Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 8, No. 3, p. 142-152, 2016
http://www.innspub.net

INNSPUB
RESEARCH PAPER

OPEN ACCESS

Fluctuating asymmetry as an indicator of ecological stress and developmental instability of *Neurothemis ramburii* (Odonata: Libellulidae) in Iligan City, Philippines

Michelle M. Barbon, Jazzie D'Zeim Arreza, Sharon Rose M. Tabugo*

Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology, Philippines

Article published on March 16, 2016

Key words: Asymmetry, *Neurothemis ramburii*, Odonata, Procrustes ANOVA, SAGE.

Abstract

Odonata species are often used for biomonitoring purposes are often influenced by environmental instability in diverse ways. The degree of developmental stability of individuals and populations is most often estimated by their level of fluctuating asymmetry (FA). FA refers to a slight number and nondirectional deviations from strict bilateral symmetry of biological objects that occur as a result of stochastic microscopic processes. In this study, analysis was done on eurytopic species *Neurothemis ramburii*. Specifically, it investigated the differences in fluctuating asymmetry (right and left wing) of three populations from different barangays (Dalipuga, Pugaan and Buruun) Iligan City, Mindanao, Philippines. It determined developmental stability via fluctuating symmetry and used FA as an indicator of stress. Analysis was based on Procrustes Method and makes comparison of FA indices of homologous points. Using landmark method for shape asymmetry, anatomical landmarks were used and analyzed using Symmetry and Asymmetry in Geometric Data (SAGE) program. Procrustes ANOVA and Principal Component Analysis (PCA) results showed considerable variation and significant evidence of FA for all populations with relatively high FA for more disturbed areas (Pugaan and Dalipuga). Significant FA may present inability of species to buffer against endogenous and exogenous stress in its developmental pathways hence, would mean developmental instability. Directional asymmetry (DA) was also significant in all populations. A significant FA and DA suggest that stress and variation could be a product of genotype-environment interaction. Awareness and understanding of adaptation and survival of Odonata species as biological indicators for wetland assessment is essential

***Corresponding Author:** Sharon Rose M. Tabugo ✉ sharonrose0297@gmail.com

Introduction

Odonata species are widely known as biological indicators for wetland assessments because of their sensitivity to human disturbances. They are important components of food web and are good subjects of behavioural, ecological studies and even evolutionary studies (Samways and Steytler, 1996, Tennessen, 2009, Kutcher and Bried, 2014). Species are widely distributed across the globe. Dragonflies like libellulids are diverse, numerous and commonly observed and studied especially for biomonitoring purposes (Ware *et al.*, 2007). Many species are expected to be influenced by environmental instability in very diverse ways according to its intrinsic biological characteristics (Pinto *et al.*, 2012).

In this respect, a means to measure developmental stability (DS), especially in organisms for biomonitoring purposes like Odonates would be very useful. This will also provide pertinent information on the nature, variation and status of a given population in an area. DS is the ability of organisms to maintain a stable state despite different environmental conditions and stressors, (Waddington, 1942, Palmer, 1994) and the converse of which is known as developmental instability (DI), that refers to the inability of species to buffer against endogenous and exogenous stress in its developmental pathways (Mpho *et al.*, 2000). Notably, It is believed that organisms exhibiting bilateral symmetrical traits will only often show very minor differences in their development because corresponding body sides presumably share the same genome and in a given homogenous environment experience similar external effects. Thus, any variation, changes, deviations or notable asymmetries around the target phenotype given its genotype and under a particular environment are perceived as a product or reflect inability of the organism to buffer its development against small random perturbations of cellular processes (Lens *et al.*, 2002). Such perturbations experienced by the organisms resulting from stochastic, cellular process, mostly act locally hence the effects often accumulate on the left and right sides of developing individuals separately then consequently, manifested as left-right asymmetries in development (Reeve, 1960, Palmer and Strobeck, 1986, Palmer, 1994, McAdams and Arkin, 1999). Thus, it is in this context that the pattern of symmetry of bilateral structures (e.g. wings) has been widely used as a marker for DI.

Moreover, for biomonitoring purposes the use of fluctuating asymmetry (FA) as a predictor for stress related changes have been promoted for conservation. FA is defined as fine and random deviations from perfect symmetry of organism's morphology. It is considered as a reliable factor for measuring DI, because it reflects both genetic and environmental stresses and this has been an important theory in evolutionary biology for decades (Parsons, 1990, Ryazanova and Polygalov, 2013). FA is important because it reflects a population's state of adaptation and coadaptation. In addition, it increases under both environmental and genetic stress (Graham *et al.*, 2010). Herewith, there is a direct relationship between FA and DI. The conditions that are identified to increase FA consist of such environmental factors as temperature, nutrients, and light, as well as such genetic factors as mutation, hybridization, and inbreeding that contribute to developmental alteration (Ho´dar, 2002). A number of cases have shown a correlation between FA and several fitness components or genetic characteristics, in particular, mating success and individual level of heterozygosity (Carchini *et al.*, 2000). The ubiquity of symmetry led to the utilization of FA over all other measures of developmental instability. FA could also provide major advantage over other indicators of stress because FA is easy to measure and cost-effective (Clarke, 1993, Tataro and Tabugo, 2015). Deviations from perfect symmetry may be measured as variances of linear dimensions, shape variation involving landmarks, or as continuous symmetry measures (Graham *et al.*, 2010, Carpentero and Tabugo, 2014). The left-right asymmetry of morphological structures implies perturbation in developmental homeostasis at the molecular, chromosomal, and epigenetic levels

(Chang *et al*., 2007).

Hence, in this study wing venation of *Neurothemis ramburii*, which is a widespread species, was studied to determine developmental stability via fluctuating asymmetry. FA levels were used also as indicator of stress. This study investigates differences in the FA (right and left wings) of three different populations in Iligan City using Symmetry and Asymmetry in Geometric Data (SAGE). FA has been suggested as a promising method for monitoring sub-lethal levels of pollutants (Chang *et al*., 2007). Hypothesis assumes that FA has costs and reflects the quality of individuals. Hence, this study may be able to generate knowledge and provide information on the nature and variation of dragonflies in the area. The use of FA as an indicator of developmental stability and a measure of ecological stress is based on the assumption that a stressful environment would result in higher FA levels than those observed in optimum environments (Palmer and Strobeck, 1986; Leary and Allendorf, 1989; Parsons, 1992; Tabugo *et al*., 2015). Thus, the relationship between FA and exogenous or endogenous stresses could be utilized especially in solving practical tasks such as biomonitoring purposes (Ryazanova and Polygalov, 2013).

Materials and methods

Study Area

Iligan City is located in the northeastern part of Mindanao (3° 29' N 124° 39'E) and faces Iligan Bay and is a highly urbanized city north of the Province of Lanao del Norte (Orbita and Gumban, 2013). The specimens were collected from three different areas in Iligan City namely: Barangay Buru-un (N 08° 11' 31.4'' E 124° 10' 37.4''), Dalipuga (N 08° 18' 26.63'' E 124° 16' 05.91'') and Pugaan (N 08° 18' 26.63'' E 124° 16' 05.91'') (see Fig. 1 and Fig. 2).

Collection of samples and Processing

Dragonflies are often used for biomonitoring studies. In this study, eurytopic species *Neurothemis ramburii,* were chosen since they were widely distributed and adapted to different environment. Samples were collected from three different sites in Iligan City: Buruun, Dalipuga and Pugaan respectively, with N=90 per site. Samples were collected using sweep nets for catching and appropriate preservation techniques were applied. Adult specimens were identified using existing illustrated keys and guides. The wings (fore- and hind wings) were removed and placed in clear glass slides for scanning. Digital images were acquired from both sides of fore-and hind wings using a HP 2400 scanner at 1200 dpi.

Landmark Assignment

Building TPS files and landmark assignment were done using tpsUtil and tpsDig2 softwares. Descriptions of landmark locations were shown in Table 1 and 2 and a total of 29 landmarks in the fore-wing and 35 landmarks in the hind wing were digitized using tpsDig2 software (Fig. 3). Landmarking was done in triplicates per specimen to quantify and minimize measurement error.

Measurement of Fluctuating Asymmetry and Principal Component Analysis

Overall- and localized FA levels of dragonflies were obtained using the SAGE (Symmetry and Asymmetry in Geometric Data) program by subjecting the paired landmark coordinates to Procrustes ANOVA (Hermita *et al*., 2013). This software analyzed the x- and y-coordinates, using a configuration protocol that corresponds to both sides of the fore- and hind wings of the dragonfly. Matching symmetry protocol was applied in this case for both the left and right fore- and hind wings. Procrustes method was used to analyze shape by superimposing configurations of landmarks into two or more specimens to achieve an overall best fit. Procrustes superimposition analysis was performed with the original and mirrored configurations simultaneously.

FA is the deviation from perfect bilateral symmetry (Graham *et al*., 2010). 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. 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 (Samuels *et al*., 1991, Palmer and Strobeck, 2003, Graham *et al*., 2010). Principal Component Analysis (PCA), of the covariance matrix associated with the component of FA variation were also performed for all populations to carry out an interpolation based on a thin-plate spline to visualize shape changes as landmark displacement in the deformation grid (Klingenberg *et al.,* 1998, Marquez, 2006, Tabugo *et al*., 2015).

Results and discussion

For biomonitoring purposes, there is growing evidence from various studies that Fluctuating Asymmetry (FA), can act as a universal measure of developmental stability (DS) and predictor of stress-mediated changes in fitness (Lens *et al*., 2002). FA is directly related to developmental instability (DI), hence, a tool in investigating DI. FA is said to be the departure from perfect symmetry. An underlying hypothesis behind FA analysis is that the development of the two sides of a bilaterally symmetrical organism is often influenced by identical genes and thus, non-directional differences between the sides must be environmental in origin and reflect accidents occurring during development (Palmer, 1994, Gangestad and Thornhill, 1999). FA is believed to be a more sensitive stress estimator than the traditional use of fitness measures (Clarke and McKenzie, 1992). FA is often used as a conservation tool and biomonitoring because of its potential to predict future, stress-mediated changes in fitness (Cairns *et al*., 1993, Clarke, 1995,).

Table 1. Description of assigned landmarks on both left and right fore-wings of *Neurothemis ramburii*.

Landmark #	Descriptive location	Landmark #	Descriptive location
1	Proximal End of the Costa (C)	16	Distal End of the Radius (R)
2	Proximal End of the Subcosta (Sc)	17	Origin of the Radial Branches (R2 and R3)
3	Proximal End of the Radius + Media (R+M)	18	Anterior End of the 2nd Crossvein between Radial Branches (R2 and R3)
4	Proximal End of the Cubitus (Cu)	19	Posterior End of the 2nd Crossvein between Radial Branches (R2 and R3); Origin of Radial Supplement (Rspl)
5	Proximal End of the 1st Anal Vein (A/IA)	20	Proximal End of Radial Supplement (Rspl)
6	Basal End of the Arculus (Arc)	21	Distal End of Radial Supplement
7	Proximal End of the Anterior Margin of the Triangle (T)	22	Distal End of Anterior Media (MA)
8	Distal End of the Anterior Margin of the Triangle (T)	23	Distal End of Radial Branch (R4)
9	Midpoint of the Triangle (T)	24	Distal End of Intercalary Radial Vein (IR2)
10	Midpoint of the Triangle (T)	25	Distal End of Radial Branch (R2)
11	Posterior End of the Triangle (T)	26	Antero-lateral and Distal End of the Pterostigma
12	Origin of Radial Branches (R2 and R4)	27	Postero-lateral and Distal End of the Pterostigma
13	Origin of Intercalary Vein (IR3)	28	Antero-lateral and Proximal End of the Pterostigma
14	Nodus (N)	29	Postero-lateral and Proximal End of the Pterostigma
15	Distal End of the Subcosta (Sc)		

It has become a popular tool to estimate the quality and health of individuals and populations (Markow, 1995, Leung and Forbes, 1996, Moller, 1997, Moller and Thornhill, 1998). The use of FA as an index of habitat quality may be especially useful for Odonata species. They are wetland assessment bioindicator with wide distributions, which tolerate markedly different conditions throughout their geographical range.

Table 2. Description of assigned landmarks on both left and right hind wings of *Neurothemis ramburii*.

Landmark #	Descriptive location	Landmark #	Descriptive location
1	Proximal End of the Costa (C)	19	Origin of the Intercalary Radial Vein (IR3)
2	Proximal End of the Subcosta (Sc)	20	Nodus (n)
3	Proximal End of the Media (m)	21	Distal End of the Subcosta (Sc)
4	Proximal End of the Cubitus (Cu)	22	Distal End of the Radius (R)
5	Posterior End of the Anal Crossing (Ac)	23	Origin of the Radial Branches (R2 and R3)
6	Basal End of the Arculus (Arc)	24	Distal End of Radial Supplement
7	Posterior and Proximal Vertex of the Hypertrigone (ht)	25	Posterior End of the 2nd Crossvein between Radial Branches (R2 and R3); Origin of Radial Supplement (Rspl)
8	Anterior and Proximal Vertex of the Subtrigone (t)	26	Distal End of the Anterior Media (AM)
9	Anterior and Proximal Vertex of the Hypertrigone (ht)	27	Distal End of the Radial Branch (R4)
10	Posterior and Proximal Vertex of the Subtrigone (t)	28	Distal End of the Intercalary Radial Vein (IR3)
11	(Cu2 + A2)	29	Distal End of the Radial Branch (R3)
12	Distal Vertex of the Subtrigone (t)	30	Distal End of Intercalary Radial Vein (IR2)
13	Anal Supplement (Aspl)	31	Distal End of Radial Branch (R2)
14	Basal end of the Anal Vein (A3)	32	Antero-lateral and Distal end of the Pterostigma
15	Second Branch of Cubital Vein (Cu2)	33	Postero- lateral and Distal end of the Pterostigma
16	Distal End of the Cubito-anal Vein (Cu2)	34	Antero-lateral and Proximal end of the Pterostigma
17	Distal End of the Posterior Cubital Vein	35	Postero-lateral and Proximal End of the Pterostigma
18	Origin of the Radial Branch (R4)		

This study investigated developmental stability and stress through FA of the fore-wing and hind wing of *Neurothemis ramburii* and, specifically looked into the FA levels of three different populations from three sites: Buruun, Dalipuga and Pugaan in Iligan City.

FA levels were determined using the coordinates of the tangential space including the product of the coordinates of the left and right homologous points in formula which provided the final result of the Procrustes ANOVA (Table 3). The mean square of the interaction of "sides" and "individuals by sides" effects revealed a high value compared to the low value of mean square measurement error which indicates a significant result for all populations. F values for "*individuals by sides*" effect for the three sites were all significant. However, Pugaan and Dalipuga has relatively high F value for both fore-wings and hind wings (F=3.7673, F=3.9926; F=4.1212, F=2.592) compared to Buruun population respectively. A higher F value would mean smaller P value (*P<0.001 is significant) thus, more significant. Only "*individuals by sides*" interaction denotes FA. Directional asymmetry (DA) ("*sides*") was also significant in all populations. A both significant FA and DA suggest that stress and variation could be a product of genotype-environment interaction. Significant FA and increase FA present inability of species to buffer stress in its developmental pathways hence, would mean developmental instability and have implications on species fitness.

In this case, results suggested that tested populations are relatively stressed. High FA implies increase DI. According to Badyaev *et al.*, 2000, environmental stress is able to increase phenotypic variation in population by disturbing the developmental stability of an individual.

Table 3. Procrustes ANOVA results of the three barangays for the fore-wings and hind wings of *N. ramburii.*

Effects	SS	DF	MS	F	SIGNIFICANCE
FOREWING					
Buru-un					
Sides	0.00177	54	3.28E-05	1.5207	Highly significant
Individuals x Sides	0.033748	1566	2.16E-05	4.1745	Highly significant
Measurementerror	0.033453	6480	5.16E-06	--	
Dalipuga					
Sides	0.003071	54	5.69E-05	2.4645	Highly significant
Individuals x Sides	0.036133	1566	2.31E-05	4.1212	Highly significant
Measurementerror	0.03628	6480	5.60E-06	--	
Puga-an					
Sides	0.006257	54	0.000116	7.1483	Highly significant
Individuals x Sides	0.025385	1566	1.62E-05	3.7673	Highly significant
Measurementerror	0.027882	6480	4.30E-06	--	
HINDWING					
Buru-un					
Sides	0.002238	66	3.39E-05	2.7433	Highly significant
Individuals x Sides	0.023654	1914	1.24E-05	2.1537	Highly significant
Measurementerror	0.045447	7920	5.74E-06	--	
Dalipuga					
Sides	0.003325	66	5.04E-05	2.7118	Highly significant
Individuals x Sides	0.035552	1914	1.86E-05	2.592	Highly significant
Measurementerror	0.092409	7920	1.17E-05	--	
Puga-an					
Sides	0.003203	66	4.85E-05	4.5224	Highly significant
Individuals x Sides	0.020541	1914	1.07E-05	3.9926	Highly significant
Measurementerror	0.021288	7920	2.69E-06	--	

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

A stressful environment could be a result of anthropogenic impacts as observed in the sampling sites. For instance, in Dalipuga, streams where the dragonflies were found had been converted already as a public washing area for the populace. Meanwhile, in Pugaan, most streams had been converted to quarry sites and in Buru-un it had been converted to local tourist's site. Table 4 shows the pH values and water temperature of the areas. It is hypothesized that human interventions resulting in ecological stress could have a potential impact on variation and developmental homeostasis of these organisms, resulting in high FA levels and high developmental instability in populations. According to studies, high environmental stress yields a low developmental stability; this is measured as a high level of FA (Bonada *et al.*, 2005).

Fig. 1. Geographical map of Iligan City showing the three barangays; Barangay Buru-un, Dalipuga and Pugaan.

Moreover, to examine variability of landmark points in tangent space principal component analysis (PCA) was done based on tangent coordinates derived from Procrustes analysis.

This is a way to visualize variation based on landmarks (Table 5 and Fig. 4 and 5). Principal component scores as exhibited by PC1 and PC2 show the total amount of overall variation for the fore-wings and hind wings respectively. The population from Brgy. Pugaan (85.06%; 60.01%) exhibited more variation, followed by Brgy. Dalipuga (85.30%; 59.52%), and both were relatively high compared to Brgy. Buru-un (56.33%; 39.06%).

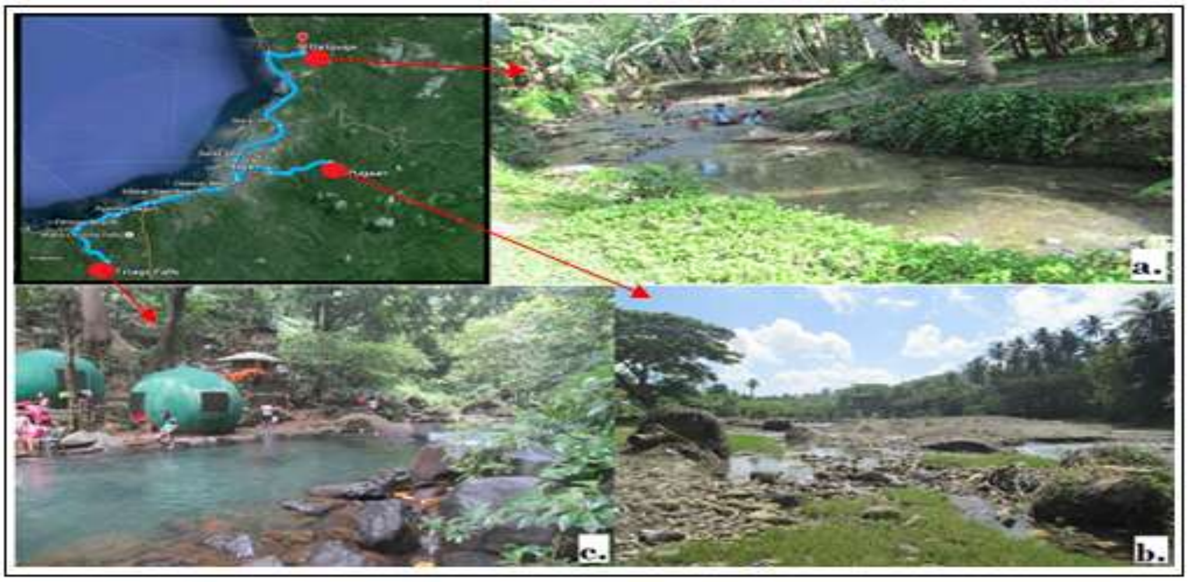

Fig. 2. Location of the three sampling sites: (a) Brgy. Dalipuga, (b.) Brgy. Pugaan, and (c) Brgy. Buru-un.

The fore-wings exhibit more variation that the hind wings. Fore-wings are more prone to adapt in environmental changes and geographic variation in wide-ranging species (Mayr, 1963). It is noted that PC1 depicts vectors at landmarks that show the magnitude and direction of the landmark as it is displaced relative to others. PC2 depicts the difference via the thin plate splines. This shows an interpolation function that models change between landmarks from the data of changes in coordinates of landmarks. Herewith, the red dots represent the morphological landmarks used in the study and the radiating 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, Ducos, 2014). The population from Pugaan exhibited more variation. Thus, higher FA was also exhibited by samples where, PC 1 accounts for most of the variation. The results obtained from principal component analysis supports the Procrustes FA analysis.

Furthermore, the analyses from the different populations provided evidence consistent with the idea that the wings may undergo compensatory development, and the fore-wings were much affected with ecological stress depicted by high value of overall principal component. Data on PCA suggested that the morphological features are said to be adaptive to changing ecological conditions. Significantly increased levels of FA in a population may indicate that individuals are having more difficulty maintaining particular development, resulting in negative effects on the population over time. Accordingly, overall, increased fluctuating asymmetry could be either related to environmental stress or with increased homozygosity (endogenous and exogenous) (Merila and Bjorklund, 1995).

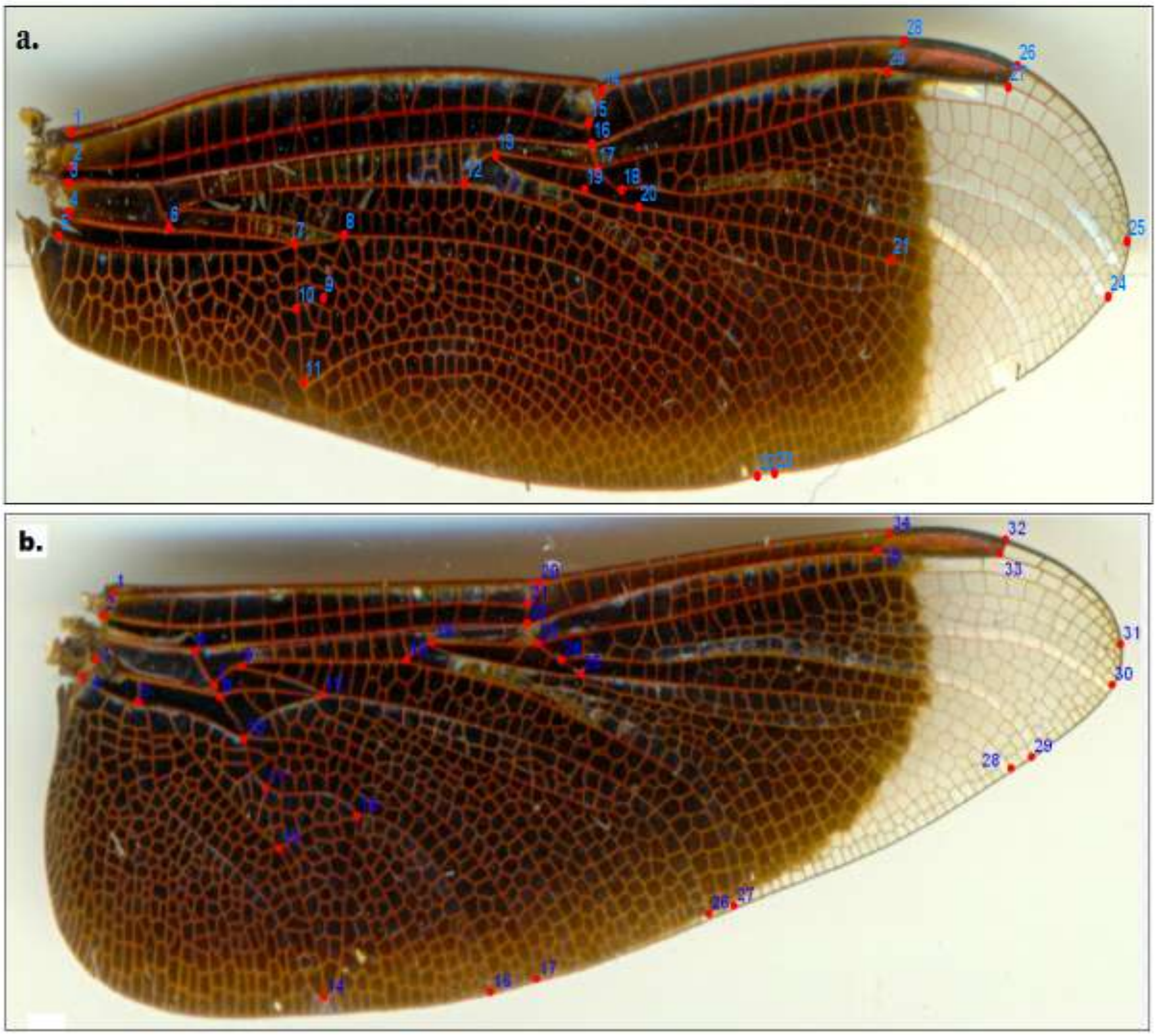

Fig. 3. Location of landmarks on the fore-wings and hind wings of *Neurothemis ramburii.*

Conclusion

This study shows the potential of FA in measuring developmental instability (DI) and stress in populations. This is based on the assumption that the right and left sides of the organism share the same genotype, and likely are influenced by the same environmental factors. Herewith, populations exhibit high FA are subject to DI based on FA analysis. Noteworthy, is that instability could be attributed to rapid and drastic changes due to increasing anthropogenic influences in habitat structure because of human interventions resulting in changes in dispersal processes and long term consequences on species distribution and morphological features of *N. ramburii.*

Thus, higher FA has been observed on areas more prone to environmental stressors such as Brgy. Pugaan and Dalipuga. Underlying reasons behind high FA may include stress as experienced by populations (endogenous and exogenous). A significant FA and DA suggest that stress and variation could be a product of genotype-environment interaction. Significant FA and increase FA present inability of species to buffer stress in its developmental pathways hence, would mean developmental instability and have implications on species fitness, adaptation and quality of individuals. Awareness and understanding on the nature, variation, adaptation and survival of odonata species which are good biological indicators for wetland assessments is essential.

References

Badyaev AV, Foresman KR, Fernandes MV. 2000. Stress and Developmental Stability: Vegetation Removal Caused Increased in Fluctuating Asymmetry in Shrews. Ecology **81,** 336-345.

Bonada N, Vives S, Rieradevall M, Prat N. 2005. Relationship between pollution and fluctuating asymmetry in the pollution-tolerant caddisfly *Hydropsyche exocellata* (Trichoptera, Insecta). Archiv für Hydrobiologie **162**, 167-185.

Cairns J, McCornick PV, Niederlehner BR. 1993. A proposed framework for developing indicators of ecosystem health. Hydrobiologia **263,** 1-44.

Carchini G, Chiarotti F, Domenico M, Mattoccia M, Paganotti G. 2001. Fluctuating asymmetry, mating success, body size and heterozygosity in *Coenagrion scitulum* (Rambur) (Odonata:Coenagrionidae). Animal Behaviour **61**, 661–669.

Carpentero ER, Tabugo SRM. 2014 Determining Developmental Instability via Fluctuating Asymmetry in the shell shape of *Arctica islándica* Linn. 1767 (ocean quahog). European Journal of Zoological Research **3,** 1-7.

Chang X, Zhaia B, Liu X, Wang M. 2007. Effects of temperature stress and pesticide exposure on fluctuating asymmetry and mortality of *Copera annulata* (selys) (Odonata: Zygoptera) larvae. Ecotoxicology and Environmental Safety **67,** 120-127.

Clarke GM. 1993. Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. Environmental Pollution **82,** 207–211.

Clarke GM. 1995. Relationships between developmental stability and fitness: application for conservation biology. Conservation Biology **9,** 18-24.

Clarke GM, McKenzie LJ. 1992. Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. Journal of Economical Entomology **85**, 2045-2050.

Ducos MB, Tabugo SR. 2014. Fluctuating asymmetry as an indicator of ecological stress and developmental instability of *Gafrarium tumidum* (ribbed venus clam) from Maak and Lagoon Camiguin Island, Philipines. AACL Bioflux **7,** 516-523.

Gangestad SW, Thornhill R. 1999. Individual differences in developmental precision and fluctuating asymmetry: a model and its implications. Journal of Evolutionary Biology **12**, 402–416.

Graham JH, Raz S, Hagit H, Nevo E. 2010. Fluctuating Asymmetry: Methods, Theory and Applications. Symmetry **2,** 466-495.

Hermita Z, Gorospe J, Torres M, Lumasag G, Demayo C. 2013. Fluctuating Asymmetry In The Body Shape Of The Mottled Spinefoot Fish, *Siganus fuscescens* (Houttuyn, 1782) Collected From Different Bays In Mindanao Island, Philippines. Science International **25**, 857-861.

Ho´dar J. 2002. Leaf fluctuating asymmetry of Holm oak in response to drought under contrasting climatic conditions. Journal of Arid Environments **52**, 233–243.

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.

Kutcher TE, Bried JT. 2014. Adult Odonata conservatism as an indicator of freshwater wetland condition Ecological Indicators **38**, 31-39.

Leary RF, Allendorf FW. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. Trends in Ecology and Evolution **4**, 214–217.

Lens L, Dongen VS, Kark S, Matthysen E. 2002. Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? Biological Reviews 77, 27-38.

Leung B, Forbes MR. 1996. Fluctuating asymmetry in relation to stress and fitness: effect of trait type as revealed by meta-analysis. Ecoscience **3**, 400-413.

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

Marquez E. 2006. Sage: symmetry and asymmetry in geometric data. Ver 1.04. Michigan: University of Michigan Museum of Zoology, 1-7.

Mayr E. 1963. Animal species and evolution. Cambridge, MA: Harvard University Press, 797.

McAdams HH, Arkin A. 1999. It's a noisy business! Genetic regulation at the nanomolecular scale. Trends in Genetics **15**, 65-69.

Merila J, Bjorklund M. 1995**.** Fluctuating asymmetry and measurement error. Systematics Biology **44,** 97-101.

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.

Moller AP. 1997. Developmental stability and fitness:a review. American Naturalist. **149,** 916-932.

Moller AP, Thornhill R. 1998. Bilateral symmetry and sexual selection: a meta-analysis. American Naturalist. **151**, 174-192.

Orbita M, Gumban N. 2013. Investigation of the community structure of seagrasses in the coastal areas of Iligan City,Mindanao, Philippines. AAB Bioflux **5,** 140-151.

Palmer RA. 1994. Fluctuating asymmetry analysis: a primer. In: MarkowTA (ed.) Developmental Instability:Its Origins and Evolutionary Implications. London: Kluwer Academic, 335–364.

Palmer AC, Strobeck C. 1986**.** Fluctuating asymmetry - measurement, analysis, patterns. Annual Review of Ecology and Systematics **17,** 391-421.

Palmer AR, Strobeck C. 2003. Fluctuating asymmetry analyses revisited. In: Polak M, Ed. Developmental Instability: causes and consequences. New York, USA: University Press, 279-280.

Parsons PA. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biological Reviews **65,** 131–145.

Parsons PA. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. Heredity **68**, 361–364.

Pinto N, Juen L, Cabette H, De Marco P Jr. 2012. Fluctuating asymmetry and wing size of *Argia tinctipennis* Selys (Zygoptera: Coenagrionidae) in relation to riparian forest preservation status. Neotropical Entomlogy **41**, 178-85.

Reeve ECR. 1960. Some genetic tests on asymmetry of sternopleural chaetae number in Drosophila. Genetical Research **1**, 151-172.

Ryazanova G, Polygalov A. 2013. Fluctuating asymmetry of wing venation in damselflies *Ischnura elegans* (V.d. Lind.) (Odonata, Coenagrionidae) and prospects of its use as a biological indicator of ecological quality of fresh-water reservoirs. Moscow University Biological Sciences Bulletin **68**, 195-199.

Samuels ML, Casella G, McCabe GP. 1991. Interpreting blocks and random factors: rejoiner. Journal of the American Statistical Association **86,** 798-808.

Samways MJ, Steytler NS. 1996. Dragonfly (Odonata) distribution patterns in urban and forest landscapes, and recommendations for riparian management Biological Conservation **78**, 279-288.

Tabugo SR, Casas PA, Pareño MT, Peñaredondo MA. 2015. Fluctuating Asymmetry and Developmental Instability in the Wings of *Neurothemis terminata* as Bioindicator of Stress. Advances in Environmental Biology **9**, 10-17.

Tataro SM, Tabugo SRM. 2015. Population Analysis via fluctuating asymmetry in the wings of *Culex quinquefasciatus* Say from selected breeding sites in Iligan City, Philippines. Journal of Biodiversity and Environmental Sciences **7**, 109-118.

Tennessen KJ. 2009. Odonata: Dragonflies, Damselflies. In: Resh VH, Cardé RT, Eds. Encyclopedia of Insects (Second Edition). MA, USA: Academic Press, 721-72.

Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. Nature **150,** 563-565.

Ware J, May M, Kjer K. 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): An exploration of the most speciose superfamily of dragonflies. Molecular Phylogenetics and Evolution **45**, 289-310.