Article

Comparative fluctuating asymmetry of spotted barb (*Puntius binotatus*) sampled from the Rivers of Wawa and Tubay, Mindanao, Philippines

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Abstract

Fluctuating Asymmetry (FA) commonly uses to evaluate environmental stress and developmental variability of different biotic elements. This study aims to describe the possible effects of pollutants on the body shapes of spotted barb (*Puntius binotatus)* with notes of physico-chemical parameters of Wawa River, Bayugan City, Agusan del Sur and Tubay River, Tubay, Agusan del Norte, Philippines. There were a total of 80 samples (40 females & 40 males) collected from each sampling areas. Digital imaging was prepared and the acquired images were loaded into tpsDig2 program. Standard landmarks on fish morphometric were employed. Using thin-plate spline (TPS) series, landmark analysis were completed and subjected to symmetry and asymmetry in geometric data (SAGE) software. Results in Procrustes ANOVA showed high significant differences of (P<0.0001) in the three factors analyzed: the individuals; sides; and the interaction of individuals and sides; indicating high fluctuating asymmetry. In Tubay River, the level of asymmetry in females were 79.06% and in males 71.69% while in Wawa River, the level of asymmetry in females were 76.60% and in males 62.64%. Therefore, indicating high level of asymmetry denotes environmental alterations. On the other hand, physico-chemical parameters were also determined in the two sampling areas. The results of One-way ANOVA showed that the mean parameters in Wawa River has significant difference of (P<0.0001), while Tubay River has no significant difference. Results of Pearson-correlation of fluctuating asymmetry between physico-chemical parameters shows no correlation which suggests that water components is not directly influenced by the fluctuating asymmetry. The approach of FA and physico-chemical parameters were significant for evaluating environmental condition as well as species state of well-being.

Keywords fluctuating asymmetry; physico-chemical; spotted barb; *Puntius binotatus*; Wawa River; Tubay River.

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1 Introduction

The shape and shape change has been a fundamental prerequisite to biological anthropology at the same time detecting shapes and copying metric observations is a challenge to distinguish the manner in which biological forms diverse from one another (Richtsmeier et al., 2002).

Fish was highly recognized as an environmental biomarker for different types of the stressor. It has been used for several scientific investigations as it provides reliable evidence of occurring pollutants. Indeed, fish serve as useful genetic models for the evaluation of pollution in the aquatic ecosystem (Mitchell and Kennedy, 1992). Along its presence, it also works as the reliable bio-indicator from many effluents and plays important role for bio-monitoring. Pollution coming from anthropogenic activities, agricultural runoff and disposing of a huge amount of waste into the aquatic ecosystem can be detrimental to the environment (Natividad et al., 2015). Hence, it may cause impairment and later affects the quality and quantity of the organisms (Duruibe et al., 2007). Thus, unbalanced state of environment merely affects the total morphology of the organisms and likely disturbed its overall symmetry (Daloso, 2014). Freshwater ecosystem plays an important role for biodiversity and as of today, it has been considered the most affected from various pollution. As well as, this type of ecosystem underneath threat and may pose a risk to the organisms. Moreover, it has been top of the list to be endangered (Jumawan et al., 2016).

Fluctuating Asymmetry (FA) was employed to evaluate the flux of pollutants in the aquatic environment and its effects on the organism. This mechanism provides the dependable result as it repeatedly used by numerous studies around the world. FA said to be an excellent means for determining environmental health (Lecera et al., 2015). It is also an effective type of approach and less expensive in evaluating the condition of the environment and the organisms (Angtuaco and Leyesa, 2004). Fluctuating Asymmetry or FA extensively used as a potential indicator for describing developmental instability (Ducos and Tabugo, 2015). The procedure of using geometric morphometrics in outlining feature FA has been recognized in several studies (Klingenberg and McIntyre, 1998; Savriama et al., 2012; Hermita et al., 2013). On the other hand, FA plays an important mechanism for distinguishing morphological similarity and differences (David Polly, 2012). As well as, FA is known to be a marker of the well-being both of the environment and organisms as it discloses morphological dissimilarities (Swaddle, 2003). It is also a significant instrument in the field of biology as it reveals a population’s state of adaptation and co-adaptation (Jumawan et al., 2016). Furthermore, fluctuating asymmetry identified to be an efficient tool for giving a vital information over another biomarker of developmental variability (Clarke, 1993).

The sampling areas might experiencing environmental disturbances that could affect its health condition as well as the inhabitants. The Wawa River situated were constant soil erosion occur along its riverbanks that causes siltation, immediate changes of the water level and also it undertaken several anthropogenic activities while Tubay River became a primary recipient of chemical effluents coming from households and the mining activities. These scenarios might be a reason that these rivers would be unsuitable for the many fish species and likely affects its morphological feature. Alongside, physico-chemical parameters were also determined in the study to further analyze its water components. The species of spotted barb (*P. binotatus*) were subjected to investigation as it is readily available and commonly found in the two sampling areas. Indeed, *P. binotatus* has been used as an essential bio-marker to environmental stress or the health condition of the aquatic habitat (Baumgartner, 2005; Mat Isa et al., 2010; Zakeyudin et al., 2012). Hence, it is important to apply scientific methods as it helps to explain the state of well-being both of the environment and the organisms. This study aims to identify the possible effects of pollutants on the morphology of *P. binotatus* and to evaluate the condition of the two rivers using physico-chemical parameters. Thus, the outcome of this study would be significant due to on-going developments in the areas and may further aggravate the rivers condition.

2 Materials and Methods

2.1 Study area

This study was conducted in the two sampling areas: Wawa River, Bayugan City, Agusan del Sur and Tubay River, Tubay, Agusan del Norte, Philippines. The fish collection was done in the month of August and September 2016.

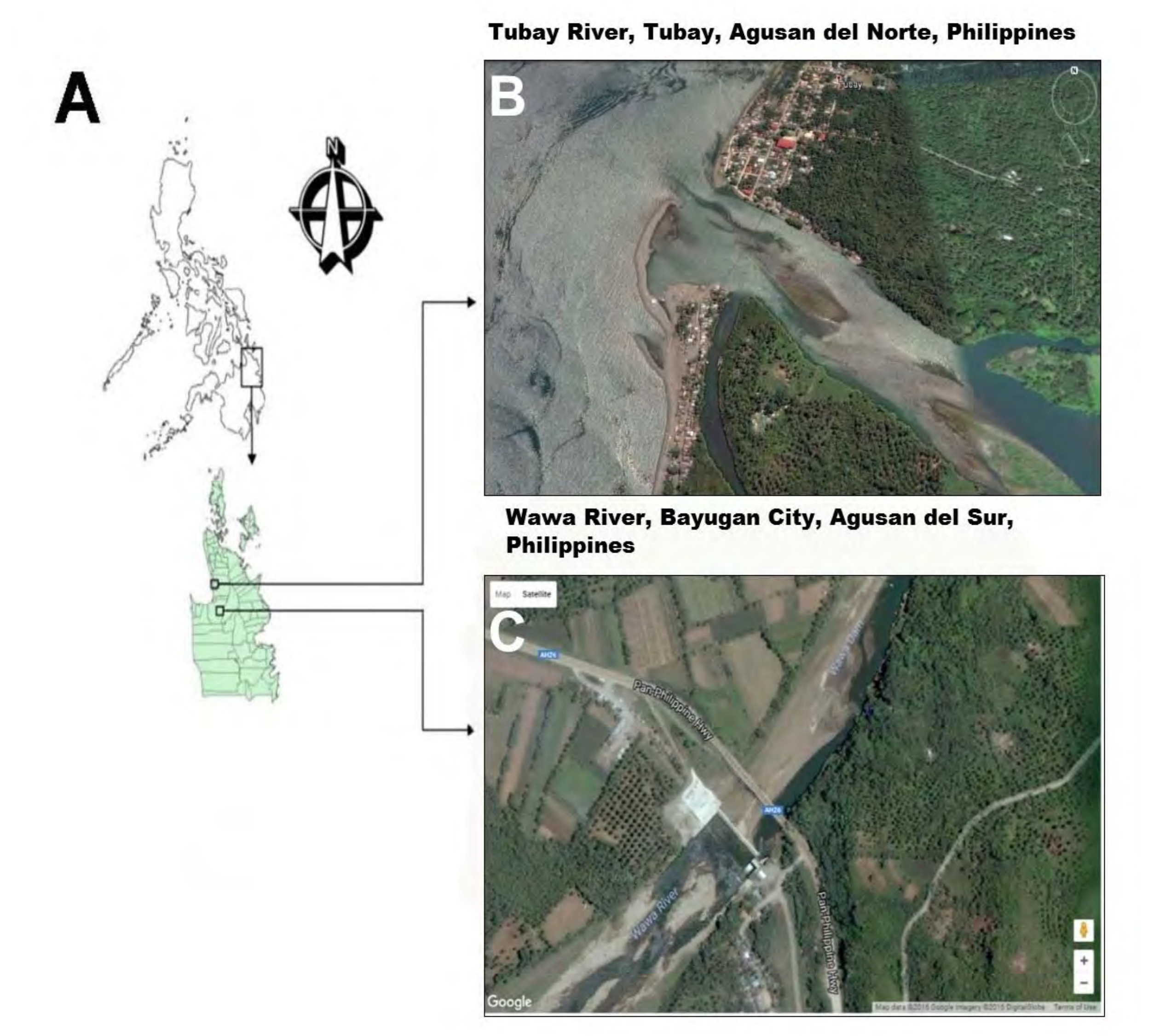

Fig. 1 Map showing **A**. Philippines **B**. Tubay River, Tubay Agusan del Norte. **C**. Wawa River, Bayugan City, Agusan del Sur, Philippines**.**

2.2 Fluctuating asymmetry of spotted barb (*Puntius binotatus*)

2.2.1 Sample processing

Eighty samples of *P. binotatus* (40 males and 40 females) were collected in each sampling areas. The fish samples was then placed in a flat Styrofoam for the pinning of its fins to make it wider and to visibly see the samples point of origin for the land-marking procedures. A 10% Formalin was applied in all the fins of the sampled fish to make it hardened with the aid of small brush. Digital imaging was done with Canon digital camera (Pixma, 14 megapixels). To acquire the length of individuals, the left and right lateral side of each sample was taken using a ruler (Natividad et al., 2015).

2.2.2 Sex Determination

Sex identification of the samples was done through its genitalia. The presence of eggs and ovaries in general yellow or orange with granular texture were females while the presence of testes which were normally non-granular in texture, whitish and smooth were males (Requiron et al 2010).

2.2.3 Landmark selection and digitization

Images captured were then sorted with accordance to its sex and converted into TPS file format using tpsUtil. Land-marking of samples was done using tpsDig2 (version 2, Rohlf 2004). A total of sixteen anatomical landmark points (Table 1) were used to digitize the symmetrical body shape of the samples (Fig. 2).

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

Coordinates	Locations
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 Landmark points of *P. binotatus* (**top**) female (**bottom**) male.

2.2.4 Shape analysis

The digitation for Left-Right of the fish samples undergone tri-replicated images through tpsDig2. The coordinate data was then subjected to (**SAGE**) Symmetry and Asymmetry in Geometric Data (version 1.04 Marquez, 2007) software (Fig. 3) and also to obtain the principal components of individual symmetry which denotes by deformation grid (Natividad et al 2015). Procrustes ANOVA test was performed to determine the significant difference in the symmetry of the three factors considered – individual, sides and interaction of individuals and side. The significance level was confirmed at P<0.0001. The differences among the side and the measure of directional asymmetry also specifies. The percentage (%) of FA were acquired and compared between the sexes (Natividad et al., 2015).

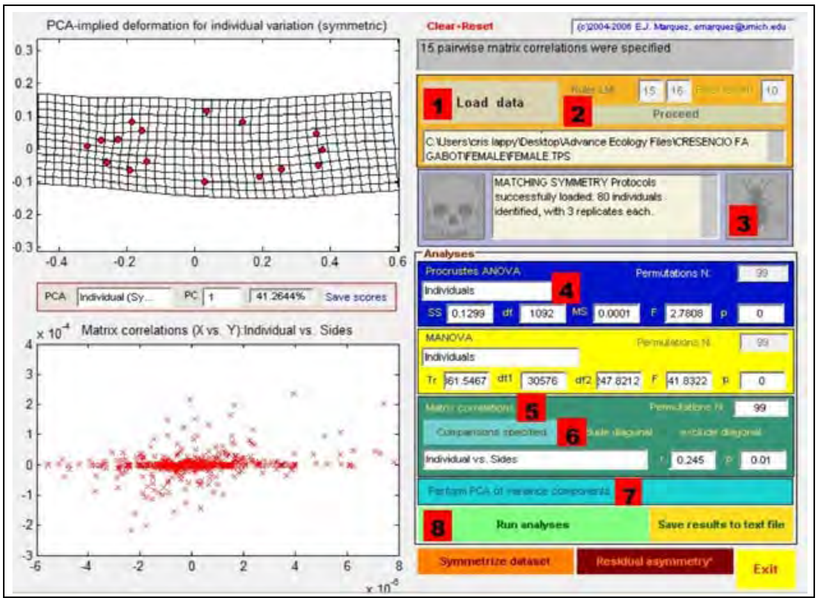

Fig. 3 Overview of the schematic flow of shape analysis using SAGE.

2.2.5 Physico-chemical parameters

The water parameters that comprises temperature, total dissolve solids (TDS), conductivity, pH, Dissolve Oxygen (DO) and salinity, were evaluated with the same date mentioned above during the fish collection. In each sampling areas three sites was established along with three replicates and randomly selected for the measurements. This was done using the multi-parameter water quality meter (EUTECH PCD). Data was presented as mean ± standard error mean (SEM) and was calculated using Paleontological Statistics and Software (PAST).One-way Analysis of Variance (ANOVA) was also used for testing the significant difference of physico-chemical parameters between the sampling areas.

2.2.6 Correlation of fluctuating asymmetry and physico-chemical parameters.

The correlation between fluctuating asymmetry and water parameters were also determined. Using the mean values of physico-chemical and the percentage of fluctuating asymmetry was subjected to analysis. Pearson correlation coefficient was used to analyze the relationship between fluctuating asymmetry and water parameters. All data were analyzed using Graph Pad Prism 5.

3 Results and Discussion

3.1 Fluctuating Asymmetry

To describe the individual shape fluctuations Procrustes ANOVA was used. The comparison of individual symmetry of Left-Right shape and size was analyzed (Tables 2a & 2b). Three factors were analyzed: the individuals; sides; and the interaction of individuals and sides. Both sexes (male and female) of the fish samples was applied for the analysis. The results indicated fluctuating asymmetry along the three factors and between the female and male (P<0.0001). It was observed that highly significant difference occurs in the individual fish resulting fluctuating asymmetry when one of the fish samples compared into another. Its sides also showed highly significant variance signifying fluctuating asymmetry in its left and right sides of the samples. In general, the relation among the individuals and sides also exhibited highly significant difference which suggested fluctuating asymmetry influenced amongst the interaction of individuals and sides. Thus, fluctuating asymmetry of all the factors was detected in both female and male fishes.

The data illustrated that the samples of *P. binotatus* collected along the two sampling areas were experienced asymmetrical in the aspects of comparison as an individual, both of the left and right side morphology, and the interaction of its individuals and sides. It was also observed that when female and male combined the results suggest asymmetry within its factors (individuals, sides and individual and sides). Since, asymmetry in the morphology of the female and male fish samples were observed, it might be a sign that fishes in the two sampling areas underneath environmental disturbances. Under normal situation, symmetrical in its morphology could be manifested. Nonetheless, seeing fluctuating asymmetry would be an indication that the existence of pollutants within its habitat affects its morphology. The continuity of the fishes in the polluted and disturbed environment causes asymmetry. By this, *P. binotatus* will also undergone asymmetrical in appearance when extended exposure to effluents in the sampling areas.). In addition, organisms that give high FA parallels with the stressors present in the environment (Hermita et al., 2013).

Table 2a Procrustes ANOVA on body shape of *P. binotatus* in terms of sexes from Wawa River, Bayugan City, Agusan del Sur, Philippines.

Factors	SS	DF	MS	F	P-VALUE
		Female			
Individuals	0.1299	1092	0.0001	2.7808	0.0001**
Sides	0.034	28	0.0012	28.4209	0.0001**
Individual x Sides	0.0467	1092	0	7.1498	0.0001**
Measurement Error	0.0268	4480	0	--	--
		Male			
Individuals	0.1763	10922	0.0002	5.0868	0.0001**
Sides	0.0466	28	0.0017	52.4557	0.0001**
Individual x Sides	0.347	1092	0	3.6106	0.0001**
Measurement Error	0.0394	4480	0	--	--

** (P<0.0001) highly significant.

The data shows the indication for FA of the fishes that can be accredited to a stressed environment probably from different types of aquatic pollutants. As a result, the ecosystem that is polluted will eventually cause morphological variation as these effluents interfere during its growth and development (Bonada and Williams, 2002). Therefore, displaying asymmetry is a sign that species is incompetent to counteract and shield from any environmental disorders (Van Valen, 1962). Hence, the developmental variability of *P. binotatus* will lead to fluctuating asymmetry.

Principal component analysis (PCA) was applied in order to define the affected landmarks using the symmetry and asymmetry scores. There were four principal components (PC) considered in female and male samples. The four highest PC scores determined landmarks which were frequently affected in fluctuating asymmetry of the samples (Tables 3a & 3b). Skewness of the histogram was reflected in every PC score alongside with the deformation grid to determine affected landmarks (Figs 4a, 4b and 5a, 5b).

In the female samples from Wawa River, has four principal components (PC) constituted 81.76% of the cumulative variation while female samples from Tubay River, has 80.34% of the five principal components. PC 1 from Wawa River has the highest variation accounted to 41.26% while in Tubay River has 44.36%. The commonly affected landmarks in female samples to the four PC score from Wawa River were landmarks 3, 4, 6, 8, and 10 while the affected landmark common to the five PC score from Tubay River were: 3, 4, 9 and 16 (Tables 3a and 3b).These were portion of the anterior and posterior insertion of dorsal fin, midpoint or lateral line, posterior and anterior insertion of anal fin, dorsal base of pelvic and pectoral fin. In male samples from Wawa River, the four PC constituted to 87.31% of the cumulative variation while males from Tubay River has 76.72%. PC 1 from Wawa River contributed the highest variation with 52% while from Tubay River accounted to 46.46%. The commonly affected landmarks in male samples to the four PC score from Wawa River were landmarks 1, 3, 4, 5, 7, 8 9, 10, 11, 12, and 16 while the affected landmarks from Tubay River were: 3, 4 9, 10 and 16 (Tables 3a and 3b). These landmarks were mainly of the snout tip, anterior and posterior insertion of dorsal pin, dorsal and ventral insertion of caudal fin, posterior and anterior insertion of anal fin, dorsal base of pelvic and pectoral fin, ventral end of lower jaw articulation, and posterior end of the premaxilla.

Table 2b Procrustes ANOVA on body shape of *P. binotatus* in terms of sexes from Tubay River, Tubay, Agusan del Norte, Philippines.

Factors	SS	DF	MS	F	P-VALUE
			Female		
Individuals	0.1802	1092	0.0002	2.23	0.0001**
Sides	0.0454	28	0.0016	21.894	0.0001**
Individual x Sides	0.0808	1092	0.0001	3.4156	0.0001**
Measurement Error	0.0971	4480	0	--	--
			Male		
Individuals	0.3041	1092	0.0003	3.9827	0.0001**
Sides	0.0321	28	0.0011	16.4117	0.0001**
Individual x Sides	0.764	1092	0.0001	4.5469	0.0001**
Measurement Error	0.0689	4480	0	--	--

It was detected that female samples from Tubay River has the highest PC compared to Wawa River while male samples from Wawa River has the highest PC compared to Tubay River (Tables 3a and 3b). On the other hand, the highest percentage of FA recorded from Tubay River compared to Wawa River. It might because samples from Tubay River were already affected by various aquatic pollutants since the river situated were runoff of effluents coming from households and considerably from mining industries. The presence of these aquatic pollutants will likely affect fish morphology. Such, females in particular were vulnerable for fluctuations as they were adaptive for environmental modifications. Indeed, study shows that females were competent to buffer ecological modifications; thus keeping its homeostasis and metabolic frequency for reproduction (Cabuga et al., 2016). It was also identified that affected landmarks were almost similar and these were further shown in deformation grid and histogram of the values revealed skewness proposing asymmetry in body form (Figs 4a, 4b & 5a, 5b).

Table 3a Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks from Wawa River, Bayugan City, Agusan del Sur, Philippines.

PCA	Individual (Symmetry)	Sides (Directional asymmetry)	Interaction (Fluctuating asymmetry)	Affected landmarks
		Female		
PC1	41.2644%	100%	47.8175%	1,3,4,5,6,7,8,9,10,11,12,14,16
PC2	20.9153%		13.6766%	8,10
PC3	10.3516%		9.801%	2,3,4,5,6,8,10
PC4	9.2301%		5.3071%	2,3,4,6,7,9,11,12,13,14
	81.7614%		**76.6022%**	
		Male		
PC1	51.996%	100%	25.2923%	1,2,4,5,6,7,8,9,10,11,12,13,16
PC2	17.757%		16.6716%	1,3,4,5,7,8,9,10,11,12, 13,14,16
PC3	11.4987%		11.3548%	3,4,6,7,8,9,10,15,16
PC4	6.0557%		9.3235%	3,4,5,11,14
	87.3074%		**62.6422%**	

Table 3b Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks from Tubay River, Tubay, Agusan del Norte, Philippines.

PCA	Individual (Symmetry)	Sides (Directional asymmetry)	Interaction (Fluctuating asymmetry)	Affected landmarks
		Female		
PC1	44.3632%	100%	53.8937%	1,2,3,4,5,6,7,8,9,10,11,12,13,16
PC2	12.8096%		10.0211%	3,4,5,8,9,10
PC3	10.0718%		6.0222%	4,10,16
PC4	7.8632%		4.6034%	1,3,4,5,6,7,8,9,11,12,15,16
PC5	5.2307%		4.5155%	1,2,3,4,9,12,15,16
	80.3385%		**79.0559%**	
		Male		
PC1	46.4605%	100%	48.3352%	1,2,3,4,5,6,9,10,11,12,13,16
PC2	13.7935%		11.7068%	1,2,3,4,5,6,7,8,9,10,11,12,1,3,14, 15,16
PC3	9.4761%		6.3728%	4,7,9,10,16
PC4	6.987%		5.2805%	3,4,8,9,10
	76.7171%		**71.6953%**	

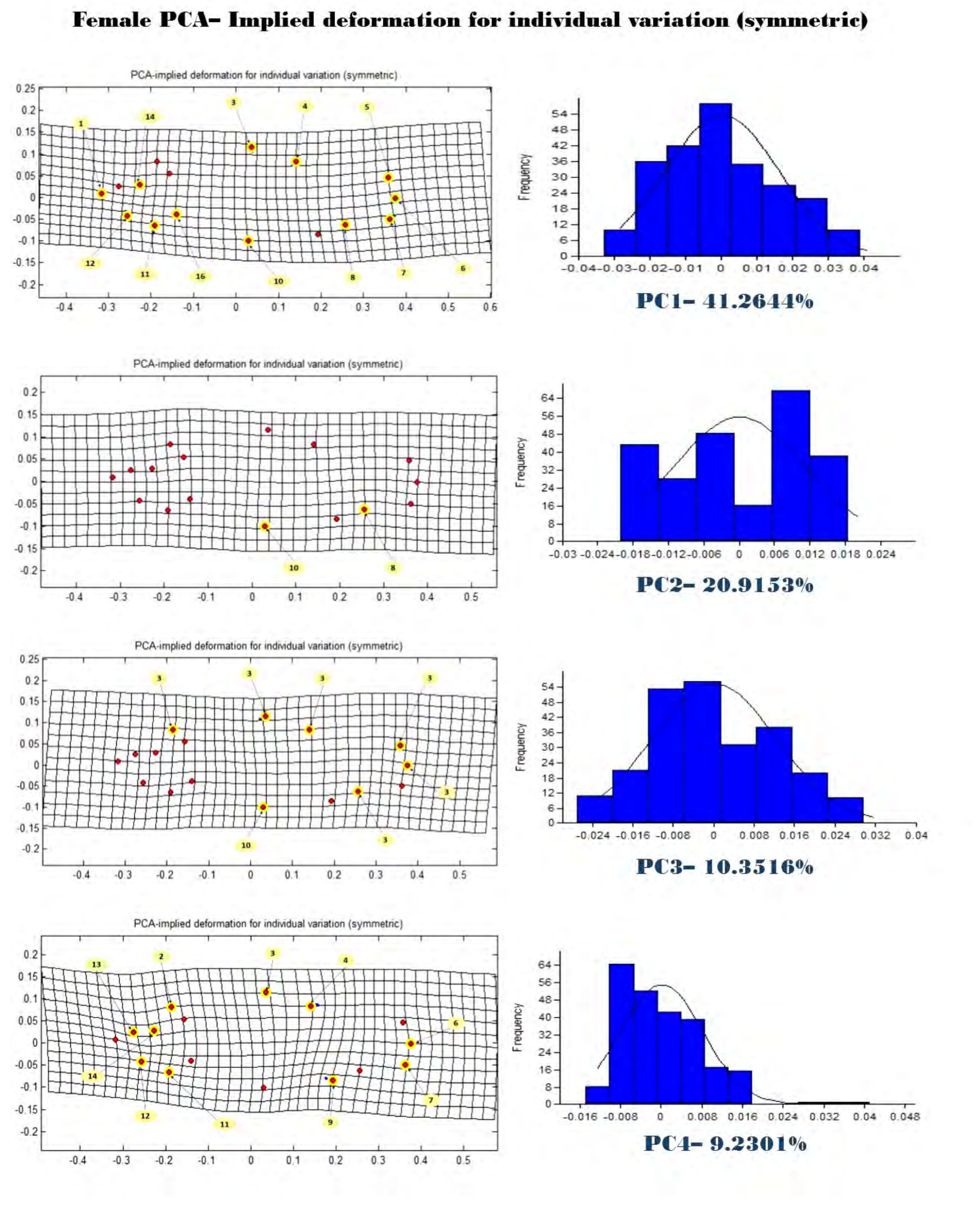

Fig. 4a Principal Component (PC) implied deformation grid and histogram of individual (symmetric) of female *P. binotatus* collected from Wawa River, Bayugan City, Agusan del Sur, Philippines.

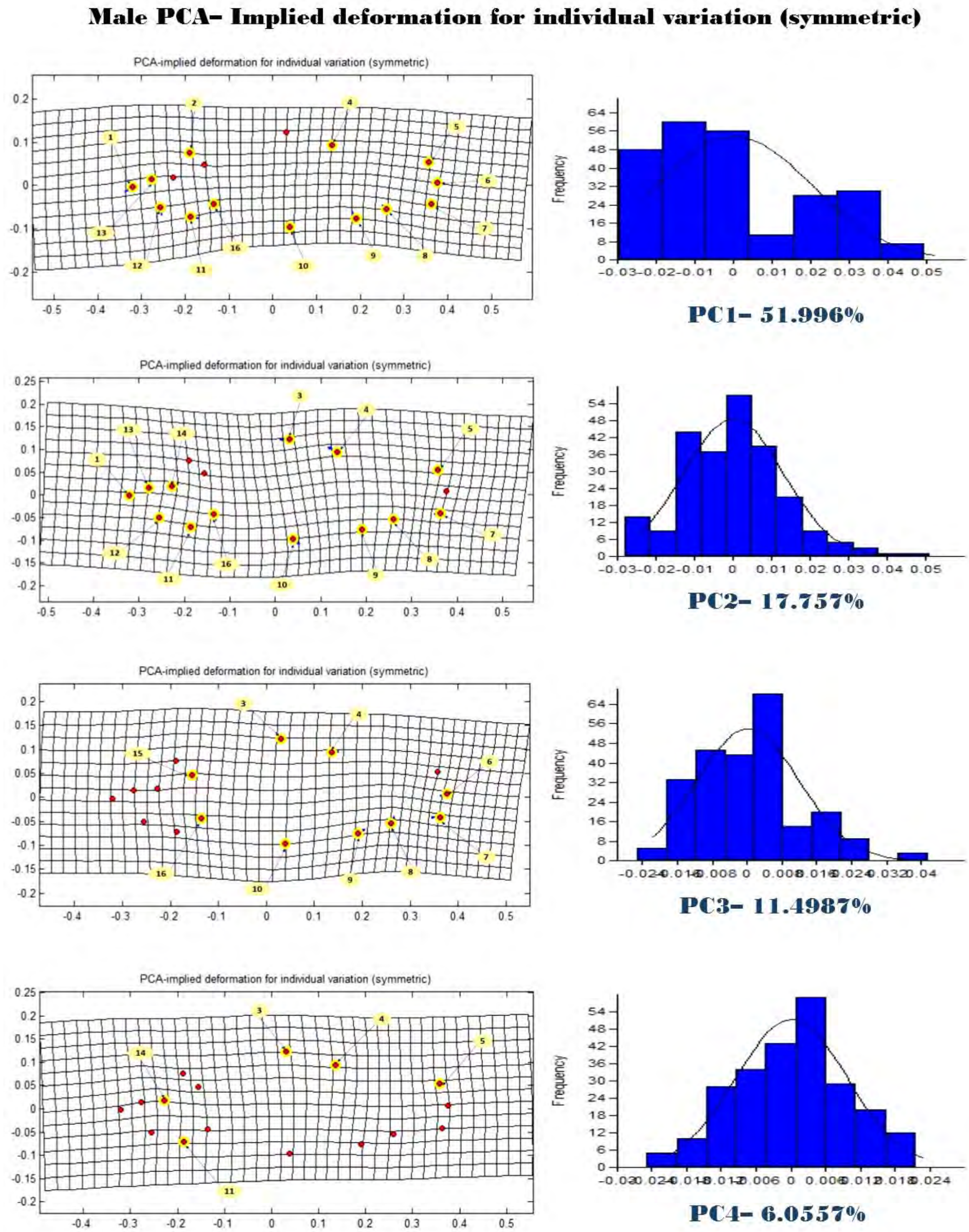

Fig. 4b Principal Component (PC) implied deformation grid and histogram of individual (symmetric) of male *P. binotatus* collected from Wawa River, Bayugan City, Agusan del Sur, Philippines.

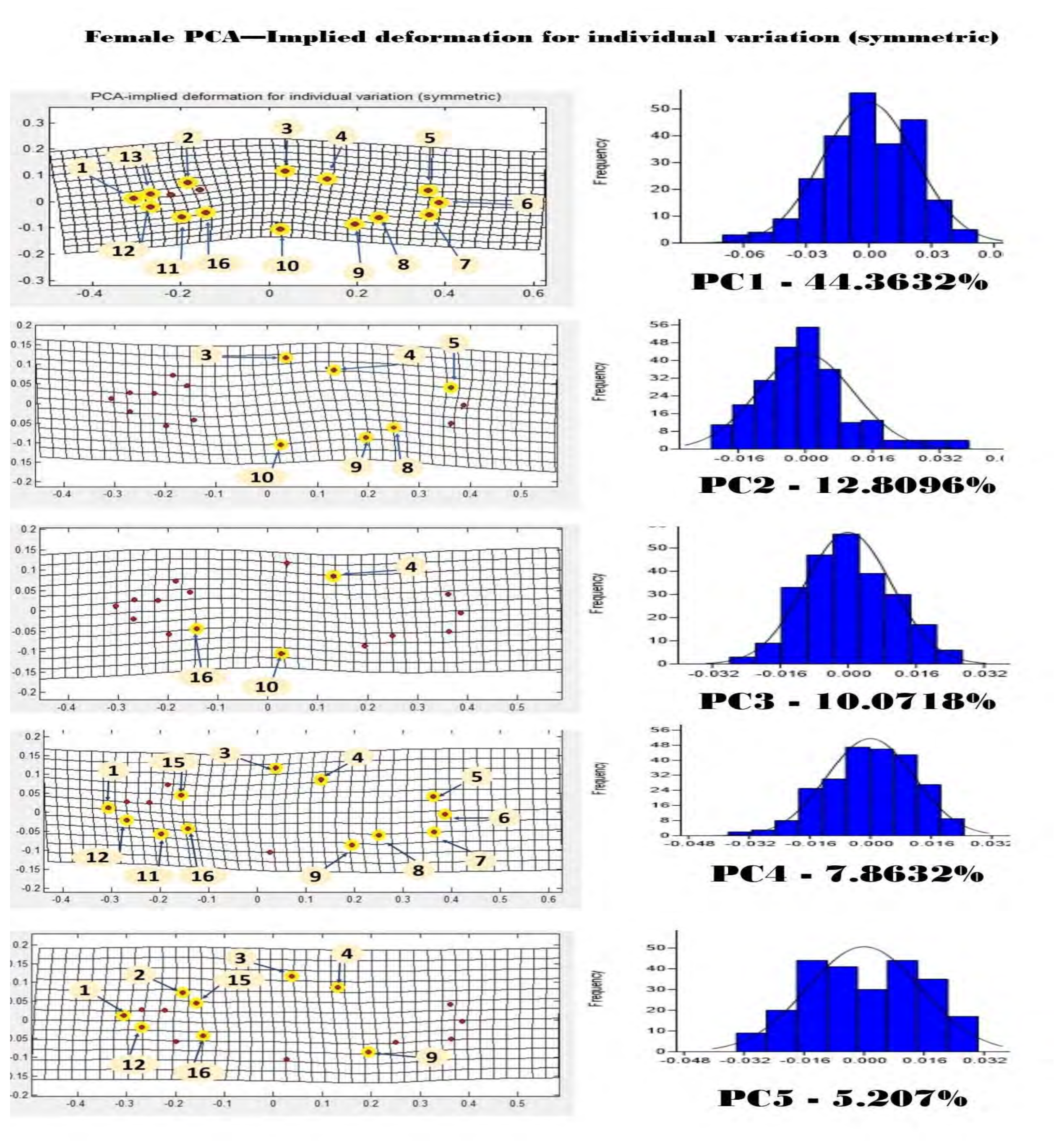

Fig. 5a Principal Component (PC) implied deformation grid and histogram of individual (symmetric) of female *P. binotatus* collected from Tubay River, Tubay, Agusan del Norte, Philippines.

The present study identifies the high fluctuating asymmetry among the samples of *P. binotatus* and might the current status of the sampling areas influences its asymmetry. Study shows that greater fluctuating asymmetry is the outcome of the species towards environmental situation (Ducos and Tubago, 2015). Differences in the percentage of FA may be attributed to the ability of the traits to buffer developmental alterations (Graham et al., 1993; Lens et al., 2002). Therefore, environmental condition takes part for the overall condition of the species and may enhance its fitness to resist alterations.

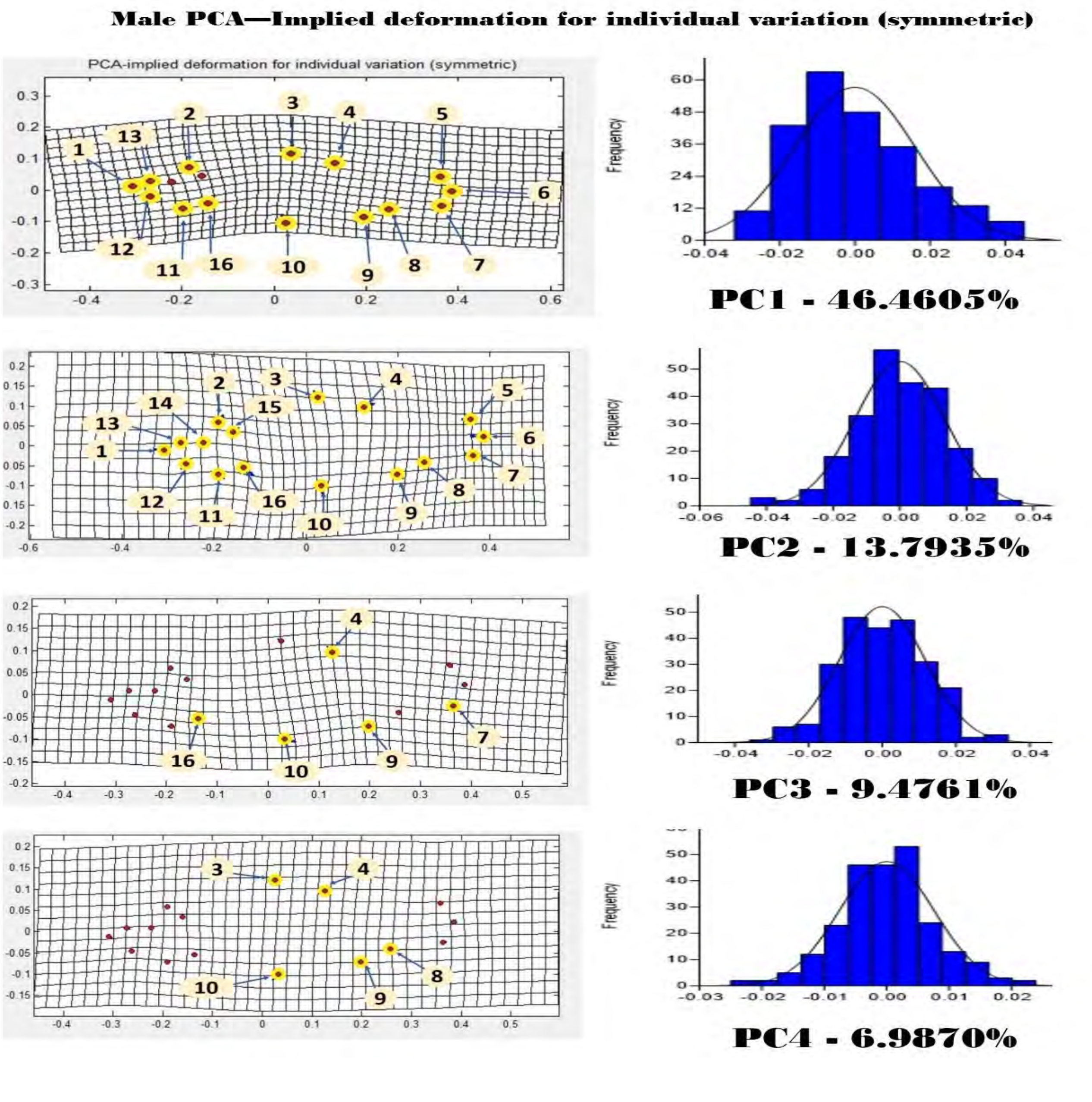

Fig. 5b Principal Component (PC) implied deformation grid and histogram of individual (symmetric) of male *P. binotatus* collected from Tubay River, Tubay, Agusan del Norte, Philippines..

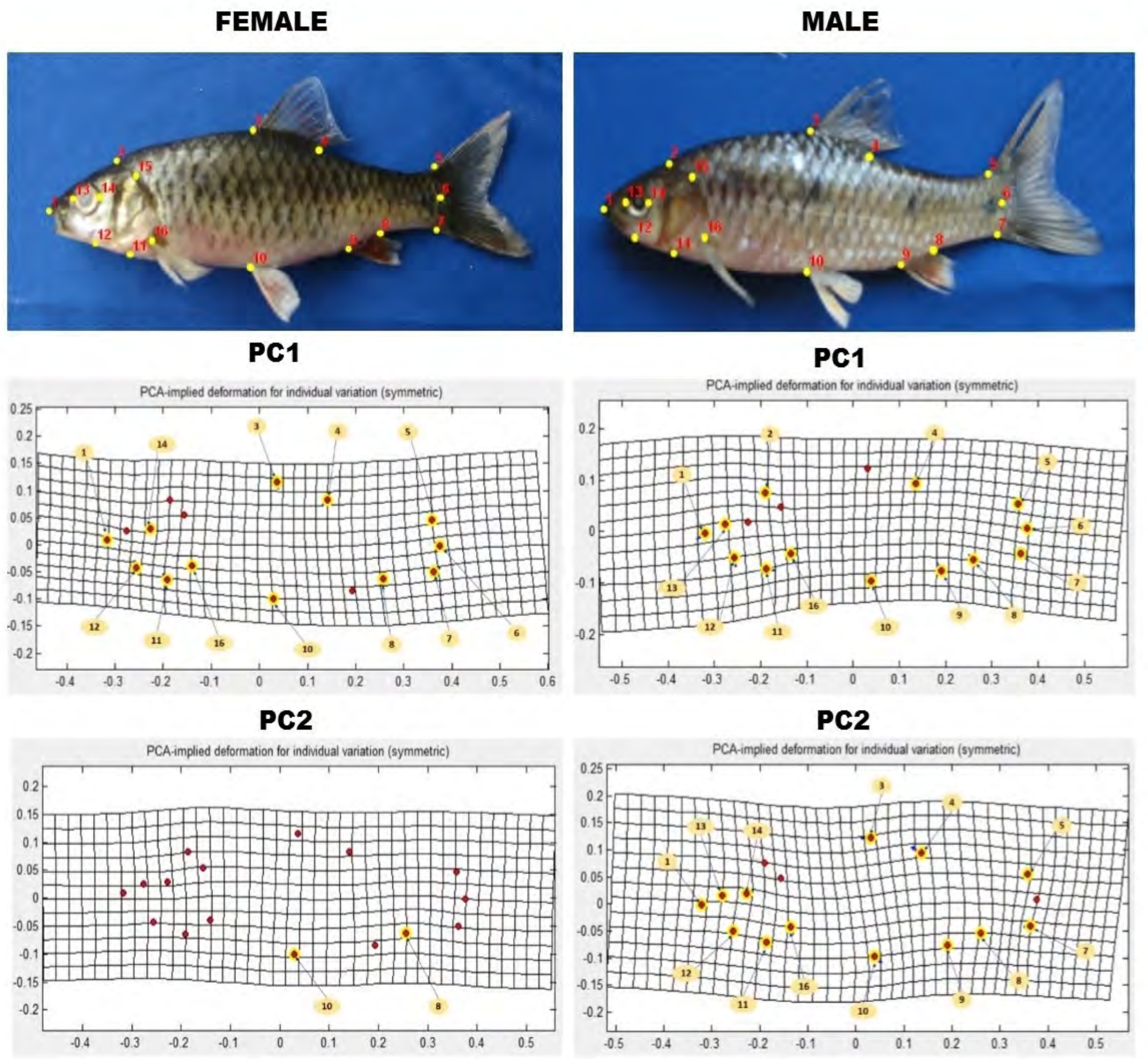

Fig. 6a Actualized picture of digitized female and male *P. binotatus* collected from Wawa River with affected landmarks shown in PCA-deformation grid for PC1 and PC2.

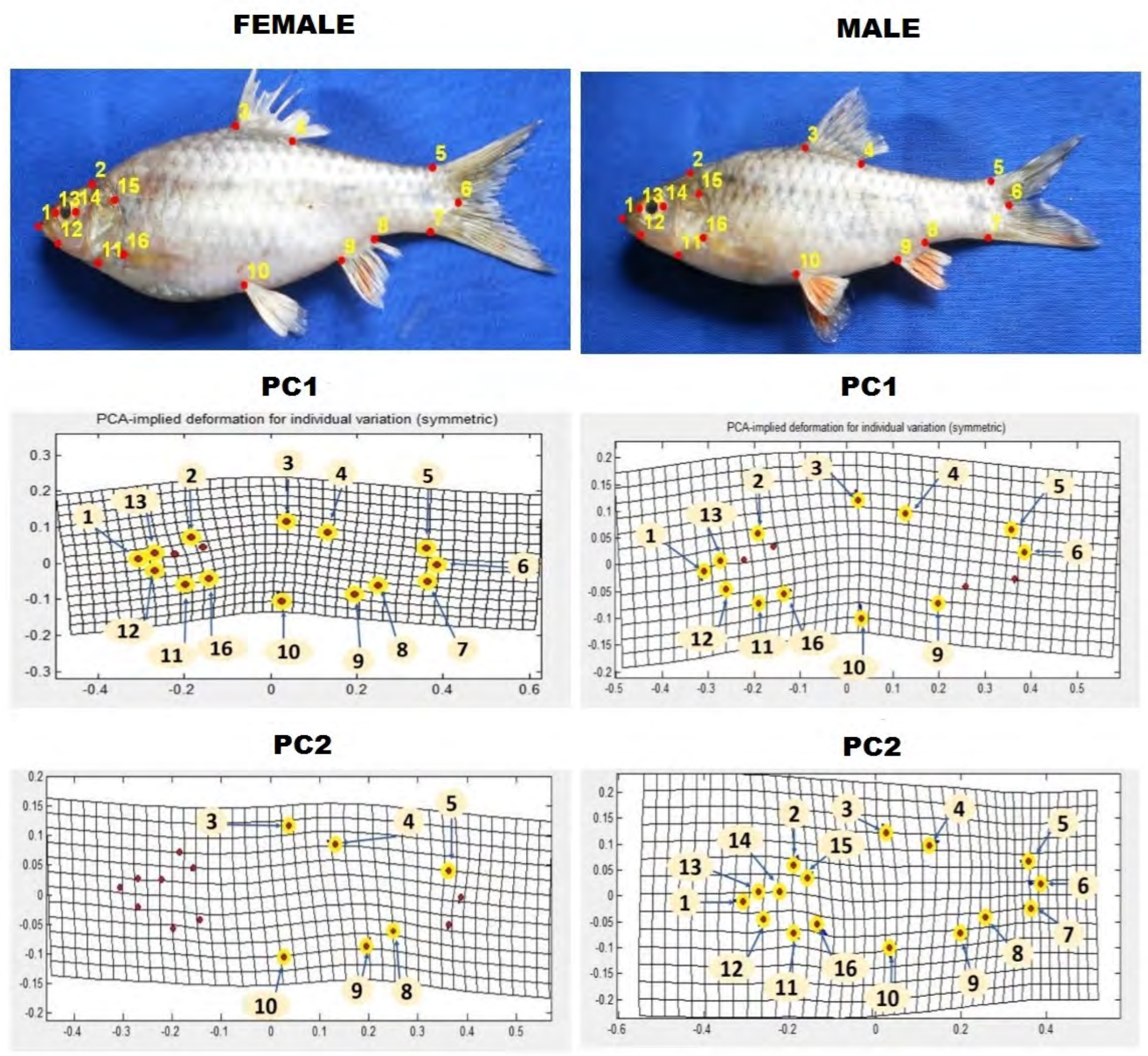

Fig. 6b Actualized picture of digitized female and male *P. binotatus* collected from Tubay River with affected landmarks shown PCA-deformation grid for PC1 and PC2.

3.2 Physico-chemical parameters

The determination of different water parameters were also determined and shown in Table 4. The mean values of water physico-chemical parameters in two sampling areas presented in the Tables 5a & 5b. The obtained values were compared to the standards recommended by the Water Watch Australia National Technical Manual 2002. Among the two rivers analyzed, Wawa River has the highest mean values for the following parameters: temperature (32 ± 0.057), conductivity (291.32 ± 7.896), pH (8.27 ± 0.145), Dissolve Oxygen (DO) (4.811 ± 0.044) and Salinity (266.011 ± 6.389). While Total Dissolve Solids (TDS) has a mean value of (434.633 ± 96.466) which is recorded from Tubay River (Fig. 8a).

The results of One-way ANOVA showed that the mean values in Wawa River has significant difference of

(P<0.05) while Tubay River has no significant difference. Although, Wawa River recorded the highest mean values it doesn't exceeded to the water quality standards.

Table 4 Physico-chemical parameters and its particular method of analysis.

Parameters	Unit	Method of Analysis	Sample Volume
Conductivity	µS/cm	Direct method (Electrode)	Det. on site
Temperature	^{0}C	Direct method (Electrode)	Det. on site
Total Dissolve Solids (TDS)	mg/L	Direct method (Electrode)	Det. on site
pH	Range (0-14)	Direct method (Electrode)	Det. on site
Dissolve Oxygen (DO)	mg/L	Direct method (Electrode)	Det. on site
Salinity	mg/L	Direct method (Electrode)	Det. on site

Table 5a Mean values of physico-chemical parameters from Wawa River, Bayugan City, ADS, Philippines.

Parameters	Standard Water Watch Australia National Technical Manual (2002)	Site 1 Mean ± SEM	Site 2 Mean ± SEM	Site 3 Mean ± SEM	Mean Mean ± SEM
Temperature	3^{0}C rise[a]	31.9 ± 0.058	32 ± 0	32.1 ± 0.379	32 ± 0.057*
TDS	<1000mg/L	13.703 ± 0.012	12.856 ± 0.023	13.563 ± 0.379	13.37 ± 0.26*
Conductivity	0-800 µS/cm	301.6 ± 1.510	275.8 ± 0.289	295.566 ± 0.775	291.32 ± 7.896*
pH	6.5-8.5	8.33 ± 0	8.48 ± 0.006	7.99 ± 0.082	8.27 ± 0.145*
DO	>5mg/L	4.767 ± 0.088	4.9 ± 0.058	4.767 ± 0.120	4.811 ± 0.044*
Salinity	<5ppt	0.272 ± 0.000	0.253 ± 0.000	0.272 ± 0.000	0.266 ± 0.006*

*Significant (P<0.05)

Table 5b Mean values of physico-chemical parameters from Tubay River, Tubay, ADN, Philippines.

Parameters	Standard Water Watch Australia National Technical Manual (2002)	Site 1 Mean ± SEM	Site 2 Mean ± SEM	Site 3 Mean ± SEM	Mean Mean ± SEM
Temperature	3^0C rise[a]	30.7 ± 0.058	29.7 ± 0.058	30.167 ± 0.524	30.189 ± 0.289
TDS	<1000mg/L	242.433 ± 1.071	516.2 ± 4.557	545.267 ± 0.994	434.633 ± 96.466
Conductivity	0-800 µS/cm	5.278 ± 0.057	11.857 ± 0.047	11.887 ± 0.024	9.674 ± 2.198
pH	6.5-8.5	7.52 ± 0.084	7.437 ± 0.121	7.423 ± 0.003	7.46 ± 0.030
DO	>5mg/L	4.567 ± 0.033	4.667 ± 0.088	4.5 ± 0.058	4.578 ± 0.048
Salinity	<5ppt	0.005 ± 0.000	0.013 ± 0.000	0.013 ± 0.000	0.010 ± 0.002

According to Ajibade et al. (2008), water temperature likely depends on the water column, climatic & topographic changes. Where, conductivity influenced by total dissolve solids & salinity; thus the higher the conductivity, the higher the salts and electrical conductivity (EC) (Lawson, 2011). The pH constitutes the acidity and basicity of aquatic environment consequently the lower the pH, likely will become acidic thus, affecting the growth and diversity of the aquatic environment. Study shows that pH is utilized to determine the alkalinity and basicity of a substance ranking the scale from 1.0-14.0 (US EPA, 1997).The Dissolve Oxygen (DO), in water is very important for aquatic species. DO, likely affects the growth, survival, distribution, behavior and physiology of all aquatic organisms (Solis, 1988 & Sharma et al, 2013). Indicating a very low value of DO (<4 mg/L) is not appropriate for aquatic life; this may be attributed to microbial decomposition from sewages (Pathak et al, 2012).The Total Dissolve Solids (TDS) is used to analyze the presence of organic salts and organic substances in the water (Lawson, 2011). Moreover, TDS is also a way of indicating an array of chemical effluents within the body of water (US EPA, 1997).

The conservation of strong aquatic ecosystem is determined on the physico-chemical components and the biotic factors (Venkatesharaju et al., 2010). In addition, vegetated riparian zones also exhibits strong influence into the chemical substances of nearby streams, lakes and rivers specifically in reducing the influx of various nutrients coming from agricultural uplands (Dosskey, 2001, Hefting et al, 2005, Baker et al 2006). Indeed, the assessment on its parameters is important as it gives indication on the current standing, productivity and sustainability (Djukie et al., 1994). Therefore, richness and distribution of a biota is dependent to the physical and chemical aspects of water body (Unanam and Akpan, 2006).

On the other hand, chemical effluents are major contributors of water pollutions that established in the event of water movement from geological processes (Kataria et al., 2011). In addition, pesticides and fertilizers are main factors to water contamination; anthropological activities, rocks weathering, soil leaching, and the mining processing these are the many ways for affecting aquatic natural resources (Manjare et al 2010). Furthermore, the pyhsico-chemical parameters assist the current condition of aquatic environment with respect to the values obtained.

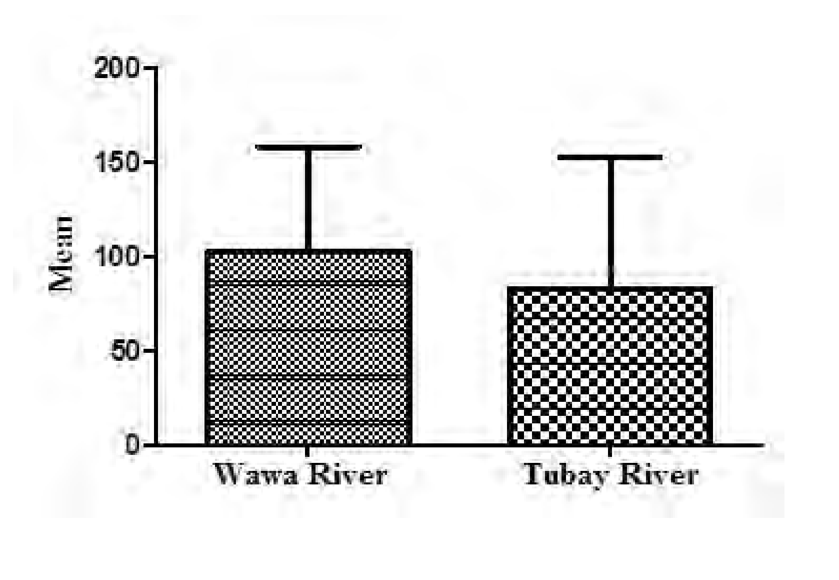

Fig. 7a Comparison of physico-chemical mean values between two sampling areas

3.3 Correlation between fluctuating asymmetry and physico-chemical parameters

Pearson-correlation revealed that fluctuating asymmetry and physico-chemical parameters shows negatively correlated (Fig. 8b). Result indicates that Tubay River has a value of (r = -0.1963) while Wawa River has a value of (r = -0.2862) this indicates that fish samples and the water components were not directly correlated. Such, that water parameters were not the direct factor that initiates asymmetry in the morphology of *P. binotatus*. Indeed, the mean values obtained from physico-chemical parameters were in the range recommend by water quality standards. In contrast, the incidence of FA in *P. binotatus* might be due to its developmental, metabolic and homeostatic processes. The mobility of the fishes is considerably a key mechanism to buffer environmental variations. In addition, Ducos and Tabugo (2015) theorized that stressed environment together with genomic aspects can build up FA, which denotes a potential weakening during developmental homeostasis of the organisms.

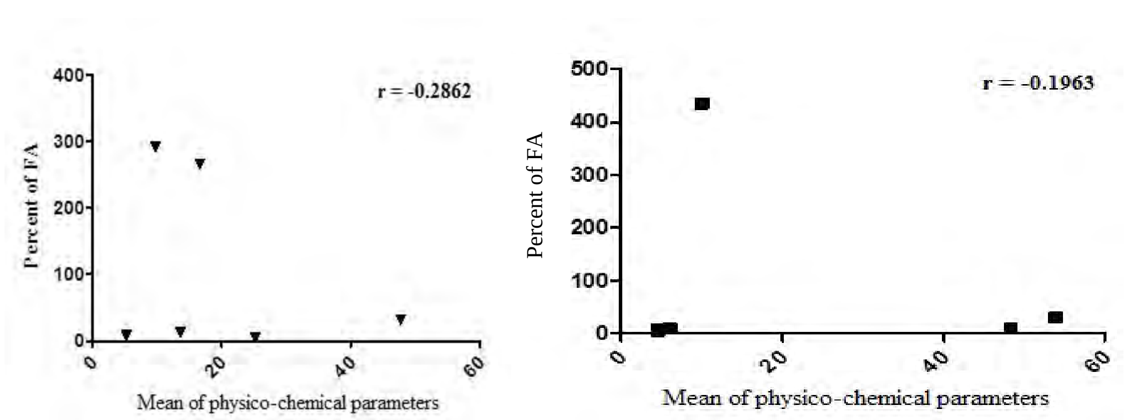

Fig. 7b Scatter plot showing the correlation of FA and Physico-chemical parameters (**Right)** Tubay River, Tubay, Agusan del Norte, (**Left**) Wawa River, Bayugan City, Agusan del Sur, Philippines.

4 Conclusion

Fluctuating asymmetry was employed to *P. binotatus* collected from the two sampling areas in order to identify the possible effects of aquatic pollutants in its morphology. Alongside. physico-chemical parameters were also established to assess the water quality in the two rivers. Procrustes ANOVA shows high FA in Tubay River compared to Wawa River. The results of One-way ANOVA showed that physico-chemical parameters in Wawa River has significant difference of ($P<0.05$) while Tubay River has no significant difference. Pearson-correlation revealed that fluctuating asymmetry and physico-chemical parameters shows negatively correlated. This study identifies the importance of evaluating environmental condition as well as the state of well-being of a species through fluctuating asymmetry and physico-chemical analysis.

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