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Marine pollution effects on the southern surf crab *Ovalipes trimaculatus* (Crustacea: Brachyura: Polybiidae) in Patagonia Argentina

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ABSTRACT

We compared the carapace shape and thickness as well as the energy density of *Ovalipes trimaculatus* inhabiting areas comprising a gradient of marine pollution: high, moderate and undetected, in the Nuevo gulf (Patagonia Argentina). The carapace shape was evaluated by means of individual asymmetry scores (=fluctuating asymmetry) whereas the carapace thickness was assessed by measuring the carapace dry weight. The energy density was analyzed through its negative relationship with water content in muscle tissue. The individual asymmetry scores as well as the percentage of water content in muscle tissue were proportional to the marine pollution gradient, whereas the carapaces thickness did not differ among sampling sites. Our results are consistent with previous findings and demonstrate the direct effect of marine pollution on other taxa different from gastropods, cephalopods and polyplacophora and add to long-standing concerns about detrimental effects caused by marine pollution on the benthic community of the Nuevo gulf.

1. Introduction

Marine pollution comprises a range of threats including oil spills (Neuparth et al., 2012), untreated sewage (Azizullah et al., 2011), nutrient enrichment (Isbell et al., 2013) and persistent organic pollutants (Harrad, 2010), among others. Contaminants at large polluted sites often share critical properties such as toxicity, high environmental persistence, high mobility leading to pollution of groundwater, and high lipophilicity, resulting in bioaccumulation in food webs (Fent, 2004). Organotins belong to the most toxic pollutants known so far to aquatic life (de Mora, 1996). Among them, tributyltin, an anti-fouling paint compound, is one of the most toxic chemicals commonly found in areas with high marine traffic. This compound is introduced in marine waters by ship traffic and scrapping activities as well as sewage disposal, and persists within the sedimentary column for years (Hoch, 2001). Molluscs species are the most sensitive group; among the widespread toxic threats induced by tributyltin, shell malformations and thickness, as well as the appearance of male characters in females (imposex) are the most common (Bryan and Gibbs, 1991).

Marine shorelines occupy 4725 km of the coast of Argentina and the state of pollution and its effects on marine fauna has been scarcely studied (Barragán et al., 2003). After the study of Penchaszadeh et al. (2001), who related the imposex incidence to tributyltin concentrations in Mar del Plata sediments, other authors have used this alteration in the reproductive systems of gastropods species to assess the state of marine pollution (e.g. Cledón et al., 2006; Bigatti et al., 2009; Arrighetti and Penchaszadeh, 2010). In the Nuevo gulf (Patagonia Argentina), Bigatti and Penchaszadeh (2005) concluded that marine pollution decreases from the Puerto Madryn piers (high tributyltin pollution, 100% imposex) towards the north (50% of imposex), whereas those beaches located south are non-polluted sites with low marine traffic and null imposex. By considering this reported differences in marine pollution in the west coast of the Nuevo gulf, Márquez et al. (2012) reported body weight loss and shell weight loss in the volutid *Odontocymbiola magellanica* inhabiting the polluted area. Malformations of the reproductive system (pseudohermaphroditism) in other taxa were also shown in earlier studies and were associated with marine pollution in the Nuevo gulf (Ortiz and Ré, 2006; Scarano and Ituarte, 2009). Apart from these few studies, the effects produced by marine pollution on members of the benthic community of the Nuevo gulf, have been scarcely researched.

Marine environments biomonitoring programs are usually focused on identifying the best biomarker and bioindicator acting as a prognostic tool for marine pollution levels (Tosti and Gallo,

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2012). The use of invertebrate populations as biomonitors of environmental quality has been extensively reported (Clarke, 1993 and references therein) and typically assessed by means of conventional life history or physico-chemical parameters (Giblock and Crain, 2013; Pereira et al., 2006), but also throughout the use of morphological indicators (Frontalini and Coccioni, 2011; Nuñez et al., 2012). Because the proportion of morphological abnormalities increases with pollution, it is sometimes considered as an indicator of the exposition of an organism to pollution (Le Cadre and Debenay, 2006). Since the pioneer work of Ludwig (1932), asymmetry in otherwise normally symmetrical traits has been used as a measure of morphological abnormalities. Specifically, among the three forms of biological asymmetries (see Palmer and Strobeck, 2003 for examples) fluctuating asymmetry, which refers to the small and completely random departures from bilateral symmetry observed in individuals (Klingenberg and McIntyre, 1998), was widely used as an overall indicator of stress in organisms inhabiting polluted environments (Parsons, 1992). It should be mentioned, however, that controversial results regarding the association between environmental stress and fluctuating asymmetry are found in the literature (for examples, see Beasley et al., 2013). Nevertheless and although some recognized disagreements (for a review, see also Leung et al., 2003), the utility of fluctuating asymmetry for measuring slight morphological alterations has been proven (Savriama and Klingenberg, 2011).

The southern surf crab, *Ovalipes trimaculatus* (De Haan 1833) (Crustacea: Polybiidae) is adapted to live in high-latitude environments (Stephenson and Rees, 1968). According to present interpretation of its taxonomic status, this species is found on the margins of the southwest and southeast Atlantic, south and southeast Pacific and Indian Oceans (Schoeman and Cockcroft, 1993) on sandy substrates (Fenucci and Boschi, 1975), from the low intertidal to about 100-m deep (Boschi et al., 1992). On the coast of South America it is reported in the western coast of the Atlantic Ocean from Brazil (Melo, 1996) to Argentina (25°–45° S; Boschi et al., 1992) and in the eastern coast of the Pacific Ocean from Peru to Chile (14°–41° S; Retamal, 1994). It is an edible resource and as such, it must be studied regarding its general biology and the effects that marine pollution could cause on it.

Because of its intertidal habitat and its close association with sediments, in addition to the reported effect of marine pollution on the aquatic fauna from the west coast of the Nuevo gulf (e.g. Bigatti and Penchaszadeh, 2005; Ortiz and Ré, 2006; Scarano and Iturarte, 2009; Bigatti et al. 2009; Márquez et al., 2012), we hypothesized that the southern surf crab inhabiting this area might be harmed by marine pollution. Therefore we predict that crabs exposed to marine pollution will exhibit higher levels of morphological alterations, carapace thickness loss and low energy density in contrast to those from not exposed sites. The main objective of this study is to assess if the levels of morphological alterations in the carapace (shape and structure) and the energy density of *O. trimaculatus* differ among sites exposed to different degrees of marine pollution in the Nuevo gulf. A close association between the marine pollution gradient and morphological/physiological effects might be considered as evidence of the fact that the southern surf crab is acting as a prognostic tool for marine pollution levels in the Nuevo gulf.

2. Materials and methods

Study area and sample collection: Sampling sites comprised three sandy beaches separated by 30 km of coastline in the west coast of the Nuevo gulf: El Doradillo (ED; 42.37° S; 64.56° W), Puerto Madryn (PM; 42.45° S; 65.02° W) and Cerro Avanzado (CA; 42.50° S; 64.52° W) (Fig. 1). The sampling sites are similar in terms of its sediment characteristics (Ferrando et al., 2010) as well as wave energy and topographic conditions (Monti and Bayarsky, 1996), but different regarding marine pollution, particularly by tributyltin. PM and CA were previously assessed by Bigatti et al. (2009) and while PM has tributyltin 1.7 ng Sn/g sediment dry weight and 100% of imposex, CA has 0% of imposex. ED, situated 15 km north of PM, presents 50% of imposex (Bigatti et al., 2009). Based on these results, which highlight that at least the tributyltin concentration varies gradually in the west coast of the Nuevo gulf, PM, ED and CA were considered as high, moderate and undetected marine polluted sites respectively.

During October and November 2012, 50 crabs (25 non-ovigerous females and 25 males; carapace width range in mm = 52.18 to 102.37 and 53.66 to 122.37 for females and males respectively) were manually collected from the sea bottom by SCUBA diving close inshore at 1–6 m deep in each sampling site concurrently. Non-parasitized and intact crabs (e.g. without any noticeable damage or additional epifauna) were selected and then transferred alive to the laboratory where they were sorted by sex based on the morphology of the pleon and dissected. In order to avoid any bias due to differences in ontogeny and/or molt stage, only mature crabs in the final stage of the intermolt period were included. Maturity was established according to P. Barón (personal communication) and the molt stage was determined according to Alvarez et al. (2009). All groups included crabs with comparable size distribution.

Data acquisition: Cephalothoraxes (henceforth carapaces) were photographed using a calibrated digital camera (Sony Cyber Shot DSC-W200, 12.1 Megapixels) according to the standard procedure reported by Lezcano et al. (2012). The dorsal view of each carapace was photographed considering the scaling procedure and the carapace orientation, thus avoiding distortions. Images were compiled, scaled and digitized in the TpsUtil and TpsDig programs (Rohlf, 2010a,b) and the carapace shapes were assessed by means of 2D geometric morphometric analysis (Bookstein, 1991). Given that the carapace has an internal plane of symmetry (Klingenberg et al., 2002) we based our analysis on object symmetry. In order to achieve a good representation of the carapace shape, twice the same observer (AHL) digitized 22 landmarks (henceforth landmarks configuration) in two separate sessions. A total of 2 midline landmarks and 10 pairs of landmarks on the left and right side of the carapace were digitized (Fig. 2). Before setting the landmarks

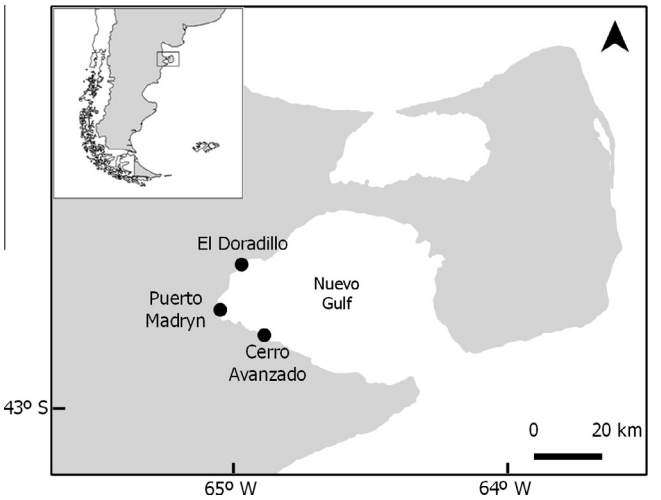

Fig. 1. Sampling sites in the west coast of the Nuevo gulf. El Doradillo (ED), located 15 km north of Puerto Madryn (PM), is the moderate marine polluted site. PM is the high marine polluted site and Cerro Avanzado (CA), which is located 17 km south of PM, is the non-detected marine pollution site.