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Fluctuating asymmetry of parasite infested and non-infested *Sardinella sp.* from Misamis Oriental, Philippines

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Abstract. Fluctuating asymmetry (FA) is defined as subtle, random deviation from perfect symmetry and is the most commonly used estimate of developmental stability. It has been found to increase with the presence of stressors including parasite infestation. In this study, potential relationships were investigated between the presence of parasite and its effect to FA as a widely employed measure of developmental stability on *Sardinella* sp. FA levels as asserted in many studies increases with developmental instability. *Sardinella* sp. from Misamis Oriental were collected and assessed for the presence of parasites in the gills. Fluctuating asymmetry in the traits were analyzed using landmark method for shape asymmetry, via Symmetry and Asymmetry in Geometric Data (SAGE) program. A total of twelve landmark points were used and a total of 200 fishes were evaluated. Procrustes ANOVA showed insignificant levels of FA for *Sardinella* sp. found without parasite while those found with parasites showed significant levels of FA. Thus, in consistency with other studies, that the presence of parasites may affect the developmental stability of an organism as seen in the asymmetry measurement of the left and right sides of the organism, which implies the extent to which the organism is able to buffer any disturbance. Hypothesis assumes that parasites can cause an increased level of FA due to the stress it induced in the development of the organism. An increase level of FA has implications on species fitness and adaptation. Presence of parasites has negative impact on host fitness.
Key Words: Adaptation, development of the organism, species fitness, developmental stability.

Introduction. Developmental stability is defined as the ability of an organism to buffer its development against genetic or environmental disturbances encountered during development to produce the genetically predetermined phenotype (Waddington 1942) and, as such, it is a fundamental characteristic of development. The most commonly used estimate of developmental stability has been the fluctuating asymmetry (FA). The underlying assumption of this measure is that the development of both sides of a bilaterally symmetric organism is influenced by identical genes, and thus non-directional differences between sides must be environmental in origin and reflect accidents occurring during development (Waddington 1942; Moller & Swaddle 1997). FA refers to a pattern of bilateral variation in a sample of individuals, where the mean of right minus left values of a trait is zero and the variation is normally distributed about that mean (Palmer 1994). Typically, one or more indexes are calculated that express FA as a variance, or an average absolute value, of the difference between the right and left elements of a bilateral pair for a sample of individuals (Palmer & Strobeck 1986). A highly significant FA (high FA value) indicates a lower developmental stability.

It is usually assumed that elevated levels of FA are the result of environmental and/or genetic stress experienced by the organism during ontogeny which perturbs the normal developmental program (Valen 1962; Palmer & Strobeck 1986; Palmer 1994; Markow 1994). FA variance of populations and absolute FA values of individuals has been found to increase with stress (Leung & Forbes 1996; Graham et al 1993). Both genomic and environmental changes can increase FA which represents a possible deterioration in developmental homeostasis apparent in adult morphology. Noteworthy, is that genetic perturbations include intense directional selection and certain specific genes. While, environmental perturbations leading to elevated levels of FA have been associated with environmental variables such as temperature extremes, protein deprivation, audiogenic stress, pollution, parasites etc. (Palmer & Strobeck 1986; Mpho et al 2000). As such, it has been proposed as a general tool for biomonitoring stress within populations regardless of the nature of stress. FA has been used as a bioindicator in a broad array of applications.

Recent studies on FA have focused on naturally selected traits and how asymmetry is associated with environmental stress (Siikamaki & Lammi 1998; Imasheva et al 1999; Reimchen & Nosil 2001). For example, fish from polluted or otherwise altered waters exhibit higher levels of asymmetry in gill rakers and fin rays than those inhabiting nonpolluted or natural habitats (Ostbye et al 1997; Campbell et al 1998), logging and consequent habitat alteration leads to increased mandibular asymmetry in shrews (Badyaev et al 2000), and food deprivation in the laboratory is associated with higher levels of asymmetry in feather growth in some birds (Swaddle & Witter 1994). Moreover, another potential source of environmental stress is parasitism by macroparasites and pathogens. Several studies have demonstrated a positive correlation between levels of parasitism and extent of asymmetry in various traits (Moller 1996), and these studies are consistent with the widespread assumption drawn from other work that parasitism does affect FA levels and can be detrimental to host individuals (Martin & Hosken 2009).

In this study, potential relationships were investigated between the presence of parasite and its effect to FA as a widely employed measure of developmental stability on *Sardinella* sp. FA levels as asserted in many studies increases with developmental instability. *Sardinella* sp. was considered of economic value because of its high nutritional content as they are rich in vitamins and minerals. They are also a natural source of marine omega-3 fatty acids, which reduce the occurrence of cardiovascular disease, and good source of vitamin D, calcium, vitamin B12, and protein. This species is commercially harvested as an important food source in Iligan City, Misamis Oriental and nearby localities (Mahrus et al 2012).

Also much interest has been devoted to the determination and examination of FA as an indicator of individual quality and fitness. Here, a hypothesis assumes that parasites can cause an increased level of FA in the organism due to the stress it induced in the development of the organism (Graham et al 1993; Martin & Hosken 2009). Hence, this study generally aims to test if the presence of parasites (e.g. in the gills) may affect developmental stability of *Sardinella* sp. through the use of fluctuating asymmetry as an estimate of developmental stability. Specifically, check the presence of parasites in *Sardinella* sp. samples from Misamis Oriental and investigate if there is any significant difference in FA measurement between infested and non-infested samples of *Sardinella* sp.

Material and Method. Samples of *Sardinella* sp. come from Misamis Oriental. Two-dimensional images of the sample were taken. The samples were then thoroughly checked in their gills for the presence of any parasite. Gills of the samples were scraped and examined using conventional microscopy. A total of 200 fishes were evaluated.

A total of 12 landmarks were digitized using the tpsDig software. The location of the landmarks and the anatomical descriptions of each are presented in Figure 1 and Table 1. Overall and localized fluctuating asymmetries were determined by subjecting the paired landmark coordinates to Procrustes ANOVA following the method of Klingenberg et al 1998 and using Symmetry and Asymmetry in Geometric Data (SAGE) software.

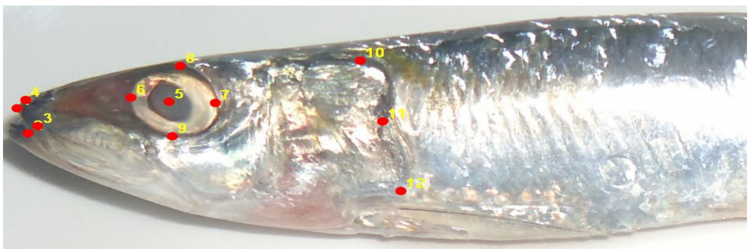

Figure 1. Location of the 12 landmark points on *Sardinella* sp.

Table 1

Position of the twelve landmarks selected in *Sardinella* sp.

Landmark #	*Position* — *Anatomical landmarks*
1	Lower anterior of the premaxilla
2	Lower posterior of the premaxilla
3	Upper posterior of the premaxilla
4	Upper anterior of the premaxilla
5	Center of eye
6	Anterior margin through the midline of the eye
7	Posterior margin through the midline of the eye
8	Superior margin of the eye
9	Inferior margin through the midline of the eye
10	Dorso-lateral angle of the operculum
11	Posterior margin of the operculum
12	Isthmus

FA levels were assessed using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.0. The software analyzed the x and y coordinates of the landmarks per individual, using a configuration protocol for both lateral sides of the head of *Sardinella* sp. Procrustes superimposition analysis was performed with the original and mirrored configurations of the right and left lateral sides simultaneously. The least squares Procrustes consensus of set of landmark configurations and their relabelled mirror images is a perfectly symmetrical shape, while FA is the deviation from perfect bilateral symmetry (Marquez 2006; Klingenberg et al 1998). 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 Procrustes ANOVA was used. Sides (directional asymmetry; DA), Individual x sides (fluctuating asymmetry; FA), and their respective error were included as effects. The ANOVA used most frequently for fluctuating asymmetry is a two-way, mixed-model ANOVA with replication. The main fixed effect is *sides* (*S*), which has two levels (left and right). The block effect is *individuals* (*I*), which is a random sample of individuals from a population. The *sides by individuals interaction* (*S x I*) is a mixed effect. Finally, 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 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 antisymmetry thus, a mixed effect. The error term is the measurement, and is a random effect. Only Individual x Sides interaction denotes fluctuating asymmetry (FA) (Samuels et al 1991; Palmer & Strobeck 1986, 2003; Carpentero & Tabugo 2014).

Moreover, to 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 to carry out an interpolation based on a thin-plate spline and then visualize shape changes as landmark displacement in the deformation grid (Albarran-Lara et al 2010; Marquez 2006).

Results and Discussion. An underlying hypothesis of FA analysis is that bilaterally symmetrical traits should be in principle identical on either side of the body since they are said to be governed by the same genes thus, non-directional differences between the sides must be environmental in origin and reflect accidents occurring during development (Palmer 1994; Valen 1962; Gangestad & Thornhill 1999; Martin & Hosken 2009). However, deviations from perfect asymmetry were described as common and thought to convey information about developmental stability of individuals and populations.

Developmental stability is defined as the ability of a genotype to resist developmental perturbations and its common estimate is through FA although, other types of symmetry may also convey to some extent information about developmental stability. As such, there is a direct relationship between FA and developmental instability and FA was thought to reflect an organism's ability to cope with genetic and environmental stress during development and considered to reflect a population's average state of adaptation, coadaptation, fitness and individual quality (Graham et al 2010; Parsons 1990). Moreover, it is thought to increase under both environmental and genetic stress (Graham et al 2010). A potential source of environmental stress is parasitism by macroparasites and pathogens and this have been demonstrated through several studies which showed a positive correlation between levels of parasitism and extent of asymmetry in various traits (Moller 1996). Such studies were consistent with the widespread assumption that parasitism does affect FA levels and can be detrimental to host individuals (Martin & Hosken 2009). FA of the right and left lateral sides of the head of *Sardinella* sp. were assessed through Procrustes method using SAGE software. It was investigated whether there was any significant difference in FA measurement between infested and non-infested samples of *Sardinella* sp.

Index of FA using the coordinates was determined and final result of the Procrustes ANOVA is shown in Table 2. It was noted that 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 antisymmetry thus, a mixed effect. The error term is the measurement, and is a random effect. Only Individual x Sides interaction denotes fluctuating asymmetry (FA) (Palmer & Strobeck 1986; Galbo & Tabugo 2014). Hereby, the interaction of '*Side x Individuals*' showed a high value of mean square and a low value of mean square measurement error. Thus, the F value suggested highly significant FA for all parasite infested samples of *Sardinella* sp. from Misamis Oriental where *P<0.001. The results of the Procrustes ANOVA indicated a random variation (FA) between the right and left lateral sides of the head of *Sardinella* sp. rather than non-random differences among sides.

Results implied that presence of parasite affects fluctuating asymmetry in host species. This is consistent with other studies where parasitism has significant effect on the degree of fluctuating asymmetry on the host (Moller 1992; Polak 1993). The mechanisms by which parasites cause increased character asymmetry in general and for *Sardinella* sp. in particular are unknown and still subject to further investigations though there were underlying pre-conceived notions. But most likely, parasite-induced nutritional deprivation of various forms destabilizes host development and elevates levels of fluctuating asymmetry (Polak 1993, 1997). Parasitism can limit host nutrient availability by reducing host food intake, digestion, absorption and nutrient assimilation (Whitefield 1979; Thompson 1983; Polak 1997). Parasites may compete with their hosts for resources, and therefore impinge upon host metabolism, growth and development (Schall et al 1982; Goater et al 1993). Some experimental studies have shown that nutritional stress directly leads to elevated asymmetry (Sciulli et al 1979; Swaddle et al 1994; Imasheva et al 1999), although others have shown no such effect (Hovorka & Robertson 2000; Bjorksten et al 2000). In this case, there is high parasitic load in *Sardinella* sp. samples examined such that more likely parasitism lead to nutritional deprivation which in turn caused increased fluctuating asymmetry. Most of the commonly encountered fish parasites were protozoans (e.g. ciliates, flagellates, myxozoans, microsporidians, and coccidians). The costs of parasitism could result in increased physiological stress during development and thus, directly induce high levels of asymmetry. Supporting this idea were some experimental manipulations of parasite loads which provide evidence for a direct causal link between FA and parasites. However, FA and parasites may be associated for several other reasons (Polak 1997; Thomas et al 1998). The higher the FA (significant FA) it is more developmentally unstable the organism (Palmer 1994). Thus, FA can be used as an indicator of individual quality and adaptation thereby, also demonstrating the potential for FA as a biomarker of stress and developmental instability of populations. Moreover, according to Mpho et al (2000), the possible causes of developmental instability were well studied and include a wide range of environmental factors (e.g. deviant climatic conditions, food deficiency, parasitism, pesticides) and genetic factors (e.g. inbreeding, hybridization, novel mutants). Such factors may also increase stress to populations.

Table 2
Procrustes ANOVA results for samples *Sardinella* sp. with- and without parasites from Misamis Oriental

Effect	*SS*	*DF*	*MS*	*F*	*P*	*Significance*
			Sardinella sp. withouth parasites			
Sides	0	20	0	0	1	ns
Individuals x Sides	1.1604e-031	1780	6.5192e-035	9.5074e-032	1	ns
Measurement error	2.4685	3600	0.0006857	-	-	-
			Sardinella sp. with parasites			
Sides	0.013025	32	0.00040703	3.1328	2.018e-008	******
Individuals x Sides	0.12057	928	0.00012993	1.6483	0	******
Measurement error	0.15234	1920	7.8825e-005	-	-	-

Side = directional asymmetry; individual x sides interaction = fluctuating asymmetry; * P<0.001 significant, ns – statistically insignificant (P>0.05); significance was tested with 99 permutations.

Another way to examine the variability of landmark points in tangent space is to run a principal component analysis (PCA) on the tangent coordinates derived from Procrustes analysis. First principal component depicts vectors at landmarks that show the magnitude and direction in which that landmark is displaced relative to the others. The second depicts the difference via the thin plate splines, an interpolation function that models change between landmarks from the data of changes in coordinates of landmarks. Here, 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 (Marquez 2006).

Based on the percentage of overall variation exhibited by PC1 and PC2, parasite infested samples exhibits higher variation compared to non infested samples. Thus, higher FA was also exhibited by parasite infested samples. Hence, the presence of parasites may contribute or increase variability (Table 3 & Figure 3).

Table 3
Variance explained by first two principal components between *Sardinella* sp. with- and without parasites from Misamis Oriental

Parasite +/-	*PC 1 (%)*	*PC 2 (%)*
+	10.7	89.3
-	52.0	23.2

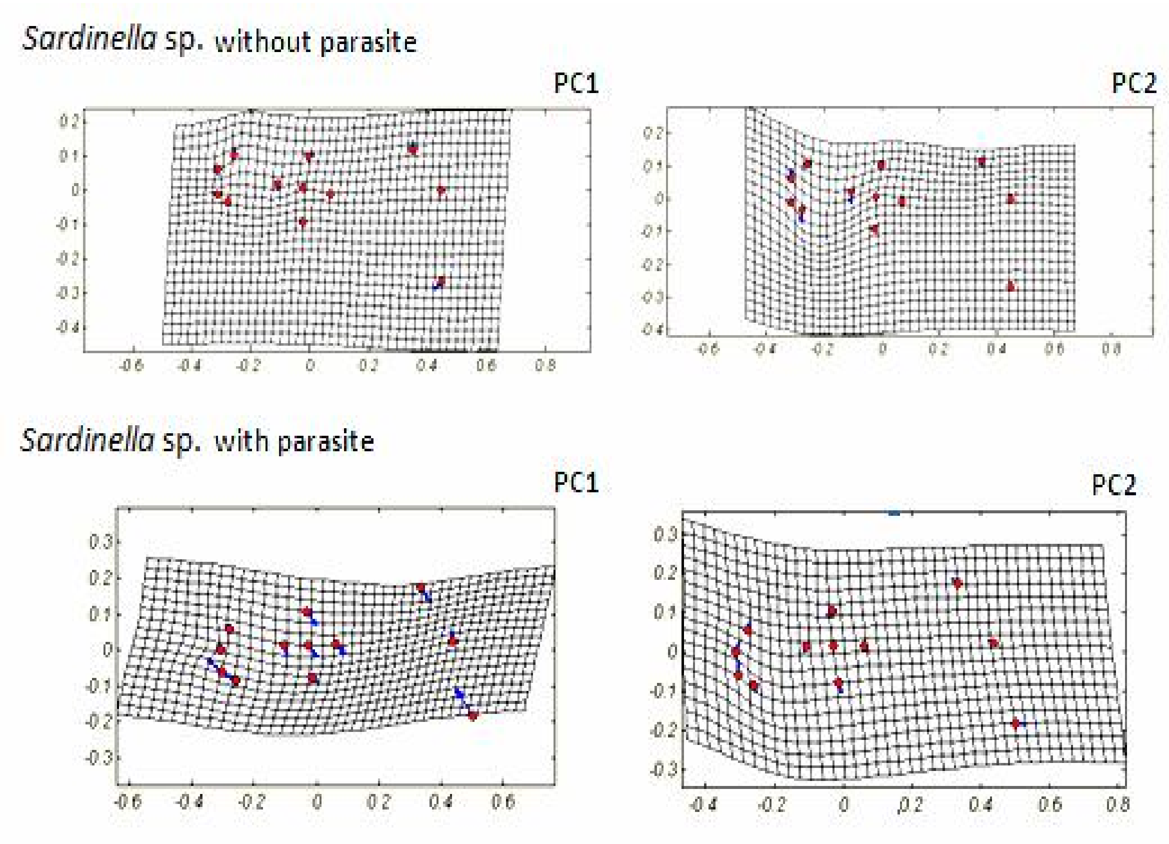

Figure 3. PCA implied deformation for individual x side interaction of fluctuating asymmetry of *Sardinella* sp. with- and without parasites.

Conclusions. Fluctuating asymmetry is defined as deviations from symmetry which may be caused by environmental stresses, developmental instability and genetic problems during development. It is thought that the more perfectly symmetrical an organism is, the better it has been able to handle developmental stress and has more developmental stability. Significant values for FA were observed in parasite infested *Sardinella* sp. suggesting deviations in its bilateral symmetry. Meanwhile, *Sardinella* sp. without parasite had insignificant value for FA. Hence, this study provides experimental evidence that parasites can affect fluctuating asymmetry in *Sardinella* sp. The results of the study demonstrates the potential of FA as a biomarker of stress and its efficacy in measuring developmental stability in *Sardinella* sp. Findings confirmed and correspond to a number of studies suggesting that fluctuating asymmetry is thus a useful tool in determining developmental stability.

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