Article

Fluctuating asymmetry and developmental instability in *Protoreaster nodosus* (Chocolate Chip Sea Star) as a biomarker for environmental stress

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Abstract
Fluctuating asymmetry (FA), pertains to small and random departures from perfect symmetry of an organism's bilateral traits and has been used as a measurement of developmental instability and as a potential indicator of stress in populations. It measures the variations from symmetry of a symmetrical structure whose sides are said to be genetically identical, with similar history of gene activity and experiencing the same environment. Symmetries are potentially the basis for studies on FA. Hence, this study assessed the potential of FA as a reliable developmental instability and environmental stress indicator in five-fold dihedral symmetrical *Protoreaster nodosus* (Chocolate chip sea fish) from three (3) different sites (Linamon, Lanao del Norte; Initao, Misamis Oriental and Jasaan, Misamis Oriental). FA for each population from every site was measured for comparison. In this study, anatomical landmarks were subjected to Procrustes superimposition and Principal Component Analysis (PCA) using "Symmetry and Asymmetry in Geometric Data" (SAGE) program. Results showed highly significant FA and significant DA for population from Jasaan and Linamon where habitat disturbance due to anthropogenic activities were prevalent. Thus, experienced more stress compared to the other populations, suggesting that significant variation in size or left-right side of each individual could be a product of genotype-environment interaction. Moreover, insignificant FA and high DA was obtained from Initao (protected seascape area) which indicated that variation among individual genotypes and asymmetry in phenotypes is mostly induced by genetics under less stressful environment. Significant FA and increase FA present inability of species to buffer stress in its developmental pathways and have implications on species fitness. Hypothesis assumes that fluctuating asymmetry has costs, reflects the quality of individuals and the level of genetic and environmental stress experienced by individuals or populations during development. Here, FA proved to be efficient when applied to five-fold dihedral symmetrical organisms.

Keywords fluctuating asymmetry; developmental instability; biomarker; *Protoreaster nodosus*; environmental stress.

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1 Introduction

Monitoring the impacts of a wide range of environmental stressors on ecosystem health is of interest to conservation biology and sustainability (Beasley et al., 2013). Stressors of interest include pollutants, changes in natural environmental parameters such as temperature, humidity, density, and shifts in resource use induced by global warming, habitat defragmentation and habitat loss, that often leads to detrimental consequences or loss of inhabitant organisms (Whiteman and Loganathan, 2001). Thus, there is a need to find reliable and suitable early-warning biological indicators of such stress for conservation programmes.

Along this line, in marine environment sea stars play an ecological role as keystone species (Paine et al., 1985). Wherein, they are not necessarily abundant in the marine community however, they exert strong control on community structure not by numerical might but according to their pivotal ecological roles or niches. Ecological data can be utilized from ecologically important species of sea stars (e.g. *Protoreaster nodosus* and *Pisaster ochraceus*) because they have a long life span, with a maximum of 34 years (Menge, 1975; Bos et al., 2008). Hence, are suitable for long term monitoring. With this, is the incessant search for easily measured biomarkers which resulted in the investigation of asymmetry of morphological characters as a possible biomarker for stress and the most widely used measure of asymmetry is Fluctuating Asymmetry (FA). Symmetry is a major trait of life and it has been suggested that more symmetrical individuals have higher developmental stability (DS), reproductive success and survival rate. Developmental stability is defined as the ability of an organism to moderate its development against genetic or environmental conditions and produce the genetically determined phenotype (Daloso, 2014; Galbo and Tabugo, 2014; Carpentero and Tabugo, 2014 and Trotta et al., 2005). DS has been used to monitor the effects of anthropogenic and natural factors of stress on living organisms (Albarra´n-Lara, 2010). Under normal conditions, development follows a genetically determined pathway, and minor perturbations are controlled by developmental stability mechanisms. Under stressful conditions (e.g., increased toxicants) and tolerance limits have exceeded, the stress leads to developmental instability such that development cannot be restored to the original pathway causing increase phenotypic variations of the organism, reduce the homeostasis of a biological system, or generate symmetry deviations in an organ or an organism's relative symmetry. In this context, stress identified at morphological level generally means that the physiological and molecular plasticity were unable to buffer the stress (Whiteman and Loganathan, 2001). The most common tool for measuring DS is FA. Generally, FA is defined as fine and random deviations from perfect symmetry of organism's morphology. Also, considered a reliable factor for measuring developmental stability because it reflects both genetic and environmental stress and this has been an important theory in evolutionary biology for decades (Palmer, 1994). In this respect, it is perceived that FA measures the capacity of the organism to buffer its developmental pathways against any environmentally derived and genetic stressors. It is believed that the presence of either of the said stressors during ontogeny may impair the effectiveness of these buffering mechanisms. This may affect normal developmental process and could be manifested as increase levels of FA of an otherwise bilaterally symmetrical character on organisms (Mpho et al., 2000). In this context, it is perceived that there is a direct relationship between FA and developmental instability (Graham et al., 2010).

In this regard, this study was done to investigate the potential of FA as a biomarker of environmental stress and determine developmental instability in the sea star (*Protoreaster nodosus*). It assessed the difference in the FA indexes of *Protoreaster nodosus* from three (3) different sites (Linamon, Lanao del Norte; Initao, Misamis Oriental and Jasaan, Misamis Oriental) portraying different environmental conditions and determine the possibility of FA as a tool to determine ecological stress and its efficiency when applied to five-fold dihedral symmetrical organisms. Fluctuating asymmetry is also a useful potential indicator of an organism's health and welfare.

2 Materials and Methods

2.1 Study sites and specimen collection

Three study sites were surveyed within the coast of Northern Mindanao region: Bobotan, Initao; Linamon, and Aplaya, Jasaan. The sites chosen differ with their proximity to human settlements and predicted to differ in terms of environmental conditions. Bobotan, Initao is a protected seascape under Initao-Libertad Protected Landscape and Seascape declared last 2002. There were more less 15 households residing near the area which take part of the local government unit (LGU) activities in protecting and preserving the area. Linamon and Aplaya were chosen as sites which differ in anthropogenic disturbance. Survey and sampling procedures were done during low tide. Samples were collected in a 30 meter by 2 meter line transect (10 m by 2 m line transect per sampling replicate) running parallel with the shore and placed randomly at each site within the low intertidal zone (see Fig. 1).

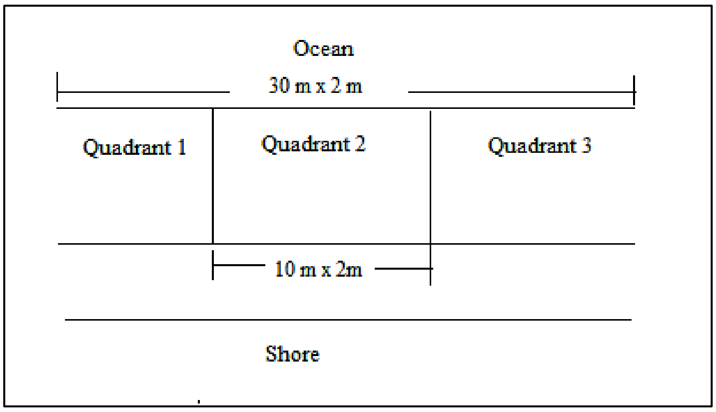

Fig. 1 Transect line used for the collection of *P. nodosus*.

2.2 Physico-chemical parameters

Measurement of ecological variables could serve as indicator for pollution or water disturbance in a particular community. The following variables were recorded at each site: water and air temperature, hydrogen ion concentration (pH) and salinity. These parameters were measured *in situ* conducted by triplicate in the 30 meter by 2 meter line transect.

2.3 Digital imaging preparation and measurement of Fluctuating Asymmetry (FA)

Thirty (30) individuals of *P. nodosus* were photographed for each site using a standard procedure. Samples were carefully removed from the water, photographed and then returned to their original nest such that no animal was harmed during the process. The digital images of the sea stars were processed and landmarked assignment was done using tpsUti1 and tpsDig2 softwares. Landmarking was done in triplicates to quantify and minimize measurement error. For morphometric analysis, forty-one (41) landmark points were assigned for each individual. Arm opposite to the madreporite was designated as Arm 1, and the others follow clockwise successively in aboral view (see Fig. 2) based on Ji et al., (2012) study. Fig. 3 shows the location of the landmarks used in the sea star. Descriptions of identified landmarks are presented in Table 1.

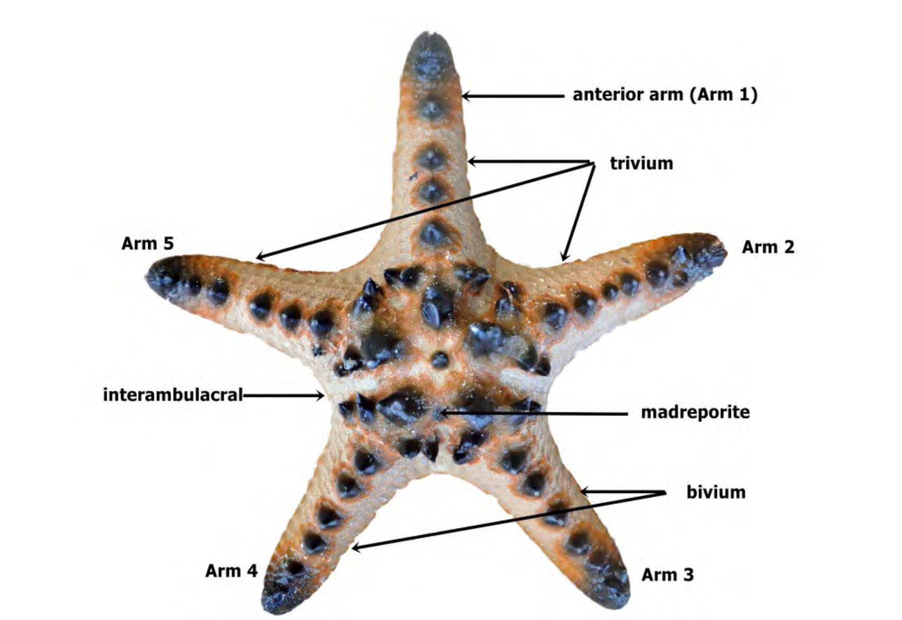

Fig. 2 Aboral view of *P. nodosus* showing the anterior arm, opposite to the madreporite, which serves as Arm 1 and other arms follow clockwise successively.

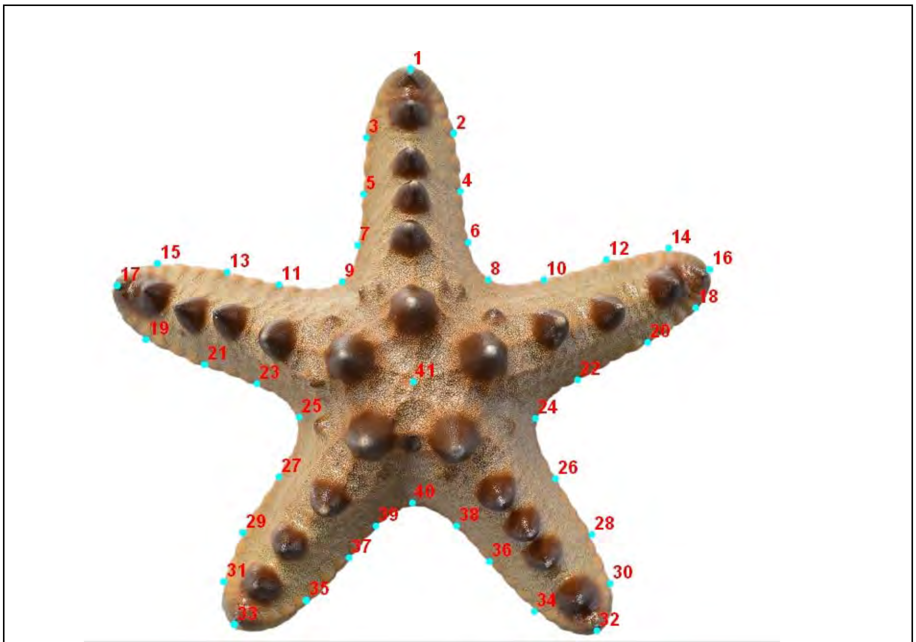

Fig. 3 Location of forty-one (41) landmarks in *P. nodosus* sea star used for fluctuating asymmetry (FA) analysis.

Table 1 Description of assigned landmarks on *P. nodosus* sea star.

Landmark points	Anatomical Description	Landmark points	Anatomical Description
1	Eyespot of anterior arm (Arm 1)	30, 34	Distal end of Arm 3
2,3	Distal end of anterior arm	32	Eyespot of Arm 3
4,5	Middle of anterior arm	40	Interambulacrum between bivium Arms
6,7	Proximal end of anterior arm	27, 39	Proximal end of Arm 4
8	Interambulacrum between Arm 1 and Arm 2	29, 37	Middle of Arm 4
9	Interambulacrum between Arm 1 and Arm 5	31, 35	Distal end of Arm 4
10, 22	Proximal end of Arm 2	33	Eyespot of Arm 4
12, 20	Middle of Arm 2	25	Interambulacrum between Arm 4 and 5
14, 18	Distal end of Arm 2	11, 23	Promixal end of Arm 5
16	Topmost/ eyespot of Arm 2	13, 21	Middle region of Arm 5
24	Interambulacrum between Arm 2 and 3	15, 19	Distal end of Arm 5
26, 38	Proximal end of Arm 3	17	Eyespot of Arm 5
28, 36	Middle of Arm 3	41	Center of central disk

Individual levels of FA were obtained using the SAGE (Symmetry and Asymmetry in Geometric Data) program. This software analysed the x- and y-coordinates, using a configuration protocol that divided both sides of the sea star body. Object symmetry was applied in this case as sea stars have five-fold dihedral symmetry. The FA theory has mostly been applied to bilaterally symmetrical organisms with only a few published studies on organisms having five-fold dihedral symmetry (most echinoderms including starfish). Herewith, breaking dihedral symmetry produces a bilaterally symmetrical object having just one reflective axis of symmetry.

Procrustes methods were used to analyze shape by superimposing configurations of landmarks into two or more specimens to achieve an overall best fit (see Fig. 4). The squared average of Procrustes distances for all specimens is the individual contribution to the FA component of variation within a sample. To detect the components of variances and deviations, a two-way, mixed model ANOVA with three replicates was used. (Marquez, 2006; Klingenberg et al., 1998).

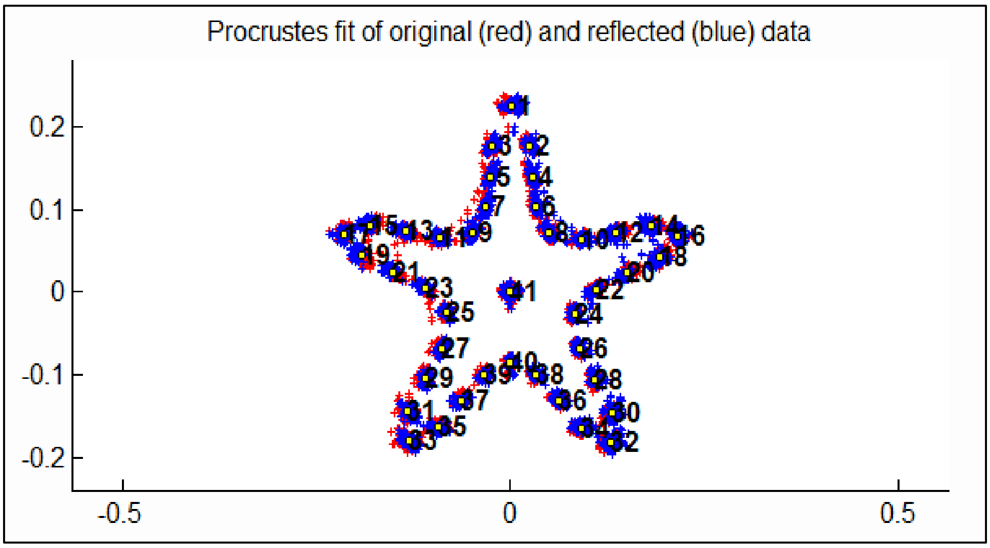

Fig. 4 Procrustes fitted image of *P. nodosus* done by SAGE software.

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 (size and shape variation). The individual’s mean square is a measure of total phenotypic variation and it is random. The ‘individual by 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 anti-symmetry; variations could be dependent to both environmental and genetic conditions (Graham et al., 2010).

3 Results and Discussion

There were many assumptions behind fluctuating asymmetry (FA) and developmental stability (DS) and the nature of the factors behind developmentally unstable phenotypes is not yet well understood. However, it is noted that developmental stability is the situation wherein an organism has adequately buffered itself against epigenetic disturbances hence, displaying its developmentally programmed phenotype. At the instance, that an organism fails to buffer such disturbances, it may display signs of developmental instability. The origin of the disturbance is assumed to be genetic, environmental or the product of genotype-environment interaction (Markow, 1995). Fluctuating asymmetry (FA), pertains to small and random departures from perfect symmetry of an organism’s bilateral traits and has been used as a measurement of developmental instability and as a potential indicator of stress in populations. Thus, investigating the link between FA and DS in ecologically important natural populations of sea stars shed light on the quest for morphological characters as a possible biomarker for stress and knowledge on gene-environment interaction (Daloso, 2014; Galbo and Tabugo, 2014; Trotta et al., 2005).

Herewith, Table 2 shows the Procrustes two-way, mixed model ANOVA table with expected mean squares. The effect called “individuals” is the variation among individual animals and can be interpreted as a size/shape variation; the “individuals” mean square is a measure of total phenotypic variation and is random. The effect called “sides” is the variation between the two sides; it is a measure of directional asymmetry (DA). The “individual by side interaction” is the failure of the effect of individuals to be the same from side to side; it is a measure of FA and antisymmetry. It is a mixed effect. The error term is the “measurement error”; it is a random effect (Parés-Casanova and Kucherova, 2013).

Results show not significant FA value for Initao site (protected seascape) yet highly significant DA value, which indicated that variation among individual genotypes and asymmetry in phenotypes is mostly induced by genetics under less stressful environment. Meanwhile, the F values of “individual x sides” suggested highly significant FA for the other two sites, Linamon and Aplaya, Jasaan as indicated by low mean square value of measurement error compared to the individual by sides mean square values. Chocolate chip sea star populations in Linamon and Aplaya, Jasaan have also shown significant scores on “individual” and “side” effects. The effect called “sides” which refer to the variation between the two sides, a measure of directional asymmetry (DA) were significant for both populations and were of the same level. A high FA and significant DA leads to generation of phenotypes interacting with the perturbed ambient. Thus, may indicate interplay of genotype and environment under more stressful environment. Noteworthy, was that Linamon and Aplaya, Jasaan sampling sites displayed some level of environmental disturbance based on the ocular site inspection. Anthropogenic disturbance were prevalent in Linamon sampling site due to human settlements along the shore such that sewage and canal run offs go directly to the bay and various litters (e.g. plastics, diapers) were often found scattered in the shoreline. While, Jasaan sampling area was situated near two industrial plants, Philippine Sinter Corporation (PSC) and Pilipinas Kao, Inc., that produce sintered ore and biodegradable chemicals, respectively. Yet, there were no official report on heavy metals or toxic contamination in the site. Results coincide with the study of Utayopas (2001) on *Trichopsi vittatus* (croaking gourami) with highest mean asymmetries were detected from the most polluted site in almost all characters. This suggests that significant variation in size or left-right side of each individual could be a product of genotype-environment interaction. Thus, *P. nodosus* individuals in these areas may have developmental instability during ontogeny which could be due to exposure to environmental or genetic stressors. The individual’s inability to buffer the stress leads to deviation in its relative symmetry. In this context, it is perceived that there is a direct relationship between FA and developmental instability (Graham et al., 2010).

Table 2 Procrustes two-way, mixed model anova results of body symmetry of *p. Nodosus.*

Effect/Site	SS	dF	MS	P	F
Linamon					
Individuals	0.13662	1131	0.0001208	0	2.4497******
Sides	0.030035	39	0.00077013	0	15.6182******
Individuals x sides	0.055769	1131	4.9309e-005	0.0020071	1.1418***
Measurement error	0.20211	4680	4.3186e-005	--	
Initao					
Individuals	0.14522	1131	0.0001284	0	2.4965******
Sides	0.049487	39	0.0012689	0	24.6727******
Individuals x sides	0.058167	1131	5.143e-005	0.76773	0.96575
Measurement error	0.2492	4680	5.3253e-005	--	
Jasaan					
Individuals	0.18584	1131	0.00016432	8.6159e-009	1.3995******
Sides	0.069279	39	0.0017764	0	15.1301******
Individuals x sides	0.13279	1131	0.00011741	0	2.285******
Measurement error	0.24046	4680	5.1381e-005		

*Significant, P< 0.05.

In addition, principal component analysis from Procustes analysis may serve as reliable tool in visualization of variations in landmarks (Galbo and Tabugo, 2014). The percentage values of PCA represent the total variation in FA (see Table 3 and Fig. 5). Based on the results exhibited by PC 1 and PC2, population from Initao express less variation compared to Linamon and Aplaya, Jasaan. Reversely, higher FA was exhibited by the individuals in Linamon and Aplaya, Jasaan compared to Initao. Generally, PC 1 accounts for most of the variation. Highest variation (PC 1= 70%) is exhibited in Jasaan followed by Linamon (PC 1= 37%). Thus, explaining the symmetry deviation observed. This could have been attributed by both genetic and, largely, of environmental stressors.

Table 3 Variance explained by first two principal components of *Protoreaster nodosus* in three sites.

Sites	PC 1 (%)	PC2 (%)	Overall (%)
Linamon	37.19	19.81	57
Initao	34.14	19.28	53.42
Jasaan	69.66	11.35	81.01

Moreover, using Canonical Correspondence Analysis (CCA) physico-chemical parameters of each sampling sites were correlated with PC values. Results revealed that individual variation in each samples were more likely related to pH stress in *P. nodosus* (Fig. 6). Study of Dupont et al. (2008) show that at low pH larvae of the ecological keystone brittlestar, *Ophiothrix fragilis*, either were abnormal, had altered skeletal proportions and asymmetry during skeletogenesis and there was a delay in development. The exposure of larvae to elevated CO2 (high pH) treatment takes longer to reach the same developmental stage. Herewith, FA has costs and reflects the degree of environmental stress, health and quality of individuals.

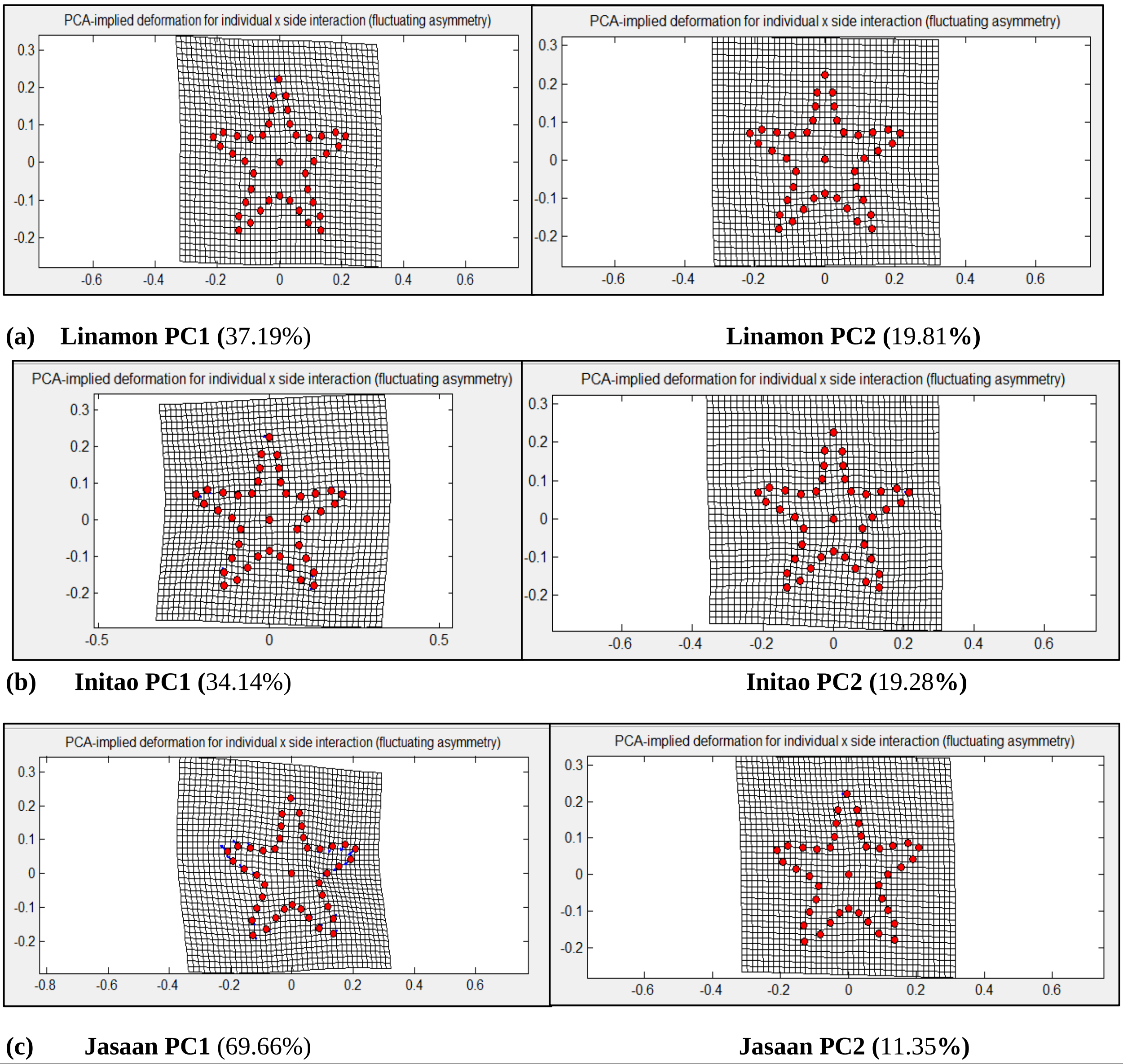

Fig. 5 Deformation grid of fluctuating asymmetry for PC1 and PC2 of (a) Linamon, (b) Initao, and (c) Jasaan *P. nodosus* populations.

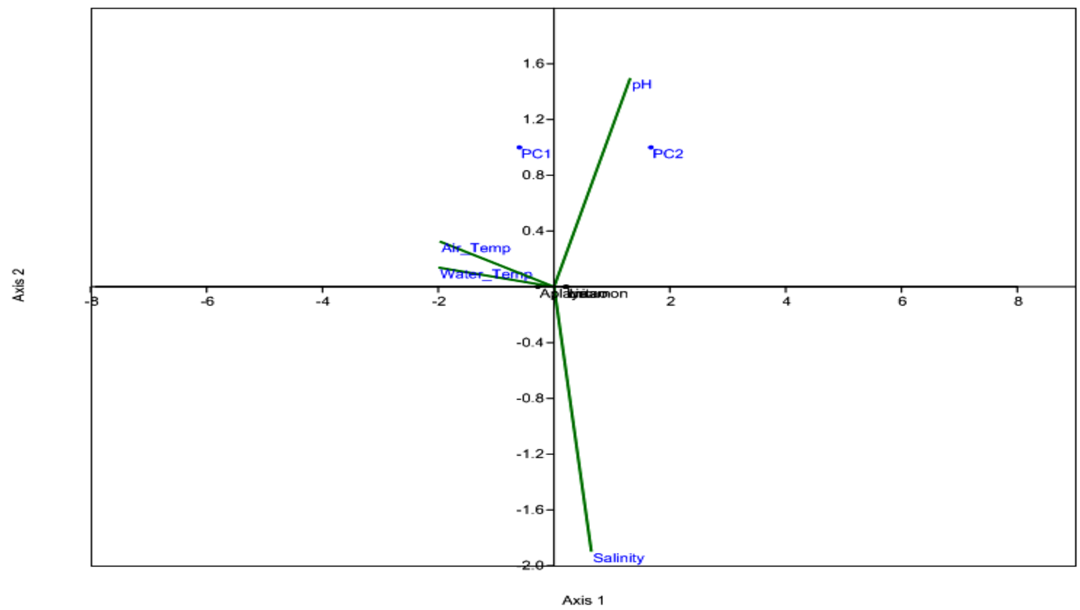

Fig. 6 Canonical Correspondence Analysis (CCA) of PCA scores and physico-chemical parameters.

4 Conclusion

Under stressful conditions, the genotypes may show some deviations in their perfect bilateral symmetry, which can be identified through the analysis of fluctuating asymmetry (FA). Results show that sampling areas which are environmentally disturbed (Linamon and Jasaan) due to various anthropogenic activities such as industrial or residential pollutants exhibited high FA and significant DS. This suggests that significant variation in size or left-right side of each individual could be a product of genotype-environment interaction. Meanwhile, Initao (protected seascape) population have exhibited insignificant FA level and high DS, such that variation among individual genotypes and asymmetry in phenotypes is mostly induced by genetics under less stressful environment. Hence, *P. nodosus* found in Linamon and Aplaya, Jasaan could be considered as developmentally unstable and its inability to buffer the environmental and genetic stressors above tolerance limit have led to deviation of its relative symmetry. It is perceived that there is a direct relationship between FA and developmental instability. Thus, the study demonstrates the potential of FA as a biomarker for environmental stress in five-fold dihedral symmetry of sea stars and a tool in detecting developmental instability. Moreover, this tool should be applied to other similar organisms and wide range of physico-chemical parameter should be included to fully assess the health of a certain habitat.

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References

Albarrán-Lara AL, Mendoza-Cuenca L, Valencia-Ávalos S, González-Rodríguez A, Oyama K. 2010. Leaf fluctuating asymmetry increases with hybridization and introgression between *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae) along an altitudinal gradient in Mexico. International Journal of Plant

Sciences, 171(3): 310-322

Beasley DA, Bonisoli-Alquati A, Mousseau T. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. Ecological Indicators, 30: 218-226

Bos AR, Gumanao GS, Alipoyo JCE, Cardona LT. 2008. Population dynamics, reproduction and growth of the Indo-Pacific horned sea star, *Protoreaster nodosus* (Echinodermata; Asteroidea). Marine Biology, 156:55-63

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

Daloso DM. 2014. The ecological context of bilateral symmetry of organ and organisms. Natural Science, 6(4): 184-190

Dupont S, Havenhand J, Thorndyke W, Peck L, Thorndyke M. 2008. Near-future level of CO2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. Marine Ecology Progress Series, 373: 285-294

Galbo K, Tabugo SRM. 2014. Fluctuating asymmetry in the wings of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) from selected barangays in Iligan City, Philippines. AACL Bioflux, 7(5): 357-264

Graham JH, Raz S, Hel-Or H, Nevo E. 2010. Fluctuating asymmetry: Methods, theory, and applications. Symmetry, 2(2): 466-540

Ji C, Wu L, Zhao W, Wang S, Lv J. 2012. Echinoderms have bilateral tendencies. PLoS ONE 7(1): e28978

Klingenberg CP, McIntyre GS, Zaklan SD. 1998. Left-right asymmetry of fly wings and the evolution of body axes. Proceedings of the Royal Society of London B, Biological Sciences, 265: 1255-1259

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

Marquez E. 2006. Sage: symmetry and asymmetry in geometric data. Ver 1.0. http://www.personal.umich.edu/~emarquez/morph/

Menge BA. 1975. Brood or broadcast? The adaptive significance of different reproductive strategies in the two intertidal sea stars *Leptasterias hexactis* and *Pisaster ochraceus*. Marine Biology, 31: 87-100

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

Paine RT, Castillo JC, Cancino J. 1985. Perturbation and recovery patterns of starfish-dominated intertidal assemblages in Chile, New Zealand and Washington State. American Naturalist, 125: 679-691

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

Parés-Casanova PM, Kucherova I. 2013. Horn asymmetry in a local goat population. International Journal of Research in Agriculture and Food Sciences, 1(2): 12-17

Trotta V, Corrado F, Calboli F, Garoia F, et al. 2005. Fluctuating asymmetry as a measure of ecological stress in *Drosophila melanogaster* (Diptera: Drosophilidae). European Journal of Entomology, 102: 195-200

Utayopas P, 2001. Fluctuating Asymmetry in Fishes Inhabiting Potluted and Unpolluted Bodies of Water in Thailand. Thammasat International Journal of Science and Technology, 6(2): 10-20

Vishalakshi C, Singh BN. 2008. Effect of environmental stress on fluctuating asymmetry in certain morphological traits in Drosophila ananassae: nutrition and larval crowding Canadian Journal of Zoology, 86(5): 427-437

Whiteman HH, Loganathan BG. 2001. Developmental stability in amphibians as a biological indicator of chemical contamination and other environmental stressors. Kentucky EPA/EPSCoR, USA