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Describing body shape variation of bugwan fish (*Hypseleotris agilis)* species using land mark-based geometric morphometric in Lake Mainit, Surigao del Norte, Philippines

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Abstract

The study primarily aimed to determine the body shape variation of *Hypseleotris agilis* using Fluctuating Asymmetry (FA) in relation to their existing population in Lake Mainit, Surigao del Norte, Philippines. The research used Procrustes ANOVA and Principal Component Analysis (PCA) by means of Symmetry and Asymmetry in Geometric Data (SAGE) software to analyze the data. Finding reveals that *Hypseleotris agilis'* individuals, sides, and individual x sides show high significant difference (P<0.0001). Females obtained high percentage of FA (83.0227%) while males have (76.3627%). It implies that females are vulnerable toward environmental alterations that could be manifested in its morphology. Further, utilizing Geometric Morphometric is essential in identifying morphological variations and co-variations of species in the same taxa.

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Introduction

Lake Mainit is located in Surigao del Norte, Philippines, holds the once endemic *Hypseleotris agilis* which is locally known as Bugwan Fish. It has been endemic to the lake since its accidental introduction along with the stocking of manuscript (2012).

This work was supported in part by the Philippines Department of Science and Technology (DOST)-ASTHRD. Among *Hypseleotris gill* 1863 (carp gudgeons) species, which have been characterized by having strongly compressed head and body, a small mouth, an elongate body cavity with several anal pterygiophores preceding the first vertebral hemal spine, and a ovoid blotch at the dorsal base of the pectoral fin, H. agilis has the most distinctive feature, a pattern of eight spots mediolaterally and three stripes radiating posteriad from the eye (Thacker and Unmack, 2005; Unmack, 2000). Hypseleotris species in Lake Mainit is of interests because their biology and taxonomy are poorly known because of their small in sizes.

Additionally, among of the many factors that affect to the variation of their species, pollutants are direct factors that could alter the morphology and physiology of many fish species; it also results to diminish environmental condition and later reduced the efficiency to uphold different organisms (Duruibe *et al*., 2007). Developmental instability of species has been associated with pollution that occurs persistently. In effect, this could be manifested in the species morphology. However, threats and disturbances often linked to causing fish killed on it. This implies that the lake is not protected and the current environmental situation could decrease the aquatic production (Mozende & Mozol, 2011).

To determine the potential influences of environmental degradation to the fish morphology, fluctuating asymmetry (FA) was employed as an indicative tool in geometric morphometry; this is a fusion of biology and geometry dealing the morphometric forms in 2D or 3D space (Bookstein, 1982). FA is known to be an excellent tool in determining morphological variations of species and efficient mechanism to evaluate environmental condition (Cabuga *et al.,* 2017). It has been used as quantitative measurements to evaluate dissimilarities based on the derived phenotypic traits. FA is an essential morphometric tool that defines the nonconformity from the left and right side of the species morphology (Moller & Swaddle 1997; Palmer &Strobeck 2003). Further, increased level of FA has been associated with environmental stressors from its growth and development (Hermita *et al.,* 2013). Thus, the significant of FA in identifying shape variations widely applied in biological investigations.

The *H. agilis* in Lake Mainit, although not that commercially viable when compared to other bigger fishes like tilapia and carp, is shown to be important in the biodiversity of the lake and has been recommended for conservation (De Guzman, Gorospe and Openiano, 2009). Studying the biology of this species could provide information on how to protect and preserve it. Without such information it is difficult to suggest appropriate management practices. Thus, this study was conducted to gather information on the variations in the morphology of *H. agilis* in Lake Mainit with the use of landmark-based geometric morphometrics. GM is a statistical tool used to quantify and analyze the overall shape based on the landmark configurations which allows hypothesis testing (Rohlf, Marcus, 1993; Adams, Cavalcanti, Monteiro and Lopes, 1999). The use of morphometrics has been also successful in relating the body shape differentiation to ecology or phylogeny and proved the effectiveness in showing differences in morphological aspects of fishes that discriminate both within and between species populations.

Materials and methods

Study Area

The study was conducted in Lake Mainit, Surigao del Norte, Philippines (Fig. 1).

Fig. 1. The map of Lake Mainit (Warm Lake), Surigaodel Norte.

It geographically lies between 08°57'25.28"N 125°32'36.63"E. The fish collection was done in the month of July 2017 through the aid of local fisherman utilizing motorized bancas and gills net as their catching gears while the physicochemical water parameters were completed with the same date stated above.

About 100 samples of *H. agilis*(50 males and 50 females) were randomly collected in the study area. The collected samples were immediately transported to the laboratory and proper preservation was applied.

The individual fish were positioned in a Styrofoam for pinning of its fins. To make it wider and visible to locate the point of origin for land-marking methods; it was applied a 10% Formalin all over its fins to make it hardened using the small brush. The samples were then captured using the digital camera (Lenovo-13 megapixels). To obtain the total

Land-marking of the samples

length of the samples, the left and the right bilateral side were taken using a ruler (Natividad *et al.,* 2015).

Landmark selection and digitization

The acquired images were then sorted according to its sex and converted into TPS file format utilizing the tpsUtil. Digitation/landmarking procedure of the samples through tpsDig2 (version 2, Rohlf 2004).

There were sixteen (16) anatomical landmark points were utilized to digitize the samples of *H. agilis* presented in Table 1.

Line diagram of the left flank of the sample (Fig. 2), showing the locations of 16 landmarks that were used for morphological analysis.

These landmarks corresponds to: (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 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, and (16) dorsal base of pectoral fin.

Shape analysis

To lessen the measurement error, the samples were replicated three times. Its bilateral symmetry (left and right) was digitized using tpsDig2. The collected coordinates were then subjected to (SAGE) Symmetry and Asymmetry in Geometric Data (version 1.04, Marquez, 2007) software (Figure 2). This data provides information about the acquired principal components of individual symmetry which indicates through a deformation grid (Natividad *et al.,* 2015). The Procrustes ANOVA test was implemented to identify the significant difference in the symmetry of the three factors examined – individual, sides and interaction of individuals and side. The significant level was verified at P<0.0001. Along with, the variances of its side and the estimation of directional asymmetry also identified. The level of FA was stipulated through percentage (%) which were analyzed and compared between male and female samples (Natividad *et al.,* 2015).

Results and discussion

Body shape analysis of H. agilis

Table 2 showed the results of the body shape in the morphology of *H. agilis*.

The symmetry (left and right) of each samples were tested to determine the asymmetry in both female (50) and male (50) in three factors measured, namely: individuals, sides and the interaction of individuals by sides. Results were shown with high fluctuating asymmetry of P<0.0001 in.

The acquired results from the collected samples of *H. agilis* revealed that the populations were undertaken asymmetrical in the aspect of individual, its left and right side and its association as individual versus the sides since asymmetry in its morphology wad observed and this might be an indicator that the fish samples from the study area underwent environmental perturbations that causes objectionable effects. Accordingly, fish that acquired high fluctuating asymmetry has been linked with contaminated aquatic environment and also occurs in Chironomid larvae and shrimp this results of the analyzed factors.

Table 1. Description of the landmark points adapted from Paña *et al.* (2015).

Coordinates	Locations/Nomenclature
C1	Snout tip
C2	Posterior end of nuchal spine
C3	Anterior insertion of dorsal fin
C4	Posterior insertion of dorsal fin
C5	Dorsal insertion of caudal fin
C6	Midpoint or lateral line
C7	Ventral insertion of caudal fin
C8	Posterior insertion of anal fin
C9	Anterior insertion of anal fin
C10	Dorsal base of pelvic fin
C11	Ventral end of lower jaw articulation
C12	Posterior end of the premaxilla
C13	Anterior margin through midline of orbit
C14	Posterior margin through midline of orbit
C15	Dorsal end of operculum
C16	Dorsal base of pectoral fin

*** (P<0.0001) highly significant.*

It was also noticed that individual samples display a high amount of fluctuating asymmetry (FA). With respect to its left and right side it demonstrates high significant difference of FA as population. Nonetheless, the component of individuals by sides relatively indicated high significant difference suggesting a fluctuating asymmetry. Furthermore, the incidence of FA was observed between sexes and within the factors analyzed.

Run-off coming from agricultural fertilizer (Tomkins and Kotiaho, 2001). Furthermore, study shows that altered environment highly affects species morphology thus results to high level of FA rather than those inhabiting natural habitats (Ostbye *et al.,* 1997; Campbell *et al* 1998). Meanwhile, study shows that most susceptible for aquatic alterations are freshwater species (Laffaille *et al.,* 2005; Kang *et al* 2009; Sarkar *et al.,* 2008). The incidence of FA in *H. agilis* revealed that it was affected from various disturbances from the area. Moreover, FA is essential in determining morphological variations and an indicative tool to assess environmental condition. Thus, utilizing geometric morphometric is efficient mechanism.

Table 2. Procrustes ANOVA on body shape of *H. agilis*in terms of sexes from Lake Mainit, Surigaodel Norte.

Factors	SS	DF	MS	F	P-VALUE
Female					
Individuals	0.5436	1372	0.0004	2.865	0.0001**
Sides	0.2013	28	0.0072	51.9883	0.0001**
Individual x Sides	0.1897	1372	0.0001	4.7996	0.0001**
Measurement Error	0.1614	5600	0	--	--
Male					
Individuals	0.5922	1372	0.0004	3.6259	0.0001**
Sides	0.0862	28	0.0031	25.868	0.0001**
Individual x Sides	0.1633	1372	0.0001	6.3655	0.0001**
Measurement Error	0.1047	5600	0	--	--

The principal component analysis (PCA) was presented in Table 3, this uses to define the affected landmarks in both female and male fish samples. The result shown that females obtained the highest Interaction or Fluctuating Asymmetry (83.0227%) and generates five principal component (PC) scores accounting to 80.2718%. While it was observed that the affected landmarks on the females were found in 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) 11 (Ventral end of lower jaw articulation) and 16 (Dorsal base of pectoral fin).

Table 3. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of *H. agilis* from Lake Mainit, Surigaodel Norte.

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
Female				
PC1	48.6155%	100%	38.7556%	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
PC2	10.2393%		21.3719%	1,2,3,4,5,8,9,11,12,13,14,15,16
PC3	8.6899%		11.3719%	1,2,3,4,5,6,7,8,9,10,11,12,16
PC4	6.6529%		6.693%	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
PC5	6.0742%		4.4561%	1,2,3,4,5,6,8,9,10,11,13,14,15,16
	80.2718%		83.0227%	
Male				
PC1	52.9437%	100%	45.2099%	1,2,3,4,6,7,11,12,14,15,16
PC2	10.2129%		15.2629%	1,2,10,11,12,14,15
PC3	8.6371%		8.9517%	1,2,4,7,8,9,10,11,12,14,15,16
PC4	7.0006%		6.9382%	2,3,4,6,7,8,9,10,13,14,15
	78.7943%		76.3627	

Table 4. Mean values of physicochemicaln analysis parameters from Lake Mainit, Surigaodel Norte.

Water Parameters	Standard DAO 90-34 &Water Watch Australia National Technical Manual (2002)	Station 1 Mean ± SEM	Station 2 Mean ± SEM	Station 3 Mean ± SEM	Mean Mean ± SEM
Conductivity	100-2000 µS/cm	243.033 ± 39. 967	510.967 ± 199.858	232.933 ± 48.557	328.978 ± 91.041
DO	>5mg/L	7.1 ± 0.3	7.1 ± 0.2	7.7 ± 0.351	7.3 ± 0.2
pH	6.5-8.5	7.087 ± 0.221	7.137 ± 0.279	7.81 ± 0.382	7.345 ± 0.233
Salinity	<0.5ppt	0.12 ± 0.02	0.247 ± 0.096	0.107 ± 0.027	0.158 ± 0.045
Temperature	3°C rise[a]	26.069 ± 0.233	26.367 ± 0.296	27.7 ± 0.954	26.712 ± 0.501
TDS	<1000mg/L	118.867 ± 18.333	246.3 ± 94.71	107.767 ± 27.274	157.645 ± 44.443

Note: DAO– DENR Administrative Order

On the other hand, males obtained (76.3627%) of Interaction or Fluctuating Asymmetry and generates four principal component (PC) scores accounting to 78.7943%. Meanwhile, the to both evaluate phenotypic plasticity of individuals in the populations and ecological status. midline of orbit) and 15 (Dorsal end of affected landmarks were 2 (Posterior end of nuchal spine), 14 (Posterior margin through operculum).

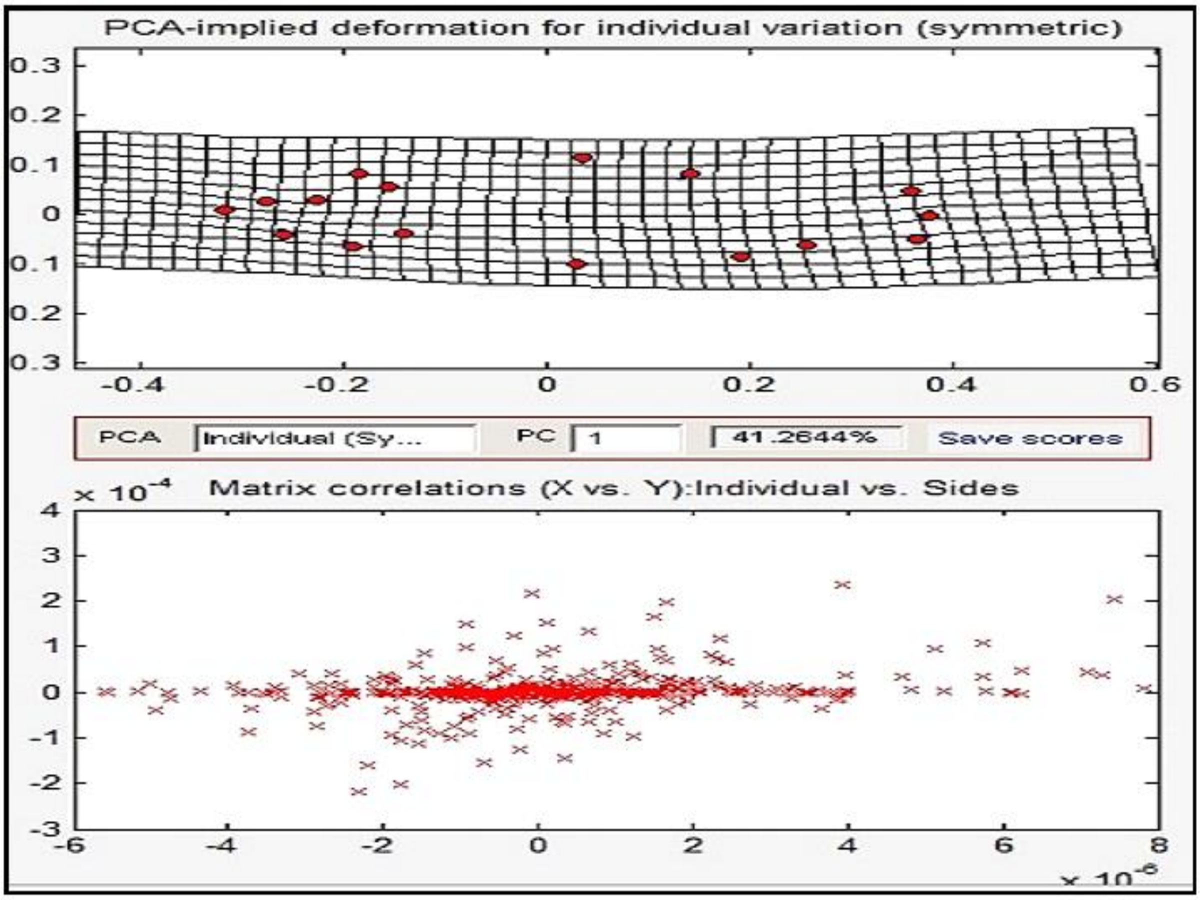

Fig. 2. Symmetry and asymmetry in geometric data.

It was also observed that the affected landmarks of female samples were different from the male samples. Accordingly, female fish were highly obtained FA level and this associated with its adaptive mechanism from changed environment and vulnerable from environmental perturbation during the reproductive season (Requiron *et al.*, 2012; Cabuga *et al.*, 2016). Therefore, the detected FA in the body shape of *H.*

agilis was acquired from its habitat and this manifested through investigating among the female and male samples. This also implies that the study area was not exempted from environmental disturbances. Continuing the human induced activities will highly affects other species found in the area.

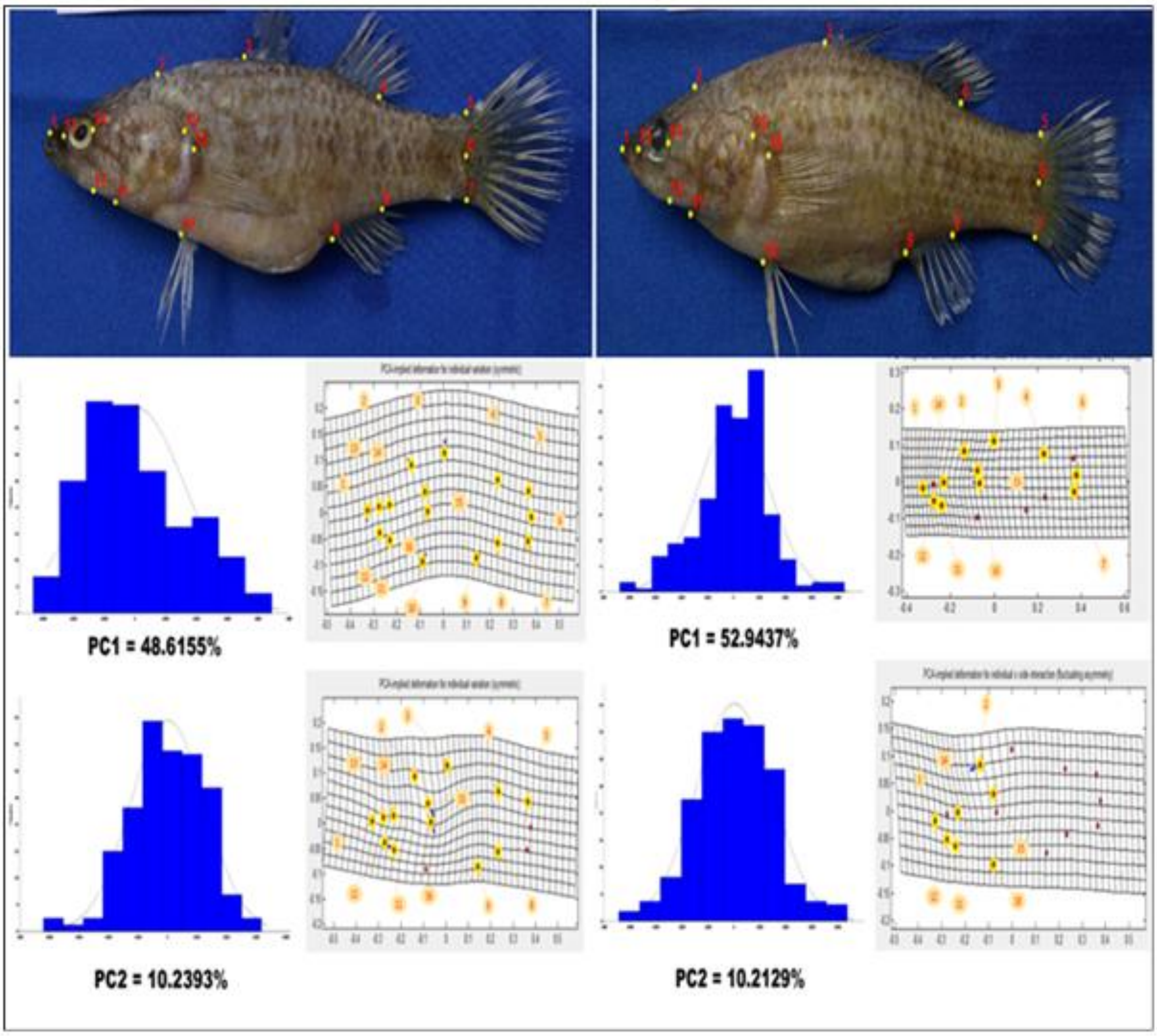

Fig. 3. Actualized picture of digitized of female and male sampled at Lake Mainit Surigao del Norte with affected landmarks shown in PCA-deformation grid for PC1 and PC2.

Results showed (Table 4) that the conductivity, dissolved oxygen, pH level, salinity, temperature and total dissolve solids were in the acceptable limits as presented in Table 4.

Hence, this value of salinity is small higher than the normal ppt of the freshwater would simply means it is less desirable for existence of the aquatic organisms (Flores, 2012). On the other hand, there was no significant difference in the parameters except the salinity of the water.

Conclusions

It is concluded that utilizing geometric morphometric explain the body shape variations of *H. agilis* or Bugwan fish species. In the study, Procrustes ANOVA shows high significant difference ($P<0.0001$). Female samples obtained the high percentage of interaction or fluctuating asymmetry (FA) (83.0227%) while males have (76.3627%).

This could happen since females are susceptible especially during reproduction period. For the conductivity, DO, pH level, salinity, temperature and total dissolve solids of Lake Mainit were in the acceptable limits except the salinity of the water which is a bit higher in its ppt.

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