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Phenotypic differentiation of the Red Sea gastropods in response to the environmental deterioration: Geometric morphometric approach

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ABSTRACT

The negative impacts of degradation in the coastal zone of the Red Sea are becoming well known in upper portions of the trophic web (e.g., humans and fish), but are less well known among the benthic primary consumers. In addition, the degree to which heavy metals are entering the trophic web can be better-quantified using macrobenthos. Two-gastropod genera encompassing *Echinolittorina subnodosa* and *Planaxis sulcatus* from three different localities on the Egyptian coast of the Red Sea were examined in order to deduce the impact of environmental deterioration on the morphology of shells. The examined sites include clean pristine, slightly polluted, and markedly polluted rocky shores. Phosphate/lead industry is the main source of pollution in this zone. Because landmarks on the rugose *Echinolittorina* are difficult to define and to ensure finer resolution of the analyses, a newly 'grid-based' landmarks was implemented. Both Canonical Variate Analysis (CVA) and Thin Plate Spline (TPS) were particularly capable to capture and terrace the minor morphological variations accurately. Two phenotypes portioned among the environmentally different populations were recognized and interpreted as ecotypes with many intermediate forms. The first ecotype has a higher spire and smaller aperture and dominating the pristine site North of Marsa Alam, whereas the second ecotype has a globular shell shape with big aperture and dominating the markedly polluted site. The intermediate forms dominating the slightly polluted site. The shape differences are interpreted as an adaptive differentiation to different metal concentrations. As the morphological variation between the two-ecotypes of both taxa is still minors, and both ecotypes occur together with many intermediate forms, the phenotypic divergence stage has not yet accomplished. The gradational shape change among the investigated populations was positively correlated with index of Pollution (IP). As the human activities were the main driver of the phenotypic changes, hence anthropogenic impact may shift the evolution and/or the extinction rates.

1. Introduction

Littorinid gastropods are dominating the high tidal areas of the temperate and tropical seas. The systematics of this group has been intensively studied over the past years, initially using morphological characters (Reid, 1989; Reid and Williams, 2004; Carvajal-Rodríguez et al., 2005) and recently by molecular phylogenetic methods (Reid et al., 1996; Williams et al., 2003). Thus, it serves as model organism for studying the global patterns of speciation and biogeography (Williams et al., 2003). Morover, the rocky intertidal shores consider as a classic model system for investigating theoretical and mechanistic models of habitat complexity (Meager et al., 2011). The genus *Echinolittorina* has been examined using a combination of phylogenetic, morphological and geographical criteria. The substantial intraspecific variation in the shell shape of most species may have a genetic basis, but ecophenotypic effects are also considered if there is a pronounced geographical pattern (Muñoz et al., 2005, 2008; Reid et al., 1996; Marshall et al., 2011; Gaete and Hidalgo, 2014). By the way, the genus *planaxis* was analyzed for the same research purposes (i.e. Hadley, 1977; McKillup and McKillup, 1993; Meager et al., 2011) occasionally in the West Indian province.

Past research has indicated a pivotal role of aquatic invertebrates in assessment of the impact of pollution on the ecosystems (Rodrigues and Pardal, 2014; Abdelhady and Fürsich, 2014, 2015). De Wolf et al. (2001) suggested that heavy metals accumulate preferentially in the animals' soft tissues and do not affect the shell morphology of the species. Cravo et al. (2004) introduced the partitioning factor (PF, defined as the ratio between the mean metal

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concentrations in soft tissues and in shells. They found that concentrations of some metals such Mn were higher in the shells than in the soft tissues, while others such as Fe was lower in shells than in soft tissues. The latter suggest that the mechanisms controlling accumulation of different metal are variable. In addition, they considered the shells of *Patella* as a good biomonitoring indicator for Mn in sewage contamination studies, while the soft tissues are a good indicator for Zn. Roberts (2012) has drawn the attention to the action of resuspension of contaminated sediments, which frequently recurring ecological threats in contaminated marine habitats.

Although many researchers have investigated the genetic variability in natural populations, only few have addressed the effects of chemical contamination on population genetics. Spite of the existence of a regulatory mechanism in gastropods for essential metals, there is a certain threshold concentration that could be maximally regulated by the periwinkles (Kang et al., 2000). Bickham et al. (2000) stated that stochastic processes resulting from chemical contamination might increase the mutation load and accelerate the process of population extirpation. The genetic diversity was significant lower in heavy-metal polluted environments compared to those unpolluted ones as a sequential effects from pollution at a population level (Kim et al., 2003). Jordaen et al. (2006) found a genetic variability between the polluted and reference areas where a certain allele or genotype frequencies were associated with the pollution. They argued the patterns of genetic variation to both genetic drift and pollution-mediated selection. Ross et al. (2002) added that the genetic diversity of isopods and prawns population from the lead smelter discharge site was significantly lower than that found in one reference population, and not significantly different from the other two reference populations. Thus, they highlighted the need to include many reference populations for comparative purposes in genetic diversity studies, and the need to assess the influence of pollution on more than one species. Based on Nested Clade analysis (NCA), the haplotype diversity was significant lower in heavy-metal polluted populations of gastropod *Littorina brevicula* along the southeast coast of Korea (Kim et al., 2003).

Estebenet and Martín (2003) indicated that shape variability of laboratory snails differ from their field counterparts, suggesting an environmental influence on shape. They added also that the genetic differentiation is the outcome of adaptive differentiation and that trophic style affected the shell thickness and body weight more than water chemistry. Neubauer et al. (2013) concluded that future research should focus on relating morphological changes to evolutionary mechanisms of gastropods (the goal of this paper). The results of Neubauer et al. (2013) demonstrated that environmental changes (such as habitat types) shift and expand the morphospace resulting on morphological changes and thus phenotypic divergence. By investigating gastropod shell, Noshita et al. (2012) demonstrated that more globular and shouldered forms dominated a low water energy environment and hence environmental changes stimulate the production of phenotypes (engine of evolution). The strong correlation between the environment and appearance of new phenotypes was interpreted as an adaptive radiation (Schluter, 2000; Losos and Mahler, 2010). The divergence towards two different phenotypes represents successful adaptation for new habitats and explains the slowly evolving action of divergent natural selection (Schluter, 2001, 2009; Rundle and Nosil, 2005; Neubauer et al., 2013).

Although the Red Sea region has remained relatively free of pollution, the environment is currently under increasing threat from a wide range of human activities. Land-based activities of phosphate/lead mining are becoming a significant source for marine pollution. Now natural habitats, such as coral reefs have been physically altered or partially destroyed (Hodgson, 1999; El-Sorogy et al., 2012; Furby et al., 2014). The selection of gastropod for this study is making sense. Gastropods are one of the most species-rich taxa and the most abundant on the coastal marine areas. Their abundance and the linear nature of their ranges along the margin, make it a powerful tool for comparative studies. Regarding past and recent shells, morphological variation may be caused by non-genetic factors such as ecophenotypy. Detailed taxonomical and ecological framework for Red Sea gastropods are extensively done (e.g., Zuschin and Hohenegger, 1998; Zuschin et al., 2001, 2009; Wronski, 2010). Impact of pollution on the gastropod was also previously investigated (see Hamed and Emara, 2006; El-Sorogy et al., 2013). El Mamoney and Khater (2004) have suggested the need to study the impact of phosphate loading on the marine environment near Safaga. The goal of this paper is to examine gastropod shells in order to delineate morphological variation and their non-genetic causes (ecophenotypy) in addition to interpreting the response of marine fauna the environmental deterioration. Therefore, I propose to address the following research questions;

1) How does water pollution influence the gastropod shells?
   a Is the gradient of heavy metal pollution reflected in the phenotypic characteristics of the gastropod population?
   b Does gastropod growth vary in concern with pollution intensity?
2) Are geometric morphometric and multivariate analyses of gastropod shells capable to terrace the minor phenotypic variations accurately?

2. Material and methods

2.1. Study area and sampling

The area under investigations is located between Quseir and Marsa Alam along the red sea coast (Fig. 1). Quaternary fossil coral form a series of rocky shores on the coast. They form either steep cliffs or reef flats. Inlets occur at intervals formed by drowned river valleys and provide areas of sheltered soft sediment forming mudflats and sand beaches (Fig. 1E). Tides are semi-diurnal and oscillate around a nodal point near 19°N with a spring range of 0.6 m in the north and 0.9 m in the southern Red Sea. Strong seasonal changes of up to 0.5 m occur throughout the Red Sea due to monsoon winds, causing a large part of the intertidal zone to be inundated during winter months (PERSGA, 2004).

Based on index of pollution (IP), the investigated area can be subdivided into three zones; the first one is pristine (12 km north of Marsa Alam southward; $IP < 3$), the second zone represents the moderately polluted area (40 km to the north of Marsa Alam; IP from 4 to 7). This district shows relative enrichment of Th and sometimes Mo and/or U. Indeed. The third zone represents the markedly polluted areas (area from Abu Darag to 20 km to the south of Hurghada; $PI > 7$) with high enrichment in many trace elements (El-Sorogy et al., 2013; El-kahawy et al., 2015). The sources of pollution are frequent phosphate/lead mining, thus beds contain high metal concentrations in addition to waste products outcropped to the surface, leached by rainfall, and drained to the sea. Sabkhatization of the coastal sediments in the polluted areas are markedly and can be seen even from a satellite image (Fig. 1B). The gastropods were collected from three localities located within different polluted zones. These sites are from north to south; Um Gheig (Markedly polluted), Um Greifat (slightly polluted), and 10 km north of Marsa Alm (pristine, Fig. 1).

The material was collected from three sites on the intertidal zone representing the different pollution zones as mentioned