Fluctuating asymmetry in the body shape of flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758) from Masao river, Butuan city, Agusan del Norte, Philippines

[1]Nanette L. Samson, [1,2]Joycelyn C. Jumawan, [1]Samantha O. M. A. L. Abastillas, [1]Manny P. Eviota

[1] Graduate School, Caraga State University, Ampayon, Butuan City, Philippines; [1,2] Biology Department, College of Arts and Sciences, Caraga State University, Ampayon, Butuan City, Philippines. Corresponding author: N. L. Samson, rnet97@yahoo.com

Abstract. Fluctuating asymmetry (FA) has been widely used as an indicator of ecological stress of an organism within a population. Stress can increase the phenotypic difference in a population thereby affecting the stability of an individual. Thus, increased stress may result to the asymmetry of each bilateral trait, especially during development. In this study, *Mugil cephalus* was used because this fish is capable surviving brackish, salt, and freshwater environments. Thin plate spline (TPS) series was used for landmark analyses of each sample and were subjected to symmetry and asymmetry in geometric data (SAGE) software. Results of the Procrustes ANOVA showed that individual symmetry of L-R sides presented in males were not significant. However, in females the individual symmetry was not statistically significant. The results of principal component (PC) scores present high percentage FA of males (73.9541%) and females (82.6848%), respectively. In males, PC1 (34.76%) and PC2 (20.92%) significant variations were found, most of the landmarks were affected except in the anterior and posterior insertion of dorsal fin. In females, PC1 (43.48%) and PC2 (15.92%) which also shows that most of the landmarks were also affected except in the midpoint of caudal border of hypural plate, anterior margin through midline orbit and the dorsal base of pectoral fin. This study aimed to determine the fluctuating asymmetry in the body shapes of *M. cephalus* as an indicator of the water quality of Masao river, Butuan city, Philippines.
Key Words: brackish, endogenous, environmental stress, ecology, estuary.

Introduction. The flathead grey mullet (*Mugil cephalus* L.) originally was described by Carl Linnaeus (1758), a Swedish naturalist. Mugil from the Latin “mugil” meaning fish and species cephalus from the Latin meaning a “mullet”. *M. cephalus* belongs to family Mugilidae. These important fish are capable surviving in brackish, salt, and freshwater environments in a variety of depth (Chang et al 2004). They were characterized by having a broad head with two dorsal fins and a cylindrical body. The first fins have soft spines and the second have 8-9 rays. Caudal fin concave to weakly forked (Hoese & Bray 2006).

M. cephalus is considered an economically important fish in Masao River, Libertad, Butuan City, Philippines and is locally known as Banak. Its size can reach up to a common maximum length of 50.0 cm SL (http:www.fishbase.org/summary/SpeciesSummary.php?ID=785).

Fluctuating asymmetry (FA) is small and random departures from perfect symmetry in an organism bilateral traits (Palmer & Strobeck 1986; Trotta et al 2005). Higher asymmetry reflects an increased in the inability to cope with the stressful situation (Palmer & Strobeck 1986; Pomiankowsky 1997). Degrees of asymmetry arise when organisms were exposed to environmental stress during development, and also endogenous factors, as breeding may also contribute to asymmetry (Wedekind & Muller 2004). Direct exposure of pollutants to an individual organism will affect the development of the species. In a case of a high level of pollutants introduced to an environment, will cause portray skewed parts of organism which is the primary basis for the measurement of FA (Natividad et al 2015). Fish is a good source for monitoring the health of an aquatic environmental because they are intimately associated with their habitats (Helfman et al 1997). Fishes also are the most abundant organisms in the aquatic habitat. The presence of *M. cephalus* in Masao River is a reliable indicator in assessing the FA of the fish. The FA of the fish helps in identifying the status of the water quality of the river.

Agusan river that drains directly into Butuan bay had carried sand, silt and clay. These formed a delta, which expanded and transformed the area from a marine environment into a coastal plain. Swamps, marshes, streams and estuaries were formed. The growth of the delta divided the direction of the flow of Agusan river and created a new river in the western part of Butuan city. Masao river serves as the direct source of freshwater for the estuary. Settlements existed along the estuaries shorelines. However, these estuaries slowly changed into a swampy and marshy environment. Sand, silt and clay blanketed the inlet with flood waters of Masao river (http://hnricbtn.tripod.com/Bhistory. html).

This study aimed to determine the FA in the body shapes of *M. cephalus* as an indicator of the water quality of Masao river in Butuan City, Philippines.

Material and Method

Study area. Masao River (9º0'0" N and 125º28'59.99" E) is one of the tributaries of Agusan river. The river is located in Barangay Masao, Butuan city, Philippines (Figure 1).

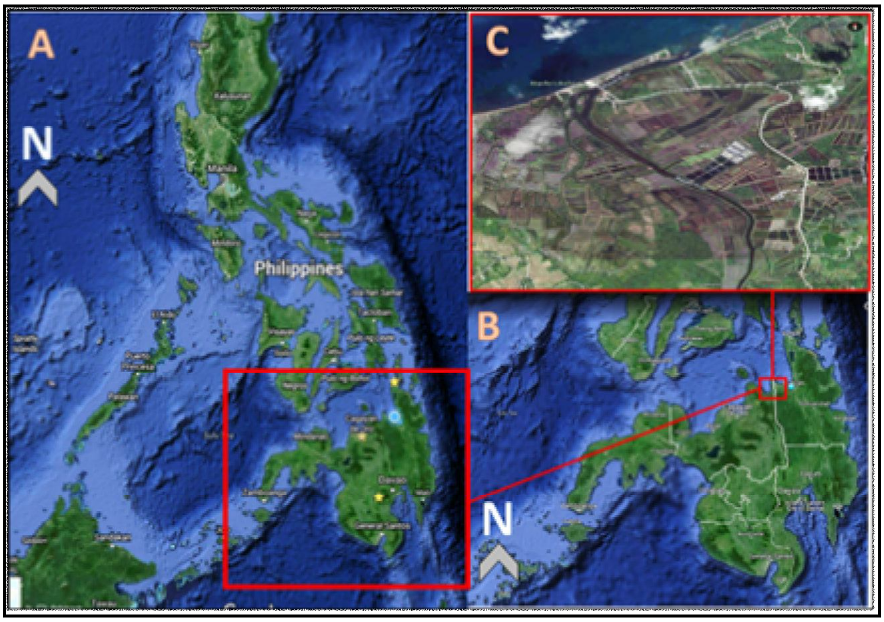

Figure 1. Map of the Philippines (A), map showing Mindanao (B), and map showing the location of the Masao river, Butuan city, Philippines (C).

Sample collection. A total of 100 samples of *M. cephalus* were collected from Masao river, Butuan city from January 20-26, 2016. The samples were immediately processed for image capture and analysis. Fifty male and fifty female individuals were analyzed using Sony camera with 16 megapixels. The samples placed in a Styrofoam and pinned the fins to show its origin. Both the left and the right lateral side of the fish was measured with a ruler parallel to the specimen for the determination of its length. Captured images were digitized, tpsDig2 program (version 2.0, Rohlf 2004) were used and were saved to TPS files. Before digital imaging, the fish were pinned, 10% solution of formalin applied to their fins for stiffening (Love & Chase 2009). Sexes of the specimens were determined by direct examination of the gonads. 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. With the use of Thin-Spline (TPS) series, landmark analyses were obtained to determine the variations in the images. Digitized landmarks in fish morphometric were used through tpsDig2. A total of 16 landmarks as described in Table 1 (Chakrabarty et al 2008) were identified in order to represent the external shape of the specimens body. Digitization was copied in triplicates for each sample (Figure 2).

Table 1

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

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 operculum
16	Dorsal base of pectoral fin

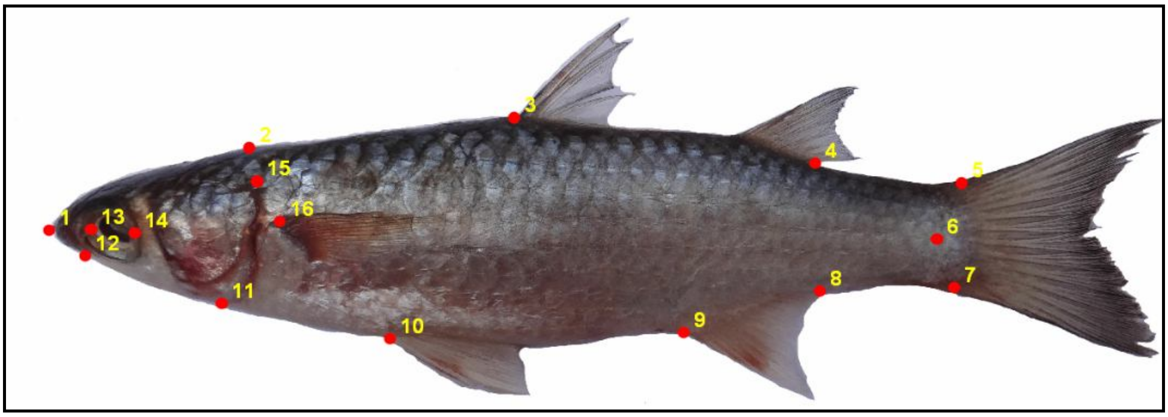

Figure 2. Landmark points of the *Mugil cephalus.*

Shape analysis. Procrustes ANOVA were employed in determining the individual symmetry. Landmark coordinates for the TPS version of the left and right sides was subjected to symmetry and asymmetry in geometric data (SAGE) software (version 1.04, Marquez 2007). This data was used to comprehend the geometric data of the sample and is useful in shape conformation of individuals variation including the possible covariance condition (Jumawan et al 2016) (Figure 3).

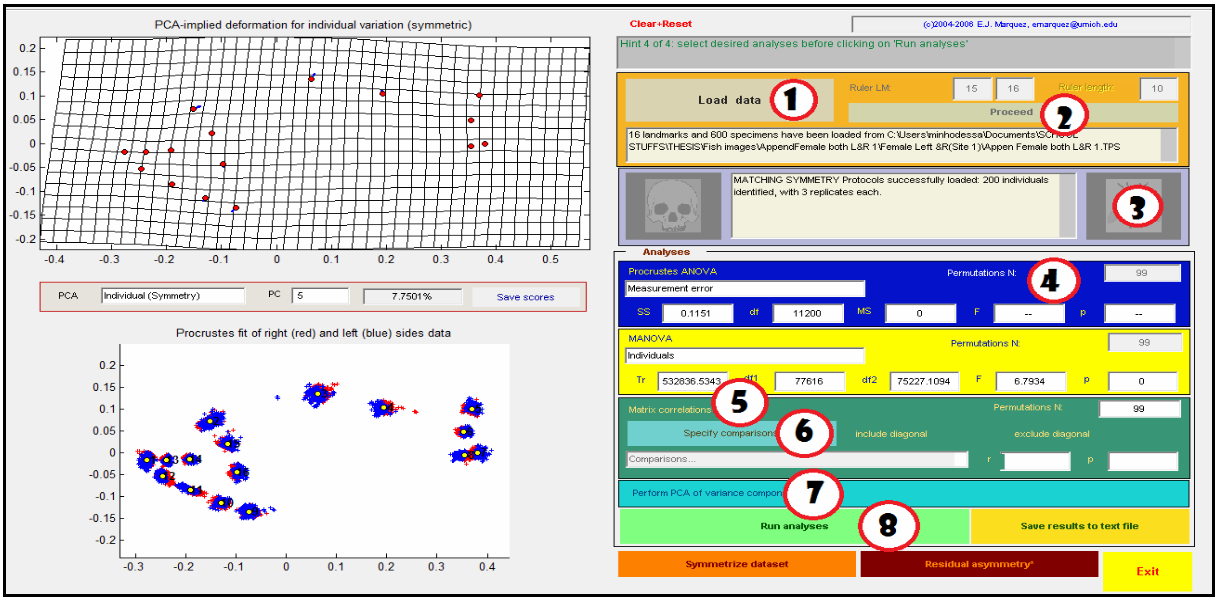

Figure 3. Summary of the process of shape analysis using SAGE software.

Intraspecific variation between sexes. Analysis through paleontological statistics (PAST) software (Hammer et al 2001) was used in comparing the individual symmetry between individuals and sexes. Relevant representations such as histogram, box plot and scattered plot were also distinguished.

Results and Discussion. Procrustes ANOVA was used to demonstrate the individual body shape fluctuations. Left and right sides were analyzed to equate its FA. Three factors were included in the analysis: individual, sides and the interaction of individuals by sides. Table 2 presents the male and female asymmetrical and symmetrical analysis of females and males *M. cephalus.* Male samples shows that all the factors were highly significant (P<0.0001) which indicates a FA in their body shape within individuals, their sides and on both individuals and sides.

However, in female samples it shows that all factors are to be highly significant except when samples were compared with 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 shows to be highly significant indicating to have FA.

Table 2

Procrustes ANOVA for the body shape of *Mugil cephalus* in terms of sexes

Effect	*Sum of squares*	*Degrees of freedom*	*Mean square*	*F value*	*P-value*
		Male			
Individuals	0.28	1372	0.0002	0.8563	<0.0001**
Sides	0.0353	28	0.0013	11.6723	<0.0001**
Individual x Sides	0.1482	1372	0.0001	11.9236	<0.0001**
Measurement Error	0.0507	5600	0	---	---
		Female			
Individuals	0.19993	1372	0.0001	1.0305	0.2892 ns
Sides	0.0157	28	0.0006	3.9787	<0.0001**
Individual x Sides	0.1934	1372	0.0001	14.6844	<0.0001**
Measurement Error	0.0538	5600	0	---	---

**highly significant (p<0.0001), ns - not significant.

In Table 3, principal component shows the values of symmetry and asymmetry scores with the summary of the affected landmarks of *M. cephalus*. Only above five percent

(5%) of the percentile were considered both left and right of the sample. A total of 73.9541%of FA interaction of male *M. cephalus* was observed. It illustrates that in PC1 which is the highest percentile constituting about 34.7625% of FA which all the landmarks are affected except in landmark 4 (posterior insertion of dorsal fin) followed by PC2 having 20.9223%. All the principal components have its FA which means that there is a difference in the samples body shape which it has its affected landmarks. Most of the affected landmarks are found mostly on the parts for its mobility.

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*
			Male	
PC1	38.9542%		34.7625%	1,2,3,5,6,7,8,9,10,11,12,13,14,15,16
PC2	30.6619%		20.9223%	1,2,5,6,7,8,9,10,11,13,15,16
PC3	8.7809%	100%	10.9626&	1,2,4,8,11,12,15,16
PC4	5.0396%		7.3067%	1,2,9,10,11,16
Total	83.4366%		73.9541%	
			Female	
PC1	36.6762%		42.4845%	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
PC2	18.6399%		15.9075%	1,2,3,4,5,6,7,8,9,11,12,13,15
PC3	11.0103%	100%	9.3257%	1,2,3,9,10,11,12,14,15,16
PC4	6.4989%		9.552%	1,2,3,4,7,8,9,10,11,16
PC5	5.9172%		5.4151%	1,2,4,5,9,10,11,14,15
Total	78.7425%		82.6848%	

The male sample has a total individual symmetry of 83.4366% which accounts for variation of FA of 73.9541%. On the other hand, 78.7425% of individual symmetry in female implies that there is a similarity of body shapes but 82.6848% in the interaction in their FA gives a hint that there is a difference in the individuals body shape.

Figure 4 and 5 shows the asymmetrical shape distribution of female and male *M. cephalus* in Masao river. Yellow marks represent the variation which indicates the affected landmark movements. Histogram is used to visualize the centroid configuration, shape and distribution of the individual asymmetry. Skewness is also observed, which is an evidence for asymmetry of *M. cephalus.*

Figure 6 shows the actualized picture of digitized male and female *M. cephalus* with the affected landmarks which is shown in PCA- deformation grid of PC1 and PC2.

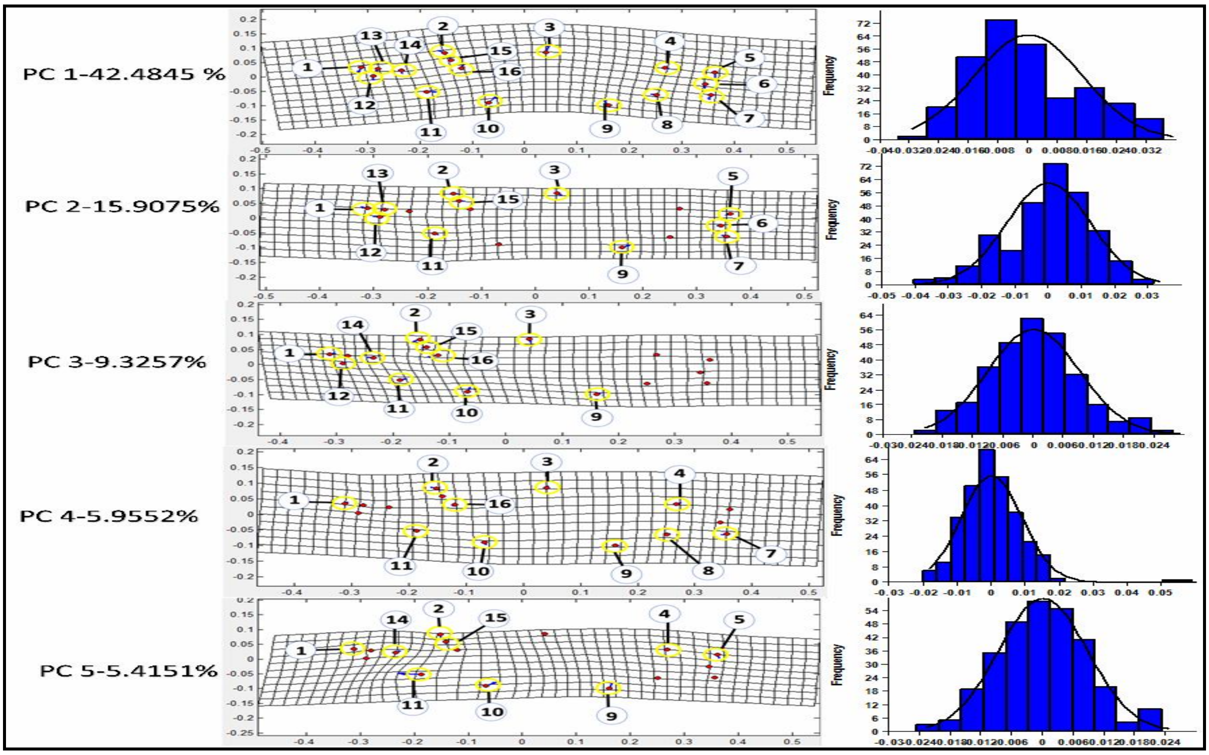

Figure 4. Principal component (PC) implied deformation grid and histogram of individual (symmetric) in female *Mugil cephalus* in Masao river.

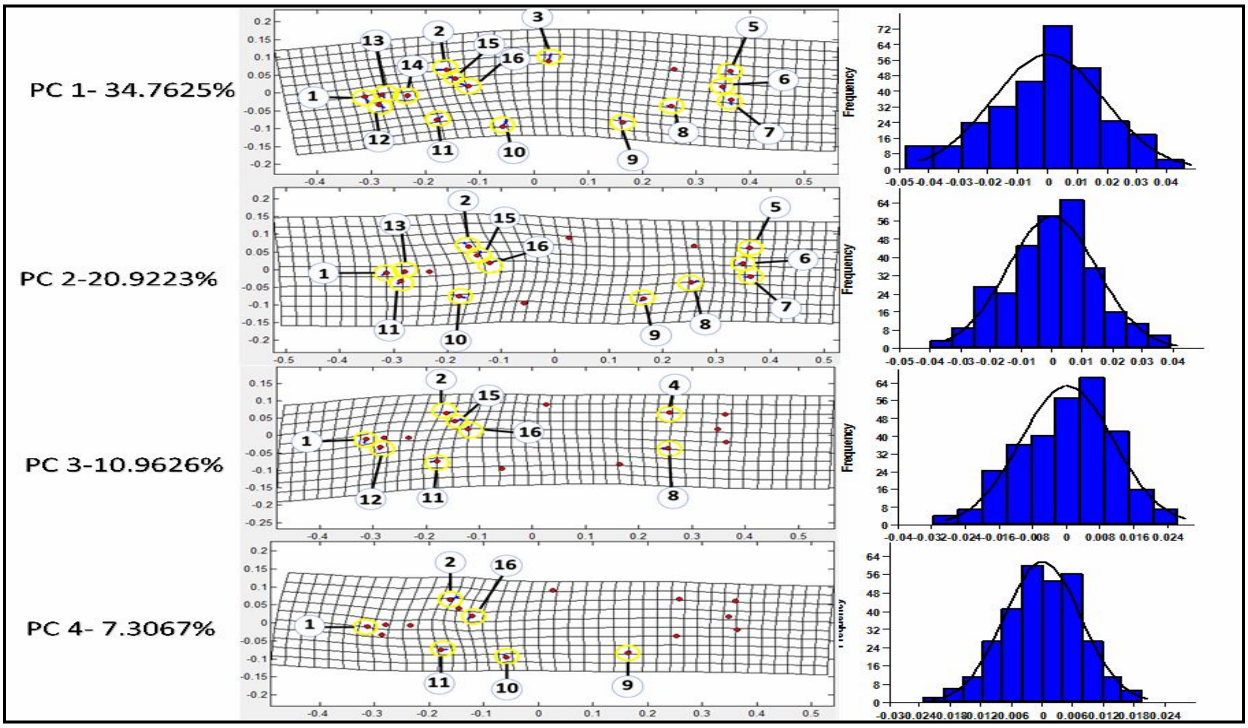

Figure 5. Principal component (PC) implied deformation grid and the histogram of individual (symmetric) in male *Mugil cephalus* in Masao river.

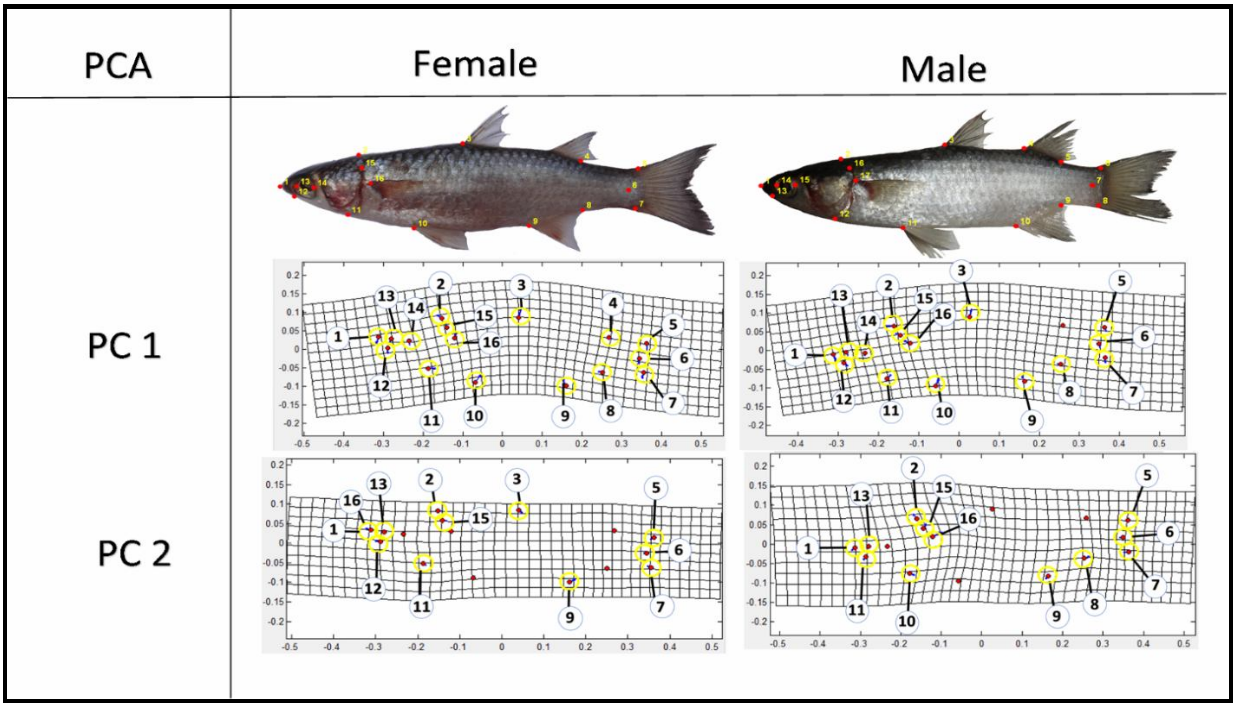

Figure 6. Actualized picture of digitized male and female *Mugil cephalus* with the affected landmarks shown in PCA- deformation grid for PC1 and PC2.

Conclusions. The result of the statistical analysis demonstrated that there is a high variation of FA in males *M. cephalus* which (73.9541%) compared to females (82.6848%). Affected landmarks were the cephalic, pectoral and the caudal regions of the individuals (majority involving mobility). The high FA on both the L-R sides of the samples indicates the poor condition of Masao river. This also indicates that the fish develops environmental agitation and stresses. This might be due to household wastes of the settlers near the river and the several fish cages that are present in the area aside from the effluents causing degradation of the river.

Acknowledgements. The researchers would like to express their gratitude to Mr. Jess H. Jumawan for giving them an opportunity to learn about this field and to Candy Claire D. Joseph for the assistance of the data analysis.

References

Chakrabarty P., Amarasinghe T., Sparks J. S., 2008 Redescription of ponyfishes (Teleostei: Leiognathidae) Shri Lanka and the status of *Aurigequula* fowler, 1918. Ceylon Journal of Science (Biological Science) 37(2):143-161.

Chang C. W., Iizuka Y., Tzeng W. N., 2004 Migratory environmental history of the grey mullet *Mugil cephalus* as revealed by otolith Sr:Ca ratios. Marine Ecology Progress Series 269:277-288.

Hammer O., Harper D. A. T., Ryan P. D., 2001 PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1):9.

Helfman G. S., Collette B. B., Facey D. E., 1997 The diversity of fishes. Blackwell Science, London, England, 528 pp.

Hoese D. F., Bray D. J., 2006 Mugiliformes, pp.. In: Zoological catalogue of Australia. Beesley P. L., Wells A. (eds), Part 1, 35:675-685, ABRS & CSIRO Publishing.

Jumawan J. H., Abastillas S. O. M. A., Dicdican M. J. A., Cabag M. B., Gamolo E. M., Velasco J. P. B., Cabuga C. C., Mordeno Y., Requieron E., Torres M. A. J., 2016 Fluctuating asymmetry in the body shapes of goby fish *Glossogobius giuris* from Agusan River, Butuan City, Agusan del Norte, Philippines. AACL Bioflux 9(1):133-142.

Love J. W., Chase P. D., 2009 Geometric morphological differences distinguish populations of scup in the Northwestern Atlantic Ocean. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 1:22-28.
Marquez E., 2007 Sage: Symmetry and asymmetry in geometric data. Ver. 1.05 (compiled 09/17/08). Available at: http://www.personal.umich.edu/marquez/morph/.
Natividad E. M. C., Dalungdong A. R. O., Ecot J., Jumawan J., Torres M. A., Requieron E. A., 2015 Fluctuating asymmetry as bioindicator of ecological condition in the body shapes of *Glossogobius celebius* from Lake Sebu, South Cotabato, Philippines. AACL Bioflux 8(3):323-331.
Palmer A. R., Strobeck C., 1986 Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17:391–421.
Pomiankowski A., 1997 Genetic variation in fluctuating asymmetry. Journal of Evolutionary Biology 10:51-55.
Requieron E., Manting M. M. E., Torres M. A. J., Demayo C. G., 2010 Body shape variation in three congeneric species of pony fishes (Teleostei: Perciformes: Leioghathidae). Transaction of the National Academy of Science and Technology 32(1):49-50.
Rohlf F. J., 2004 TpsDig version 2.0. Department of Ecology and Evolution, State University of NY.
Trotta V., Calboli F. C. F., Garoia F., Grifoni D., Cavicchi S., 2005 Fluctuating asymmetry as a measure of ecological stress in *Drosophila melanogaster* (Diptera: Drosophilidae). Europian Journal of Entomology 102:195-200.
Wedekind C., Muller R., 2004 Parental characteristics versus egg survival: towards an improved genetic management in the supportive breeding of Lake Whitefish. Finnish Zoological and Botanical Publishing Board 41:105-115.
*** http:www.fishbase.org/summary/SpeciesSummary.php? ID=785
*** http://hnricbtn.tripod.com/Bhistory.html

Received: 04 April 2016. Accepted: 21 August 2016. Published online: 30 August 2016.
Authors:
Nanette Lampad Samson, Caraga State University, Graduate School, Philippines, Butuan City, 8600, Ampayon, Brgy. Ampayon, e-mail: rnet97@yahoo.com
Joycelyn Cagatin Jumawan, Caraga State University, Graduate School, Philippines, Butuan City, Ampayon; Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Butuan City, 8600, Brgy. Ampayon, e-mail: joycejumawan@gmail.com
Samantha Odisa Marie Anne Lumapas Abastillas, Caraga State University, Graduate School, Philippines, Butuan City, 8600, Ampayon, Brgy. Ampayon, e-mail: sammy_popcool@yahoo.com
Manny Plaza Eviota, Caraga State University, Graduate School, Philippines, Butuan City, 8600, Ampayon, Brgy. Ampayon, e-mail: many.eviota1986@gmail.com

How to cite this article:
Samson N. L., Jumawan J. C., Abastillas S. O. M. A. L., Eviota M. P., 2016 Fluctuating asymmetry in the body shape of flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758) from Masao river, Butuan city, Agusan del Norte, Philippines. AACL Bioflux 9(4):929-936.