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Dissecting the distribution of brittle stars along a sewage pollution gradient indicated by organic markers

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ABSTRACT

We have assessed variation in brittle star distribution patterns along a contamination gradient identified by fecal steroids and aliphatic hydrocarbons in Paranaguá Bay, southern Brazil. A hierarchical design using multiple spatial scales (centimeters-kilometers) was applied. Generalized linear mixed models (GLMMs) were used to investigate the spatial and temporal variability of brittle stars. Main principal components from the contamination and environmental matrices were used to investigate the best explanatory dataset. The abundance of brittle stars was significantly lower in sites with high concentrations of fecal steroids and aliphatic hydrocarbons. The best model fitting always included components from the contamination gradients, which precludes a purely environmental driving of brittle star abundance. Variability in spatial scales lower than kilometers was probably driven by sediment characteristics. We highlighted the importance of a robust multi-scale sampling design for a better biological indication of coastal contamination.

1. Introduction

Coastal regions are currently subject to increasing anthropogenic pressures worldwide (Halpern et al., 2008; Borja et al., 2011; Duarte et al., 2013). Eutrophication and contamination, mainly caused by the input of nutrients, sewage and petroleum hydrocarbons, are among the main drivers of such global changes (Paerl, 2006; Borja and Dauer, 2008). This raises an immediate need for consistent diagnoses of environmental quality, and concern for the control and reduction of current degradation rates (Borja et al., 2013; Duarte et al., 2013).

Molecular markers, such as hydrocarbons and sterols, are useful for assessing human impact and sources of organic matter (OM) in coastal areas, due to their source specificity and resistance to degradation (Martins et al., 2011a, 2011b, 2012; Abreu-Mota et al., 2014). For instance, coprostanol (5β-(H)-cholestan-3β-ol) and epicoprostanol (5β-(H)-cholestan-3α-ol), named fecal sterols since they occur in human feces, are present in sewage effluents reaching coastal areas from nearby urban centers (Martins et al., 2014). Conversely, other sterols, such as cholesterol (cholest-5en-3β-ol) and cholestanol (5α-(H)-cholestan-3β-ol), are the main sterols to be found in marine environments due to their ubiquitous occurrence in zooplankton and phytoplankton (Volkman, 2005). Higher values of cholestanol may also indicate a large influx of nutrients, high bacterial activity and the development of hypoxia conditions in the sediment. In addition, aliphatic hydrocarbons (AHs), such as n-alkanes and unresolved complex mixtures (UCM) are used for assessing the input of OM and petroleum and their by-products in sediments (Silva and Bícego, 2010).

Benthic animals are commonly used as indicators of human impacts in coastal regions (Neto et al., 2010; Sturdivant et al., 2014). However, it is difficult to develop causal disturbance models by correlating faunal patterns to contamination, due to the marked background variations associated with estuarine gradients themselves (Dauvin, 2007; Teixeira et al., 2008; Dauvin and Ruellet, 2009). This task becomes increasingly complex, as biological variability occurs on distinct spatial and temporal scales (Levin, 1992; Morrisey et al., 1992; Hewit and Trush, 2007). The application of more precise and robust sampling designs, able to detect complex changes in complex coastal systems under human influence, may lead to better monitoring actions and management polices (Borja et al., 2008).

Brittle stars, which are the most diverse group of echinoderms, occur from tidal regions to the deep ocean (Stöhr et al., 2012). Their abundance and diversified feeding habits and reproductive strategies make them good models for testing ecological hypotheses (O'Hara et al., 2011). Brittle stars are usually seen as reliable indicators of pristine environments (Rosenberg et al., 2004; Solis-Weiss et al., 2004). Variations in the quantity and quality of OM (Gunnarson et al., 1999; Selk et al., 2005), contamination by petroleum and by-products (Newton and Mckenzie, 1995), and metals (Deheyn et al., 2000; Solis-Weiss et al., 2004), or hypoxic conditions (Dauer et al., 1992; Gray et al., 2002) may lead to marked variations in their populations.

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We investigated the variability in abundance of brittle stars along a 20-km sewage contamination gradient in the Paranaguá Bay, one of the largest subtropical estuarine systems in the southwestern Atlantic. A hierarchical sampling design, ranging from centimeters to kilometers scales, was applied to assess the environmental and contamination effects, evidenced by molecular markers, on brittle stars. The hierarchical mixed model approach also aimed to avoid confounding results due to random effects of spatial variability. We tested the hypotheses that: i) the abundance of brittle stars will be lower near Paranaguá City, where sewage is discarded *in natura*; ii) best models should include effects at the kilometer scale that account for the contamination gradient; iii) the abundance of brittle stars along to the Cotinga Channel will be best explained by predictive models including contamination variables than by a purely environmental dataset.

2. Materials and methods

2.1. Study area

Paranaguá Bay (Fig. 1), comprising about 612 km^2, is a Unesco Biosphere Reserve since 1995. The bay is part of the largest remaining sectors of the Atlantic Rain Forest along the Brazilian coast. Its human population is about 191,700 people, most of them living in Paranaguá City (IBGE, 2009). The Bay hosts the third largest port in Brazil and currently the largest grain exporter in Latin America. Over the last decades, the region has passed through significant social-economic growth (Lana et al., 2001). Despite good preservation indicators, there is ample evidence of increasing anthropogenic disturbance, indicated by the detection of organic pollutants, such as polychlorinated biphenyls (Combi et al., 2013), polycyclic aromatic hydrocarbons (Froehner et al., 2010; Abreu-Mota et al., 2014), trace elements (Sá et al., 2006) and sewage, as indicated by fecal steroids (Martins et al., 2010, 2011b; Abreu-Mota et al., 2014). There is also growing evidence of corresponding anthropogenic effects on estuarine benthic fauna of Paranaguá Bay (e.g. Choueri et al., 2010; Egres et al., 2012; Barboza et al., 2013; Souza et al., 2013; Brauko et al., 2015).

The local climate is regulated by the South Atlantic semi-permanent anti-cyclone, and by the passage of polar fronts during the winter (Lana et al., 2001). Estuarine hydrodynamics is mainly regulated by tidal currents, and markedly seasonal fresh-water input (Machado et al., 2000). During the summer (rainy season — October to March), average rainfall rates can reach three times more than that the winter (Lana et al., 2001).

Most of the domestic sewage of Paranaguá City does not receive even primary treatment (Kolm et al., 2002). Sewage discharge in the Itiberê River, at the inner sector of the Cotinga Channel, near Paranaguá harbor, creates a 20-km contamination gradient (Fig. 1), previously investigated by Barboza et al. (2013) and Abreu-Mota et al. (2014).

2.2. Sampling design, field and lab routines

To assess variability in brittle-star distribution at multiple scales (sectors:areas:sites:replicates), the Cotinga Channel was first subdivided into inner, middle and outer *sectors*, spatially separated within a scale of kilometers (Fig. 1). Three *areas*, separated within scales of hundreds of meters, were randomly sampled in each sector. Three *sites* were randomly sampled at the scale of dozens of meters within each area. Five *replicates* were taken at each site within scales of centimeters (total n = 135). Sampling was applied twice, in August 2008 (austral winter) and March 2009 (austral summer). Samples from each seasonal survey were collected in a single day, thereby avoiding small-scale temporal variability that could mask spatial variability. Sampling was carried out by scuba divers using PVC cores measuring 20 cm in diameter and 10 cm in height (0.031 m2). Samples were preserved in a 5% saline formalin solution, and subsequently washed and sieved through a 0.5-mm mesh.

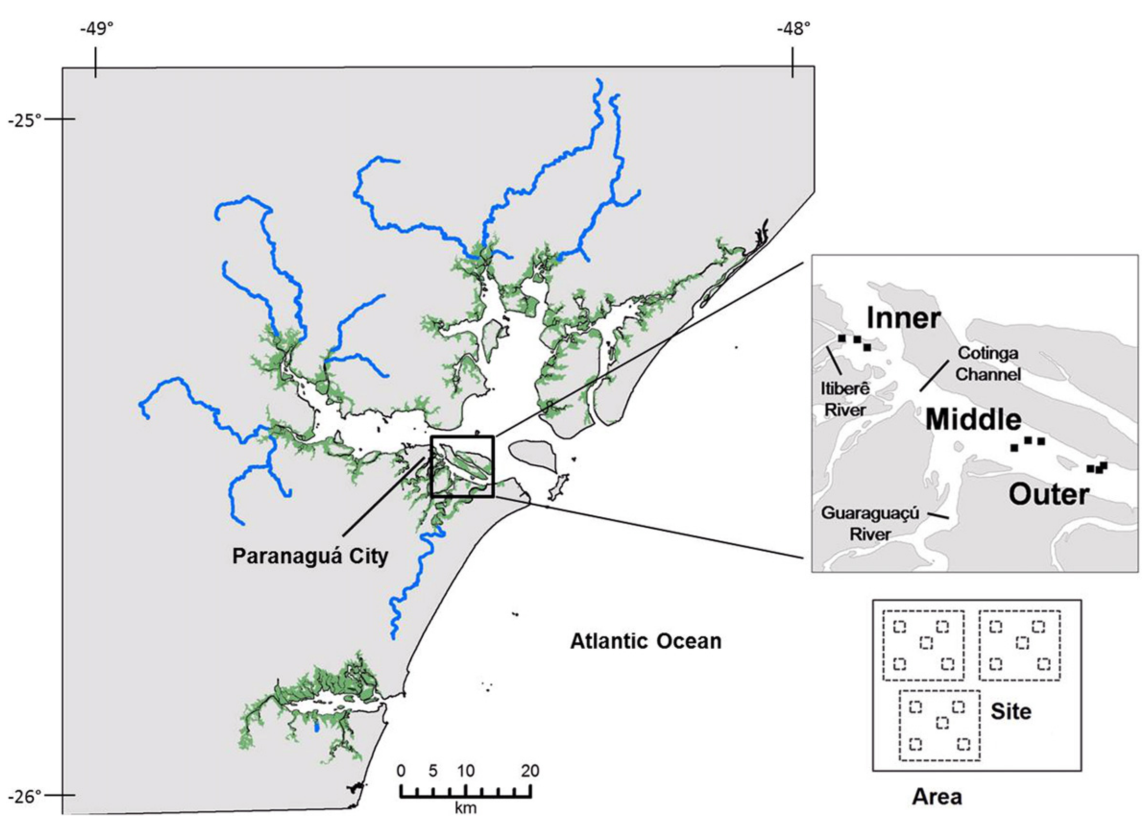

Fig. 1. Map of Paranaguá Bay. Upper panel is sectors (inner, middle, outer) and areas within each sector (black squares) along the Cotinga Channel. Lower panel is sites within each area, as well as the five replicates (empty squares) within each site. Green areas are mangrove vegetation.