Probing the exposure to environmental stress using fluctuating asymmetry of metric traits in *Johnius vogleri*, (Bleeker, 1853) from lower Agusan River basin, Butuan City, Agusan del Norte, Philippines

[1]Jess H. Jumawan, [1]Cresencio C. Cabuga Jr, [1]Joycelyn C. Jumawan, [1]Ellaine M. B. Cortez, [1]Sheila M. N. Salvaleon, [1]Karen J. S. Gamutan, [1]Mary G. G. Dollisen, [1]Ana L. G. Suico, [2]Elani A. Requieron, [3]Mark A. J. Torres

[1] Biology Department, College of Arts & Sciences, Caraga State University, Ampayon, Butuan City, Philippines; [2] Mindanao State University, General Santos City, Philippines; [3] Department of Biological Sciences, College of Science and Mathematics, Mindanao State University, Iligan Institute of Technology, Iligan City, Philippines. Corresponding author: C. C. Cabuga Jr, cresenciocabugajr@gmail.com

Abstract. Fluctuating asymmetry (FA) was used to determine the possible effects on the morphological symmetry of *Johnius vogleri* sampled in lower Agusan river basin. The river as an aquatic system was known to be contaminated with bacteria and heavy metals. Numerous studies have demonstrated that FA can be used to assess the fish morphology exposed in various environmental conditions. However, no similar study was conducted in the area nor any assessment on fish conditions. There were a total of 100 samples collected (50 males and 50 females). Digital imaging was done and the captured images were loaded into tpsDig2 program. Standard landmarks on fish morphometrics were applied. Using thin-plate spline (TPS) series, landmark analysis were attained and subjected to symmetry and asymmetry in geometric data (SAGE) software. Results in Procrustes ANOVA revealed that the factors as individuals, sides and interaction of individuals and sides showed highly significant differences ($P<0.0001^{**}$). Similar results were observed to both male and female samples indicating FA of *J. vogleri* in lower Agusan river basin. The asymmetry in the morphology of the fish indicated exposure to environmental stress. Asymmetrical appearance was the manifestation in buffering environmental disturbance. Principal component analysis on the symmetry and asymmetry scores was applied to determine the affected landmarks. Ordination of the resulting data was displayed in deformation grids and histograms to both female and male samples. In females, affected landmarks were portion of the head and fins. In males, affected landmarks were largely from the head and caudal fin. The result of the study is highly important in future management actions of lower Agusan river basin.
Key Words: geometric morphometry, environmental health, bioindicators, developmental instability, ecological stress.

Introduction. Many of the organisms provide a vital role in predicting biological conditions. The empirical knowledge of evaluating the extent of effects through bioindicators will be an advantage to a better understanding to its morphology and the environment they inhabit.

Fishes play a fundamental role in human diet because of its high protein content, low saturated fat and sufficient omega fatty acids to maintain good health (Kumar et al 2011). Fish are often used as indicators of pollutants in the aquatic ecosystem since they occupy high trophic levels and an essential food source (Blasco et al 1998; Agah et al 2009). Understanding the biological importance of fishes is consequently significant for sustaining our efforts to advance our use and exploitation of fish resources.

Freshwater ecosystems were considered as the most endangered ecosystems in the world. Biodiversity was considerably greater in freshwaters than in the most affected terrestrial ecosystems (Sala et al 2000). It was also noted that freshwater ecosystems particularly the rivers were commonly affected by direct and indirect disturbances. These include households, agricultural and industrial runoffs that unswervingly affect the rivers. This would be reflected as pollutants that are direct components altering the morphological, behavioral, and physiological condition of many fish species. It causes objectionable effects, impairing the condition of the environment and reducing the quality of life and later may increase species mortality (Duruibe et al 2007). Ecological impairment would cause imbalances which forces species to exert resistance and adapt to the environment (Chau 2008). Furthermore, the most threatened groups are freshwater fishes because of high susceptibility to aquatic alteration (Laffaille et al 2005; Kang et al 2009; Sarkar et al 2008). Adverse conditions of an environment bring unfavorable effect to the species morphology or even raise the rate of death in the population.

To explore the possibility of environmental effects to the morphology of fishes, fluctuating asymmetry (FA) was employed as a diagnostic tool in geometric morphometry. It is considered as a fusion of geometry and biology (Bookstein 1982) dealing with the morphometric forms in two or three-dimensional space. FA performs to be an excellent bioindicator to understand the fitness of environmental condition (Lecera et al 2015). This is an efficient approach and costless manner of evaluating if the environment is proficient of ecological development (Angtuaco & Leyesa 2004). As well as, FA demarcated as intelligent dissimilarities between the left and the right sides of bilateral traits (Swaddle 2003) is said to be a marker of the well-being of the inhabitants and environmental standing.

Additionally, a great trait in FA corresponds with distress throughout the growth of different fishes (Hermita et al 2013). FA extensively used as an instrument for determining developmental variability (Ducos & Tabugo 2015). The usage of geometric morphometries in defining feature FA has widely recognized in various studies (Klingenberg & McIntyre 1998; Savriama et al 2012; Hermita et al 2013). According to Sommer (1996), stress is a direct component that can lessen the energy requirement of organisms to resist environmental condition. On the other hand, high FA reflects reduced developmental homeostasis at the epigenetic, chromosomal and molecular levels (Pursons 1990).

These morphological asymmetries were postulated to result from imperfect development and the inability of the genome to buffer developmental processes against random intrinsic factors (Waddington 1957; Zakharov 1992). FA is essential to biology because it reflects a population's state of adaptation and co-adaptation. FA is important morphometrics instrument to lesser, unsystematic aberration from the perfect morphology of organism because to its proficiency of giving total variance among the left and right side of a bilaterally symmetrical species (Moller & Swaddle 1997; Palmer & Strobeck 2003). Subsequently, it has been applied to theoretical and practical biology such as genetics, conservation biology, anthropology, agriculture and medicine (Graham et al 2010). Thus, FA provides vital information over another bioindicator of developmental instability due to its low cost and efficiency (Clarke 1993).

The significance of this study will determine the possible effects of pollutants in the morphology of *Johnius vogleri* locally known as "Guama". This fish species widely found in the area and consumed by local communities. At present, there were no conducted studies on *J. vogleri* as the biological indicator to assess environmental stress in the area. Consequently, FA has not been applied to define developmental instability in the morphology of *J. vogleri*. The study area was located in lower Agusan River basin which has reports on heavy metals contamination and pollution from sewages. Thus, this study would be highly significant in probing the possible effects to environmental stress on the morphology *of J. vogleri*. Furthermore, the study will be beneficial to environmental planners of the about possible exposure of the fish species to environmental stress.

Material and Method

Study area. The study area was part of Lower Agusan River basin. It was located in

Brgy. Pagatpatan, Butuan City and geographically lying between $8^{0}59^{'}26.35^{"}$ N $125^{0}31^{'}31.16^{"}$ E (Figure 1). The sampling of fishes was conducted in the month of August, 2015.

Figure 1. Map of the sampling area showing the Philippines (A), Butuan City (B), and Brgy. Pagatpatan (C) (http://maps.google.com).

Processing of fish samples. The collected fish samples were using fish identification manuals and verified based on www.fishbase.org. Fifty samples of *J. vogleri* per sexes (males and females) were collected and analyzed. *J. vogleri* individuals (50 males and 50 females) were preserved using 10% formalin and placed in a styrofoam box. Digital imaging was done using Canon digital camera (Pixma, 10 megapixels). To obtain the length of individuals, the left and right lateral side of each sample was taken using a ruler (Natividad et al 2015). The captured images were digitized using the tpsDig2 program (version 2.0, Rohlf 2004) and saved as a TPS file. The identification of males and females was done based on its external morphology and later confirmed by direct examination of the gonads. The male fishes had whitish soft textured gonads while yellowish coarsely textured gonads with eggs for female specimens (Requieron et al 2010).

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. Digitization was copied in triplicates for each sample to reduce the discrepancies and errors in plotting the landmark points (Natividad et al 2015).

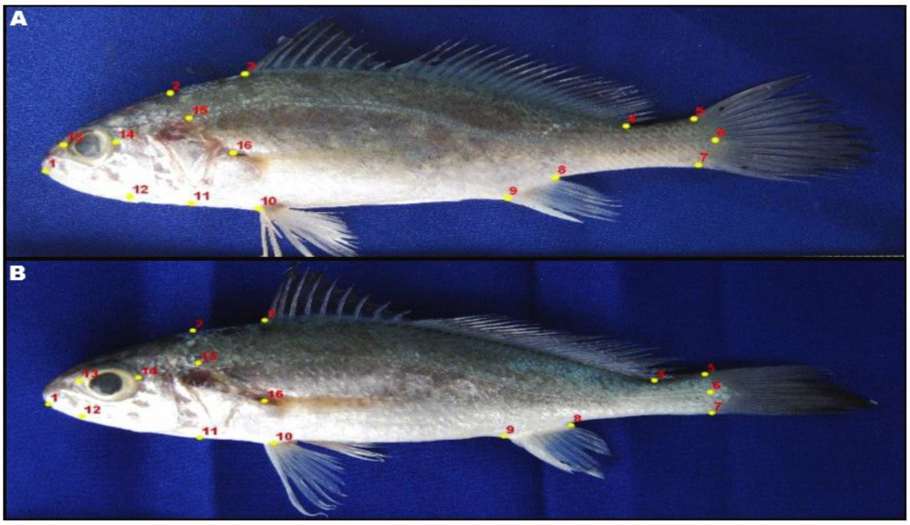

Figure 2. Landmark points of *Johnius vogleri* female (A) and male (B) (Original).

Table 1
Description of the landmark points in the body shape of *Johnius vogleri* (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
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 FA 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 symmetry and asymmetry in geometric data (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 *J. vogleri*. 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 or (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 is shown in Table 2.

Table 2

Procrustes ANOVA on body shape of *Johnius vogleri* in terms of sexes

Factors	*SS*	*DF*	*MS*	*F*	*P-VALUE*
			Female		
Individuals	0.2846	1372	0.0002	4.5763	<0.0001**
Sides	0.0156	28	0.0006	12.2981	<0.0001**
Individual x Sides	0.0622	1372	0	6.2649	<0.0001**
Measurement Error	0.0405	5600	0	-	-
			Male		
Individuals	0.2853	1372	0.0002	2.1611	<0.0001**
Sides	0.0192	28	0.0007	7.1379	<0.0001**
Individual x Sides	0.132	1372	0.0001	8.0414	<0.0001**
Measurement Error	0.067	5600	0	-	-

** (P<0.0001) highly significant.

There were 3 factors considered in the analysis: the individuals, sides, and the interaction of individuals and sides. The analysis was implemented to both male and female of the fish species. The results showed FA for the 3 factors considered and also between male and female specimens (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.

The data showed that *J. vogleri* samples in Agusan River were already asymmetrical 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 was both similarly observed when samples were pooled as males and females. The asymmetry in the morphology of the three factors among males and females could be an indication 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 environment might contain pollutants affecting the morphology of the fish. The prolonged exposure to disturbed and polluted water conditions eventually will lead to asymmetrical appearance of *J. vogleri* in the sampling area.

The data illustrated the evidence for FA of the fishes that can be attributed to a degrading condition in the environment possibly from pollutants. As a consequence, the disturbed 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 *J. vogleri* eventually resulted to this FA.

Principal component analysis was implemented to determine 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).

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	65.7583%	100%	29.415%	1,2,3,5,6,7,8,9,10
PC2	7.9016%		16.9888%	1,2,3,6,7,8,9,10, 11
PC3	5.3519%		11.3923%	1,2,4,6,7,8,9,10, 11
	79.0081%		57.7961%	
		Male		
PC1	55.489%	100%	51.4384%	1,2,3,5,6,7,8,9,10 11,12,13,14,15,16
PC2	15.8195%		11.459%	1,2,5,6,7,8,9,10 12,13,15
PC3	7.2258%		9.3028%	1,2,5,8,11,12,13 14,15
	78.5343%		72.2002%	

Skewness of the histogram was reflected in every PC score along with the deformation grid to determine affected landmarks (Figures 4 & 5).

In the female samples, the three principal components (PC) constituted 79% of the cumulative variation. PC 1 has the highest variation accounted to 69.76%. The affected landmarks common to the three PC score were landmarks 1, 2, 6, 7, 8, 9 and 10 (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 78.53% of the cumulative variation. PC 1 contributed the highest accounted variation with 51.49%. The commonly affected landmarks in male samples were landmarks 1, 2, 5, 12, 13 and 15 (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.

It was observed that in females, the heavily affected landmarks were portion of the head and fins while in males were largely the head and caudal fin. It was interesting to note that affected landmarks were different 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).

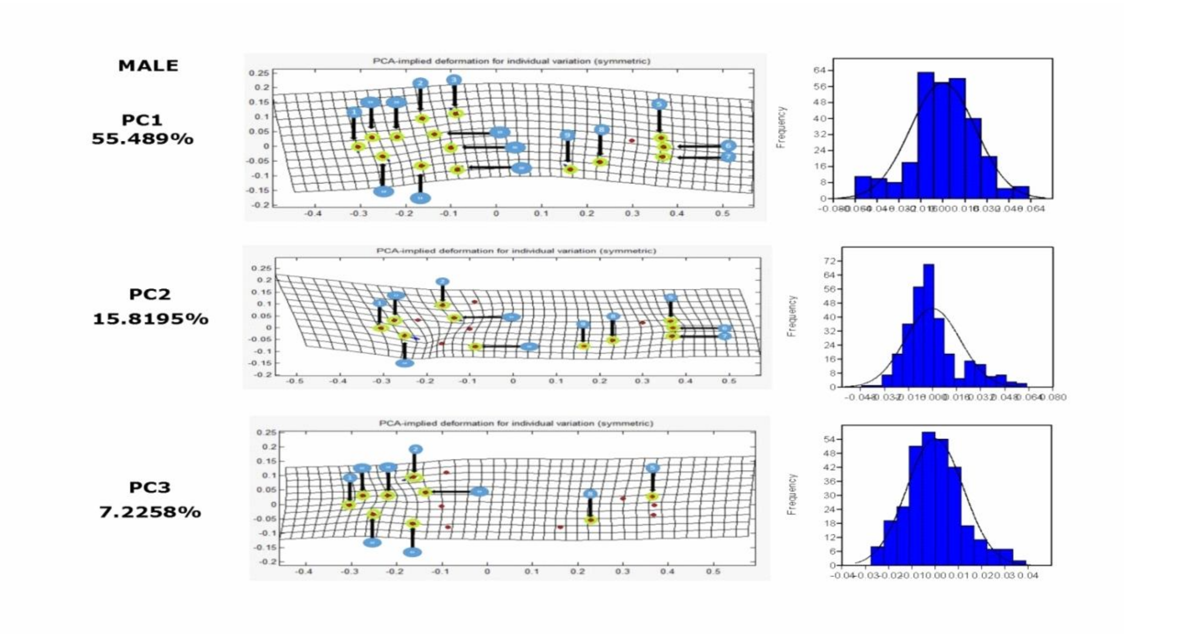

Figure 4. Principal components (PC) implied deformation grid and a histogram of individual (symmetric) in *Johnius vogleri* male species.

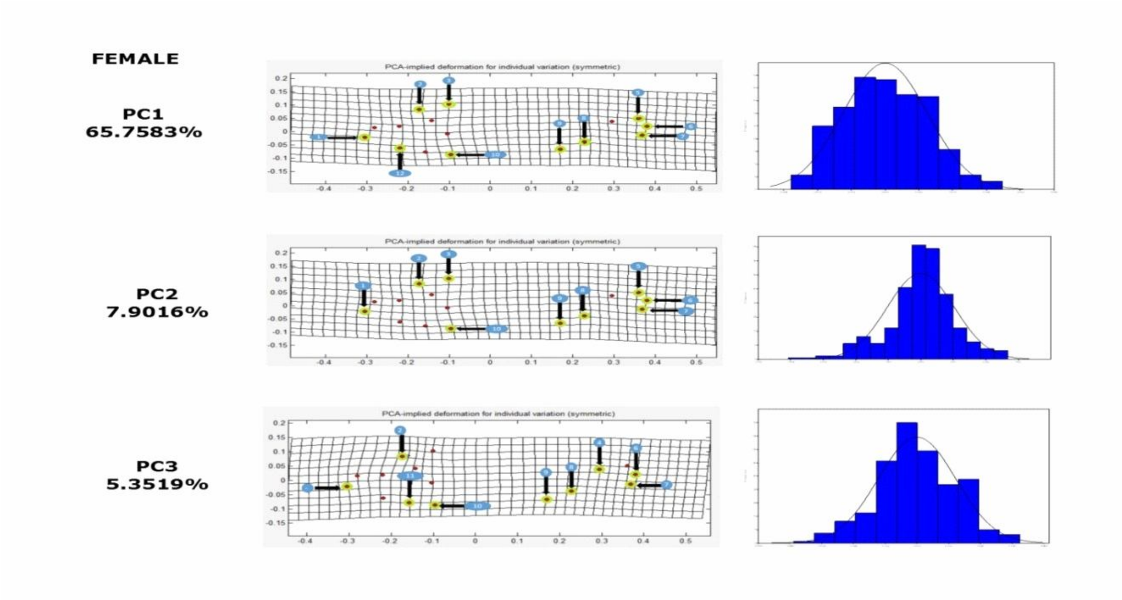

Figure 5. Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *Johnius vogleri* female species.

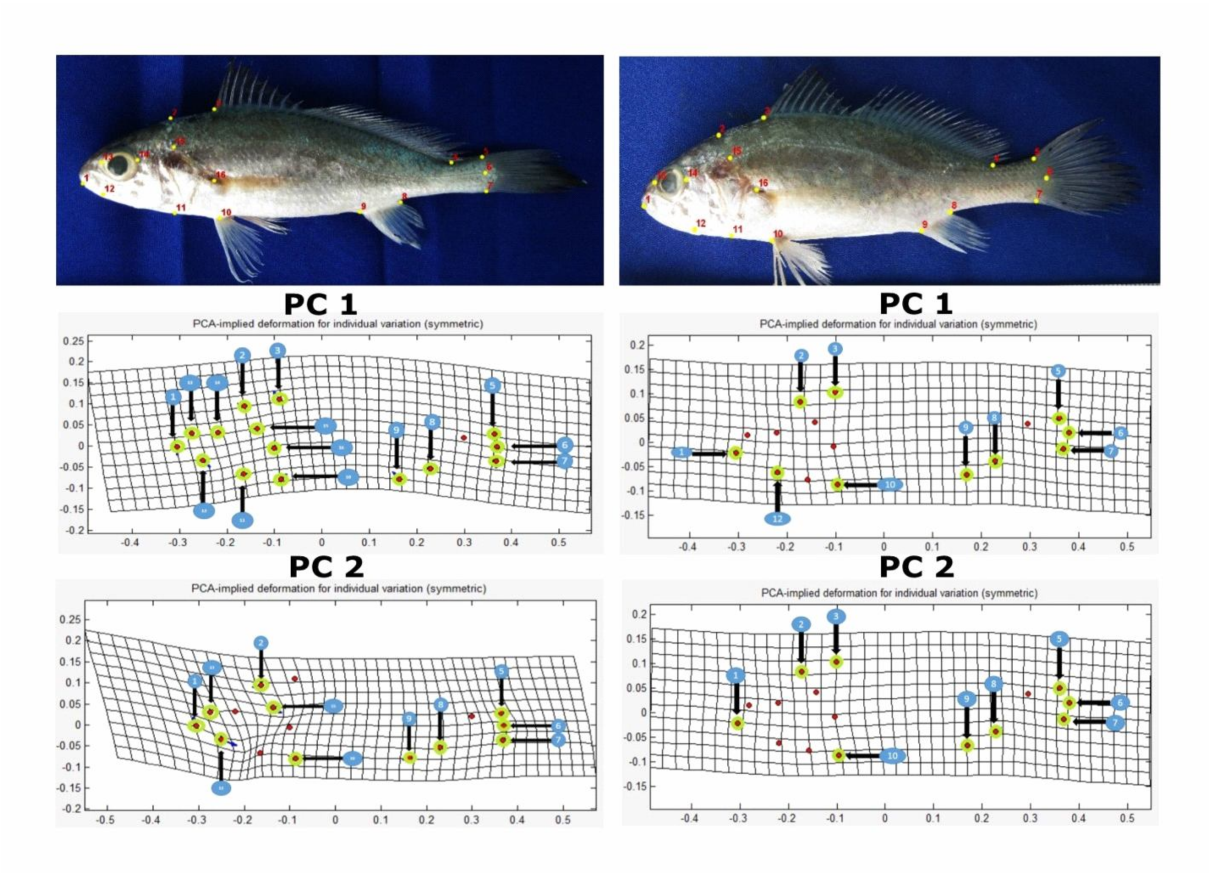

Figure 6. Actualized picture of digitized male and female fish with the affected landmarks shown in PCA- deformation grid for PC1 and PC2.

This study shows elevated FA that only certifies that lower Agusan river basin ultimately contents pollutants affecting the asymmetry of *J. vogleri*. Similar studies conducted in the Philippines shows a comparable result. According to Ducos & Tabugo (2015) with *Gafrarium tumidum* as a bioindicator had confirmed that the greater the F value the higher the stress a species are undertaken due to the environmental situation. It was stated that not all character examined in the fish exhibited FA (Swaddle 2003; Costa & Cataudella 2007; Graham et al 1993). Dissimilarities in the levels of FA validated that different traits may vary the ability to shield developmental stress and homeostasis. (Graham et al 1993; Lens et al 2002). Similar studies also confirmed that in female *Leiopotherapon plumbeus* high significant level of FA specifies difficulty of sustaining specific development that results to adverse effects on the population through time (Markow 1995). Elevated FA suggests the organisms poor developmental ability (Palmer 1994). It also proposes that among the possible bases of FA is the presence of the parasite in the host species affecting its state of well-being and adaptation (Pojas & Tabugo 2015). 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 results of this study will be an important information to confirm the current condition of lower Agusan River basin and showed that FA is said to be effective as an indicator of ecological stress and developmental instability.

Conclusions. The study demonstrated the use of FA as a tool in probing the exposure to environmental stress of *J. vogleri* samples in lower Agusan river basin. The sampling area was known as catching basin from tributaries in upper Agusan River basin. Mining effluents, industrial wastes, and sewages were possible sources of pollutants. Heavy metal and bacterial contamination were known issues in the management of this valuable aquatic system. Yet, there were no studies conducted to assess the metric traits in *J. vogleri* exposed to possible pollutants from lower Agusan river basin. The results demonstrated that the species in the sampling area showed FA in both female and male samples. This non-symmetrical observation in the species morphology was observed in the three measured factors: individuals, sides, and interaction of individuals and sides. The affected landmarks for females were portion of the head and fins. In males, affected landmarks were largely from the head and caudal fin. The results of the study are highly important in future management action of lower Agusan River basin.

Acknowledgements. The researchers would like to express their gratitude to Mafi Kamille A. Angco, Christian James R. Presilda and Owen Lloyd P. Obenza for their assistance in processing the fish samples for analysis.

References

Agah H., Leermakers M., Elskens M., Fatemi S. M., Baeyens W., 2009 Accumulation of trace metals in the muscles and liver tissues of five fish species from the Persian Gulf. Environmental Monitoring and Assessment 157:499-514.

Angtuaco S. P., Leyesa M., 2004 Fluctuating asymmetry: an early warning indicator of environmental stress. Asian Journal of Biology Education 2:3-4.

Blasco J., Rubio J. A., Forja J., Gomez-Parra A., Establier R., 1998 Heavy metals in some fishes of the muglidae family from salt-pounds of Codiz Bay SW Spain. Ecotoxicology and Environmental Research 1:71-77.

Bonada N., Williams D. D., 2002 Exploration of utility of fluctuating asymmetry as an indicator of river condition using larvae of caddisfly *Hydropsychemorosa* (Trichoptera: Hydropsychidae). Hydrobiologia 481:147-156.

Bookstein F. L., 1982 Foundations of morphometrics. Annual Review of Ecology and Systematics 13:451–470.

Chakraborty P., Amarasingle T., Sparks J. S., 2008 Rediscription of ponyfishes (Teleostei: Leiognathidae) of Sri Lanka and the status of *Aurigeguula* fowler 1918. Ceyion Journal of Science (Biological Sciences) 37(2):143-161.

Chau P., 2008 Ecosystem balance and biodiversity. Chapter 2, Ecology, Part C. ENVR 30.

Clarke G. M., 1993 Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. Environmental Pollution 82:207–211.

Costa C., Cataudella S., 2007 Relationship between shape and trophic ecology of selected species of Sparids of the Caprolace coastal lagoon (Central Tyrrhenian sea). Environmental Biology of Fishes 78:115-123.

Ducos M. B., Tabugo S. R. M., 2015 Fluctuating asymmetry as bioindicator of stress and developmental instability in *Gafrarium tumidum* (ribbed venus clam) from coastal areas of Iligan Bay, Mindanao, Philippines. AACL Bioflux 8(3):292-300.

Duruibe J. O., Ogwuegbu M. O. C., Egwurugwu J. N., 2007 Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences 2(5):112-118.

Graham J. H., Freeman D. C., Emlem J. M., 1993 Antisymmetry, directional symmetry and dynamic morphogenesis. Genetica 89:121-173.

Graham J. H., Raz S., Hel-Or H., Nevo E., 2010 Fluctuating asymmetry: methods, theory, and applications. Symmetry 2(2):466-540.

Hammer O., Harper D. A. T., Ryan P. D., 2001 Past: Paleontological statistics software package for education and data analysis. Palaeontological Electronica 4(1):1-9.

Hermita J. M., Gorospe J. G., Torres M. A. J., Lumasag J. L., Demayo C. G., 2013 Fluctuating asymmetry in the body shape of the mottled spinefoot fish, *Siganus fuscescens* (Houttuyn, 1782) collected from different bays in Mindanao Island, Philippines. Science International (Lahore) 25(4):857-861.

Kang B., Daming H., Perrett L., Wang H., Hu W., Deng W., Wu Y., 2009 Fish and fisheries in the Upper Mekong: current assessment of the fish community, threats and conservation. Reviews in Fish Biology and Fisheries 19(4):465-480.

Klingenberg C. P., McIntyre G. S., 1998 Geometric morphometrics of developmental instability: analysing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52(5):1363-1375.

Kumar B., Mukherjee D. P., Kumar S., Mishra M., Dev Prakash S., Singh K., Sharma C. S.,

2011 Bioaccumulation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in east Kolkata wetlands. Annals of Biological Research 2(5):125-134.
Laffaille P., Acou A., Guillouet J., Legault A., 2005 Temporal change in European eel, *Anguilla anguilla*, stocks in a small catchment after installation of fish passes. Fisheries Management and Ecology 12:123–129.
Lecera J. M. I., Pundung N. A. C., Banisil M. A., Flamiano R. S., Torres M. A. J., Belonio C. L., Requieron E. A., 2015 Fluctuating asymmetry analysis of trimac *Amphilophus trimaculatus* as indicator of the current ecological health condition of Lake Sebu, South Cotabato, Philippines. AACL Bioflux 8(4):507-516.
Lens L., Van Dongen S., Kark S., Matthysen E., 2002 Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies. Biological Reviews of the Cambridge Philosophical Society 77(1):27-38.
Markow T. A., 1995 Evolutionary ecology and developmental instability. Annual Review of Entomology 40:105–120.
Marquez E., 2007 Sage: symmetry and asymmetry in geometric data version 1.05 (compiled 09/17/08). Available online at: http://www.personal.umich.edu/~ emarquez /morph/
Moller A. P., Swaddle J. P., 1997 Asymmetry, developmental stability, and evolution. Oxford University Press, Oxford, U.K.
Natividad E. M. C., Dalundong A. R. O., Ecot J., Jumawan J. H., Torres M. A. J., 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., 1994 Fluctuating asymmetry analysis: a primer. In: Developmental instability: its origins and evolutionary implications. Markow T. A. (ed), London: Kluwer Academic.
Palmer A. R., Strobeck C., 2003 Fluctuating asymmetry analyses revisited. In: Developmental instability. Causes and consequences. Polak M. (ed), pp. 279-319, Oxford University Press, Oxford.
Parsons P. A., 1990 Fluctuating asymmetry: an epigenetic measure of stress. Biological Review 65:131–145.
Pojas R. G., Tabugo S. R. M., 2015 Fluctuating asymmetry of parasite infested and non-infested *Sardinella sp*. from Misamis Oriental, Philippines. AACL Bioflux 8(1):7-14.
Requieron E. A., Torres M. A. J., Manting M. M. E., Demayo C. G., 2010 Relative warp analysis of body shape variation in three congeneric species of ponyfishes (Teleostei: Perciformes: Leiognathidae). Transactions 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 New York.
Sala O. E., Chapin F. S., Armesto J. J., Berlow R., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L. F., Jackson R. B., Kinzig A., Leemans R., Lodge D., Mooney H. A., Oesterheld M., Poff N. L., Sykes M. T., Walker B. H., Walker M., Wall D. H., 2000 Global biodiversity scenarios for the year 2100. Science 28:1770–1774.
Sarkar U. K., Pathak A. K., Lakra W. S., 2008 Conservation of freshwater fish resources of India: new approaches, assessment and challenges. Biodiversity and Conservation 17:2495–2511.
Savriama Y., Gomez J. M., Perfectti F., Klingenberg C. P., 2012 Geometric morphometrics of corolla shape: dissecting components of symmetric and asymmetric variation in *Erysimum mediohispanicum* (Brassicaceae). New Phytologist 196:945-954.
Sommer C., 1996 Ecotoxicology and developmental stability as an in situ monitor of adaptation. Ambio 25:374–376.
Swaddle J. P., 2003 Fluctuating asymmetry, animal behavior and evolution. Advances in the Study of Behavior 32:169-205.
Van Valen L., 1962 A study of fluctuating asymmetry. Evolution 16:125–142.
Waddington C. H., 1957 The strategy of the genes. Allen & Unwin, London.
Zakharov V. M., 1992 Population phenogenetics: analysis of developmental stability in

natural population. Acta Zoologica Fennica 191:7-30.
**http://maps.google.com
**http://www.fishbase.org

Received: 12 October 2015. Accepted: 18 February 2016. Published online: 24 February 2016.
Authors:
Jess Hornijas Jumawan, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: jehoju78@yahoo.com
Cresencio Cata-ag Cabuga Jr, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: cresenciocabugajr@gmail.com
Joycelyn Cagatin Jumawan, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: joycejumawan@gmail.com
Ellaine Mae Bayron Cortez, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: ellainecortez227@gmail.com
Sheila Mae Namoc Salvaleon, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: sheilamaesalvaleon96@gmail.com
Karen Joy Siarez Gamutan, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: mykarenjoy@gmail.com
Mary Grace Guiritan Dollisen, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail:dollisen_marygrace@yahoo.com
Ana Liza Gascon Suico, Caraga State University, College of Arts and Sciences, Biology Department, Philippines, Ampayon, Butuan City, Agusan del Norte, 8600, e-mail: suico021987@gmail.com
Elanie Antonio Requieron, Mindanao States University College of Natural Sciences and Mathematics, Science Department, Philippines, General Santos City, Brgy. Fatima General Santos City, 9500, e-mail: elanie_requieron2003@yahoo.com
Mark Anthony Jariol Torres, Mindanao State University-Iligan Institute of Technology, College of Science and Mathematics, Department of Biological Sciences, Philippines, Iligan City, 9200, Andres Bonifacio St, e-mail: torres.markanthony@gmail.com

How to cite this article:
Jumawan J. H., Cabuga C. C. Jr, Jumawan J. C., Cortez E. M. B., Salvaleon S. M. N., Gamutan K. J. S., Dollisen M. G. G., Suico A. L. G., Requieron E. A., Torres M. A. J., 2016 Probing the exposure to environmental stress using fluctuating asymmetry of metric traits in *Johnius vogleri*, (Bleeker, 1853) from lower Agusan River basin, Butuan City, Agusan del Norte, Philippines. AACL Bioflux 9(1):122-132.