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Assessing the suitability of five benthic indices for environmental health assessment in a large subtropical South American estuary

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ABSTRACT

Despite the increased and widespread usage of benthic indices for environmental health assessment, some methodological ambiguities remain to be solved. We tested the congruence and consistency of the benthic indices ITI, BO2A, BENTIX, AMBI and M-AMBI in a subtropical estuary (Paranaguá Bay, Brazil). Indices were applied to non-vegetated tidal flats increasingly contaminated by sewage to test: (i) correlations with molecular biomarkers of sewage (consistency); and (ii) evaluate the overall agreement/similarity of responses (congruence). The responses of the benthic indexes ITI, AMBI and BO2A were congruent among themselves and consistent with molecular biomarkers values. BENTIX and M-AMBI were less consistent and congruent and possibly need a readjustment of boundaries for subtropical habitats. The indices seemed robust to natural background yearly variations not related to contamination. Faecal sterols associated to nutrient contents suitably supported the validation of indices and could integrate reference conditions for sewage impacted coastal habitats. Benthic indices can successfully integrate management guidelines, but their suitable application demands further research on tolerance shifts of key indicator species.

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1. Introduction

Ecological indicators for marine health assessment such as benthic indices rely on the response of populations and assemblages to pollution-induced changes. Many indices based on macrobenthic faunal responses are currently employed as real-world tools for health assessment in coastal waters. Different indices should function and respond similarly, but the estimation of uncertainty of assessments remains a challenge (Hering et al., 2010). Inconsistencies in indices' responses may be caused by the indicative ambiguous value of chosen species, erroneous index group assignment or simply non-adjusted boundaries of different indices (Gillett et al., 2015; Simboura and Reizopoulou, 2008). The adjustment of boundaries or intercalibration of indices is reached when the numerical interval of each ecological status (e.g. *good* or *poor*) of different indices is adjusted to achieve a maximum level of agreement.

Regardless of the nature of inconsistencies, the suitability of indices must be investigated before their application. Testing of indices is an exercise aiming not only to select the most appropriate or consistent index to be applied in distinct geographical areas but also to assure that results are comparable to two or more indices (Simboura and Reizopoulou, 2008). Intercalibration of indices commonly rely on reference conditions, habitats that soundly correspond to good ecological status determined by complex and subjective criteria. Nevertheless, as indices are expected to respond to non-biological measures of contamination (Benyi et al., 2009), the direct correlation of indices responses to molecular markers (e.g. petroleum hydrocarbons and faecal sterols) may be a simpler and more satisfactory approach to suitability assessment. Many studies have evaluated the relative performance of different indices but although less subjective, indices are not always clearly correlated with reliable abiotic markers of contamination (Brauko et al., 2015). Indices should be constantly tested for boundaries adjustments, metrics changes and algorithm enhancements towards simplification, stability and robustness (Borja et al., 2008). As yet, no multi-integrative attempts using highly specific

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markers of sewage input as coprostanol, the most abundant sterol in human faeces and, consequently, used as an urban sewage tracer (Daughton, 2012), have been conducted to assess the adequacy of such indices in South American coastal habitats.

Indices should also integrate different temporal scales, translated into robustness to natural temporal changes (Simboura et al., 2014). The capacity to distinguish human-induced and natural disturbance is a desired feature of any index for quality assessment. In this sense, temporal variability could imply that the index is more sensitive to natural variation instead of the variation attributed to human impact when the contamination source does not vary (Culhane et al., 2014). The choice of indices must involve comparison techniques as objective and integrative as possible so that temporal variations are as well taken into account.

In this study, we tested the suitability of five biotic indices to assess estuarine environmental health, all based on the varying sensitivity of species groups to organic contamination, using a multivariate approach to address congruence and consistency patterns in a southern Brazilian subtropical estuary. We applied the indices ITI (Infaunal Trophic Index), BO2A (Benthic Opportunistic Annelida Amphipods Index), BENTIX, AMBI (AZTI marine biotic index) and its multivariate extension M-AMBI in non-vegetated tidal flats under distinct levels of sewage contamination in order to: (i) test for correlations with molecular markers of contamination (index consistency as real-world tools); and (ii) evaluate the overall agreement/similarity of their responses (index congruency among themselves). All analyses were performed along two consecutive years, to assess the consistency of trends over time. Hence, suitable indices are expected to be highly correlated with sewage molecular markers and to display congruent responses to increasing contamination levels over time and space.

2. Materials and methods

2.1. Study area

The Paranaguá Estuarine Complex (PEC) is one of the largest (612 km^2) and most preserved coastal areas along the South American coast, despite increasing port and tourist activities (Fig. 1). The surveys were conducted in the polyhaline Cotinga sub-estuary, about 20 km long, close to the mouth of the estuary. About 34% of the surface area of the sub-estuary, strongly influenced by tidal currents and freshwater discharges, is covered by mangroves and marshes or remain unvegetated (Noernberg et al., 2006). The inner sector of the sub-estuary receives most of the anthropogenic input of sedimentary organic matter or sewage-derived material from Paranaguá city (Souza et al., 2013). A compressed gradient of sewage contamination from the inner sector to the outer part of the sub-estuary was evidenced by *Escherichia coli* sediment concentrations (Kolm et al., 2002) and levels of faecal steroids, highly stable and specific organic markers (Martins et al., 2010). The sewage contamination indicated by coprostanol levels may vary from high to moderate, and are confined to sites close to Paranaguá city (Abreu-Mota et al., 2014).

2.2. Sampling and laboratory procedures

The data used in this study correspond to twelve sampling surveys undertaken along two consecutive summers and winters in 2011 and 2012. In each survey, the macrofauna was sampled in four plots with three replicates each along four tidal flats (two in the Non-contaminated and two in the Contaminated site), covering 576 samples in two years. The benthic samples were collected during low spring tides using plastic core tubes (10 cm diameter, 15 cm deep, 78.5 m^2), and all plots were placed parallel

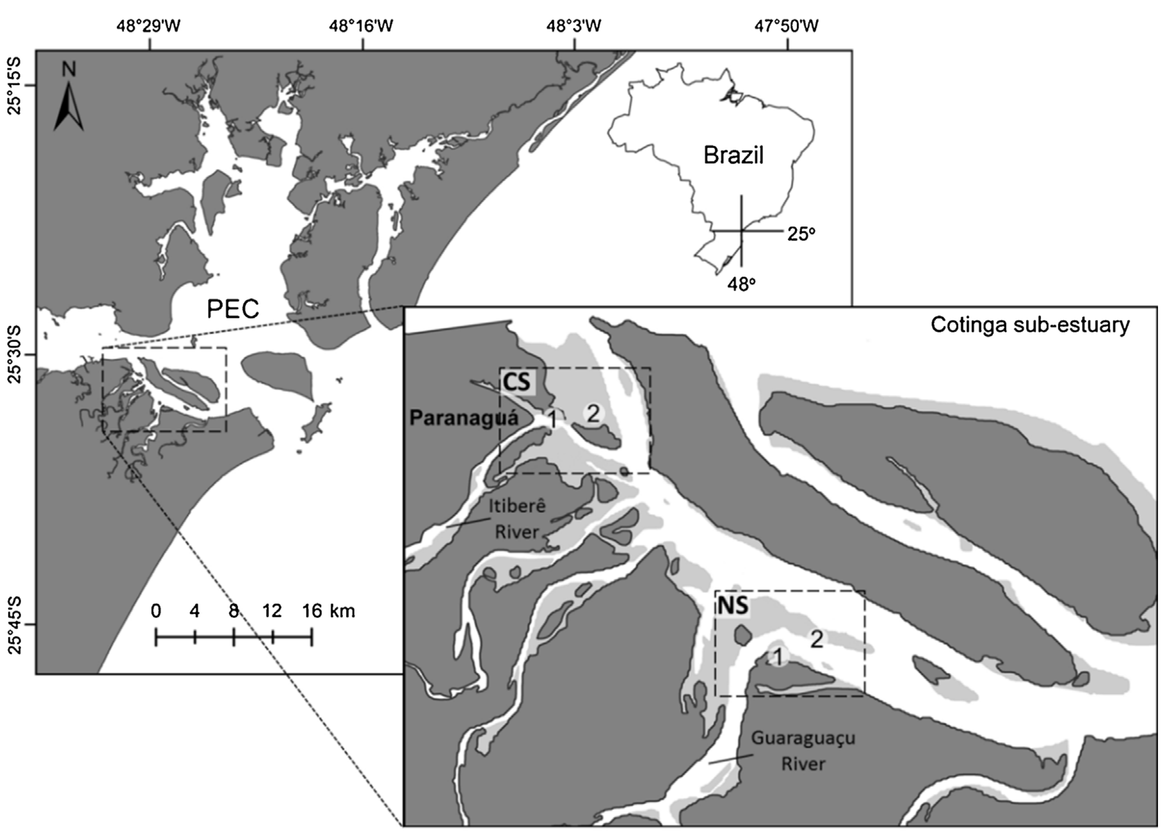

Fig. 1. Study sites in Paranaguá Estuarine Complex (PEC), Brazil. Indices were applied to four tidal flats within Contaminated sites (CS) and Non-contaminated sites (NS) of Cotinga sub-estuary in 2011 and 2012.

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