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The effects of salinity changes on the Polychaete *Diopatra neapolitana*: Impacts on regenerative capacity and biochemical markers

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ABSTRACT

Polychaetes have been identified by several authors as a group of marine invertebrates that respond rapidly to anthropogenic stressors. However, studies investigating alterations in Polychaetes affected by climate changes are scarce. Thus, the present study aimed to assess the impact of salinity changes (14, 21, 28, 35, 42 g/L) on the physiological and biochemical performance of the Polychaete *Diopatra neapolitana*, evaluating the species regenerative ability and biochemical alterations.

The results obtained demonstrated that organisms exposed to extreme salinity conditions (14, 21 and 42 g/L) presented higher mortality rates, needed more days to completely regenerate the missing body region and also regenerated less chaetigers, when compared to organisms exposed to salinities 28 and 35 g/L. The present study further demonstrated that *D. neapolitana* presented significantly lower glycogen and protein content at salinities 21 and 42 g/L, which can be explained by higher energy expenditure in the physiological and biochemical processes. A marked impairment of the glutathione redox status was also recorded at salinities 21 and 42 g/L. Increased antioxidant enzyme activities were observed at salinity 21 g/L while LPO levels were increased at salinity 42 g/L.

Overall the present study demonstrated that the regenerative capacity of *D. neapolitana* can be used as a tool to assess environmental changes, namely salinity shifts. Moreover, stress related biomarkers revealed to be useful to evaluate the alterations in Polychaetes due to salinity changes. *D. neapolitana* revealed to be a good bioindicator to salinity alterations.

1. Introduction

Coastal marine habitats are of major importance as they house a large number of benthic species with considerable economic and ecological importance, which makes these environments a major object of concern regarding the predicted global alterations (e.g., climate change, biological invasions, the presence of emergent contaminants). In recent years, there has been an upsurge of attention in climate change impacts in coastal systems, with most of the literature being focused on the effects of temperature (e.g., Chen et al., 2007; Harley et al., 2006; Hiebenthal et al., 2013; Monari et al., 2007) and water acidification (e.g., Barros et al., 2013; Dupont et al., 2013; Range et al., 2011; Timmins-Schiffman et al., 2013). However, there is also concern about the effects of future alterations in seawater salinity, mainly in estuarine areas (Cardoso et al., 2008; Kay et al., 2006; Matozzo and Marin, 2011; Reid et al., 2003). Frequent flood events are hypothesized to lead to prolonged periods of reduced salinity with increased frequency in estuarine areas (Bussell et al., 2008). Recent studies revealed that rainfall events are increasing on average worldwide, which in marine coastal systems may act as a disturbing agent, promoting responses of organisms. In fact, studies have shown that increases above usual rainfall levels may strongly decrease environmental salinity with implications for the functioning of ecosystem (Cardoso et al., 2008; Chollett and Bone, 2007; Norkko et al., 2002; Salen-Picard and Arlhac, 2002; Salen-Picard et al., 2003; Zajac and Whitlatch, 2003). Besides extreme rainy events, salinity shifts in aquatic systems can also be a consequence of drought periods, related to the reduction of freshwater inputs into estuaries, with effects at the community level. Attrill et al. (1996) investigated the effect of low flows on tidal freshwater macroinvertebrates at the head of an estuary and noted dramatic changes in community composition with small increases in salinity.

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Since salinity is a major force regulating the structure and function of the ecological communities in an estuary, as when this factor fluctuates mobile species may move, whereas sedentary biota in the estuary tolerates normal salinity changes. However, when strong disturbances occur, sedentary biota may suffer impacts, including physiological and/or biochemical alterations (Anthony and Patel, 2000; Bussell et al., 2008; Carregosa et al., 2014a,b; Hamer et al., 2008; Pfeifer et al., 2005; Matozzo et al., 2007).

Among benthic species, Polychaetes have been identified by several authors as a group of marine invertebrates that respond rapidly to stressful conditions. Essentially due to their life-history characteristics, Polychaetes have frequently been used to evaluate the impact of environmental disturbances in estuarine systems (see for review Dean, 2008). Among the most commonly used as bioindicator species (*Arenicola marina*, Casado-Martinez et al., 2011; Ramos-Gómez et al., 2011a,b; *Eurythoe complanat*, Nusetti et al., 2005; *Laeonereis acuta*, Geracitano et al., 2004; Ventura-Lima et al., 2011; *Perinereis rullieri*, Nesto et al., 2010; *Perinereis gualpensis*, Diaz-Jaramillo et al., 2010, 2011a,b, 2013; *Sabella spallanzanii*, Bocchetti et al., 2004; *Hediste diversicolor*, Catalano et al., 2012; Durou et al., 2007a,b; Gomes et al., 2013; Pérez et al., 2004; Moreira et al., 2006; Solé et al., 2009; Tankoua et al., 2012) is *Diopatra neapolitana* (Delle Chiaje, 1841) (Freitas et al., 2012; Carregosa et al., 2014a). This species is a carnivorous, 15–50 cm long, sedentary marine species that lives inside a membranous tube buried in the sediment (Fauvel, 1923; Leguerrier et al., 2004). In the Ria de Aveiro (Portugal) *D. neapolitana* appears buried on muddy sediment around 0–4 m water depth. This species has been reported in intertidal and shallow subtidal habitats, namely in the Red Sea and Indian Ocean (Wehe and Fiege, 2002), the Mediterranean Sea (Arvanitides, 2000; Dagli et al., 2005; Gambi and Giangrande, 1986), and the Atlantic Ocean (Fauvel, 1923; Lourido et al., 2008; Moreira et al., 2006). *D. neapolitana* is an euryhaline species with the capacity to live in a wide range of salinities (among others, Menendez et al., 1984; Raman and Ganapati 1983). Furthermore, as marine invertebrates in general, this Polychaete is an osmoconformer, allowing the osmolarity of the body fluids to be similar to that of the surrounding seawater. However, no studies are known on the impacts of salinity fluctuations on the regenerative ability of this species, which was investigated in the present study. When submitted to anthropogenic and natural stressors organisms may experience oxidative stress, due to the production of reactive oxygen species (ROS). Thus, the biochemical alterations (indicators of cellular damage, the activities of antioxidant and biotransformation enzymes) imposed due to salinity changes were also evaluated in *D. neapoliatana*.

2. Methodology

2.1. Sampling and experimental conditions

D. neapolitana specimens were collected in the Mira channel, the southern shallow arm of the Ria de Aveiro lagoon, an area considered as relatively pristine (Castro et al., 2006; Freitas et al., 2014). Organisms for biochemical analysis and for regenerative capacity evaluation were transported to the laboratory in plastic containers inside their tubes.

In the laboratory, the specimens were removed from their tubes, rinsed with seawater and placed in aquaria, filled with sediment (6 L) and artificial seawater (18 L, salinity 28 ± 1 g/L) for acclimatization during 10 days. Salinity was set up by the addition of artificial sea salt to deionized water. Taking into consideration the physic-chemical characteristics of the sampling site, temperature was maintained at $20 \pm 1\,^{\circ}C$, pH ranged between 7.7 and 7.8 and photoperiod was set up at 12 h light:12 h dark. During this period the aquaria were continuously aerated and organisms were fed ad libitum with small fragments of frozen cockles, every two–three days (Pires et al., 2012).

After acclimatization, the specimens were removed from their tubes and anaesthetized with a solution of 4% $MgCl_2 \cdot 6H_2O$. Under a stereomicroscope organisms were amputated at the 60th chaetiger. Amputation at chaetiger 60 was selected because it corresponds to the end of the branchiae. A previous study (Pires et al., 2012) conducted with *D. neapolitana* demonstrated that 100% of the individuals survived when amputated at the posterior end after branchial region.

After amputation, organisms were submitted to salinity assay, consisting on six individuals/container, two containers/aquarium and two aquaria/condition. The salinities tested were: 14, 21, 28, 35 and 42 g/L, which were selected taking into account the salinity range found at the sampling site (25–35 g/L), in the Ria de Aveiro, where *D. neapolitana* specimens were harvested, and salinity changes predicted to occur due to strong rainy events and longer drought periods. In summer and winter periods these values can reach salinities of 38 and 10 g/L, respectively (Santos et al., 2007). During the experimental period, temperature was maintained at $20 \pm 1\,^{\circ}C$, pH ranged between 7.7 and 7.8, and photoperiod was set up at 12 h light:12 h dark. The temperature selected for acclimatization and exposure periods was based on values found at the Ria de Aveiro lagoon in spring and summer (between 17 and 21 °C, Vaz et al., 2005).

Througout the experiments, water of each container was continuously aerated and individuals were fed ad libitum with frozen cockles every two–three days. Dead organisms were removed from the containers whenever identified. During the acclimatization and experimental periods water in aquaria was completely renewed every five days.

After 28 days of exposure, 12 organisms for biochemical analyses were frozen at $-80\,^{\circ}C$ and the remaining individuals were maintained under the same laboratory conditions until regeneration was completed.

2.2. Laboratory analysis

2.2.1. Regenerative capacity

To assess the regenerative ability of *D. neapolitana,* exposed to different salinities, 12 specimens per salinity condition were analyzed every week: the percentage of the regenerated body part was measured, corresponding to the width of the new segments regenerated and this value was compared with the width of the old body part, and the number of regenerated chaetigers was counted. Regenerated chaetigers were identified by the lighter color and/or their narrower width when compared to the rest of the body. The regenerative capacity was assessed considering the number of days needed to achieve full regeneration, i.e., when no differences could be noticed between the width of the older and the new regenerated segments.

2.2.2. Biochemical responses

After 28 days of exposure, specimens from every condition were pulverized with liquid nitrogen and for each biochemical analysis 0.5 g of soft tissue was used. Extraction was performed with the specific buffer for each biochemical parameter. For this, samples were sonicated for 15 s at 4 °C and centrifuged (10, $000 \times g$) for 15 min at 4 °C. Supernatants were stored at $-20\,^{\circ}C$ or used immediately to measure the protein and glycogen content, Lipid peroxidation (LPO), reduced (GSH) and oxidized (GSSG) glutathione and the activities of antioxidant and biotransformation enzymes (catalase, CAT; superoxide dismutase, SOD; glutathione *S*-transferases, GSTs).