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## Can biotic indices detect mild organic enrichment of the seafloor?

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#### ABSTRACT

Synthesis indices have been developed in order to implement the Water Framework Directive (WFD) goal of achieving a good ecological status for European waters and so need to be validated and inter-calibrated. This study was conducted in a coastal area under mild organic enrichment from a sewage outfall where no other anthropogenic point source disturbance exists. Results are presented from an integrated assessment in order to test if biotic indices would give a response comparable to that of other descriptors. The study included the analysis of sediment descriptors, stable carbon and nitrogen isotopes in benthic species, benthic community species composition and abundance, and biotic indices. The various groups of descriptors were tested under the null hypothesis of no significant alterations with increasing distance from the outfall. The sediment grain-size, median and total organic matter did not cause rejection of the null hypothesis, in contrast to the sediment redox potential and the stable carbon isotopic composition. The benthic community species composition and abundance and their stable carbon isotopic composition also rejected the null hypothesis. In areas closer to the outfall, the redox potential showed negative values, the macrofauna showed the dominance of opportunistic species and the stable carbon isotope composition showed depletion, in accordance with a terrestrial origin of organic matter. These three independent groups of descriptors diagnosed a coherent alteration scenario associated with organic enrichment. However, the biotic indices failed to reject the null hypothesis. The analysis of the full species composition dataset gave a more reliable picture of the environmental disruption and specific descriptors such as stable isotopes allowed a direct measure of the spatial extent of the organic enrichment. In this study, the biotic indices were not effective in showing benthic alterations associated with the mild organic enrichment despite some being based on species tolerance/sensitivity thresholds to this type of disturbance. Their use caused the loss of essential information and hence impaired the diagnostic capability.

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#### 1. Introduction

The Water Framework Directive (WFD, 2000) and the Marine Strategy Framework Directive (MSFD, 2008) delineate the legal basis for the conservation of European offshore, coastal, transition and fresh waters. Both directives aim to protect and improve the aquatic environment and stimulate a progressive reduction of discharges and emissions. The initial implementation steps of the WFD included the integrative assessment of the current ecological status of these water bodies, by using several hydro-morphological, physicochemical and biological elements. This procedure requires the establishment of indices (metrics) that should enable the detection and quantification of induced alterations to the ecosystem and allow the classification of their ecological quality status. Such classi-

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fication requires the comparison of data obtained from monitoring studies to reference conditions and deriving an Ecological Quality Ratio (EQR) that must be consistent with the normative definitions of the class boundaries specified in the WFD: High, Good, Moderate, Poor or Bad. This ratio must be expressed as a numerical value between zero and one, with 'High' status represented by values close to one and 'Bad' status by values close to zero (Vincent et al., 2002).

In coastal and transition waters, one of the biological quality elements to be considered is the benthic invertebrate fauna, particularly the soft-bottom benthos. The benthic fauna is considered ideal for environmental quality assessment in sedimentary habitats because most species live for several years and their sedentary life makes most animals unable to escape adverse conditions. Benthic species encompass a range of feeding guilds, life-history traits and tolerance/sensitivity thresholds to stress, which enhances their capacity to integrate the benthic habitat and near-bottom water quality (Gray and Elliott, 2009). The community level approach based on species composition has thus long been regarded as a valid diagnostic tool for environmental assessment. The ben-

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thic macrofauna succession along organic enrichment gradients namely is among the most studied examples in the marine environment of community level response to disturbance, following the Pearson-Rosenberg paradigm (Pearson and Rosenberg, 1978). It is at the basis of the tolerance/sensitivity classification of species used in recently developed biotic indices aiming to portray environmental responses/alterations to anthropogenic effects (Borja et al., 2000). Biotic indices aim to summarize the community data into a single number. This oversimplification, although appealing to managers and decision makers, has both gained followers and opponents in the scientific community (Gray and Elliott, 2009). Several biotic indices based on the benthic macrofauna assemblages have been recently proposed (AMBI, Borja et al., 2000; BENTIX, Simboura and Zenetos, 2002; BQI, Rosenberg et al., 2004; BOPA, Dauvin and Ruellet, 2007) and considered useful diagnostic tools in different coastal and transitional areas (see reviews in Diaz et al., 2004; Salas et al., 2006; Gray and Elliott, 2009; Pinto et al., 2009). The most widely used, alone or in conjunction with other metrics, is the AZTI Marine Biotic Index, AMBI. It has been integrated with measures of species richness, abundance and/or diversity, originating multi-metric indices, which accommodate the class ranges to satisfy the WFD. Examples include the Multimetric AMBI, Basque Country, Northern Spain (M-AMBI, Borja et al., 2004; Muxika et al., 2007), the Infaunal Quality Index, United Kingdom (IQI, Quintino et al., 2006), the DKI, Denmark (Borja et al., 2007), the Benthic Assessment Tool, Portugal (BAT, Teixeira et al., 2009). AMBI, either alone or in conjunction with other indices, has been tested in a variety of geographic areas around the world, subject to different types of perturbations (Salas et al., 2004; Muniz et al., 2005; Muxika et al., 2005; Labrune et al., 2006; Dauvin et al., 2007; Borja et al., 2007; Bigot et al., 2008; Callier et al., 2008; Grémare et al., 2009). Some studies question the performance of biotic indices as quality indicators (Zettler et al., 2007; Blanchet et al., 2008; Lavesque et al., 2009), and it is clear that they require testing in areas under the influence of single disturbance agents, which is the aim of the present study.

In order to test the performance of biotic indices in a situation of mild organic enrichment of the seafloor, these were compared to a series of independent descriptors using an integrated assessment approach. The study included the analysis of sediment baseline descriptors, stable carbon and nitrogen isotopes in sediment and benthic species, benthic community species composition and abundance, and biotic indices. The assessment here conducted differs from the more conventional sediment quality triad concept (SQT, Long and Chapman, 1985) given that no sediment ecotoxicological component was included. It was instead replaced with a stable carbon and nitrogen isotope analysis for the sediment and benthic species, given their ability to identify the terrestrial vs. marine origin of the organic matter and hence provides a direct measure of the spatial extent of the organic enrichment (Sweeney et al., 1980; Van Dover et al., 1992). Borja et al. (2008a) did not include the SQT as an integrative tool in assessing ecological integrity since they consider that this methodology is focused on assessing pollution. However, Chapman and Hollert (2006) pointed to the possibility of expanding the SQT concept by including other components that could strengthen the assessing power of this integrative tool. This could help directing SQT not only to focus at pollution assessment situations but also to a more ecosystem-based approach, given the use of the right components (see also Chapman, 2009).

This study was conducted in a dispersive coastal shelf area under organic enrichment from a sewage point source and tests the null hypothesis that with increasing distance from the outfall no significant differences exist in baseline sediment descriptors, macrofauna assemblages species composition and abundance, their stable carbon and nitrogen isotopic compositions and synthesis biotic indices. The various descriptors were tested using permutation

multivariate analysis of variance (Anderson, 2001), using areas at increasing distance from the outfall as a fixed factor and sites nested in areas. Because no other anthropogenic point source emission exists in the area, this study interrogates biotic indices under a scenario for which their sensitivity/tolerance species classifications were initially developed, i.e. organic enrichment.

#### 2. Methodology

#### 2.1. Study area and sampling

The study area is located at depth ranging from 40 to 60 m on the coastal shelf off Lisbon, Western Portugal. The main sources of organic material to this coastal area include a sewage outfall point discharge and the outflow from the Tagus estuary. This outfall is the largest in Portugal with an average flow of  $1.6 \, \mathrm{m}^3 \, \mathrm{s}^{-1}$  and consists of a double branch system placed about 15 km west of the mouth of the Tagus estuary. The effluents are diffused at a mean depth of 40 m, in the final 400 m of both outfall branches, approximately between 2350 and 2750 m offshore. At present, the system includes a preliminary treatment with screening and removal of grit and non-degradable materials, soon to be upgraded to an enhanced primary treatment with UV disinfection.

The operation of the outfall was initiated in 1994 and a regular monthly monitoring of the effluent is held since 1997, based on composite daily samples. Between 1997 and 2007, the effluent flow rate ranged a mean value from 99,926 (1998) to 186,490  $\rm m^3/day$  (2003), with an overall mean value of 142,222  $\rm m^3/day$  (Santos et al., 2008). In 2007, the outfall discharged a mean value of approximately 140,000  $\rm m^3/day$ . Between 1997 and 2007, the mean annual biological oxygen demand (BOD5) value ranged from 210 mg/L (2003) to 381 mg/L (2007) and the chemical oxygen demand (COD) from 480 mg/L (2003) to 687 mg/L (2005). In 2007, the mean BOD5 and COD were approximately 381 and 680 mg/L respectively (Santos et al., 2008).

The sewage outfall is installed in an area of fine sand with a low proportion of silt and clay. With increasing depth and towards the estuary, the amount of fine particles (<0.063 mm) in the sediment increases and reaches a proportion close to 80% of the total sediment (Freitas et al., 2006). This sediment structure has remained stable through time as was the same before the operation of the outfall (Silva et al., 2004; Sampaio et al., 2010). Previous studies have also shown that the highest sediment contamination in polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) follows closely the sediment fines' content. Thus the more contaminated sediments, both before and after the outfall operation, are the deeper muddy sediments influenced by the Tagus estuary outflow whereas the outfall is installed in the shallower parts, over fine sand with low silt and clay content and very low PCB and PAH contents (Quintino et al., 2001; Silva et al., 2004). Moreover, in 2007 and more recent years, PAHs, PCBs and metals, followed the same pattern (unpublished data).

This study is based on samples taken in 2007, at nine sites arranged in three areas placed at increasing distance from the outfall, three sampling sites per area with three replicates per site and descriptor (Fig. 1). Previous sediment studies in this coastal area allowed placing the three study areas over the same sediment type, fine sand with low silt and clay content and low persistent organics and metal contamination. All sediment samples were obtained with a  $0.1\,\mathrm{m}^2$  Smith–McIntyre grab, at the reason of nine replicates per site, three for the macrofauna community study, three for the baseline sediment characterization and sediment stable isotope analysis and three for the stable isotope analysis in the biological material. The samples for the study of the benthic community were washed on board over a 1 mm-mesh sieve and the residue was pre-

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