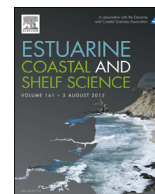




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# Temporal variability of biodiversity patterns and trophic structure of estuarine macrobenthic assemblages along a gradient of metal contamination

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## ABSTRACT

The present study aimed to investigate the response of macrobenthic assemblages along a gradient of metal contamination using a combination of uni- and multivariate methods focusing on their composition, structure and function. A total of six sites were established based on a preliminary survey, which identified three areas with different levels of contamination. These areas were defined as slightly contaminated (SC), moderately contaminated (MC) and highly contaminated (HC). Each area comprised two sites, sampled in four sampling surveys (September 2012, February, May and October of 2013). To investigate the response of the macrobenthic assemblages the number of individuals (N), number of taxa (S), Shannon–Weaver diversity ( $H'$ ), Pielou's equitability ( $J'$ ) and different distance-based multivariate measures of  $\beta$ -diversity (complementarity) were analysed.  $\beta$ -diversity as turnover was also analysed together with spatial and temporal changes in the trophic structure. A clear gradient of increasing contamination was consistently detected, but comparisons with available sediment quality guidelines indicated that adverse biological effects may be expected in all areas. This result suggests measuring concentrations of contaminants in the sediment per se may be insufficient to establish a clear link between ecological patterns and the contamination of the system. Also it highlights the difficulty of identifying reference areas in highly urbanized and industrialized estuaries. Only multivariate analysis (dbRDA; both using the taxonomic and trophic composition) and  $\beta$ -diversity as turnover showed a consistent response to metal contamination. Higher heterogeneity, mainly due to contribution of rare species (i.e. species present in a single sampling period), was observed in the least contaminated area (SC), decreasing towards the HC. In terms of the trophic function, a shift from a dominance of carnivores in the SC to the dominance of deposit-feeding organisms (and associations) along the contamination gradient was evident.

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## 1. Introduction

Estuaries are among the most socio-economically relevant coastal ecosystems. However, they have been exposed to a variety of pollutants from numerous sources, which tend to become

trapped in sediments (Serafim et al., 2013). Among the typical array of contaminants found in estuarine sediments, trace metals, resulting from the rapid industrial development over the past century, represent a major environmental problem worldwide (Iwasaki et al., 2009; Subida et al., 2013). Since sediments can act as a sink or source of contaminants to the water column (Adams et al., 1992), the presence of trace metals in estuarine systems can have ecological repercussions for both benthic and pelagic fauna and flora.

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Several worldwide studies have tried to evaluate the ecological impact of trace metals on local fauna using macrobenthic organisms as biological indicators (e.g. MacFarlane and Booth, 2001; Warwick, 2001; Muniz et al., 2004; Thompson and Lowe, 2004; Cheggour et al., 2005; Mucha et al., 2005; Dauvin, 2008; Josefson et al., 2008; Ryu et al., 2011). Macrobenthos is a key biological component of the ecosystem, playing a relevant role in detritus decomposition, nutrient cycling and on the energy flow to upper trophic levels (Wallace and Webster, 1996; Nunes et al., 2008). Additionally, their sedentary lifestyle (Dauvin, 2007; Patrício et al., 2012), relatively long life-spans (Reiss et al., 2006) and high taxonomic diversity increase the ability of these organisms to reflect anthropogenic and/or natural disturbance gradients over space, time and trophic levels. However, community level responses in estuarine field studies can be unpredictable or difficult to detect and interpret due to the physical, chemical and ecological complexity of these systems (Dauvin, 2008). The lack of clear relationships between metal contamination in the environment and the response of macrobenthic organisms at the community level may reflect the complexity of the biological responses in a community of diverse taxa (Newman and McIntosh, 1991). Three main factors accounted for the inconsistent patterns between macrobenthic communities and metal levels in sediment: i) metals from natural and anthropogenic origins coexist in marine sediments (Chapman and Wang, 2001; Mucha et al., 2005; Dauvin, 2008); ii) the total concentrations of metals alone do not indicate that they are bioavailable (Bryan and Langston, 1992; Burton, 2010); and iii) the exposure to metals lead to the expression of tolerance mechanisms in aquatic organisms (Bryan and Langston, 1992; Warwick, 2001; Nunes et al., 2008). Moreover, several macrobenthic species showed tolerance to high levels of accumulated trace metals due to a range of physiological detoxification mechanisms (i.e. metallothionein like proteins, metal rich granules) (Mouneyrac et al., 2003). Such protective mechanisms could prevent alterations at community level due to metal contamination.

Ecologically significant responses of estuarine benthic assemblages to anthropogenic pollution are usually measured by changes in the diversity of the macrobenthos (Ryu et al., 2011), based on the assumption that such disturbance will induce changes in species composition, abundance and biomass (Pearson and Rosenberg, 1978). However, the majority of diversity studies addressing the spatial and temporal patterns of variability in communities facing anthropogenic pressures have been focused on local and regional diversity (alpha- and gamma-diversity, respectively). Therefore, beta-diversity (complementarity or turnover) has received less attention (Gray, 2000; Magurran, 2004). This diversity component provides a measure of biological dissimilarities among environments and is mainly influenced by the environmental conditions and geographical distance (Costa and Melo, 2008). Thus, measures of regional and local diversity combined with multiple beta-diversity metrics can be used to disentangling the spatial and temporal changes in the diversity patterns of macrobenthic assemblages under contrasting disturbance pressures (Bevilacqua et al., 2012).

The Tagus estuary is one of the largest European estuarine systems, serving a population of over 2 million people. The estuary also supports approximately 18,000 industries with an annual industrial load of  $75.5 \times 10^6 \text{ m}^3$  (Vasconcelos et al., 2007). The Tagus estuary has been classified as the most impacted estuary in Portugal (Vasconcelos et al., 2007), with some areas exhibiting extremely high concentrations of trace metals in the sediment, reaching levels 20 times higher than the natural background (Figüeres et al., 1985). Mercury (Hg), known as an extremely toxic element for biota (Boening, 2000; Cardoso et al., 2013), has been shown to have an extremely high accumulated concentration (both

total Hg and methylmercury) at a specific area located near the cities of Barreiro and Seixal (Canário et al., 2005). This area represents a historic contamination by Hg, mainly resulting from outflows of a pyrite roasting plant and smelter works (Figüeres et al., 1985). This area also supports large industry complexes (e.g. chemical, petrochemical, metallurgic, shipbuilding and cement plants) (Caçador et al., 1996), which contribute additional contamination, including cadmium (Cd), lead (Pb) and copper (Cu). A recent study has successfully attributed the metal enrichment of Tagus estuary delta to an anthropogenic origin resulting from the industrialization starting in 1930s (Mil-Homens et al., 2009). These authors also suggest that the enhancement of industrial and domestic treatment, as well as the actual absence of older industrial point sources, has not yet resulted in a reduction of trace metal enrichment. The high residence time of trace metals in the environment associated with persistent anthropogenic metal sources in this area of the Tagus estuary makes it a relevant case study.

The present study aims to investigate whether the composition and structure of macrobenthic assemblages, as well as multiple components of biodiversity (alpha- and beta-diversity), differ across and among areas with different levels of contamination. Given the renewed interest in beta-diversity measures and their potential to provide complementary information to the alpha-diversity measures (Terlizzi et al., 2009; Carvalho et al., 2013; Legendre and De Cáceres, 2013), this component of diversity was also analysed. Beta-diversity helps to detect possible losses of ecological heterogeneity, which can be affected by anthropogenic disturbances (Magurran and Henderson, 2010; Bevilacqua et al., 2012; De Juan et al., 2013). Therefore, classical and multivariate measures of beta-diversity will be applied in order to better understand the variability in macrobenthic patterns in response to metal contamination. We hypothesized that: i) the macrobenthic community composition and structure will change across a gradient of metal contamination; ii) trophic structure as a measure of impacts on the ecosystem function will change across a gradient of metal contamination; and iii) the alpha- and beta-diversity will increase with the distance to the main source of contamination.

## 2. Material and methods

### 2.1. Sampling area and strategy

The Tagus estuary is one of the largest European estuarine systems covering an area of approximately 325 km<sup>2</sup>. This mesotidal estuary, comprised of several channels and islands (Vale et al., 1998), has a tidal range from 1 m (neap tides) to 4 m (spring tides) (Conde et al., 2013). The mean depth is <10 m and the deepest parts, ca. 40 m, are found near the mouth of the estuary. The mean river flow is 400 m<sup>3</sup> s<sup>-1</sup>, although this is highly variable both seasonally and inter-annually. Salinity varies from 0 (50 km upstream from the mouth) to nearly 37 at the mouth of the estuary (França et al., 2005).

Following the findings of previous studies concerning metal contamination in this estuary (Vale, 1990; Canário et al., 2005; França et al., 2005), three areas with different contamination levels were selected and hereafter entitled as “slightly contaminated” (SC), “moderately contaminated” (MC) and “highly contaminated” (HC) (Fig. 1). SC is located upstream near the Vasco de Gama Bridge while MC and HC are located close to the margins of Montijo and Barreiro (Fig. 1). The selection of the areas also took into consideration the existence of similar salinity regimes, depth and sediment grain-size composition. In each area, two sites were established within each area, totalling six sites per sampling period. The GPS location of each site was recorded. Four sampling surveys were carried out, specifically in September of 2012, and in February,

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