Developmental instability in Odonata larvae in relation to water quality of Serdang River, Kedah, Malaysia

Salman A. Al-Shami[1,2]*, Siti Nurhanani Hishamuddin[1], Che Salmah Md Rawi[1], Nur Huda Abdul[1,] Abu Hassan Ahmad[1]

[1]School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
[2]Biology Department, Faculty of Science, University of Tabuk, P.O. Box 1458, Tabuk 7134, Saudi Arabia.
Email: alshami200@gmail.com

Abstract: We examined the fluctuating asymmetry (FA) in larvae of two Odonata genera; *Pseudagrion* sp. (Zygoptera: Coenagrionidae) and *Onychothemis* sp. (Anisoptera: Libelulidae) living in a relatively polluted river as a tool for water quality assessment. Larval and water samples were collected monthly from January to June 2008. Various water parameters including pH, temperature, velocity, nitrate, phosphate, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), and ammonium-N content were recorded. Composite effect of selected water parameters expressed as Water Quality Index (WQI) was also calculated. FA indices [(FA), absolute asymmetry (AbsFA), composite fluctuating asymmetry (CFA)] of the first and second antennal segments of *Onychothemis* sp. and and last tarsal segment of *Pseudagrion* sp. hind legs were calculated. We found that high FA levels in the selected traits for the two Odonata species were associated with deterioration in the water quality (WQI). BOD and pH were positively correlated with high FA indices in the antennal segments of *Onychothemis* sp. The FA levels calculated as FA indices of last tarsal segment of *Pseudagrion* sp. hind legs were positively correlated with ammonium-N, phosphate, and COD. We concluded that selected traits of the odonate taxa are useful bioindicators as the incidence of fluctuating asymmetry in their larvae was strongly associated with deterioration in the water quality of the river.
[Al-Shami S., Siti Nurhanani H., Che Salmah M. R., Salman A., Nur Huda A., Abu Hassan A. **Developmental instability in Odonata larvae in relation to water quality of Serdang River, Kedah, Malaysia.** *Life Sci J* 2014;11(7):152-159]. (ISSN:1097-8135). http://www.lifesciencesite.com. 19

Keywords: Odonata; Malaysia; fluctuating asymmetry; water quality.

1. Introduction

Developmental instability in the final morphology of organisms has been highlighted as a reliable indicator for environmental quality. Changes in the morphology of individuals including deformities, phenodeviations, and asymmetries have been included in recent environmental assessment studies in aquatic (Almeida et al. 2008; Al-Shami et al. 2010a, b; Al-Shami et al. 2011a, b; Arambourou et al. 2012) and terrestrial ecosystems (Pinto et al. 2012).

The final morphology characteristics of an organism are a result of delicate balance of various physiological, biochemical, genetics and environmental processes which may interfere during any stage of individual development and growth (Clarke 1993; Servia et al. 2004; Al-Shami et al. 2011a, b). Thus, developmental stability of any organism is indicated by its capability to maintain a normal shape under effects of certain conditions (Kozlov et al. 2002). Therefore, any deviation in the shape and/or size of any morphological traits is considered to be disruption in the developmental mechanisms due to genetic (Clarke 1993) or environmental factors (Al-Shami et al. 2011a).

Fluctuating asymmetry (FA) a common tool applied for measuring the developmental instability (Clarke 1993). According to Palmer (1994), FA is defined as the random deviation from typical symmetry of any bilateral anatomical character. Hence, FA always presents a normal distribution around the mean of zero (Palmer 1994). These departures apparently occur on the left or right side of an organism under stresses of environmental or genetic conditions which disrupt the proper development (Parson 1990).

Consequently, FA may offer an early warning tool to determine the effects of environmental stresses on organisms prior to critical changes in the population and community structures (Clarke 1993). Evidently, the association between occurrence of morphological and developmental instability and environmental quality has been addressed in aquatic ecosystems by using aquatic insects (chironomids: Al-Shami et al. 2010a; Al-Shami et al. 2011a, b; Arambourou et al. 2012; mayflies: Dobrin and Corkum1999; dragonflies: Carchini et al. 2000; Chang et al. 2007a, b) and fish (Almeida et al. 2008) as models.

Contamination with pesticides was suggested to be the most influential factor to cause FA in aquatic organisms. For example, Valentine and Soule (1973) had shown increasing FA of fin rays in grunion fry, a marine fish, exposed to increasing levels of p,p’-DDT in the laboratory. Similarly, Dobrin and Corkum

(1999) found that contamination of aquatic ecosystems with PCB may result with occurrence of FA in adult burrowing mayflies (*Hexagenia rigida*). However, they concluded that contaminant effects were difficult to detect in the field leading to unconfirmed results. Besides chemical toxicants, environmental factors such as temperature stress have been suggested to cause fluctuating asymmetry in Odonata larvae (Chang et al. 2007b).

Although tropical streams of South East Asia including Malaysia have been highlighted as hotspot for global diversity (Al-Shami, in press), pollution due to intensive agricultural and industrial development in this region continues at critical levels that readily endangering the natural biodiversity of aquatic flora and fauna (Dudgeon 2008). During the last decade, very few studies have been dedicated to investigate the adverse effects of pollution on aquatic insects at community (Azrina et al. 2006; Al-Shami et al. 2011c), individual (Al-Shami et al. 2010a; Al-Shami et al., 2011a, b) and molecular levels (Al-Shami et al. 2012). Indeed, such studies help in improving the bioindication principles as well as the general knowledge about the insects-toxicants response.

Odonata is one of the primitive and ancient insect orders. They are very diverse and considered as the second largest aquatic insect order in terms of species number as well as abundance (Nur Huda 2012). In Malaysia, several ecological studies have highlighted the potential of odonates (at community level) as indicators for environmental health assessment and conservation management (e.g. Che Salmah et al. 2004; Wahizatul Afzan 2004; Nur Huda 2012). This was facilitated by relatively complete taxonomical information of this group of aquatic insects in Malaysia (see Orr and Hamalainen 2003; Orr 2005). Although, application of the bioindication principles using Odonata at individual level has been studied worldwide (for example see Chang et al. 2007b), no such information is available from Malaysian streams.

Previously, we revealed that FA in chironomid larvae is associated with deterioration in the water quality and heavy metal pollution (Al-Shami et al. 2011a). However, no effort was made to investigate the relationship between FA levels and water quality in Malaysian streams by using larger aquatic insects such as Odonata. We assume that the FA occurrence as well as severity levels may show different patterns in larger organism (i.e. odonates) compared to small organisms (i.e. chironomids). Thus, the present study aimed at investigating the FA patterns in two tolerant Odonata species (*Pesudagrion* sp. and *Onychothemis* sp.) (Orr 2005) which were common in Malaysian rivers. We intended to relate the levels of FA (expressed as FA indices in two traits for each Odonata species) with water quality in a relatively polluted Serdang River, Kedah to confirm their suitability as a water quality monitoring tool in such river.

2. Materials and Methods

A relatively small Serdang River (11 m to 12.5 m wide) is one of the tributaries of Kerian River that borders three states in the northern Peninsular Malaysia; Penang, Kedah and Perak (Fig. 1).

The study area, located at N05° 12’ 20.84” longitude and E100° 36’ 34.27” latitude passed through urban and residential areas receiving many anthropogenic wastes from both places (Rawi et al. 2001; Che Salmah et al. 2004; Wahizatul Afzan 2004; Nur Huda 2012). The water quality was further worsened by discharges from an aquaculture pond located at one side of the river. Semi aquatic weeds such as para grass (*Branchiara* sp.) and alligator weed (*Altenanthera* sp.) grew on both sides of the river banks. Enriched river substrate was covered by dense growths of a submerged aquatic weed, *Hydrilla verticillata*. Water surface of this area was partly shaded by leaves of oil palm (*Elaeis guineensis*) trees planted on one side of the river bank. The water was relatively shallow with a depth ranging from 0.1 m to 1.5 m during raining season.

Physico-chemical parameters and water quality index

Water pH, water temperature, velocity, depth and width of the river and dissolved oxygen (DO) were measured *in situ* on each sampling occasion. Dissolved oxygen and water temperature were measured with a YSI-57 meter, whereas the pH of water was taken with a Thermo-Orion model 210 pH meters. Depth and width of the river were measured by using a metal ruler and a measuring tape respectively, while water velocity was measured by a Hydroprob flow-meter (MK11-90cms^{-1}). Three replicates of water samples were collected at random places along the river into 500ml plastic bottles for analyses of biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, ammonium-N, and total suspended solids (TSS). Each bottle was thoroughly rinsed out with river water immediately prior to collecting a sample. The samples were transported under cool condition in a Coolman® chest, and in the laboratory, they were stored at 4^0C for further processing. COD was analyzed by using a calorimeter HDR 890 which was also used to measure nitrate and phosphate contents. Meanwhile, BOD_3 was measured with a YSI Pro-BOD Probe (YSI 550A). The Water Quality Index (WQI) was calculated to categorize the water quality based on previous description (see Al-Shami et al. 2011c).

Sampling of Odonata Larvae

Odonata larvae were collected from Serdang River on monthly basis using a modified kick sampling technique (Merritt and Cummins 1996) from January to June 2008 by sampling benthos along an approximately 300m stretch of the river using a D-shaped aquatic net (20 cm radius, 40 cm wide, and 300-μm mesh size). All benthic materials collected during each sampling effort were transferred into labelled plastic bags and transported to the laboratory for sorting and identification. In the laboratory, Odonata larvae were sorted and preserved in 75% ethanol for subsequent taxonomic identification and measurement.

All odonate specimens in the collections were identified using taxonomic keys of Lieftincks (1964), Fonseka (2000), Okudaira *et al.* (2001),Yule and Yong (2004), Orr (2005) and Morse et al. (1994). In the present study, anisopteran *Onychothemis* sp. (Libellulidae) and zygopteran *Pseudagrion* sp. (Coenagrionidae) were found to be the most dominant at the study site thus they were selected for fluctuating asymmetry investigation. *Onychothemis* sp. was mainly represented by *O. testacea* and there were at least two species of *Pseudagrion* (*P. pruinosum* and *P. microcephalum*) reported from Serdang River (Wahizatul Afzan 2004). During larval stages these species were difficult to separate consequently they were only identified to genera.

Measuring of Fluctuating Asymmetry

When possible, late larval instars were included in this study. Two morphological traits of *Onycothemis* sp., lengths of first and second antennal segments and length of the last tarsal segment on the hind legs of *Pseudagrion* sp. were examined. For all the traits, the measurements were taken twice following the general procedures as demonstrated by Palmer (1994). The specimens were examined under an Olympus CX41 microscope equipped with a digital camera (Olympus M-TV0.63XC). We measured all selected traits on the right and left sides of the specimens using the Digital Imaging and Measurement System, VIS ver. 3.00 (PLUS).

Measurement Error

Prior to analysis of the FA data, several statistical assumptions were considered. The data were examined visually for possible outliers that are common sources of skewness or leptokurtosis using Box plots of FA as demonstrated by Palmer (1994) to distinguish the presence of a deformity from an asymmetry (Bonada and Williams 2002). Then, ANOVA for directional asymmetry (DA) and antisymmetry that might associate with FA and measurement error (ME) (Palmer 1994) were conducted. The ME was calculated for each trait using two-way ANOVA with *side* (left/right) and *individual* as random factors at significance level of 0.05. Significance of the factor *side* in the two-way ANOVA (P<0.05) would reveal the existence of directional asymmetry. Antisymmetry was detected through testing the normality of signed fluctuating asymmetry (R-L) distribution using Kolmogrov-Smirnov test at P<0.05. Unreliable FA level would result if dependence of FA on trait size was detected (Palmer 1994). For this purpose, linear regression of FA [|R-L|] versus trait size [(R+L)/2] was conducted.

According to Pither and Taylor (2000), the error in photographic procedures should be examined. Hence, ten individuals were randomly selected and photographed in duplicates (for both right and left sides). Then, data obtained for each trait were analysed independently using a nested-factorial ANOVA with *photo* and *individual* as random-effect factors (*photo* nested within *individual*), *side* as factorial or crossed factor, and *repeated measurement* as the residual error. In this investigation, we found no significant effect of *photo* (ANOVA, P>0.05).

Calculations of FA Indices and Statistical Analysis

Basically, FA levels were calculated as the mean difference between right and left sides measurments [R-L] for each trait (Palmer 1994). However, Absolute FA (AbsFA) was expressed as $|R\text{-}L|/\ [(R+L)/2]$, and composite index of asymmetry was provided by Leung et al. 2000) as CFA=$|R\text{-}L|$/AVG $|R\text{-}L|$.

The ME_{SI} is the mean square of (*side* x *individual*) interaction and MS_{error} is the average difference between repeated measurements (error). All ANOVAs were carried out using the SPSS software (Version 17). Meanwhile, the redundancy analysis (RDA) of CANOCO program (ter Braak 1989) was used to examine the relationship between FA indices and water quality.

Calculation of water quality index (WQI)

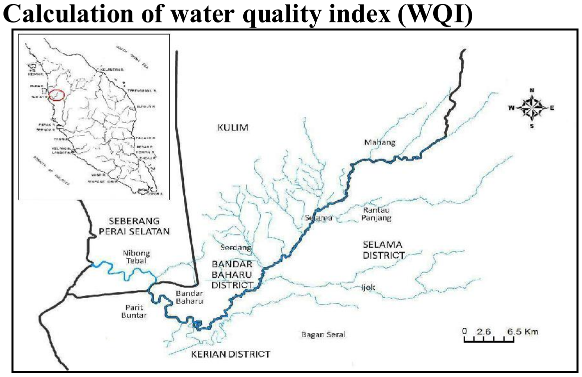


Figure 1 Locality of Serdang River (SR) in Kerian River Basin, Kedah, Malaysia.

The mean values of the six variables and parameters, namely DO (dissolved oxygen), BOD (biochemical oxygen demand), COD (chemical oxygen demand), pH, ammonium-N (AN) and total suspended solids (SS) were used for the computation of the water quality index (WQI). The values were converted to the sub indices (SIs) using the best-fit equations and aggregated to compute the WQI according to the following equation:

$$WQI = 0.22 \times SI_{DO} + 0.19 \times SI_{BOD} + 0.16\times SI_{COD} + 0.15 \times SI_{AN} + 0.16 \times SI_{TSS} + 0.12 \times SI_{pH}$$

where SI is the sub-index of each parameter.

3. Results

A total of 164 larvae of *Onychothemis* sp. and 120 of *Pseudagrion* sp. were collected from Serdang River and examined for FA occurrence (January-June 2008). In this study, we reported 9.33% of outliers. For each trait and each genus the two way ANOVA results showed significant difference between-side variation ($P<0.05$). For all traits and species, results of two-way ANOVA indicated no significant difference between sides implying lack of directional asymmetry (DA). Kolmogrov–Smirnov tests on signed (R-L) differences for both species showed that all traits were normally distributed ($P>0.05$). Linear regression results revealed absoulte independence of measured FA on trait size for both species.

For each trait, results of the two-way ANOVA on the interaction measurements [*side* x *individual*] confirmed that between-side variations were significant ($P<0.01$). As shown in Tables 1 and 2, the two-way ANOVA revealed that there was no significant difference ($P>0.05$) between-sides (right and left) in all the investigated traits for the two species. However, there was always significant differences in the between-individuals variations ($P<0.05$). Table 4 summarizes the data of various water physical and chemical parameters including calculated WQI in Serdang River.

Table 1 Results of two-way ANOVA (error) performed for each traits of *Onychothemis* sp. larvae (length of the first and second segments of antennae) collected from Serdang River, Malaysia from January to June 2008. Significance in the interaction of *side* x *individual* indicates presence of FA.

Month	Trait	Mean square (ANOVA)		
		side (ME_s)	*individual* (ME_I)	*side x individual* (ME_{SI})
January	Seg I	128.74	322.95**	209.18*
	Seg II	759.05	629.37**	155.26**
February	Seg I	14514.88	12789.23**	12905.11*
	Seg II	3117.34	668.18*	47.61*
March	Seg I	199.15	64.76**	11.76**
	Seg II	970.25	275.55**	21.62**
April	Seg I	22.49	119.14**	81.3*
	Seg II	3.392	102.87**	8.04**
May	Seg I	390.67	336.7**	27.07**
	Seg II	111.33	447.85**	8.81**
June	Seg I	6.603	146.93**	10.16**
	Seg II	246.96	86.1**	11.68**

*$P<0.05$, **$P<0.01$.

Table 2 Results of two-way ANOVA (error) performed for the last tarsal segment on hind leg of *Pseudagrion* sp. larvae collected from Serdang River, Malaysia from January to June 2008. Significance in the interaction of *side* x *individual* indicates presence of FA.

Month	Trait	Mean square (ANOVA)		
		side (ME_s)	*individual* (ME_I)	*side x individual* (ME_{SI})
January	Last tarsus	11.54	9322.94**	63.51**
February	Last tarsus	1130.7	1775.11**	192.34**
March	Last tarsus	6374.57	2950.83**	36.36**
April	Last tarsus	800.54	7380.99**	13.62**
May	Last tarsus	232.85	1158.25*	24.61*
June	Last tarsus	4515.01	4490.68*	40.98**

*$P<0.05$, **$P<0.01$.

Monthly descriptive results of selected traits of *Pseudagrion* sp. and *Onychothemis* sp. were presented in Appendices 1 and 2. All fluctuating asymmetry indices including FA |R-L|, AbsFA |R-L|/ [(R+L)/2] and Composite Fluctuating Asymmetry (CFA) |R-L|/AVG|R-L| were tabulated in Table 3.

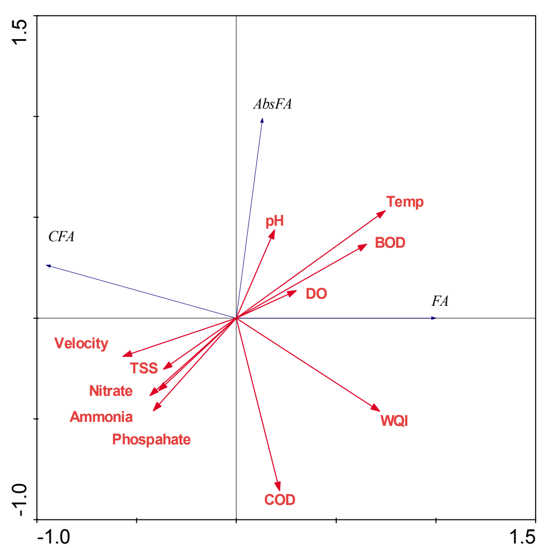
The results of multivariate analysis (i.e. RDA) and the FA indices of the investigated traits in the two species are presented in Fig. 2. Fig. 2a shows the ordination biplot of redundancy analysis (RDA) between various FA indices for the first antennal segment in *Onychothemis* sp. larvae and environmental parameters including WQI (Fig. 2a). The first two axes of RDA explained together more than 70% of the total variation in the relationship. As depicted in Fig. 2a, BOD, temperature, DO and pH were positively correlated with the AbsFA. However, there were negative correlation between WQI and COD with CFA. Fig. 2b displayed the ordination biplot of RDA between environmental parameters including WQI and FA indices for the second antennal segment of *Onychothemis* sp. larvae. The first two RDA axes explained more than 75%. BOD, temperature and pH showed strong and positive association with AbsFA. Similarly, TSS, nitrate, ammonium-N and phosphate were positively correlated with CFA. The water quality expressed as WQI was negatively correlated with CFA.

The ordination biplot of RDA of the relationship between FA indices of last tarsal segment for hind leg of *Pseudagrion* sp. and environmental parameters is illustrated in Fig. 2c. We found that RDA revealed remarkable and positive correlation between phosphate, ammonia and COD and CFA. The WQI showed weak yet negative relationship with AbsFA.

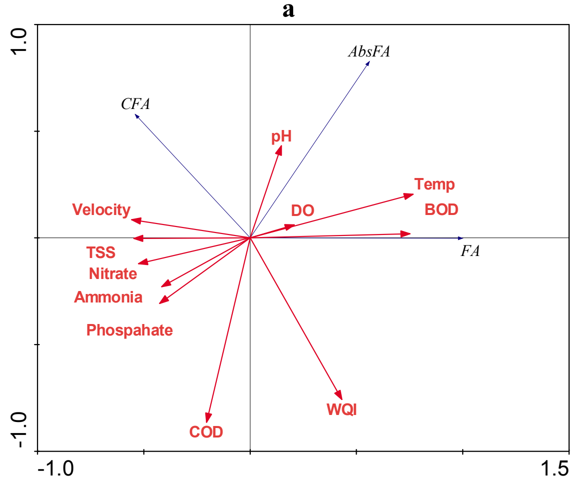
Table 3 Fluctuating asymmetry indices (Mean±SE) for each traits of *Onychothemis* sp. (length of the first and second segments of antennae) and the last tarsal segment on the hind leg of *Pseudagrion* sp. collected from Serdang River, Malaysia from January to June 2008.

Month	*Onychothemis* sp. traits	FA	AbsFA	CFA	*Pseudagrion* sp. traits	FA	AbsFA	CFA
January	Seg I	13.51±1.72	0.67±0.01	1.0±0.13	Last tarsus	41.05±13.96	0.07±0.02	1.0±0.34
	Seg II	17.06±2.42	0.77±0.11	1.0±0.94				
February	Seg I	28.41±16.61	0.10±0.02	0.99±0.58	Last tarsus	44.43±7.29	0.08±0.01	1.0±0.16
	Seg II	17.34±2.27	0.08±0.01	0.99±0.13				
March	Seg I	7.02±0.85	0.06±0.01	1.0±0.12	Last tarsus	27.99±4.45	0.062±0.01	1.0±0.16
	Seg II	10.12±1.98	0.07±0.01	1.0±0.20				
April	Seg I	6.62±1.69	0.06±0.01	1.0±0.26	Last tarsus	31.71±12.78	0.05±0.02	1.0±0.4
	Seg II	7.40±0.99	0.05±0.01	1.0±0.13				
May	Seg I	10.25±2.12	0.07±0.02	1.0±0.21	Last tarsus	24.07±6.59	0.03±0.01	1.0±0.27
	Seg II	14.22±2.07	0.09±0.01	1.0±0.15				
June	Seg I	8.62±1.65	0.05±0.01	1.0±0.19	Last tarsus	51.23±14.58	0.07±0.02	1.0±0.28
	Seg II	7.09±1.41	0.04±0.001	1.0±0.20				

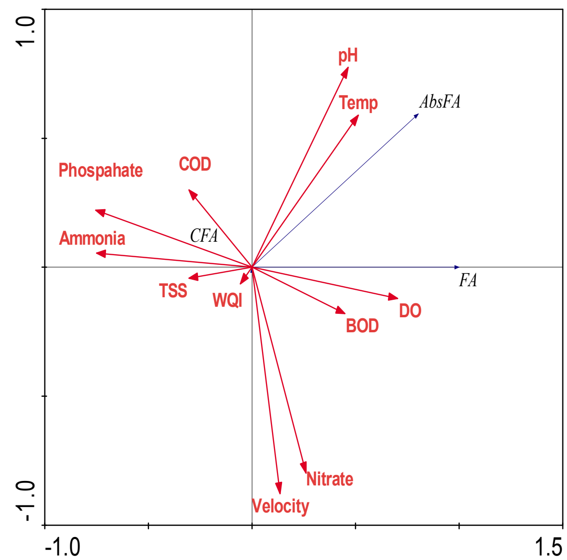
FA= |R-L|, Absolute asymmetry, AbsFA = |R-L|/ [(R+L)/2], Composite Fluctuating Asymmetry, CFA = |R-L|/AVG|R-L|.



a



b



c

Figure 2 Ordination plots of redundancy analysis (RDA) showing the relationship between environmental parameters including WQI with FA indices in *Onychothemis* sp. (**a:** length of the first and **b:** second segments of antennae) and *Pseudagrion* sp. (**c:** length of the last tarsal segment for hind leg) collected from Serdang River, Malaysia from January to June 2008.

Table 4: Mean of WQI and environmental parameters measured from January to June 2008 in Serdang River, Kedah.

Parameter	January	February	March	April	May	June
Temperature (°C)	26	26	24.7	23.6	24	24.5
pH	6.09	6.02	6.09	5.91	5.8	6
DO (mg/l)	6.58	6.58	6.29	5.7	6.37	7.03
BOD (mg/l)	1.85	1.87	0.45	0.31	1.65	1.42
COD (mg/l)	41	52	51	50	48	46
TSS (mg/l)	10	22	40	164	16	15
Ammonia (mg/l)	0.05	0.07	0.13	0.18	0.11	0.04
Phosphate (mg/l)	1.9	2.6	4.3	4.5	3.2	1.9
Nitrate (mg/l)	0.07	0.07	0.11	0.15	0.35	0.55
velocity (m/sec)	0.36	0.28	0.34	0.4	0.49	0.57
WQI	62.08	93.26	61.54	82.78	94.61	66.02
WQI category	Moderate	Excellent	Moderate	Good	Very good	Moderate

4. Discussion

We found high percentage (9.33%) of outliers which far exceeded those observed in similar studies by Bechshoft et al. (2008) who identified only 2.1% while Palmer and Strobeck (2003) reported only 5.7% of their data were outliers. However, this percentage was lower than previously recorded outliers by Al-Shami et al. (2011a) in chironomid larvae (16.18 %) collected from Juru River in Malaysia. It was suggested that high percentage of outliers were related to occurrence of deformities (Servia et al. 2002, 2004; Al-Shami et al. 2011a). In that case, lower percentage of outliers in this study might indicate lesser occurrence of deformities in Odonata larvae inhabiting (moderately polluted) Serdang River compared to chironomid larvae in (severely polluted) Juru River.

During the last decade, there are increasing efforts to design and develop more reliable and practical bioindication tools to monitor the pollution aquatic environments. Although application of Odonata larvae as a bioindicator for ecosystems health and integrity assessment in Malaysia has a relatively long history (c.f. 20 years), all of biomonitoring efforts was exclusively dedicated to examine changes in the community diversity and structure (Che Salmah et al. 2004; Nur Huda 2012). Examination of changes at individual level are known to be more useful and reliable for monitoring programmes compared to those conducted at community level (Petersen and Petersen 1983). Except for chironomid larvae (Al-Shami et al. 2010a, b) no study was attempted to investigate the effect of pollution in Malaysian rivers using other aquatic insects (e.g. Odonata) at individual level.

In Malaysia, several studies have demonstrated that the association of morphological changes in individuals with ecosystem disturbance is due to organic and inorganic pollution (Al-Shami et al. 2010a, b; Al-Shami et al. 2011a). Our recent reports (Al-Shami et al. 2011c, Al-Shami et al. 2012) revealed that the contamination with heavy metals is the major cause of damage in the DNA of aquatic organisms. Thus, we here suggested that the genotoxic effects due to pollution for the organismal cells would result with inappropriate development and functioning of various organs which ultimately lead to developmental instability (fluctuating asymmetry or morphological deformation).

Generally, the direct relationship between FA and environmental pollution and stresses remains unclear due to the fact that it was always difficult to prove which pollutant is responsible for developmental instability in aquatic organism. Despite that, some studies proved that contamination with heavy metals as well as with organic pollutants are the major factors influencing the normal development of aquatic insects (Al-Shami et al. 2011a). For example, several studies (see Bonada and Williams 2002; Chang et al. 2007a; Ambo-Rappe et al. 2008) revealed a clear relationship between fluctuating asymmetry of aquatic organisms and environmental pollution or stressors. The present findings showed that FA indices in the selected traits of Odonata genera (*Onychothemis* sp. and *Pseudagrion* sp.) were negatively correlated with water quality (expressed as WQI) in Serdang River. This trend was similar to what we reported earlier using chironomid larvae (Al-Shami et al. 2011a). Furthermore, other studies documented the effects of several organic and inorganic substances, including heavy metals on wing pads of Odonata (Chang et al. 2007a). Similarly, Chang et al. (2007a) reported that there were significant and negative associations between mortality of *Copera annulata* (Odonata: Zygoptera) and fluctuating asymmetry levels reflecting the adverse effects of environmental toxicants. Bonada and Williams (2002) found that high levels of fluctuating asymmetry in caddisfly larvae was associated with increasing concentrations of aquatic pollutants including nitrogen compounds.

As shown in RDA outputs, FA indices of the selected traits in odonates *Onychothemis* sp. and *Pseudagrion* sp. were strongly and positively associated with BOD and COD. Hence, this would support the evidence that organic pollution (indicated by high levels of BOD and COD) would induce the incidence of FA in Odonata larvae. Specifically for *Onychothemis* sp., the developmental instability indicated by the CFA index of the antennal segments (1st and 2nd) showed positive correlation with total suspended solids (TSS), nitrate, phosphate and ammonia. Thus, this implies that the fluctuating asymmetry in *Onychothemis* sp. antennae was strongly induced by organic pollution. Similar findings were reported on *Chironomus* spp. larvae by Al-Shami et al. (2011a) as they found that the fluctuating asymmetry in *Chironomus* spp. antennae was positively correlated with high concentrations of TSS, ammonia and nitrate. According to Servia et al. (2004), polluted water caused high percentages of antennal deformities in aquatic chironomid larvae. Thus, the antennae can be considered as the most susceptible structure in aquatic insect immatures and their development is readily affected by pollution. However, relatively strong associations of AbsFA and FA with water temperature, pH, BOD and COD were observed in *Psedagrion's* last tarsal segment of the hind legs.

Our results from this investigation strongly suggested the application of fluctuating asymmetry in aquatic insects as one of reliable biomonitoring tools for assessing environmental pollution and stresses. In this context, however, we emphasized that the developmental instability (fluctuating asymmetry incidence) depended on three main factors; 1) type of pollutants, 2) concentration of the pollutants and 3) the traits examined as structures of different organisms may respond to environmental stress differently.

References

1. Almeida, D., Almodóvar, A., Nicola, G. & Elvira, B. (2008). Fluctuating asymmetry, abnormalities and parasitism as indicators of environmental stress in cultured stocks of goldfish and carp. *Aquaculture,* 279, 120-125.
2. Al-Shami, S. A., Heino, J., Che Salmah, M. R., Abu Hassan, A., Suhaila, A. H. & Madrus, M. R. (In press). Drivers of beta diversity of macroinvertebrate communities in tropical forest streams. Freshwater Biology.
3. Al-Shami, S., Rawi, C.S.M., Nor, S.A.M., Ahmad, A.H. & Ali, A. (2010a). Morphological deformities in *Chironomus* spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in the Juru River system, Penang, Malaysia. *Environmental Entomology,* 39, 210-222.
4. Al-Shami, S.A., Rawi, C.S.M., Abu Hassan, A. & Nor, S.A.M. (2010b). Distribution of Chironomidae (Insecta: Diptera) in polluted rivers of the Juru River Basin, Penang, Malaysia. *Journal of Environmental Sciences,* 22, 1718-1727.
5. Al-Shami, S.A., Che Salmah, M.R., Abu Hassan, A. & Siti Azizah, M.N. (2011a). Fluctuating asymmetry of *Chironomus* spp.(Diptera: Chironomidae) larvae in association with water quality and metal pollution in Permatang Rawa River in the Juru River Basin, Penang, Malaysia. *Water, Air and Soil Pollution,* 216, 203-216.
6. Al-Shami, S.A., Salmah, M.R.C., Hassan, A.A. & Azizah, M.N.S. (2011b). Evaluation of mentum deformities of *Chironomus* spp.(Chironomidae: Diptera) larvae using modified toxic score index (MTSI) to assess the environmental stress in Juru River Basin, Penang, Malaysia. *Environmental Monitoring and Assessment,* 177, 233-244.
7. Al-Shami, S.A., Md Rawi, C.S., Ahmad, A.H., Abdul Hamid, S. & Mohd Nor, S.A. (2011c). Influence of agricultural, industrial, and anthropogenic stresses on the distribution and diversity of macroinvertebrates in Juru River Basin, Penang, Malaysia. *Ecotoxicology and Environmental Safety,* 74, 1195-1202.
8. Al-Shami, S.A., Rawi, C.S.M., Ahmad, A.H. & Nor, S.A.M. (2012). Genotoxicity of heavy metals to the larvae of *Chironomus kiiensis* Tokunaga after short-term exposure. *Toxicology and Industrial Health,* 28, 734-739.
9. Ambo-Rappe, R., Lajus, D.L. & Schreider, M.J. (2008). Increased heavy metal and nutrient contamination does not increase fluctuating asymmetry in the seagrass *Halophila ovalis. Ecological Indicators,* 8, 100-103.
10. Arambourou, H., Beisel, J.N., Branchu, P. & Debat, V. (2012). Patterns of Fluctuating Asymmetry and Shape Variation in *Chironomus riparius* (Diptera, Chironomidae) Exposed to Nonylphenol or Lead. *PloS one,* 7, e48844.
11. Azrina, M., Yap, C., Rahim Ismail, A., Ismail, A. & Tan, S. (2006) Anthropogenic impacts on the distribution and biodiversity of benthic macroinvertebrates and water quality of the Langat River, Peninsular Malaysia. *Ecotoxicology and Environmental Safety,* 64, 337-347.
12. Bechshoft, T.O., Riget, F.F., Wiig, O. & Sonne, C. (2008). Fluctuating asymmetry in metric traits; a practical example of calculating asymmetry, measurement error, and repeatability. In: *Annales Zoologici Fennici.* 32-38.
13. Bonada, N. & Williams, D.D. (2002). Exploration of the utility of fluctuating asymmetry as an indicator of river condition using larvae of the caddisfly *Hydropsyche morosa* (Trichoptera: Hydropsychidae). *Hydrobiologia,* 481, 147-156.
14. Carchini, G., Chiarotti, F., Di Domenico, M. & Paganotti, G. (2000). Fluctuating asymmetry, size and

mating success in males of *Ischnura elegans* (Vander Linden)(Odonata: Coenagrionidae). *Animal Behaviour,* 59, 177-182.
15. Chang, X., Zhai, B., Liu, X. & Wang, M. (2007a). 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.
16. Chang, X., Zhai, B., Wang, M. & Wang, B. (2007b). Relationship between exposure to an insecticide and fluctuating asymmetry in a damselfly (Odonata, Coenagriidae). *Hydrobiologia,* 586, 213-220.
17. Che Salmah, M.R., Abu Hassan, A. & Ameilia, Z.S. (2004). Odonate communities (Odonata: Insecta) in a tropical river basin, Malaysia. *Wetland Science,* 2, 1-9.
18. Clarke, G.M. (1993). Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality. *Environmental Pollution,* 82, 207-211.
19. Dobrin, M. & Corkum, L.D. (1999). Can fluctuating asymmetry in adult burrowing mayflies (*Hexagenia rigida*, Ephemeroptera) be used as a measure of contaminant stress? *Journal of Great Lakes Research,* 25, 339-346.
20. Dudgeon, D. (2008). *Tropical stream ecology,* Academic Press.
21. Fonseka, T. (2000). *The Dragonflies of Sri Lanka,* WHT Publications.
22. Kozlov, M., Niemelä, P. & Mälkönen, E. (2002). Effects of compensatory fertilization on pollution-induced stress in Scots pine. *Water, Air and Soil Pollution,* 134, 305-316.
23. Leung, B., Forbes, M.R. & Houle, D. (2000). Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *The American Naturalist,* 155, 101-115.
24. Lieftinck, M. (1964). Some Gomphidae and their larvae, chiefly from the Malay Peninsula (Odonata). *Zoologische Verhandelingen,* 69, 1-38.
25. Morse, J.C., Yang, L. & Tian, L. (1994). *Aquatic insects of China useful for monitoring water quality,* Hohai University Press.
26. Nur Huda, A. (2012). Macroinvertebrates distribution in relation to water quality in the middle reach of Kerian River Basin. Unpublished M. Sc. thesis, Universiti Sains Malaysia, Malaysia.
27. Okudaira, M., Sugimura, M., Ishida, S., Kojima, K., Ishida, K. & Aoki, T. (2001). *Dragonflies of the Japanese archipelago in color,* Hokkaido University Press.
28. Orr, A.G. (2005). *Dragonflies of Peninsular Malaysia and Singapore,* Natural History Publications (Borneo).
29. Orr, A.G. & Hamalainen, M. (2003). *A guide to the dragonflies of Borneo: their identification and biology,* Natural History Publications (Borneo).
30. Palmer, A. (1994). *Fluctuating asymmetry: A primer*. In T. A. Markow (Ed.), Developmental instability: Its origins and evolutionary implications (pp. 335–364). Dordrecht: Kluwer.
31. Palmer, A.R. & Strobeck, C. (2003). Fluctuating asymmetry analyses revisited. In: *Developmental instability: causes and consequences.* (Ed. M. Polak), pp. 279-319. Oxford University Press.
32. Parsons, P. (1990). Fluctuating asymmetry: an epigenetic measure of stress. *Biological Reviews,* 65, 131-145.
33. Petersen, L.B.M. & Petersen Jr, R.C. (1983). Anomalies in hydropsychid capture nets from polluted streams. *Freshwater Biology,* 13, 185-191.
34. Pinto, N., Juen, L., Cabette, H.S.R. & De Marco, P. (2012). Fluctuating Asymmetry and Wing Size of *Argia tinctipennis* Selys (Zygoptera: Coenagrionidae) in Relation to Riparian Forest Preservation Status. *Neotropical Entomology,* 41, 178-185.
35. Pither, J. & Taylor, P.D. (2000). Directional and fluctuating asymmetry in the black-winged damselfly Calopteryx maculata (Beauvois)(Odonata: Calopterygidae). *Canadian Journal of Zoology,* 78, 1740-1748.
36. Rawi, M., Salmah, C., Zs, A. & Ahmad, A.H. (2001). Preliminary Distribution of Ephemeroptera, Plecoptera and Trichoptera (EPT) in Kerian River Basin, Perak, Malaysia. *Pertanika Journal of Tropical Agricultural Science,* 24, 101-107.
37. Servia, M.J., Cobo, F. & Gonzalez, M.A. (2002). Ontogeny of individual asymmetries in several traits of larval *Chironomus riparius* Meigen, 1804 (Diptera, Chironomidae). *Canadian Journal of Zoology,* 80, 1470-1479.
38. Servia, M., Cobo, F. & González, M. (2004). Multiple-trait analysis of fluctuating asymmetry levels in anthropogenically and naturally stressed sites: a case study using *Chironomus riparius* Meigen, 1804 Larvae. *Environmental Monitoring and Assessment,* 90, 101-112.
39. ter Braak, C.J.F. (1989). CANOCO—an extension of DECORANA to analyze species-environment relationships. *Hydrobiologia,* 184, 169-170.
40. Valentine, D. & Soule, M. (1973). Effect of p, p'-DDT on developmental stability of pectoral fin rays in the grunion, *Leuresthes tenuis*. *Fishery Bulletin,* 71, 921-926.
41. Wahizatul Afazan, A. (2004). Some aspects of ecology of running water odonates as indicator of environmental quality. Unpublished M. Sc. thesis, Universiti Sains Malaysia, Malaysia.
42. Yule, C.M. & Yong, H.S. (2004). *Freshwater Invertebrates of the Malaysian Region* Akademi Sains Malaysia, Kuala Lumpur.

4/11/2014