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## Baseline

## The effect of urban sewage on benthic macrofauna: A multiple spatial scale approach

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## ABSTRACT

We investigated the spatial scales of variation of macrofauna in intertidal flats subjected to different levels of contamination from urban effluents in two areas sampled in the Paranaguá Estuarine Complex. The scales considered were: Conditions; Tidal flats and Plots. Although the numerically dominant taxa showed the greatest variability at a scale of Tidal flats, the variability at the Condition scale was also significant. *Tubificinae* sp. 1, *Laeonereis culveri* and *Heteromastus* sp. were the most abundant organisms in the Contaminated area, while *Heleobia australis* was most abundant in the Non-contaminated area. Our results, contrary to those frequently observed in the literature, showed that the variability was significant at the scale of hundreds of metres (Tidal flats). At this scale, the intrinsic characteristics of each tidal flat are more important in determining macrofaunal distribution, while the effects of the urban sewage contamination represent the primary forces acting at a greater spatial scale.

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Understanding the structure and dynamics of benthic communities requires knowledge of their spatial variability at different scales, which can vary from centimetres to dozens of kilometres (Levin, 1992; Norén and Lindgarth, 2005). Benthic fauna responds to changes in both water and sediment quality according to each species' susceptibility and tolerance levels (Dauer, 1993; Lancellotti and Stotz, 2004).

Organic enrichment is one of the most common anthropogenic disturbances and main causes of alterations in the occurrence, distribution and abundance of benthic fauna in coastal and estuarine areas (Ferreira et al., 2011; Pearson and Rosenberg, 1978). The model of Pearson and Rosenberg (1978) predicts positive responses to moderate organic enrichment because these responses are moderated by food availability. However, excessive discharge of organic material can lead to declines of sensitive and long-lived species, while more tolerant and short-lived species are favoured. In grossly polluted areas, the loss of diversity and consequent dominance by a few tolerant species may modify ecological processes and reduce the complexity of the food web (Lerberg et al., 2000). Short-scaled biological interactions such as predation, competition and bioturbation may also play an important role in maintaining the spatial heterogeneity of benthic associations (Anderson, 2008; Snelgrove and Butman, 1994).

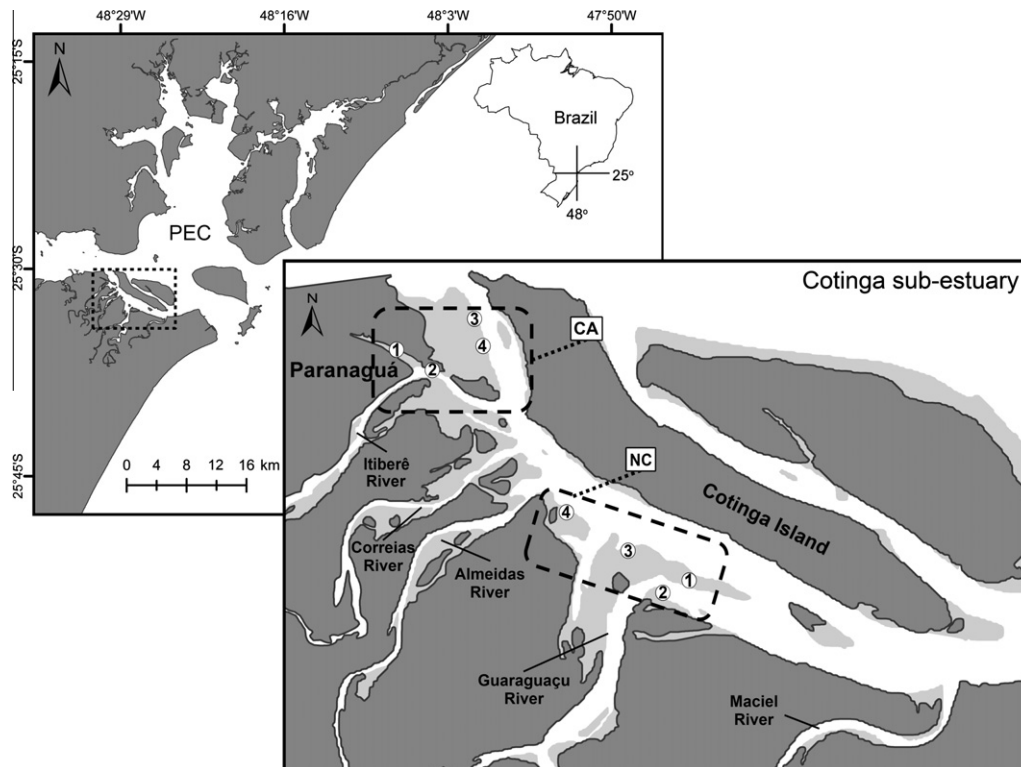
Investigating the scales at which communities vary could help in identifying the ecological processes that determine their observed distribution patterns (Morrisey et al., 1992; Underwood

and Chapman, 1996). The scales at which species and communities vary can be identified through non-manipulative experiments, which should be replicated at multiple nested scales (Chapman et al., 2010). Replication of sampling at different scales is an efficient tool for understanding variations in community structure that can mask a response to anthropogenic disturbances (Underwood, 1997).

We aim herein; (a) to identify the spatial scales showing the greatest macrofaunal variability in non-vegetated intertidal flats subjected to two different levels of contamination by urban effluents in a major subtropical estuarine system and, (b) to evaluate the influence of the effluents on the structure of local benthic associations. We tested the hypothesis that if organic contamination alters benthic structure along a km gradient, then the spatial variability will be greater between Contaminated and Non-contaminated conditions (at the 10<sup>3</sup> m scale) than within each Condition.

The study was conducted in the tidal flats of the Paranaguá Estuarine Complex (PEC), on the southern coast of Brazil (25°03'S, 48°25'W). The PEC covers an area of 612 km<sup>2</sup> and is one of the most important estuaries on the southern coast of Brazil in terms of its port and tourist activities. The Cotinga sub-estuary, which is nearly 20 km long, is located in the polyhaline section, near the mouth of the estuary (Fig. 1). Approximately 34% of the area of the sub-estuary is composed of tidal flats (Noernberg et al., 2006) formed by various systems of relevant ecological importance, such as mangroves, marshes and non-vegetated banks, with dynamics that are strongly influenced by tidal currents (Bigarella et al., 1978).

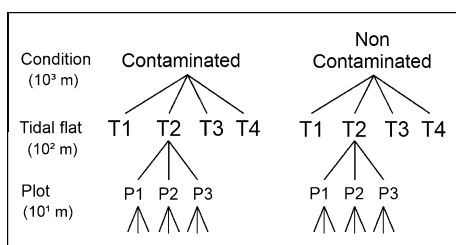
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**Fig. 1.** Paranaguá Estuarine Complex (PEC) and Cotinga sub-estuary. The Contaminated (CA) and Non-contaminated (NC) sampling areas are highlighted at a  $10^3$  m spatial scale. In each area, four unvegetated tidal flats were sampled (distant at a scale of  $10^2$  m), with three plots per tidal flat ( $10^1$  m) with three replicates ( $10^0$  m) each.

The Cotinga sub-estuary receives a large amount of waste produced by Paranaguá city. The waste of nearly 50% of the city's population undergoes treatment, while the rest is released *in natura* to the environment (Companhia de Águas do Brasil: CAB Águas de Paranaguá, 2010). The Paranaguá region is characterised by high concentrations of faecal sterols and high bacterial activity, which are associated with the discharge of domestic waste (Kolm et al., 2002; Martins et al., 2010). However, these effluents are fastly diluted in the water column, which displays a gradient throughout the Cotinga sub-estuary (Knoppers et al., 1987; Lana et al., 2000).

To identify the scales of variability, we applied a mixed linear model with three factors: Conditions – fixed, with two levels (Contaminated and Non-contaminated –  $10^3$  m); Tidal flats – random, with four levels ( $10^2$  m), nested in Conditions; and Plots – random, with three levels ( $10^1$  m), nested in Tidal flats, with three replicates performed for each Plot ( $10^0$  m) (Fig. 2). The plots were positioned parallel to the water line to avoid possible effects of macrofaunal zoning patterns due to the slope of the tidal flat. The following nested design was employed:



**Fig. 2.** Diagram of the experimental design and the scales of spatial variability corresponding to the factors of the linear model: Conditions (Contaminated and Non-contaminated); Tidal flats (T1, T2, T3 and T4); and Plots (P1, P2 and P3), with three replicates each.

Samples were collected during the spring low tide, using a PVC core with 10 cm in diameter and 15 cm in height. The fauna samples were fixed in a 4% formaldehyde solution, washed through a 0.5 mm sieve and preserved in 70% alcohol with Rose Bengal dye. The organisms were sorted and identified to the lowest taxonomic resolution. Biomass values were estimated by dry weight on a digital scale accurate to 0.001 g after drying in an oven at 60 °C for 48 h. Mollusc shells were removed. Additional samples were taken at each plot for sediment characterisation and to determine the levels of total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC) and calcium carbonate ( $\text{CaCO}_3$ ).

Sediment samples were processed according to Suguio (1973), and granulometric parameters were determined in the R environment using the *rysgran* package (Gilbert et al., 2011), following the Folk and Ward (1957) method. The  $\text{CaCO}_3$  content was calculated as the difference between the initial and final weights of each sample after chemical attack using a solution of 1 mol  $\text{L}^{-1}$  hydrochloric acid. The concentrations of TN and TP were determined through the method described by Grasshoff et al. (1983), and the concentrations of TOC were measured with the oxidation method described by Strickland and Parsons (1972).

The scales of spatial variability were evaluated using a mixed nested ANOVA model for the total density (N), total biomass, number of species (N), Shannon–Wiener diversity index ( $H'$ ), density and biomass of the five numerically dominant species, which together represented more than 80% of the total abundance (Table 4). A permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was applied to the same univariate linear model to identify variation in the community spatial patterns, complemented by an nMDS ordination (non-metric multidimensional scaling). The multivariate analyses were based on a Bray–Curtis dissimilarity matrix (Bray and Curtis, 1957). No transformation of the data was needed because the skewness effect was not pronounced. Additionally, components of variation were calculated

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