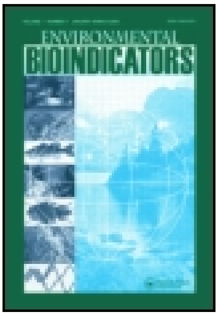

Environmental Bioindicators
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uebi20

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Available online: 31 Jul 2008

To cite this article: Rohani Ambo-Rappe, Dmitry L. Lajus & Maria J. Schreider (2008): Higher Fluctuating Asymmetry: Indication of Stress on *Anadara trapezia* Associated with Contaminated Seagrass, Environmental Bioindicators, 3:1, 3-10

To link to this article: http://dx.doi.org/10.1080/15555270701779460

Environmental Bioindicators, 3:3–10, 2008

ISSN: 1555-5275 print/ 1555-5267 online
DOI: 10.1080/15555270701779460

Higher Fluctuating Asymmetry: Indication of Stress on *Anadara trapezia* Associated with Contaminated Seagrass

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Seagrasses are marine angiosperms that colonize near-shore environments. Concern has arisen over increasing concentrations of heavy metals in these systems resulting from industrial and urban development due to the ability of seagrass to accumulate trace metals from the environment without showing any impact on their productivity. This may pose a threat to a coastal community because the polluted seagrass will then provide a source of contamination to seagrass consumers. The main aim of this study was to determine whether there was any detectable effect of heavy metal pollution in seagrass on associated fauna. Fluctuating asymmetry of shell structure of a bivalve, Anadara trapezia, *were employed as biomarkers for this environmental study. The result from this study revealed that* A. trapezia *showed distinct morphological characters and high shell asymmetry in the polluted location. Thus,* A. trapezia *associated with seagrass may be responsive to heavy metal stress and possibly a good indicator of heavy metal pollution in this system. The present study discusses the possibility of using a more cost-effective biomarker to define areas of heavy metal pollution.*

Keywords fluctuating asymmetry, seagrass, bivalve, bioindicator

Introduction

Seagrasses constitute an important component of consumer's diet in the coastal environment (Hemminga and Duarte 2000). However, the ability of seagrass to accumulate a high concentration of heavy metals without showing any impact on its productivity (Ward 1989; Marin-Guirao et al. 2005), may pose a threat to a coastal or estuarine community. This is because the polluted seagrass will then provide a source of contamination to seagrass consumers, which have the potential to sequester metals from leaf, root-rhizome and detrital material, posing threats to coastal resources through bioaccumulation and subsequent effects on trophic relationship (Klumpp et al. 1989).

Received 1 June 2007; accepted 30 October 2007.
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Anadara trapezium live in the contaminated seagrass of Lake Macquarie were found to accumulate high concentrations of heavy metals (Batley 1987; Barwick and Maher 2003). It has been generally known that some bivalve molluscs have the ability to accumulate trace metals without lethal effects. This has led to universal acceptance of this fauna as an indicator of the biologically available trace metals in the water column (Klumpp and Burdon-Jones 1982; Phillips 1990; Spooner et al. 2003; Nicholson and Lam 2005).

The impact of heavy metal pollution can be detected through morphological changes of organisms as appears to be common in metal-tolerant species (Martin and Coughtrey 1982). Morphological characteristics such as shell dimensions of bivalve molluscs have been used to indicate the environmental variability (Alexander 1974; Stanley 1981). Fluctuating asymmetry is another morphological characteristic that has been proposed as a tool for monitoring the quality of the environment and is being considered as a sensitive monitor of stress (Graham et al. 1993; Anne et al. 1998). Fluctuating asymmetry (FA), i.e. random deviations from symmetric structure, is a measure of developmental instability (DI) (Van Valen 1962; Palmer and Strobeck 1986; Moller and Shykoff 1999). DI was described as the inherent noisiness of a developmental pathway, which results in the failure of a genotype to consistently produce the same phenotype under a given set of environmental conditions (Zakharov 1992). Measures of developmental instability usually involve some aspect of symmetry, which is the feature of the organism that does not normally change during development (Graham et al. 1993). The asymmetry is detected in bilaterally symmetrical traits, as the difference between the right and left measurements. The presence of environmental and/or genetical stress during ontogeny may reduce the efficiency of normal developmental processes, this being reflected by an increase in the level of fluctuating asymmetry (Clarke 1993). This leads to the use of the increased level in FA as a detector of chronic, sub-lethal environmental stress. At the same time, as any other method of stress assessment, fluctuating asymmetry has its limitations. Thus, the most effective methodology for assessing stress, including anthropogenic stress, is a multiple approach based on number of techniques, and fluctuating asymmetry represents one of them (Leung et al. 2003).

In this study, morphological variations of the shell of a bivalve, the ark cockle *Anadara trapezia*, were employed as biomarkers of heavy metal pollution. For this bivalve, fluctuating asymmetry and shape of shell were studied.

Materials and Methods

Study Site

The study was carried out at Lake Macquarie, New South Wales, Australia (151º66'E, 33º08'S). Lake Macquarie has a long history of trace metal enrichment from anthropogenic activities (Roy and Crawford 1984). Toxic heavy metals have been accumulated in this lake since 1897 with the operation of the lead-zinc smelter, resulting in the contamination of Cockle Creek and the northern reaches of Lake Macquarie with heavy metals. This lead-zinc smelter produced lead bullion, zinc, cadmium, selenium and sulphuric acid with slag being a by-product of the production process (Hunter Health 2003). The concentrations of cadmium, copper, lead, zinc, and selenium are much higher in sediments of Cockle Bay, which is closest to the smelter, and the metal concentrations tend to decrease to the southern end of the lake (Roy and Crawford 1984). It was found that seagrass from the contaminated location (Cockle Bay) also contained a significantly higher concentration of heavy metal in its tissues compared to other location within the lake (Ambo-Rappe et al. *in press*).

Samplings were conducted in one polluted location (Cockle Bay) and six relatively clean locations (Fennel Bay, Killaben Bay, Wangi Bay, Myuna Bay, Bonnels Bay, and Wyee Bay; southern locations of the lake). Depth ranged from 40 to 60 cm and types of sediment were sand and mud. Three sites in each location were sampled at distance 100–200m.

Shell Dimension and Fluctuating Asymmetry of Anadara Trapezia

Twenty individual cockles with similar size range (10–20 gr weight) were collected in each site. However, in some sites (e.g. Cockle Bay), the density of the bivalve was very low and some bigger and/or smaller individuals were retained for analyses. Therefore, the size range of this bivalve was different in different locations.

Shells of *Anadara* were measured for characters as follows:

1. The maximum dimension across the shell hinge and the ventral side of the shell.
2. The maximum dimension along the anterior-posterior axis of the shell.
3. Length of hinge along the ventral side of the shell.
4. Distance from apex to umbo.
5. Length of umbo.

Character 1 was measured using a calliper with an accuracy of 0.02 mm, while, other characters were measured digitally using Image Tool software packages (University of Texas Health Science Centre at San Antonio, Texas and freely available at ftp://maxrad6.uthscsa.edu). Characters 2–4 were measured twice on left and right valves for fluctuating asymmetry and measurement error calculation. All the measurements were made by one operator. Additionally, animal weight, which included shell and its soft tissues, were recorded to the nearest 0.01 g.

The procedure for the calculation of size-independent indices of variation was done according to Lajus and Alekseev (2000) and included: (1) Calculation of the first principal component (PC1) based on all characters, which was considered as general size; (2) Regression of each character in PC1; (3) Dividing residuals by predicted values. Thus, data obtained were size-independent residuals. These data were used for testing the significance of differences between samples in principal components, following Lajus and others (2003a; 2003b).

Before conducting the fluctuating asymmetry calculation, Palmer (1994) suggested eliminating other types of asymmetry (directional asymmetry and antisymmetry) that might occur together with fluctuating asymmetry (FA) in the same character. Directional asymmetry (DA) is characterized by consistently greater trait development on one particular side, either left or right, resulting in mean values of the distribution of left minus right sides (L – R) deviating from zero (Van Valen 1962; Palmer and Strobeck 1986). Antisymmetry is characterized by consistently larger trait development on one side than on the other, but the larger side may be either the right or the left, at random, resulting in a platykurtic (broad-peaked or bimodal) distribution of left minus right sides (L – R) about a mean of zero (Van Valen 1962; Palmer and Strobeck 1986).

Deviations from symmetry are often rather small; therefore, measurement errors (ME) can be particularly important in fluctuating asymmetry measures (Palmer 1994; Merila and Biorklund 1995; Lajus and Alekseev 2000). The measurement error was evaluated for each trait by conducting two-way analysis of variance (ANOVA) with “Side” and “Individual” as random factors. The two-way ANOVA also tested the presence of directional asymmetry (DA), the significant of asymmetry on trait size, and the presence of non-directional asymmetry which is related to FA or antisymmetry (Palmer and Strobeck 1986; Palmer 1994).

The two-way ANOVA revealed that there was no significant difference in factor "Side" in Characters 3–5, which indicates the lack of directional asymmetry (DA) in these characters. Character 2 exhibited directional asymmetry. Thus, only characters which do not exhibit DA were chosen for further asymmetry analyses. Significant interaction of the factors "Side x Individual" indicates that there is a non-directional asymmetry, which may relate to FA.

To ensure that observed non-directional asymmetry is FA, it is necessary to test for the presence of antisymmetry. To test for antisymmetry, the distributions of left minus right within each character selected were checked for the departure from normality using Kolmogorov-Smirnov Test (Sokal and Rohlf, 1995) and the data was also checked for statistical outliers.

The normality test revealed that (R–L) distributions of Character 4 did not deviate from normal distribution, but the distribution deviated from normality for Character 3 and 5. Significant positive kurtosis was detected for (R–L) distribution of these characters ($p < 0.001$), indicating leptokurtic distribution (Sokal and Rohlf 1995).

There was a dependence of asymmetry to the size/shape of the shell indicated from significance in the factor "Individual" for all selected characters. Therefore, following Palmer (1994), the FA index that simultaneously forms the size correction was used for FA analysis as follows: FA = mean [|R-L|/((R + L)/2)].

Statistical Analyses

Asymmetrical analysis of variance (ANOVA) was used for all variables in order to compare between one polluted location and more than one control location. This analysis was designed to deal with the environmental impact assessment when no data have been obtained before the impact and, thus, only 'after' data are available. The method to construct the asymmetrical analyses used in this study followed that described in Underwood (1993) and consisted of combination of the sum of squares values from separate analyses of variance. Glasby (1997) provided a detailed description of how to deal with asymmetrical data and a discussion of the problems associated with detecting impacts when only 'after' data are available.

FA of bilateral characters for the shell of *A. trapezia* was compared between the polluted (Cockle Bay) and unpolluted locations with a two-factor asymmetrical ANOVA: Location (1 polluted and 5 control) and Site (3 levels, nested in Location) with 40 measurements per combination of factors. The similar design with only 20 measurements was used for analyzing data of morphological characters of *Anadara* shell.

Results

Cockles from Cockle Bay were on average larger than in control locations (average weight was 36.51 g ranged from 2.34 – 134.18 g in Cockle Bay, in control locations average weight was 15.01 g varying from 2.79 – 53.98 g). Density of cockles in Cockle Bay was lower than control locations, although quantitative analyses were not performed. No cockles were found in the site close to the Cockle Creek, where the heavy metals from the smelter were discharged into Lake Macquarie.

The principal components of the shell dimensions explained the following proportion of total variance: PC1 – 89.3 %, PC2 – 6.8 %, PC3 – 2.9 %, PC4 – 0.7 %, PC5 – 0.4 %. Most of characters were highly and positively correlated with PC1 ($r > 0.75$), thus this principal component was interpreted as a general size. The other principal components

(PC2 to PC5) refer to residual variation and were interpreted as related to variation in shape. Regarding the association of principal components with the heavy metal pollution, asymmetrical analyses were performed on PC2 to PC5. This test revealed that only PC4, which showed maximum correlation with character 4, was significantly different between Cockle Bay (polluted location) and control locations ($F_{1,4} = 11.20$, $p < 0.05$; Figure 1).

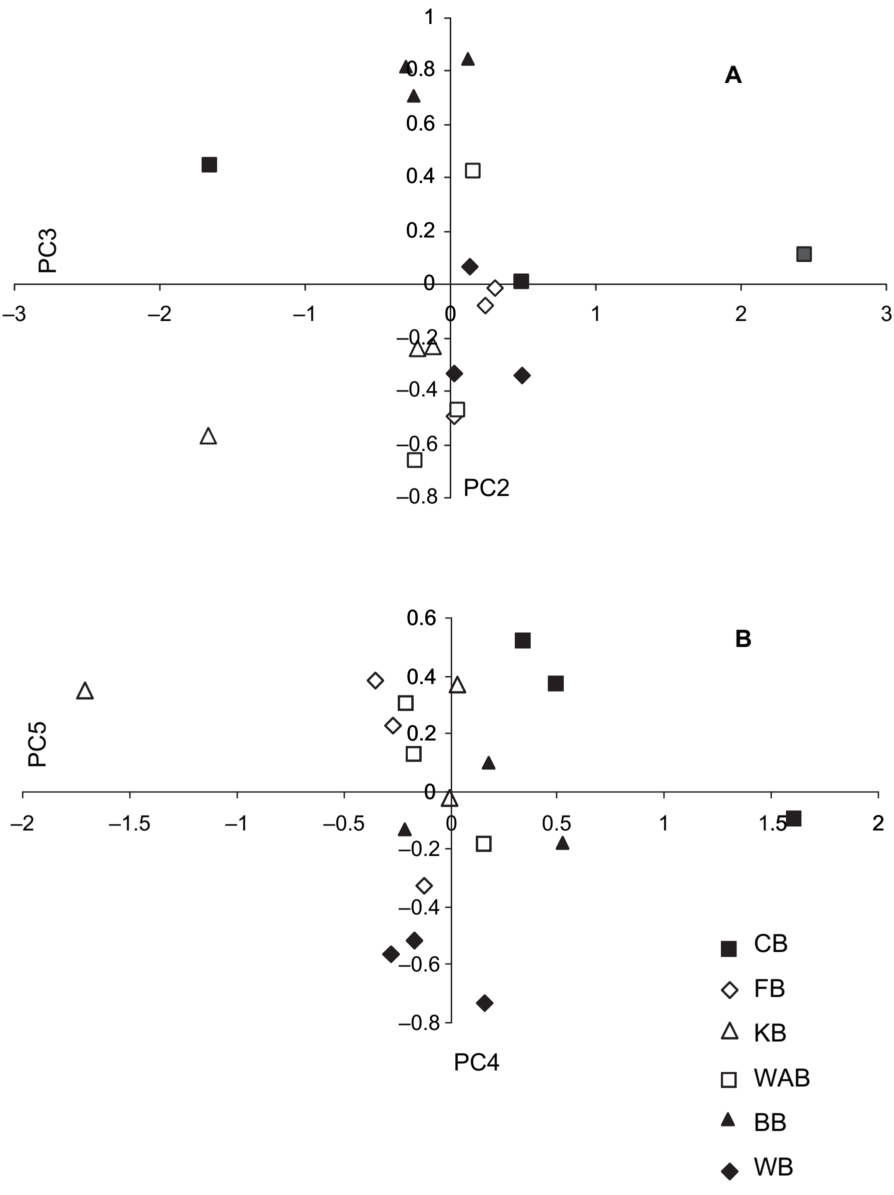

Figure 1. Distribution of individuals in coordinates of (a) PC2-PC3 and (b) PC4-PC5. Sampling locations: contaminated location (CB, Cockle Bay), uncontaminated locations (FB, Fennel Bay; KB, Killaben Bay; WAB, Wangi Bay; BB, Bonnels Bay; WB, Wyee Bay).

Fluctuating asymmetry of distance of apex to umbo (Character 4) was also significantly higher in the polluted location (Cockle Bay) than control locations ($F_{1,4} = 9.67$, $p < 0.05$). FA of this character varied among the sites within the polluted location, but no variations in this FA trait were observed among the sites within the control locations. Length of umbo (Character 5) had a similar pattern with Character 4; however, there were no significant increase of FA of this character in polluted location. Moreover, FA of hinge-length (Character 3) did not increase in the polluted location and this character varied among the sites within control locations (Table 1).

Discussion

The morphological characters of a bivalve associated with seagrass, the ark cockle *Anadara trapezia*, from the polluted area were distinctive, and even though abundance was not quantified, the cockles in Cockle Bay (polluted location) appeared much less abundant compared to the abundance of the cockles in other locations. It can also be taken into consideration that this location was named after the cockle, which can be evidence of a historically high abundance of this species here. If so, the decrease in abundance of the cockles might be an effect of environmental factors in Cockle Bay. Alexander (1974) studied the effect of salinity and substrate on size of *Anadara* and found that a higher level of salinity resulted in increase of size in this species. Also, *Anadara* were smaller in muddy sediments than in those having a larger percentage of sand. In this study, however, the variation in size of the cockle between Cockle Bay and other locations did not appear to be related to the substrate.

Anadara trapezia found in the polluted location (Cockle Bay) also showed significantly different shape and exhibited higher shell asymmetry, in terms of the distance from apex to umbo, compared to the ones in relatively unpolluted locations.

Thus, *A. trapezia* associated with seagrass may be more responsive to heavy metal stress and possibly a good indicator of heavy metal pollution in a marine system. Shape and shell asymmetry of the bivalve might be considered as a cheap and time-efficient indicator of metal pollution in seagrasses.

Table 1
Summary of asymmetrical analyses of variance (ANOVAs) comparing FA of *A. trapezia* between polluted (CB) and control locations.

		Character 3		Character 4		Character 5	
Source of variation	df	MS	F	MS	F	MS	F
Location	5	0.002	2.29 *ns*	0.024	0.40 *ns*	0.040	0.59 *ns*
CB vs Control	1	0.000	0.15 *ns*	0.086	9.67*	0.039	0.97 *ns*
Control	4	0.002	2.64 *ns*	0.009	0.32 *ns*	0.040	1.95 *ns*
Site (Location)	12	0.001	2.51**	0.061	1.79*	0.067	1.45 *ns*
Site (CB)	2	0.001	0.74 *ns*	0.222	7.84**	0.301	14.64**
Site (Control)	10	0.001	2.83**	0.028	0.93 *ns*	0.021	0.48 *ns*
Residual	702	0.000		0.034		0.046	
Residual (CB)	117	0.000		0.050		0.063	
Residual (Control)	585	0.000		0.030		0.043	

ns-not significant; $^*p < 0.05$; $^{**}p < 0.01$.

Acknowledgements

The study was funded by an Australian Agency for International Development (AUS-aid) scholarship to R. A-R. and a visitor's grant from University of Newcastle to D.L.

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