



## How functional traits of estuarine macrobenthic assemblages respond to metal contamination?



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### ABSTRACT

The effects of metal contamination on estuarine macrobenthic communities were investigated using the Biological Traits Analysis (BTA). The study was carried out in the Tagus estuary (western Portugal). Samples of macrobenthic communities and associated environmental variables were taken in four surveys (September 2012, and February, May and October 2013) across the contamination gradient from three main zones: a slightly contaminated, a moderately contaminated and a highly contaminated zone. Functional traits for the most abundant species were assigned using seven categories based on “Feeding mode”, “Life span”, “Body size”, “Motility”, “Position in sediments”, “Larval type” and “AMBI ecological group”. To investigate whether the macroinvertebrate community structure was associated with the environmental parameters and biological traits an integrative multivariate analysis, combining the RLQ analysis and the fourth-corner method, was applied. Within this analysis, human-induced estuarine variables (metals) were rendered independent from natural ones (sediment fine particles) through partial correlations. Following this approach, it was possible to decouple the effects of two typically highly correlated environmental descriptors with different origins. Overall, the study identified significant relationships between sediment environmental descriptors and the functional traits of macrobenthic communities. Further, RLQ/Fourth-corner combined analysis successfully isolated the traits and corresponding species that were most correlated with the measured concentration of trace metals in sediments, supporting the knowledge that benthic organisms exhibit distinct responses to different levels of disturbance. A shift in species dominance occurred along the contamination gradient with epifaunal tolerant species with very small size, long life span, and crawling motility dominating the highest contaminated area. This area was also related with surface deposit-feeder species. The most representative species associated with this area was the gastropod *Peringia ulvae*. Less contaminated sites revealed large-sized specimens, carnivores and swimmers, mainly represented by the polychaete *Nephtys hombergii*. This finding is consistent with other studies addressing different kinds of disturbance, where a shift in dominance from carnivore/predators, long-lived and large animals to the predominance of small-size, short-lived and deposit-feeders has been observed across increasing gradients of disturbance. Our results reinforce the importance of macrobenthic functional traits analysis to assess human disturbances driven impacts in multi-stressed estuarine ecosystems. By analysing the environmental variables with different origins independently, we were able to draw conclusions about the effects of human pressures (metals) on macrobenthic traits. Such distinction can be particularly useful to isolate different environmental descriptors and assess their effects on functional diversity, making the current approach promising in evaluation the ecological effects of anthropogenic stressors in estuarine areas.

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## 1. Introduction

Estuaries are among the most dynamic and complex natural ecosystems in the world (Liu et al., 2003) but at the same time, they are amongst the most threatened by anthropogenic inputs coming from upstream and metropolitan areas located at their margins (Mucha et al., 2005). Trace metals are one of the most common anthropogenic pollutants that impact estuaries (Riba et al., 2003) mainly resulting from twentieth-century industrialization of cities that typically surround estuarine areas (Kennish, 2002). Despite the reduction in emissions in recent years, metals are still stored in estuarine sediments, which may represent an alternative source of metal contamination for benthic organisms (Cheggour et al., 2005). Sediment metal content is usually up to five-times higher concentrations than the overlying water, and therefore significant ecological effects are expected even under the presence of a partially bioavailable metal fraction (Bryan and Langston, 1992). Typically, under metal contaminated scenarios, community responses reflect the impact of metals on the local species, both at the individual and population levels (Luoma and Carter, 1991).

Macrobenthic communities are a well-known component of estuarine ecosystems, playing an important role in the system dynamics (Herman et al., 1999). Due to their direct contact with the sediment and their limited mobility, these communities are susceptible to metal contamination (Gray, 1974), which can favour the occurrence of tolerant/opportunistic species and affect the overall biodiversity or number of species (Stark, 1998; Mucha et al., 2003; Nunes et al., 2008; Ryu et al., 2011). Furthermore, the relatively short-lived character of macrobenthic organisms, contrary to other taxonomic groups (e.g. fishes, birds), enables an assessment of health relative to recent ecosystem history rather than earlier events. However, identifying metal effects on macrobenthic communities can be difficult, particularly in estuaries, which are influenced by highly physical, chemical and geological dynamics and complex ecological interactions (Dauvin, 2008). Estuarine complexity is particularly well reflected in macrobenthic community dynamics. Seasonal (Ysebaert and Herman, 2002; Veríssimo et al., 2013), spatial (Mucha et al., 2005; Barnes and Ellwood, 2012) and even small spatial scale (Giménez et al., 2014; Brauko et al., 2015) oscillations in the structure of macrobenthic communities have been linked with higher level physical factors as well as biological interactions.

Biotic and abiotic factors that influence the benthic environment result in a wide variety of functional adaptations in benthic organisms (Vernberg, 1981). Even though each species is uniquely adapted to its niche and show specific functional performances, groups of benthic organisms have common functional attributes (Pearson, 2001). Hence, the functional structure of a community can be represented by a set of traits describing behavioural and morphological characteristics displayed by the observed species (Paganelli et al., 2012). These traits are selected by biotic factors such as the identity, richness, evenness and abundance of the inhabitant species as well as by their interactions and how these community features vary over time and space (Culhane et al., 2014).

The Biological Traits Analysis (BTA) can be a useful analytical method for addressing ecological functioning (Bremner et al., 2003; Veríssimo et al., 2012). The link between traits used by BTA and ecosystem processes is a considerable advance over traditional methods aiming to analyse changes at the ecosystem functioning (Díaz and Cabido, 2001; Dolédec et al., 2006). They integrate a broad range of information on biological traits regardless of the taxonomic group (Statzner and Bêche, 2010) in addition to the existing strong links between traits and ecosystem processes (Díaz and Cabido, 2001). The relevance of the BTA is that it is an objective measure of functional diversity, gathering information on a range

of ecological characteristics exhibited by the whole species pool (Bremner et al., 2003).

The difficulty in assessing trait detailed information, lacking for the majority of marine invertebrate species, has been pointed out as the main limitation of a more widespread use of the functional traits concept in benthic ecology (Pearson, 2001). More recently, the higher availability and accessibility of studies and the creation of web databases have improved the information on biological traits (van der Linden et al., 2012) leading to a growing interest in the BTA approach. Therefore, the increasing number of studies using this approach is not surprising (Bremner et al., 2003; Statzner et al., 2004; Cochrane et al., 2012; Paganelli et al., 2012; van der Linden et al., 2012; Veríssimo et al., 2012; Munari, 2013).

The functional trait analysis has been already applied to identify patterns of variation in macrobenthic functional traits under different scenarios of disturbance such as organic enrichment (Villnäs et al., 2011; Gusmao et al., 2016), physical disturbance (Tillin et al., 2006) or toxic sediment pollution (Archambault et al., 2010). The central issue in this analysis, faced by ecologists in recent years, is how to relate data of species composition, environmental descriptors and functional traits (Rachello-Dolmen and Cleary, 2007).

The multivariate ordination method – RLQ analysis (Dolédec et al., 1996) directly relates habitat and environment oscillations to differences in functional diversity (Rachello-Dolmen and Cleary, 2007) allowing the assessment of both environmental conditions and biological traits affected by disturbance, as well as their inter-relationship (Dolédec et al., 1996; Ribera et al., 2001; Hausner et al., 2003; Gámez-Virués et al., 2015). Hence, the use of species traits and RLQ scores can be used in conservation management, to monitor and predict the effects of changes in estuarine macrobenthic communities. This method has been successfully applied in studies focusing on different biological entities such as plants (Bernhardt-Römermann et al., 2008; Minden et al., 2012), birds (Hausner et al., 2003; Cleary et al., 2007), fishes (Pease et al., 2012), insects (Ribera et al., 2001; Gámez-Virués et al., 2015) stream macroinvertebrates (Díaz et al., 2008), coral reefs (Rachello-Dolmen and Cleary, 2007) and marine macroinvertebrate communities (Culhane et al., 2014). Despite the potential of the RLQ in relating environmental descriptors with organisms' functional traits, to date no study has used it to assess the effects of metal contamination on the functional structure of estuarine macrobenthic communities. To our knowledge, the assessment of metal contamination and specific effects on estuarine macroinvertebrates through BTA has never been carried out. Overall, the evaluation of trace metal effects on the receiving biota has received little attention (Bryan and Langston, 1992).

Biological traits of macrobenthic estuarine communities are expected to respond to both natural and anthropogenic pressures that influence these ecosystems (van der Linden et al., 2012). Since environmental descriptors can act as filters and thus allowing only a limited spectrum of traits to persist, coexisting species are more similar to one another than would be expected by chance (Zobel, 1997). Taking into account that functional traits and their interactions determine the functioning and stability of communities and ecosystems (Loreau et al., 2001), and provide information about how communities respond to environmental stress (Lavorel and Garnier, 2002), in the present study we aim to assess the response of estuarine macrobenthic communities to different levels of metal contamination based on the BTA. We hypothesized that: a) benthic macroinvertebrate traits are differently expressed in time and space; b) metal contamination is a main driver of the macrobenthic community regarding their functional structure and c) with increasing disturbance, a shift toward opportunistic traits will be observed as reported before for example regarding sewage discharges.

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