Evaluation of fluctuating asymmetry and sexual dimorphism of *Channa striata* using landmark-based geometric morphometric analysis from Agusan Marsh and Lake Mainit in Caraga Region, Philippines

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Abstract. *Saura EBD, Falcasantos GC, Andante RJM, Munda LC, Alimorong MM, Hernando BJ. 2021. Evaluation of fluctuating asymmetry and sexual dimorphism of* Channa striata *using landmark-based geometric morphometric analysis from Agusan Marsh and Lake Mainit in Caraga Region, Philippines. Nusantara Bioscience 13: 100-110.* Evaluation was conducted to determine the impact of ecological condition to *Channa striata* using fluctuating asymmetry (FA) employing the landmark-based geometric morphometric analysis, using TPS software, SAGE, PAST-Hotellings Test, including Physico-chemical analysis, cyanide and four base metals. Results of Physico-chemical analysis of the two habitats showed Agusan marsh with low oxygen level (3.37-4.55) and high in Iron and TDS as compared to DENR Standards. The statistical results showed high variations (p < 0.0001) on left and right-side having percentage of 79.22% in Agusan Marsh and 72.35% in Lake Mainit which is relatively high Fluctuating Asymmetry. In terms of sex, Agusan marsh had 84.27% and 85.41% FA in males and females while Lake Mainit having 85.12% and 80.89% in males and females. The fairly similar percentage of Fluctuating Asymmetry confirms no sexual dimorphism and no shape differences between male and female, and no peculiar characters to distinguished between sexes based on the 17 landmark points. Present study concluded that FA observed in the snout tip was due to breeding behavior, the landmark points in cephalic, pectoral, and caudal region were associated with burrowing and mobility factors within the lateral line system in these fishes which thrive in high turbid habitat and low oxygen. Furthermore, it was found that the level of FA tends to increase in disturbed environs.

Keywords: Phenotypic Plasticity, Physico-chemical parameters, Procrustes shape coordinates, Variation

INTRODUCTION

Character variation among populations describing the external morphology and accompanied differences between sexes are basic concepts in systematics. The magnitude of phenotypic differences such as body shapes, sexual dimorphism, and allometry is perhaps a reflection of the partitioning between sexes, availability of food, seasonal or climatic patterns, inter-relationship among organisms in their respective habitat which is often correlated to geographical factors (Broadley 1965; Sullivan and Best 1997).

The variability of attributes such as body shape, color, sizes and related to sex phenotypic characteristics among species are annotated as "sexual dimorphisms" (McCombe and Greer 2014). Such characteristics lead to an instinct of rivalry amongst individuals in the interior of each sex and mate selection based on morphological and behavioral traits (Ralls and Mesnick 2009; Vitt and Caldwell 2014). Geographical variation denotes dissimilarities between species in inherently founded characters through the habitat's natural topography. Studies on geographic variation of a number of organisms highlight clarity on causes and significance of microevolution (Olvido 2012; Wu et al. 2015). At the global level, geographic variation in phenotypic plasticity is in part due to genetic differences acquired via long-term natural selection to produce adaptation to different environments (Niskanen et al. 2017). The degree of phenotypic plasticity in life-history traits differs among species and these differences are adaptive and non-adaptive in various habitats (Lymbery 1992; Seigel and Ford 2001). Habitat conditions have a tendency to reduce equilibrium stability in the developmental aspect of aquatic organisms e.g. small and random deviations from bilaterally symmetrical traits leading to Fluctuating Asymmetry (FA) (Santos et al. 2013). Stressed marginal habitats tend to influence increasing asymmetry as well as genetic perturbations (Parsons 1992). FA has been used as an ecological indicator in studies of natural and sexual selection and as a reliable bio-indicator tool relevant in environmental monitoring and conservation biology (Leary and Allendorf 1989; Tomkins and Kotiaho 2001; Beasley et al. 2013; Coda et al. 2017).

Furthermore, landmark-based Geometric morphometrics offers a powerful approach in computing morphological variables and covariation of shape (Rohlf 2000; Adams et al. 2009; Webster and Sheets 2010). Such techniques utilize statistical analysis on morphological variables based on Cartesian landmark coordinates. The traditional morphometrics separates shape from overall size, position, and orientation of the landmark configurations and the resulting Procrustes shape coordinates are used for statistical analysis (Mitteroecker and Gunz 2009).

The present study was conducted on the fish species *Channa striata* of Channidae (Bloch 1973) commonly known as Chevron Snakehead which is a non-native and invasive species to Philippines. Though in wild a burrowing dweller of bottom of mud lakes, canals, and swamps and living and breeding in turbid and low levels (Phen et al. 2005; Cagauan 2007), in other cases cultured for commercial food production (Guerrero III 2014). These fish are capable of limited movement overland by the use of a wriggling motion which they use to disperse to new water bodies on rainy nights (Sayer 2005). *C. striata* have eyes, but given the turbid habitats they live in, they may also use other sensory mechanisms to find prey and mates. They have a lateral line system that detects small changes in water pressure that help them to be aware of the objects moving in the water around them (Kasumyan 2003; Musikasinthorn 2004). The capacity of the folds of skin to distend is an important adaptation enabling the jaws to protrude and assist the fish to engulf relatively large-size prey (Mittal and Agrawal 1994).

Present study aims to compare the levels of FA, sexual dimorphism, and body variations of *C. striata* collected from two separate inland waters Agusan Marsh and Lake Mainit of Caraga Region Philippines using landmark-based geometric morphometric analysis. These include the assessment of Physico-chemical parameters, Cyanide and four base metals; Copper, Lead, Zinc, Iron of the study area. The study would provide knowledge on the character variation of *C. striata* and give relevant information useful in policy making and formulation of ecosystem-based management plan to the immediate agency for environmental protection in Agusan Marsh and Lake Mainit.

MATERIALS AND METHODS

Study Area

The study was conducted in two different types of inland waters namely Agusan Marsh, Agusan del Sur, and Lake Mainit, Surigao del Norte of Caraga Region, Philippines. The ecological aspects were recorded and coordinates of the six sampling stations on each area were obtained using geocam mobile app, as presented in Table 1 and a map was generated (Figure 1).
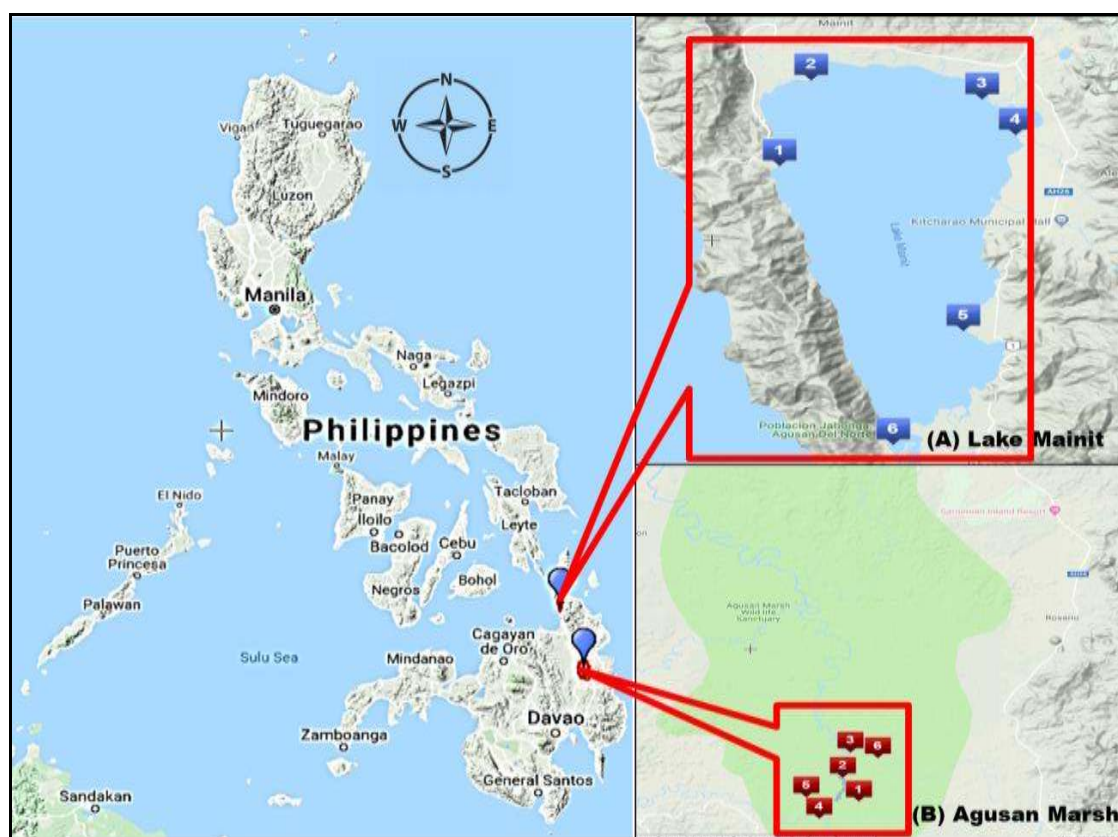
Figure 1. Map of geographically separate habitat of *Channa striata* (Chevron Snakehead) samples in Caraga Region, Philippines: A. Lake Mainit (17,060 hectares showing the six (6) water sampling stations, B. Agusan Marsh (14,835 hectares) showing the six (6) sampling stations water on the southern portion.

Table 1. Site Characteristics of the sampling stations established in Lake Mainit and Agusan Marsh, Philippines

Sampling areas	Coordinates	Site characteristics
Lake Mainit		
Station 1	9° 28' 59" N 125° 28' 49" E	River mouth of Tagbuyawan, few residents, rocky riverbanks and substrate, diverse plants in the sloping areas of the Malimono Mountain range Active fishing and navigation within the Lake. Previous site of Cyanide gold processing area in nearby areas.
Station 2	9° 31' 35" N 125° 29' 27" E	River mouth of Mayag sedges and grass dominant in the riparian area and shrub plants to trees are present in the upland areas, active fishing and navigation were observed, residential areas and agricultural land
Station 3	9° 31' 1" N. 125° 33' 2" E	River mouth of Magpayang, sedges and grass dominant in the riparian area and shrub plants to trees, coconut plants in the upper stream areas and obvious landscape of large scale mining, few live stocks, and agricultural lands like rice fields, several residential areas, and establishments. Active fishing and navigation.
Station 4	9°29'54" N 125°33'45" E	River mouth of Magtiaco with sandy river banks, sedges and grass dominant in the riparian area and shrub plants to trees, coconut plants are present in the upper stream areas and obvious landscape of small-scale gold mining, few live stocks, agricultural land like rice fields, several residential areas. Active fishing and navigation.
Station 5	9°23'51"N 125°32'40" E	River mouth of Jaliobong, cattails plants dominant in the littoral zone with floating water lilies and water hyacinth, Agricultural land and residential areas in the nearby upstream. Active fishing and navigation within the Lake.
Station 6	9°20'20"N 125°31'11" E	Kalinawan river as outlet of the lake, cattails dominated the littoral zone, near to the resort, nearby areas are present with residents, on-going operation for flood control project in the upper stream.
Agusan Marsh		
Station 1	8°11'16"N 125°54'50" E	The lake basin of Mihaba dominated by water hyacinth and few disperse trees agricultural land and mining in the nearby environs
Station 2	8°12'15"N 125°54'16" E	Creek outlet of Lake Mihaba riparian vegetation is cattails and sedges with few trees and agricultural land and mining in the upper streams. Active navigation was observed.
Station 3	9°13'16"N 125°54'29" E	Near to the intersection of by cattails and sedges with few trees and agricultural land and mining in the upper streams. Active navigation was observed
Station 4	8°10'41"N 125°53'40" E	The Loreto river end of mixing to Agusan river, river waters flowing from the northeastern of Bukidnon
Station 5	8°10'48"N 125°53'44" E	The neck of three headwaters from Bukidnon, Simulao river and Lake Mihaba drainage creek, riparian dominated by tall grass and sedges. Active navigation was observed
Station 6	9°13'22"N 125°54'14" E	Downstream of Simulao River, riparian dominated by cattails and sedges with few trees and agricultural land and mining in the upper streams. Active navigation was observed

Establishment and collection of water and Physico-chemical analysis

The water samples were collected from two different types of inland waters in CARAGA region in Lake Mainit and Agusan Marsh respectively. Six sampling stations were established in each area. The water samples were collected through depth of 1-50 cm of the water column of each sampling site. The physicochemical parameters of each sampling station in every area were done using HANNA HI98194 multi-parameter test. Water samples were collected @ 0.5 L per sample and placed directly in calibrated PET bottles. The bottles were sealed tightly and labeled with information of area, station, and date of collection and photographed for documentation. A total of six bottles with 0.5 L water samples from Lake Mainit were collected on 26.11.2018 and another six bottles from Agusan Marsh were collected on 05.12.2018. The samples were chilled in ice bucket and delivered to Philsaga Mining Assay Laboratory, Rosario, Bunawan, Agusan del Sur for the analysis of Cyanide and Four Base Metals.

Collection of fish samples and preparation for landmarking

The fresh and live samples of *C. striata* were obtained from the local fishermen folks of Agusan Marsh and Lake Mainit. A total of sixty fish samples, out of which thirty males and thirty females were collected during 13-15.11.2018 from Lake Mainit. Another sixty fish samples, with equal sex proportion, were obtained on 22.11.2018 from Agusan Marsh. The length of each fish sample was determined by measuring the specimen from its snout down to the caudal fin using measuring tape and weighed using BATVOX ACS-40 digital weighing scale with 40 kg capacity and 1/3000 F.S. precision. The individual fish was flanked on a Styrofoam and fins were pinned showing its point of origin. Photographs of the left and right side of each specimen were taken with canon eos 1200D for land-marking. Specimens were dissected individually for their sex determination.

Table 2. Description of the landmark points in the body shape of *Channa striata* adopted and modified from Saura and Andante (2018).

Coordinates	Location
1	Snout Tip
2	End of head length
3	anterior junction of the dorsal fins
4	Posterior junction of the dorsal fins
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	Posterior insertion in the dorsal base of pelvic fin
11.	Anterior insertion in the dorsal base of pelvic fin
12	Ventral end of lower jaw articulation
13	Posterior end of maxilla
14	Anterior margin through midline of orbit
15	Posterior margin through midline of orbit
16	Dorsal end of operculum
17	Dorsal base of pectoral fin
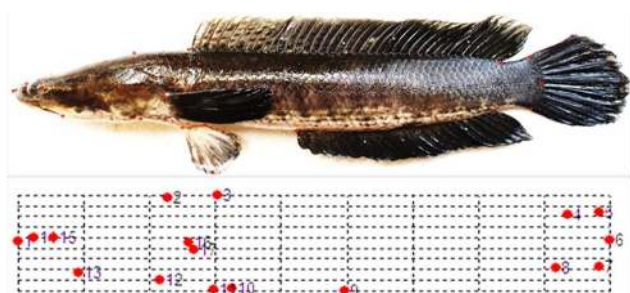
Figure 2. Actualized image of digitized selected landmark point of *Channa striata*.

Selection of Land-marking selection and digitizing

The images of fish specimens were sorted by sexes and the images processed in tpsUtil64 to create *.tps files. The resultant tps files were digitized with 17 landmarks using tpsDig2 software. The location of the landmarks and anatomical descriptions were represented in Table 2 and Figure 2. Landmarking was referred to on stickleback fish model of anyfish.com.

Shape analysis

The Procrustes superimposition was performed on the digitized image samples through tps relwarp software by subsequently pressing the clickable buttons of the consensus, partial warps, and the relative warp scores report and was saved for the statistical analysis for sexual dimorphism. Further, the digitized images were subjected to Symmetry and Asymmetry Geometric Data (SAGE) v.1.21 software following the protocols for object symmetry which is the landmark pairing protocol and matching symmetry, a side identification protocol to determine the shape conformation of individual variations (symmetric, asymmetric and error) and counting the possible covariance. In addition, the SAGE software platform provided options for the Principal Component Analysis (PCA) which was utilized to determine the affected landmark, and only the first four PCA% and deformation grid results of *C. striata* from Agusan Marsh and Lake Mainit and respective sexes were obtained.

Statistical analysis

The cumulative report saved from relative warp analysis was subjected to PAST 3 software for discriminant analysis and to derived plot for sexual dimorphisms and Hotellings T^2 test was used to determine significant differences of the sex shape differences. Further, the Procrustes method was employed and the significant differences of fluctuating asymmetry values respective to habitat and sexes were assessed using SAGE v.1.21 software. Permutation default statistical analysis i.e. Procrustes ANOVA procedure for understanding interaction and side effects were carried out in SAGE software.

Data analysis

The physicochemical parameters, content of cyanide, and four base metals, Lead, Copper, Zinc, Iron in water samples were compared to DENR standards (national water quality standards) above or below the maximum contamination level. Furthermore, the data of relative warp analysis, discriminant analysis, sexual dimorphism, and FA obtained from both the sexes of *C. striata* both Lake Mainit and Agusan Marsh were describe and compared.

RESULTS AND DISCUSSIONS

Fluctuating asymmetry

A total of one hundred twenty *C. striata* fish samples were obtained for this study, sixty from Lake Mainit and another sixty (60) from Agusan marsh. The length and weight of the fish samples from pond and Lake Mainit was presented in Table 3.

Table 3. Total Length and total weight of *Channa striata* from Lake Mainit and Agusan Marsh collected in the month of November 2018.

Fish samples	N	Total length (cm)			Total weight (g)		
		Range	Mean	SD $\pm$ SE	Range	Mean	SD $\pm$ SE
Lake Mainit	60	30.0-41.9	34.96	2.3 $\pm$ 0.3	205-480	318.83	64.3 $\pm$ 8.3
Agusan Marsh	60	29.5-41.1	36.2	2.8 $\pm$ 0.3	180-565	391.1	89.7 $\pm$ 11.6

Note: N-total number of samples

Furthermore, Procrustes method was used to assess the FA value of the right and left side of *C. striata* pooled samples both from Agusan Marsh and Lake Mainit are presented in Table 4. The individual symmetry and sides of Left and Right body shape in Agusan Marsh and Lake Mainit were not statistically significant. Out of the three effects considered, two effects (Individual and Sides) showed high significance (p < 0.0001) in both Agusan Marsh and Lake Mainit pooled samples. It indicated the incidence of FA in *C. striata* which was attributed to the effects of environmental stressors which can cause developmental instability to the species. Similar reports of developmental instability caused by the effects of stressed environment were observed in goby fish *Glossogobius giuris* from Agusan river samples (Jumawan et al 2016) and in the wings of *Aedes albopictus* from selected barangays in Iligan City, Philippines (Quirog and Tabugo 2015).

The principal component analysis (PCA) was employed on both pooled samples Agusan Marsh and Lake Mainit to determined landmark points affected (Table 5). Samples of *C. striata* from Agusan Marsh showed a total of 79.22% of FA from PC1-PC4. PC1 showed that all areas covered by these landmarks were found to have greater asymmetry. On the other hand, Lake Mainit *C. striata* showed a total FA interaction of 72.35% from PC1 to PC4. Affected landmarks were greater in PCA 3. This indicated the bilateral asymmetry of both male and female species of each habitat. The Agusan marsh *C. striata* population experienced a higher level of environmental stress. The asymmetrical shape of *C. striata* from Agusan Marsh and Lake Mainit fishes were compared and shown in Figure 3.

The individual symmetry and sides of left and right body shape of male and female in Agusan Marsh and Lake Mainit were not statistically significant shown in Table 6 and Table 7. Both male and female from two populations, out of the three effects considered, two shows high significance (p < 0.0001) both individuals and sides which is depicted.
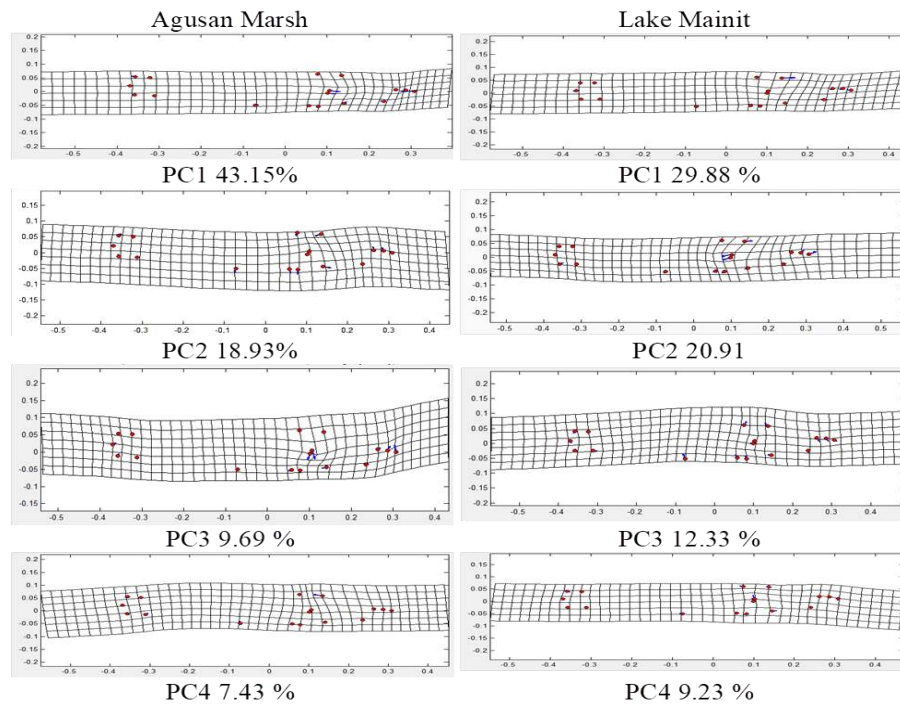
Table 4. Procrustes ANOVA results of pooled fish sample of *Channa striata* from two habitats.

Effect	Sum of Squares	Degrees of freedom	Mean square	F-value	p-value
Agusan Marsh					
Individuals	0.1216	1770	0.0001	3.2236	0*
Sides	0.0134	30	0.0004	20.8811	0*
Individual x Sides	0.0377	1770	0	0.06928	1 ns
Measurement Error	0.11075	3600	3.0764	---	---
Lake Mainit					
Individuals	0.1922	1770	0.0001	5.8043	0*
Sides	0.0044	30	0.0001	7.8103	0*
Individual x Sides	0.0331	1770	0	0.4114	1 ns
Measurement Error	0.16366	3600	4.5462	---	---

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

Table 5. The results of first four (4) Principal Components of *Channa striata* from Agusan Marsh and Lake Mainit.

PCA	Individual (symmetry)	Sides (directional asymmetry)	Interaction (fluctuating asymmetry)	Landmarks affected
Agusan Marsh				
PC1 (%)	44.31	100%	43.15	1, 2, 5, 6, 7, 9, 11, 12, 13, 14,15, 16, 17
PC2 (%)	17.61		18.93	1, 2, 3, 5, 7, 9, 10, 11, 12, 14, 15, 16
PC3 (%)	13.93		9.69	1, 6, 7, 10, 11, 14, 15, 16, 17
PC4 (%)	5.83		7.43	2, 5, 7, 8, 9
Overall%	81.68		79.22	
Lake Mainit				
PC1 (%)	61.01	100%	29.88	1, 2, 3, 6, 10, 13, 14, 15,16, 17
PC2 (%)	15.69		20.91	1, 2, 7, 9, 10, 11, 12, 14, 16, 17
PC3 (%)	6.56		12.33	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17
PC4 (%)	5.17		9.23	1, 2, 3, 5, 9, 11, 12, 16, 17
Overall%	88.52		72.35	

Figure 3. PCA implied deformation for individual × side interaction of fluctuating asymmetry of *Channa striata* from Agusan Marsh (first column) and Lake Mainit (second column) (zoom in to see blue arrows implies degree)

Table 6. Procrustes ANOVA results of *Channa striata* from Agusan Marsh in terms of sexes.

Effect	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Male					
Individuals	0.0652	870	0.0001	3.7276	0*
Sides	0.0075	30	0.0002	12.418	0*
Individual × Sides	0.0175	870	0	0.6188	1ns
Measurement Error	0.0585	1800	3.2497	---	---
Female					
Individuals	0.0524	870	0.0001	2.69	0*
Sides	0.0064	30	0.0002	9.8481	0*
Individual × Sides	0.0195	870	0	0.7712	1ns
Measurement Error	0.0523	1800	2.9031	---	---

Note: *highly significant (p < 0.0001), ns not significant

Table 7. Procrustes ANOVA results of *Channa striata* from Lake Mainit in terms of sexes

Effect	Sum of Squares	Degrees of Freedom	Mean Square	F-value	p-value
Male					
Individuals	0.0761	870	0.0001	4.6312	0*
Sides	0.0036	30	0.0001	6.2693	0*
Individual × Sides	0.0164	870	0	0.388	1ns
Measurement Error	0.0876	1800	4.8652	---	---
Female					
Individuals	0.1071	870	0.0001	7.4611	0*
Sides	0.0032	30	0.0001	6.3652	0*
Individual × Sides	0.0144	870	0	0.3905	1ns
Measurement Error	0.0761	1800	4.227	---	---

Note: *highly significant (p < 0.0001), ns not significant

Furthermore, Table 8 and 9 show the results of Principal Component Analysis of *C. striata* samples in terms of sexes from Agusan Marsh and Lake Mainit respectively. Both sexes of *C. striata* sample from Agusan Marsh showed close percentage of FA (Male, PC1, 84.41%) and (Female, PC4,85.41%). PC1 showed that all areas covered by these landmarks were found to have greater asymmetry in males and PC3 in females. On the other hand, Lake Mainit *C. striata* male showed a total FA of 85.12% from PC1 to PC4 and its affected landmarks were greater in PCA 1. While in female *C. striata* had a total FA of 80.89% from PC1 to PC4 in which PC3 had greater affected landmarks. The asymmetrical shape of *C. striata* from Agusan Marsh and Lake Mainit were compared and depicted in Figures 4 and 5.

The degree of closeness of FA is merely on behavior and characteristics and their preferred habitat. As observed in the deformation grid and PCA most of the affected portion of the body with many and variation was the anterior part of the body extended up to the middle areas which may be due to burrowing behavior and association of the anterior part with the lateral line system of the fish which enable them with their predatory skills and ability to live in stressed swampy and muddy habitat. The increase of Fluctuating Asymmetry variation of *C. striata* populations from two sites was followed by influence of their habitat

quality. Noteworthy, that the samples from Agusan Marsh had higher (7%) difference from Lake Mainit due to the fact that observed habitat had high turbidity, and local residence also attested that selected sampling rivers and creeks within the Agusan Marsh remained turbid throughout the year (Primavera and Tumanda Jr. 2008), unlike Lake Mainit that had clear waters (LMDA 2018).

Sexual dimorphism

Based on the cumulative report retrieved from relative warp analysis and discriminant analysis using PAST software, the resultant graphs showed overlap in the discriminant plot (Fig. 6) meaning shape differences between two sexes of *C. striata* specimens from Agusan Marsh and Lake Mainit are minimal. Hotellings T^2 test results also revealed no significant sexual dimorphism for the body shape of the *C. striata* in all specimens from Agusan Marsh and Lake Mainit (Table 10). This is in line with the study of Saura and Andante (2018) who showed similar less significant sexual dimorphism in *C. striata in* Bunawan, Agusan River. Parker (1992) has pointed towards sperm competition, size buffering among other features in answer to the sexes being often so alike in size. However, some studies reported *C. striata* females are larger than males (Chaudhry 2010; Dumalagan 2017).

Table 8. The results of Principal Components of *Channa striata* from Agusan Marsh in terms of sexes

PCA	Individual (symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Landmarks affected
Male				
PC1 (%)	44.68	100%	43.81	1, 2, 5, 6, 7, 9, 11, 12, 13, 14,15, 16, 17
PC2 (%)	21.90		20.45	1, 2, 3, 5, 7, 9, 10, 11, 12, 14, 15, 16
PC3 (%)	14.62		12.94	1, 6, 7, 10, 11, 14, 15, 16, 17
PC4 (%)	5.31		7.07	2, 5, 7, 8, 9
Overall%	86.51		84.27	
Female				
PC1 (%)	52.62	100%	47.80	1, 2, 3, 6, 10, 13, 14, 15,16, 17
PC2 (%)	16.57		20.31	1, 2, 7, 9, 10, 11, 12, 14, 16, 17
PC3 (%)	10.09		9.07	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17
PC4 (%)	5.31		8.23	1, 2, 3, 5, 9, 11, 12, 16, 17
Overall%	84.59		85.41	

Table 9. The results of Principal Components of *Channa striata* from Lake Mainit in terms of sexes.

| PCA	Individual (symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Landmarks affected
Male				
PC1 (%)	54.20	100%	32.81	1, 2, 5, 6, 7, 9, 11, 12, 13, 14,15, 16, 17
PC2 (%)	19.25		26.02	1, 2, 3, 5, 7, 9, 10, 11, 12, 14, 15, 16
PC3 (%)	8.05		13.09	1, 6, 7, 10, 11, 14, 15, 16, 17
PC4 (%)	5.62		7.43	2, 5, 7, 8, 9
PC5 (%)	---		5.77	
Overall%	87.12		85.12	
Female				
PC1 (%)	67.25	100%	30.39	1, 2, 3, 6, 10, 13, 14, 15,16, 17
PC2 (%)	14.68		22.07	1, 2, 7, 9, 10, 11, 12, 14, 16, 17
PC3 (%)	6.59		11.82	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17
PC4 (%)	---		10.12	1, 2, 3, 5, 9, 11, 12, 16, 17
PC5 (%)	---		6.49	
Overall%	88.52		80.89	
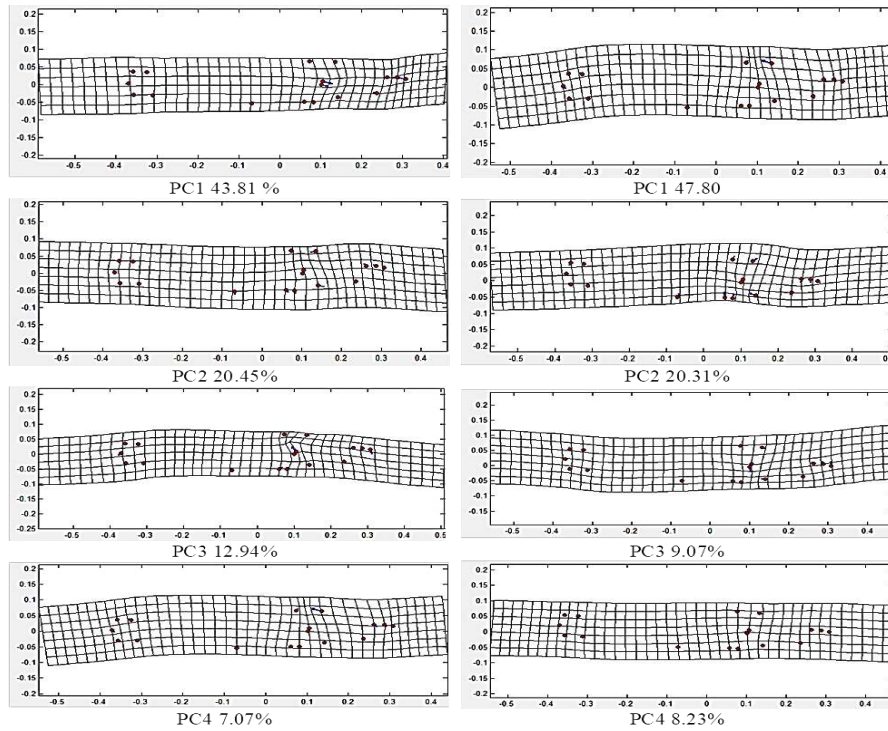
Figure 4. The male (first column) and female (second column) fishes from Agusan Marsh with the affected landmarks shown in PCA-deformation grid.
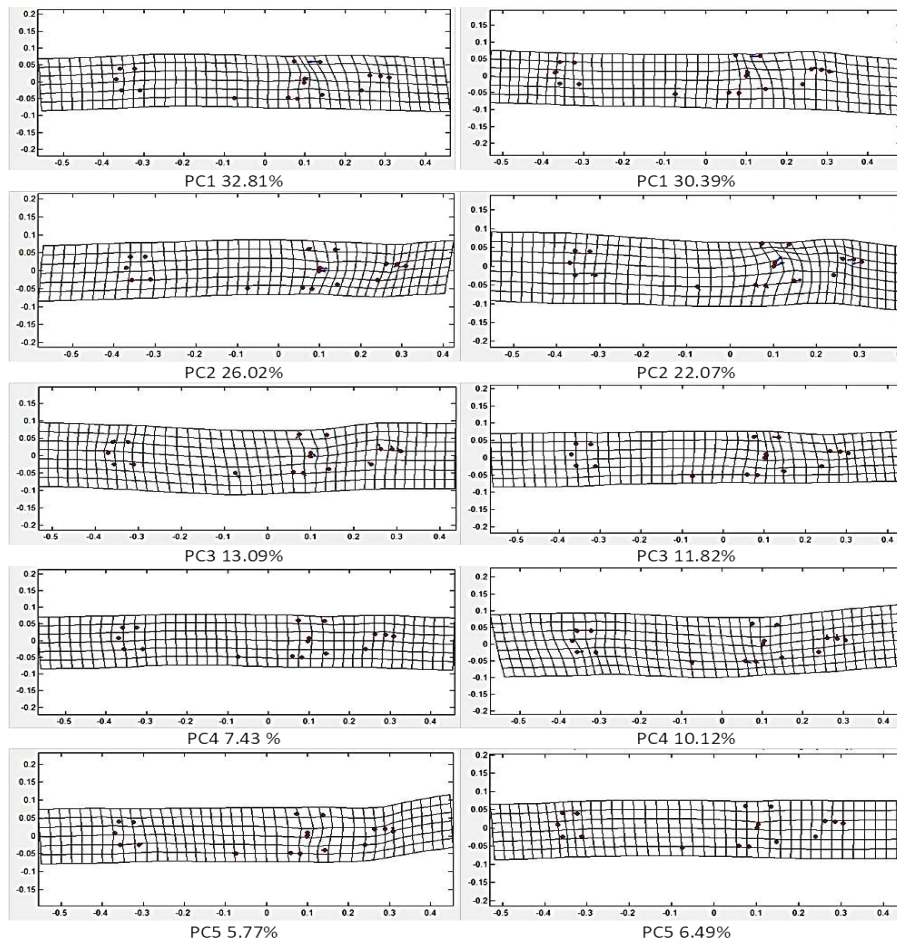
Figure 5. The male (first column) and female (second column) fishes from Lake Mainit with the affected landmarks shown in PCA-deformation grid.
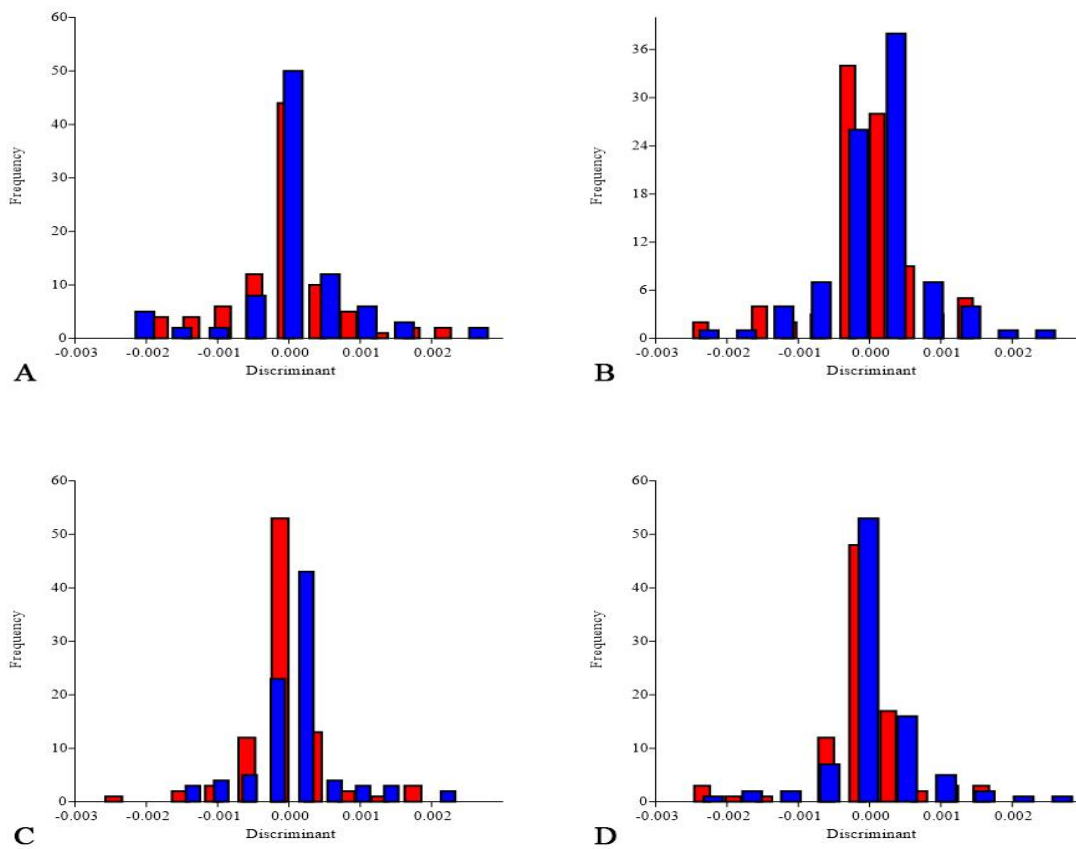
Figure 6. Discriminant plot of sexual dimorphism: A (*left* flank) and B (*right* flank) from Agusan Marsh; C (*left* flank) and D (*right* flank) from Lake Mainit.

Table 10. Result of Discriminant analysis of Sexual Dimorphism of *Channa striata*.

Discriminant analysis	*Channa striata*			
	Agusan Marsh		**Lake Mainit**	
	Left flank	**Right flank**	**Left flank**	**Right flank**
Hotelling's T^2	2.9337	2.1325	1.7599	2.1011
F-value	3.1131	2.263	1.8676	2.2296
p-value	1	1	1	1

Table 11. Physico-Chemical Parameters of both sampling areas gathered in the month of December 2018.

Sampling area	Parameters					
	TSS (ppm)	**TDS (ppm)**	**DO (ppm)**	**pH**	**Salinity (ppm)**	**Temperature (^{O}C)**
*Tagbuyawan	10.5.	101	7.86	7.73	0.03	29.35
*Mayag	3.4	100	7.52	8.07	0.03	29.50
*Magpayang	11.8	113	8.93	7.07	0.03	29.30
*Magtiaco	72.4	125	12.1	7.35	0.01	28.95
*Jaliobong	15.0	101	7.73	7.67	0.01	29.66
*Kalinawan	31.0	105	7.97	7.48	0.01	29.16
**Lake Mihaba	47.8	92	3.37	6.9	0.05	28.52
**Lake Mihaba Creek (outlet)	37.4	73	3.42	6.93	0.04	28.81
**Agusan river (downstream)	61.0	153	3.44	8.14	0.11	28.94
**Loreto river (downstream)	30.0	145	3.46	7.87	0.11	28.65
**Agusan river (upstream)	63.2	226	3.42	8.02	0.17	29.06
** Sumilao (downstream)	57.60	104	4.55	7.8	0.07	28.72

Note: Legend: *Lake Mainit sampling stations, ** Agusan Marsh sampling stations

Table 12. Comparisons of Physico-chemical parameters and concentrations of CN, Fe, Zn, Cu, Pb to DENR standards (as sampled in Lake Mainit sampling stations on November 11, 2018, and Agusan Marsh sampling stations on December 05, 2018.

Parameters	Available range		DENR
	Lake Mainit	Agusan Marsh	
TSS (ppm)	3.4-10.5	30-63.2	80
TDS (ppm)	100-125	73-226	96-100
DO (ppm)	7.52-12.1	3.37-4.55	5
pH	7.07-8.07	6.9-8.14	6.5-9.0
Salinity (ppm)	0.01-0.03	0.04-0.17	Nd
Fe (ppm)	<0.01-0.09	0.46-3.39	2
Zn(ppm)	<0.01	<0.01	1.5
Cu (ppm)	<0.01	<0.01	0.02
Pb (ppm)	<0.01	<0.01-0.01	0.02
CN$^-$(ppm)	<0.01	<0.01	0.1

Note: nd: no data

Physico-chemical, cyanide and four base metals

Table 11 shows the result of the physicochemical parameter in the sampling area of Lake Mainit and Agusan Marsh using multiparameter test. In Lake Mainit sampling stations, Magtiaco river mouth recorded the highest total suspended solids, total dissolved solids and dissolved oxygen level. It may be due to the heavy coarse load and runoff from the landslide in small-scale mining areas of upstream mountainous areas. during Typhoon (Sanba) Basyang of February 2018. Salinity, pH and temperature were mostly the same among other sampling sites. In Agusan Marsh sampling area, upstream and downstream of Agusan river were observed with high total suspended solids, total dissolved solids, pH and temperature due to the fact that Agusan River is the receiver of almost eleven tributaries including the other sampling sites. The dissolved oxygen and salinity were mostly the same among other sampling sites in Agusan Marsh sampling area.

Furthermore, the analysis of four base metals and Cyanide indicated that Zinc, Copper, Lead, and Cyanide levels were in less than detection limit of 0.01 ppm in Lake Mainit and Agusan Marsh sampling stations, except the value of Lead was 0.01 in Lake Mihaba Creek (outlet) of Agusan Marsh. In terms of Iron content, Magtiaco (0.09 ppm) in Lake Mainit had the highest value while other locations of the said sampling site had values ranging from <0.01-0.01 ppm. Agusan Marsh sampling stations had varied Iron content all above the detectable limit of 0.01 ppm ranging from 0.46 in Agusan river (upstream) to 3.39 in Lake Mihaba.

Table 12, shows the range and comparison of the water parameters that were tested in the selected sampling sites to the DENR recommended water quality standards and contamination limit on toxic and deleterious substances. The range of the parameter values was observed high mostly in Agusan Marsh TSS, TDS, Salinity and Iron (Fe) while Dissolve oxygen had low values compared to Lake Mainit. Out of the four base metals, only Iron (Fe) was recorded above detectable limit and observed high in Agusan Marsh. Cyanide was below detectable limit and acceptable to the DENR standards. Total dissolved solids in both Lake Mainit and Agusan Marsh were not acceptable to the standard set by DENR that should not

surpass 96-100 ppm except in Lake Mihaba and its outlet creek that was in the acceptable limit (see table 3). Total suspended solids, TSS, pH, Zn, Cu, Pb, CN$^-$values of Lake Mainit and Agusan Marsh were acceptable to the recommended standards. On the other hand, in terms of Dissolved Oxygen level, Agusan Marsh is not acceptable to the recommended standards. At the same time, Iron (Fe) level specifically in Lake Mihaba and its outlet in Agusan Marsh were not acceptable to the DENR standards. Salinity however owns at the level characterized by freshwater.

The values of selected physicochemical parameters differed from each habitat such as dissolved oxygen level and Iron which recorded higher in Agusan Marsh compared to Lake Mainit. These differences due to the surroundings and the ecosystem type of each habitat. The Agusan Marsh is an extensive floodplain in the middle of the Agusan River Basin in eastern Mindanao which served as stopped drainage of waters from rivers, creeks and tributaries mainly in the provinces of Agusan del Norte, Agusan del Sur and Compostela Valley and development for agriculture, construction of dams and reservoirs for irrigation, deliberate or accidental introductions of exotic species, e.g., tilapia, carps, janitor fish, and golden apple snail, and logging in the watershed areas. (Primavera and Tumanda Jr. 2008). While Lake Mainit is an oligotrophic lake based on its physicochemical, natural productivity and optical properties and morphometric characteristics (Tumanda Jr. et al. 2003). The study provided that *C. striata* which is a food source and bioresources for livelihood generation can be propagated in freshwater aquaculture even in a stressful environment.

In conclusion, the landmark-based geometric morphometric was a useful tool in describing the fluctuating asymmetry and sexual dimorphism of *Channa striata*. The statistical results showed high variations (p < 0.0001) on the left and right side of the bilateral represented by 79.22% in Agusan Marsh specimens and 72.35% in Lake Mainit specimens which may be considered as relatively high Fluctuating Asymmetry. The affected landmark points and variation are observed in the cephalic, pectoral, and caudal regions which may be due to burrowing behavior and association of the lateral line system among fishes that thrive in high turbid waters and

low oxygen. Further, the physicochemical analysis reveals that Agusan Marsh had low dissolved oxygen, TDS, and Iron deviates to the national water quality standards while Lake Mainit is within permissible limits. On the other hand, the total FA in terms of sexes, Agusan marsh had 84.27% and 85.41%, while Lake Mainit had 85.12% and 80.89% respectively in males and females. The fairly similar FA% based on the 17 landmark points of all *C. striata* population confirms to less or no sexual dimorphism of fish in the study area. The present study contributes essential information about the body variations of *C. striata* and the condition of Agusan marsh basin and Lake Mainit and importance of FA in determining the habitat condition the fact that organisms, *C. striata* preferred high turbidity and thrived in stress condition. Further study is needed regarding Fluctuating Asymmetry of *C. striata* in relation to seasonal variations.

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