Article

A geometric morphometric study in the population of Sharpnose Hammer Coacker (*Johnius borneensis,* Blecker 1851) from Butuan Bay, Caraga, Philippines

C.C. Cabuga Jr.[1], **M. K. A. Angco**[2], **Y. G. Codaste**[3], **S. M. N. Salvaleon**[4], **J. M. D. Pondang**[5]
[1]General Education Department, ACLC College of Butuan, Butuan City, Agusan Del Norte, 8600, Philippines
[2]Department of Environment and Natural Resources, CENRO, Nasipit, Agusan Del Norte, 8602, Philippines
[3]Department of Education, Taligaman National High School, Butuan City, Agusan Del Norte, 8600, Philippines
[4]General Education Department, Saint Francis Xavier College, San Francisco, Agusan Del Sur, 8501, Philippines
[5]Department of Education, Del Pilar National High School, Cabadbaran City, Agusan del Norte-8605, Philippines
E-mail: cresenciocabugajr@gmail.com

Received 25 October 2021; *Accepted 30 November 2021; Published 1 March 2022*

Abstract
Geometric morphometric is an efficient and systematic tool to identify unnoticeable shape differences among biological entities. Sharpnose Hammer Coaker (*Johnius borneensis)* is a brackish fish and serves as a resource commodity in the study area. The study aims to investigate the body shape variations among its population. 100 individuals comprising 50 males and 50 females of the same size were collected and subjected to Symmetry and Asymmetry Geometric Data Software (SAGE). Procrustes ANOVA shows that males have a significant difference (P<0.0001) among the factors analyzed (Individuals, Sides, and Individuals x sides). While females two factors (Sides and Individuals x Sides) show a significant difference (P<0.0001), however, the individuals show non-significant. Principal Component Analysis in male fish shows a total of (82.92%) while female shows (84.55%). This implied body shape variation from the collected samples. Thus, it represents a difference in morphology. While indicating a subtle detail on how male populations differ from female populations and vice versa. The importance of geometric morphometric analysis provides a vital tool to define discreet morphological variations among species of the same taxa.

Keywords estuary; itchyofauna; morphology; shape discrimination.

Computational Ecology and Software
ISSN 2220-721X
URL: http://www.iaees.org/publications/journals/ces/online-version.asp
RSS: http://www.iaees.org/publications/journals/ces/rss.xml
E-mail: ces@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

In Biology, structure and changes in form are primary considerations for anthropological study. Previous and up to the present, identifying shapes and duplicating quantitative interpretations is a challenge to distinguish how biological entities are diverse from one another. Various systematic approaches were employed to prove the connection between form and function. Indeed, trait differences are distinctive factors used for identifying species (Richtsmeier et al., 2002). The shape is an utmost morphological characteristic that offers phenotypic information linking the genotype to the environment (Ricklefs and Miles, 1994). Further, phenotypic variability is contributing factor that defines the shape variations and co-variations of organisms within the environment (Cabuga et al., 2016).

Fishes serve as a biomarker of stressors in the aquatic environment. They are the best sample for detecting environmental conditions since they inhabit where most of the effluent occurs. Fish indicating a healthy environment will be heavier than the others since heavier fish with extra weight means extra energy is a reserve. While, lighter fish don't have extra energy and are more susceptible to ecological stressors indicating an unfavorable environment (Courtney, 2011). The fish growth and development is indicative information for any aquatic system since it integrates all effects in the fish (Shakir and Qazi, 2013). The presence of this run-off can affect its physiological activities and later may express its morphology. Ecological risks such as agricultural, industrial sewages, and anthropogenic activities may pose unfavorable conditions among the environment and organisms (Natividad et al., 2015). Over the decades, aquatic pollution has contributed as a problem that causes a wide range of effects (Dikshit et al., 1990). In existence, pollutants can be a factor to modify the genetic makeup of an organism and result in diversity and variation in the population (Trono et al., 2015). Intolerable effects damage the environmental state and lead to mortality (Duruibe et al., 2007). Pollutants are components that can alter the morphological traits of the organism. These are contributing factors that directly affect its state of well-being. The effect of these pollutants suggests morphological asymmetries through imperfect development (Jumawan et al., 2016). Alongside, the environment highly affects morphological traits involving the shape and manifesting phenotypic plasticity (Via and Lande, 1985; Schlichting and Pigliucci, 1998).

To identify morphological variation in fishes, Geometric Morphometric (GM) is an application to demonstrate the different characteristic traits. Indeed, it serves as an effective tool to evaluate the developmental variability of an individual species as it represents the total population (Bergstrom and Reimchen, 2002). It aids as a mechanism to assess pollution in the environment that altered the species traits (Tomkins and Kotiaho, 2001). It is an efficient instrument for quantifying environmental conditions (Lecera et al., 2015). Also, a potential and quantitative method in assessing if the environment can provide ecological growth towards species (Angtuaco and Leyesa, 2004). In addition, GM is an efficient and reliable means of identifying developmental instability (Ducos and Tabugo, 2015). An extensive way to describe and discriminate nonconformities based on morphological traits (Swaddle, 2003). This application is widely recognized as it can deliberately identify the effects of several effluents through species morphology (Jumawan et al., 2016). GM is one of the most recognized scientific mechanisms because it can represent the quantitative function and analyze morphological shape (Polly, 2012). Nonetheless, the purpose of geometric morphometric is to define subtle information concerning discreet modifications in the species morphology.

This study uses *J. borneensis*, brackish fish and commonly found in the area. A previous study was conducted and utilized the same sample (Jumawan et al., 2016). However, the present study would make current information about the latter and compare the previous data. It is the significance of the study to investigate the asymmetry in the metric traits of the sample. Economically, the fish contributes as a resource commodity and livelihood by the locals. Thus, the study aims to investigate the body shape variations among the female and male populations.

2 Model

2.1 Study area

The study area (Butuan Bay) is a brackish portion of the Agusan River System of Butuan City, Agusan Del Norte. Geographically it is positioned (8059’26.35” N; 125031’31.16” E) Northern part of Caraga, Region. Strategically, it serves as an important passage for the locals and products transportation (Fig. 1).

2.2 Fish collection, processing and sex determination

The collection of the fishes was done in September 2020 by the local fisherman utilizing motorized Banca and gillnet as their catching materials. About 100 samples of (*J. borneensis*) (50 males; 50 females) were collected. After, it was sorted according to their weight and size using a weighing scale and a ruler. The latter was a position at the top of the Styrofoam. Individually, fish fins were pinned and applied with a 10% formaldehyde solution by a small paintbrush. The samples were capture using a digital camera (Natividad et al., 2015). For sex determination, each of the fish samples was examined for genitalia identification. Females were identified with the presence of ovaries and eggs with yellow to orange color and granular textures. Males were identified with the presence of testes with whitish color and smooth in form (Requieron et al., 2012).

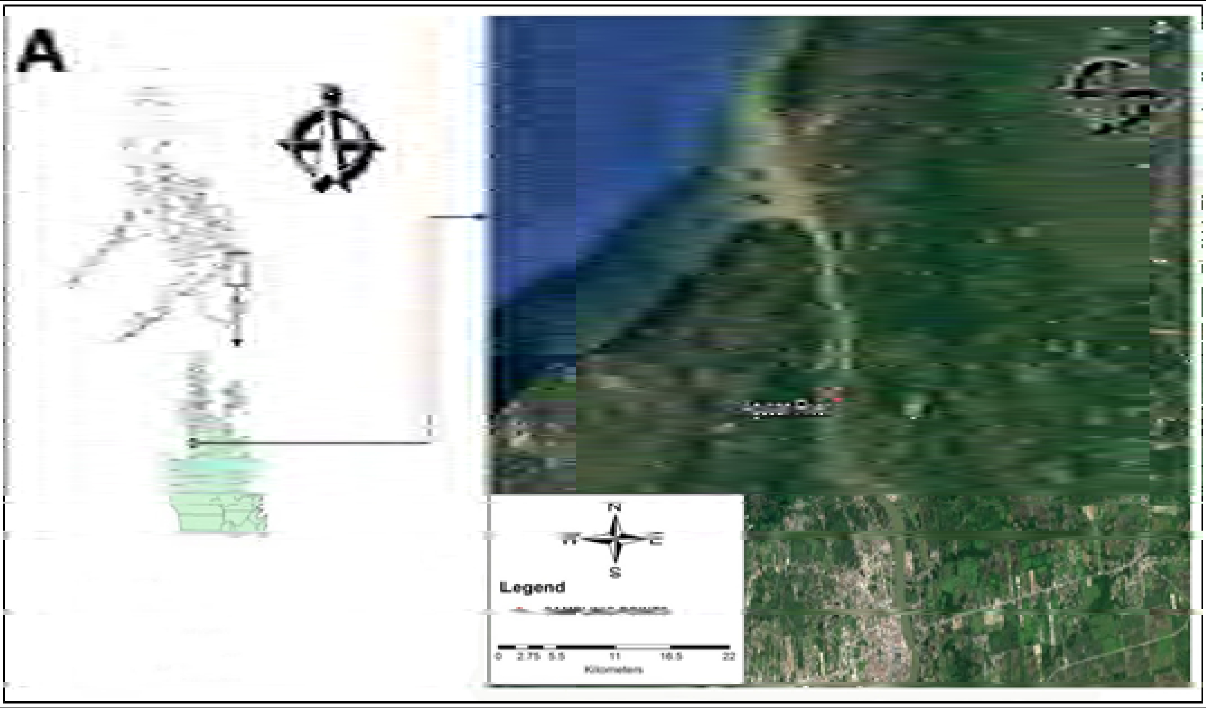

Fig. 1 Map of the study area, Butuan Bay, Agusan Del Norte, Philippines.

2.3 Landmark selection and digitation

The photographs were sorted according to their sexes (male and female) samples. Then it was loaded and converted to TPS files using the tpsUtil. The landmarking process was done employing tpsDig2 (version 2, Rohlf, 2004). The digitized left and right sides of fish samples were subjected to tri-replicated images using the same application. There were sixteen anatomical landmark points (Table 1) used to digitize the body shape of fish samples (Fig. 2).

2.4 Shape analysis and data generation

The obtained coordinates were used and subjected to Symmetry and Asymmetry in Geometric Data (SAGE) (software version 1.04, Marquez, 2007) (Fig. 3). The Procrustes ANOVA test was employed to define the significant difference ($P<0.0001$) among the factors tested (Individual, Sides, and Interaction of Individual and Sides). Further, it generates the Principal Component Analysis (PCA) of individual symmetry and interaction of the samples (Natividad et al., 2015).

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

Coordinates	Locations/Nomenclature
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
16	Dorsal base of pectoral fin

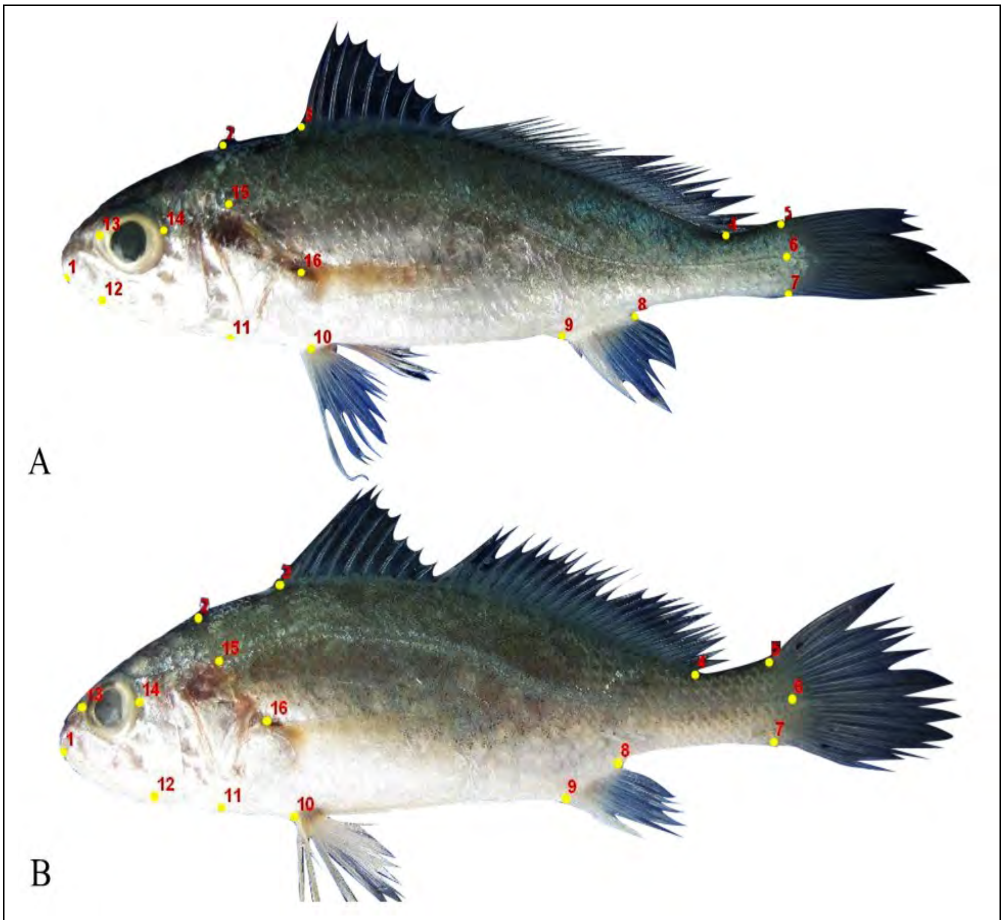

Fig. 2 Digitized anatomical landmark points of *J. borneensis* (**A**) Male; (**B**) Female.

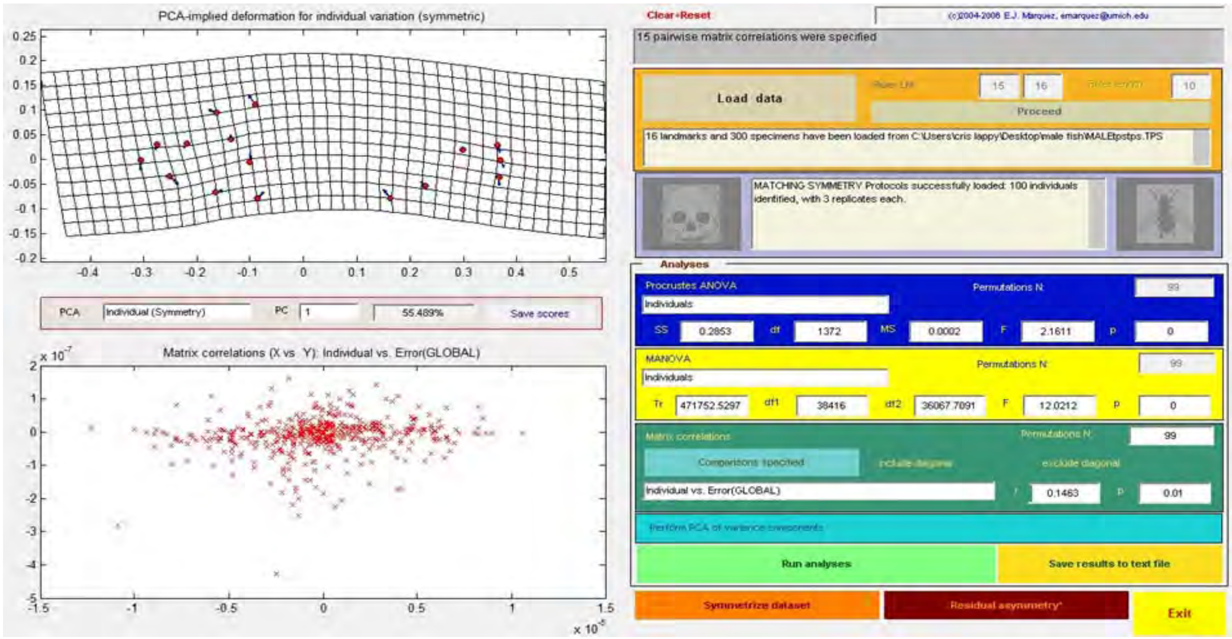

Fig. 3 Symmetry and Asymmetry Geometric Data Software (SAGE).

3 Results and Discussion

The Procrustes ANOVA test was used to determine the body shape variations of *J. borneensis* among the male and female populations (Table 2). The application widely used for fluctuating asymmetry (FA) is a two-way, mixed-model ANOVA with replication. To explain, the main fixed effect is the side (***S***) which takes two levels (left & right). The block effect is individuals (***I***) explains a random individual sample from a population. The side by individual's interaction (***S x I***) is a mixed effect. Lastly, measurement error (***M***), defined as the replication within sides by the individuals. The effect called sides is the deviation between the two sides; a measure of directional asymmetry. The effect called individuals is the deviation between individual genotypes; the individual's mean square is a measure of total morphological deviation and is by chance. The individual by sides interaction is the failure of the effect of individuals to be the same from side to side. (Samuels et al., 1991; Palmer and Strobeck, 1986, 2003; Carpentero and Tabugo, 2014).

The result shows that female has a significant difference (P<0.0001) among the factors (Sides, Individuals x Sides) while the factor (Individuals) shows the non-significant difference (P<0.6194). On the other hand, the male has a significant difference (P<0.0001) among the factors analyzed (Individuals, Sides, and Individuals x Sides). These implied that body shape variations were evident in the collected fish samples. Thus, it represents a difference in morphology. In comparison, five years ago, the same study was also conducted on *J. borneensis.* The results obtained all significant differences (P<0.0001) from the factors analyzed (Jumawan et al., 2016). It implies significant changes occurred in the morphology of the samples over time. The environment highly contributes to the growth and development of organisms. From the ontogeny stage and up to its development, fishes utilized both biotic and abiotic components. A manner that affects them genetically and is observable through their metric traits. Individually, fishes correspond to the nature where they exist to survive and thus acquire the influences that surround them.

Table 2 Procrustes ANOVA test for samplesof *J. borneensis* in terms of sexes.

EFFECT	SS	DF	MS	F	P-VALUE
Female					
Individuals	0.0793	812	0.0001	0.9789	0.6194^{ns}
Sides	0.0168	28	0.0006	6.0075	0.0001*
Individual x Sides	0.081	812	0.0001	4.6683	0.0001*
Measurement Error	0.0718	3360	0	-	-
Male					
Individuals	0.1298	812	0.0002	2.3873	0.0001*
Sides	0.0222	28	0.0008	11.8106	0.0001*
Individual x Sides	0.0544	812	0.0001	2.5725	0.0001*
Measurement Error	0.0875	3360	0	-	-

Side = directional asymmetry; individual x sides interaction = fluctuating asymmetry; * P<0.0001 significant, ns – statistically insignificant (P>0.05); significance was tested with 99 permutations.

The observed dissimilarities were associated with an environmental condition were unable to thrive and buffer (Van Valen, 1962). Developmental instability was linked to climatic conditions, food insufficiency, inbreeding, and hybridization (Mpho et al., 2000). Previous studies reported that dietary stress openly exhibits high asymmetry (Sciulli et al., 1979; Swaddle et al., 1994; Imasheva et al., 1999). In contrast, others have no known effect on the latter (Hovorka and Robertson, 2000; Bjorksten et al., 2000). Ecological stress is a contributing factor that generates shape change. This development leads to fluctuating asymmetry (Parsons, 1961, 1962, 1990, 1992; Van Valen, 1962; Palmer and Strobeck, 1986; Leary and Allendorf, 1989). Alongside, bilaterally symmetrical characters should be instigated identically on each in the body sides where they are supposed to be ruled by the same genetic factor. The non-directional deformities among the sides are originally environmental and manifested during developmental stages (Palmer, 1994; Valen, 1962; Gangestad and Thornhill, 1999; Martin and Hosken, 2009). This consequence had become interesting for many studies to undergo several works to recognize such causes in between species within the same lineage. As well as, they identify that fluctuating asymmetry lessens energy for growth, development, and reproduction that affects the population in the long run (Koehn and Bayne, 1989). Stress is a causative factor that affects organisms holistically (Sommer, 1996). Differences in the morphology are correlated to its adaptive mechanism to withstand the environment (Cabuga et al., 2019). The unsteady environmental condition contributes to the development of the organisms resulting asymmetrically (Natividad et al., 2015). Considerable deformation in the body shape of the organisms is an indicative factor of how they respond towards the ecological settings. Phenotypic variation usually begins with hereditary components and shows through its traits. However, because of intolerance, these changes would manifest in its characteristics.

Further, the ecology of the fishes has been disturbed through the efforts of anthropogenic activities. Such aquatic environment is merely affected by industrial, agricultural, and household run-off. The study area is situated where urbanization increased. Also, constitute environmental health affects the inhabitants. The rivers, lakes, and marshes are vital freshwater resources for the food supply. However, they received the most adverse effects. In this case, the Agusan River in Butuan City, Agusan del Norte known to be a fishery resource of the region but constantly declining the catch due to poor environmental mitigations. The adverse effects will lead to species conditions affecting its morphology and even mortality. In the long run, this builds anatomical differences and results in fluctuation of the shape. Thus, ecological perturbations highly influence the state of well-being among living organisms.

Table 3 Principal Component Analysis of *J. borneensis*in terms of sexes.

PCA	Individual	Sides	Interaction	Affected Landmarks
		Female		
1	55.8037%	100%	62.0478%	1,2,3,5,6,7,8,9,10,12,13,16
2	8.6707%		7.8649%	1,6,9,10,12,15
3	8.1573%		5.6463%	2,9,11
4	7.0285%		5.0777%	1,2,3,4,10,15
5	5.3359%		3.9147%	9,15
	84.99%		**84.55%**	
		Male		
1	54.0554%	100%	40.4882%	1,2,3,5,6,7,8,9,10,11,12,13 15,16
2	13.2897%		20.5062%	1,2,3,4,5,6,7,9,10,11,12,13 14,15,16
3	8.3415%		17.0661%	1,2,4,5,6,7,8,9,10,11,12,13 15,16
4	5.6255%		4.8555%	1,2,4,6,7,8,9,10,11,12,13 14,15,16
	81.31%		**82.92%**	

To further identify a body shape variation among the samples, Principal Component Analysis (PCA) was performed to show the distinct evidence of the so-called fluctuating asymmetry (FA) or the Interaction (Table 3). As observed, the female population is composed of five PCA contributing (84.99%) of the total scores generated while the Interaction or Fluctuating Asymmetry (84.55%). Meanwhile, the male population is composed of four PCA, contributing (81.31%) of the total scores generated and Interaction or Fluctuating Asymmetry (82.92%) lower compared to the female population. The affected landmarks in male samples were 1 (Snout tip), 2 (Posterior end of the nuchal spine), 6 (Midpoint or lateral line), 7 (Ventral insertion of caudal fin), 9 (Anterior insertion of anal fin), 10 (Dorsal base of pelvic fin), 11 (Ventral end of lower jaw articulation), 12 (Posterior end of the premaxilla) and 13 (Anterior margin through midline of orbit). On the contrary, there were no common affected landmarks observed in female samples. To be known, the affected landmarks must be common and present among the PCA generated. The male fishes have the most affected landmarks when compared to female samples. This phenomenon might suggest that individual fish have mechanisms to buffer ecological conditions. Anatomically snout tip is a portion of the mouth where fishes are utilized for food hunting and predator defense. The other anatomical traits that are commonly affected are those portions for the movement and mobility of the fishes. For example, benthic species like frogfishes and scorpionfishes habitually perform synchronized movement using the pectoral and pelvic fins to move over the substrate or to sustain static position in alert and even for defense mechanisms (Gosline, 1994; Yamanoue et al., 2010).

On the other hand, fluctuating asymmetry suggests incomparable shape alterations in the species morphology. This study identified that female samples have a higher percentage of FA when compared to male samples. From the data of Jumawan et al. (2016) of the same fish samples, the FA of the male population obtained (72.20%) greater than that of the female (57.80%). It is contrary to the data obtained in the present study and shows an inconsistency of the sex-related frequency of FA. This information is essential for comparing FA results of the same samples and location but different time settings. Indeed, in biology time plays a significant role in the evolutionary complexities of all living organisms. Female fishes are more susceptible to body changes during the reproduction stages (Cabuga et al., 2019). At the same time, *Leiopotherapon plumbeus* was employed to a similar analysis. The result shows a high level of FA thus, representing species difficulty in sustaining morphological progress (Markow, 1995). The dissimilarities among members of the same species are associated with seasonal gonad development (Reiss et al., 2012). While foraging, predation, and niche conditions impacted species characteristics.

Further, geometric morphometry is a tool that provides essential information to scrutinize and draw differences in the body shape of the organisms belonging to the same taxa. Therefore, a valuation of fish assemblages where distinct morphologies exhibit within the groups, providing this method to consider anatomical characteristics in the landmark coordination. Also, it supports the accurate representation of the three-dimensional position of the species. It serves to develop the detection of the structural complexity and the ecological developments of fish communities. Equally, morpho-graphical methods are in a wide array and made easy to recognize differences. The diversity in the integration of analytical approaches, consisting of graphs and numbers, is the most effective and complete choice for measuring the internal position between morphology and space where organisms are occupied (Adams et al., 2013). Thus, the present study shows the importance of using quantitative analysis to identify shape variations and correlating ecological status to the development of the species.

4 Conclusion

The study utilized geometric morphometric analysis to investigate the body shape variations in the population of *J. borneensis* collected in Butuan Bay, Caraga Phils. A significant difference (P<0.0001) was observed in the samples and thus representing shape variances. Principal Component Analysis revealed high fluctuating asymmetry in the female sample compared to male sample and implied morphological dissimilarities of the same species. While indicating a subtle detail on how male populations differ from female populations and vice versa. Using geometric morphometric provides a vital tool to define discreet morphological variations among species of the same taxa.

References

Adams DC, Rohlf FJ, Slice DE. 2013. A field comes of age: geometric morphometrics in the 21st century. Italian Journal of Mammalogy, 24: 7-14

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

Bergstrom CA, Reimchen TE. 2002. Geographical variation in asymmetry in *Gasterosteus aculeatus.* Biological Journal of the Linnean Society, 77: 9-22

Bjorksten T, David P, Pomiankowski A, Fowler K. 2000. Fluctuating asymmetry of sexual and nonsexual traits in stalk-eyed flies: a poor indicator of developmental stress and genetic quality. Journal of Evolutionary Biology, 13: 89-97

Cabuga CCC Jr, Milloria MB, Lanes JR, Varona CJL, Pondang JMD, Raules JJ. 2019. Intraspecific evaluation in the morphology of *Glossogobius guiris* using geometric morphometric analysis from Lake Mainit, Agusan del Norte, Philippines. International Journal of Biosciences, 14(1): 379-387

Cabuga CCC Jr, Masendo CBE, Hernando BJH, Joseph CCD, Velasco JPV, Angco MKA, Ayaton MA, Obenza OLP, Jumawan JH, Jumawan JC, Requieron EA, Torres MAJ, Havana HC. 2016. Relative warp analysis in determining morphological variation and sexual dimorphism between sexes of flathead goby (*Glossogobius giuris*). Computational Ecology and Software, 6(3): 109-119

Carpentero ER, Tabugo SRM. 2014. Determining developmental instability via fluctuating asymmetry in the shell shape of *Arctica islándica* Linn. 1767 (ocean quahog). European Journal of Zoological Research, 3(3): 1-7

Courtney J. 2011. Relative Condition Factors of Fish as Bioindicators One Year after the Deepwater Horizon Oil Spill. BTG Research, 1-13

David Polly P. 2012. Geometric Morphometrics. Biology and Anthropology University, Department of Geology, Indiana, USA

Dikshith TSS, Raizada RB, Kumar MK, Shrivastava SK, Kulshrestha Adholia UN. 1990. Residues of DDT and HCH in major sources of drinking water in Bhopal. Indian Bulletin of Environmental Contaminants and Toxicology, 45: 389-393

Ducos MB, 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. AACL Bioflux, 8(3): 292-300

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN. 2007**.** Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences, 2(5): 112-118

Gangestad SW, Thornhill R. 1999. Individual differences in developmental precision and fluctuating asymmetry: a model and its implications. Journal of Evolutionary Biology, 12: 402-416

Gosline WA. 1994. Function and structure in the paired fins of scorpaenifom fishes. Environment Biology of Fishes, 40: 219-226

Hovorka MD, Robertson RJ. 2000. Food stress, nestling growth, and fluctuating asymmetry. Canadian Journal of Zoology, 78: 28-35

Imasheva AG, Bosenko DV, Bubli OA. 1999. Variation in morphological traits of *Drosophilamelanogaster* (fruit fly) under nutritional stress. Heredity, 82: 187-192

Jumawan JH, Requieron EA, Torres MAJ, Velasco JPB, Cabuga CCC Jr, Joseph CCD, Lador JEO, dela Cruz, HD, Moreno M, Dalugdugan RO, Jumawan JC. 2016. Investigating the fluctuating asymmetry in the metric characteristics of tilapia *Oreochromis niloticus* sampled from Cabadbaran River, Cabadbaran City, Agusan del Norte, Philippines. AACL Bioflux, 9(1): 113-121

Jumawan JH, Cabuga CCC Jr, Jumawan JC, Cortez EMB, Salvaleon SMN, Gamutan KJS, Dollisen MGG, Suico ALG, Requiron EA, Torres MAJ. 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

Koehn RK, Bayne BL. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. Biological Journal of Linnaean Society London, 37: 157-171

Leary RF, Allendorf FW. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. Trends in Ecological Evolutionary, 4: 214-217

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. AACL Bioflux, 8(4): 507-516

Markow TA. 1995. Evolutionary ecology and development instability. Annual Review of Entomology, 40: 105-120

Marquez E. 2007. Sage: Symmetry and asymmetry in geometric data version 1.05 (complied 09/17/08). Available online at http://www.personal.umich.edu/~emarquez/morph/

Martin OY, Hosken DJ. 2009. Longevity and development stability in the dung fly *Sepsis cynipsea*, as affected by the ectoparasite mite, *Pediculoides mesembrinae*. Journal of Insect Science, 9(66): 1-9

Mpho M, Holloway GJ, Callaghan A. 2000. The effect of larval density on life history and wing asymmetry in the mosquito *Culex pipiens*. Bulletin of Entomological Research, 90: 279-283

Natividad EMC, Dalundong ARO, Ecot J, Jumawan JH, Torres MAJ, Requieron EA. 2015. Fluctuating Asymmetry in the body shapes of Gobies *Glossogobius celebius* (Valenciennes, 1837) from Lake Sebu, South Cotabato, Philippines

Palmer AR. 1994. Fluctuating asymmetry analysis: a primer. In: Developmental Instability: Its Origins and Evolutionary Implications (Markow TA, ed). Kluwer Academic, London, UK

Palmer AR, Strobeck C. 1986. Fluctuating asymmetry - measurement, analysis, patterns. Annual Review Ecological Systematics, 17: 391-421

Paña BHC, Lasutan LGC, Sabid JM, Torres MAJ, Requieron EA. 2015. Using geometric morphometrics to study the population of the silver perch, *Leiopotherapon plumbeus* from Lake Sebu, South Cotabato, Philippines. AACL Bioflux, 8(3): 352-361

Parsons PA. 1961. Fly size, emergence time and sternopleural chaeta number in Drosophila. Heredity, 16: 447-455

Parsons PA. 1962. Maternal age and developmental variability. Journal Experimental Biology, 39: 251-260

Parsons PA. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biological Reviews of Cambrian Philosophical Society, 65(2): 131-145

Parsons PA. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. Heredity, 68: 361-364

ReissP, Able KW, Nunes MS. Hrbek T. 2012. Color pattern variation in Cichlatemensis (Perciformes: Cichlidae): Resolution based on morphological, molecular, and reproductive data. Neotropical Ichthyology, 10: 59-70

Requiron EA, Torres MAJ, Demayo CG. 2012. Applications of relative warp analysis in describing of scale morphology between sexes of the snakehead fish *Channastriata.* International Journal of Biological, Ecological and Environmental Sciences, 1(6): 205-209

Richtsmeier JT, Deleon VB, Lele SR. 2002. The Promise of Geometric Morphometrics. Yearbook of Physical Anthropology, 45:63–91.

Ricklefs RE, Miles DB. 1994. Ecological and evolutionary inferences from morphology: An ecological perspective. In: Ecological morphology: Integrative Organismal Biology 34 (Wainwright PC, Reilly SM, eds). 13-41

Rohlf FJ. 2004. TpsDig Version 2.0. Department of Ecology and Evolution, State University of New York, USA

Samuels ML, Casella G, McCabe GP. 1991. Interpreting blocks and random factors: rejoiner. Journal of American Statistics Association, 86: 798-808

Schlichting CD, Pigliucci M. 1998. Phenotypic evolution: A reaction norm perspective. Heredity, 387

Sciulli PW, Doyle WJ, Kelley C, Siegel P, Siegel MI. 1979. The interaction of stressors in the induction of increased levels of fluctuating asymmetry in the laboratory rat. American Journal of Physiology

Anthropology, 50: 279-284

Shakir HA, Qazi JI. 2013. Impact of industrial and municipal discharges of growth coefficient and condition factor of major carps from Lahore Stretch of River Ravi. The Journal of Plant Sciences, 23(1): 167-173

Sommer C. 1996. Ecotoxicology and developmental stability as an in situ monitor of adaptation. American Biology, 25: 374-376

Swaddle JP. 2003. Fluctuating asymmetry, animal behavior and evolution. Advances in the Study of Behavior, 32: 169-205

Swaddle JP, Witter MS. 1994. Food, feathers and fluctuating asymmetries. Proceeding of the Royal Society of London Biological Science, 255: 147-152

Tomkins JL, Kotiaho JS. 2001. Fluctuating asymmetry. Encyclopedia of Life and Sciences. 1-5, Macmillan Publishers Ltd. Nature Publishing Group, UK

TronoDJV, Dacar R, Quinones L, Tabugo SRM. 2015. Fluctuating asymmetry and developmental instability in *Protoreasternodosus*(Chocolate Chip Sea Star) as a biomarker for environmental stress. Computational Ecology and Software, 5(2): 119-129

Valen V. 1962. A study of fluctuating asymmetry. Evolution, 16: 125-142

Via S, Lande R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution, 39: 505-522

Yamanoue Y, Setiamarga DHE, Matsuura K. 2010. Pelvic fins in teleosts: structure, function and evolution. Journal of Fish Biology, 77: 1173-1208