Complex spatial and temporal variation of subtropical benthic macrofauna under sewage impact

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ABSTRACT

To better understand variation in sewage-impacted benthic macrofauna from subtropical tidal flats over time and space, we applied a five-factor linear model at a hierarchy of spatial (Condition − Contaminated or Non-Contaminated, Tidal Flat and Plot) and temporal scales (Season and Fortnight). The Contaminated site showed high levels of coprostanol and the presence of *Paranais* cf *frici* as markers or indicators of organic enrichment. Differences between Seasons were more pronounced for the faunal variation patterns than for the other parameters, with lower species richness and abundance in summer. There were significant interactions between Fortnight and Tidal Flat for most variables, reflecting marked heterogeneity within Tidal Flats. Benthic community has significantly changed over short periods of time. These rapid changes may lead to erroneous interpretations and mask the true sources of variation. Our results clearly demonstrate the need to better understand benthic temporal variability even at small scales.

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1. Introduction

Benthic infauna are widely used as indicators of water and sediment quality due to the variable degrees of sensitivity and tolerance of individual species or assemblages (Borja and Dauer, 2008; Dauer et al., 2000; Lancellotti and Stotz, 2004). Macrobenthic assemblages are known to vary at many spatial and temporal scales, which may range from days to years and from centimeters to dozens of kilometers (Bessa et al., 2014; Díaz-Tapia et al., 2013; França et al., 2009). To better understand macrofaunal distribution patterns and their driving factors, experimental studies must incorporate adequate replication at multiple nested scales (Chapman et al., 2009). Experimental designs without adequate replication may mask the true sources of variation and may lead to erroneous interpretations. Replicated sampling across different scales is an efficient tool for understanding structural variation in ecological communities and the background sources of variation, which are often confounded with community responses to anthropogenic disturbance (de Knegt et al., 2010; Underwood, 1997).

Hierarchical models are increasingly used in marine environments to assess the scales at which species and communities vary (Muniz et al., 2012; Williams et al., 2015). Nevertheless, relationships between temporal and spatial variability patterns of living and non-living components of ecological systems are still not well understood. Improving the understanding of such variability would allow to distinguish between changes caused by human activities and the natural variability, leading to a more realistic evaluation of environmental impacts (Souza et al., 2013; Thrush et al., 1999; Underwood and Chapman, 2013).

Environmental pollution creates extensive and urgent problems for the conservation and restoration of degraded habitats. Solving these problems requires the application of high-quality scientific research, including the use of improved sampling designs for assessing faunal patterns of variation associated with geochemical markers of the impacts themselves (Underwood and Chapman, 2013).

Fecal sterols (coprostanol and epicoprostanol) are widely used as markers to assess levels of sewage contamination. Coprostanol (5β(H)-cholestan-3β-ol) is produced during the degradation of cholesterol in digestive tracts of higher vertebrates, including humans. It remains in the feces and, consequently, in sewage inputs (Grimalt et al., 1990; Readman et al., 2005; Martins et al., 2008). Epicoprostanol (5β(H)-cholestan-3α-ol) is formed during the anaerobic treatment of wastewater and can be used to assess the
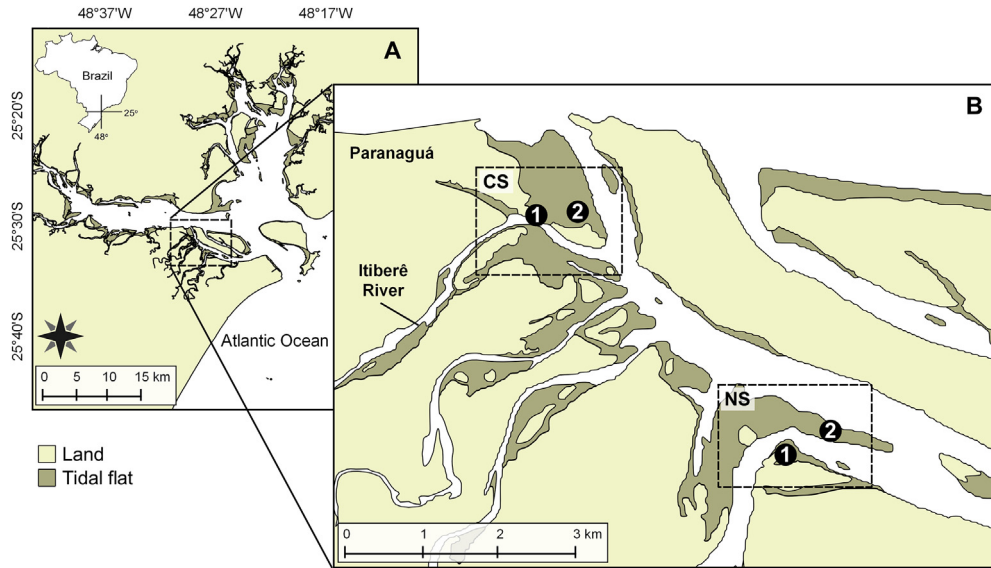

Fig. 1. Paranaguá Bay (A) and Cotinga sub-estuary (B). Tidal flats 1 and 2, sampled at the Contaminated (CS) and Non-Contaminated sites (NS).

level of treatment applied to the fecal matter in sewage before discharge (Mudge and Seguel, 1999). These markers are used as alternatives to microorganism analyses such as fecal coliform analysis because they are organic compounds that accumulate in marine sediments and are less susceptible to environmental degradation than other sewage contamination indicators (Carreira et al., 2004; Martins et al., 2014).

In this context, spatio-temporal variability patterns of benthic assemblages should fluctuate according to the scales at which the sewage discharges affects a given system, and this variation will differ between Contaminated and Non-Contaminated habitats. To test this assumption, we have assessed the structural responses of benthic fauna to key contamination drivers at tidal flats with varying levels of sewage markers along a subtropical estuary from southern Brazil, by applying a sampling design hierarchized in time and space.

2. Materials and methods

2.1. Study area

The Paranaguá Bay, centered 25°03′S, 48°25′W, is one of the largest and best preserved estuarine systems in southern South America (Lana et al., 2001). Despite the bay keeps some pristine sectors, largely protected by conservation units, human impacts have grown over the last decades, mainly associated with port activities, agricultural activities and the input of domestic and industrial sewage (Lana et al., 2001; Martins et al., 2010).

Sampling was carried out at the Cotinga polyhaline sub-estuary, a channel surrounded by mangroves forests and salt marshes, close to Paranaguá city (Fig. 1). The sub-estuary has a surface area of 21.38 km², of which 34% made up by tidal flats (Noernberg et al., 2006). One of the main tributaries of the Cotinga sub-estuary is the Itiberê River, into which urban sewage and effluents from the city of Paranaguá are discharged.

According to the local water and sanitation company, only 50% of the Paranaguá's wastewater is treated, and the remaining is discharged *in natura*. A clear gradient of organic pollution is evidenced by high concentrations of fecal sterols (Abreu-Mota et al., 2014; Martins et al., 2010), high bacterial activity (Kolm et al., 2002) and abundance of macrobenthic bioindicators of organic

enrichment (Brauko et al., 2015; Souza et al., 2013) near the Itiberê River.

2.2. Sampling design and sample processing

The sampling design was based on a five-factor model (two temporal and three spatial factors). Temporal factors included Season (Summer and Winter) and three consecutive Fortnights within each Season. Spatial factors included Condition (Contaminated and Non-Contaminated − 10³; m apart), with each Condition, sampled in two Tidal Flats (10² m apart) at four sampling Plots, arranged parallel to the waterline and spaced at 10 m. The temporal and spatial factors were arranged orthogonally (Fig. 2). Season and Condition were fixed, and all the other factors were random.

Sampling was performed in 2011 during austral summer (January and February) and winter (June and July), always at low spring tides. From each plot (1² m), three sediment samples were collected for faunal abundance and richness analysis, and one sample was collected for environmental variables analysis (redox potential discontinuity layer, grain-size distribution (according to
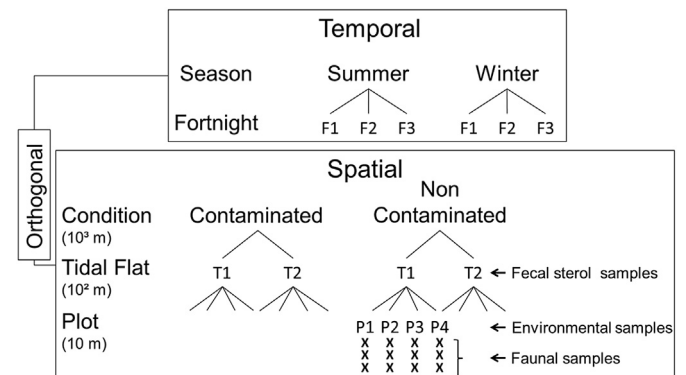

Fig. 2. Sampling design diagram. Temporal and spatial scales correspond to the factors of the linear model. Two Seasons were included (Summer and Winter), with three consecutive Fortnights per Season (F1, F2 and F3). In each Fortnight, two Conditions were sampled (Contaminated and Non-Contaminated) 10³; m apart, with two Tidal flats per Condition (T1 and T2) 10² m apart, four Plots per Tidal flat (P1, P2, P3 e P4) (10 m apart) and three replicates each (1² m plot).