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Is the microcosm approach using meiofauna community descriptors a suitable tool for ecotoxicological studies?

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ABSTRACT

The usual approaches used in ecological risk assessment have been based on individual and population level standard procedures. Although these have been important tools to assess adverse effects on ecosystems, they are generally simplified and therefore lack ecological realism. Microcosm studies using meiobenthic communities offer a good compromise between the complexity of the ecosystem and the often highly artificial settings of laboratory experiments. An experiment was designed to investigate the potential of the microcosm approach using meiofauna as a tool for ecotoxicological studies. The experiment tested the ecological effects of exposure to sewage-impacted pore water simultaneously at the community level using meiofauna microcosms and at the individual level using laboratory fecundity tests with the copepod *Nitokra* sp. Specifically, the experiment tested the toxicity of pore water from three sites according to a contamination gradient. Both approaches were efficient in detecting differences in toxicity between the less and more contaminated sites. However, only multivariate data from community analysis detected differences in the gradient of contamination. In addition to information about toxicity, the community level microcosm experiment gave indications about sensitive and tolerant species, indirect ecological effects, as well as raised hypothesis about contamination routes and bioavailability to be tested. Considering the importance of meiofauna for benthic ecosystems, the microcosm approach using natural meiobenthic communities might be a valuable addition as a higher tier approach in ecological risk assessment, providing highly relevant ecological information on the toxicity of contaminated sediments.

1. Introduction

Marine ecotoxicological studies have traditionally used individual and population level parameters by means of single "indicator" species tests as a standard approach to predict the biological effects of a particular chemical and identify how dangerous it could be by showing a specific response (Chapman, 2002; Vighi and Villa, 2013). Studies at these levels of biological organization have the advantage of monitoring lethal and sublethal effects so that environmental damage may be controlled before causing dramatic consequences to the ecosystem (e.g. loss of species, disturbances on ecosystem functions). However, they have the disadvantages of being species specific and eventually the biological response of the selected model species won't necessarily promote a cascade effect through the upper levels of biological organization (Kimball and Levin, 1985; Vighi and Villa, 2013). In addition, single-species tests exclude the interactions and indirect effects that regulate biological communities which difficults the extrapolation to field conditions. Considering the preservation of ecosystems as the main aim of environmental conservation, understanding how contaminants affect high levels of biological organization considering the complexity of the ecosystems is of foremost importance for more realistic ecological risk assessments.

Microcosm tests using natural communities offer a good compromise between the complexity of the ecosystem and the often highly artificial settings of laboratory experiments (Rohr et al., 2016; Van den Brink et al., 2005). In addition to being more representative by considering the entire community with many species with different life traits and sensitivities, the microcosm approach preserves species interactions and considers the physico-chemical environment as well as different exposure routes. In this way, biotic and abiotic interactions characteristic of natural conditions are allowed to mediate the outcome of the toxicity test (Rohr et al., 2016). Therefore, community-based

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microcosm tests are usually more ecologically relevant than single-species tests and represent a potentially powerful tool for improving ecological realism of risk assessment (Cairns and Pratt, 1993; Chapman, 2002; Schratzberger et al., 2002; SCENIHR, SCCS and SCHER, 2013; Höss et al., 2014).

Marine sediments are sinks for large numbers and amounts of anthropogenic substances that enter the marine environment, which may exert toxic effects on the benthic biota. The use of the single-species toxicity tests is of particular concern in the assessment of the sedimentary environment which is highly complex and biodiverse. In addition, benthic organisms used in such tests are often restricted to specific habitats and not very representative in marine sediments. This is why the importance of integrating multiple lines of evidence in sediment quality assessments, including the benthos, has long been recognized (Carr et al., 1996; Chapman and Hollert, 2006; Choueri et al., 2010; Krull et al., 2014; Long and Chapman, 1985).

Within sedimentary environments, meiofauna communities comprise the most abundant and species-rich metazoans (Coomans, 2000; Heip et al., 1985; Lambshead and Boucher, 2003). They are ubiquitous in virtually all marine and estuarine environments and due to rapid generation time and fast metabolic rates, they play significant roles in the provision of energy to higher trophic levels (Woodward, 2010). They also significantly contribute to important ecosystem functions such as decomposition and nutrient recycling processes (Bonaglia et al., 2014; Hubas et al., 2010). Despite their ecological importance, this group represents an often neglected component of ecotoxicological studies. Intriguingly, however, is that meiofauna communities are particularly well-suited to microcosm studies. Due to the general lack of planktonic larvae, the manipulation and maintenance of natural communities in laboratory microcosms is relatively simple (Austen and McEvoy, 1997, Austen and Sommerfield, 1997, Schatzberger et al., 2000, Millward et al., 2004). Microcosms holding less than 200 g of sediment typically contain many thousands of individuals and up to several dozen of species belonging to different trophic levels (e.g. bacterivores, microvores, herbivores, omnivores, and predators). In the last decades, the development of such microcosm setups have allowed the investigation of the isolated effects of different pollutants in natural communities under controlled and reproducible conditions (Austen et al., 1994; Gallucci et al., 2015; Schratzberger et al., 2000).

The aim of the present study was to evaluate the suitability of using meiofauna microcosm assays to assess marine sediment quality. This was done through the exposure of a meiobenthic natural community to sewage impacted pore-water and comparison of the responses of community structure with (i) effects observed by a single-species fecundity test with the meiobenthic copepod *Nitokra* sp. reared in the laboratory and (ii) sediment chemistry data. Adverse effects of sewage contaminated water have already been reported as a result from significant concentrations of pharmaceuticals and personal care products – PPCPs (Bila and Dezotti, 2003; Rodgers-Gray et al., 2000; Shareef et al., 2008), trace elements (Tjandraatmadja et al., 2008), surfactants (Jensen, 2004) and hydrocarbons (Jensen and Sverdrup, 2002). Therefore, we expected to find lower fecundity rates and changes in community structure as a response to pollutants present in the extracted pore water. The experiment evaluated the response of the different endpoints in two different time periods. We expected to see direct toxic effects in the short-term (7 days) and a result of direct and indirect effects on the longer term (30 days).

2. Methods

2.1. Experimental design

A microcosm experiment was designed to assess the effects of exposure to sewage-impacted pore water on the fecundity of the meiobenthic copepod *Nitokra* sp. reared in the laboratory and on the structure of a meiobenthic community. Defaunated sediments from a reference site were individually contaminated with pore water from sediments collected at three sites located at different distances from the sewage outfall located at Araçá Bay, Brazil (Suppl. Fig. 1). The sites were selected based on previous data from a field investigation (Gallucci et al., unpublished data) that established regions affected by an inverse gradient of distance from the outfall, i.e., the further the more contaminated (within a radius of 150 m), due to the major deposition at distant points (Suppl. Table 1). Sediments contaminated with pore water were used for microcosm set ups designed for the analysis of meiobenthic communities and for the set up of individual bioassays for the analysis of *Nitokra* sp. fecundity.

2.2. Sediment collection and defaunation

Sediments used in the microcosm experiment were collected at the shallow subtidal zone (ca. 1 m) of a sandy beach located at São Sebastião Channel, on the coast of São Paulo, southeastern Brazil (23° 49. 58′ S, 45° 25. 31′ W). This was considered as a reference site because it is a “zone of special management” in the marine spatial zoning of the *APA Marinha Litoral Norte* (North Shore Marine Protected Area). Sediment sampling and defaunation followed Gallucci et al. (2015). Another sampling was performed at the same site fifty days later to obtain sediment containing meiofauna (first 5 cm layer) to set the experiments (see Section 2.4). These sediments were transferred to boxes and slightly mixed to ensure an even distribution of the meiofauna among microcosms.

2.3. Extraction of pore water and contamination

The sediments used for the extraction of pore water were collected at the three sites (Suppl. Fig. 1) using a Van Veen grab sampler and were taken to the lab where they were gently homogenized and stored in plastic bags at a refrigerator (8 °C) until extraction (approximately 3 h after collection). The extraction was done by centrifugation of ca. 130 g of sediment at 400 rpm, for 20 min (Rachid, 2002) in a refrigerated room (20 °C). The process was repeated until the required amount (500 mL) was available. The extract obtained was kept in the refrigerator (8 °C) for a few hours. Redox potential and temperature were measured before and after each step and the variations followed the accepted standard for toxicity testing (Environmental Protection Agency, 2001). The contamination occurred through the saturation of the defaunated sediments with the extracted pore water, i.e. 35 mL of pore water for each 67 g of sediment.

2.4. Microcosm essays

2.4.1. Microcosm set up

Microcosms consisted of 500 mL Beakers (12 × 9 cm) filled with 4 cm sediment layers (spiked sediments + sediments containing meiofauna) covered with 350 mL of filtered seawater (equivalent to an 8 cm layer). The microcosms were constantly aerated and covered by parafilm to prevent evaporation and salinity increase. Microcosms were randomly assigned on the bench and maintained under constant temperature (20 °C) and dark conditions to reduce microalgal growth (Schratzberger et al., 2002) and photodegradation of any pollutants.

2.4.2. Sampling of microcosms

Four replicates of each microcosm treatment (sites 1, 2 and 3) were randomly sampled at time intervals of 5, 15 and 30 days (T5, T15 and T30). The overlying water was first removed and the redox potential measured at the sediment surface (ca. 1 cm depth) using an electrode connected to *Hanna Instruments HI 991003* m. Then, 3 cm^3 of sediment were sampled for the analysis of chlorophyll *a* and pheopigments concentrations as a proxy of the microphytobenthos biomass and 2 cm^3 were sampled for *Nitokra* sp. fecundity tests. The remaining sediment was fixed with formaldehyde 4% for analysis of meiofauna (meiofaunal