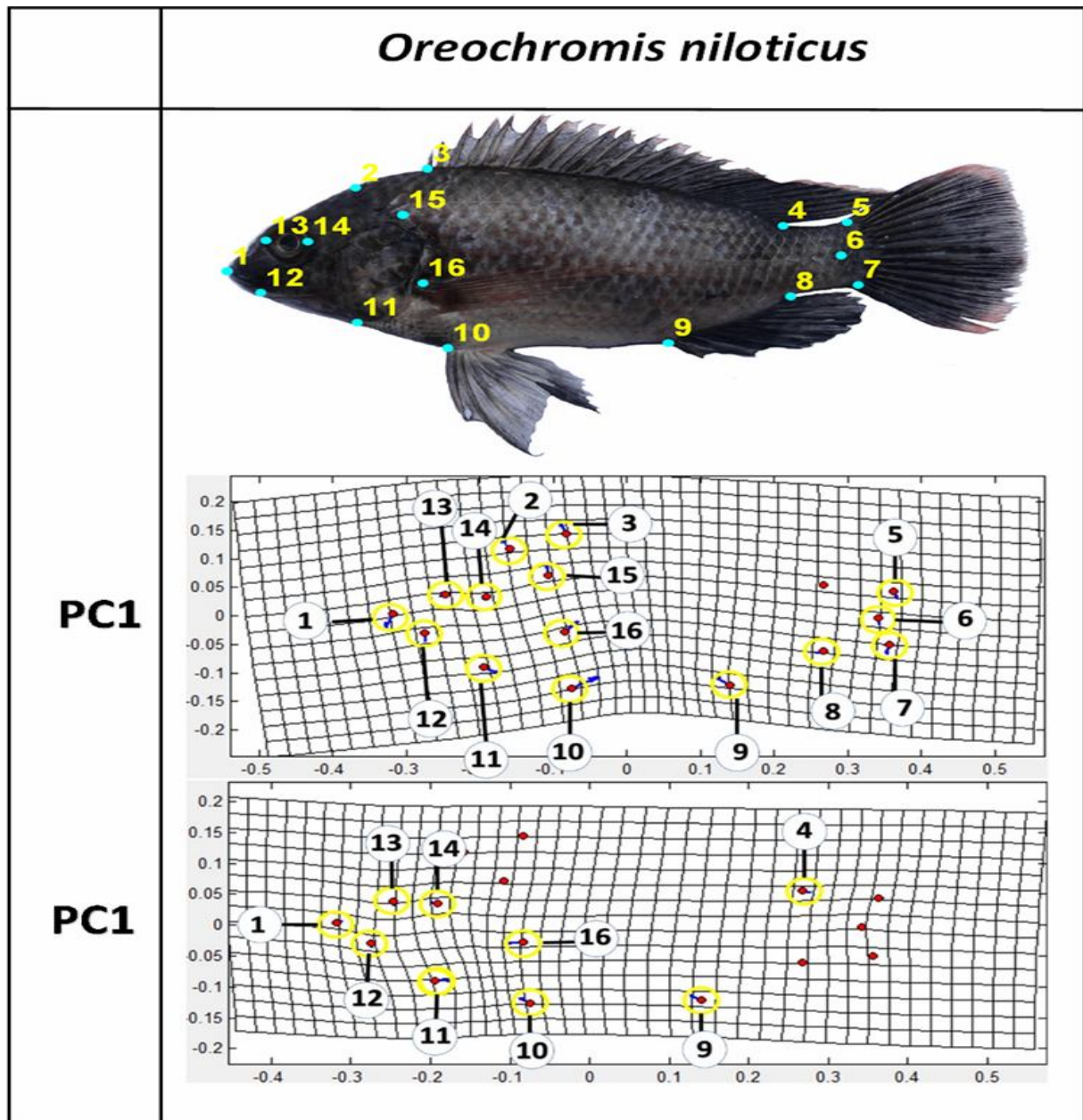


Figure 4. The highest affected landmarks in *Oreochromis niloticus*.

Conclusions. Based on the results of the study, analysis revealed *O. niloticus* in Masao river, Butuan city, Philippines exhibited significant values of FA. There was a high significant difference in the sides and the interaction of individuals and sides of *O. niloticus* using Procrustes ANOVA ($p < 0.0001$). It suggests deviations in its bilateral symmetry with a percentage of 71.4051% (PC1-PC5). Affected landmarks due to FA were found mostly at the head parts because these parts were used mostly for mobility. Thus, possibly mean that Masao river is in poor environmental status. The fishes in the study area experienced greater developmental perturbations and stresses and might be due to the pollutants in the river. Findings of this study validated the use FA to be a useful tool in determining the current ecological status of Masao river. It also confirmed to the numerous studies suggesting FA a suitable instrument in determining developmental stability. Hence, the result of this study will serve as the baseline information to the local government in the management of the river system since this is the first study to report the ecological condition of the river. Furthermore, analyses of the water quality of the river will be necessary to illustrate further the water as some tributaries to Masao river are coming from small and large scale mining areas.

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