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Ecophenotypic variation of the common cockle *Anadara maculosa* populations: Implication to microhabitat bio-indication

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Abstract

Assessment of ecophenotypic variation in response to microhabitat adaptation is currently one of the prospective approaches to bio-indication. This present study has evaluated conchological variations in the arcid bivalve *Anadara maculosa* (common cockle) populations in two varying micro geographically-isolated microhabitats (mangrove estuarine and residential intertidal areas) of Margosatubig, Zamboanga del Sur, Mindanao, Philippines. Ecological parameters known to affect the bivalve's phenotype were obtained in each site such as spatial distribution, edaphic and climatic factors, and resource use. Results of Multi-Analysis of Variance (MANOVA) on the conchological characters (length, width, height, umbo distance, ridge number and total body weight) reveal significant variations ($p=1.898e^{-7}$ at $\alpha = 0.0001$). Further, shape variations based on elliptic Fourier descriptors using SHAPE v.1.3 software tool reveal significant variations ($p=0.0268$ at $\alpha = 0.05$). This variation analysis is indicative of high ecological plasticity of this species which may be attributed to ecological condition of its microhabitat.

Keywords: conchological differentiation, microhabitat variation, morphometrics, plastic responses

Introduction

Benthic organisms such as bivalves are important indicators of the coastal ecosystem. Being indicator species means they can be sentinels of environmental degradation [1]. They are considered as keystone species, which can determine the health of a water body [2]. Accordingly, it can accumulate environmental contaminants in their tissues to which their growth are mainly related to the quality and quantity of food from the environment [3].

The common cockle (*Anadara maculosa*) is an economically important arcid bivalve belonging to Phylum Mollusca. It is characterized by their trapezoidal ribbed shells with a heavy periostracum and the taxodont hinge. In terms of its ecology, it can easily adapt to their microhabitat despite any environmental inputs such as agricultural effluence and residential wastes [4]. Despite being a valuable fishery resource among local residents with its cosmopolitan distribution and a major candidate for aquaculture across the globe, little work has been done to collect and standardize data on its growth to predict yield under different ecological conditions [4, 5].

Morphological variation analysis in response to microhabitat adaptation is currently one of the prospective approaches to bio-indication. Morphometrics can be used to quantify a trait of ecological significance by detecting phenotypic plastic responses [6]. Morphometrics variation is determined by biotic [7] and abiotic environment variability [8], genetic factors [9] and also the interaction of environment and genetic factors [10]. It is argued that cockles display metamorphic life cycle specifically meroplankton in the larval stages which transforms to adult cockle, thus allowing exchange of genotypes between regions due to ocean currents [4].

In addition, factors such as overharvesting and predation could lead to ecological plastic responses where changes in the structure of a shell are a reflection of first visible changes [11]. Thus, its morphology is strongly linked with the major adaptive trends centered on its epibyssate or burrowing life habits [12]. The changing of the habitat could accordingly cause variation in the morphometry of organisms [13]. In response to this great pressure on the biology of A. maculosa, it may exhibit morphological variability which is an indication of adaptive potential in response to microhabitat changes [14].

The study of ecophenotypic variation in response to its microhabitat is of particular interest, because their adaptation potential manifests itself primarily in changes in their morphological parameters [15].

These microhabitat factors include spatial distribution factors (longitude and latitude), edaphic factors (substrate type and pH, organic matter content, potassium content and phosporus), climatic factors (air and water temperature) and other physico-chemical factors (salinity, tidal cover, and total suspended solids).

In the Philippines, rural communities such as Margosatubig, Zamboanga del Sur, bivalve harvesting is considered a major part of their livelihood (52%) next to agriculture. The place got its name by the manifested swift river current draining towards the mouth of Dumanquillas Bay which in a way represents a complex and dynamic aquatic estuarine (Barangay Tulog-Bato) and residential intertidal coastal environment (Samboang, Barangay Poblacion) for cockles [16, 17]. Both of these areas represent major concern through increased human population, which increased chances of overharvesting and large amounts of pollution through agricultural effluents and residential wastes [18].

Thus, this present study sought to assess ecophenotypic variation in the common cockle *Anadara maculosa* populations from two different sites (Barangay Tulog-Bato, a mangrove estuarine environment and Samboang, Barangay Tiguian which is a residential intertidal area) in Margosatubig, Zamboanga del Sur with an end to bio-indication implication of the current status of its microhabitat.

2. Materials and Methods

2.1 Description of the Sampling Sites

Tulog-Bato, Barangay Tiguian, Margosatubig, Zamboanga del Sur is located at 7°34’N, 123°10’E and is characterized by estuarine habitat dominated by mangroves. On the other hand, Samboang Barangay Poblacion, Margosatubig, Zamboanga del Sur lies at 7°35’N, 123°10’E and is dominated by residential houses, steep coastline, so there is relatively little topographic area available for mangroves to grow (Fig. 1)

Fig 1: Map showing the two coastal sites in Margosatubig, Zamboanga del Sur, Mindanao, Philippines; (A) Tulog-bato, Barangay Tiguian and (B) Samboang, Barangay Poblacion.

2.2 Physico-chemical Parameter Assessment

Physico-chemical parameters were measured and mean readings were recorded. Spatial distribution factors (longitude and latitude) were obtained using Geographic Positioning System (GPS); edaphic factors such as substrate type, substrate pH, organic matter (OM), potassium and phosphorus content were analyzed at the Department of Agriculture’s Bureau of Soils; climatic factors such as air and water temperature analyses were obtained by immersing the laboratory thermometer for three times (with a 1 minute duration each trial) and mean readings were recorded. Tidal cover was measured using a meter stick during low tide and readings in centimeter (cm) were obtained for three times within each site. Water pH readings were determined by computing the average of three trials by dipping the digital pH meter’s probe. Water salinity was analyzed at the Bureau of Fisheries and Aquatic Resources-Regional Fisheries Laboratory; and Total Suspended Solids (TSS) was measured by filtering a 1-L water sample from each site with a filter paper wherein their initial and final weights were obtained thereafter. In terms of the site’s resource use, a description of the study site was done.

2.3 Collection of Samples

A total of 30 samples of *A. maculosa* were handpicked purposively from two different sites in Margosatubig, Zamboanga del Sur, Philippines.

2.4 Morphometrics

Six conchological characteristics were measured such as shell length, shell width, shell height, umbo distance, shell ridge number and total body weight for meristic measurements (Fig. 2).

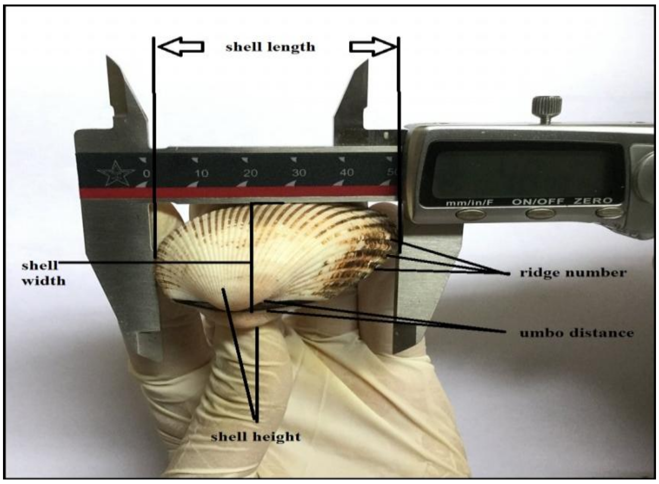

Fig 2: Conchological characteristics obtained meristically.

Shell length, shell width, shell height and umbo distance were measured in millimeters (mm) using digital Vernier caliper, while shell ridge number was determined by manually counting the ridges using dissecting needle. Total body weight was measured in grams (g) using a digital balance. A Multivariate Analysis of Variance (MANOVA) was used to compare if there is a significant difference between the two *Anadara* populations in terms of the meristically obtained conchological characters.

On the other hand, for geometric analysis using shape analysis, image acquisition was done in each of the 30 individuals from the two sites. The outline of the bivalves were analyzed in chain coding technique using the software package SHAPE v.1.3 [19] to examine the variation in shapes. All images were saved in. bmp format (24bit) and were binarized with Chain Coder. Chain code is a coding system for describing geometric information about contours in numbers from 0 to 7. Chain coder converts the full color image into a binary (black and white) image, reduces noise, traces the contours of objects and describes the contour information as chaincode. Then, the Chain-code file was transformed into a Normalized Elliptic Fourier file with Chc2Nef, using 20 harmonics. These Elliptic Fourier descriptors (EFDs), originally proposed by Kuhl and Giardina

[20], can delineate any type of shape with a closed two-dimensional contour.

Normalization of data obtained from chain codes used the first harmonic ellipse as a basis which corresponds to the first Fourier approximation and utilized the 20 harmonics number to be calculated as suggested by Iwata and Ukai [19]. It is based on the methodology of Elliptic Fourier descriptors which allows describing each type of two-dimensional shape with a closed outline, in terms of harmonics [21] Subsequently, a Principal Component Analysis (PCA) was performed on the variance-covariance matrix of normalized coefficients (elliptic Fourier descriptors) using PrinComp, which gives a graphical output of the average shape of the standard deviation [22]. The data were then subjected to MANOVA using PAST (Paleontological Statistics) Software Package v. 1.91 to determine if the populations differ significantly from one another based on the shape of its shell. Wilks' lambda, Pillai trace values and p values were also obtained.

3. Results and Discussion

Estuarine & coastal areas are complex and dynamic aquatic environments. With the growth of human populations and commercial industries, these ecosystems have received large amounts of pollution effluents [23]. Table 1 shows the mean micro-biogeoclimatic characterization of the two sampling sites in Margosatubig, Zamboanga del Sur, Philippines.

Table 1: Mean micro-biogeoclimatic characterization of the two sites in Margosatubig, Zamboanga del Sur, Philippines.

Sampling Sites	**Spatial Distribution**		**Edaphic Factors**						**Climatic Factors**					**Resource Use**
	Latitude	**Longitude**	**Substrate Type**	**Subs-trate pH**	**OM %**	**P ppm**	**K ppm**	**Air Temp. (°C)**	**Water Temp. (°C)**	**Tidal cover (cm)**	**Wa-ter pH**	**Sali-nity (ppm)**	**Total Suspended Solids(TSS) (g)**	
Tulog-bato	7°34'N	123°10'E	Sandy	7.71	0.2	10.0	230	31.3	31.3	216.7	7	32	0.50	Mangrove
Samboang	7°35'N	123°10'E	Muddy	8.30	0.8	16.0	330	32.7	33	140	8	30	0.55	Residential

Physicochemical parameters vary between the two sites of Margosatubig, Zamboanga del Sur in terms of latitudinal spatial distribution, edaphic factors, climatic factors and resource use. These microhabitat variations could affect the morphometry of *A. maculosa*. Biological characterization of *Anadara maculosa* in terms of its morphological characteristics is shown in Table 2.

Table 2: Mean data on biological characteristics of *Anadara maculosa* found in the two sites in Margosatubig, Zamboanga del Sur, Philippines.

Sampling Sites	**Shell length (mm)**	**Shell width (mm)**	**Shell height (mm)**	**Umbo distance (mm)**	**Ridge number**	**Total body weight (g)**
Tulog-bato	45.25	27.09	32.31	2.60	34.6	23.51
Samboang	38.07	22.28	28.02	1.37	35.5	12.54

With the exception of ridge number, all mean biological characteristic values of *A. maculosa* were greater in Tulog-bato, Barangay Tiguian, Margosatubig. The coastal waters are micro-geographically-isolated from each other as reflected in their spatial distribution. With a differing substrate type and resource use, variation in morphometry was also reflected. Bivalves such as *Anadara* preferred a sandy substrate. With the characteristic estuarine condition of the Tulog-bato mangrove area, they can be subjected to pronounce salinity fluctuations because of evaporation, rainfall and inflow from rivers. This indicates their ability to survive in a wide range of salinities [24].

Further, a higher water depth or tidal cover and higher salinity are higher in Tulog-bato. The shallow water as characterized by the Samboang's intertidal residential area leads to a permanently turbid condition. Tidal cover may affects feeding which could be a major restriction on growth at higher shore levels. These factors may result in an elevated metabolic rate coupled with restricted filtration which might combine to produce poor growth. Body weight may be reduced in an area with warm water and subjected to prolonged intervals of high turbidity [24].

Although a higher organic matter content (OM), potassium (K) and phosphorus (P) of the substrate is reflected in Samboang, the strong overharvesting in Samboang's residential microhabitat may also be one important factor affecting its low average harvested cockle sizes relative to the less exploited mangrove area in Tulog-bato [25, 26]. Qonita *et al.* [4] found that climatic factors such as temperature can affect the shape and size of shells. This is supported by Zhongming *et al.* [27] and Moss *et al.* [28] who state that temperature is the most important ecological factor for marine organisms which affects the growth of bivalves through its metabolic rates. These arguments support the findings in this study wherein a smaller shell size was demonstrated in Samboang's cockle population with a relatively higher air and water temperatures than in Tulog-bato.

Thus, the interplay of the micro-biogeoclimatic factors may affect the morphology of organisms. Several studies have stated that environmental factors would likely cause different morphologies resulting from phenotypic plasticity [29,30]. Multi-variate Analysis of Variance (MANOVA) reveals significant phenotypic variations in terms of the obtained conchological characters (Tables 3 & 4).

Table 3: MANOVA results between the two populations of *A. maculosa* based on meristically obtained conchological characteristics.

Wilks lambda	**df1**	**df2**	**F**	**p(same)**
0.4656	6	53	10.14	1.898e^{-7}*
Pillai trace	df1	df2	F	p(same)
0.5344	6	53	10.14	1.898e^{-7}*

*significant at α = 0.0001

Table 4: Pairwise comparison between populations of *Anadara maculosa* based on meristic characters.

	Tulog-bato
Samboang	1.89815e^{-7*}

*significant at α = 0.0001

Further, quantitative evaluation of the cockles' shell shape based on elliptic Fourier descriptors revealed the principal components (PC): 50.52% in PC1; 70.61% in PC2; 77.77% in PC3; 82.18% in PC4; 85.73% in PC5; 88.31% in PC6; 90.28% in PC7; 91.84% in PC8 and 93.16% in PC9. The first PC showed the highest variability in the shell shape. Figure 3 shows the contour shapes of the shell of *A. maculosa*.

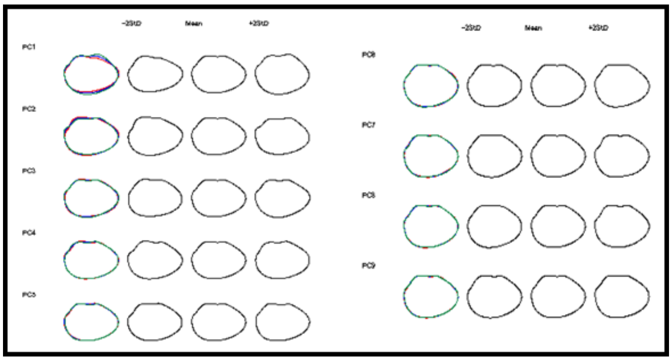

Fig 3: Contour shapes of the shell of *A. maculosa*

The normalized ER in the analysis of its PC showed 9 significant principal components. In the determination of subtle variations between the two populations, the principal component scores (PCs) that defined shape differences were used. This became an exploratory procedure in order to create comparison between shapes and elucidate possible biological significance. Tables 5 & 6 show the MANOVA results between the two populations of *A. maculosa*.

Table 5: Manova results between the two populations of *A. maculosa* based on significant Relative Warp (RW) obtained using SHAPE Analysis

Wilks lambda	**df1**	**df2**	**F**	**p(same)**
0.6932	9	48	2.361	0.02684*
Pillai trace	df1	df2	F	p(same)
0.3068	9	48	2.361	0.02684*

*significant at α = 0.05

Table 6: Pairwise comparison between populations of *A. maculosa* based on significant RW

	Tulog-bato
Samboang	0.0268*

*significant at α = 0.05

MANOVA results show significant differences (p = 0.0268 at α = 0.05) in terms of the shell shape between the two populations of *A. maculosa*. This indicates that the two varying micro-geographically isolated populations of *A. maculosa* reveal variations in terms of its shape using geometric coordinates.

Overall results from both meristic and geometric morphometrics support eco-phenotypic variation in the shells of *A. maculosa*. As supported by the works of Aydin *et al.*, [30], there is a strong correlation between shell thickness and shell width (R^2=1.0). Further, in the study by Qonita *et al.* [4], morphological characteristics of the pill ark cockle (*A. pilula*) from three different sites on the northern coast of Java Island, Indonesia reveal significant differences in three morphometric traits (umbo height, symmetry of the right valve and symmetry of left valve). Accordingly, environmental factors such as temperature, salinity and tidal level among the sites are suspected to be the cause of variation in morphometric characteristics. The shell therefore, is the most distinct feature of bivalves which exhibits a large degree of variation in terms of its morphology [31, 32].

4. Conclusion

Larger length and width, greater height and umbo distance and heavier weight were reflected in Tulog-bato's cockle population, an estuarine mangroove area with a sandy substrate than those cockles collected from Samboang, a residential intertidal area characterized by muddy substrate. Significant differences obtained from meristic measurements (shell length, shell width, shell height, umbo distance, shell ridge number and total body weight) and geometric morphometrics through shape analysis indicates ecophenotypic variation in response to varying microhabitat conditions. Thus, the interplay of the micro-biogeoclimatic factors such as substrate type, temperature, pH and water depth may affect the morphology of organisms. This analysis of variability in terms of its conchological characters is indicative of high ecological plasticity of this eurybiotic species, thus ecophenotypic variation analysis may be significant in bio-indication implication of the current status of its microhabitat. This implies that with varying habitat condition whether disturbed by human settlements such as depicted in Samboang Barangay Poblacion, Margosatubig, Zamboanga del Sur or altered naturally by environmental factors as characterized by Tulog-Bato, Barangay Tiguian, Margosatubig, Zamboanga del Sur, *A. maculosa* may tend to develop alternative phenotypes to suit the current condition of its environment.

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