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Developmental stability via Fluctuating Asymmetry in the shell shape of *Gafrarium tumidum* (Tumid Venus Clam) found in Panguil bay

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Abstract

Fluctuating asymmetry is the extent at which an organism departs from the ideal symmetry. It increases as the environmental stress increases and thus referred to as a tool for measuring developmental stability and environment quality. The purpose of the study is to demonstrate the use of fluctuating asymmetry as a tool for monitoring developmental stability of *Gafrarium tumidum* (Tumid venus clam) and environment. This study determined the developmental stability of *G. tumidum* via fluctuating asymmetry collected from two coastal areas around Panguil Bay. Thirteen anatomical landmarks were used and were subjected to Procrustes superimposition and Principal Component Analysis (PCA) using "Symmetry and Asymmetry in Geometric Data" (SAGE) program. Procrustes ANOVA result showed no significant evidence of fluctuating asymmetry on both populations. But yield significant result for directional asymmetry, 'sides' interaction which suggests that observed variation in populations examined is of genetic origin and not due to environmental stress. It also suggests that populations in the area have the ability to buffer stress. Thus, *G. tumidum* is relatively developmentally stable and the environment that it inhabits is fairly tolerable. Hence, relatively fit for cultivation to maximize yield for food production.

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Introduction

Bivalves, commonly known as clams, shells or locally known as "kinhasun," are typically marine mollusk that is bilaterally symmetric and is protected by a shell which is composed of two valves. They lack a distinct head and are usually filter feeders. In the Philippines, some bivalves are gathered as another source of living and cheap food for most local fishermen and coastal people. Aside from being able to fill the guts of hungry people, clams are also used as a bioindicator of environmental stress particularly heavy metal pollution (Yusof, 2004). Herewith, this study was conducted to study the nature of the bivalve species *Gafrarium tumidum* (Tumid venus clam), and assess developmental stability by measuring fluctuating asymmetry. It investigates differences in the fluctuating asymmetry (right and left valves) of two populations.

This study also provides some information about the environmental condition of Panguil Bay, as express in the fluctuating asymmetry of the bivalves. In this respect, developmental instability can be measured by degrees of fluctuating asymmetry or the extent at which an organism randomly departs from the ideal symmetry. It could be used to represent the population's state of adaptation and coadaptation as it could also measure developmental noise (Mather, 1953; Jones, 1987). Fluctuating asymmetry has been proposed as a tool for monitoring the quality of the environment and is being considered as a sensitive monitor of stress (Graham *et al.*, 1993; Anne *et al.*, 1998).

Moreover, Panguil Bay is a small but economically important fishing ground that supports the livelihood of small-scale fishermen in the northern part of Mindanao. Raw-an, Lanao del Norte and Clarin, Misamis Occidental coastal areas are known fishing grounds that surround Panguil Bay.

The area is rich in marine animals and is home to a number of marine goods. Due to its diversity and productivity, Panguil Bay has been exploited for decades by a large fishing population using a wide variety of gears (Jimenez *et al.*, 2009). Incidences of blast or dynamite fishing on these areas, as well as on adjacent coastal areas were rampant in the past few years (Jimenez *et al.*, 2009; Leong *et al.*, 2013). The bay is also situated adjacent to Iligan Bay in which practically all factories are oriented to the bay and effluents constantly drained directly into the water.

The maintenance of seawater quality in the area is generally poor and has been characterized as unfit for recreational and fishing activities (Ologuin, 2009; Tampus *et al.*, 2014). Recent report also pointed out a decline in the overall habitat condition of Panguil Bay (Roxas *et al.*, 2009). Thus, in this study it is hypothesized that the presence of environmental stress or genetic stress during its ontogeny would disrupt the normal developmental processes and may cause developmental instability or the failure of a genotype to consistently produce the same phenotype which would ultimately lead to variations of morphological features among an organism's species (Clarke, 1993; Escós *et al.*, 2000) and this will be manifested as levels of fluctuating asymmetry. Hence, this study is worthwhile since it looked into the developmental stability via fluctuating asymmetry of the bivalve species *G. tumidum*.

Materials and methods

Study Area, Specimen Collection, Identification and Image Processing

Panguil Bay is located in the northwestern part of Mindanao. It covers approximately 18,000 hectares of water area and has a total coastline measuring 116 km from Clarin, Misamis Occidental to Brgy. Liangan, Maigo, Lanao del Norte. Situated along this coastline are the two cities of Tangub and Ozamiz and the municipalities of Clarin and Bonifacio in the province of Misamis Occidental; the municipalities of Aurora and Tambulig in the province of Zamboanga del Sur; and the municipalities of Maigo, Kolambugan, Tubod, Baroy, Lala and Kapatagan in Lanao del Norte (Fig.1). Altogether, 78 barangays belonging to these cities and municipalities are strategically located around the bay. Administratively, the bay is divided among the above-mentioned provinces and among Regions 9

and 10 where these provinces are politically located (Roxas *et al.*, 2009).

Adjacent to Panguil Bay is Iligan Bay. It is located at the southern part of Mindanao Sea and west of Macajalar Bay. It is bounded at the northeast by the coastal areas of Gitagum, Misamis Oriental and at the northwest by Plaridel, Misamis Occidental. The southernpart is bounded by Clarin, Misamis Occidental at the west and Maigo, Lanao del Norte at the east (Leong *et al.*, 2013).

Fig. 1. Map Sampling areas:Clarin and Raw-an along Panguil Bay.

Gafrarium tumidum, a small but sturdy bivalve characterized by large squarish beads that line to form the ribbed pattern of the shell, belongs to the family Veneridae. It is common in Southeast Asian countries and is one of the commercially important bivalves in India as well as in the Philippines (Jagadis and Rajagopal, 2007). Bivalves were randomly collected from Raw-an, Lala, Lanao del Norte and at Lupagan, Clarin, Misamis Occidental. There were 94 samples collected from Clarin and 34 samples collected from Raw-an. Identification was done using taxonomic keys, guides and consultation of experts. Soft tissues were removed leaving the shells totally clean then sundried. The two valves of each sample were slowly separated by carefully tearing their ligament. The left and right valves were documented dorso-ventrally. Using a standard procedure, the ventral aspect was documented where all internal shell structures are clear and distinct with the umbo oriented vertically and upward. Digital images of the left and right valves of clams were taken using standard procedure and landmark assignment was done using tpsDig2 software. This was done in triplicates in order to quantify and minimize measurement error. Thirteen landmarks were assigned in the inner valves. The 13 landmarks, were comprised of 9 anatomical landmarks and 4 mathematical landmarks. Descriptions of identified landmarks are presented in Table 1 and Fig. 2.

Fluctuating Asymmetry Analysis (FA) and Principal Component Analysis (PCA)

The FA levels were assessed per population using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.0 (Marquez, 2006). The software analyzed the x and y coordinates of the landmarks per individual, using a configuration protocol for both left and right valves of the

Gafrarium tumidum (tumid venus clam). Afterwards, Procrustes superimposition analysis for both valves was performed with the original and mirrored configurations of the right and left inner valves simultaneously. The least squares Procrustes consensus set of landmark configurations and their relabelled mirror images is a perfectly symmetrical shape, while FA is the deviation from perfect bilateral symmetry (Klingenberg *et al.,* 1998; Marquez, 2006). Hence, the squared average of Procrustes distances for all specimens is the individual contribution to the FA component of variation within a sample. In this respect, to detect the components of variances and deviations, a Procrustes Analysis of Variance (ANOVA) was used where, *Sides* (DA), *individual x sides* (FA), and their respective error were included as effects.

Table 1. Position of the thirteen landmarks selected in the interior valve of *Gafrarium tumidum*.

Landmark #	Position
	Anatomical Landmarks
1	Umbo
2	End of ligament
3	Junction of posterior retractor and posterior adductor
4	Junction of posterior adductor and pallial sinus
5	Inside of pallial sinus
6	Outside of pallial sinus
7	Junction of anterior adductor and pallial line
8	Junction of anterior retractor and anterior adductor
	Mathematical Landmarks
9	Near umbo
10	Dorsal margin maxima
11	Posterior margin maxima
12	Ventral margin maxima
13	Anterior margin maxima

Table 2. Procrustes ANOVA result of *G. tumidum* collected from Clarin and Raw-an.

Effect	*SS*	*dF*	*MS*	*F*	*P*	*Remarks*
Clarin						
Sides	0.010684	22	0.00048562	2.3245*	0.0005211	significant
Individuals x Sides	0.21142	1012	0.00020891	0.87548	0.99671	Insignificant
Measurement Error	1.4804	6204	0.00023863	--	--	
Raw-an						
Sides	0.0063791	22	0.00028996	1.697*	0.026967	significant
Individuals x Sides	0.060144	352	0.00017086	0.96225	0.67457	Insignificant
Measurement Error	0.39846	2244	0.00017757	--	--	

Note: side=directional asymmetry; individuals x sides= fluctuating asymmetry, *P< 0.001, ns= statistically insignificant (P>0.05); significance was tested with 99 permutations.

The ANOVA used most frequently for fluctuating asymmetry is a two-way, mixed-model ANOVA with replication. In this study design, the main fixed effect is *sides* (*S*) has two levels (left and right). The block effect is *individuals* (*I*) is a random sample of individuals from a population. The *sides by individuals interaction* (*S x I*) is a mixed effect. Moreover, an error term (*m*) represents measurement error (replications within *sides by individuals*). The effect called *sides* is the variation between the two sides; it is a measure of directional asymmetry. The effect called *individuals* is the variation among individual genotypes; the individuals mean square is a measure of total phenotypic variation and it is random. Meanwhile, the *individual x sides* interaction is the failure of the effect of individuals to

be the same from side to side. It is a measure of fluctuating asymmetry and antisymmetry thus, a mixed effect. The error term is the measurement, and is a random effect (Samuels *et al.,* 1991; Palmer and Strobeck, 2003; Graham *et al.*, 2010). In addition, to further detect the components of variances and deviations, Principal Component Analysis (PCA) of the covariance matrix associated with the component of FA variation were also performed for the samples per population. This procedure is carried out as an interpolation based on a thin-plate spline and then visualizes shape changes as landmark displacement in the deformation grid (Marquez, 2006; Albarran-Lara *et al.,* 2010; Cabiluna and Tabugo, 2017).

Table 3. Variance explained by first two principal components of Clarin and Raw-an.

Location	PC 1 (%)	PC 2 (%)	Overall (%)
CLARIN	20	15	35
RAW-AN	32	17	49

Results and discussion

Measurement of FA levels

Levels of Fluctuating asymmetry (FA) were evaluated per population of samples. Through Procrustes method using SAGE software, FA of the right and left valves of *Gafrarium tumidum* (tumid venus clam) collected from Raw-an, Lala, Lanao del Norte and Lupagan, Clarin, Misamis Occidental respectively were evaluated.

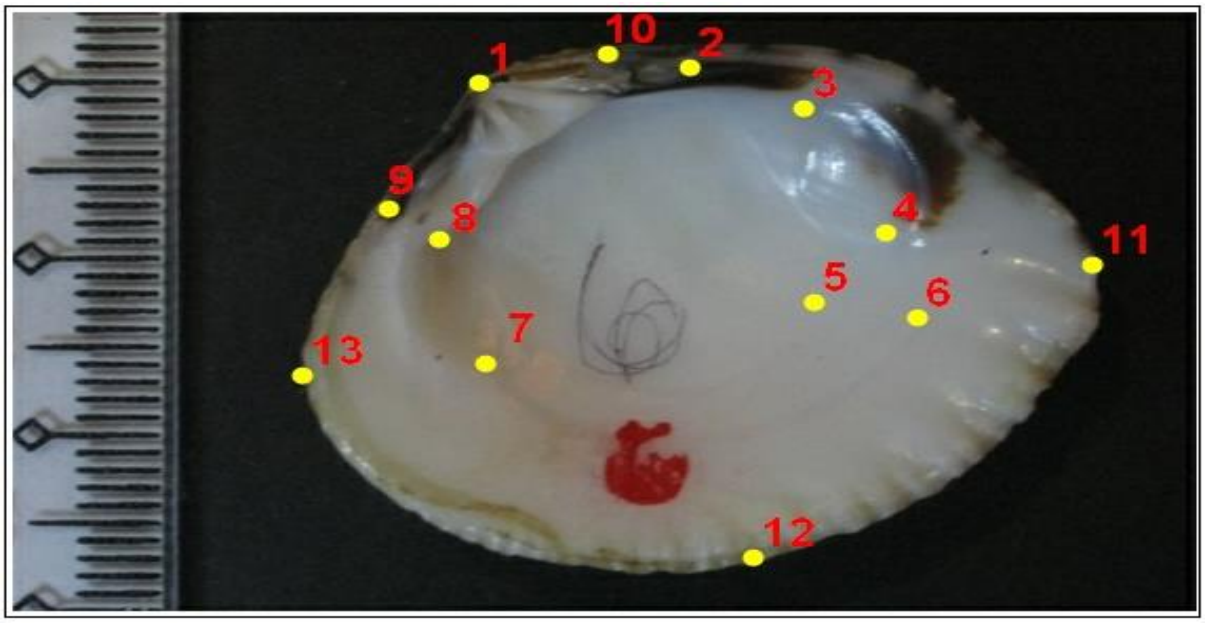

Fig. 2. Location of the 13 landmarks on the bivalve interior of *Gafrarium tumidum*.

It is noted that FA is related to developmental stability (DS), thus, a tool in investigating the DS. Not significant, low FA values are perceived to be developmentally stable phenotypes such that they can easily buffer stress and maintain developmental homeostasis (Graham *et al.*, 2010). Together with the product of the coordinates of the left and right homologous provided the final results of the Procrustes ANOVA the index of FA using the coordinates was determined (Table 2).

The interaction 'Individual x sides' presented a low value of mean square and a high value of mean square measurement error for both Clarin and Raw-an population thus, indicate insignificant fluctuating asymmetry for all samples. However, it yield a significant result for directional asymmetry, 'sides' interaction which suggests that observed variation in

populations examined is of genetic origin and not due to environmental stress and that populations have ability to buffer such stress and maintain homeostasis. The results of the Procrustes ANOVA showed a non-random variation (FA) between the left and the right sides of the landmark parts of the bivalve shells, rather than random differences among sides.

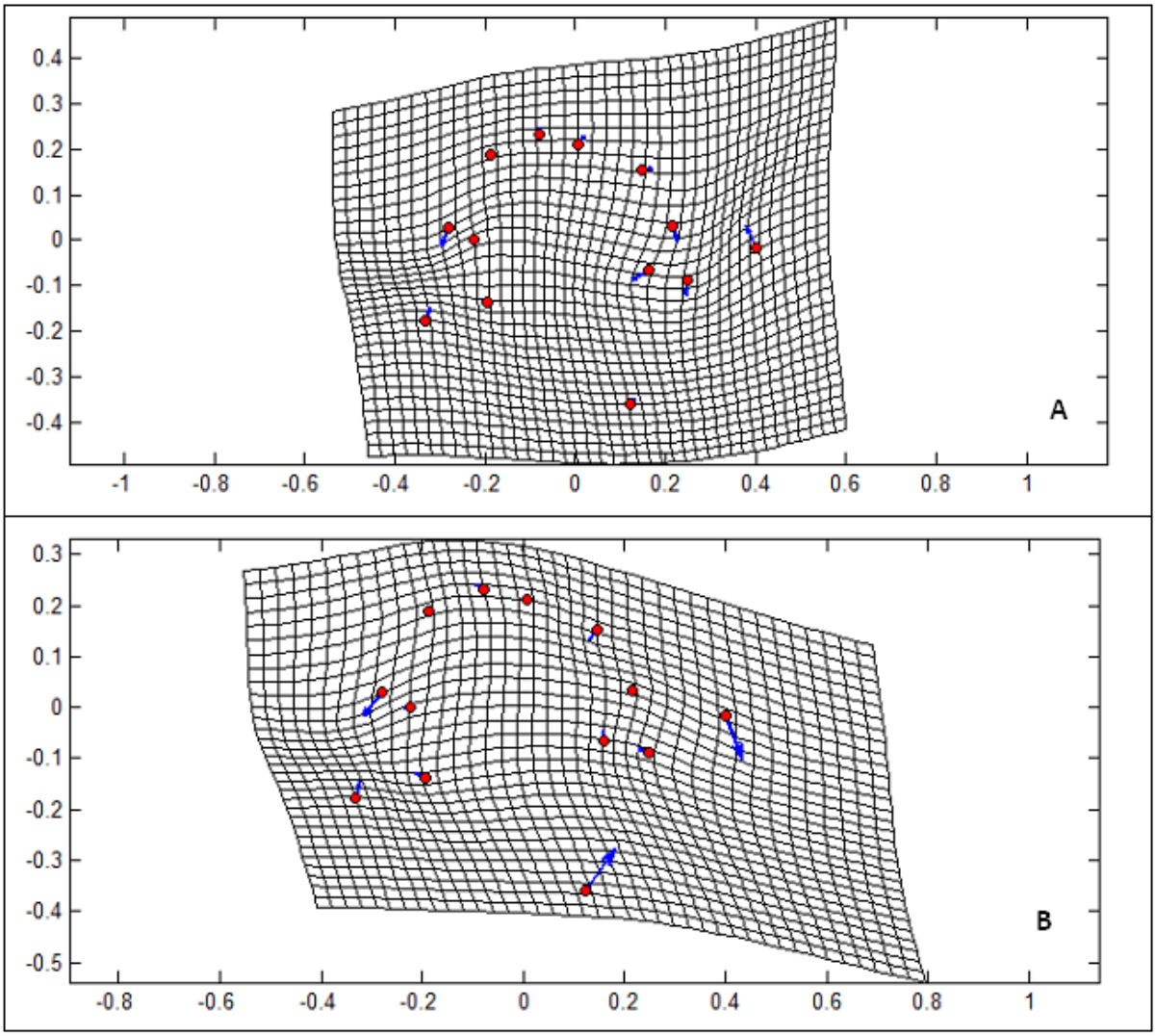

Fig. 3. Deformation grid for PC1 and PC2 (*A* and *B*, respectively) of shell shape variation of *G. tumidum* collected from Clarin with PCA values of (A) 20% and (B) 15%.

Therefore, the F value show highly insignificant FA for all populations where *P >0.001. Based on the observed values, it is hypothesized that possible reason for insignificant FA values is that the amount of environmental stress in Raw-an and Clarin may not be enough to cause disruption of the developmental processes of *G.tumidum* (Graham *et al.*, 2010) such that developmental homeostasis seems not easily disturbed. This suggests that populations in the area have the ability to buffer stress. Thus, *G. tumidum* is relatively developmentally stable and the environment that it inhabits is fairly tolerable (Ambo-Rappe *et al.*, 2008).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was also performed in order to visualize the covariance shape change for each principal component and to see the general direction and magnitude of the fluctuation for each landmark. The red dots represent the morphological landmarks used in the study while the blue arrows indicate the direction as well as the

magnitude of the fluctuation. The percentage values of PCA represent the level of variability in the data. Here, the amount of overall variation exhibited by PC1 and PC2 of samples from Raw-an showed more percentage of variation than samples from Clarin. The total percentage for samples from Raw-an was 49% and for samples from Clarin was only 35%. The overall percentage of variation is less than 50% which further indicate less observed level of variability in the samples (Table 3; Figure 3 and 4). This supports the notion that *G. tumidum* is relatively developmentally stable and that the environment is tolerable.

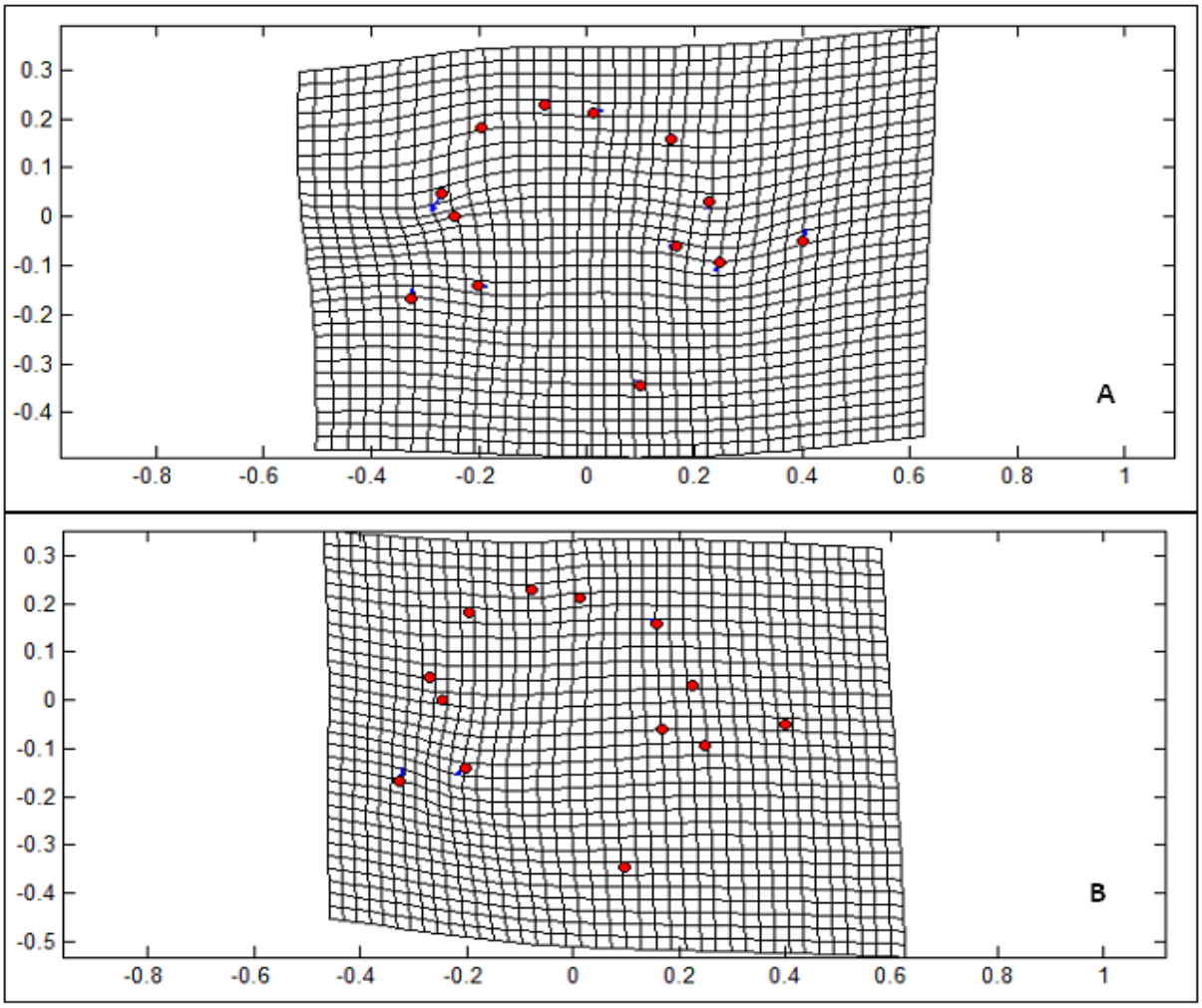

Fig. 4. Deformation grid for PC1 and PC2 (*A* and *B*, respectively) of shell shape variation of *G. tumidum* collected from Raw-an with PCA values of (A) 32% and (B) 17%.

Moreover, one possible contributing factor to insignificant result of fluctuating asymmetry, despite the recent reports on the decline of the overall habitat in Panguil Bay, is the direction of the water currents. The presence of an eddy, a circular pattern of ocean current, on the northern portion of Iligan Bay and the location of the bay itself could be the reason why Panguil Bay was not affected by the pollution coming from the adjacent Iligan Bay. Effluents and other pollutant particles that could possibly cause environmental stress to the bivalves are driven northwestward instead of towards Panguil Bay by the ocean current (Cabrera *et al.*, 2011). Despite the knowledge that the overall habitat quality of Panguil Bay is declining the results show otherwise (Jimenez *et al.*, 2010).

The decline may be due to other factors such as increase in the number of inhabitants, increase in the number of fishermen and efficiency of fishing gears.

Conclusion

Fluctuating asymmetry was obtained using Procrustes ANOVA. Insignificant values for FA were observed in both populations examined. Hence, indicates that *G.tumidum* found in Clarin, Misamis Occidental and Raw-an, Lala, Lanao del Norte are relatively developmentally stable.

The amounts of environmental stress found on both areas are fairly normal enough for the bivalve to buffer and maintain developmental homeostasis. Environmental conditions on both areas can still be considered as practically fit.

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