International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 3, p. 214-227, 2021

INNSPUB

RESEARCH PAPER OPEN ACCESS

Morphological Analysis on the Body Shape of *Oreochromis niloticus* using Fluctuating Asymmetry as an Indicator of Stress in Major River Tributaries of Bayog Watershed

Rosanilio M. Yagos[1,2]

[1]*School of Agriculture, Forestry and Environmental Sciences, J.H. Cerilles State College, Mati, San Miguel, Zamboanga del Sur, Philippines*

[2]*Mindanao State University - Iligan Institute of Technology, Tibanga, Iligan City, Philippines*

Key words: Fluctuating Asymmetry, Bioindicator, Morphology, Tilapia.

http://dx.doi.org/10.12692/ijb/18.3.214-227 Article published on March 30, 2021

Abstract

This study utilized the fluctuating asymmetry as a biomarker of stress in the morphology of *O. niloticus* from the river tributaries of Bayog Watershed, Bayog, Zamboanga del Sur. A total of 136 samples were used in this study, distributed into 17 males and 17 females for each of the sampling sites. Then, it was landmarked with the use of thin plate spline (TPS) series. Eighteen anatomical landmarks were selected on fish images and were subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software. Procrustes two-way mixed model ANOVA showed significant levels of both directional and fluctuating asymmetries (FA) among populations and between sexes. However, Principal Component Analysis (PCA) revealed that populations from Depore, Dipili and Guinoman rivers have relatively higher fluctuating asymmetry compared to Sibugay river based on localized trait asymmetry and the number of affected landmarks. Thus, it indicates more stress or disturbances experienced by the three populations of *O. niloticus* compared to the Sibugay river. Understanding the relationship between the species and its environment would be useful to determine the health of a given freshwater ecosystem. This might be essential to the local government for inclusion in the policies for the management of the river system and future studies in the area.

* **Corresponding Author:** M. Yagos ✉ rmyagos@gmail.com

Introduction

Freshwater bodies were considered the most endangered ecosystems in the world. It was also noted that freshwater ecosystems particularly the rivers were commonly affected by direct and indirect disturbances. This includes household, farm and industrial runoffs that have an unwavering effect on rivers (Natividad *et al*., 2015). Collective pollution of aquatic ecosystems had become a major concern (Green and Lochmann, 2006; Ozsoy *et al*., 2007). These were direct factors influencing the physiological processes of some aquatic species. More subtle effects include reduced growth, increased disease susceptibility, and increased rates of morphological anomalies. Early detection of sub-lethal effects can allow remedial measures to be taken by biologists and resource managers to avoid further habitat destruction and species population declines.

Many organisms provide a vital role in predicting biological conditions. The scientific understanding of determining the magnitude of impacts through bioindicators would be an advantage to better understand the environment they inhabit. Fishes served as an indicator of environmental conditions because of their tolerance to pollution in an aquatic environment (Pascual and Abollo, 2005). Fishes are often used as indicators of pollutants in the aquatic ecosystem since they occupy high trophic levels and are intimately associated with their habitats (Agah *et al.,* 2009).

Oreochromis niloticus, also known as tilapia, is a widely caught fish in the Bayog watershed. This species is considered to have economic value in the region due to its abundance and is consumed and sold on the market by local communities. It is also considered to be capable of tolerating different environmental conditions (Jumawan *et al*., 2016). Some studies have used it to determine the ecological state of the river system (Peligro *et al.,* 2016).

One of the morphological characteristics associated with habitat change and environmental stress is fluctuating asymmetry (FA). The use of FA to detect environmental disturbances and as an ecological condition indicator was suggested by numerous studies (Jawad *et. al.,* 2011; Natividad *et. al*., 2015; Lecera *et al*., 2015; Tabugo *et al*., 2016). FA is important to reflect a population's state of adaptation and co-adaptation (Lens *et al*., 2001; Hermita *et al*., 2013). Also, it is frequently used as a measure of developmental stability (Van Valen, 1962). In the Utayopas study (2001), a strong association between FA and environmental disruptions was found. In addition, due to its ability to elucidate changes in the shape on both the left and right side of a bilaterally symmetrical organism, FA is an important tool in morphometric (Palmer and Strobeck, 2003). The assumption of the FA study is that the formation of the two sides of a bilaterally symmetrical organism is regulated by similar genes and, thus, the non-directional variations between the sides must be of environmental origin and represent accidents occurring during development.

Thus, this study aimed to assess the fluctuating asymmetry in the body shape of *O. niloticus* as an indicator of the ecological health of the major river tributaries in Bayog watershed.

Materials and methods

Study area

The study was conducted in the Bayog Watershed, which extends from Bayog, Zamboanga del Sur (7.9281° N, 123.0564° E) towards Diplahan (7.744989N, 122.9685E) and Imelda (7.6578° N, 122.9444°E), Zamboanga Sibugay, Mindanao, Philippines. A total of four sampling stations were established to represent the river systems of the watershed. Site 1 is located in Depore River, Site 2 is located in Dipili River, site 3 is located in Sibugay River and site 4 is located in Guinoman River (Fig. 1).

Sample collection

Various methods have been used to gather samples such as electrical shock systems, seine nets, hand nets, fish traps and the hook-and-line process. A total of 136 samples were used in this study distributed into 17 males and 17 females for each of the sampling sites. Only sexually matured samples were chosen for digitalization. The samples were then transported to the laboratory for further processes using styropore box with ice. The sex of individual samples was determined using external morphology and further confirmed by gonads examination (Dorado *et al.*, 2012).

Data acquisition

The samples were measured and photographed taking the left and right side of its body using a Cannon 450D DSLR camera, while it is being flanked on white styrofoam and pinning its fins to show its points of origin. It was then processed for digitization, landmarking and further statistical analysis.

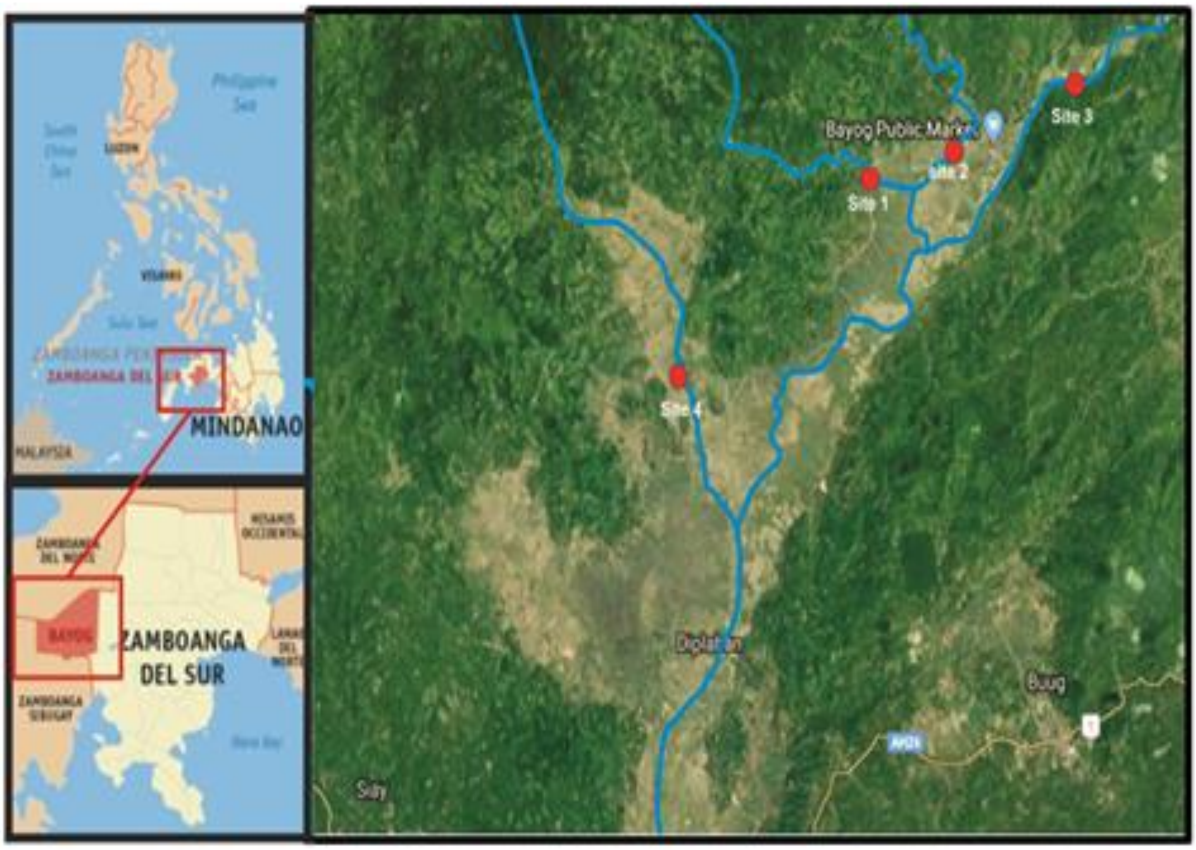

Fig. 1. Map location of the study sites in Bayog Watershed, Bayog Zamboanga del Sur. In set is the map of the Philippines and map of Zamboanga del Sur.

Landmark selection and digitization

A landmark analysis was used to obtain its distorted features within the images using TPS series. Twenty landmarks (Fig. 2 and Table 1) were chosen to give a detailed summary of the morphology of the fish. The digitized landmarks used in this study were the standard points for fish morphometric and have evolutionary and functional importance (Turan, 1999; Turan *et al.*, 2004; Costa and Cataudella, 2007; Buitrago-Suarez and Brooks, 2007; Vasconcellos *et al.*, 2008). The landmarks were digitized using the TpsDig ver. 2.10 (Rohlf, 2006). Digitization was copied in triplicates for each fish sample, to minimize the inconsistencies or errors in plotting the landmark points.

Shape analysis

The x and y coordinates will serve as primary data in calculating the fluctuating asymmetry of *O. niloticus*. Flat form landmark coordinates from the left and right images of the TPS version were subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software version 1.04, (Marquez, 2007) to determine geometric data of object with emphasis on its asymmetry. SAGE software is useful in shape confirmation of individuals' variation (symmetric, asymmetric and error) including the probable covariance condition. Procrustes ANOVA was used with triplicates and with 99 permutations to analyse and measure the remaining asymmetry. This specifies the difference between sides and is the degree of indicator asymmetry. The effect called "*sides*" is the variation between the two sides of the individual; it is a measure of directional asymmetry (DA). The individual's mean square is a measure of total phenotypic variation and it is random. The interaction of "individual by sides" is the failure to be the same from side to side with the effect of individuals; it is a measure of fluctuating asymmetry and antisymmetry; differences could depend on both environmental and genetic conditions.

In addition, PCAs of the covariance matrix associated with the FA variation portion was carried out to simultaneously perform an interpolation based on a thin-plate spline analysis to visualize shape changes as landmark displacement in deformation grids.

Results and discussion

In this study, fluctuating asymmetry was evaluated in the left and right body shape of *O. niloticus*. Here, fluctuating asymmetry measures nonconformities from the normal state of symmetry and is, therefore signifies to illustrate the level of genetic and environmental disturbances experienced by the species during development.

It tests the differences in the symmetry of bilateral organisms' right and left sides since; both sides of a symmetrical structure are said to be identical genetically, a similar history of gene activity and experiencing the same environment. Hence, FA is considered the most common tool used for measuring developmental instability (Graham *et al.*, 2010). Here, fluctuating asymmetry was used as a biomarker of stress and as an indicator of developmental instability.

Table 1. Landmarks' description of *O. niloticus*.

No.	Description
1	Snout tip
2	Posterior end of nuchal spine
3 and 4	Anterior and posterior insertion of the 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 operculum
16	Point of maximum extension of operculum on the lateral profile
17 and 18	Superior and inferior insertion of the pectoral fin

To elucidate the individual morphology variation, Procrustes ANOVA was used. Results from Procrustes ANOVA yield significant FA values in terms of sides and interaction, and, individual and sides for all the sites of both male and female (Table 2). This result suggests that there are a deviation from perfect symmetry among *O. niloticus* populations in all sites and these were significantly due to directional (DA) and fluctuating asymmetries (FA). Directional asymmetry is a possibility for a trait to be constantly developed in a different way on the left and right body sides (Klingenberg, 2015). However, it seemed that asymmetry was largely due to FA in most of the sites except Depore female population, in which DA contributed much of its asymmetry. It means that there is a highly significant asymmetry in terms of the left and right sides of each sample in Depore female population. This may be due to the ongoing mining activity in the upper mountain, which is directly linked to this river. It is known that heavy metals and acidification could affect the morphology of organisms such as fishes (Mazzi and Bakker, 2001; Vollestad and Hindar, 2001). Leachate from mining operations contains heavy metals and other substances that could affect the health of the streams and rivers.

Table 2. Procrustes ANOVA results witihing sexes of *O. niloticus* populations from four sites.

Factors	SS	DF	MS	F	P-VALUE
DEPORE					
Female					
Sides (DA)	0.03654	32	0.0011419	12.2856	<0.0001**
Individual x Sides (FA)	0.047588	512	9.2945e-05	6.5358	<0.0001**
Male					
Sides (DA)	0.011872	32	0.000371	4.5208	6.0729e-14**
Individual x Sides (FA)	0.042017	512	8.2064e-05	4.9096	<0.0001**
DIPILI					
Female					
Sides (DA)	0.016286	32	0.00050894	4.2399	9.8832e-13**
Individual x Sides (FA)	0.061458	512	0.00012004	7.6703	<0.0001**
Male					
Sides (DA)	0.018907	32	0.00059086	6.3468	<0.0001**
Individual x Sides (FA)	0.047665	512	9.3095e-05	9.0176	<0.0001**
SIBUGAY					
Female					
Sides (DA)	0.016576	32	0.00051799	4.2418	9.7011e-13**
Individual x Sides (FA)	0.062523	512	0.00012212	9.8754	<0.0001**
Male					
Sides (DA)	0.01432	32	0.0004475	7.0887	<0.0001**
Individual x Sides (FA)	0.032322	512	6.3129e-05	8.0035	<0.0001**
GUINOMAN					
Female					
Sides (DA)	0.0057588	32	0.00017996	1.9356	0.0018685*
Individual x Sides (FA)	0.047604	512	9.2976e-05	9.2861	<0.0001**
Male					
Sides (DA)	0.0091356	32	0.00028549	5.2855	<0.0001**
Individual x Sides (FA)	0.027655	512	5.4014e-05	6.6333	<0.0001**

*DA - directional asymmetry; FA - fluctuating asymmetry; ** (P<0.0001) highly significant.*

Notably, human interventions were present in the four sampling sites. All of the sites were affected by agricultural effluents which served as direct drainage of water from agricultural areas such as rice fields. Pesticides and fertilizers are common in farming practices, thus excessive use of these chemicals may contaminate the freshwater system.

Under normal conditions, a symmetrical appearance on the fish species could be expected (Daloso, 2014). However, the observed FA would suggest that the aquatic environment is highly disturbed or might contain pollutants affecting the morphology of the fish (Natividad *et al.*, 2015; Cabuga *et al.*, 2016). The asymmetry in the morphology of the two factors (sides and interaction, and individual and sides) among males and females could be an indication that the species in the study area was under environmental stress.

This means that the degree of FA shows the failure in retaining homeostasis when exposed to stress during development (Dongen, 2006) as shown in the individual effect among individual genotypes. The prolonged exposure to disturbed and polluted water conditions eventually will lead to asymmetrical appearance (Galloway *et al.*, 2004; Allenbach, 2011; Ducos and Tabugo, 2015; Jumawan *et al.*, 2016) of *O. niloticus* in the sampling area. Barrett (2005) stated that stressors in the environment affect the species' ability to develop the desired path phenotypically.

Table 3. First and second PC scores with the symmetry and asymmetry values and summary of the affected landmarks.

PCA	Individual (symmetry)	Sides (directional asymmetry)	Interaction (fluctuating asymmetry)	Affected Landmarks
		DEPORE		
		Female		
PC1	34.1776%	100%	35.5246%	1,2,3,9,10,11,12,13,14,15,16,17,18
PC2	22.6991%		18.676%	1,2,3,4,5,6,7,8,9,10,11,12,15,16
		Male		
PC1	50.6351%	100%	33.7306%	1,2,3,8,9,11,12,14,15,16,17
PC2	24.8094%		22.7893%	1,2,3,4,7,9,11,14,15,16
		DIPILI		
		Female		
PC1	46.3376%	100%	33.4141%	1,2,10,11,12,13,14,15,16,17,18
PC2	17.0777%		21.5747%	1,2,3,10,11,12,15,16,17,18
		Male		
PC1	47.2945%	100%	36.5615%	1,2,5,6,7,9,11,12,14,15,16,17,18
PC2	15.6445%		22.6021%	1,5,6,8,9,10,11,13,14,16,17,18
		SIBUGAY		
		Female		
PC1	45.8949%	100%	48.0305%	1,2,3,7, 9,10,11,12,17,18
PC2	18.9962%		20.5298%	1,2,3,5,6,9,10,12,15,16
		Male		
PC1	50.9642%	100%	39.4905%	1,2,11,14,15,16,17,18
PC2	15.4782%		19.7933%	1,2,3,10,12,15,16
		GUINOMAN		
		Female		
PC1	29.6885%	100%	41.5658%	1,2,3,4,7,8,9,10,12,14,15,16,17,18
PC2	21.2025%		16.6287%	2,3,8,9,10,11,12,13,14,15,16
		Male		
PC1	65.5472%	100%	25.9411%	1,5,6,7,8,9,10,12,13,14,15,16,17,18
PC2	15.1457%		23.6502%	1,4,8,9,10,11,12,13,14,15,16,18

Another way to determine and examine variability in the body shape for each population was through Principal Component Analysis (PCA) derived from Procrustes analysis. This analysis illustrates shape variation in accordance to the landmark points (Table 3 and Fig. 3 & Fig. 4). The first principal component portrays vectors at landmarks that reflect the scale and course in which that landmark is displaced compared to the others. The second illustrates the variance from the thin plate splines, an interpolation function that models change among landmarks from the data of variations in coordinates of landmarks. The percentage values of PCA represent the level of variability in the data.

Table 3 shows the first and second principal component illustrating the percentage values of symmetry and asymmetry with the details of the affected landmarks and is further illustrated and summarized in Fig. 3, Fig. 4 and Table 4 through implied deformation plot for individual and side interaction. It further showed that the most common landmarks affected and can be found in both of the first two PCs in Depore female population are 1,2,3,9,10,11,12, 15, 16 and in the male population are 1, 2,3,4,9,11, 14,15 and 16 that can be explained 54.20% and 56.52% of fluctuating asymmetry respectively. In Dipili female population, landmarks 1,2,10,11,12,15,16,17 and 18 (54.99% FA) while in male population, landmarks 1,5,6,9,11,14,16,17 and 18 (59.16% FA) were the common affected landmarks. In sibugay population, the affected landmarks are, 1,2,3,9,10,12 (68.56% FA) in female and1,2,15,16 (59.28% FA) in male. Lastly, in Guinoman population, the most common landmarks affected and can be found in both of the first two PCs are 2,3,8,9,10 and 12 in female (58.19% FA), while landmarks 1,8,9,10,12,13,14,15,16 and 18 (49.59% FA) in male.

Table 4. Presence of localized trait asymmetry in the populations of the O. niloticus.

	Character	Depore river		Dipili river		Sibugay river		Guinoman river	
		F	M	F	M	F	M	F	M
1	Snout tip	**	**	**	**	**	**	*	**
2	Posterior end of nuchal spine	**	**	**	*	**	**	**	-
3	Anterior insertion of the dorsal fin	**	**	*	-	**	*	**	-
4	posterior insertion of the 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 operculum	**	**	**	*	*	**	**	**
16	Point of maximum extension of operculum on the lateral profile	**	**	**	**	*	**	**	**
17	Superior insertion of the pectoral fin	*	*	**	**	*	*	*	-
18	inferior insertion of the pectoral fin	*	-	**	**	*	*	*	**
	TOTAL	18	13	12	16	14	11	16	15

*First two PCA test for the presence of traits asymmetry: *(present at least in a PCA), ** (present in first 2 PCA).*

Table 4 shows the presence of localized trait asymmetry in the populations of the *O. niloticus*. It further showed that females in all populations have greater trait asymmetry based on the first two PCs except in Dipili River.

Within the population, a sex difference was also observed (Table 3 and Table 4). For instance, in Guinoman population, only females exhibit FA in the posterior end of the nuchal spine, anterior insertion of the dorsal fin and superior insertion of the pectoral fin. In Sibugay population, males do not show FA in Dorsal insertion of the caudal fin, Midpoint of lateral line and anterior insertion of the anal fin. On the other hand, only males in Dipili population showed FA in Dorsal insertion of the caudal fin, Midpoint of the lateral line, Ventral insertion of the caudal fin, Posterior insertion of the anal fin and Anterior insertion of anal fin (Table 4). The differences observed in the patterns of FA between sexes might show that there is an existence of variation in the level of developmental homeostasis between males and females (Demayo *et al.*, 2013).

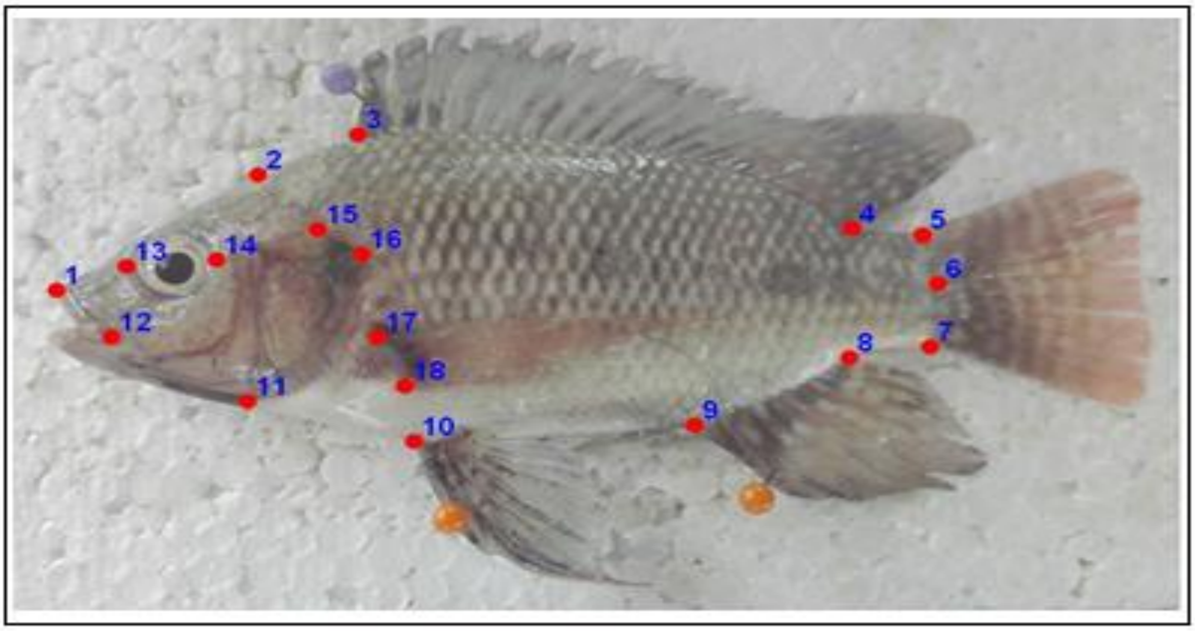

Fig. 2. Landmark points of *O. niloticus*.

Moreover, in the basis of the total landmarks affected per site base on the first two PCs (Table 3 and 4), Depore and Guinoman population has the highest frequency with a total of 31 landmarks affected from female and male populations followed by Dipili (28) and Sibugay (25).

These results suggest that Depore and Guinoman populations exhibit more FA than Dipili and Sibugay populations, but Sibugay have less FA compared to Dipili population. Guinoman, Depore and Dipili rivers are geographically located near the area where there is intensive agricultural farming like rice farming and passes through densely populated areas. In addition, the high FA in Depore population might be due to the ongoing mining operation in the upper mountain that is directly connected to this river. Thus, this population experienced more stress compared to the others. On the other hand, only half part of the Sibugay River is massively used in agriculture and the other side was still a forested area. These might explain the fewer landmarks affected in this population but still, FA was observed.Agricultural activity is one of the causes for the degradation of the water bodies.

The excessive use of fertilizer may result in the eutrophication of many aquatic habitats because precipitation carries dissolved nutrients into them. Water is also used to flush irrigated land to get rid of excess salt and other chemicals in the soil that degrades the water bodies. Pesticides can enter aquatic habitats through surface runoff, leaching, and/or erosion (Dubus *et al.*, 2000). Pesticide runoff can persist for days in the environment but their action on tissues of non-target organisms such as fishes and other aquatic fauna may last for weeks and even months (Assis *et al.*, 2012). Furthermore, solid and liquid domestic wastes from the community along the rivers could contribute to the water quality of the rivers.

These results support previous studies in fish at individual and population levels of bilateral asymmetry which have been shown to relate positively to a wide range of stresses such as acidification (Vollestad and Hindar, 2001; Oxnevad *et al.*, 2002), toxic chemicals, or heavy metals (Franco *et al.*, 2002; Romanov and Kovalev, 2004; Estes *et al.*, 2006). Stresses such as chemical contaminants like heavy metals or pesticides can cause developmental disruptions that can dramatically increase the FA level in invertebrates (Green and Lochmann, 2006; Chang *et al.*, 2007).

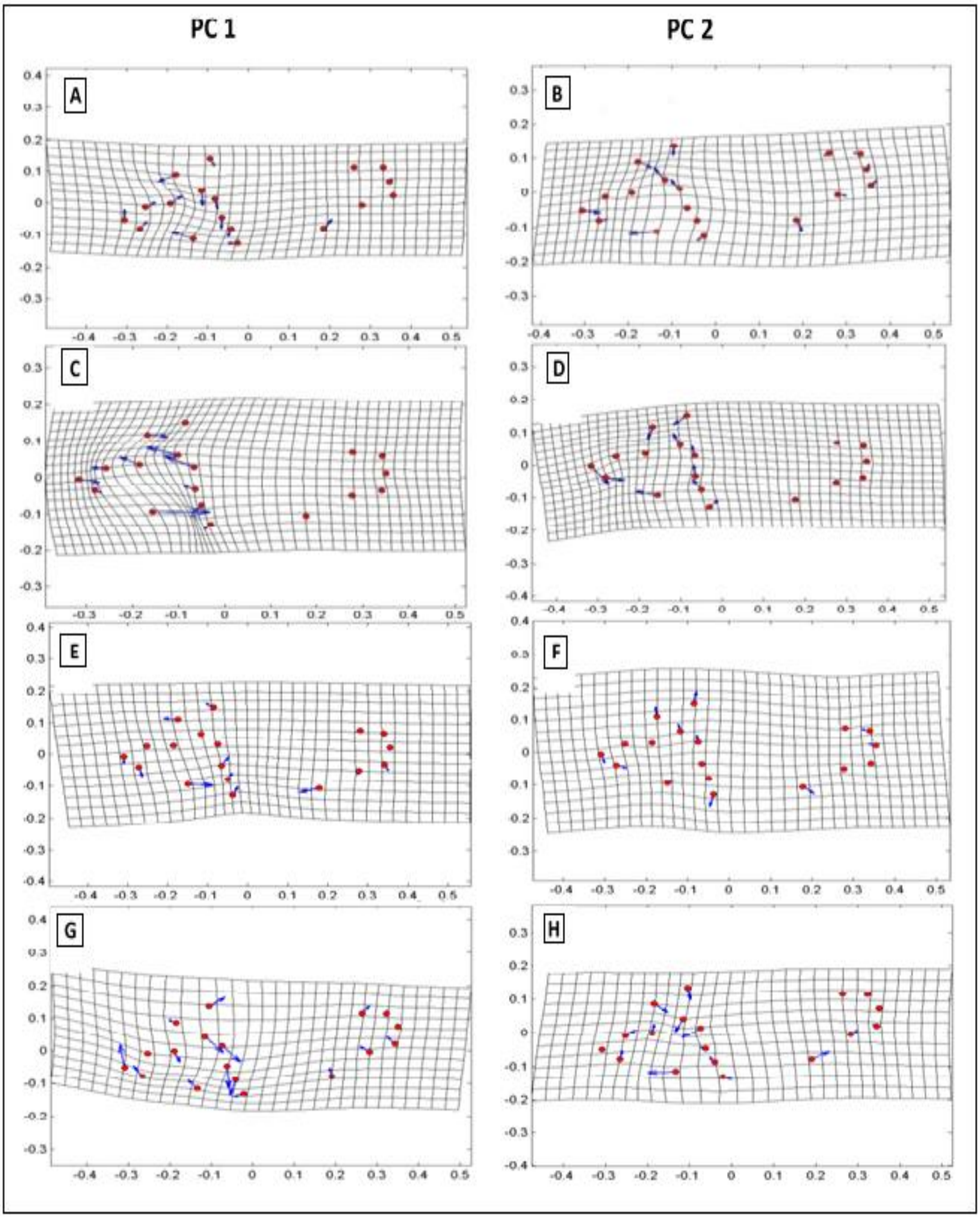

Fig. 3. PCA implied deformation for individual x side interaction (fluctuating asymmetry), as explained by two principal components (PC1, left; PC2 right) of Female Tilapia populations from four sites: A,B) Depore river, C,D) Dipili river, E,F) Sibugay river and G,H) Guinoman river. Red dots represent landmark points whereas blue arrows represent magnitude and direction of variation.

Fluctuating asymmetry is a helpful indicator of stress, not because it is more susceptible to stress than phenotypic changes, but because it elucidates the robustness and resilience of the average individuals in the face of stress (Graham *et al.*, 2010). Stress is an environmental factor that causes a reduction in the efficient use of energy, causing a reduction in developmental homeostasis, and finally reducing long-term, total inclusive fitness (Escos *et al.*, 2000).

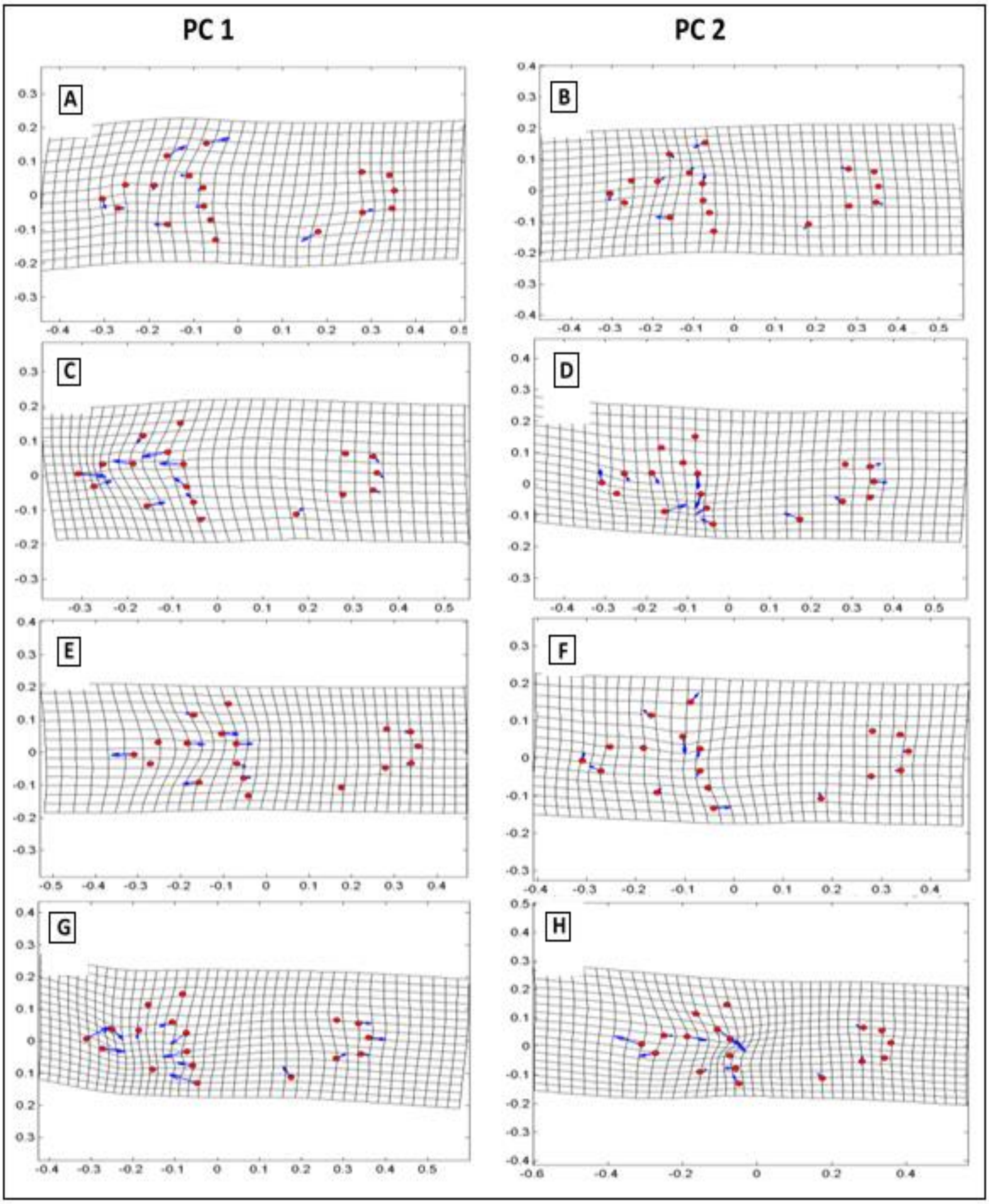

Fig. 4. PCA implied deformation for individual x side interaction (fluctuating asymmetry), as explained by two principal components (PC1, left; PC2 right) of Male Tilapia populations from four sites: A,B) Depore river, C,D) Dipili river, E,F) Sibugay river and G,H) Guinoman river. Red dots represent landmark points whereas blue arrows represent magnitude and direction of variation.

Conclusion

Results have demonstrated a highly significant fluctuating asymmetry (FA) for all the populations examined. Significant levels of fluctuating asymmetry across populations may indicate that individuals' developmental homeostasis is disturbed. These populations might have experience developmental disturbances early in life which manifested in the observed difference from bilateral symmetry in many of the traits examined. However, populations from Depore river, Dipili river and Guinoman river have relatively higher fluctuating asymmetry compared to Sibugay river based on localized trait asymmetry and the number of affected landmarks. Thus, indicates more stress or disturbances experienced by these populations of *O. niloticus*. Herewith, stresses or disturbances present could be attributed to agricultural wastes (pesticides and fertilizers), heavy metals, household waste and illegal and overfishing as well. FA has been considered as a good indicator of developmental instability (DI) and thus acts as a biomarker for environmental stress. Thus, understanding the relationship between the species and its environment would help determine the health of a given ecosystem. The results of this study will be vital to the local government for the inclusion of local policies in the management of the river system and future research in the area.

Acknowledgment

The Researchers would like to acknowledge the Department of Science and Technology (DOST) – Accelerated Science and Technology Human Resource Development Program for the funding of the research and the Local Government Unit of Bayog for allowing the researcher to conduct the study in the area.

References

Agah H, Leermakers M, Elskens M, Fatemi SM, 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. https://doi.org/10.1007/s10661-008-0551-8

Allenbach DM, 2011. Fluctuating asymmetry and exogenous stress in fishes: a review. Reviews in Fish Biology and Fisheries **21,** 355-376. https://doi.org/10.1007/s11160-010-9178-2

Assis CRD, Linhares AG, Oliveira VM, França RCP, Carvalho EVM, Bezerra RS, De Carvalho LB. 2012. Comparative effect of pesticides on brain acetylcholinesterase in tropical fish. Science of the Total Environment **441,** 141–150. https://doi.org/10.1016/j.scitotenv.2012.09.058

Barrett C. 2005. Fluctuating dental asymmetry as an indicator of stress in prehistoric native Americans of the Ohio River Valley. (Electronic Thesis or Dissertation). Retrieved from https://etd.ohiolink.edu/

Buitrago–Suarez UA, Burr BM. 2007. Taxonomy of the catfish genus Pseudoplatystoma Bleeker (Siluriformes: Pimelodidae) with recognition of eight species. Zootaxa **1512,** 1-38. http://dx.doi.org/10.11646/zootaxa.1512.1.1

Cabuga CC, Jumawan JH, Jumawan JC, Cortez EMB, Salvaleon SMN, Gamutan KJS, Dollisen MGG, Suico ALG, Requieron EA, Torres MAJ. 2106. 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. Aquaculture, Aquarium, Conservation & Legislation **9,** 122-132.

Chang X, Zhai B, Liu X, Wang M. 2007. Effects of temperature stress and pesticide exposure on fluctuating asymmetry and mortality of *Copera annulata* (selys) (Odonata: Zygoptera) larvae. Ecotoxicology and Environmental Safety **67**, 120-127. https://doi.org/10.1016/j.ecoenv.2006.04.004

Costa C, Cataudell 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.
https://doi.org/10.1007/s10641-006-9081-9

Daloso DM. 2014. The ecological context of bilateral symmetry of organ and organisms. Natural Science **6,** 184-190.
https://doi.org/10.4236/ns.2014.64022

Demayo CG, Harun SA, Torres MAJ. 2013. Procrustes analysis of wing shape divergence among sibling species of Neurothemis dragonflies. Australian Journal of Basic and Applied Sciences **5,** 748-759.

Dongen SV. 2006. Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. Journal of Evolutionary Biology **19(6),** 1727-1743.
https://doi.org/10.1111/j.1420-9101.2006.01175.x

Dorado EL, Torres MAJ, Demayo CG. 2012. Sexual dimorphism in body shapes of the spotted barb fish, *Puntius binotatus* of Lake Buluan in Mindanao, Philippines. Aquaculture, Aquarium, Conservation & Legislation **5**, 321-329.

Dubus IG, Hollis JM, Brown CD. 2000. Pesticide in rainfall in Europe. Environmental Pollution **110,** 331–344.
https://doi.org/10.1016/S0269-7491(99)00295-X

Ducos MB and Tabugo SRM. 2015. Fluctuating asymmetry as bioindicator of stress and developmental instability in *Gafrarium tumidum* (ribbed venus clam) from coastal areas of Iligan Bay, Mindanao, Philippines. Aquaculture, Aquarium, Conservation & Legislation **8,** 292-299.

Escos JM, Alados CL, Pugnaire FI, Puigdefábregas J, Emlen JM. 2000. Stress resistance strategy in an arid land shrub: interactions between developmental instability and fractal dimension. Journal of Arid Environment **45,** 325-336.
https://doi.org/10.1006/jare.2000.0641

Estes EC, Katholi CR, Angus RA. 2006. Elevated fluctuating asymmetry in eastern mosquitofish (*Gambusia holbrooki*) from a river receiving paper mill effluent. Environmental Toxicology and Chemistry **25,** 1026-1033.
https://doi.org/10.1897/05-079R1.1

Franco A, Malavasi S, Pranovi F, Nasci C, Torricelli P. 2002. Ethoxyresorufin O-deethylase (EROD) activity and fluctuating asymmetry (FA) in *Zosterisessor ophiocephalus* (Teleostei, Gobiidae) as indicators of environmental stress in the Venice lagoon. Journal of Aquatic Ecosystem Stress and Recovery **9,** 239-247.
https://doi.org/10.1023/A:1024010813669

Galloway TS, Brown RJ, Browne MA, Dissanayake A, Lowe D, Jones MB, Depledge MH. 2004. A multibiomarker approach to environmental assessment. Environmental Science Technology **38,** 1723–1731.
https://doi.org/10.1021/es030570+

Graham JH, Raz S, Hel-Or H, Nevo E. 2010. Fluctuating Asymmetry: Methods, Theory, and Applications. Symmetry **2,** 466-540.
https://doi.org/10.3390/sym2020466

Green CC, Lochmann SE. 2006. Fluctuating asymmetry and condition in golden shiner (Notemigonus crysoleucas) and channel catfish (Ictalurus punctatus) reared in sublethal concentrations of isopropyl methylphosphonic acid. Environmental Toxicology and Chemistry 25, 58–64.
https://doi.org/10.1897/04-554R.1

Hermita Z M, Gorospe JG, Torres MAJ, Lumasag GJ, Demayo CG. 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,** 857-861.

Jawad L, Sadighzadeh Z, Al-Mamary D. 2011. Fluctuating asymmetry in the otolith length, width and thickness in two pelagic fish species collected from the Persian Gulf near Bandar Abbas. Scientific and Research Center of the Republic of Slovenia. Annales. Series Historia Naturalis **22,** 83-88.

Jumawan JH, Abastillas SOMAL, Dicdican MJA, Cabag MB, Gamolo EM, Velasco JPB, Cabuga Jr CC, Mordeno Y, Requieron EA, Torres MAJ. 2016. Fluctuating asymmetry in the body shapes of goby fish *Glossogobius giuris* from Agusan River, Butuan City, Agusan del Norte, Philippines. Aquaculture, Aquarium, Conservation & Legislation **9,** 133-142.

Klingenberg CP. 2015. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry **7,** 843-934. https://doi.org/10.3390/sym7020843

Lecera JMI, Pundung NAC, Banisil MA, Flamiano RS, Torres MAJ, Belonio CL, Requieron EA. 2015. Fluctuating asymmetry analysis of trimac *Amphilophus trimaculatus* as indicator of the current ecological health condition of Lake Sebu, South Cotabato, Philippines. Aquaculture, Aquarium, Conservation & Legislation **8,** 507-516.

Lens L, Dongen SV, Mathysen E. 2001. Fluctuating asymmetry as an early warning system in the criticality endangered Taita Thrush. Conservation biology **16,** 479-487. https://doi.org/10.1046/j.1523-1739.2002.00516.x

Marquez E. 2007. SAGE: symmetry and asymmetry in geometric data. Ver 1.04. http://www-personal.umich.edu/~emarquez/morph/

Natividad EMC, Dalundong ARO, Ecot J, Jumawan JH, Torres MAJ, Requieron EA. 2015. Fluctuating asymmetry as bioindicator of ecological condition in the body shapes of *Glossogobius celebius* from Lake Sebu, South Cotabato, Philippines. Aquaculture, Aquarium, Conservation & Legislation **8**, 323-331.

Oxnevad SA, Heibo E, Vollestad LA. 2002. Is there a relationship between fluctuating asymmetry and reproductive investment in perch (*Perca fluvialtilis*)? Canadian journal of zoology **80,** 120-125. https://doi.org/10.1139/z01-215

Ozsoy DE, Erkmen B, Ozeren C, Kolankaya D. 2012. Detection of aquatic pollution in meric river by a measure of developmental instability, fluctuating asymmetry in the fish, common carp, *Cyprinus carpio L., 1758*. Journal of Animal and Veterinary Advances **11,** 1213-1216. https://doi.org/10.1016/j.toxlet.2007.05.413

Palmer AR, Strobeck C. 2003. Fluctuating asymmetry analyses revisited. In: Developmental instability. Causes and consequences. Polak M. (ed), Oxford University Press, Oxford, 279-319.

Pascual S, Abollo E. 2003. Accumulation of heavy metals in the whaleworm *Anisakis simplex s.l.* (Nematoda: Anisakidae). Journal of the Marine Biological Association of the United Kingdom **83,** 905-906. https://doi.org/10.1017/S0025315403008038h

Peligro VC, Jumawan JC. 2016. Assessment of fluctuating asymmetry in the body shapes of Nile tilapia (*Oreochromis niloticus*) from Masao river, Butuan city, Philippines. Aquaculture, Aquarium, Conservation & Legislation **9,** 604-613.

Rohlf FJ. 2006. TpsDig Version 2.10. Department of Ecology and Evolution, State University of New York at Stony Brook, New York, http://life.bio.sunysb.edu/morph/

Romanov NS, Kovalev MY. 2004. Fluctuating asymmetry in goldfish *Carassius auratus gibelio* (Cyprinidae) from some water bodies of the Far East. Journal of Ichthyology/Voprosy Ikhtiologii **44,** 109-114.

Tabugo SRM, Yuto CMM, Lumogdang L. 2016.

Fluctuating asymmetry as an Indicator of Ecological Stress in *Rhinocypha colorata* (Odonata: Chlorocyphidae) in Iligan City, Mindanao. Philippines. Entomology and Applied Science Letters **3,** 13-20.

Turan C. 1999. A note on the examination of morphometric differentiation among fish populations: the Truss System. Tropical Journal of Zoology **23,** 259-263.

Turan C, Den DE, Rlek MG, Babusta N, Turan F. 2004. Morphometric structuring of the anchovy (*Engraulis encrasicolus* L.) in the Black, Aegean and Northeastern Mediterranean Seas. Turkish journal of veterinary and animal sciences **28,** 865-871.

Utayopas P. 2001. Fluctuating Asymmetry in Fishes Inhabiting Potluted and Unpolluted Bodies of Water in Thailand. Thammasat International Journal of Science & Technology **6,** 10-20.

Van Valen L. 1962. A study of fluctuating asymmetry. Evolution **16,** 125–142.
https://doi.org/10.2307/2406192

Vasconcellos AV, Vianna P, Pavia PC, Schama R, Sole-Cava R. 2008. Genetic and morphometric differences between yellow tail snapper (*Ocyurus chrysurus*, Lutjanidae) populations of the tropical West Atlantic. Genetics and Molecular Biology **31,** 308-316.
https://doi.org/10.1590/S1415-47572008000200026

Vollestad LA, Hindar K. 2001. Developmental stability in brown trout: are there any effects of heterozygosity or environmental stress?. Biological Journal of the Linnean Society **74,** 351-364.
https://doi.org/10.1006/bijl.2001.0585