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A TRAIT-BASED APPROACH TO INVESTIGATE MACROBENTHIC COMMUNITY FUNCTIONING IN ESTUARINE AND COASTAL ECOSYSTEMS

Tese de doutoramento em Biociências, ramo de especialização em Ecologia Marinha,
orientada pelo Professor Doutor João Carlos Marques, pelo Doutor Angel Borja e pela Doutora Joana Patrício,
apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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A trait-based approach to investigate macrobenthic community functioning in estuarine and coastal ecosystems

Doctoral thesis in Biosciences, scientific area of Marine Ecology, supervised by Prof. Dr. João Carlos Marques, Dr. Angel Borja and Dr. Joana Patrício, presented to the Faculty of Sciences and Technology of the University of Coimbra

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For my mother

Ours was the marsh country, down by the river, within, as the river wound, twenty miles of the sea…that this bleak place overgrown with nettles was the churchyard… and that the dark flat wilderness intersected with dikes and mounds and gates, with the scattered cattle feeding on it, was the marshes; and that the low leaden line beyond was the river; and that the distant savage lair from which the wind was rushing, was the sea.

Charles Dickens, *Great Expectations*

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Abstract

Research based on species traits can lead to a deeper understanding on how anthropogenic disturbance and environmental gradients may impact communities and ecosystem functioning, thereby improving ecosystem-based management and conservation, which is vital in the current era of rapid environmental change. Because of this potential, trait-based studies are rapidly expanding. Yet, the utilisation of trait-based approaches in estuaries and coastal zones is still largely unexplored, despite the increasing demand for environmental assessments of these systems to include more functional orientated approaches.

This context sets the main objective of this study, which was to explore whether trait-based approaches can provide an improved picture on how environmental change may impact macrobenthic community functioning in estuarine and coastal environments. Therefore, this study aims to contribute to a deeper knowledge of the overall functioning of these ecosystems, helping to improve ecosystem-based management.

To achieve this goal, we specifically focussed on testing and evaluating multiple complementary and novel trait-based indices. These include the: “community-weighted mean trait values” (CWM) defining the dominant traits in a community; several functional diversity (FD) indices expressing the extent of trait differences in a community; and a new index developed during this study that is based on the relation between taxonomic and functional diversity. The indices were tested for their ability to reflect anthropogenic disturbance and/or environmental gradients on macrobenthic communities in estuaries and coastal zones located in different biogeographical regions, i.e. a temperate European estuary (Mondego estuary, Portugal) and coastal zone (Basque coast, Bay of Biscay, Spain), and in two tropical estuaries (Paraíba and Mamanguape, N-E Brazil).

One of the main lessons learned from the Mondego estuary case study, is that abundance-weighted FD indices should be used with caution in estuarine systems where few and dominant species naturally occur. Within this respect, non-weighted indices, reflecting the extinction of rare species with often-rare combinations of traits, such as the new developed redundancy index, are potentially more useful, particularly when used in combination with the CWM index.

The Basque coastal zone case study showed us primarily that trait-based indices can face difficulties assessing anthropogenic seafloor disturbance caused by effluents, when traits simultaneously respond to multiple other sources of environmental change (anthropogenic and naturally induced) existing in the area.

In the final case study of this thesis, we explored the trait-based approach in two tropical Brazilian estuaries. An important lesson learned from this study is that different taxonomic groups (Polychaetes and Molluscs) within the macrobenthic community might behave differently in response to environmental change. Therefore, we need to be cautious when evaluating the results given by only one macrobenthic group in isolation. Preferably, the entire macrobenthic community should be included in environmental assessment studies.

Overall, our results showed that trait-based approaches have potential to complement the classical taxonomic-based approaches for benthic assessment in estuarine and coastal systems. By using both approaches we could better determine changes in community structure (i.e. taxonomic and the underlying functional structure) that has, potentially, key consequences in the functioning of these ecosystems. Trait-based approaches, despite not being very helpful for detecting subtle gradients in the form of anthropogenic disturbance, were capable of detecting strong environmental gradients. There is, nevertheless, sufficient room for improvement. Particularly important is the acquisition of abundant and accurate trait data on marine macroinvertebrates, which is currently missing for many species, especially for tropical species. Trait-based research is very recent, and new approaches, frameworks, indices and tools are swiftly being developed. All this is promising news for the future of ecosystem-based management and a sustained conservation of estuarine and coastal ecosystems.

Keywords - biological traits, ecosystem functioning, functional diversity, taxonomic diversity, functional redundancy, benthic macroinvertebrates, estuaries and coastal zones, anthropogenic disturbance and environmental gradients.

Resumo

O estudo da variação dos atributos (traits) das espécies poderá permitir uma compreensão mais aprofundada em relação à forma como perturbações antropogénicas e gradientes ambientais podem afetar as comunidades biológicas e o funcionamento dos ecossistemas. Tal contribuirá para uma mais eficiente gestão e conservação destes, o que assume uma importância particular num período de alterações ambientais muito rápidas. O potencial deste tipo de ferramentas de avaliação tem, de forma geral, justificado o rápido aumento dos estudos sobre “traits”. Em estuários e zonas costeiras, no entanto, apesar da crescente necessidade de inclusão da perspetiva funcional na avaliação de qualidade ambiental destes ecossistemas, a utilização de abordagens baseadas em “traits” seja ainda reduzida.

Foi neste contexto que o objetivo principal deste estudo foi definido: Analisar se as abordagens baseadas em “traits” conseguem reflectir de maneira detalhada e precisa a forma como as alterações ambientais s-ao susceptíveos de afetar o funcionamento das comunidades macrobentónicas em ambientes estuarinos e costeiros, visando dar uma contribuição relevante para o conhecimento do funcionamento geral desses ecossistemas, assim como para melhorar a informação utilizável na sua avaliação e gestão de qualidade ambiental.

Com esse objetivo, o trabalho desenvolvido focou-se essencialmente em avaliar a resposta/desempenho de vários índices complementares e inovadores baseados na análise de “traits”, a saber: a) o "community-weighted mean trait value" (CWM), que define os traços dominantes numa comunidade, b) vários índices de diversidade funcional (FD), que expressam a presença dos diversos “traits” numa comunidade, e c) um novo índice desenvolvido durante o presente estudo, que se baseia na relação entre diversidade taxonómica e a diversidade funcional. Os índices foram testados quanto à sua capacidade de refletir o impacto de perturbações antropogénicas e/ou gradientes ambientais nas comunidades macrobentónicas de estuários e zonas costeiras situadas em diferentes regiões biogeográficas, tendo sido utilizados como casos de estudo, além do estuário do Mondego, a Baía de Biscaia, em Espanha, e dois estuários tropicais, os do Paraíba e do Mamanguape, no NE do Brasil.

Uma das principais conclusões do caso de estudo do estuário do Mondego foi que os índices FD ponderados pela abundância devem ser usados com cautela em sistemas estuarinos, onde tendem a ocorrer naturalmente poucas espécies mas com elevada dominância. Neste contexto, os índices não

ponderados, que refletem o desaparecimento de espécies raras com combinações de “traits” frequentemente raras, são potencialmente mais úteis (o novo índice de redundância desenvolvido é um bom exemplo), particularmente quando utilizados em combinação com o índice CWM.

Os resultados obtidos no caso de estudo da zona costeira basca mostraram claramente que os índices testados não conseguiram capturar adequadamente o estado das comunidades sujeitas a efluentes. Este resultado mostrou a dificuldade de utilizar “traits” que respondem simultaneamente a múltiplas fontes de pressão ambiental (antropogénicas e naturalmente induzidas) existentes na área.

Finalmente, da aplicação da abordagem baseada em na análise de “traits” nos dois estuários tropicais brasileiros, resultou a conclusão importante de que diferentes grupos taxonómicos (Poliquetas e Moluscos) integrantes das comunidades macrobentónicas podem apresentar comportamentos distintos face às pressões ambientais. Devemos pois ser cautelosos na generalização dos resultados obtidos quando são analisados grupos taxonómicos isoladamente. De preferência, toda a comunidade macrobentónica deve ser incluída em estudos de avaliação ambiental.

No geral, os resultados desta tese indicam que as abordagens baseadas na análise de “traits” têm potencial para complementar as abordagens taxonómicas clássicas no contexto da avaliação da condição das comunidades macrobentônica estuarinas e costeiras. Usando simultaneamente ambas as abordagens, podemos capturar de forma mais precisa a resposta das comunidades a diferentes tipos de pressão, tanto no que respeita a alterações na estrutura taxonómica como em termos das funções que lhe estão subjacentes. Apesar das abordagens baseadas na análise de “traits” não terem sido muito bem sucedidas a detectar o impacto de perturbações antrópicas ligeiras, foram capazes de detectar o impacto de fortes gradientes ambientais nas comunidades macrobentónicas. Existe ainda bastante margem para o aperfeiçoamento destas ferramentas, sendo particularmente importante o investimento na aquisição de dados precisos sobre os “traits” de espécies de macroinvertebrados marinhos, particularmente de espécies tropicais. Sendo a investigação baseada na análise de “traits” bastante recente, novas abordagens, índices e ferramentas têm sido alvo de propostas e testados nos últimos tempos. Tal representa uma direção promissora para o futuro da gestão ambiental baseada nos ecossistemas, visando nomeadamente a conservação e o uso sustentável dos ecossistemas estuarinos e costeiros.

Palavras-chave – “Traits” biológicos, funcionamento dos ecossistemas, diversidade funcional, diversidade taxonómica, redundância funcional, macroinvertebrados bentónicos, estuários e zonas costeiras, perturbação antropogénica e gradientes ambientais.

Contents

General Introduction

Human impacts have pushed estuarine and coastal environments far from their historical baseline of productive, rich and diverse ecosystems (Lotze et al., 2006). Anthropogenic impacts including, overfishing, habitat destruction, pollution and climate change, have increased in frequency, extent and intensity, modifying the rates of natural replacement and exchange of species, and increasing species invasions and species extinctions (Loreau et al., 2001; Lotze et al., 2006; Worm et al., 2006; Halpern et al., 2008; Thrush et al., 2009; Barbier et al., 2011). These changes and loss in biodiversity represent a great influence over natural balance and dynamics and have a strong potential to alter the functioning of estuarine and coastal ecosystems (Vitousek et al., 1997; Lotze et al., 2006; Worm et al., 2006; Norkko et al., 2013). Healthy communities are essential for retaining the sustained functionality of natural ecosystems (Hooper et al., 2012; Villnäs et al., 2012).

Ecosystem functioning

“Ecosystem functioning” is a broad concept, that refers in essence, to the overall performance of ecosystems (Jax, 2005). It includes many ecosystem processes (e.g. nutrient cycling and biomass production), the services that these processes provide for society (e.g. climate regulation and food production), as well as the resilience and resistance of these processes and services over time or in response to disturbance (Díaz and Cabido, 2001; Hooper et al., 2005; Jax, 2005; Duffy and Stachowicz, 2006; Bremner, 2008). The overall functioning of an ecosystem is complex and involves many factors relating to the abiotic (physical and chemical) and biotic components of the system (Bremner, 2008). Of these biotic components, the effect of biodiversity on ecosystem functioning is widely cited as being influential (Hooper et al., 2005; Cardinale et al., 2012; Lefcheck et al., 2015) and is generally being referred to as the “biodiversity and ecosystem functioning (BEF) relationship” (Loreau et al., 2001). Good ecosystem functioning represents a significant component of ecosystem health (Tett et al., 2013).

Ecosystem-based management and conservation

The increasing requirement to preserve biodiversity, and thus, to ensure a sustainable functioning of coastal and marine systems has triggered the necessity for conservation and management policies (Strong et al., 2015). Legislation initiatives such as the global Convention on Biological Diversity (CBD, 1992), the Clean Water Act (CWA) and Oceans Act in the United States of America, and its European counterparts, the Water Framework Directive (WFD, 2000/60/EC, European Commission, 2000) and Marine Strategy Framework Directive (MSFD, 2008/56/EC, European Commission, 2008; 2010) have been adopted to protect and restore the health of aquatic systems. Borja et al. (2008a) provide a rather complete overview on worldwide legislation.

In the scope of the European directives, the WFD deals with ground and surface waters (i.e. rivers, lakes, estuaries and coasts). In the case of coasts, the application extends on the landward side of a line, every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured. In turn, the MSFD applies to marine waters, from the baseline of the territorial waters up to 200 miles (Borja et al., 2010).

The working principle of both directives is very similar, and broadly consist of assessing and tracking the health status of European waters and the required interventions to bring them back to their desired good status (van Hoey et al., 2010; Rice et al., 2012). Hereby, both directives adopted the “ecosystem-based management (EBM) approach” as a central part of their objectives (van Hoey et al., 2010; Berg et al., 2015). The EBM approach is a framework for integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way (Levin et al., 2009; Atkins et al., 2011; CBD, 1992), and as such it particularly targets the protection of ecosystem processes and services delivered (Berg et al., 2015).

Therefore, to evaluate and track the health of coastal and marine environments, understanding how anthropogenic impacts affect biodiversity and subsequently how biodiversity affects ecosystem functioning is an important requirement (Borja et al., 2016).

Taxonomic-based approaches

In general, biodiversity, and how it relates to environmental change and ecosystem functioning and health, has often been measured and quantified using “traditional” taxonomic-based approaches, which include taxonomic-diversity indices and multivariate methods, based on the taxonomic structure (i.e. abundance, biomass and diversity) of species communities (Tilman, 2001; Vandewalle et al., 2010; Strong et al., 2015).

Among the taxonomic-diversity indices, taxonomic-richness (i.e. total number of taxa in the community) is the simplest index. It is very widely used, often in combination with indices, that incorporate both richness and evenness (expresses how evenly the individuals in the community are distributed over the different taxa) into a single index, such as the popular Shannon-Wiener index and Simpson index (Help et al., 1998; Magurran, 2004).They are quick and easy to interpret and can be used to compare different conditions/locations. The underlying assumption is that environmental stress or disturbance results in reduced taxonomic diversity, indicating an impacted system with reduced ecosystem functioning and health as a consequence (Loreau et al., 2001; Hooper et al., 2005). However, disturbance does not always result in reduced taxonomic-diversity (Rapport et al., 1985) and can, in some cases, have a positive effect (e.g. Connell, 1978; Gotelli and Colwell, 2001). Moreover, some systems, estuaries in particular, may be naturally species-poor (Whitfield et al., 2012). As a consequence, studies that describe a low-diversity community may erroneously categorise it as impacted (Elliott and Quintino, 2007).

Benthic macroinvertebrates as bio-indicators

Taxonomic-based approaches have been applied to describe changes in different species communities (e.g. plants, birds, fish, etc.) within different environments (e.g. aquatic and terrestrial). With regards to coastal and marine assessment, it is often based on benthic macroinvertebrate communities (Birk et al., 2012, Borja et al., 2015, Teixeira et al., 2016). This could be because benthic macroinvertebrates have a crucial role in regulating the functioning of aquatic systems and benthic-pelagic coupling by contributing to habitat engineering, nutrient cycling and primary productivity, and by being an essential part of the food web (Piersma et al., 1993; Lohrer et al., 2004; Villnäs et al., 2012). This group of organisms usually has well-defined responses to environmental changes, in particular those stressors that influence the sediment structure and its chemistry (Newell, 2004; Quintino et al., 2006). For this reason, research on macroinvertebrates has achieved a fundamental role in aquatic ecosystem assessment and management worldwide (McLusky and Elliott, 2004; Borja et al., 2015).

This has resulted in a rapid growing number of so-called “benthic indices” used for evaluating ecosystem health (Díaz et al., 2004; Pinto et al., 2009; Villnäs et al., 2015; Teixeira et al., 2016).These type of indices generally strive to convey an easily understandable assessment of complex ecological data to resource users and decision makers, by combining several benthic variables into a single number (Borja and Dauer, 2008). Most of the recently developed benthic indices are “multi-metric” indices that are based on variables describing the taxonomic structure of macrobenthic communities and the relative abundance of stress sensitive and tolerant species (Villnäs et al., 2015). Examples of multi-metric benthic indices that were considered into the regulations of several European countries in the context of the aquatic directives, are the Benthic Quality Index (BQI: Rosenberg et al., 2004; Leonardsson et al., 2009) and the Multivariate-AZTI’s Marine Biotic Index (M-AMBI: Muxika et al., 2007). These indices have been successfully used to indicate various types of anthropogenic disturbances in different environments and biogeographical regions worldwide (Borja et al., 2015). However, increased caution is required when applying these indices, developed for marine areas, to estuarine systems (Diaz et al., 2004; Blanchet et al., 2008; Dauvin and Ruellet, 2009; Villnäs et al., 2015). Estuaries are highly variable, naturally disturbed environments, and the communities that are adapted to these conditions often show features that are also typical for those exposed to anthropogenic disturbance (i.e. few dominant, highly abundant opportunistic species, which often express a high tolerance to stress). As such, these indices have difficulties in distinguishing changes due to natural stress from human induced disturbances. As a consequence, they may erroneously categorise these systems as impacted (Elliott and Quintino, 2007; Villnäs et al., 2015).

Biological traits

A complementing approach to study how biodiversity relates to environmental change and ecosystem functioning is by analysing changes in the biological trait (i.e. functional) structure of communities. A biological trait (or simply “trait”) is any measurable feature of an organism that potentially affects its performance or fitness (e.g. growth, reproduction or survival ability) in a given environment (Violle et al., 2007; Mouillot et al., 2013a). As such, a trait determines the organism’s response to the environment and its effect on ecosystem functioning (Díaz and Cabido, 2001; Loreau et al., 2001; Hooper et al., 2005; Cardinale et al., 2012). Traits are typically assigned at the species level and the faunal traits most relevant to macroecology and large-scale community ecology include: morphology (e.g. body-size), life-history (e.g. life-span), reproduction (e.g. fecundity), resource-use (e.g. feeding-strategy, living-position) and behaviour traits (e.g. dispersal ability) (Webb et al., 2009; Tyler et al., 2012).

Trait-based approaches

The functional structure of communities can be quantified using trait-based approaches. Similar to traditional taxonomic-based approaches, which include indices and multivariate methods based on the taxonomic structure, trait-based approaches include indices and multivariate methods based on the underlying functional structure of a certain biological community.

Trait-based approaches are based on the habitat-templet and environmental-filtering concepts (Southwood, 1977; Townsend and Hildrew, 1994; Verberk et al., 2013). These concepts state that the environment dictates community assembly through species traits, because only species with specific traits can persist under certain environmental conditions. When disturbance acts as an environmental filter, trait combinations in the disturbed community are reduced to a subset of those existing in the undisturbed community (Boersma et al., 2016).

Thus, trait-based approaches have been proposed as potentially useful tools to identify community responses to disturbances or environmental gradients that may not be revealed by taxonomic-based approaches alone (Vandewalle et al., 2010; Lepš et al., 2011; Mouillot et al., 2013a; Verberk et al., 2013). And, since traits strongly influence the rate and magnitude of ecosystem processes, they are expected to improve understanding of ecosystem functioning (de Bello et al., 2010; Vandewalle et al., 2010; Lavorel et al., 2013; Gagic et al., 2015). Further, communities that have no species in common will share traits (e.g. body size), and trait values can be compared among individuals within and among communities. Therefore, trait-based approaches may highlight patterns across ecosystems that are not apparent using taxonomic-based approaches (Verberk et al., 2013; Boersma et al., 2016).

Because of the potential of trait-based approaches to determine changes in community structure, and potential consequences for ecosystem functioning, the use of trait-based approaches in basic and applied ecology is a rapidly expanding research area (Vandewalle et al., 2010; Verberk et al., 2013; Boersma et al., 2016). Hence, much of the research interest now lies in understanding what organisms do in ecosystems, rather than which and how many species are present (Petchey and Gaston, 2006; Reiss et al., 2009).

Trait-based indices

The functional structure of communities is composed of two main components that can be quantified with different indices (de Bello et al., 2013; Dias et al., 2013). The first component, the “community-weighted mean trait value” (CWM) can be calculated for each species trait as the average trait value in a community weighted by the relative abundance of the species carrying each value (Garnier et al., 2004), for example, the biomass of large-sized species. This index is often used to define the dominant traits in a community and can be particularly useful to understand the response of communities to changes in disturbance and environmental gradients due to environmental selection for certain traits (Vandewalle et al., 2010; Ricotta and Moretti, 2011). It is also directly related to the mass-ratio hypothesis, which states that the traits of dominant species exert a key effect on many ecosystem processes (Lepš et al., 2011; Dias et al., 2013). CWM is considered a single-trait-based index as it can only be calculated for a single trait (Dias et al., 2013). However, it can be calculated for any kind of trait, making quantitatively comparisons between traits possible (van der Linden et al., 2016a).

The other component describes the functional diversity (FD) within a community, i.e. the extent of trait differences among coexisting species (Petchey and Gaston, 2006). FD can be further decomposed into three families of complementary components, i.e. functional richness, functional evenness and functional divergence (Mason et al., 2005; Villéger et al., 2008). A large variety of FD indices have been proposed to quantify one or more of these components (Petchey and Gaston, 2002; Botta-Dukát, 2005; Villéger et al., 2008; Laliberté and Legendre, 2010). Rao’s quadratic entropy index (Botta-Dukát, 2005; Lepš et al., 2006) is one of the most used indices and incorporates all FD components (Mouillot et al., 2013a). FD indices are considered multi-trait indices as they can be calculated with multiple traits at once (Dias et al., 2013). The general assumption for the use of FD indices is that the prevalence of environmental filtering in stressed communities should cause decreased FD (Mason et al., 2013; Mouchet et al., 2010), thereby decreasing the diversity in resource use strategies (Strong et al., 2015). Vandewalle et al. (2010) showed in their review based on four case studies with four different organism types (river benthic invertebrates, soil fauna, terrestrial insect and birds) that both CWM and FD responded to different environmental gradients. Existing FD indices and the methods to calculate them are constantly being upgraded and new indices, or forms of computing them, are being developed (Villéger et al., 2008; Laliberté and Legendre, 2010; Mouchet et al., 2010; Schleuter et al., 2010; Mason et al., 2013; Mouillot et al., 2013a; Podani et al., 2013; Boersma et al., 2016; Carmona et al., 2016).

Quantifying the different components (CWM and FD) of the functional structure of communities are expected to provide complementary information on how communities change along disturbance and environmental gradients that has, potentially, key consequences on ecosystem functioning (Vandewalle et al., 2010; Ricotta and Moretti, 2011; Dias et al., 2013). Yet the extent to which these multiple components and indices respond to disturbance and environmental gradients remains poorly understood (de Bello et al., 2013).

Integrating taxonomic- and trait-based approaches

With the rapid expansion of trait-based research, many recent studies seem to focus on the sole use of FD indices, with the classical taxonomic diversity indices sometimes being overlooked. However, the integration of both approaches may provide important ecological information (Díaz and Cabido, 2001; Vandewalle et al., 2010). For instance, it allows to determine the degree of “functional redundancy” in communities, which is the relationship between taxonomic and functional diversity (Micheli and Halpern, 2005; Sasaki et al., 2009). Functional redundancy is defined as the extent to which a community is saturated with species with similar traits (Petchey and Gaston, 2006). This relation can be of various forms, including an increasing linear relationship (with varying slopes possible), a saturation relationship wherein functional diversity increases at a decreasing rate to reach an plateau at high taxonomic diversity, or even a negative relationship where functional diversity increases with decreasing taxonomic diversity (Díaz and Cabido, 2001; Micheli and Halpern, 2005; Petchey and Gaston, 2006; Sasaki et al., 2009). These forms highlight that taxonomic diversity is not always a good surrogate for functional diversity as different species can share similar traits, i.e. they are functionally identical (Díaz and Cabido, 2001). A certain level of redundancy is considered important to ensure ecosystem resilience to perturbations (Díaz and Cabido, 2001). Functional redundancy is an insurance policy against the loss of ecosystem processes in the event that species are lost. The larger the number of functionally similar species in a community, the greater the probability that at least some of these species will survive to changes in the environment and maintain ecosystem functioning (Díaz and Cabido, 2001; Loreau et al., 2001).

Therefore, determining the relationships between species diversity and functional diversity is critically important for predicting the consequences of species loss on ecosystem functioning (Micheli and Halpern, 2005; Sasaki et al., 2009). For example, Micheli et al. (2014) demonstrated that comparisons between a large marine reserve and fished reefs confirm that fishing significantly reduces functional redundancy of fish communities by removing whole functional groups, causing high vulnerability of ecosystem processes and services to species loss, and that protection of multi-species communities is needed to maintain ecosystem functioning.

Application of trait-based approaches in estuarine and coastal systems

The application of trait-based approaches has a long tradition in terrestrial and freshwater ecology (Vandewalle et al., 2010). Especially with regards to terrestrial plants (see Garnier and Navas, 2011 for a review) and freshwater macroinvertebrates (see Statzner and Bêche, 2010 for a review).For example, Dolédec et al. (1999) provided evidence to link changes in the functional structure of freshwater macroinvertebrate communities to the influence of particular anthropogenic activities within a large European river.

Steadily, trait-based approaches found their way in marine ecology, mostly applied to macrobenthic communities to describe patterns of benthic functioning, mainly with respect to environmental gradients (Bremner et al., 2003, 2006; Ellingsen et al., 2007; Pacheco et al., 2010; Neumann and Kröncke, 2011; Bolam and Eggleton, 2014; Darr et al., 2014; Berthelsen et al., 2015; Törnroos et al., 2015), impacts of bottom trawling (Tillin et al., 2006; de Juan et al., 2007; Olsgard et al., 2008; Clare et al., 2015; Muntadas et al., 2016), and dredging activities (Cooper et al., 2008; Wan Hussin et al., 2012; Bolam et al., 2016). In marine and coastal ecology, trait-based approaches are often collectively known under the name "biological trait analysis" (BTA).

Around the same time, these approaches started to be applied to coastal zones, mainly to evaluate the consequences of increasing hypoxic disturbance (a result of anthropogenic effluents) on macrobenthic community functioning (Marchini et al., 2008; Papageorgiou et al., 2009; Boström et al., 2010; Villnäs et al., 2011, 2012, 2013; Oug et al., 2012; Paganelli et al., 2012; Culhane et al., 2014; Khedri et al., 2016; Krumhansl et al., 2016), and to report the functional consequences of invasive non-native macroinvertebrates (Hewitt et al., 2016; Weigel et al., 2016).

A similar progression occurred in estuarine systems with trait-based studies rapidly emerging (van der Linden et al., 2012; Veríssimo et al., 2012; Dolbeth et al., 2013, 2015, Barnes and Hendy, 2015a, 2015b; Wong and Dowd, 2015), although the number of studies are still disproportional behind those of the other realms. Most of these studies showed that trait-based approaches are potential useful tools for environmental assessment purposes, by revealing changes in macrobenthic communities along disturbance and environmental gradients that could often not be detected with classic taxonomic-based approaches alone. While most trait-based studies have been conducted in regions with a temperate climate (mostly in Europe), in tropical estuaries these studies are rarer (Gusmao et al., 2016; Jimenez et al., 2015; Leung, 2015; Sivadas et al., 2013). As such, there is a considerable gap in knowledge on how tropical macrobenthic communities function in respects to disturbance and environmental gradients (Barros et al., 2012).

Novel trait-based approaches in combination with classical taxonomic-based approaches have proven to improve our mechanistic understanding on how disturbance and environmental gradients impacts biodiversity and also on how it may affect ecosystem functioning. Existing legislation schemes are however still predominantly based on taxonomic approaches, while there is an increasing demand for environmental assessments to also include functional (trait-based) approaches (Strong et al., 2015; Barton et al., 2016).

General objective and thesis outline

The general objective of this thesis was to explore the ability of trait-based approaches to provide a better understanding on how anthropogenic disturbance and environmental gradients may impact benthic macroinvertebrate communities in estuarine and coastal ecosystems. Therefore, this study aims to contribute to a deeper knowledge of the overall functioning of these ecosystems, helping to improve ecosystem-based management, which is vital in the current era of rapid environmental change.

To fulfil the main goal of this work, we structured the thesis into five sections. The first section is a general introduction setting the context of the studies, which is followed by three chapters that explore different aspects of this work. In particular, we focussed on testing and evaluating multiple complementary and novel trait-based indices. The indices were evaluated depending on their ability to reflect anthropogenic disturbance and/or environmental gradients on macrobenthic community functioning in estuaries and a coastal zone located in different biogeographical regions (i.e. a temperate European estuary and coastal zone, and two tropical Brazilian estuaries. The final section is a general conclusion where we summarised the key findings of this thesis. The contents of each section are briefly summarized below:

General introduction - In this section we set the context and revise the state-of-the-art regarding the topics covered by the other sections of the thesis.

Chapter 1 - In this chapter we critically compare the performance of several taxonomic- and novel complementary trait-based indices within an estuarine environment, the Mondego estuary, Portugal. We proposed and developed a new index to measure redundancy based on the relation between taxonomic and functional diversity. This chapter has been published as:

van der Linden, P., Marchini, A., Dolbeth, M., Patrício, J., Veríssimo, H., Marques, J.C., 2016. The performance of trait-based indices in an estuarine environment. Ecol. Indic. 61, 378–389.

Chapter 2 - This chapter assesses how two complementary trait-based indices responded to anthropogenic seafloor disturbance in a coastal system (the Basque coast, Bay of Biscay, Spain), relative to the performance of two of the most widely used and established benthic indices (i.e. AMBI and M-AMBI). Based on the results, we recommended on whether these "novel" trait-based indices have the potential to effectively be used for management purposes. This chapter has been published as:

van der Linden, P., Borja, A., Rodríquez, J.G., Muxika, I., Galparsoro, I., Patrício, J., Veríssimo, H., Marques, J.C., 2016. Spatial and temporal response of multiple trait-based indices to natural- and anthropogenic seafloor disturbance (effluents). Ecol. Indic. 69, 617–628.

Chapter 3 - In this chapter we extended the geographical scope of our analysis. So far, most studies are based upon macrobenthic communities in environments situated within temperate regions. While, there is relatively little known on how these communities function in tropical ecosystems. With this in mind, we combined taxonomic and trait-based approaches to assess how estuarine polychaetes and mollusc communities within two tropical estuaries (Paraíba and Mamanguape) in N-E Brazil responded to disturbance in the form of anthropogenic effluents and to estuarine environmental gradients.

van der Linden, P., Marchini, A., Smith C.J. , Dolbeth, M., Simone, L.R.L., Marques, J.C., Molozzi, J., Medeiros, C.R., Patrício, J., 2017. Functional changes in polychaete and mollusc communities within two tropical estuaries. Estuar. Coast. Shelf S. Doi: 10.1016/ j.ecss.2016.12.019. (*in press*).

General conclusion - In this final section we provide a birds-eye view on each chapter, a synthesis of key findings regarding all chapters, and a synoptic summary responding to the study's main question: are trait-based approaches useful for estuarine and coastal assessment? Finally, we suggest some new directions that will improve future trait-based research for estuarine and coastal assessment.

Chapter 1

The performance of trait-based indices in an estuarine environment

Abstract

The performance of several indices of benthic functioning, based on the traits of estuarine macro-invertebrates, was tested in the lower Mondego estuary (Portugal), whose two arms exhibit different disturbance levels related to hydromorphology. The results showed that some indices responded clearly to this type of disturbance and others not so well. We argue that the community-weighted mean (CWM) trait values in combination with the newly developed SR-FRED index provided the best overall picture of how the benthic communities might have been affected by hydromorphological disturbance. This study also showed that certain indices should be used with caution when dealing with communities with few and dominant species, such as in estuarine environments.

Keywords - species traits, species diversity, functional diversity, functional redundancy, benthic invertebrates, environmental disturbance.

1. Introduction

Traditionally, species-environment relationships have often been studied using taxonomic-based indices (e.g. richness, diversity and abundance of species) (Tilman et al., 2001; Vandewalle et al., 2010). These indices may accurately describe spatial and temporal differences in the composition and structure between species communities. However, they do not capture the causal mechanisms underlying species-environment relationships (Statzner and Bêche, 2010; Mouillot et al., 2013a; Stuart-Smith et al., 2013; Verberk et al., 2013). Trait-based indices are based upon the richness, diversity and abundance of species ‘traits’ (morphological, physiological and life-history characteristics of species), and offer a useful alternative approach, since a species’ ability to deal with environmental disturbance is at least partly prompted by its traits (e.g. Dolédec et al., 1996; Townsend et al., 1997; Statzner and Bêche, 2010; Mouillot et al., 2013a). The concept behind this approach is based upon

Southwood's 'habitat templet theory' (1977), which states that the habitat provides the template upon which evolution forges species traits. When disturbance increases, only species with specific combinations of traits suitable for survival pass through the environmental filter.

Since the 1990s, the number of studies using trait-based indices to investigate the effects of environmental disturbance on different species communities has been steadily increasing (Statzner and Bêche, 2010; Vandewalle et al., 2010; Verberk et al., 2013). Many of these studies have shown that species traits are, to some extent, predictably affected by disturbance (e.g. Statzner and Bêche, 2010; Vandewalle et al., 2010; Stuart-Smith et al., 2013). The functional structure of communities (the traits displayed by the species in a community) have often been described quantitatively by calculating two trait-based indices: (a) the dominant trait-categories in a community, which can be measured by calculating the community-weighted mean trait values (CWM) and/or (b) functional diversity (FD) (Petchey and Gaston, 2006; de Bello et al., 2010; Vandewalle et al., 2010). FD has been defined as the extent of trait differences among species in a community (Petchey and Gaston, 2006), and can be further partitioned into three components: 1) functional richness, i.e. the number of species traits in a community; 2) functional evenness, i.e. the distribution of traits in a community weighted by the relative abundance of species; and 3) functional divergence, i.e. the degree of dissimilarity among traits weighted by the relative abundance of species (Mason et al., 2005; Villéger et al., 2008). Each component provides independent information on the trait structure, and a separate index is required to quantify each component (Mouchet et al., 2010; Mason et al., 2013).

To date, there are about a dozen trait-based indices, most of which measure one component of FD, while only a few integrate more components. Existing trait-based indices and the methods to calculate them are constantly being upgraded and new indices, or forms of computing them, have been developed (e.g. Villéger et al., 2008; Laliberté and Legendre, 2010; Mouchet et al., 2010; Schleuter et al., 2010; Mason et al., 2013; Mouillot et al., 2013a). Most of these studies used theoretical models as surrogates for biological communities along a hypothetical stress gradient, and their general conclusion is that many of the tested FD indices are complementary, each one illustrating its own unique information of community functioning. Mouchet et al. (2010) and Mason et al. (2013) recommended that any study examining changes in assembly processes along disturbance gradients should employ several complementary FD indices.

As with taxonomic-based indices, trait-based indices also have their pitfalls (e.g. Petchey and Gaston, 2006; Verberk et al., 2013). For example, indices that take abundance into account (e.g. Rao’s quadratic entropy) measure the amount of trait dissimilarity between two random individuals in a community (Botta-Dukát, 2005) and by so doing, might give a differential weight to the traits of the dominant species (Petchey and Gaston, 2006). This could provide a distorted picture of functioning in environments where a few species are naturally dominant, as in estuaries. One possible solution when addressing this issue is to measure the amount of trait ‘dissimilarity’ between ‘species’ in a community, instead of measuring it between two random ‘individuals’. One way of doing so is by measuring the amount of ‘functional redundancy’ (FRED) among species in a community, i.e. the relationship between species diversity (SD) and FD (*sensu* Rosenfeld, 2002; Sasaki et al., 2009). FRED is defined as how much a community is saturated by species with similar trait-categories (Petchey and Gaston, 2006) and can range from being non-existent, when all species display different trait-categories (FD = SD), to maximum, when all species share the same trait-categories (i.e. they are functionally identical: FD = 0) (de Bello et al., 2007).

As such, FRED has the potential to be used as an indicator of disturbance (Micheli and Halpern, 2005; Sasaki et al. 2009). The concept behind it goes back to the ‘habitat templet theory’ (Southwood, 1977). FRED is expected to increase (to a certain extent) with increasing disturbance due to the environmental filtering of traits, i.e. rare species with rare trait-categories unsuitable for survival are the first to be filtered out, being substituted by species with less dissimilar trait-categories that can cope with the increase in disturbance. In coastal and marine benthic communities, FRED has been used rather as an indicator of ‘ecosystem resilience’ (e.g. van der Linden et al., 2012; Törnroos and Bonsdorff, 2012; Darr et al., 2014; Dolbeth et al., 2013; Rodil et al., 2013), assuming that FRED acts as a natural ‘buffer’ against the loss of function in the event that species are lost: the higher FRED is, the greater the probability that at least some of these species will survive changes in the environment and maintain ecosystem functioning (Díaz and Cabido, 2001; Loreau et al., 2001). These two seemingly contrasting concepts, as an indicator of disturbance or as an indicator of resilience, make FRED difficult to interpret. More so, because FRED can increase or decrease regardless of the number of species in the community (see also Sasaki et al., 2009), and here we argue that this relationship between FRED and species richness (SR) is important when investigating the effects of disturbance on species communities. Based on this relationship, we developed a new index, the SR-FRED index.

The aim of this study was to test the performance of the SR-FRED index in an estuarine environment, alongside nine other indices, seven of which were based on species traits. As descriptors we used subtidal benthic invertebrate communities from the Mondego estuary, Portugal. The lower estuary consists of a north and a south arm, each with different hydromorphological features, causing differences in the hydrodynamics and benthic community composition between both arms (Teixeira et al., 2009; Veríssimo et al., 2013a). The indices were tested according to the hypothesis that the benthic communities in the north arm are more disturbed, mainly due to the stronger hydrodynamic conditions, than the south arm communities. Thus, we expected to find a higher proportion of traits able to cope with that disturbance for the north arm communities, lower values of taxonomic and functional diversity indices, and lower values of the SR-FRED index.

2. Methods

2.1 Study site

The study was conducted in the lower Mondego estuary which is located on the west central Atlantic coast of Portugal (40º08’N, 8º50’E). The downstream part of the estuary consists of two arms with dissimilar hydromorphological features: the north and the south arms (Fig.1).

The north arm has been subjected to several physical interventions over the last few decades, such as river embankment (canalisation) and the construction of the Figueira da Foz harbour, as it is the estuary’s main shipping channel. As a result, the north arm is deeper (4-8 m during high tide), handles most of the Mondego river’s freshwater discharge, and the combination with fast tidal penetration of seawater results in strong hydrodynamic conditions, i.e. current velocities, turbidity and tidal salinity changes (Teixeira et al., 2008; Veríssimo et al. 2013b). The daily intensity of these conditions is further amplified by seasonal and annual changes in rainfall. The hydrological conditions in the north arm’s downstream areas do not change much, i.e. water depth, bottom salinity (30-35) and sediment characteristics (mostly medium-sized sand with low organic matter content (Teixeira et al., 2008). Dredging activities take place only in the most downstream area of the north arm maintain an optimum depth for shipping activities (Ceia et al., 2011).

The morphology of the south arm was less changed, with most of its area (75%) being covered by intertidal mudflats, including seagrass and salt marsh areas. Between the end of the 1980s and 1998, eutrophication was a major threat to the ecological quality in the south arm. During this period, the riverhead connection with the north arm completely silted up, resulting in high water resident time, followed by eutrophication symptoms, which led to several negative impacts on the seagrass and benthic communities in the south arm’s upstream stations (Patrício et al. 2009; Dolbeth et al., 2011). In 1998, limited communication between the two arms was re-established which led to a reduction in the water residence time and a general improvement in the ecological quality in the south arm (e.g. Grilo et al., 2010; Dolbeth et al., 2011). In 2006, the riverhead connection was completely restored, resulting in a further reduction of the water residence time (Veríssimo et al., 2013b). Still, most of the river's freshwater discharge flows through the north arm and, as a result, the hydrodynamic conditions are weaker in the south arm.

2.1.1 Disturbance in the benthic communities

Previous studies have pointed out that the benthic communities in the north arm are less diverse than those in the south arm (e.g. Teixeira et al., 2008, 2009; Veríssimo et al 2013a) and the main causes are the strong hydrodynamic conditions in this arm, making it difficult for species to settle. The communities in the south arm are faced with milder hydro dynamic conditions, and the higher habitat heterogeneity of this arm allows the settlement of different species and higher species diversity compared to the north arm (Teixeira et al., 2008, 2009; Veríssimo et al., 2013a). Although eutrophication has not been a major threat since 1998, specific weather events such as floods (Winter 2006), droughts (summer 2005) and occasional engineering works have also impacted the benthic communities of each arm in different ways (e.g. Grilo et al., 2010; Dolbeth et al., 2011; Veríssimo et al., 2013b).

2.2 Data collection

2.2.1 Biological data

We used benthic community data collected from six different subtidal stations in the north and south arms of the Mondego estuary, from 2004 to 2008: three stations in the north arm (10, 11 and 12) and three stations in the south arm (4, 6 and 7) (Fig. 1).

At each station, three benthic samples (replicates) were taken with a van Veen grab (0.1 m^2) and sieved in situ through a 1 mm mesh bag. Subsequently, the content was preserved in a 4% buffered formalin solution. In the laboratory, the benthic invertebrates were sorted and identified to species level. Biomass was estimated as ash-free dry weight (g AFDW m-2). Mysids and decapods (crabs and shrimps) were removed from the analyses because the sampling method underestimates the size of their populations (Couto et al., 2010; Neto et al., 2010).

We only considered spatial differences, since the different level of disturbance between the two arms is for the most part related to the particular hydrodynamic conditions of each arm. Our datasets contained the biological data collected during the spring months (March, April, May) to avoid the months in which extreme climatic events occurred (summer 2005 and winter 2006), and to remove the effects of temporal variations.

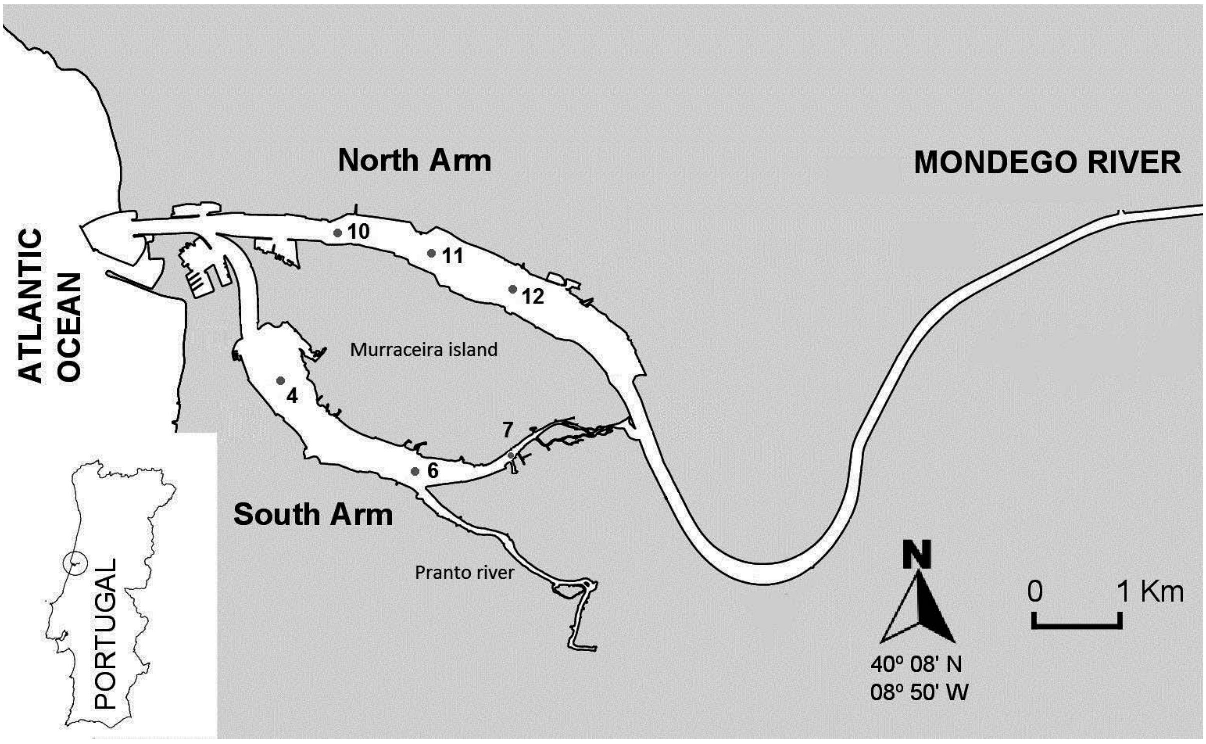

Figure 1. The Mondego estuary and the sampling stations 10, 11, 12 (north arm) and 4, 6, 7 (south arm).

2.2.2 Species traits

We gathered the species traits from a variety of published sources (e.g. species identification guides, scientific papers and established online databases such as MarLIN, 2006 and WoRMS Editorial Board, 2014). A total of four traits containing 15 trait-categories were chosen for their potential ability to indicate environmental disturbance (Table 1). Each species was assigned to the trait-categories using a ‘fuzzy coding’ approach (Chevenet et al., 1994). The trait-categories were given an affinity score between ‘0’ and ‘3’, with ‘0’ indicating no affinity of a species to a trait-category, and ‘3’ indicating a high affinity to the trait-category. The fuzzy coding procedure makes it possible to capture variation in the affinity of a given species to the different categories of a given trait, thereby addressing spatial or temporal differences in the traits of a given species (Statzner and Bêche, 2010). We compiled these scores into the ‘species by traits matrix (47 species and 15 trait-categories). To give the same weight to each species and each trait in further analyses, the scores were standardised so that their sum for a given species and a given trait equalled 1 (or 100%).

Table 1. Species traits (categories and rationale behind the trait selection).

Traits	**Categories**	**Rationale behind the trait selection**
Feeding strategy	Scavenger Grazer Filter Predator Deposit	Feeding traits determine the species abilities to utilise/tolerate different hydrodynamic conditions, with a switch from predominantly filter-feeders to deposit-feeders indicating a potential reduction in the hydrodynamic conditions (Rosenberg,1995; Dolbeth et al., 2009). Grazers are more abundant in areas with high levels of primary producers, usually found in areas with low depth (Dolbeth et al., 2009) and potentially low hydrodynamics. Predators and scavengers will be associated to areas with high availability of prey (Dolbeth et al., 2009), and not specifically with hydrodynamic disturbance.
Living position	Burrow-dweller Tube-dweller Free-living	Tube-dwellers and burrow-dwellers are potentially less vulnerable to strong hydrodynamic disturbance, anoxic conditions and water pollution as opposed to free-living species because they can hide in their fixed tubes or burrows (Reise, 2002).
Body size	Very-small (< 1 cm) Small (1-3 cm) Medium (3-10 cm) Large (> 10 cm)	Small-bodied species may characterise environments with high instability, the result of environmental/anthropogenic disturbances imposed on the organisms (Mouillot et al., 2006).
Life span	Short (< 1 year) Medium (1-5 years) Long (> 5 years)	Short-lived species increase in richness and abundance as disturbance increases (Pearson and Rosenberg, 1978).

2.2.3 Data analysis

Two basic matrices were used to perform all analyses and to compute the indices: the ‘species-biomass-by-station’ matrix and the ‘species-by-traits’ matrix. We used R statistical software to run the statistical procedures (R Core Team, 2013). Data in the ‘species-biomass-by-station’ matrix was transformed by log (1 + x) in order to reduce the influence of dominant species on the samples. The standard affinity scores for each species in the ‘species-by-trait’ matrix were multiplied by the species biomass at each station (‘species-biomass-by-station’ matrix), which resulted in the ‘trait-by-station’ matrix. Ten indices were tested, including the new SR-FRED index (see Table 2). For the computation of the indices, we used two types of software: R statistical software, (including various packages) and an Excel macro file from Lepš et al. (2006) (available from http://botanika.prf.jcu.cz/suspa/FunctDiv.php) (see Table 2 for details).

We tested all indices for significant differences between the stations and the two zones using the Kruskal-Wallis test (Kruskal and Wallis, 1952). Two separate Kruskal-Wallis tests were performed; one using the stations as a factor, and the other using the zones as a factor. A p-value ≤ 0.05 indicated significant difference. Correlation among the indices was tested with a Pearson correlation test. We adjusted the reported p-values using the Holm method (Holm, 1979) (significantly correlated when p value is ≤ 0.05). Table 3 summarises the expected behaviour of the indices according to the literature, and according to the hypothesis tested in this study.

Table 2. List of taxonomic and trait-based indices that were used for this study, with some distinctive features: weighted by abundance, range of values, and the software used to compute these indices.

#	Labels	Index name	References	Weighted by abundance ?	Range of values	Software used to compute the indices (reference)
1	SR	Species richness	Gotelli and Colwell (2001)	No	0 –∞	R: vegan package (Oksanen et al., 2011)
2	SIMD	Simpson diversity	Simpson (1949)	Yes	0 – 1	Excel macro (Lepš et al., 2006) http://botanika.prf.jcu.cz/suspa/FunctDiv.php
3	CWM	Community-weighted mean trait values	Garnier et al. (2004)	Yes	0 – 1	R: Ade4 (Thioulouse et al., 1997)
4	FRIC	Functional richness	Villéger et al. (2008)	No	0 –∞	R: FD package (Laliberté and Legendre, 2010)
5	FEVE	Functional evenness	Villéger et al. (2008)	Yes	0 – 1	FD
6	FDIV	Functional divergence	Villéger et al. (2008)	Yes	0 – 1	FD
7	FDIS	Functional dispersion	Laliberté and Legendre (2010)	Yes	0 –∞	FD
8	FRAO	Rao's Quadratic Entropy	Botta-Dukát (2005)	Yes	0 – 1	Excel macro (Lepš et al., 2006)
9	FRED	Functional redundancy	de Bello et al. (2007); van der Linden et al. (2012)	No	0 – 1	-
10	SR-FRED	Species richness-Functional redundancy	Present study	No	-1 – +1	-

1. Species richness (SR) and 2. Simpson diversity index (SIMD)

SR measures the number of different species within a community, while SIMD takes into account both the number of species and their abundance. SIMD measures the probability that two randomly selected individuals within a community will belong to the same species, with values constrained between 0 and 1. We expected a decrease in SR and SIMD after disturbance (Gotelli and Colwell, 2001).

3. Community-weighted mean trait values (CWM)

CWM is the mean trait value in a community weighted by the relative abundance (in our case study, biomass) of the species in a community (e.g. the biomass of filter-feeding species)

(Garnier et al., 2004; Ricotta and Moretti, 2011). We calculated the CWM values using the ‘trait-by-station’ matrix. This metric has often been used to define the dominant trait-categories in a community and is directly related to the mass ratio hypothesis, which considers the traits of the most abundant species to largely determine ecosystem processes (Ricotta and Moretti, 2011). CWM can also be a useful indicator of disturbance, because it makes it possible to perceive the shifts in the mean trait values within the community due to environmental selection for certain traits (Vandewalle et al., 2010). This index can only be used to analyse single traits separately, making it possible to quantitatively compare trait-categories. A higher proportion of disturbance sensitive traits is expected after disturbance (Statzner and Bêche, 2010).

4. Functional richness (FRIC)

FRIC measures the amount of trait space filled by the species in the community (Villéger et al., 2008). FRIC is independent from species abundance (Mason et al., 2005), has no upper limit and requires at least three species to be computed (Laliberté and Legendre, 2010). FRIC is expected to decrease after disturbance (Mouillot et al., 2013a).

5. Functional evenness (FEVE)

FEVE measures the evenness in the distribution of abundance in the trait space (Villéger et al., 2008). FEVE will be maximised by an even distribution of both species and abundances in the trait space. FEVE decreases either when abundance is less evenly distributed among trait-categories or when some parts of the trait space are empty while others are densely populated. FEVE values are constrained between 0 and 1 and need at least three species to be computed. FEVE is expected to decrease after disturbance because species traits will become more unevenly distributed among species (Villéger et al., 2008; Mouillot et al., 2013a).

6. Functional divergence (FDIV)

FDIV measures the degree to which abundance distribution in the trait space maximises the divergence of trait-categories within the community, i.e. FDIV relates to how trait-categories are distributed among individuals (Mason et al., 2005; Villéger et al., 2008). FDIV is low when the most abundant species have trait-categories that are close to the centre of the trait space and high when the most abundant species exhibit extreme trait-categories (Mason et al., 2005). FDIV values are constrained between 0 and 1 and need at least three species to be computed (Villéger et al., 2008). FDIV is expected to decrease after disturbance (Mouillot et al., 2013a).

7. Functional dispersion (FDIS)

FDIS measures the mean distance of individual species to the centre of the trait space occupied by species (Laliberté and Legendre, 2010) and accounts for both FRIC and FDIV (Mason et al., 2013). FDIS has no upper limit and requires at least two species to be computed (Laliberté and Legendre, 2010). FDIS is expected to decrease after disturbance (Mouillot et al., 2013a).

8. Rao's quadratic entropy (FRAO)

FRAO is a generalised form of the SIMD index that measures the amount of trait dissimilarity between two random entities (individuals) in the community (Botta-Dukát, 2005; Lepš et al., 2006). In fact, if dissimilarity among all species pairs is maximum, then FRAO is identical to SIMD (Botta-Dukát, 2005). As a result, the SIMD index represents the maximum potential value that FRAO can reach in a given community where the species completely differ in their trait-categories. FRAO values are constrained between 0 and 1 and need at least two species to be computed (Lepš et al., 2006). FRAO is conceptually similar to FDIS and simulations have shown high positive correlations between the two indices (Laliberté and Legendre, 2010). FRAO is expected to decrease after disturbance (Mouillot et al., 2013a).

9. Functional redundancy (FRED)

FRED is the relationship between FD and SD (Sasaki et al., 2009) and measures the amount of trait similarity between species in a community. FRED is defined as the extent to which a community is saturated with species with similar traits (Petchey and Gaston, 2006). FRED can range from being non-existent, in which case all species have different trait-categories, to maximum, in which case all species display the same trait-categories. FRED can be measured by subtracting SD – FD, i.e. the potential FD minus the observed FD (as in de Bello et al., 2007), or it can be measured by dividing FD/SD (as in van der Linden et al., 2012). For this study, we calculated FRED as FD/SD, with FD computed as Rao's quadratic entropy (FRAO) and SD computed as Simpson diversity (SIMD). In order to obtain a regularly increasing index, it is necessary to invert the formula into: 1 – (FRAO/SIMD). This way, maximum FRED is indicated by a value of '1' and minimum FRED by a value of '0'. FRED is unaffected by dominant or rare species in the community, since it measures trait similarity between species and not between individuals. In a community of only 1 species, FRED will be 0. Based on its formulation, FRED is expected to increase with disturbance (environmental filtering) until it reaches an asymptote (maximum FRED, occurring when the surviving species share similar traits) (Micheli and Halpern, 2005; Sasaki et al., 2009). For this reason, FRED is incapable of discriminating among levels of disturbance.

10. Species richness-functional redundancy (SR-FRED)

We hypothesise that in cases of non-disturbance, SR will be high and FRED will be low; when disturbance increases, SR will decrease while FRED will increase until reaching an asymptote. When disturbance increases even further, only SR will decrease, while FRED will remain maximal. Based on this relationship between SR and FRED, a new indicator is being introduced, the SR-FRED index, which measures the relationship between SR and FRED. The formula for SR-FRED is the following: SR-FRED = SR' – (1 – (FRAO/SIMD)), where SR' = SRobs / SRmax is scaled between 0 and 1 by dividing the observed SR value (SRobs) by a value of SR (SRmax) assumed to represent the "reference condition" of no disturbance within the dataset. Following the literature on the assessment of reference conditions for the evaluation of ecological quality (e.g. Andersen et al., 2004; Paganelli et al., 2011), SRmax was computed as the 90th percentile of SR distribution within the dataset; this procedure makes it possible to avoid misrepresentation of index results due to outliers (i.e. very high SR values in a single sample). In case of no disturbance, SR (1) – FRED (0) = 1; in case of medium disturbance SR (.5) – FRED (.5) = 0; in case of maximum disturbance SR (0) – FRED (1) = -

1. This index requires at least two species to be computed. Since the observed SR has to be scaled by taking into account a percentile SR value, this index is only suitable for relative comparisons within a dataset.

Table 3. Expected (general) behaviour of the index values according to the literature, and their expected outcome according to this study's hypothesis that the north arm communities are more disturbed due to the stronger hydrodynamic conditions, than the south arm communities.

#	Labels	Index name	Expected (general) behaviour of the index values after environmental disturbance, according to the references	Expected outcome of the index values, according to this study's hypothesis; that the north arm communities are more disturbed due to stronger hydrodynamic conditions, than the south arm communities	
				north arm	**south arm**
1	SR	Species richness	Decrease (Gotelli and Colwell, 2001)	Lower	Higher
2	SIMD	Simpson diversity	Decrease (Simpson, 1946)	Lower	Higher
3	CWM	Community-weighted mean trait values	Higher proportion of trait-categories that are able to cope with the disturbance conditions (see Table 1 for details)	Higher proportion of trait-categories that are able to cope with the disturbance conditions	More even distribution of trait-categories
4	FRIC	Functional richness	Decrease (Mouillot et al., 2013a)	Lower	Higher
5	FEVE	Functional evenness	Decrease (Mouillot et al., 2013a)	Lower	Higher
6	FDIV	Functional divergence	Decrease (Mouillot et al., 2013a)	Lower	Higher
7	FDIS	Functional dispersion	Decrease (Mouillot et al., 2013a)	Lower	Higher
8	FRAO	Rao's quadratic entropy	Decrease (Mouillot et al., 2013a)	Lower	Higher
9	FRED	Functional redundancy	Increase (Micheli and Halpern, 2005; Sasaki et al. 2009)	Higher	Lower
10	SR-FRED	Species richness-functional redundancy	Decrease (present study)	Lower	Higher

3. Results

3.1 Species biomass distribution

The two arms show some marked differences in the spatial distribution of species mean biomass (Fig. 2). The dominant species in the north arm is the polychaete *Nephtys cirrosa* and the bivalve *Cerastoderma edule* (most dominant at station 10), while the dominant species in the south arm are: *C. edule*, *Cyathura carinata, Hediste diversicolor* and *Scrobicularia plana.* There are also differences among the stations, for example, *C. carinata* and *H. diversicolor* are more dominant at stations 6 and 7, and *C. edule* and *S. plana* are more dominant at station 4.

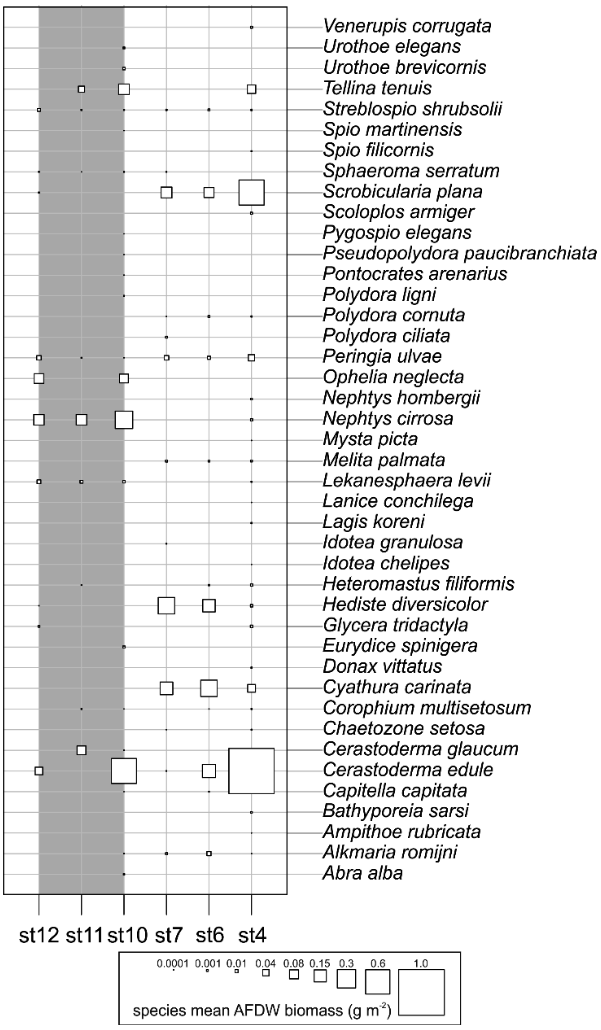

Figure 2. Spatial distribution of species mean AFDW biomass within the north arm (station 10, 11 and 12) (indicated by the grey background) and the south arm (station 4, 6 and 7) of the Mondego estuary. The size of the squares is proportional to the amount of mean biomass (larger squares equal higher biomass).

3.2 Performance of the indices

3.2.1 Community-weighted mean trait (CWM)

The CWM values, i.e. the proportion of species biomass in the community with a given trait category, highlighted differences in the trait structure among stations and the two arms (Fig. 3). Most trait-categories showed significant differences between the stations and the two arms, except for predators (not significantly different between the two arms) and very-small sized species (less than 1 cm). In the north arm, most of the community is composed of medium-sized species (3 to 10 cm), with long-life spans (more than 5 years), burrow-dwellers, and scavengers and predators. This tendency maintains practically throughout the three stations of the north arm, with the exception of station 12, which shows a higher percentage of free-living, very small-size species and feeding traits more similar to the ones found in the south arm. Regarding the south arm, there is a higher proportion of small- (1 to 3 cm) and large-sized (more than 10 cm) species, with short (less than 1 year) and medium (1 to 5 years) life-spans, and a higher proportion of tube-dwelling and free-living species. These species are mostly grazers, deposit-feeders and filter-feeders. These tendencies were similar for the three stations, except for station 7, which has the highest proportion of short-lived, large-sized species.

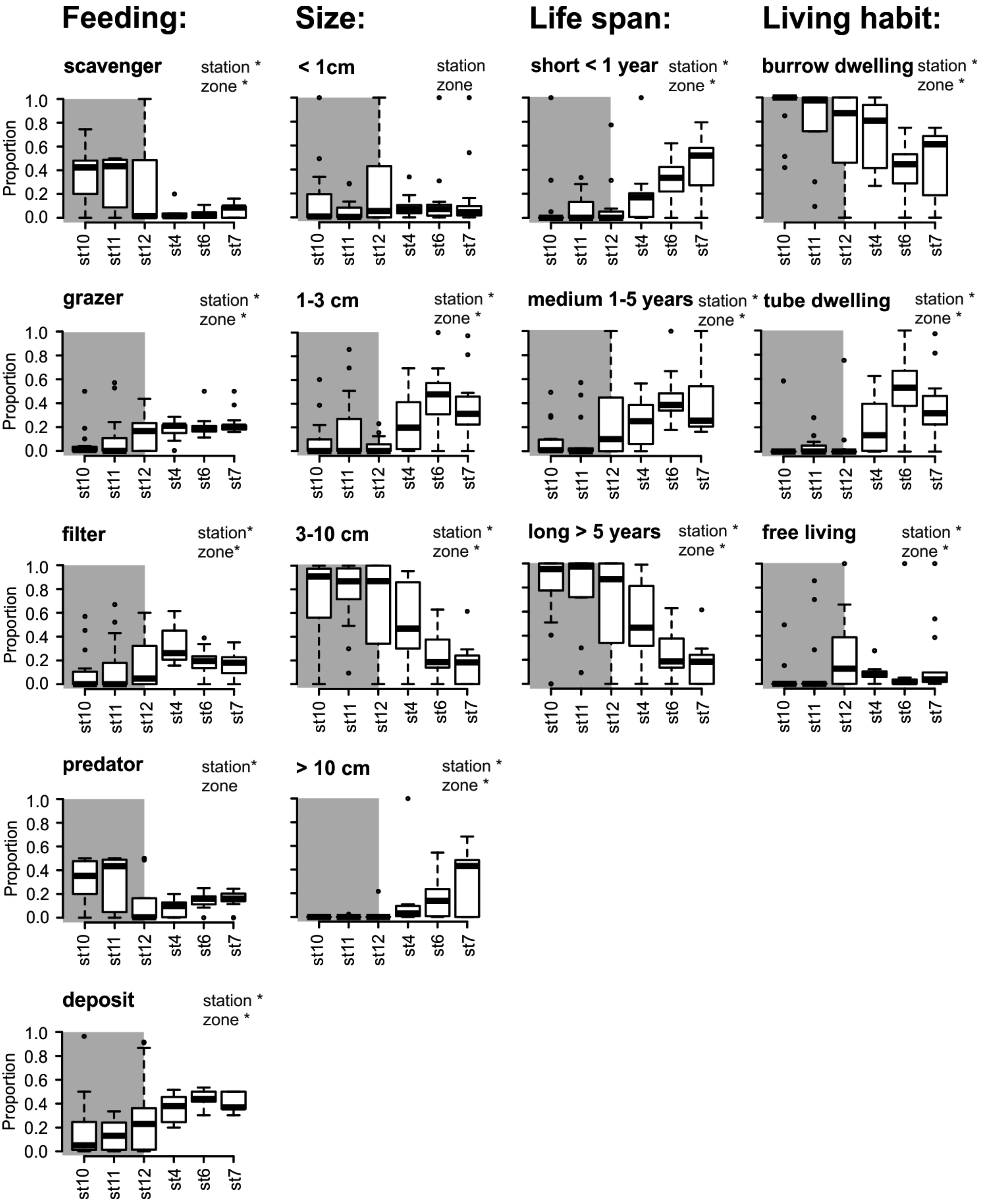

Figure 3. The spatial variability of Community-weighted mean trait values (CWM). The grey background indicates the three stations in the north arm; the white background indicates the three stations in the south arm. * indicates significant differences between the stations and/or the two zones: north and south arm (p-value ≤ 0.05).

3.2.2 Other indices

Fig. 4a-i shows the spatial variability of the index values. Functional dispersion (Fig. 4f) and functional divergence (Fig. 4e) were unable to detect significant differences between stations or zones, respectively. Functional evenness (Fig. 4d) and functional redundancy (Fig. 4h) were unable to detect significant differences between stations and zones; conversely, species richness (Fig. 4a), Simpson diversity (Fig. 4b), functional richness (Fig. 4c), Rao's quadratic entropy (Fig. 4g) and the SR-FRED index (Fig. 4i) made it possible to detect both these differences and all of them provided higher values in the south arm.

Most indices, except functional divergence (FDIV) were significantly correlated to two or more other indices (Table 4). For example, species richness (SR) was significantly correlated to Simpson diversity (SIMD), functional richness (FRIC), functional dispersion (FDIS), Rao’s quadratic entropy (FRAO) and the SR-FRED index, but this relation was most powerful with FRIC (0.75) and SR-FRED (0.78).The SR-FRED index was also significantly correlated to several indices. The relationship between this index and SR was stronger (0.78) than with FRED (-0.56). There was also a highly significant and positive correlation between FRAO and FDIS (0.96).

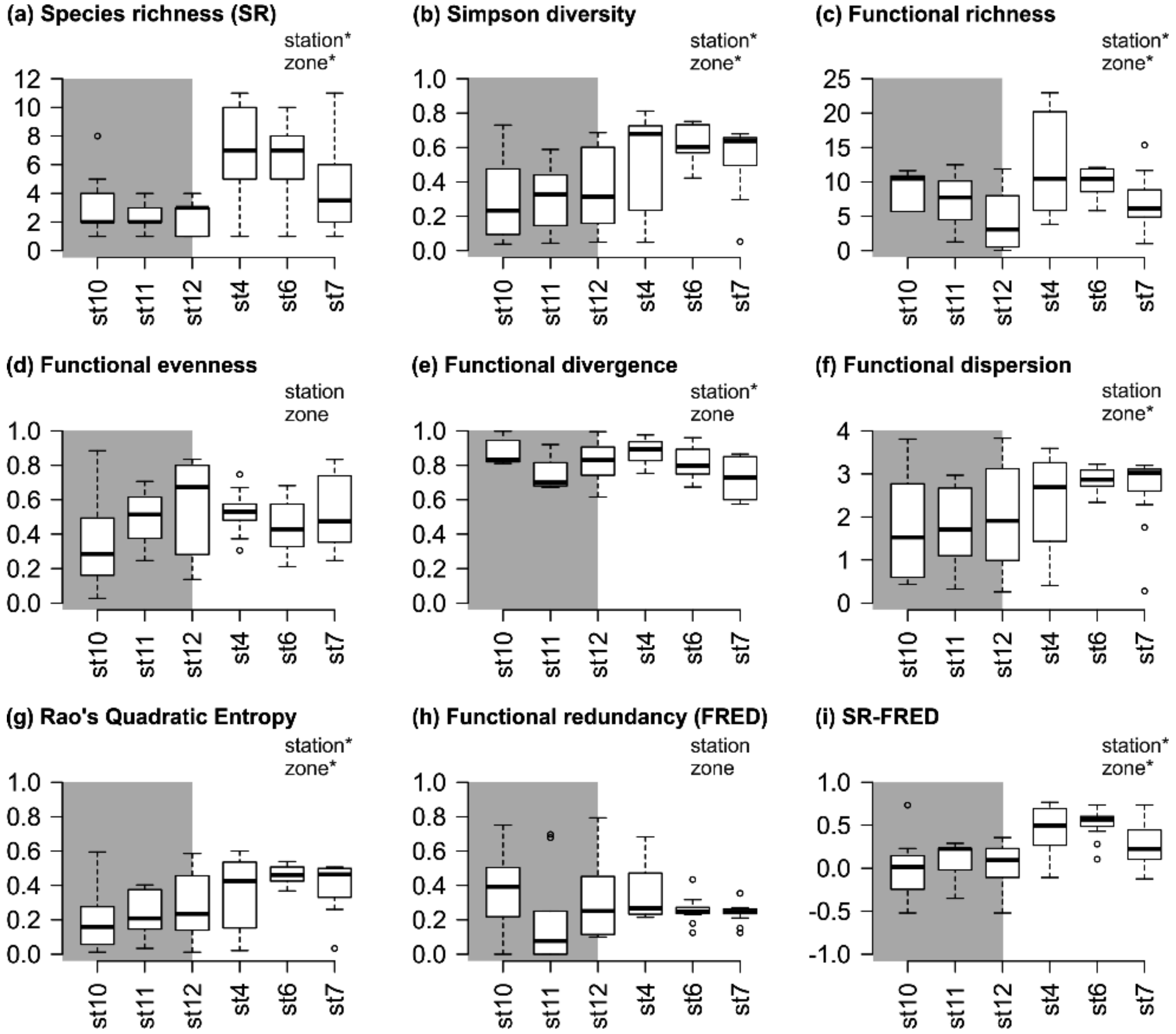

Figure 4a-i. Spatial variability of the index values. The grey background indicates the three stations in the north arm; the white background indicates the three stations in the south arm. * indicates significant differences between the stations and/or the two zones: north and south arm (p-value ≤ 0.05).

Table 4. Pearson correlation values between the indices. Reported p-values were adjusted using the Holm method (significantly correlated when p value is ≤ 0.05*).

	SR	SIMD	FRIC	FEVE	FDIV	FDIS	FRAO	FRED
SIMD	0.6**5***							
FRIC	**0.75***	0.28						
FEVE	-0.07	**0.47**	-0.07					
FDIV	0.04	-0.23	0.10	-0.12				
FDIS	**0.46***	**0.93***	0.21	**0.51***	-0.19			
FRAO	**0.56***	**0.92***	0.26	0.39	-0.17	**0.96***		
FRED	0.06	-0.23	-0.03	-0.20	0.08	**-0.46***	**-0.52***	
SRFRED	**0.78***	**0.70***	**0.57***	0.05	-0.02	**0.68***	**0.80***	**-0.56***

4. Discussion

The aim of this study was to test the performance of the SR-FRED index alongside nine other indices, seven of which were based on species traits, in two estuarine arms subjected to different hydrological conditions. We tested the indices against the hypothesis that the benthic communities in the north arm of the Mondego estuary are more disturbed than the south arm communities due to the stronger hydrodynamic conditions in the north arm. We expected that the indices would indicate this different level of disturbance between the arms. As a result, some indices clearly showed this difference, and others not so well. This section addresses the performance of the indices, having featured the most noteworthy outcomes.

4.1 The community-weighted mean trait (CWM)

The CWM revealed patterns in the spatial distribution of the trait-categories associated to each arm with a different disturbance level. Nonetheless, not all trait-categories responded to the disturbance as expected. The trait-categories' long life-span, very small and medium body size, burrow-dwellers and scavengers were most important in the north arm, while the trait-categories short life-span, small and large body-size, tube-dwellers, grazers, deposit-feeders and filter-feeders were most important in the south arm.

As expected, deposit-feeders were more important in the south arm, as the milder hydrodynamic conditions allowed their food source (i.e. organic matter) to accumulate. Thus, deposit-feeders potentially indicated a reduction in hydrodynamic conditions, as also discussed by Rosenberg (1995) and Dolbeth et al. (2009). For the same reason, grazers (mostly *Peringia ulvae*) were also more important in the south arm. Grazers feed mostly on benthic algae and epiphytes, which are potentially more abundant in this part of the estuary due to its lower depth, large intertidal areas, seagrass and salt marsh areas (Baeta et al., 2009).

We also expected filter-feeders to be more dominant in the north arm, because strong currents usually provide favourable feeding conditions for bivalves (Rosenberg, 1995; Gosling, 2004). However, they were more dominant in the south arm; in fact, they were the second-largest feeding group, after deposit-feeders. Several of its dominant species may act both as deposit and filter-feeders (e.g. *Scrobicularia plana,* Baeta et al., 2009), explaining in part the results obtained. Verdelhos et al. (2015) also found this species to be more abundant in the south arm and reasoned that its sediment preference is probably the main reason for this spatial distribution. This burrowing clam has a preference for fine sand or mud, or sand/mud mixtures, such as those found in the south arm (Verdelhos et al., 2015). Another important reason why this species might prefer the south arm is its milder hydrodynamic conditions. Very strong currents may lead to excessive sediment resuspension and water turbidity, which might affect species performance and survival, namely through the clogging up of the feeding structures of these bivalves (Verdelhos et al., 2014).

Scavengers and predators were the most dominant trait-categories in the north arm. These traits were mostly expressed by one single dominant species, the polychaete *Nephtys cirrosa*. This species usually prefers more coarse sediments (as those found in the north arm) over more muddy and fine-sand sediments (Clark and Haderlie, 1960).

The very small-sized (<1cm) and short-lived species (<1 year) were also associated with the north arm, as they may characterise environments with higher instability due to hydrodynamic disturbance. However, the medium-sized (3-10 cm) and long-lived species (>5 years) were the dominant trait-categories in the north arm. Again, these categories were mostly expressed by *N. cirrosa*, which contradicts the former assumption. In fact, small-sized (1-3 cm) and short-lived species were highly abundant in the south arm, when we expected the opposite due to the milder hydrodynamic conditions. The species that mostly exhibited these trait-categories were *Cyathura carinata* and *Peringia ulvae,* whose preferential habitats occur in the muddy intertidal areas, like those within the south arm, where they can attain high production levels (Dolbeth et al., 2011). Finally, we expected free-living species to be more dominant in the south arm because of the less stressful hydrodynamic conditions. Instead, tube-dwellers and burrow-dwellers were the dominant groups. Here too, the dominance of certain species clearly affected these results, and the relationship between these traits and hydrodynamic disturbance was not totally clear. In this estuarine system, deposit-feeders and grazers seem to be the best indicators of hydrodynamic disturbance, whereas size and in particular life-span are not.

4.2 Functional evenness (FEVE) and functional divergence (FDIV)

It was expected that FEVE and FDIV would decline in the presence of disturbance (Mouillot et al., 2013a), and yet we found no significant differences between the two arms. FEVE and FDIV values were unexpectedly high in the north arm, suggesting that the traits were regularly distributed in the community (especially obvious at stations 11 and 12) and that the most abundant species had dissimilar combinations of traits in comparison to the rare species. In the case of FEVE, its high values could have been related to the low amount of SR. Podani et al. (2013) found FEVE to be negatively correlated with SR, and reasoned that fewer species are more likely to produce an even distribution of traits than many species, due to decreasing functional redundancy (FRED) (see also Mouillot et al., 2013a). This study seems to support their reasoning; SR and FRED values were low, which was especially obvious at station 11. In the case of FDIV, its high values might have also been related to the low amount of SR in combination with the high abundance of a few dominant species. The chance that the most abundant species have dissimilar combinations of traits in comparison to the few rare species will be high. Another reason for the high values of both these indices can be related to their mathematical algorithm.

For the computation of these indices (and for functional richness–FRIC), at least three species are required. However, some of the communities, especially the ones from the north arm, often have fewer than three species, and in these cases, FRIC, FEVE and FDIV values could not be computed. As in this study, several other studies also found FEVE to perform poorly. Mouchet et al. (2010) demonstrated that the power of FEVE to detect assembly patterns occurring in (theoretical) species communities was poor with SR values lower than 40, and very poor when SR was 10. Mason et al. (2013) also found low variation in FEVE, and mentioned the possibility that FEVE is simply not associated with changes in assembly processes. In the case of FDIV, Mouchet et al. (2010) observed medium to high power among all SR values: (FDIV was most powerful when SR was higher than 30). Contrary results were obtained in the studies by Mason et al. (2013) and Pavoine and Bonsall (2010), who found low power using FDIV.

Taking the above into consideration, our main message is that caution is required when using these indices in environments where SR is naturally low, such as in estuarine environments.

4.3 Taxonomic versus trait-based indices

Functional-richness (FRIC) and Rao's quadratic entropy (FRAO), the functional counterparts of species-richness (SR) and the Simpson-diversity index (SIMD), were able to indicate the different level of disturbance between the two arms. As expected, all these indices showed higher values in the south arm than in the north arm, and spatial patterns were significantly similar. The only exceptions were the slightly different FRIC values in comparison to the SR values in the north arm. This indicated that FRIC can increase or decrease regardless of SR; FRIC will show a higher rate of increase or decrease when rare species with rare traits are added to or lost from the community (Mouillot et al., 2013a). Most other studies also found a strong positive relation between these two indices with different types of communities (Villéger et al., 2008; Schleuter et al., 2010; Mason et al., 2013; Podani et al., 2013). The same accounts for FRAO and SIMD, which showed similar patterns (Lepš et al., 2006; Vandewalle et al., 2010). FRAO was also significantly correlated with functional-dispersion (FDIS), which was expected, because both these indices have a similar mathematical background (Laliberté and Legendre, 2010; Mason et al., 2013).

Overall, the trait-based indices FRIC and FRAO performed similarly to their taxonomic-based counterparts SR and SIMD, which indicated that with the loss or addition of a species, unique traits were being lost or added to the community. Again, in communities with few and dominant species, such as those occurring in estuarine environments, abundance-weighted diversity indices like SIMD, FRAO and FDIS should be interpreted with caution. These indices measure the amount of (trait) dissimilarity between two random individuals in a community. As a result, the chance of these individuals belonging to a particular dominant species is very high, resulting in low (trait) dissimilarity (low FD and SD). By so doing, these indices fail to take into account rare species and their traits, which could lead to an underestimation of FD and SD. Rare species often have distinct combinations of traits, thus increasing the FD of communities (Mouillot et al., 2013b).

According to Southwood's 'habitat templet concept' (1977), rare species with rare combinations of traits are the first to be filtered out when disturbance increases, with the remaining species usually being the ones with traits that can cope with this increased disturbance. Therefore, the extinction of rare species and their traits might provide an advance warning to increasing disturbance (Mouillot et al., 2013a). Considering this, indices that indicate the extinction of these rare species and their traits might be especially useful to investigate the effects of disturbance, especially in environments where a few dominant species naturally occur.

4.4 Functional redundancy (FRED and SR-FRED)

FRED measures the amount of trait-dissimilarity among 'species' and not among 'individuals' the way that FRAO and FDIS do. As a result, FRED is not weighted by abundance, i.e. all species are equally important. When rare species with rare combinations disappear because of increasing disturbance, FRED will increase, because the remaining species share traits that are more similar. Thus, FRED might be a potential 'early warning' indicator for increasing disturbance. Nonetheless, FRED should be used with caution as a disturbance indicator. In highly disturbed environments where very few species can survive (low SD), the FD of the community might rapidly approach the value of SD, thus resulting in low FRED. For this reason, FRED was not significantly different between the two arms, in contrast to expectations (higher FRED was expected for the north arm). This result was especially due to station 11, featuring low FD in combination with low SD. Moreover, FRED can increase or decrease regardless of the amount of SR in the community; for this reason, we developed the new SR-FRED index which takes into account the nonlinear relationship between FRED and SR. SR is a critical variable for the interpretation of FRED (Petchey and Gaston, 2002; Sasaki et al., 2009).

The SR-FRED index succeeded in indicating potentially higher levels of disturbance in the north arm, as opposed to the south arm. We argue that this new index provided the clearest picture of the potential changes in the benthic functioning regarding the different level of hydrodynamic disturbance. The strength of this index is that it is based on both SD and FD, it is not affected by dominant species and it is able to give an indication of the level of disturbance. However, one important limitation of this index is that it uses reference values to normalise SR into the range 0-1. Consequently, results strongly rely on the size and quality of the available dataset. To test the full potential of this index, it has to be tested in other environments subjected to different types and levels of disturbance conditions.

4.5 Concluding remarks

Some indices responded clearly to the different level of hydrological disturbance in this estuarine ecosystem and others not so well. We argue that the community-weighted mean trait values (CWM) in combination with the new SR-FRED index provided the best overall picture of how the benthic communities might have been affected by a different level of disturbance. The CWM index is useful for revealing patterns in the spatial distribution of the trait-categories, while the SR-FRED index makes it possible to combine and synthesise the taxonomic and functional structure of the communities. This study also showed that some indices should be used with caution when dealing with communities with few and dominant species, which often occurs in estuarine ecosystems.

Acknowledgements

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Chapter 2

Spatial and temporal response of multiple trait-based indices to natural- and anthropogenic seafloor disturbance (effluents)

Abstract

To support ecosystem-based management and achieve the Good Environmental Status (GES) of marine waters it is important to better comprehend the relationships between biodiversity and environmental disturbance (anthropogenic and natural). Biotic indices are widely used in studies to help understanding these relationships and to assess the environmental status of waters. In recent years, trait-based indices rapidly emerged as an alternative ‘functional’ approach to serve this purpose. In this study, we analysed how two indices based upon the mean (community-weighted mean trait value - CWM) and the diversity of multiple traits (Rao’s quadratic entropy - Rao) in a macroinvertebrate community respond to natural- and anthropogenic seafloor disturbance (effluents) and we compared their performance with the widely used AMBI and M-AMBI.

Our results demonstrate that CWM and Rao were not effective in indicating anthropogenic disturbance in the Basque coast, Bay of Biscay. The main reason was probably that many traits did not have a strong link with this type of disturbance. Besides, the mechanistic links between certain traits and their response to anthropogenic seafloor disturbance in marine environments is currently not well understood. From a management perspective: the CWM does not provide a single value indicating a quality status, which makes it a difficult tool to use and interpret. This index is probably more useful for scientists who want to explore and understand different aspects of community functioning. On the other hand, Rao and other indices expressing trait diversity do provide a single value of functioning; therefore they could potentially be effectively used for management purposes. However, to improve its performance, detailed and accurate trait data is required, which is currently lacking for many marine species.

Keywords - ecosystem functioning, impact assessment, Marine Strategy Framework Directive, macroinvertebrates, biodiversity, ecological indicators.

1. Introduction

Understanding how biodiversity relates to environmental disturbance has been one of the hot topics of aquatic environmental research over the past 40 years (e.g. Pearson and Rosenberg, 1978; Warwick, 1986). A better understanding of this relationship can ultimately help us to preserve and improve the quality of marine ecosystems. During this period, indices based on species traits emerged as an alternative approach to study this relationship (e.g. Bremner et al., 2006; Bremner, 2008), as opposed to the use of mostly structural approaches (e.g. taxonomic-based indices, see Borja et al., 2015). Indeed, increasing evidence suggests that a species ability to deal with environmental disturbance is at least partly driven by its traits (Pearson and Rosenberg, 1978; Bremner et al., 2003; Culhane et al., 2014). As such, trait-based indices have the potential to determine the cause of change in systems by investigating the type of traits affected (Dolédec et al., 1999).

In 2008, the European Union (EU) approved the Marine Strategy Framework Directive (MSFD: European Commission, 2008). The main goal of the MSFD is to protect efficiently the marine environment across European seas; in particular, it aims to achieve Good Environmental Status (GES) of the EU's marine waters by 2020. To assess the current environmental status, the European Commission (2010) has indicated different indicators. Among these are the indices to assess benthic community condition and functionality, in relation to seafloor integrity (see van Hoey et al., 2010; Rice et al., 2012). As the MSFD follows an ecosystem-based approach, the selected indices should be oriented not only to determine structural changes in species assemblages, but also functional (Borja et al., 2013). The inclusion of trait-based indices could help to study these functional changes and, by doing so, they potentially allow to better assess the response of species communities to disturbance (Vandewalle et al., 2010).

Nowadays, one of the most used and established disturbance indices, on benthic invertebrate communities in marine environments (Borja et al., 2015), is the AZTI’s Marine Biotic Index (AMBI: Borja et al., 2000) and its multivariate version: M-AMBI (Muxika et al., 2007). Since their introduction, both indices have been successfully used to indicate various types of disturbances in different environments and biogeographical regions worldwide (Borja et al., 2015), and are officially incorporated into the regulations of several European countries in the context of aquatic directives (Borja et al., 2009). AMBI is based on the sensitivity (response) of benthic invertebrate species to anthropogenic pressures, and species are allocated to five sensitivity (ecological) groups ranging from sensitive to opportunistic (Borja et al., 2000). M-AMBI incorporates AMBI with species richness and Shannon diversity (Muxika et al., 2007). This index is based on the observation that benthic communities respond to an improvement in environmental quality in three stages. Firstly, species abundance increases, subsequently species diversity rises, and finally the opportunistic species become dominant with the subsequent reduction in species abundance and diversity (Pearson and Rosenberg, 1978; Paganelli et al., 2011).

Both indices can essentially be classified as trait-based indices, because the AMBI ecological groups (EG’s) are mostly determined by the response of multiple species traits (e.g. feeding strategy, size, life span, larval development) to anthropogenic disturbance (e.g. Marchini et al., 2008; Culhane et al., 2014). However, these traits are ‘fixed’ within these EG’s, meaning that these indicators cannot be used to analyse each of these ‘individual’ traits separately. Yet, a number of studies demonstrated that analysing each of these individual traits separately, might also be useful for detecting anthropogenic disturbance (e.g. Reise, 2002; Bremner et al., 2003; Cooper et al., 2008; Paganelli et al., 2012; van der Linden et al., 2012; van Son et al., 2013; Törnroos et al., 2015; Weigel et al., 2016).

Two trait-based indices in particular have been increasingly used to assess the response of species communities to disturbance that can handle ‘multiple’ different types of traits (Vandewalle et al., 2010; Ricotta and Moretti, 2011). These are the ‘community-weighted mean trait value’ – CWM (Garnier et al., 2004) and ‘Rao’s quadratic entropy’ – Rao (Botta-Dukát, 2005). CWM can be adequately used to analyse shifts in mean trait values within communities due to environmental selection for certain traits. While, Rao can be effectively used to analyse patterns of trait (functional) diversity, i.e. a decrease or increase in trait diversity compared to a random expectation (Vandewalle et al., 2010; Ricotta and Moretti, 2011). The employment of these indices to assess disturbance is based upon the ‘habitat templet concept’ of Southwood (1977), which states that the habitat provides the template upon which evolution forges species traits. When disturbance increases, only species with specific combinations of traits suitable for survival pass through the environmental filter. Ricotta and Moretti (2011) showed that these two indices may be used to describe two complementary aspects of community structure, such as the mean and the diversity of traits within a given species assemblage, and that using them simultaneously can provide an effective framework to assess the effects of environmental disturbance on species communities. Despite the potential utility of these two trait-based indices, surprisingly few studies used them simultaneously (as a framework) to assess disturbance on benthic communities in marine environments (e.g. Paganelli et al., 2012; Culhane et al., 2014; de Juan et al., 2015; Barnes and Hendy, 2015; Weigel et al., 2016).

Taking this into consideration, the main purpose of this study was to assess how the community-weighted mean trait value (CWM) and trait diversity (expressed by Rao) responded to seafloor disturbance relative to the performance of AMBI and M-AMBI. We only analysed disturbance caused by anthropogenic effluents and wave impact, although many other factors may contribute to its disturbance, namely fisheries, dredging and sediment deposit, among others. Based on the obtained results, we could give a recommendation on whether CWM and Rao might be implemented as useful seafloor disturbance indices for the MSFD.

2. Materials and Methods

2.1 Study area, anthropogenic- and natural seafloor disturbance

Environmental and benthic community data were collected annually in winter, between 1995 and 2012, from sixteen marine off-shore sampling stations along the Basque coast, in northern Spain, Bay of Biscay (Fig. 1A, B). All stations are located at sedimentary areas and situated at a depth of around 30 m, ranging from muddy to sandy. In general, there are not important sources of anthropogenic disturbance in the area. However, there is one particular station (identified as L_UR20) that is located in an area where urban and industrial wastewaters are discharged (driving to increases in organic matter content in sediment and consumption of oxygen) (Borja et al., 2009). This station is regarded as the most disturbed of the study area, especially between 1995 and 2001, when untreated wastewaters were directly discharged in the close vicinity of this station (Fig. 1C), affecting the benthic communities due to poor quality of the sediment. In 2001, a submarine outfall was constructed which, to date, transports the already biologically treated wastewater (since 2006) to a location approximately 1.2 km offshore. Since then, sediment quality steadily improved (Borja et al., 2009). Other stations that are subjected to an above average level of anthropogenic disturbance are L_N20 and L_OI20. Station L_N20 is situated close to the Nervion estuary, which was historically disturbed, but in recuperation since 1989 (Borja et al., 2006). In addition, this station is close to a historical disposal site, which can, to some extent, affect the condition of the benthic assemblages of this area (Borja et al., 2008b). On the other hand, station L_OI20 is situated in the vicinity of the other disturbed estuary (Oiartzun). In addition, close to this station there are some disposal sites of dredged sediments (see Galparsoro et al., 2010). For the whole area, all stations are more or less affected by natural disturbance (e.g. wave activity that can affect the sediment dynamics - Galparsoro et al., 2013).

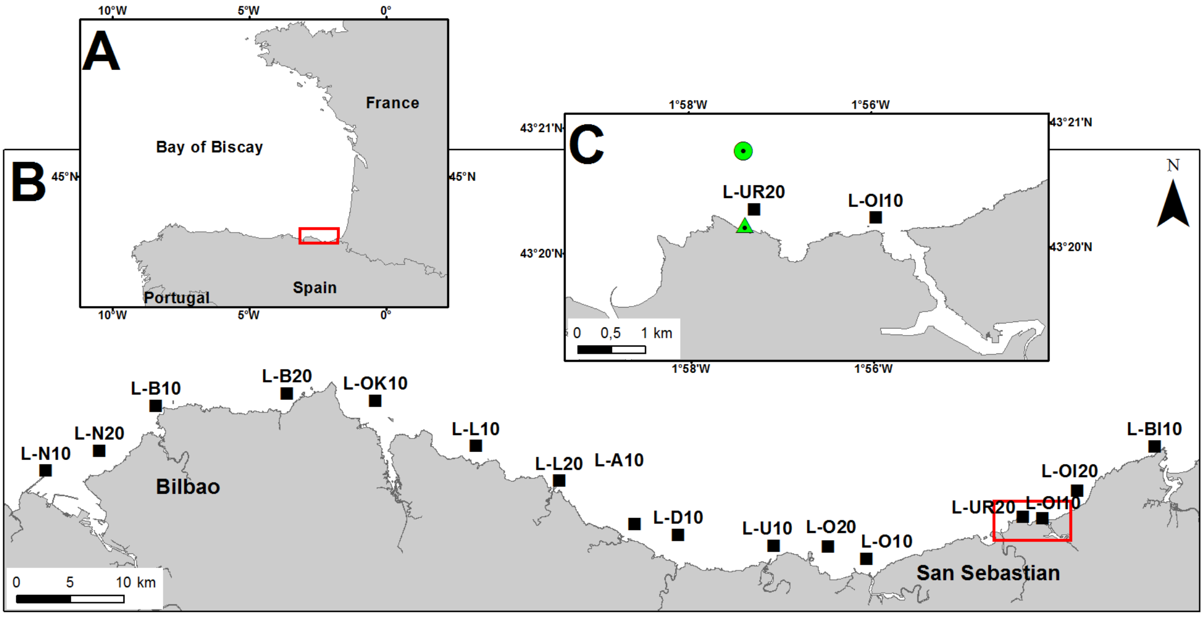

Figure 1. Study area within the Bay of Biscay (A) and the position of the 16 off-shore sampling stations along the Basque coast (Spain) (B). Diagram C shows the urban wastewater discharge locations (the green triangle points out the discharge location prior to 2001, and the green circle points out the current location, which became operational in 2001).

2.2 Data collection

At each station, three benthic samples (replicates) were taken with a van Veen grab (0.1 m^2) and sieved in situ through a 1 mm mesh. Subsequently, the benthic invertebrates were sorted and identified to the lowest possible taxonomic level. Biomass was initially estimated as dry weight (g m^{-2}), but subsequently converted to ash-free dry weight by using the conversion factors as in Ricciardi and Bourget (1998). This benthic community data was then compiled into a ‘taxa-biomass-by-sample’ matrix.

An additional sediment sample was taken at each station to analyse the variables: mud-content (%), organic-matter-content (%) and redox-potential values (mV). The correspondent limit for organic-matter-content is usually considered to be 5% (Holmer et al., 2005). Redox-potential values indicate the oxidation-reduction status of the sediments, with high values (>300) indicating aerobic sediments, and negative values indicating anaerobic sediments (Pearson and Stanley, 1979). We also measured ‘wave-flux’ as an environmental variable producing natural induced disturbance. Wave-flux (kW/m) is a measure of energy per meter of wave front (for further details, see Galparsoso et al., 2013). The above mentioned environmental variables were used to explain possible spatial and temporal variation in species assemblages. Organic-matter content and redox-potential served as a proxy to indicate anthropogenic disturbance. We considered mud-content as a proxy to indicate the potential natural characteristics of the study area, and wave-flux to indicate natural induced disturbance. These environmental variables were compiled into an ‘environmental-variables-by-sample’ matrix.

2.3 Species traits

Species traits were gathered from a variety of published sources (e.g. species identification guides, scientific papers and established online databases such as MarLIN (2006) and WoRMS Editorial Board (2014)). A total of six traits containing 28 trait categories were chosen for their potential ability to reflect anthropogenic- and natural induced environmental disturbance conditions (see Table 1 for details). The lack of available traits information in the literature, prevented our assignment of the trait categories for many taxa at the ‘species’ level. Instead, the trait categories were adjusted at the ‘genus’ level and data was coded using a ‘fuzzy coding’ approach (Chevenet et al., 1994). Records of taxa not identified to at least ‘genus’ level (6.9% of records) were excluded. The trait categories were given an affinity score between ‘0’ and ‘3’, with ‘0’ indicating no affinity of a species to a trait category, and ‘3’ indicating a high affinity to the trait category. The fuzzy coding procedure allows to capture variation in the affinity of a given taxa to the different categories of a given trait, thereby addressing spatial or temporal variation in the traits of a given taxa (Statzner and Bêche, 2010). These scores were then compiled into the ‘taxa-by-trait’ matrix (336 genus and 28 trait categories). To give the same weight to each taxa and each trait in further analysis, the scores were standardised so that their sum for a given taxa and a given trait equalled 1 (or 100%).

Table 1. Species traits (categories), labels and their *a-priori* expected response after disturbance.

Trait	Category	Labels	Expected response after disturbance
Feeding-strategy	Suspension	F_SUS	The proportion of suspension feeders in a community is expected to decrease after disturbance caused by organic pollution[2]
	Deposit	F_DEP	The proportion of deposit-feeders and grazers in a community are expected to increase after disturbance caused by organic pollution[2,4]
	Grazer	F_GRA	
	Scavenger	F_SCA	No particular response expected for scavengers and predators after disturbance
	Predator	F_PRE	
	Omnivore	F_OMN	The proportion of omnivores in a community is expected to increase after disturbance caused by organic pollution (i.e. better resilience capacity)[2]
	Parasite	F_PAR	No particular response expected after disturbance
Maximum size	Very small (< 1 cm)	S_1	The proportion of smaller sized taxa in a community is expected to increase after disturbance (i.e. better resilience capacity)[1,4]
	Small (1-3 cm)	S1_3	
	Medium (3-10 cm)	S3_10	No particular response expected after disturbance
	Medium-Large (10-20 cm)	S10_20	The proportion or larger sized taxa in a community is expected to decrease after disturbance (e.g. fine sediment deposits)[1,4]
	Large (> 20 cm)	S_20	
Life-span	Very short (< 1 year)	L_1	The proportion of short-lived taxa in a community is expected to increase after disturbance (i.e. better resilience capacity)[2,4]
	Short (1-3 years)	L1_3	
	Medium (3-10 years)	L3_10	No particular response expected after disturbance
	Long (> 10 years)	L_10	The proportion of longer-lived taxa in a community is expected to decrease after disturbance (e.g. fine sediment deposits)[2,4]
Living-position	Tube dwelling	LH_TD	The proportion of tube dwellers and burrow dwellers in a community are expected to increase after disturbance (e.g. anoxic conditions, organic pollution and fine sediment deposits), as opposed to free living species and species that are attached to the substratum, because they can hide in their fixed tubes or burrows[3,4]
	Burrow dwelling	LH_BD	
	Free living	LH_FL	
	Attached	LH_ATT	
Larval-development	Planktotrophic (feeding at least in part on materials captured from the plankton)	DT_PLAN	The proportion of taxa with a planktotrophic larval development (high dispersal potential) are expected to increase after disturbance, because the extinction risk of taxa with a lecithotrophic (medium dispersal potential) and direct larval development (no dispersal potential) is higher[5]
	Lecithotrophic (development at the expense of internal resources, i.e. yolk)	DT_LEC	
	Direct (development without larval stage)	DT_DIR	
AMBI ecological (sensitivity) groups (EG's)	(I) very sensitive species	EG_I	The proportion of taxa belonging to EG III, IV and V in a community are expected to increase after disturbance, while EG I is expected to decrease[6]
	(II) indifferent	EG_II	
	(III) tolerant	EG_III	
	(IV) 2nd order opportunists	EG_IV	
	(V) 1st order opportunists	EG_V	

[1] Townsend and Hildrew, 1994
[2] Pearson and Rosenberg, 1978
[3] Reise, 2002
[4] Statzner and Bêche, 2010
[5] McHugh and Fong, 2002
[6] Borja et al., 2000

2.4 Data analysis

For the data analysis and the computation of the indices, three matrices were used: 1) 'taxa-biomass-by-sample' matrix; 2) the 'environmental-variables-by-sample' matrix; and 3) the 'taxa-by-trait' matrix. Data in the 'taxa-biomass-by-station' matrix were explored by means of Correspondence Analysis (CA), after log-transforming (log 1 + x) the biomass values, using R-package 'ade4' (Chessel et al., 2004). The standard affinity scores for each taxa in the 'taxa-by-trait' matrix were multiplied by the taxa biomass in each sample (taxa-biomass-by-sample matrix), which resulted in the 'trait-by-sample' matrix.

2.5 Calculation of the indices

The main purpose of this study was to assess how CWM and Rao responded to natural- and anthropogenic seafloor disturbance relative to the response of AMBI and M-AMBI. Therefore, we assessed their response at the spatial scale (between all stations) and temporal scale (using station L_UR20 as a test case). To better interpret the response of M-AMBI, the responses of its individual components were also assessed. These are: genus richness (the standard procedure is to use species richness in the M-AMBI calculation), the Shannon index and AMBI. To better interpret the response of Rao, the Simpson index was included because Rao is a generalised form of the Simpson index (Botta-Dukát, 2005). This allowed understanding the relationship between species diversity and functional diversity (Stuart-Smith et al., 2013). Genus richness and the Shannon index (log x) were calculated using R-package 'ade4' (Chessel et al., 2004).

2.5.1 AMBI and M-AMBI calculation

Usually, AMBI and M-AMBI are calculated with species density, however, in order to make a viable comparison between all indices, AMBI and M-AMBI had to be calculated using genus biomass. Warwick et al. (2010) and Muxika et al. (2012) already tested the usefulness of AMBI using species biomass instead of species density. Moreover, two studies by Cai et al. (2014, 2015) also aimed to assess environmental disturbance by using both species density and species biomass in the calculation of AMBI and M-AMBI. These authors found a significant correlation between both methods in regards to environmental disturbance. However, we are not aware of studies that tested the correlation between M-AMBI calculated with species density and genus biomass. Therefore, we tested this correlation using an Spearman's rank correlation analysis. Moreover, we tested how both calculation methods responded to the temporal variation in disturbance conditions at station L_UR20. The non-parametric Wilcoxon signed-rank test was used for this purpose. These outcomes are excluded from the results section of this paper as it was not the main purpose of this study. Instead, they are presented in Appendix Chapter 2 as Fig. A.1 and Fig. A.2. These outcomes indicated a significant correlation between both calculation methods in their response to disturbance. Taking this into account, we were confident enough to use AMBI and M-AMBI, calculated with genus biomass, for the purpose of this study. These indices were calculated using AMBI 5.0 software (freely available from http://ambi.azti.es) and the November 2014 species list. Since the reference conditions for the area are based on species, the reference conditions for the M-AMBI calculation based on genus were set as following: genus richness was set as the 0.95 percentile of its maximum observed value in the dataset, the Shannon index was set at the 0.95 percentile of its maximum observed value in the dataset and AMBI was set as lowest observed value in the dataset. As for the ‘bad’ status, the reference values used were 0 for diversity and richness, and 6 for AMBI.

2.5.2 CWM and Rao calculation

The CWM was calculated for each of the 28 trait categories. The trait values were weighted by genus biomass (e.g. the biomass of filter-feeding taxa identified at genus level) (Garnier et al., 2004; Ricotta and Moretti, 2011). This index can be adequately used to summarize shifts in mean trait category values within communities due to environmental selection for certain traits (Ricotta and Moretti, 2011). As such, the calculation of this index allowed us to test how each trait category responded to the environmental variables. This index was calculated, using R-package ‘ade4’ (Chessel et al., 2004).

As mentioned before, Rao is a generalised form of the Simpson index, which measures the amount of trait diversity between two random individuals in the community (Botta-Dukát, 2005; Lepš et al., 2006). In fact, if diversity between all species pairs is maximum, then Rao is identical to the Simpson index (Botta-Dukát, 2005). The Simpson index, as a result, represents the maximum potential value Rao can reach in a given community where the species completely differ in their trait categories. This index can be effectively used to analyse patterns of trait (functional) diversity, i.e. a decrease or increase in trait diversity compared to a random expectation (Vandewalle et al., 2010; Ricotta and Moretti 2011). An Excel macro file (available from http://botanika.bf.jcu.cz/suspa/FunctDiv.php; Lepš et al., 2006) was used to calculate the Simpson and Rao index. Rao provided the mean dissimilarity values for each of the six traits (feeding-strategy, size, life-span, living-position, larval-development, and the AMBI ecological groups - EG's) for each station and subsequently a mean of the index values calculated across all these six traits.

2.6 Statistical treatment

Non-parametric Kruskal-Wallis tests were performed in order to test whether the median values for environmental variables and indices showed significant differences between the stations and between the periods 1995-2001 (non-diverted and untreated discharges) and 2002-2012 (diverted, and since 2006, treated discharges) at station L_UR20 (α: 0.05). Correlations among indices and between the indices and the environmental variables were tested with a Pearson correlation test. When testing for correlation between the indices and the environmental variables, the reported pairwise p-values (α: 0.05) were adjusted using the 'false discovery rate' (Benjamini and Hochberg, 1995).

3. Results

3.1 Environmental conditions

The sediment conditions within most stations were relatively similar, despite mud-content, organic-matter content and redox-potential displaying significant differences between stations (Kruskal-Wallis, p-value: < 0.01). Nevertheless, station L_UR20 stood out from the rest because of higher mud content and lower redox potential values (Fig. 2). Wave-flux values were also significantly different between stations (Kruskal-Wallis, p-value: < 0.01), with the highest values at stations L_N20, L_OI20, L_BI10 and the lowest values at stations L_L20, L_A10 and L_O20 (Fig. 2). Regarding the temporal variation of the sediment conditions at station L_UR20, only organic-matter content showed a significant difference (Kruskal-Wallis, p-value: 0.0005) between the two periods, with higher values in the period with the non-diverted and untreated discharges (1995-2001) (Fig. 3).

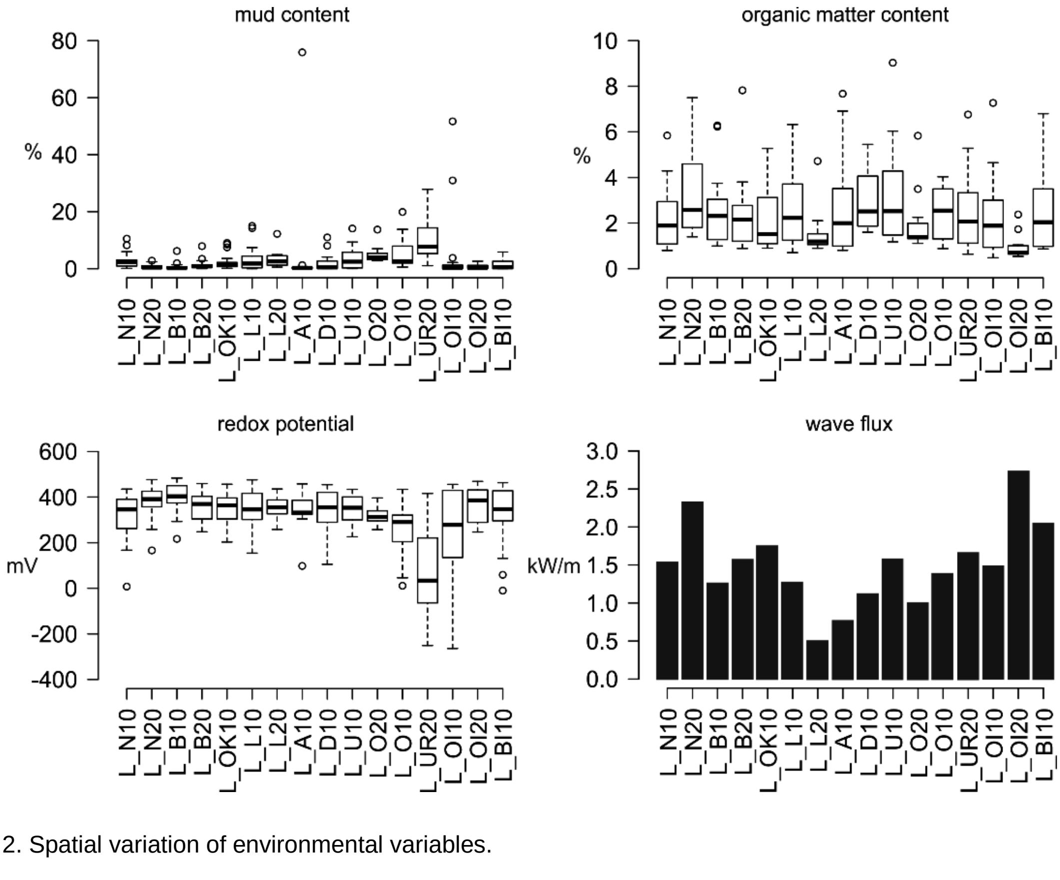

Figure 2. Spatial variation of environmental variables.

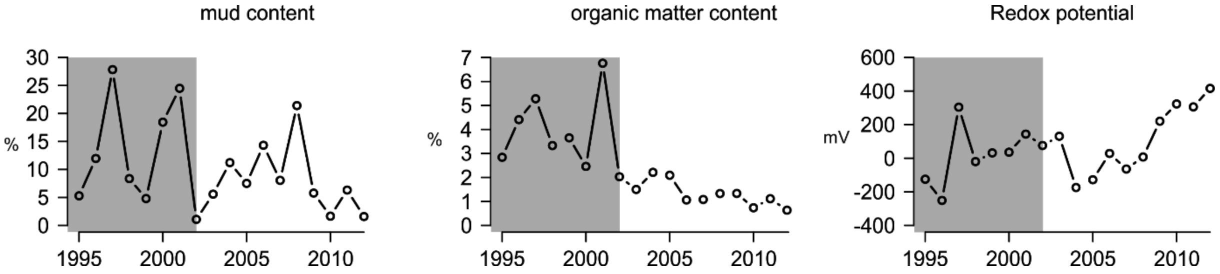

Figure 3. Temporal variation of environmental variables measured in the surficial sediment at station L_UR20. The period with the non-diverted and untreated discharges (1995-2001) is highlighted in grey.

3.2 Indices

The list of taxa (genus level) identified in this study, together with the associated traits can be consulted in Table A.1, in Appendix Chapter 2.

3.2.1 Spatial variation

Almost all indices (except for CWM) displayed significant differences in their median values between stations (Kruskal-Walllis, p-value: < 0.01). Besides, many of these indices showed a very similar spatial variation pattern (Fig. 4). They were all significantly correlated with each other. These correlations were mostly positive, with the exception of genus richness versus AMBI, and AMBI versus M-AMBI, which were negatively correlated, since the scale of AMBI is opposite to the others (lower values indicate better status, whilst for the others this is indicated by higher values). Noticeable are the bell-shaped patterns in the spatial variation of most indices median values (except for AMBI), i.e. generally lower median values at the outer stations, and higher median values at the inner stations. This shape is especially clear for genus richness. The CWM showed considerable variation in their values for most of the trait categories (see Figure A.3, in Appendix Chapter 2).

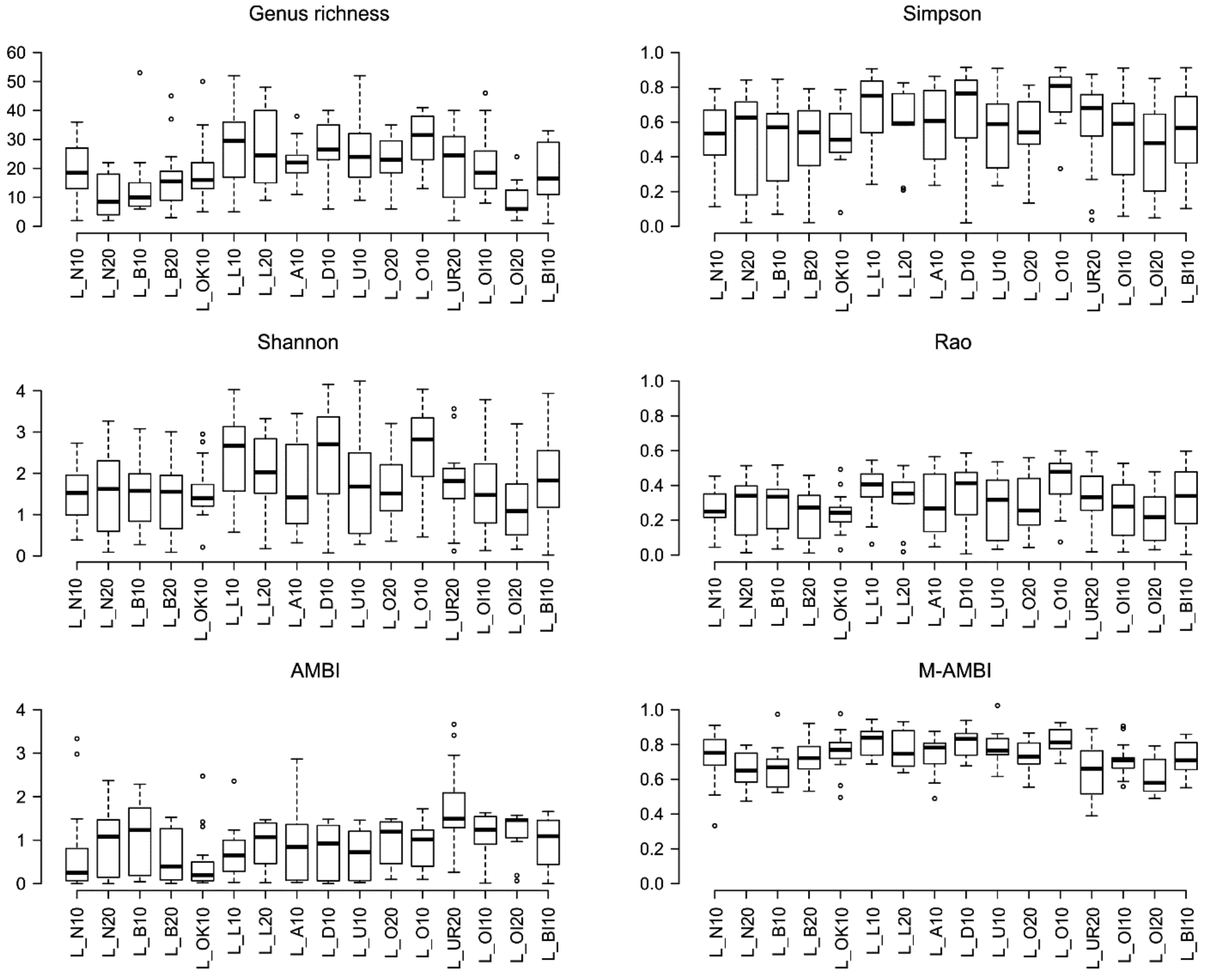

Figure 4. Spatial variation in the indicator values (for the results of the CWM index, see Figure A.3 at Appendix Chapter 2).

3.2.2 Temporal variation

The temporal variation of the indices mean values were assessed at station L_UR20 (Fig. 5 and 6). Genus richness and M-AMBI showed a slight increase towards the latter years, while AMBI showed a general decrease. Simpson, Shannon and Rao did not show slope patterns. Genus richness and M-AMBI were positively correlated (Pearson, r: 0.87, df: 16, p-value: < 0.0001). Rao was positively correlated with Simpson (Pearson, r: 0.93, p-value: < 0.0001) and Shannon (Pearson r: 0.94, df: 16, p-value < 0.0001). AMBI and M-AMBI were negatively correlated (Pearson r: -0.84, df: 16, p-value < 0.0001) and neither of them were significantly correlated with Rao. When comparing the values between the period with the non-diverted and untreated discharges (1995-2001) and the period with the diverted, and since 2006, treated discharges (2002-2012), significant differences were found for genus richness (Kruskal-Wallis, p-value: 0.04), AMBI (Kruskal-Wallis, p-value: 0.003) and M-AMBI (Kruskal-Wallis, p-value: 0.004). All these three indices indicated higher seafloor disturbance during the period with the non-diverted and untreated discharges.

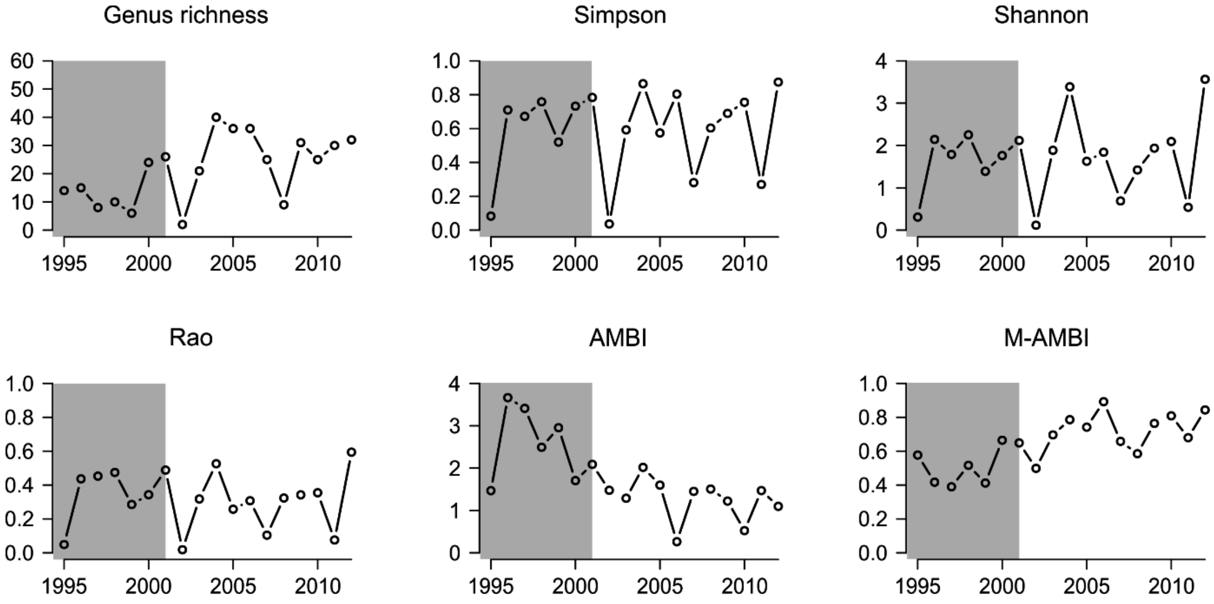

Figure 5. Temporal variation in the indicator mean values at station L_UR20 (for the results of the CWM index, see Fig.6). The period with the non-diverted and untreated discharges (1995-2001) is highlighted in grey.

The CWM index showed some subtle differences in the mean trait values between the two periods (non-diverted and untreated discharges: 1995-2001 versus diverted, and since 2006, treated discharges: 2002-2012) (Fig. 6). When comparing the values between these two periods, significant differences were found for deposit-feeders (Kruskal-Wallis, p-value: 0.05), scavengers (Kruskal-Wallis, p-value: 0.002), very small sized species (Kruskal-Wallis, p-value: 0.03), short lived species (Kruskal-Wallis, p-value: 0.04) and opportunistic species–EG V (Kruskal-Wallis, p-value: 0.002). CWM for deposit-feeders, short lived-, and opportunistic species were higher during the period with the non-diverted and untreated discharges, and the

CWM for scavengers and very small sized species were higher during the period with the diverted, and since 2006, treated discharges.

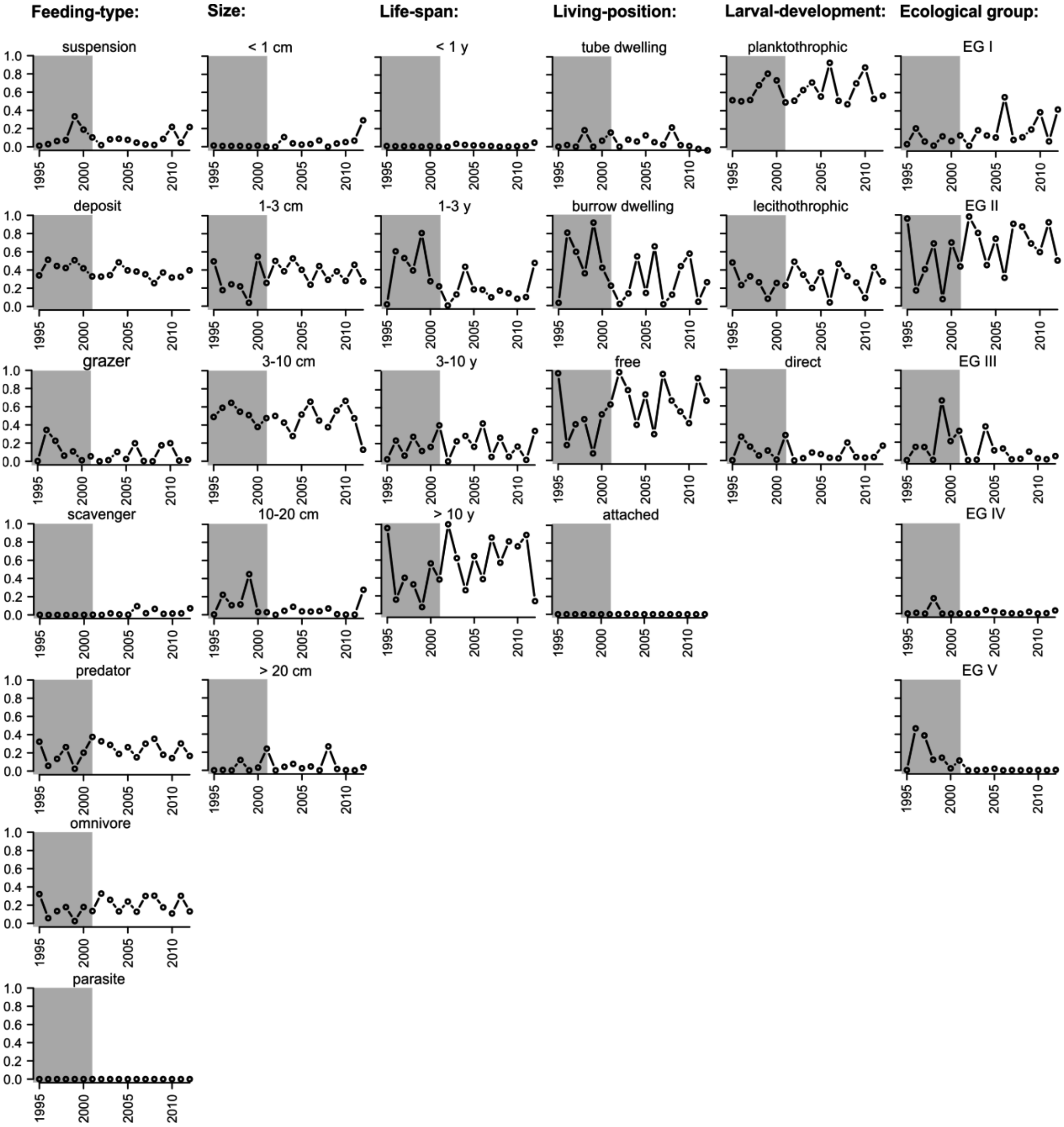

Figure 6: Temporal variation in the CWM values at station L_UR20. The period with the non-diverted and untreated discharges (1995-2001) is highlighted in grey.

3.3 Correlation between the CWM and the AMBI ecological groups (EG's)

Table 2 summarises the results of the correlation analysis between all trait categories and the EG's. For all traits, the category with the highest positive correlation is shown. Sensitive species (EG I) correlated with suspension-feeders, medium size, medium life-span, burrow-dwellers and species with a planktotrophic larval-development. Opportunistic species (EG V) correlated with species displaying a very short life-span and a direct larval-development.

Table 2. Correlations (Pearson, df: 260, pairwise p-values) between the EG's and the CWM (traits) (* p < 0.05, ** p < 0.01, *** p < 0.001). Only the highest correlation for each cell is presented.

CWM (traits)	**EG I (sensitive species)**	**EG II (indifferent species)**	**EG III (tolerant species)**	**EG IV (2nd order opportunistic species)**	**EG V (1st order opportunistic species)**
Feeding	suspension (0.61***)	omnivore (0.71***)	deposit (0.42***)	suspension (0.14*)	-
Size	medium (0.33***)	very small (0.16*) large (0.16*)	very large (0.16*)	small (0.13*)	-
Life-span	medium (0.16*)	Short (0.13*)	short (0.27***)	-	very short (0.42***)
Living-position	burrow-dwelling (0.51***)	free (0.55***)	-	-	tube-dwelling (0.18**)
Larval-development	planktotrophic (0.26***)	lecithotrophic (0.34***)	direct (0.21**)	-	direct (0.19**)

3.4 Correlation between the indices and the environment

Considering the spatial variation, all indices showed significant correlations with one or more environmental variables (see Table 3 for details). The taxonomic indices (genus richness, Simpson and Shannon) were all negatively correlated with wave flux (genus richness showed the strongest correlation). Simpson, Rao and AMBI were positively correlated with mud-content (AMBI showed the strongest correlation). Only AMBI and M-AMBI were correlated with redox-potential. Regarding the CWM, most size traits were correlated to either organic-matter content, redox-potential or wave-flux, but not with mud-content. Short- and long life-span and a variety of feeding traits were mostly correlated with organic-matter content and wave-flux, while the living-habit traits (tube-dwelling and attached) and the larval-development traits (planktotrophic and lecithotrophic) correlated with organic-matter content and redox-potential. EG's I, III, IV and V were correlated with mud-content and redox potential. Considering the temporal variation at station L_UR20, AMBI, direct larval-development and EG V were positively correlated with organic-matter content, while M-AMBI was negatively correlated (see Table 4).

Table 3. Significant correlations (Pearson, df: 260, adjusted pairwise p-values) between the indices and the spatial variation of environmental variables (* p < 0.05, ** p < 0.01, *** p < 0.001).

Index	**Trait (categories)**	**Mud-content**	**Organic-matter content**	**Redox-potential**	**Wave-flux**
Genus richness	-				-0.33***
Simpson	-	0.17*			-0.16*
Shannon	-				-0.15*
AMBI	-	0.28***		-0.35***	
M-AMBI	-			0.19**	-0.27***
Rao	-	0.16*			
CWM	Size (very small: < 1 cm)			0.16*	0.25***
CWM	Size (small: 1-3 cm)		0.27***	-0.15*	-0.16*
CWM	Size (medium: 3-10 cm)		-0.22**		
CWM	Size (large: > 20 cm)		0.26***		
CWM	Life-span (short: 1-3 year)		0.15*		0.24***
CWM	Life-span (long: > 10 year)		-0.14*		
CWM	Feeding-strategy (suspension)				-0.16*
CWM	Feeding-strategy (deposit)		-0.14*		
CWM	Feeding-strategy (grazer)		-0.16*		
CWM	Feeding-strategy (scavenger)			0.17*	0.24***
CWM	Feeding-strategy (predator)		0.19**		0.20**
CWM	Feeding-strategy (omnivore)		0.15*		0.17*
CWM	Living-position (tube-dwelling)			-0.19**	
CWM	Living-position(attached)		0.17*		
CWM	Larval-development (planktotrophic)		-0.23***	0.15*	
CWM	Larval-development (lecithotrophic)		0.21**	-0.25***	
CWM	EG I	-0.14*		0.22***	
CWM	EG III			-0.19**	
CWM	EG IV	0.28***			
CWM	EG V	0.225***		-0.314***	

Table 4. Significant correlations (Pearson, df: 16, adjusted pairwise p-values) between the indices and the temporal variation of environmental variables measured in the surficial sediment at station L_UR20 (* $p < 0.05$, ** $p < 0.01$).

Index	Trait (categories)	Mud-content	Organic-matter content	Redox-potential
AMBI	Ecological groups		0.76**	
M-AMBI	Ecological groups		-0.63*	
CWM	Larval-development (direct)		0.61*	
CWM	EG V		0.69*	

4. Discussion

4.1 AMBI and M-AMBI

AMBI was able to indicate the effects of anthropogenic seafloor disturbance. According to this index, the seafloor was most disturbed at station L_UR20, and more than average disturbed at stations L_N20 and L_OI20 (also disturbed by anthropogenic pressures). At station L_UR20 it was also able to distinguish between the two periods with different levels of disturbance (i.e. higher disturbance during 1995-2001 and lower disturbance during 2002-2012). Station L_UR20 is regarded as the most disturbed of the dataset, especially between 1995 and 2001, when untreated urban wastewater was directly discharged in the close vicinity of this station, affecting the benthic communities due to poor sediment quality (i.e. high organic matter content and low redox potential values). In 2001, a marine outfall was constructed, which, to date, transports the biologically treated (since 2006) wastewater to a location approximately 1.2 km offshore. Since then, sediment quality steadily improved by reducing the organic matter and increasing the redox potential (Borja et al., 2009), as can be seen in Figure 3.

M-AMBI showed a slightly different response. According to this index, not station L_UR20 but stations L_N20 and L_OI20 were the most disturbed over the whole period (1995-2012). This response can be attributed to the influence of richness and diversity in its calculation. In particular, genus richness, but also the Shannon index, showed very low values at stations L_N20 and L_OI20. Also, the method used to calculate M-AMBI for this study influenced its performance. M-AMBI was calculated at genus level. Therefore, genus richness and the Shannon index were slightly different from those calculated based on species level. In fact, M-AMBI detected the worst seafloor quality at station L_UR20 when based on species level identification, after Borja et al. (2009).

To adequately compare the performance of all indices, both AMBI and M-AMBI were calculated with ‘genus biomass’ instead of ‘species density’ which is the common calculation method used in most studies (e.g. Borja et al., 2009; Paganelli et al., 2011). The results demonstrated a strong correlation between both calculation methods regarding their response to anthropogenic seafloor disturbance in this marine environment. However, some performance loss did occur due to the exclusion of certain taxa at a lower resolution (nematodes, oligochaetes, etc.) that mostly belonged to ecological group (EG) V (1st order opportunists). Previous studies by Warwick et al. (2010), Muxika et al. (2012) and Cai et al., (2014) already demonstrated a strong relationship between AMBI (the two former studies) and M-AMBI calculated with ‘species biomass’ versus ‘species density’.

In summary, both AMBI and M-AMBI were able to adequately assess the effects of anthropogenic seafloor disturbance in this coastal environment. They responded to changes in the redox-potential (spatial variation) and organic-matter content (temporal variation at station L_UR20). However, the performance of the indices was influenced by other factors. AMBI, for instance, also responded to mud-content, which can be considered a natural characteristic of the area. M-AMBI responded to wave-flux, which is a natural type of disturbance. The impact of wave-flux on the seabed was generally higher at the stations that are more exposed to the most common swell direction (coming from the north-west, e.g. L_N20 and L_OI20). These stations are situated in front of the stretch of coastline that is most perpendicular orientated towards this swell direction.

4.2 Community-weighted mean trait values (CWM)

The CWM was used to summarize shifts in the mean trait category values within communities due to environmental selection for the traits (Ricotta and Moretti, 2011). As such, we expected that all six-trait groups (28 trait categories) would be indicative of anthropogenic- and natural seafloor disturbance.

In general, the EG's were the most indicative of anthropogenic seafloor disturbance, which was obviously reflected in the performance of AMBI and, subsequently, M-AMBI. EG's I (sensitive species), III (tolerant species) and V (1st order opportunists) all responded to the spatial variation of redox-potential values, while the latter also responded to the temporal variation of organic-matter content (station L_UR20 showed a relatively high mean for EG V, especially during the period with the non-diverted and untreated discharges).

The strength of the EG's, and therefore AMBI and M-AMBI as anthropogenic disturbance indicators, is that they synthesise information regarding functioning based on multiple traits (Marchini et al., 2008). Indeed, each EG was correlated with at least two or more individual traits. For example, EG V was positively correlated with short-lived, tube-dwelling species with a direct larval-development. This wide spectrum of traits might have caused an advantage over each individual trait. Each individual trait does not always contribute with unique information on functioning (Verberk et al., 2013). In this respect, the use of a smaller number of strategies capturing the most relevant differences in trait combinations could help improve the signal-to-noise ratio, resulting in higher discriminatory power (Verberk et al., 2013).

The individual traits that seemed most indicative of anthropogenic seafloor disturbance were tube-dwelling, lecithotrophic- and direct larval-development. Tube-dwelling and lecithotrophic larval-development showed the strongest correlation with the spatial variation of redox-potential values. Direct larval-development was correlated with the temporal variation of organic-matter content at station L_UR20. Besides, these traits responded solely to anthropogenic disturbance and not to natural disturbance in the form of wave-flux.

At first glance, also the traits that correlated with the spatial variation of organic-matter content appear to be indicative of anthropogenic disturbance. However, this correlation was only observed regarding the spatial variation, which did not change much. Considering the temporal variation at station L_UR20, none of these traits responded to the considerable decrease of organic-matter content. Besides, some of these traits were also influenced by natural disturbance (small size, short life-span, predators and omnivores). This suggested that these traits were not particularly indicative of anthropogenic seafloor disturbance in this environment. However, a number of studies observed an increase of small-sized species with increasing organic-matter content (e.g. Dauer et al., 1992; Pacheco et al., 2010; van Son et al., 2013).

As mentioned before, tube-dwellers, lecithotrophic- and direct larval-development categories seemed the most indicative of anthropogenic disturbance. Indeed, for tube-dwellers this response was expected (Reise, 2002) but not for lecithotrophic and direct larval-development. Taxa with a planktotrophic larval-development was *a-priori* expected to increase in abundance with seafloor disturbance (Table 1). High larval mobility usually indicates an unstable habitat (Paganelli et al., 2012). However, Villnäs et al. (2011) and van Son et al. (2013) found that lecithotrophic larval-development characterised organic enriched environments. This study does not support their findings because it was not correlated with organic-matter content. As such, a clear mechanistic link for why lecithotrophic- and direct larval-development might be used to indicate anthropogenic seafloor disturbance is missing.

In summary, the CWM of most individual traits was not indicative of anthropogenic seafloor disturbance in this coastal ecosystem. This might have been due to different reasons: the links between the traits and the environmental variables that are associated with anthropogenic seafloor disturbance were weak; the mechanistic links between certain traits (e.g. larval-development) and their response to seafloor disturbance in marine environments is currently not well understood (Berthelsen et al., 2015). Besides, other anthropogenic pressures exist in the area, like fishing and dredging or sediments deposits, which may have contributed to mask the results obtained. Moreover, many traits were also influenced by wave-flux (natural disturbance), which made it difficult to understand whether they were influenced by anthropogenic- or natural disturbance, or by a combination of both.

4.3 Trait diversity (Rao)

We *a-priori* expected that trait diversity, which was expressed by the Rao, would be lowest at the most disturbed stations (L_N20, L_UR20 and L_OI20), especially at station L_UR20 during the period with the non-diverted and untreated discharges (1995-2001). However, this was not the case, Rao values at these stations were similar to those of most other stations, and its values during 1995-2001 were not much different from the period with the diverted, and since 2006, treated discharges (2002-2012). Based upon these results, Rao was not a useful indicator to detect anthropogenic seafloor disturbance in this particular environment. However, this outcome does not necessarily mean that Rao or any other measure for trait diversity is useless for detecting seafloor disturbance. A number of studies demonstrated a clear response of Rao to anthropogenic seafloor disturbance (e.g. Cooper et al., 2008; Paganelli et al., 2012; Wan Hussin et al., 2012). As previously mentioned when discussing the CWM results, also the performance of Rao depends on which types of traits are considered. Rao will perform better if traits have more strong and clear links with the particular type of disturbance that is being studied, and if there is none or little distortion between anthropogenic- and natural disturbance. The performance of Rao was also similar to that of genus richness and the Simpson index (strongly correlated). This reflects the relationship between species richness and trait (functional) diversity in that with the loss or addition of a species, unique traits were being lost or added to the community (Culhane et al., 2014; van der Linden et al., 2016). Most studies found a strong correlation between Rao and Simpson (e.g. Vandewalle et al., 2010; Culhane et al., 2014; van der Linden et al., 2016a).

4.4 From a management perspective

AMBI and M-AMBI were able to adequately assess the effects of anthropogenic seafloor disturbance in the form of organic-matter enrichment and oxygen depletion of the surficial sediments in this marine system. Their strength lies in the ability of their ecological groups to capture a wide range of information about the response of multiple individual traits to this particular type of disturbance. The CWM of the individual traits and the diversity of these traits, as expressed by the Rao index, were not effective in indicating this disturbance. The main reason was probably that many of the individual traits did not have a very strong and clear mechanistic link with this type of disturbance. Besides, some traits also responded to natural disturbance in the form of wave-flux, which makes it difficult to unravel the effects of both types of disturbance. A clear advantage by using the CWM of the individual traits is that it gave a more detailed understanding on how the two types of disturbances (anthropogenic and natural) affected the individual traits, and thus the functioning of species communities as a whole. This knowledge might aid in the development of existing- or to be developed indices. For instance, if you know that small sized species will respond to natural disturbance in your study area, one might exclude this trait from that particular index. However, from a management perspective, which aims to simply monitor the quality and health of the site, a full understanding of a site may not necessarily be required (Culhane et al., 2014). Moreover, the CWM of multiple traits does not provide a single number that indicates a quality status, which makes it a difficult tool to use and interpret, especially for managers. It is probably more useful for scientists who really want to explore and understand different aspects of community functioning. In this aspect, AMBI and M-AMBI are easier and more straightforward to use. That is why several European Member States have used them in the first MSFD phase of GES assessment.

Unlike the CWM, trait diversity (Rao in this case) provides a single value of functioning, having therefore real potential to effectively be used for management purposes. However, to improve its performance, detailed and accurate traits data are required. This is currently lacking for many marine species (Munari, 2013; Berthelsen et al., 2015). We therefore suggest that more research is needed into quantifying a larger number of traits and to understand their links with anthropogenic seafloor disturbance, before effectively utilising trait (functional) diversity for this purpose. Perhaps, when doing so, trait diversity will not be as strongly correlated to species diversity, which is now questioning the use of trait diversity as an effective tool for management purposes.

Acknowledgements

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Chapter 3

Functional changes in polychaete and mollusc communities within two tropical estuaries

Abstract

Currently, there is limited knowledge on how benthic communities function in relation to changing environmental conditions in tropical estuaries. This study contributes to filling this knowledge gap by analysing structural and functional community patterns within two tropical estuaries in the northeast of Brazil. Replicate macrofaunal samples were taken in different environmental zones along the estuary, together with physico-chemical measurements. The faunal analysis focussed on the trait analysis of polychaete and mollusc communities as both these groups have a crucial role in regulating the functioning of aquatic systems. The separate analyses of polychaetes and molluscs allowed evaluating how each group responded to the environmental gradients.

The results demonstrate that community functioning for the polychaetes depended greatly on the spatial environmental gradients within these systems, with generally higher functional diversity in the lower zones suggesting increased diversity in resource use strategies, whereas the upper zones showed very little functional diversity due to the prevalence of environmental filtering. Considering the molluscs, their functioning remained more similar along the estuarine gradient, with low taxonomic and functional diversity throughout the estuaries. In general, polychaetes displayed higher levels of taxonomic and functional diversity, suggesting that in comparison to the molluscs, they potentially utilised the available resources more efficiently, which may enhance the processes and overall functioning of these systems. We advocate that more studies are needed to obtain a generalised pattern of estuarine function within and across biogeographical regions.

Keywords - estuarine invertebrates, disturbance and environmental gradients, biological traits, trait-based approach, functional diversity, tropical estuaries.

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1. Introduction

Estuarine ecosystems are subjected to a high degree of variability in their environmental conditions due to freshwater and seawater inputs, as well as climatic changes and anthropogenic impacts. These changing environmental conditions play an important role in structuring benthic macroinvertebrate communities and, thus, the functioning of these ecosystems (Elliott and Quintino, 2007). A better understanding on these species-environment relationships can help us to preserve and improve the quality of these ecosystems. Studies investigating these relationships are increasingly making use of species biological traits as an complementing approach to quantify community patterns in response to anthropogenic impacts (Krumhansl et al., 2016; van der Linden et al., 2016b) and environmental gradients (e.g. Berthelsen et al., 2015; Otegui et al., 2016). Analysis of species traits, using information on life history, morphological and behavioural characteristics, gives a greater mechanistic understanding on how communities may respond to environmental change (Pearson and Rosenberg, 1978; Bremner et al., 2003). And, since traits strongly influence the rate and relative importance of particular ecosystem processes, they are expected to further improve understanding of ecosystem functioning (Vandewalle et al., 2010). Further, communities that have no species in common will share traits, and trait values can be compared among individuals within and between communities (Boersma et al., 2016). Thus, trait-based approaches may highlight patterns within and across ecosystems that are not apparent in taxonomic-based approaches (Boersma et al., 2016; Weigel et al., 2015). Considering estuarine systems, most trait-based studies have been carried out in estuaries located in regions with a temperate climate (mostly in Europe), whereas in tropical estuaries, these studies are relatively rare (Gusmao et al., 2016; Jimenez et al., 2015; Leung, 2015; Sivadas et al., 2013). There is limited knowledge on how benthic communities function with regard to spatial and temporal patterns in tropical estuaries (Barros et al., 2012). This study contributes to filling this knowledge gap by using trait-based approaches to analyse estuarine invertebrate communities within two estuaries in N-E Brazil. We focus on estuarine polychaetes and molluscs, as both are dominant faunal groups in the benthos, exhibiting a wide range of representative traits, which have a crucial role in regulating the functioning of aquatic systems and benthic-pelagic coupling, by contributing to nutrient cycling and energy flows (Martin and Bastida, 2006; Newell, 2004; Otegui et al., 2016). Both groups have long been considered as good indicators of marine environmental conditions, being included in a number of indices or approaches for ecological status assessment (e.g. Bellan, 1980; Warwick and Clarke, 1994; Olsgard et al., 2003; Dimitriadis and Koutsoubas, 2008). Moreover, they can represent an important food source for crustaceans, fish and birds (Piersma et al., 1993; Omena and Amaral, 2000; Martin and Bastida, 2006). In particular, we studied how the traits of polychaetes and molluscs communities of the Paraíba and the Mamanguape estuaries responded to spatial gradients, seasonal influences and disturbance in the form of anthropogenic effluent. We also examined structural patterns by means of taxonomic-based approaches and explored the relationship between taxonomic and functional structure of the investigated communities.

2. Materials and methods

2.1 Study sites

The study was carried out in two estuarine ecosystems located in Paraíba state (north-east coast of Brazil): the Paraíba and Mamanguape estuaries (Figure 1). According to the Köppen-Geiger climatic classification, the climate type in the two estuaries can be classified as ‘equatorial with dry summers’ (Alvares et al., 2013). In both estuaries, the wet season lasts from February to August, with most rainfall occurring in June and the least in November. Historical records show that on average the Paraíba estuary receives more rainfall (1717 mm year^{-1}) than the Mamanguape estuary (1392 mm year^{-1}) (data from 1999-2014; CPTEC/INPE, 2015). Both estuaries have an extensive mangrove area composed of *Rhizophora mangle, Avicennia schaueriana, Avicennia germinans, Laguncularia racemosa* and *Conocarpus erectus* (Nishida et al., 2006). Along the coast of the Paraíba State, tides are semi-diurnal, i.e.

two high tides and two low tides every 24 hours with a maximum tide of 2.80 m (Nishida et al., 2006). Despite these similarities, the environmental context of the two estuaries is remarkably different. The Paraíba is the main river of the state and is highly impacted due to nutrient loadings coming from run-off from sugarcane plantations, shrimp aquaculture and urban waste water discharge coming from its surrounding cities (Santa Rita, Lucena, Bayeux, João Pessoa and Cabedelo: total of approximately 1.1 million inhabitants). In contrast, the Mamanguape estuary is an environmental protected area (IUCN Protected Area category V), created in 1993 for the purpose of protecting ecosystems along the Brazilian coast (Atlantic forest, mangroves, coastal reefs, restinga forests, dunes and cliffs) and to guarantee a place for feeding and reproduction for the West Indian manatee (*Trichechus manatus*). The Mamanguape estuary is not exempt from human impact, with anthropogenic effluents coming mostly from sugar cane plantations and from its nearby small cities (approximately 66.000 inhabitants in total). Local fishermen blame agrochemicals for a decrease in fish production along this river (Nishida et al., 2006).

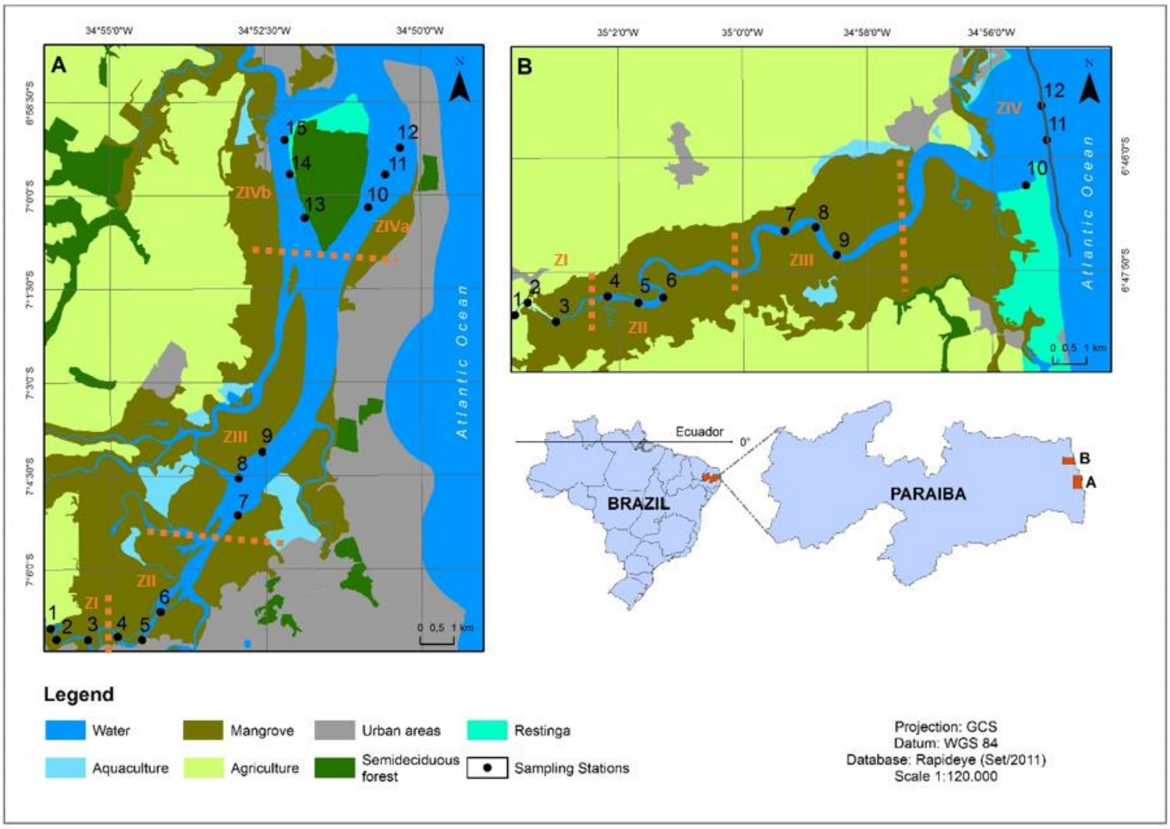

Figure 1. Study sites (northeast Brazil): the Paraíba (A) and Mamanguape (B) estuaries. Each estuary is divided into four zones (I, II, III and IV) according to the location of the sampling stations. Zone IV in the Paraíba was divided into IVa (representing sampling stations 10, 11 and 12) and zone IVb (representing sampling stations 13, 14 and 15).

2.2 Data collection

2.2.1 Biological data

Sediment samples were collected from 15 sampling stations in Paraíba and 12 sampling stations in Mamanguape (Fig. 1). These subtidal sampling stations were subdivided into four zones, to encompass the variety of benthic communities that inhabit the estuarine salinity gradient, from the most brackish reaches to marine-like conditions: zone I (stations 1, 2, 3), zone II (stations 4, 5, 6), zone III (stations 7, 8, 9) and zone IV (stations 10, 11, 12). In the Paraíba, zone 4 was divided into sub-zones IVa (stations 10, 11, 12) and IVb (stations 13, 14, 15). The samples were collected during the dry season in 2013 (November) and during the wet season in 2014 (July). In each station, three sediment samples (replicates) were taken with a van Veen grab (0.1 m^2). Samples were stored in 4% buffered formalin solution and washed in the laboratory through 0.5 mm mesh sieves. Afterwards, the specimens were sorted and preserved in 70% ethanol. Polychaetes and molluscs were separated and identified at the lowest possible taxonomic level (mostly genus level). The polychaetes were identified, following the taxonomic keys provided by Amaral and Nonato (1996), while for the identification of the molluscs, we used the keys provided by Rios (1985, 2009), Mikkelsen and Bieler (2008) and Tunnell et al. (2010). To estimate biomass (mg AFDW · 0.1 m^{-2}), the organisms were placed in an oven at 60º for 72 hours and weighed, and then subjected to combustion in a muffle furnace at 550°C for 8 hours to determine the ash-free dry weight (AFDW). The taxa biomass (mg AFDW · 0.1 m^{-2}) in each sample was converted to 1.0 m^{-2}, and subsequently displayed in a numerical matrix ('taxa-biomass-by-sample' matrix).

2.2.2 Environmental data

During the collection of the sediment samples, salinity, temperature (°C), transparency (m), turbidity (NTU) and pH were measured *in situ*, along the river's main channel at the bottom of the water column. The water column was quite homogeneous in both seasons and along the spatial gradient (i.e. almost no stratification). Samples were taken during high tide and water depth never exceeded 10 metres. An additional water sample was taken at each sampling station to quantify total-N (µg L^{-1}), total-P (µg L^{-1}) and chlorophyll-*a* (µg L^{-1}) in the laboratory, following the procedures described in Alves et al. (2016). An extra sample of sediment was collected at each station to determine sediment size classes. These classes were determined through sieving using mechanical separation, employing a column with six different-size sieves for clay (<38 µm), silt (38-63 µm), fine sand (63–250 µm), medium sand (250–500 µm), coarse sand (500–1000 µm), very coarse sand and gravel (>1000 µm). The environmental variables in each sample were compiled into an 'environmental-variables-by-sample' matrix.

2.2.3 Species traits

Six traits and their associated categories were selected for their potential ability to reflect patterns in community assembly due to changes in the environmental conditions: ‘body-mass’, ‘feeding-strategy’, ‘habitat’, ‘life-span’, ‘larval-development’ and ‘fecundity’ (Table 1). The categories for the trait ‘body-mass’ have different ranges for polychaetes and molluscs, and were defined as the mean body mass (mg AFDW) for each taxon per estuary (total population biomass/population density). Each taxon was assigned to the trait categories using a ‘fuzzy coding’ approach (Chevenet et al., 1994). The trait categories were given an affinity score between ‘0’ and ‘3’, with ‘0’ indicating no affinity of a species to a trait category, and ‘3’ indicating a high affinity to the trait category. The fuzzy coding procedure allows the capture of the variability in the affinity of a given taxon to the different categories of a given trait, thereby addressing spatial or temporal variation in the traits of a given taxa (Statzner and Bêche, 2010). To give the same weight to each taxa and each trait in further analysis, the scores were standardised so that their sum for a given taxa and a given trait equalled 1 (or 100 %). These scores were then compiled into four different ‘taxa-by-trait matrices’: a separate matrix for each taxa group per estuary (Table A.1- A.4; Appendix Chapter 3). The trait information for the polychaetes were mainly gathered from online databases such as MarLIN (MarLIN, 2006) and Polytraits (Faulwetter et al., 2014). Due to a lack of trait information for tropical molluscs in online databases and in the literature, the traits for this group of invertebrates were assigned on the basis of expert knowledge of one of the authors (LS).

Table 1. Biological traits and their categories for polychaetes and molluscs, their labels and the importance of each trait to species-environment relationships. For the trait 'body-mass', categories in normal type refer to polychaetes, in bold type to molluscs.

Traits	Categories	Labels	General importance (see also Tyler et al., 2012)
Body-mass	Very small: (< 0.01 mg), **(< 1 mg)** Small: (0.01-0.1 mg), **(1-10 mg)** Medium: (0.1-1 mg), **(10-50 mg)** Large: (> 1 mg), **(> 50 mg)**	B_VS B_S B_M B_L	Key trait, related to many ecosystem functions (e.g. energy flow and nutrient cycling), that may indicate disturbance. Species with smaller body-mass are expected to increase after disturbance (i.e. better resilience capacity) (Norkko et al., 2013)
Feeding-strategy	Predator Scavenger Grazer Deposit Suspension	F_PRE F_SCA F_GRA F_DEP F_SUS	Indicates food source availability, influences energy flow and nutrient cycling and may indicate disturbance. Deposit-feeders are expected to increase after disturbance caused by organic pollution (Pearson and Rosenberg, 1978)
Habitat	Tube dwelling* Burrow dwelling Surface dwelling	H_T H_B H_S	Indicates food source availability, influences bioturbation processes and may indicate disturbance. Tube and burrow dwellers are expected to increase after disturbance (e.g. anoxic conditions, organic pollution), as opposed to surface dwelling species, as they have some protection from tube or burrow linings and are more likely to have pumping/irrigation features for oxygenation (Reise, 2002)
Life-span	Short (< 1 year) Medium (1-3 years) Long (> 3 years)	LS_S LS_M LS_L	Influences community dynamics through response to disturbance. Short-lived species are expected to increase after disturbance (i.e. better resilience capacity) (Pearson and Rosenberg, 1978)
Larval-development	Direct (development without larval stage) Lecithotrophic (development at the expense of internal resources, i.e. yolk) Planktotrophic (feeding at least in part on materials captured from the plankton)	LD_DIR LD_LEC LD_PLA	Related to the dispersal potential of species that may indicate disturbance. Species with a high dispersal potential are expected to increase after disturbance, because the extinction risk of taxa with a lecithotrophic (medium dispersal potential) and direct larval development (no dispersal potential) is higher (McHugh and Fong, 2002)
Fecundity	Low (1-2500 eggs) Medium (2500-100.000 eggs) High (>100.000 eggs)	FE_L FE_M FE_H	Related to rate of community increase and may indicate disturbance. Species with high fecundity are expected to increase after disturbance (i.e. better resilience capacity) (Williams, 1996)

* The trait category 'tube dwelling' was not included for trait analysis on the molluscs.

2.3 Multivariate data analysis

2.3.1 Environmental analysis

Environmental differences within the estuaries and between the seasons were explored with normalised Principal Component Analysis (PCA). For each estuary, a separate PCA was performed using the R-package 'ade4' (Thioulouse et al., 1997). Prior to the analyses, the environmental variables, whose absolute values are shown in Fig. A.1 (Appendix Chapter 3), were transformed by $\log_{10}$ whenever data were moderately skewed in distribution. Subsequently, the variables were checked for co-linearity by calculating the Variance Inflation Factor (VIF), using the R-package 'usdm' (Naimi et al., 2014). Following Zuur et al. (2010), we considered a VIF value > 3 as an indication for a strong correlation between each pair of variables, in which case one of these variables was excluded from the analyses. The remaining variables (i.e. salinity, grain size, temperature, transparency, turbidity, pH, total-P, total-N and chlorophyll-*a*) were then normalized and processed in a PCA for ordination. To ordinate the samples on a bi-dimensional plane, we calculated the Euclidean distances between samples. None of the variables were strongly correlated (VIF < 3), and therefore we decided to plot them all. In the resulting bi-dimensional plots, the variables were grouped for zones per season (dry and wet). Zones close to one another in the context of the plot coordinates therefore have similar environmental conditions.

2.3.2 Taxonomic analysis

The taxonomic structure within each estuary was explored with Correspondence Analysis (CA). The 'taxa-biomass-by-sample' matrices were analysed separately for polychaetes and molluscs, after log-transforming the biomass values. The R-package 'ade4' (Thioulouse et al., 1997) was used to perform the analyses.

2.3.3 Trait analysis

The trait structure of the polychaetes and molluscs in the two estuaries were explored with Fuzzy Correspondence Analysis (FCA) (Chevenet et al., 1994). FCA is a correspondence analysis method appropriate for fuzzy coded data. For each estuary and taxa group (polychaetes and molluscs) a separate FCA was performed using the R-package ‘ade4’ (Thioulouse et al., 1997). Euclidean distances were calculated from the relative frequencies of the community weighted (biomass) mean (CWM) trait values in each sample, which were then used to ordinate the samples on a multidimensional space. FCA provided the variability contained in every axis, and FCA scores for each season and each zone were plotted on bi-dimensional plots. In the resulting plots, zones are located at the weighted average of the trait categories present in those zones. Zones close to one another in the context of the plot coordinates therefore have a similar trait structure.

2.4 Taxonomic and functional diversity

The distribution of taxa biomass and the CWM trait values as explored with taxonomic and trait analysis describe a different aspect of the taxonomic and functional community structure, however these differences in distribution should be reflected in the taxonomic and functional diversity indices (Heip et al., 1998; Ricotta and Moretti 2011).

Structural and functional community patterns were further analysed by means of taxonomic and functional diversity indices. Taxonomic diversity was estimated by measuring taxonomic richness (the number of different taxa in a community) and Simpson’s diversity index (hereafter ‘Simpson’). Simpson takes into account both the number of different taxa and their abundances and requires at least two species to be computed. Functional diversity was estimated by measuring functional richness (as in Villéger et al. 2008) and Rao’s quadratic entropy index (Botta-Dukát, 2005; hereafter ‘Rao’). Functional richness measures the number of different traits in a community and was calculated using the R-package ‘FD’ which requires at least three species to compute (Laliberté and Legendre, 2010). Rao is a generalised form of the Simpson index, which measures the amount of trait dissimilarity between two random individuals in the community (Lepš et al., 2006). At least two species are required to compute this index. An Excel macro file (available from http://botanika.bf.jcu.cz/suspa/FunctDiv.php; Lepš et al., 2006) was used to calculate the Simpson and Rao index. Rao provided the mean dissimilarity values for each of the six trait groups (body-mass, feeding-strategy, life-span, habitat, larval-development and fecundity) for each sample and subsequently a mean of the index values calculated across all these six trait groups. When we could not calculate the indicator's value due to species richness being too low in certain samples, we did not assign a value to that particular sample.

2.5 Statistical treatment

Variation patterns within taxonomic and functional diversity were further explored with PERMANOVA based on a Euclidean distance matrix (taxonomic richness) and a Bray-Curtis similarity matrix (Simpson and Rao) to test differences between estuaries, seasons and zones for each index. Three factors were considered: 'estuary', with two fixed levels (Paraíba and Mamanguape); 'season', with two levels (wet and dry); and 'zone', with four to five random levels (zones I, II, III and IV for Mamanguape and zones I, II, III, IVa and IVb for Paraíba, nested in 'estuary'). These analyses were performed with PRIMER v6 and PERMANOVA+, which is suitable for multiple factor comparisons and unbalanced designs (Anderson et al., 2008), such as the case for number of samples for 'zones'. Correlations among indices were tested with a Pearson correlation test (α: 0.05).

3. Results

3.1 Environmental conditions

In Paraíba, there were clear spatial differences in the environmental conditions between the upper estuarine zones (I and II) and the lower zones (IVa and IVb) along the main PCA axis (Fig. 2A). Salinity, pH and transparency values were higher in the lower zones, whereas the upper zones were mainly characterised by larger grain size and higher concentrations of nutrients and chlorophyll-*a*. The factor 'season' separated samples along the 2nd PCA axis, demonstrating that in this estuary seasonal differences provided a weaker contribution to the global variability of the dataset. Nonetheless, seasonal differences were more obvious in the upper zones. Turbidity, water temperature and chlorophyll-*a* levels were higher in the dry season. Nutrient levels (mainly N) were higher in the wet season.

In Mamanguape, spatial differences in the environmental conditions were less defined (Fig. 2B), instead seasonality was more important (main PCA axis). Salinity, turbidity, temperature and pH levels were lower in the wet season. For more details on the spatial and seasonal distribution of the environmental variables in terms of their absolute values from both estuaries, please see Fig. A.1 in Appendix Chapter 3.

When comparing both systems (Mamanguape versus Paraíba), there were several differences: salinity was higher in the upper zones of Mamanguape in the dry season, and turbidity levels were higher in the middle and lower zones of Mamanguape in the dry season. Nutrient concentrations were considerably lower in Mamanguape in both seasons, as well as lower chlorophyll-*a* levels in the upper zones of Mamanguape in the dry season.

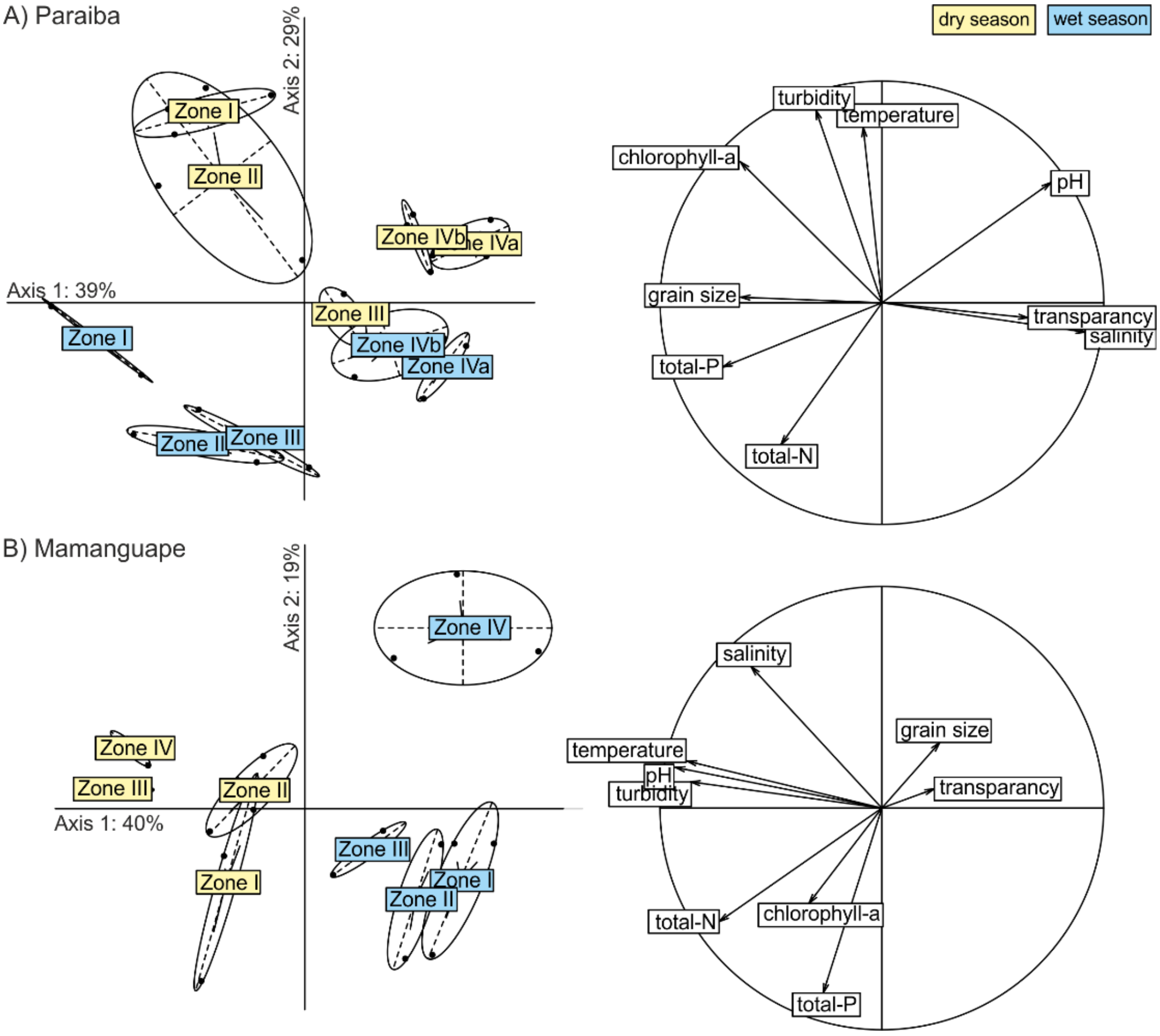

Figure 2. Results of PCA analysis of environmental variables, for the Paraíba (A) and the Mamanguape (B) estuaries. On the left: 2D ordination of samples (points) grouped (circles) for zones per season (dry-wet). On the right: 2D ordination of environmental variables.

3.2 Taxonomic structure

In both estuaries, the polychaetes in particular displayed a clear spatial gradient in terms of taxonomic richness, with generally higher richness in the lower zones (zone IV) versus lower richness in the upper zones (zones I and II) (Fig. 3A and B) (p-perm: <0.05). The taxon *Laeonereis* dominated the few taxa inhabiting the upper zones (see Fig. 4), which generally translated into low Simpson values. Seasonal differences in environmental conditions seem to mostly affect the polychaetes in the Paraíba, particularly in zone IVb, with higher richness (e.g. the addition of *Notomastus, Kinbergonuphis* and *Isolda*, see Fig. 4A) in the wet season (despite season not being a significant factor in the main test). The mollusc diversity was much lower than the polychaete diversity, and in contrast to the polychaetes, they did not demonstrate this clear spatial gradient between lower and upper zones, but they seem to respond to seasonal influences in the lower estuarine zones (Fig. 3B). In a similar result to the polychaetes, the most obvious seasonal differences occurred in zone IVb in the Paraíba, with higher richness (e.g. the addition of *Chione*, see Fig. 5A) in the wet season (despite season not being a significant factor in the main test). When comparing the two estuaries, the most obvious difference is the generally higher polychaete richness in the lower zones of the Paraíba, however differences between the two estuaries were not statistically significant.

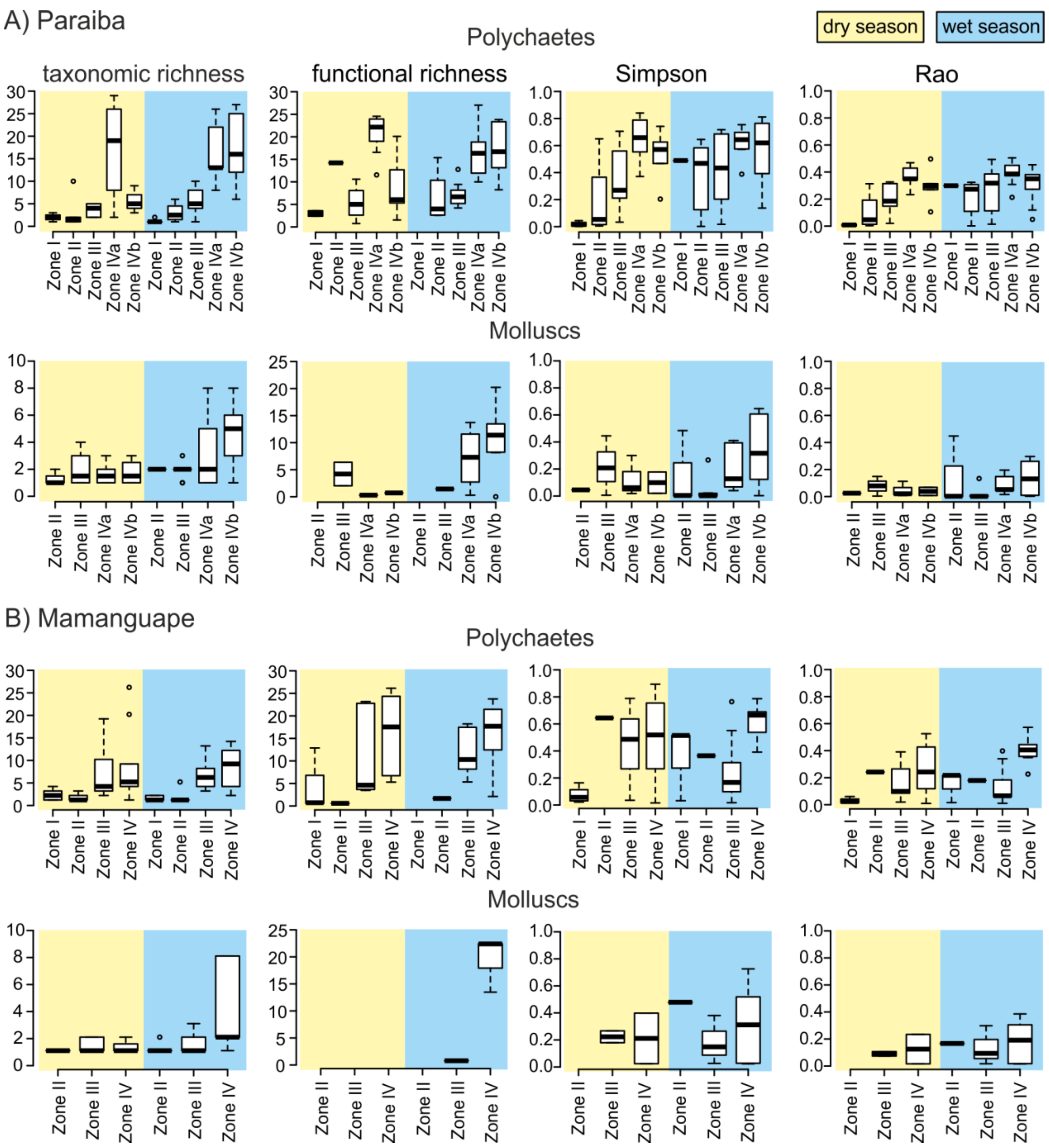

Figure 3. Box-whisker plots showing the spatial (zones) and seasonal (dry-wet) variation within taxonomic richness, functional richness, Simpson and Rao of both taxa groups (polychaetes and molluscs) in the Paraíba (A) and the Mamanguape (B) estuaries (sample, n=9). Mid-line of box, 50th percentile; extremities of box, 25th and 75th percentiles; maximum length of each whisker, 5th and 95th percentiles; outliers shown individually. Values for functional richness, Simpson and Rao are sometimes missing (especially for the molluscs in Mamanguape), because a minimum amount of taxa is required to compute these indices (see the Materials and methods section for details).

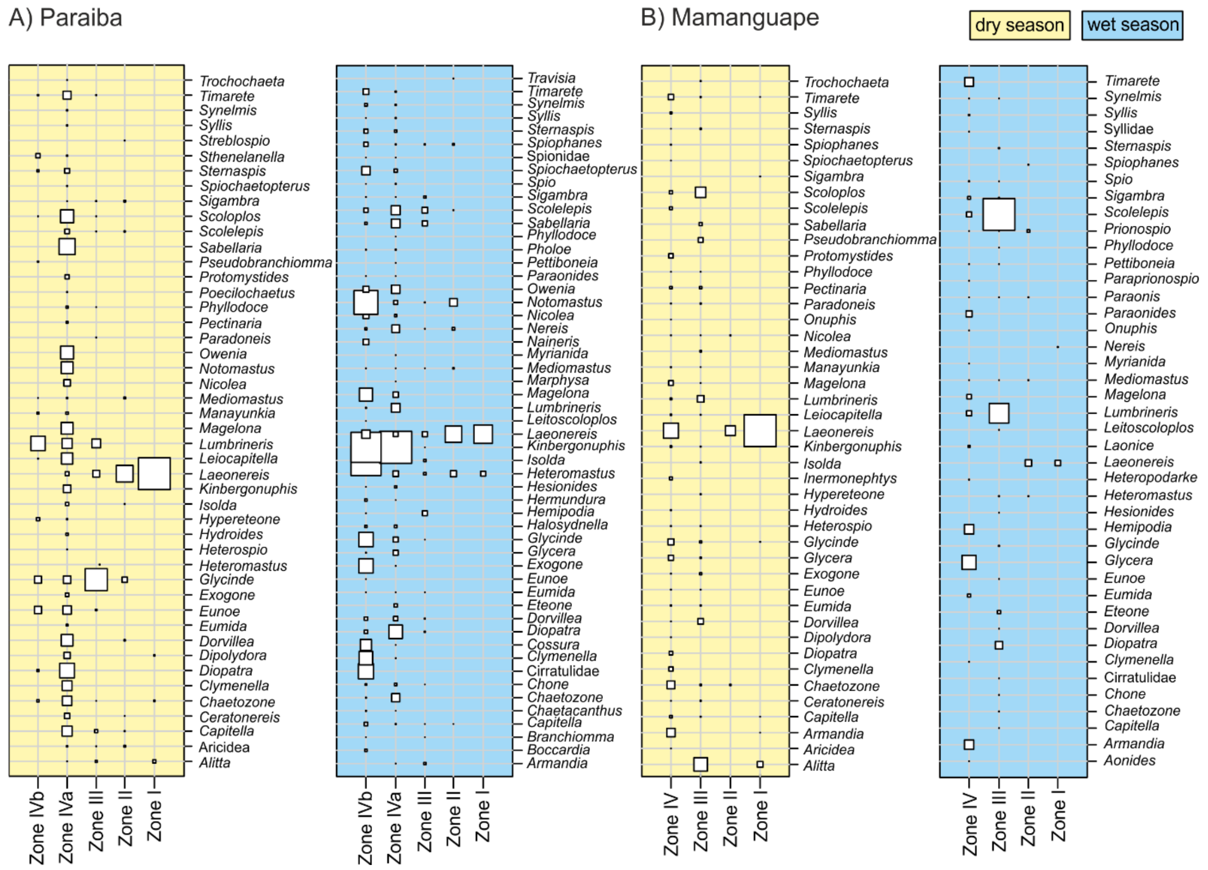

Figure 4. Spatial (zones) and seasonal (dry-wet) distribution of the mean biomass (AFDW) of polychaetes within the Paraíba (A) and Mamanguape (B) estuaries. Differences in biomass between species/zones/season is indicated on a relative scale by the size of each square (larger square equals higher biomass).

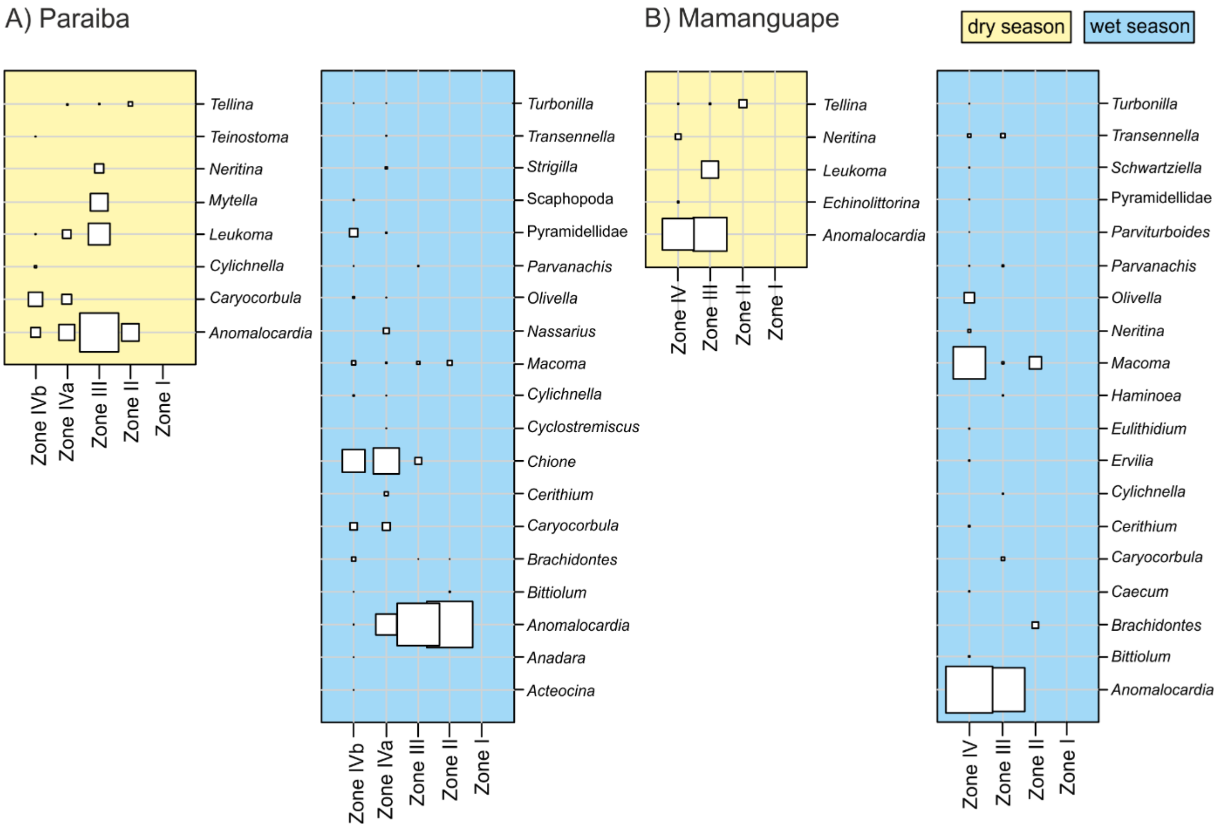

Figure 5. Spatial (zones) and seasonal (dry-wet) distribution of the mean biomass (AFDW) of molluscs within the Paraíba (A) and Mamanguape (B) estuaries. Differences in biomass between species/zones/season is indicated on a relative scale by the size of each square (larger square equals higher biomass).

3.3 Functional structure

In both estuaries, the functional structure of polychaetes and molluscs, in terms of functional diversity (functional richness and Rao), responded to the spatial gradients and seasonal influences in a similar way to taxonomic diversity (Fig. 3). Functional richness correlated with taxonomic richness (Pearson, $\alpha<0.05$) and Rao correlated with Simpson (Pearson, $\alpha<0.05$). Only the polychaetes demonstrated significant spatial differences (p-perm: 0.001) in the functional diversity values (functional richness and Rao) for both estuaries, with generally higher values for the lower estuarine zones versus lower values for the upper estuary. Conversely, differences in the spatial variation of Rao values were not detected for molluscs (p-perm >0.05; functional richness was not tested for molluscs due to insufficient data).

When comparing the functional structure of both polychaetes and molluscs between the two estuaries, there were no significant differences (p-perm >0.05 for functional richness and Rao).

The changing environmental conditions also caused shifts in their functional profiles (i.e. the community weighted mean values of each single trait-category). Polychaetes, in particular, demonstrated a distinct difference between the upper (I and II) and lower (IV) estuarine zones, as demonstrated by the FCA's main axis in Fig. 6. Upper zones were characterised by small to medium sized, surface dwelling polychaetes with a predatory/scavenging feeding strategy, with medium life-spans, direct/lecithotrophic larval-development and medium fecundity, while in the lower zones, the following traits were more present: large body-mass, tube/burrow dwellers, suspension feeders, long life-span, planktotrophic larval development and low/high fecundity.

Seasonal influences were less apparent (the factor season separated the samples along the 2nd FCA axis). Nevertheless, seasonal influences were more evident in the lower estuarine zones, with a higher proportion of tube-dwelling suspension-feeders with a large body-mass occurring in the wet season. Unlike polychaetes, the functional profiles of molluscs responded less obviously to the spatial gradients or seasonal influences (Fig. 7). For more details on the spatial and seasonal distribution of the community weighted mean (CWM) trait values, see Figures A.2 and A.3 in Appendix Chapter 3.

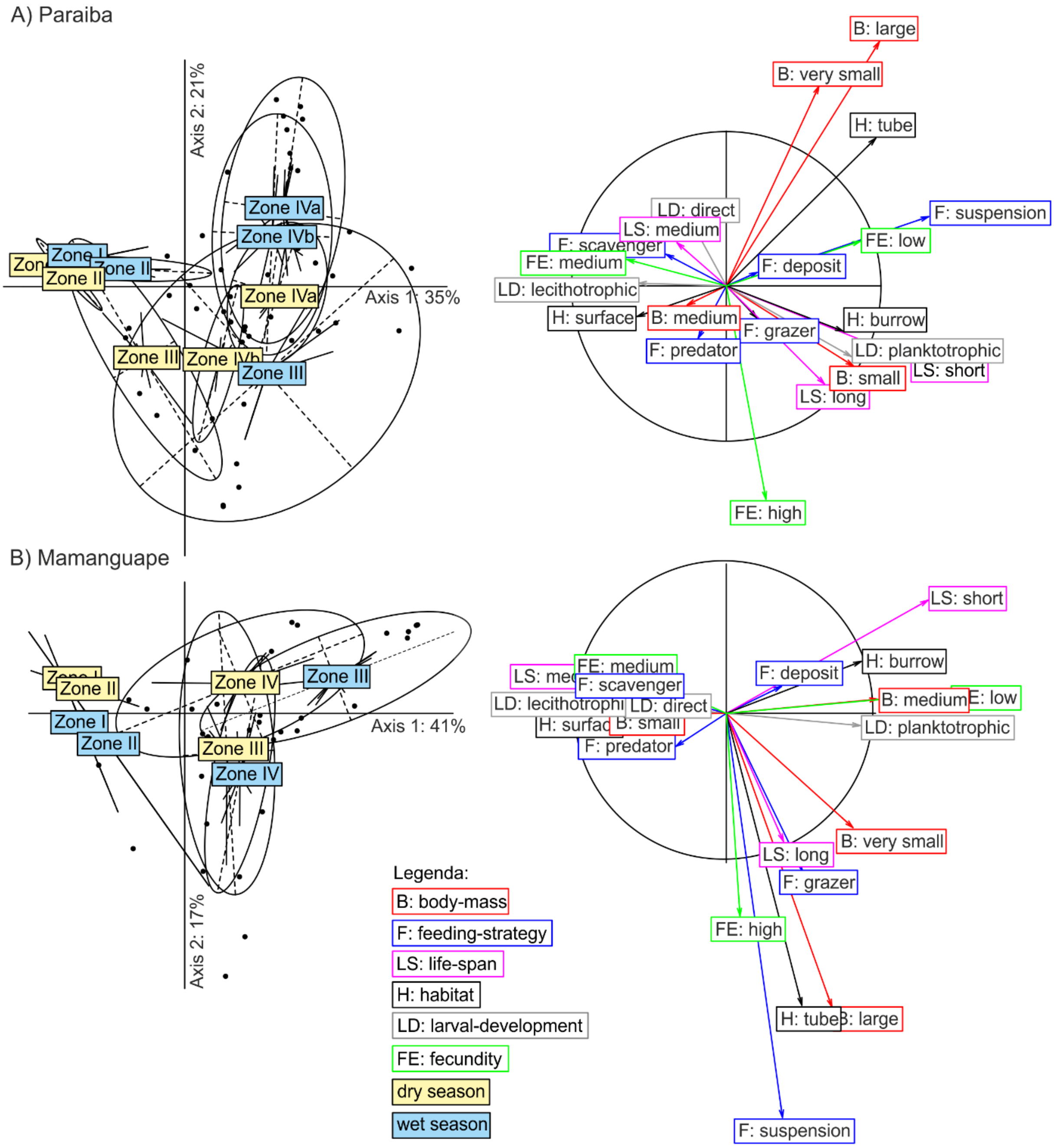

Figure 6. Results of Fuzzy Correspondence Analysis on polychaete data for the Paraíba (A) and the Mamanguape (B) estuaries. On the left: 2D ordination of samples based on traits categories exhibited by the polychaete communities (weighted by the community mean biomass); samples (points) are grouped (circles) for zones per season (dry and wet). On the right: 2D ordination of traits categories based on the samples where these categories are exhibited. Arrows point to the centre of gravity of the samples presenting that category.

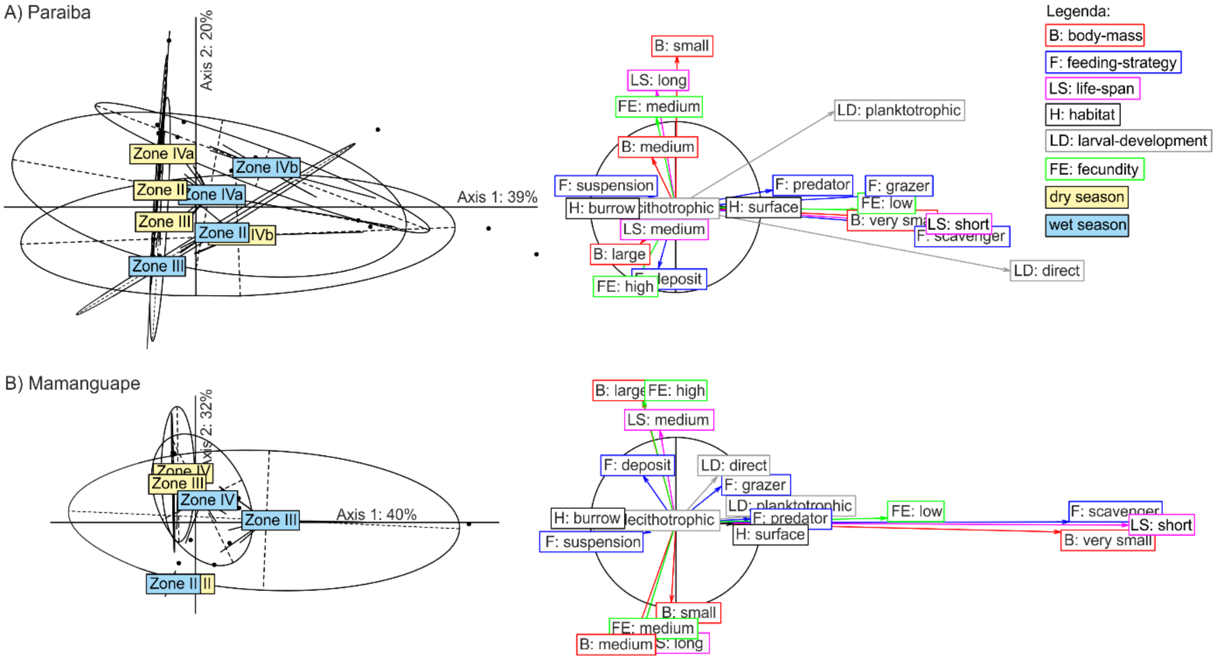

Figure 7. Results of Fuzzy Correspondence Analysis on mollusc data for the Paraíba (A) and the Mamanguape (B) estuaries. On the left: 2D ordination of samples based on traits categories exhibited by the mollusc communities (weighted by the community mean biomass); samples (points) are grouped (circles) for zones per season (dry and wet). On the right: 2D ordination of traits categories based on the samples where these categories are exhibited. Arrows point to the centre of gravity of the samples presenting that category.

4. Discussion

Trait-based studies performed in marine/transitional environments are often challenged by the need to find available, detailed and accurate trait information for marine macroinvertebrates (Tyler et al., 2012). This study faced this difficulty, especially with regard to the molluscs: the traits of many of the molluscs in our samples are neither described in scientific literature, nor in online trait databases such as MarLIN (MarLIN, 2006), which mostly include trait information of macroinvertebrates from European waters. The trait information of molluscs in this work is therefore based on expert knowledge and can be consulted in the 'trait-by-taxa' matrices available at Appendix Chapter 3 (Tables A.3, A.4).

Spatial environmental gradients, i.e. the combination of environmental variables that differ in the upper versus lower zones, were evident in both estuaries. Both polychaetes and molluscs responded to this gradient, with low taxonomic and functional diversity in the upper zones, as also observed in temperate estuaries (van der Linden et al., 2012; Barnes and Hendy, 2015a). The lower salinity in combination with the larger sedimentary grain size does not provide a suitable habitat for most polychaetes and molluscs. The polychaete communities in particular exhibited clear differences in their functional profiles (i.e. the community weighted mean values for each single trait-category) between the upper and the lower zones of both estuaries. The few polychaetes that are able to survive in the upper zones were characterised by predatory/scavenging surface dwellers, with a small/medium body-mass, a medium life-span, a medium fecundity and a direct/lecithotrophic larval-development. The genus *Laeonereis* (family: Nereididae) dominated this habitat in terms of biomass and contributed most to this trait profile. Polychaetes belonging to this genus are surface dwellers, which may partially explain its success as most other polychaetes are burrow dwellers that prefer sediments with a smaller grain-size, similar to those more commonly found within lower estuarine zones (Martin and Bastida, 2006). The high salinity tolerance of this genus is another reason for its colonization success in estuaries (Omena and Amaral, 2000; Santos et al., 2003; Barros et al., 2008).

The effect of seasonal changes in the environmental conditions (dry versus wet season) on the benthic communities were less defined, and can mainly be seen in a slight increase of functional richness during the wet season, which was more evident for molluscs in the lower estuarine zones. The functional profiles of molluscs however remained more constant, because of dominant taxa occurring in these lower zones (*Anomalocardia*, *Chione* and *Macoma*) which exhibited similar traits, in particular those related to resource use capabilities (i.e. they are burrow-dwelling, suspension feeders). Functional diversity as expressed by the Rao index, showed less seasonal variation in comparison with functional richness. This is because Rao was much more influenced by the previously mentioned dominant taxa, as this index is weighted by abundance (Lêps et al., 2006). Functional richness is not weighted by abundance, so rare taxa (and their traits) have more expression in this index. Rare species often have traits that are distinct from those of common species (Mouillot et al., 2013b). For example, the predatory surface dwelling gastropod *Olivella* sp. only appeared in the wet season: despite being relatively unimportant in terms of biomass, it added traits otherwise unrepresented. These ‘rare’ traits may be a reflection of a richer variety of available food resources and microhabitats created by the environmental conditions in the wet season (Ellingsen et al., 2007). A similar effect of the wet season was also observed for the functional richness of polychaetes, particularly in one of the arms (zone IVb) of the lower Paraíba. Here, the proportion of tube-dwelling suspension-feeding polychaetes with a large body-mass increased in the wet season. The genus *Isolda* contributed most to this trait display.

When comparing the communities between estuaries, we did not observe major differences, both in terms of their taxonomic and functional structure. This contrasted with the *a-priori* expectations of lower taxonomic and functional diversity for the communities in the more impacted Paraíba estuary, especially within the upper zones where nutrient and chlorophyll-*a* levels were much higher in comparison to the upper zones of the Mamanguape. In this environment, we also expected to find a higher proportion of *r*-selected (opportunistic) traits (i.e. very small/small body-mass, short life-span, high fecundity, planktotrophic larval development) reflecting the disturbance conditions (Pianka, 1970; Warwick, 1986). In general, it has been hypothesized that more pristine estuarine systems present a higher turnover (species replacement) than impacted systems (Barros et al., 2014). However, both polychaete and mollusc communities were more driven by intra-estuary spatial gradients and seasonal effects than by inter-estuary differences. Similar results were reported by Barros et al. (2012), who compared the taxonomic structure of three estuaries with differing environmental conditions in the Brazilian state of Bahia: the observed patterns of colonisation by polychaetes and molluscs along the estuarine gradients were consistent among estuaries, irrespective of the different catchment sizes and pollution levels of the three rivers.

By using a trait-based approach, we were able to give new quantitative insights on how the functional structure of these tropical estuarine communities responded to environmental gradients. We advocate that more studies are needed to obtain a generalised pattern of estuarine function within and across biogeographical regions, similarly to what has been performed on the basis of taxonomic diversity (Barros et al., 2014). In our study, taxonomic diversity proved to be a worthy surrogate for functional diversity, suggesting that with the loss or addition of taxa, potentially important ecosystem processes (e.g. resource dynamics) were lost or added (Culhane et al., 2014; van der Linden et al., 2016a). Most studies have found a strong correlation between taxonomic and functional diversity (Vandewalle et al., 2010; Culhane et al., 2014; van der Linden et al., 2016a, 2016b). Nevertheless, there were also signs supporting the hypothesis that these taxonomic components are not always related to their functional counterparts (Díaz and Cabido, 2001). Hence, in one of the upper zones (II) of the Paraíba, the taxonomic richness of polychaetes was low, whereas their functional richness was relatively high in comparison to the other zones, suggesting a high trait dissimilarity between species, which is typical of estuarine systems where species exhibit a high degree of specificity along a gradient and are adapted to local conditions (Barros et al., 2014). The separate analyses of polychaetes and molluscs allowed the evaluation of how each group responded to the environmental gradients. In the case of estuarine polychaetes, the degree to which they function depended greatly on the spatial gradients within these systems (i.e. different functional role in the upper versus the lower estuarine zones). The higher levels of functional diversity in the lower estuarine zones, especially in relation to added feeding and habitat related traits, suggested a potential increase in resource use strategies in comparison to the upper zones, where the low diversity values indicated the strong effect of environmental filtering. Considering the estuarine molluscs, their functional role remained more similar along the estuarine gradient. In general, polychaetes displayed higher levels of taxonomic and functional diversity, suggesting that in comparison with the molluscs, they may be utilizing the available resources more efficiently, which may reflect in changes in the functioning of the systems.

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General conclusion

Quantifying species traits promises improved understanding on how disturbance and environmental gradients may impact communities and ecosystem functioning. Yet, the utilization of trait-based approaches in estuaries and coastal zones remains largely unexplored, despite the increasing demand for environmental assessments of these systems to include more functional orientated approaches, thereby fulfilling the requirements for ecosystem-based management and conservation. This context determined the main purpose of this thesis, which was to explore the ability of trait-based approaches to provide a better insight on how environmental change may impact macrobenthic community functioning in threatened estuarine and coastal ecosystems. This study specifically focussed on testing and evaluating multiple complementary trait-based indices. These indices include the: "**community-weighted mean trait values**" (CWM) defining the dominant traits in a community; the **functional diversity** (FD) indices expressing the extent of trait differences in a community; and a **new index to quantify redundancy** that was developed based on the relation between taxonomic and functional diversity. The indices were evaluated depending on their ability to reflect anthropogenic disturbance and/or environmental gradients on macrobenthic community functioning in three different systems, i.e. (i) a temperate European estuary (Mondego estuary, Portugal), (ii) a temperate coastal zone (Basque coast, Bay of Biscay, Spain), (iii) and in the largely unexplored waters of two tropical estuaries (Paraíba and Mamanguape, N-E Brazil). Each of these systems is characterised by distinct ecological features, such as natural differences in benthic community structure (e.g. higher richness in coastal zones versus estuaries), physical and chemical gradients (e.g. strong gradients in estuaries), and different types of anthropogenic disturbances, all of which proved to be important for the interpretation and evaluation of the indices performance.

Before formulating a synoptic summary on whether trait-based approaches may or may-not be useful for estuarine and coastal assessment, we first need to dive deeper into each system (case-study) and break down the indices performance, i.e. which indices performed according to previous expectations, which did not, and why did they behave as they did?

Temperate estuary: assessing distinct hydromorphological features

We first explored the indices performance in the Mondego estuary, where we tested their ability to reflect differences in macrobenthic community functioning between two estuarine arms, each with distinct hydromorphological features. The results showed that some indices responded clearly to this difference and others not so well. One of the main findings was that caution is needed when interpreting indices that are weighted by abundance when dealing with communities with few and dominant species, as typical in estuaries. This is commonly known for taxonomic diversity indices like Simpson, but we demonstrated that it also applies to weighted functional indices.

By design, these indices give a differential weight to dominant species and their traits, thereby neglecting rare species with often distinct combinations of traits, which could lead to a underestimation of diversity and consequently a misleading picture of functioning. Dominant species often exert a key effect on many ecosystem processes (Lepš et al., 2011; Dias et al., 2013). However, rare species tend to disproportionally increase the potential breadth of ecosystem processes, making them functionally important and, therefore, should not be disregarded (Ellingsen et al., 2007; Mouillot et al., 2013b). Furthermore, rare species with rare traits are often the first to be filtered out when disturbance increases, leaving the remaining species to share traits that are more similar. Taking this into consideration, indices reflecting the extinction of rare species and their traits may be particularly useful to assess disturbance, especially in environments were few dominant species naturally occur.

For this study, we developed and tested such an index (i.e. SR-FRED). We argue that this new index, in combination with the CWM index, provided the clearest picture of how hydromorphological differences may have affected benthic functioning in this estuary. The

CWM index revealed community patterns due to environmental selection for certain traits, however, the relationship between most traits and differences in hydromorphological conditions was not entirely clear. In this system feeding traits seemed to best reflect this difference, whereas body-size and in particular life-span were not.

Temperate coastal zone: response to natural disturbance and effluents

The Basque coast provided the second setting for testing the trait-based approach. We tested how two complementary indices based on the mean (CWM) and diversity of multiple traits (FD expressed with the Rao index) responded to natural disturbance (wave impact) and anthropogenic seafloor disturbance (effluents). Moreover, we compared the performance of these indices with two of the most widely used and established benthic indices, AMBI and M-AMBI. These indices were originally developed and thoroughly tested in these coastal waters, which provided a solid background for testing the performance of the novel trait-based indices versus the established “benchmark indices”.

Benthic macroinvertebrate communities in coastal zones are usually more diverse and less populated by dominant species as in estuaries. Taking into account what we learned in the Mondego estuary case study regarding the interpretation of the weighted diversity indices, we expected *a-priori* that these indices would be better suited for assessing the effects of environmental disturbance in this coastal environment. The results showed that CWM and Rao were not effective in indicating anthropogenic disturbance (effluents) in this coastal system, in contrast with AMBI and M-AMBI that were able to do it. Different factors may have influenced this result. The links between most traits and the environmental variables associated with this type of disturbance were weak and poorly understood. Many traits were influenced by natural induced disturbance (e.g. wave-impact), and most likely by other existing anthropogenic pressures in the studied area (e.g. fishing, dredging, sediment deposits). Besides, the traits (body-size and larval-development) showed different responses in comparison with some other studies investigating trait responses to seafloor disturbance caused by organic enrichment. A clear advantage of using the CWM index is that it gave a more detailed picture on how the two types of disturbances (anthropogenic and natural) affected each trait, thereby contributing to a deeper knowledge on community functioning.

Tropical estuaries: different levels of human impact and the role of environmental gradients

In the final chapter of this thesis, we tested whether trait-based approaches reflected environmental change on macrobenthic communities in two tropical Brazilian estuaries, which have been less studied from a functional perspective. In fact, these estuaries were recently studied for the first time, and information is now also available on fish (Dolbeth et al., 2016a, 2016b) and zooplankton (de Moura et al., 2016) functioning.

In particular, we studied how the functional structure of polychaetes and molluscs communities responded to environmental gradients and anthropogenic effluents. Trait-based approaches included multivariate analysis based on CWM, and two FD indices, i.e. functional richness and Rao. As in temperate estuaries, these tropical systems were also characterized by dominant taxa, which clearly affected the Rao index (as in the Mondego estuary). Functional richness, on the other hand, is not weighted by abundance; so rare taxa and their traits have more expression in this index. Unfortunately, functional richness (as in Villéger et al., 2008) needs a minimum of three taxa to be computed (Laliberté and Legendre, 2010). Since, the communities in these estuaries (and also in the Mondego) were frequently composed of less than three taxa, this particular index needs to be used with caution in environments were few species are naturally common. In general, the polychaetes displayed higher levels of FD, suggesting that in comparison with the molluscs, they potentially utilised the available resources more efficiently. However, the indices failed to reflect functional differences between estuaries, while otherwise expected (i.e. lower FD and a higher proportion of taxa with typical opportunistic traits in the more impacted Paraíba). Macrobenthic community functioning seemed more driven by intra-estuarine gradients than by different catchment sizes and pollution levels between estuaries

.

Synthesis of key findings

- Abundance-weighted FD indices should be used with caution in estuarine systems where few and dominant species naturally occur. Within this respect, non-weighted indices such as the new developed SR-FRED index that reflect the extinction of rare species with often rare combinations of traits, are potentially more useful.
- For all studied systems, CWM contributed to a more profound knowledge on the functioning of macrobenthic communities in response to environmental change. However, most traits were not effective in indicating disturbance in the form of anthropogenic effluents in the Basque coast and the Paraíba estuary, which also reflected in the performance of FD indices.
- A clear mechanistic link between many traits and environmental change was often missing, probably because of the masking effect of multiple types of disturbances (anthropogenic and naturally induced), and the sometimes contradictory results with other studies.
- CWM is an index difficult to interpret from a management perspective, as it does not provide a single number indicating a quality status.
- Unlike CWM, FD indices (like Rao) do provide a single number of functioning, therefore potentially more effective for management purposes.
- To improve the performance of CWM and FD indices, and trait-based approaches in general, abundant and accurate trait data is required, which is currently lacking for many marine macroinvertebrates, especially for tropical marine species.
- Different taxonomic groups within the macrobenthic community (e.g. Molluscs and Polychaetes) might function differently, as seen in our study. It is therefore necessary to be cautious while interpreting results given be only one group in isolation. If possible,

the entire macrobenthic community should be included in environmental assessment studies.

- More trait-based studies are needed to obtain a generalised pattern of estuarine and coastal function within and across biogeographical regions, similarly to what has been performed on the basis of taxonomic-based research.

Synoptic summary

This study showed that, overall, trait-based approaches have potential to complement the classical taxonomic-based approaches for benthic assessment in estuarine and coastal systems. Trait-based approaches, however, may work better along strong physical and chemical gradients as demonstrated in the estuarine systems, while they were not able to assess more subtle gradients in the form of anthropogenic effluents along the Basque coast and in the Paraíba estuary. We need to consider that trait-based research is relatively new and still under development, and that there is plenty room for improvement. Particularly important is the acquisition of abundant and accurate trait information for estuarine and coastal macroinvertebrate species, which will improve the relationship between environment and traits.

New directions

New directions and challenges emerged from this study and here we suggest the most important ones that I believe could help to improve future trait-based research for estuarine and coastal assessment:

1. Development of a publicly available open-source global macroinvertebrate trait database, filled with abundant and accurate trait data for all macroinvertebrate species existing in estuarine, coastal and marine habitats at a global geographical scale.
2. Increase field- and laboratory measurements on traits (e.g. maximum specific growth rates, temperature/salinity tolerances etc.). Many important traits are difficult to

measure, especially in the field. This knowledge would help to improve the relationship between environment and traits (Barton et al., 2016).

3. More studies to differentiate between response and effect traits are needed. “Response traits” determine the community response to environmental change, while “effect traits” determine the effect of that change on ecosystem processes and services, i.e. ecosystem functioning (Díaz and Cabido, 2001). Trait-based studies performed in estuarine, coastal and marine systems have rarely considered the distinction between these two types of traits (Bolam et al., 2016). Yet, understanding their distinction is of utmost importance as response traits may vary independently from effect traits (Bolam et al., 2016). The extent to which response and effect traits can be linked within taxa, either via trait correlations or trade-offs, is still largely lacking (Moretti et al., 2016). A framework for differentiating response and effect traits similar to what have been proposed for plants (Suding et al., 2008; Lavorel et al., 2013) will improve our ability to predict changes in ecosystem functioning under environmental change (Bolam et al., 2016; Moretti et al., 2016).
4. Linking effect traits to multiple ecosystem processes and services is lacking. Trait-based studies have linked effect traits of benthic fauna to specific ecosystem processes such as: production (Bolam and Eggleton, 2014; Dolbeth et al., 2015; Jänes et al., 2016), and sediment oxygen-nutrient fluxes (Mermillod-Blondin et al., 2005; Norling et al., 2007; Solan et al., 2008; Braeckman et al., 2010; Janson et al., 2012; Villnäs et al., 2012; Norkko et al., 2013). However, traits of benthic fauna have yet to be linked to multiple ecosystem processes and services. Such assessments of trait-service linkages allow for more accurate estimations of (benthic) ecosystem functioning under environmental change, and would be a crucial step forward in ecosystem-based management.
5. Development of a global trait-based tool is needed. This tool should be easy to interpret (also from a management perspective), and should allow quantitative comparison of traits within and between environments at a global scale. For coastal and marine

systems, it would be interesting to compare, for example, benthic functioning across impacted areas, marine protected areas, harbours, fish farms, etc.

6. Increase the use of trait-based models. These are rarely used regarding macrobenthic fauna, however, they can be important tools for combining numerous and diverse data sources for testing and generating hypotheses that link traits to (global) patterns of environmental change, ecosystem structure/functioning, and other fundamental controlling mechanisms (Strong et al., 2015; Barton et al., 2016).

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Appendix Chapter 2

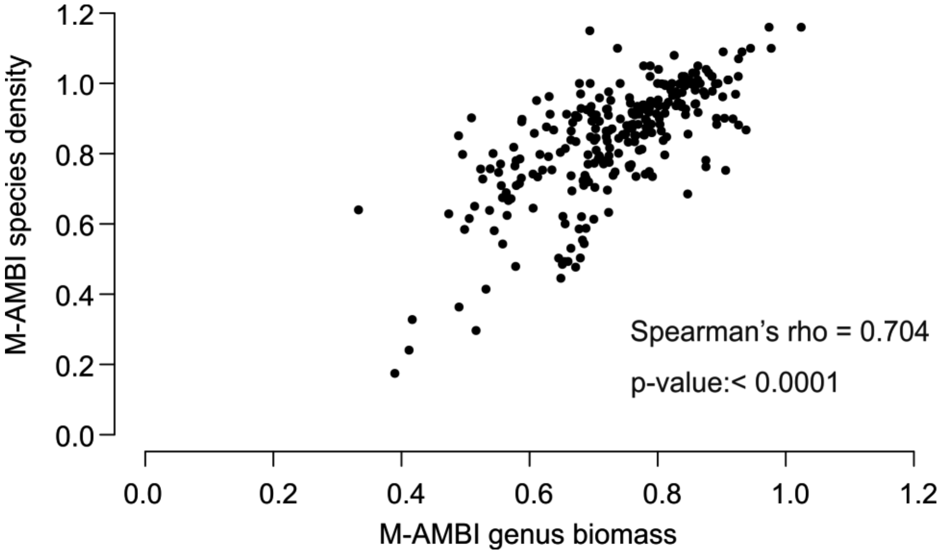


Figure A.1 Relation between M-AMBI calculated with species density and genus biomass. Results of the Spearman's rank correlation analysis are shown.

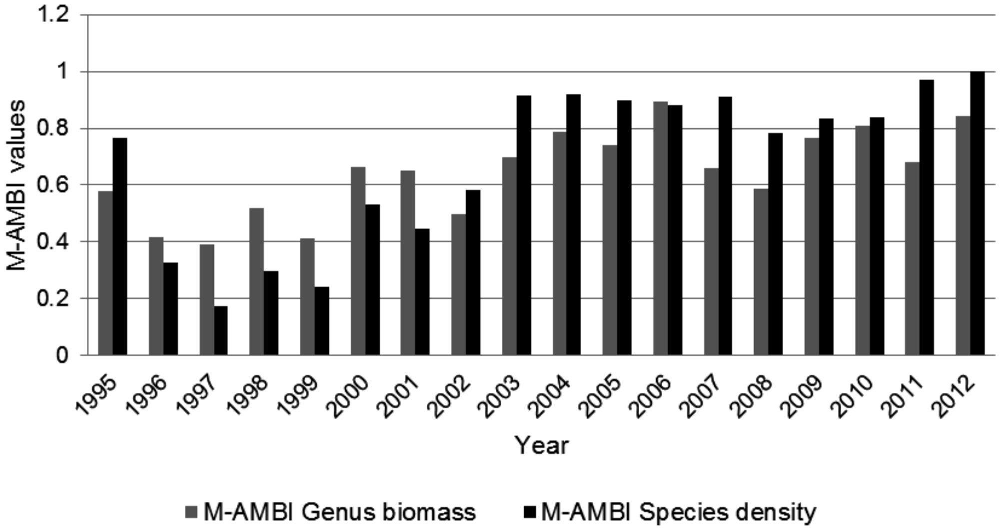
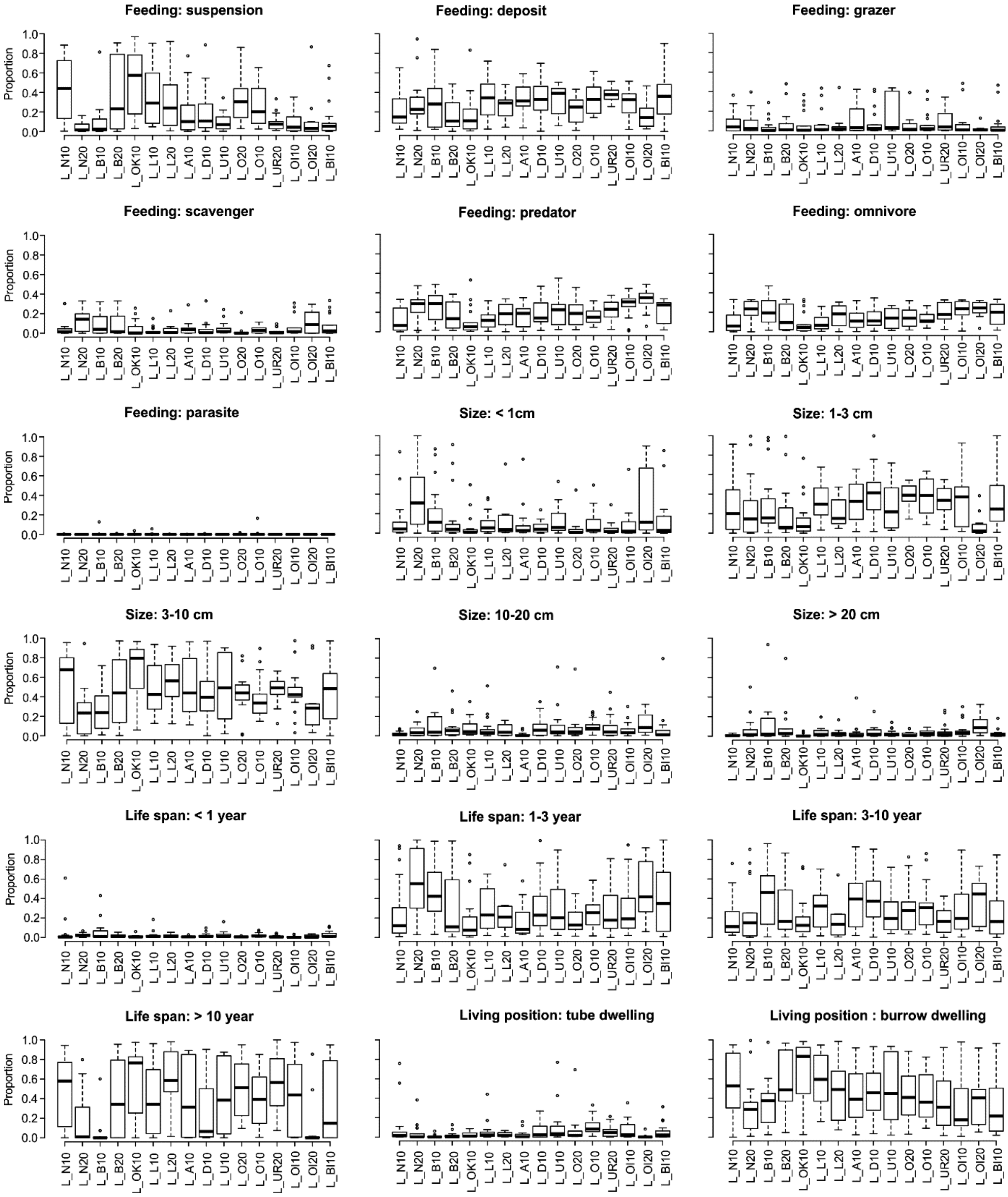


Figure A.2 M-AMBI values calculated with species density and genus biomass at station L_UR20. From 1996 to 2001 (and in 2006), M-AMBI values calculated with genus biomass exceeded the values of M-AMBI calculated with species density. The opposite can be observed for the other years. The reason for this difference is that M-AMBI calculated with species density (standard calculation of M-AMBI) included taxa at a lower resolution than genus (nematodes, oligochaetes, etc.). As most of these taxa belong to ecological groups IV and V (opportunists), M-AMBI calculated with species density responded more obviously to disturbance during the initial years, which is in accordance with the expected disturbance pattern at this station. Nevertheless, both calculation methods showed similar patterns. The Wilcoxon signed-rank test results indicated non-significant differences between the two calculation methods (p-value: 0.369)


Feeding: suspension
Feeding: deposit
Feeding: grazer
Feeding: scavenger
Feeding: predator
Feeding: omnivore
Feeding: parasite
Size: < 1cm
Size: 1-3 cm
Size: 3-10 cm
Size: 10-20 cm
Size: > 20 cm
Life span: < 1 year
Life span: 1-3 year
Life span: 3-10 year
Life span: > 10 year
Living position: tube dwelling
Living position : burrow dwelling
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
L_N10
L_N20
L_B10
L_B20
L_OK10
L_L10
L_L20
L_A10
L_D10
L_U10
L_O20
L_O10
L_UR20
L_OI10
L_OI20
L_BI10

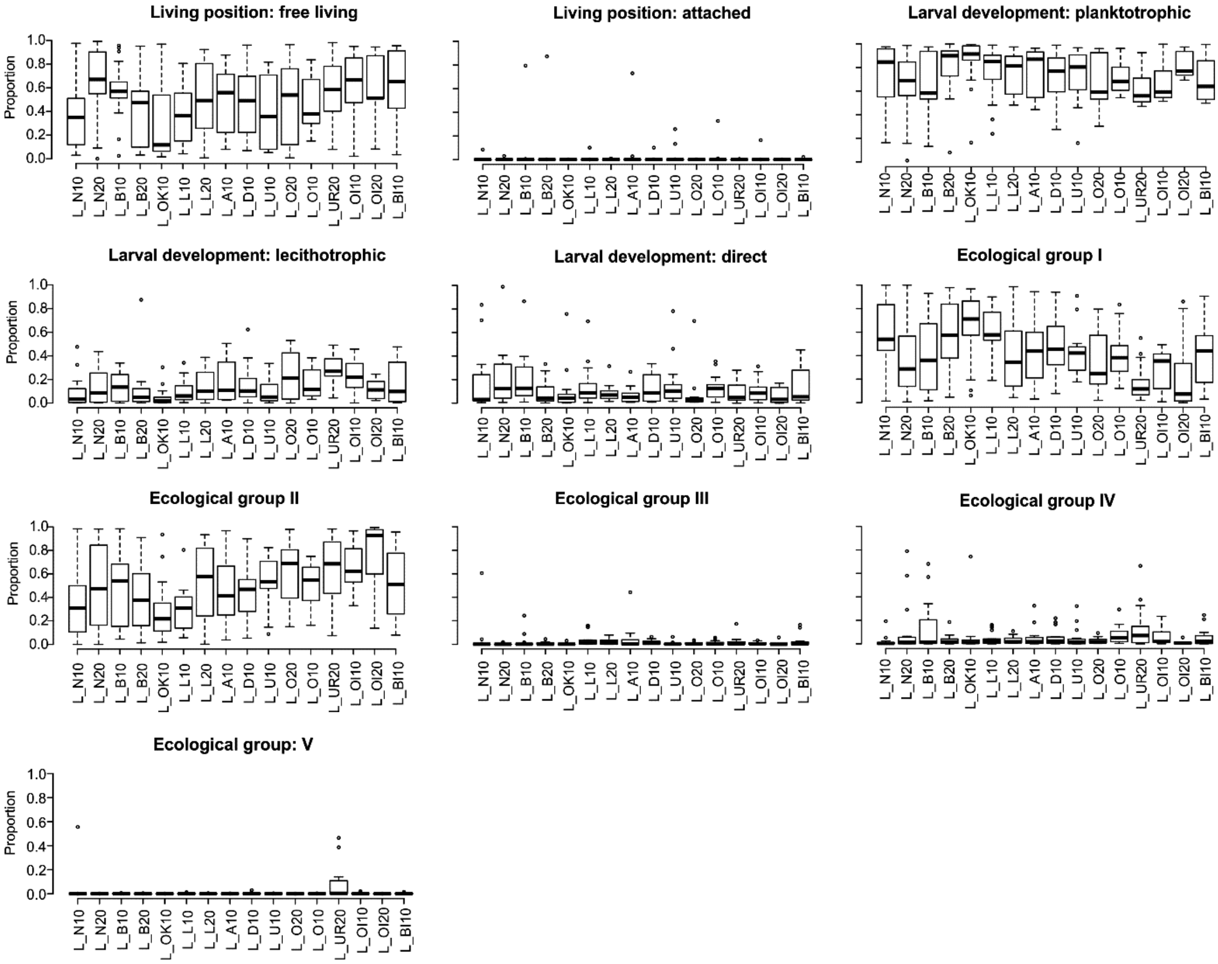


Figure A.3 The community weighted mean trait values for each of the 28 trait categories for each station.

Table A.1 The list of taxa (genus level) identified in this study, together with the associated traits (See Table 1 for the meaning of the labels).

Genus	S_1	S1_3	S3_10	S10_20	S_20	L_1	L1_3	L3_10	L_10	F_SUS	F_DEP	F_GRA	F_SCA	F_PRE	F_OMN	F_PAR	LH_TD	LH_BD	LH_FL	LH_ATT	DT_PLAN	DT_LEC	DT_DIR	EG_I	EG_II	EG_III	EG_IV	EG_V
Abarenicola	0	0	0	1	0	0	0	1	0	0	2	1	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0
Abludomelita	1	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0
Abra	0	1	0	0	0	0	3	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
Abyssoninoe	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0
Acanthocardia	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Acrocnida	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
Acteon	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	0	0
Aglaophamus	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0
Alvania	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Amathia	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Ampelisca	1	1	1	0	0	1	3	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0
Ampharete	1	1	2	0	0	0	1	1	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0
Amphibalanus	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Amphictene	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
Amphipholis	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Amphiura	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Anapagurus	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Anchialina	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0
Animoceradocus	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
Anoplodactylus	1	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Aonides	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
Aora	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
Aphelochaeta	0	0	1	0	0	0	2	1	0	1	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0
Apherusa	1	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0
Apistobranchus	2	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Apohyale	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
Aponuphis	0	0	1	0	0	0	1	1	0	0	0	0	1	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Aporrhais	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Apseudes	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Aricidea	1	2	0	0	0	3	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0
Armandia	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
Ascorhynchus	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Aspidosiphon	0	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Astarte	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0
Astropecten	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	1	0	1	0	0	0	0
Atylus	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0
Autonoe	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
Barleeia	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Bathyporeia	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0
Bela	2	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Bittium	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Bodotria	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0
Branchiomaldane	1	1	1	1	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Branchiostoma	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Brania	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Caecum	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0
Callianassa	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
Capitella	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	1
Caryophyllia	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0
Caulleriella	0	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0
Cellepora	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0
Cerastoderma	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
Cerebratulus	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Chaetozone	0	1	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0
Chamelea	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Cheirocratus	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
Chone	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0
Cirolana	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Cirrophorus	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0
Clausinella	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0
Cleantis	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Clytia	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Colomastix	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0
Comarmondia	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0
Corbula	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0
Coryne	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Corystes	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Cossura	0	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	1	0
Cumella	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Cylichna	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Cymia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cymodoce	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Dardanus	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Diastylis	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0

Digitaria	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0
Diogenes	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Diopatra	0	0	0	0	1	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	1	1	1	0	0	0
Dioplosyllis	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0
Diplocirrus	0	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
Dispio	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0
Donax	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Dosinia	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Dynamene	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Ebalia	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Echinocardium	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0
Echinocyamus	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	0
Echiurus	0	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0
Edwardsia	0	1	0	0	0	0	0	1	3	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
Embletonia	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
Ensis	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Eocuma	1	0	0	0	0	2	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Epitonium	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Epizoanthus	0	1	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0
Erinaceusyllis	1	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0
Euclymene	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	1	2	2	0	0	0	0	0
Eulalia	0	0	1	1	0	0	1	3	0	0	0	0	1	1	1	0	0	0	1	0	3	1	0	0	1	0	0	0
Eulimella	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0
Eumida	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Eunice	0	0	1	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Eunicella	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0
Eupolymnia	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Eurydice	1	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0
Eurysyllis	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	1	0	0	0
Euspira	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Eusyllis	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0
Exogone	0	1	0	0	0	3	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0
Galathea	0	1	1	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Galathowenia	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
Gammarella	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Gammaropsis	1	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0
Gammarus	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0	0
Gari	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0
Gastrosaccus	0	1	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	1	2	0	0	0	1	0	1	0	0	0
Glycera	0	0	1	1	1	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0
Glycinde	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Glycymeris	0	0	1	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0
Goniada	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Goniadella	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Goodallia	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
Gouldia	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Grania	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1
Gregariella	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Guernea	1	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Gyptis	1	2	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Haplostylus	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0
Harmothoe	0	1	1	0	0	0	1	1	0	0	0	0	0	2	1	0	1	0	3	0	1	0	0	0	1	0	0	0
Harpinia	1	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	1	3	0	0	0	1	1	0	0	0	0
Hediste	0	0	0	1	0	1	0	0	0	1	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0
Hesionura	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Hesiospina	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Heterodrilus	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Hiatella	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Hippomedon	1	1	0	0	0	1	2	0	0	0	1	0	0	2	1	0	0	0	1	0	0	0	1	1	0	0	0	0
Hyalinoecia	0	0	1	1	1	0	1	3	0	0	1	0	0	0	1	0	3	1	0	0	0	0	1	0	1	0	0	0
Idotea	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Idunella	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0
Iphinoe	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	3	0	0	0	1	1	0	0	0	0
Jaera	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0
Jassa	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
Kefersteinia	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Kurtiella	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0
Lacydonia	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Laevicardium	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Lagis	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0
Lanassa	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Lanice	0	0	0	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0
Laonice	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
Lekanesphaera	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Lepidepecreum	1	0	0	0	0	1	2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0

Lepidonotus	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Leptochelia	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Leptoplana	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Leptosynapta	0	0	0	0	1	0	1	2	0	0	1	1	0	0	0	0	0	2	1	0	0	0	1	1	0	0	0	0
Leucothoe	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Levinsenia	0	0	1	0	0	0	1	1	0	1	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Limaria	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Limatula	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0
Liocarcinus	0	1	1	0	0	0	0	3	0	0	0	0	0	3	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Lophogaster	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	0
Loripes	0	1	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0
Lucinella	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0
Lumbrinerides	0	0	1	0	0	0	1	1	0	0	1	0	0	2	1	0	0	1	3	0	0	1	1	0	1	0	0	0
Lumbrineriopsis	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0
Lumbrineris	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0
Lutraria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Lysidice	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	3	0	1	0	0	0	1	0	0	0
Lysilla	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0
Macrochaeta	1	0	0	0	0	0	1	1	0	1	2	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	0
Mactra	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Maerella	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Magelona	0	0	1	1	0	0	0	1	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0
Malacoceros	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
Malmgreniella	0	1	1	0	0	0	0	1	0	0	0	0	0	2	1	0	1	1	3	0	1	0	0	0	1	0	0	0
Mangelia	1	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Marphysa	0	0	1	2	2	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	0	0	0
Mediomastus	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	3	1	0	0	1	0	0	0	0	1	0	0
Megaluropus	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Megamphopus	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0
Melanella	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0
Melinna	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	3	0	0	0	0	1	0	0	0	1	0	0
Mesochaetopterus	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0	0
Mesonerilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microdeutopus	1	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
Microjaera	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0
Micromaldane	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0
Microspio	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
Modiolula	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Moerella	0	1	1	0	0	0	0	1	0	1	3	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Monoculodes	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1	3	0	0	0	1	1	0	0	0	0
Montacuta	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	3	1	0	1	0	0	0	1	0	0	0
Monticellina	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0
Musculus	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0
Myrianida	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Myriochele	0	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Mysta	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	3	0	0	1	1	0	0
Mystides	1	2	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Mytilaster	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Nassarius	0	1	1	0	0	0	0	0	1	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	0	0	0
Natatolana	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Nebalia	0	1	0	0	0	1	0	0	0	3	1	0	0	0	0	0	0	0	1	0	3	0	1	0	0	0	0	1
Necallianassa	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
Nephasoma	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
Nephtys	0	0	1	1	1	0	1	3	0	0	1	0	0	2	1	0	0	1	1	0	3	1	0	0	1	0	0	0
Nereimyra	0	1	0	0	0	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	1	0	0	0	0	1	0	0
Nereiphylla	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Nereis	0	0	1	1	0	0	0	1	0	0	1	0	0	2	1	0	0	1	0	0	0	1	2	0	0	1	0	0
Nicomache	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
Nothria	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
Notocirrus	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Notomastus	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0	1	2	0	0	0	1	0	0
Nucula	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	3	0	1	0	0	0	0
Odontosyllis	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Onuphis	0	0	0	1	0	0	1	1	0	0	0	0	0	2	1	0	1	0	0	0	0	0	1	0	1	0	0	0
Ophelia	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0
Ophiopsila	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Ophiura	0	1	1	0	0	0	0	1	0	1	1	0	0	3	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Ophryotrocha	1	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0
Opisthodonta	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	1	0	0
Orbinia	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Orchomene	1	0	0	0	0	1	2	0	0	0	0	0	0	2	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Owenia	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
Oxydromus	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0
Pagurus	0	1	1	0	0	0	0	1	0	1	1	0	0	2	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Pandora	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0

Papillicardium	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Paradialychone	1	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0	1	0	0	0	1	1	0	1	1	0	0	0
Paradoneis	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Paraehlersia	0	1	0	0	0	0	1	0	0	0	1	0	0	2	1	0	0	1	0	0	0	0	1	0	1	0	0	0
Parahaustorius	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0
Paranaitis	0	1	2	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Paraonis	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Parapionosyllis	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	1	0	1	0	0	0
Pariambus	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Perioculodes	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	3	0	0	0	1	0	1	0	0	0
Phascolion	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0
Phaxas	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Pherusa	0	0	1	0	0	0	0	1	0	1	2	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0
Philine	0	1	1	0	0	0	0	1	0	0	0	0	0	2	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Philocheras	0	0	1	0	0	0	0	1	0	0	0	0	0	2	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Phoronis	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0
Phtisica	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0
Phyllodoce	0	0	1	1	0	0	3	1	0	0	0	0	0	1	1	0	0	1	2	0	1	1	0	0	1	0	0	0
Pinnotheres	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0
Pionosyllis	1	0	0	0	0	0	1	0	0	0	0	0	0	2	1	0	0	0	1	0	0	1	1	0	1	0	0	0
Pisione	0	0	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Pista	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0
Pistella	0	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0
Plakosyllis	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	1	0	1	0	0	0
Plumularia	0	1	1	2	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	0
Podarkeopsis	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Podocoryna	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Poecilochaetus	0	0	1	0	0	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
Polybius	0	0	1	0	0	0	0	1	0	0	0	0	0	2	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Polycirrus	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	3	0	0	1	0	0	0	0	1	0
Polydora	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	3	1	0	0	1	0	1	0	0	0	1	0
Polygireulima	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0
Polygordius	0	0	1	0	0	1	3	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Polyophthalmus	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	3	0	0	0	1	1	1	0	0	0	0
Pontocrates	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	3	0	0	0	1	0	1	0	0	0
Portumnus	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	1	0	0	0	0
Prionospio	0	1	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1	1	0
Processa	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Prosphaerosyllis	2	1	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	0	1	0	1	0	1	0	1	0	0	0
Protodorvillea	0	1	1	0	0	0	1	0	0	0	0	0	0	2	1	0	0	0	1	0	0	0	1	1	1	0	0	0
Protodriloides	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Protodrilus	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Protomystides	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	0	0	0
Psamathe	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0	0
Psammechinus	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1	0	0	0	0
Pseudocuma	1	0	0	0	0	1	3	0	0	1	1	0	0	0	0	0	0	1	3	0	0	0	1	0	1	0	0	0
Pseudomystides	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Pseudopolydora	1	1	1	0	0	0	1	0	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	0
Pseudoprotella	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Pseudopythina	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Retusa	1	1	0	0	0	0	0	1	0	0	0	0	0	2	1	0	0	1	0	0	1	0	0	0	1	0	0	0
Rissoa	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Rocellaria	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Sabella	0	0	1	1	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0
Sabellaria	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
Saccocirrus	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Salvatoria	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Sarsinebalia	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Scalibregma	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0
Scaphander	0	2	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0
Schistomeringos	0	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	0	0	0
Sclerocheilus	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Scolaricia	0	0	0	1	0	0	1	3	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0
Scolelepis	0	1	1	1	0	3	1	0	0	2	3	0	0	1	1	0	0	1	1	0	3	0	1	0	0	1	0	0
Scoletoma	0	0	1	1	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0	1	0	0	0
Semivermilia	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0
Sertularella	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Sigalion	0	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Siphonoecetes	1	0	0	0	0	1	0	0	0	2	2	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0
Sipunculus	0	1	1	1	0	0	1	0	0	0	2	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Socarnes	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0
Spadella	1	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0
Spatangus	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Sphaerosyllis	1	0	0	0	0	0	1	0	0	0	3	0	0	1	1	0	0	0	1	0	0	0	1	1	1	1	0	0
Sphenia	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0

Spio	0	1	1	0	0	1	1	0	0	1	2	0	0	0	0	0	1	0	0	0	3	0	1	0	0	1	0	0
Spiochaetopterus	0	0	1	0	0	0	0	1	0	1	2	0	0	0	0	0	3	1	0	0	1	0	0	0	0	1	0	0
Spiophanes	0	0	1	0	0	0	1	0	0	1	2	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0
Spirobranchus	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0
Spisula	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Sthenelais	0	0	1	1	0	0	0	1	0	0	0	0	0	2	1	0	0	0	1	0	1	0	0	0	1	0	0	0
Streblospio	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Streptosyllis	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Sycon	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Syllides	1	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0
Syllis	0	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	3	0	1	0	0	0
Synchelidium	1	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	0	1	3	0	0	0	1	1	0	0	0	0
Tellimya	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	3	1	0	1	0	0	0	1	0	0	0
Tellina	1	1	0	0	0	1	1	2	0	1	2	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Tharyx	0	1	1	0	0	0	1	3	0	1	2	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0
Thia	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0
Thracia	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Thyasira	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0
Timoclea	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Tricolia	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Triphora	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0
Trypanosyllis	1	1	2	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0
Tryphosella	1	0	0	0	0	1	2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Tryphosites	0	1	0	0	0	1	2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Tubulanus	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0
Tubularia	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0
Turbonilla	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0
Turritella	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
Uca	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0
Unciola	0	1	1	0	0	1	0	0	0	1	2	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0
Urothoe	1	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	0	1	3	0	0	0	1	1	0	0	0	0
Vaunthompsonia	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Venus	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Verruca	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Volvulella	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0
Websterinereis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Xenosyllis	1	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0

Appendix Chapter 3

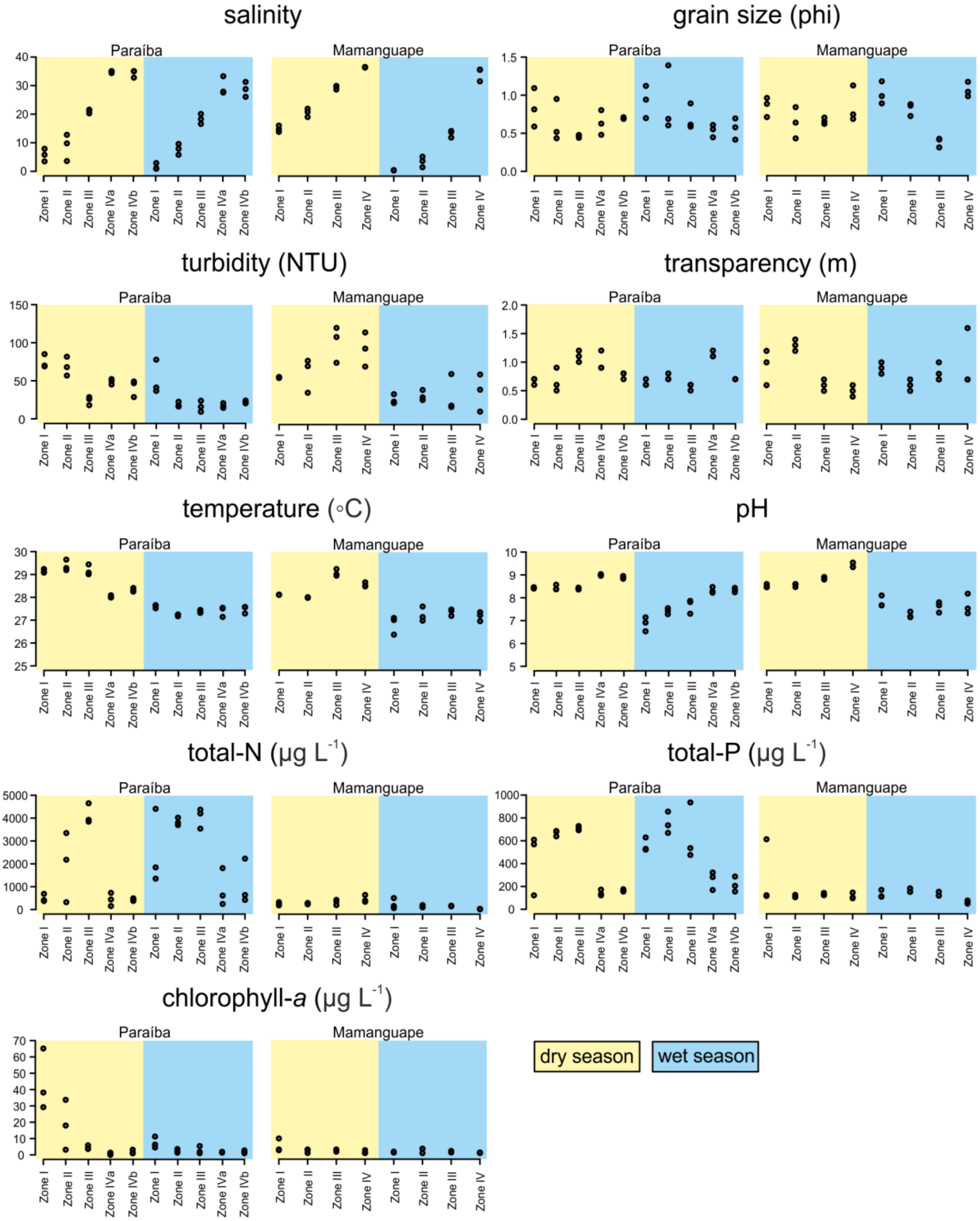
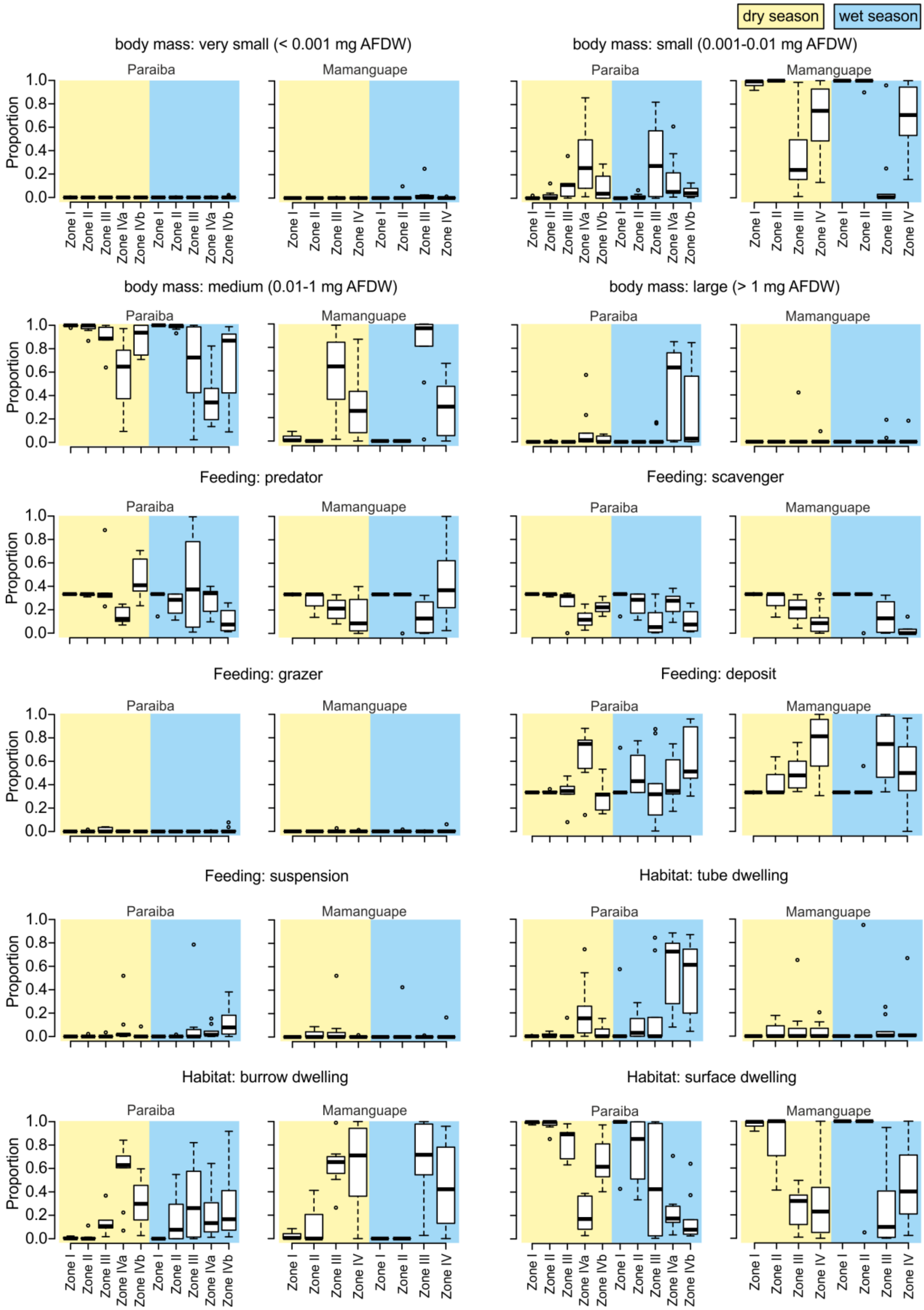


Figure A.1. Individual data points (sample, n=3) showing the spatial (zones) and seasonal (dry-wet) variation of environmental variables within the Paraíba and Mamanguape estuaries.


dry season
wet season
body mass: very small (< 0.001 mg AFDW)
body mass: small (0.001-0.01 mg AFDW)
body mass: medium (0.01-1 mg AFDW)
body mass: large (> 1 mg AFDW)
Feeding: predator
Feeding: scavenger
Feeding: grazer
Feeding: deposit
Feeding: suspension
Habitat: tube dwelling
Habitat: burrow dwelling
Habitat: surface dwelling
Paraiba
Mamanguape
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Zone I
Zone II
Zone III
Zone IVa
Zone IVb
Zone IV

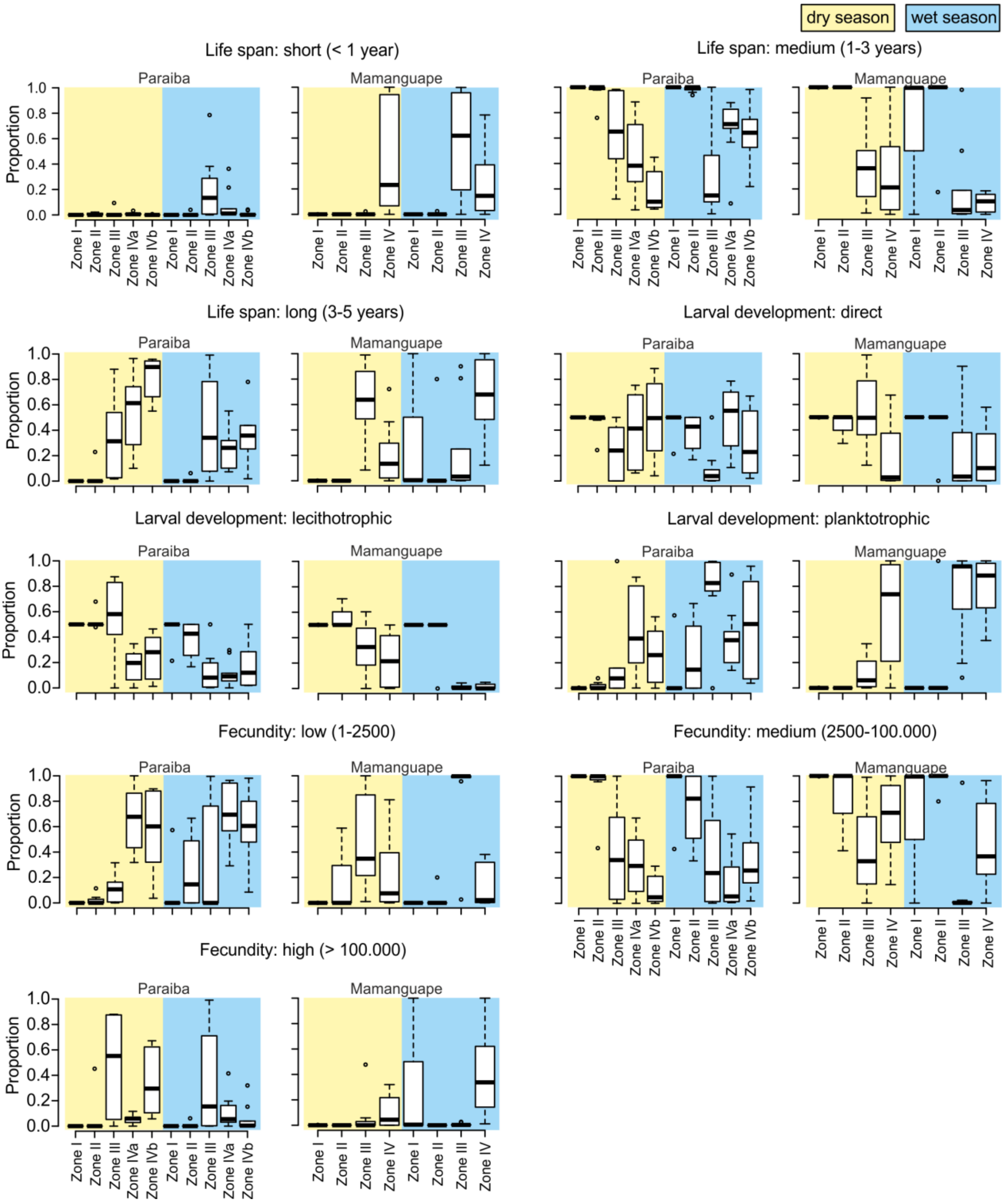
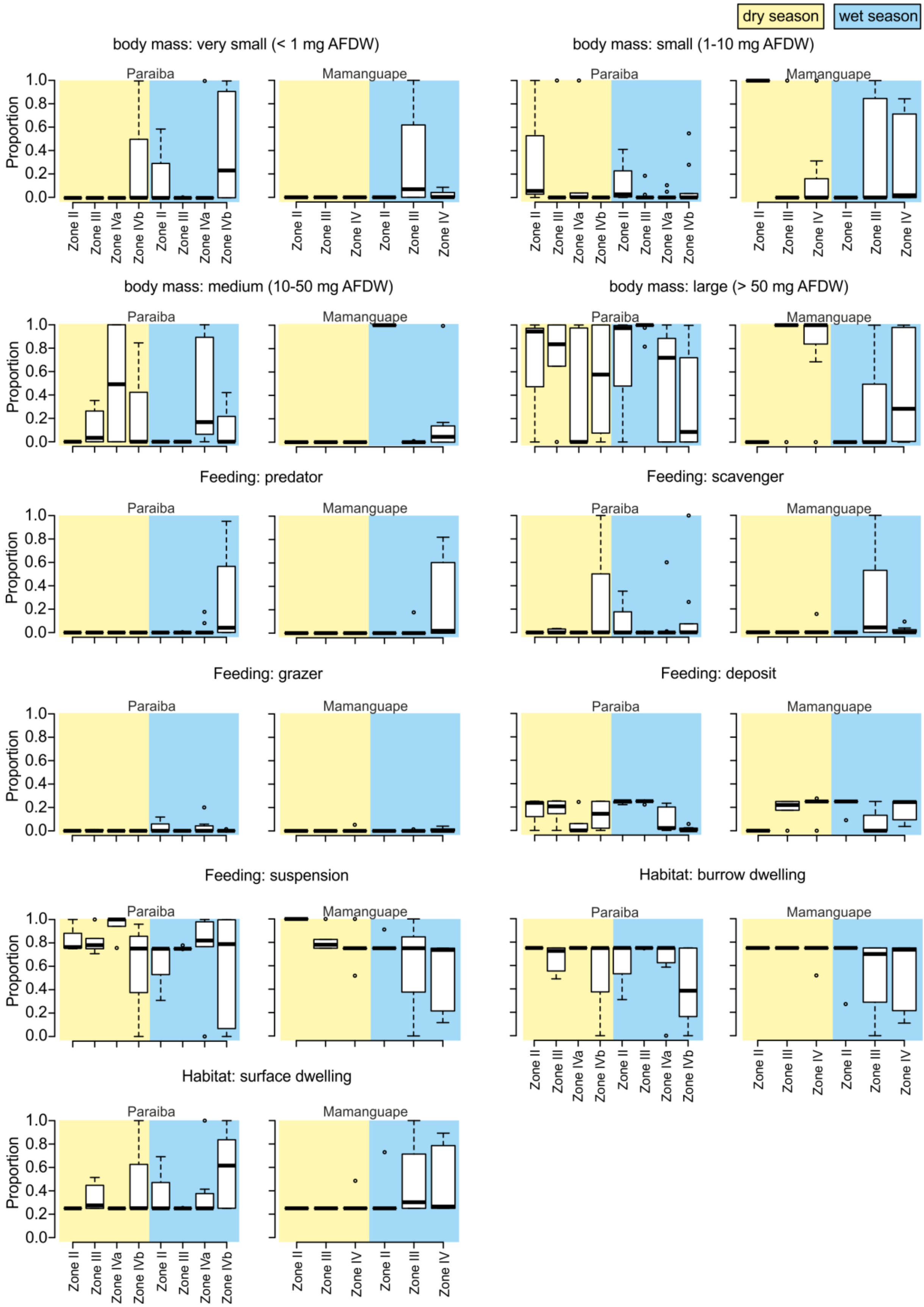


Figure A.2. Box-whisker plots showing the spatial (estuaries and zones) and seasonal (dry-wet) variation of community weighted mean (CWM) trait category values for polychaetes (sample, n=9). Mid-line of box, 50th percentile; extremities of box, 25th and 75th percentiles; maximum length of each whisker, 5th and 95th percentiles; outliers shown individually.



body mass: very small (< 1 mg AFDW)

body mass: small (1-10 mg AFDW)

body mass: medium (10-50 mg AFDW)

body mass: large (> 50 mg AFDW)

Feeding: predator

Feeding: scavenger

Feeding: grazer

Feeding: deposit

Feeding: suspension

Habitat: burrow dwelling

Habitat: surface dwelling

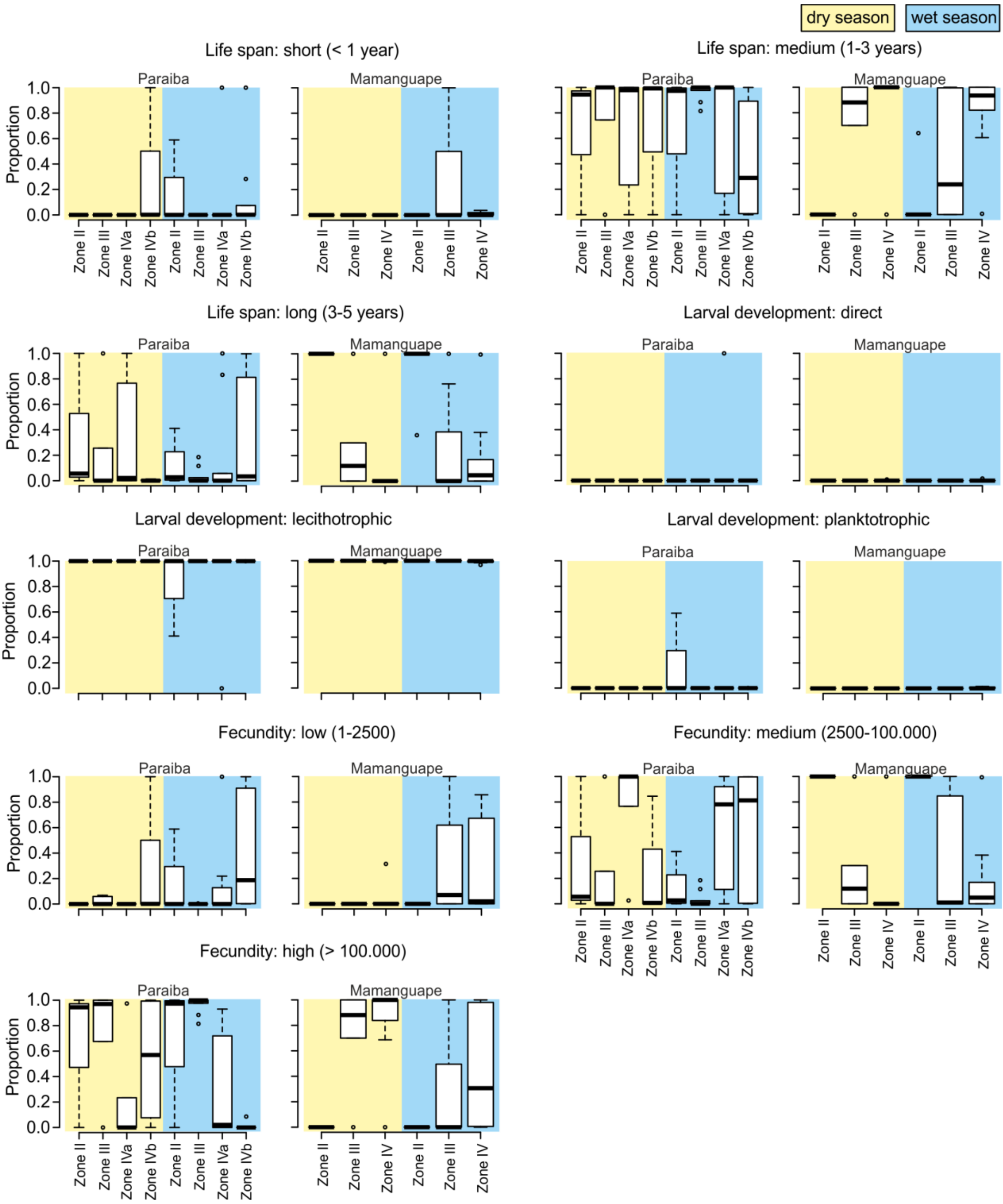


Figure A.3. Box-whisker plots showing the spatial (estuaries and zones) and seasonal (dry-wet) variation of community weighted mean (CWM) trait category values for molluscs (sample, n=9). Mid-line of box, 50th percentile; extremities of box, 25th and 75th percentiles; maximum length of each whisker, 5th and 95th percentiles; outliers shown individually.

Table A.1. Matrix taxa-by-trait for the polychaetes in the Paraíba. The 21 trait categories for the 72 different taxa are standardised so that their sum for a given taxa and a given trait equalled 1 (or 100 %). See Table 1 for the definition of the labels.

Taxa	B_VS	B_S	B_M	B_L	F_PRE	F_SCA	F_GRA	F_DEP	F_SUS	LD_DIR	LD_LEC	LD_PLA	LS_S	LS_M	LS_L	H_T	H_B	H_S	FE_L	FE_M	FE_H
Alitta	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Aricidea	0.00	1.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Armandia	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Boccardia	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.50	0.50	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00
Branchiomma	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.33	0.33	0.33	0.00	0.50	0.50	1.00	0.00	0.00	0.00	1.00	0.00
Capitella	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Ceratonereis	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Chaetacanthus	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Chaetozone	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Chone	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
Cirratulidae	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Clymenella	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.50	0.00	0.50	0.50	1.00	0.00	0.00	1.00	0.00	0.00
Cossura	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Diopatra	0.00	0.00	0.00	1.00	0.33	0.33	0.00	0.33	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Dipolydora	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00
Dorvillea	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Eteone	0.00	0.00	0.00	1.00	0.33	0.33	0.00	0.33	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Eumida	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Eunoe	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Exogone	0.00	0.00	1.00	0.00	0.25	0.25	0.25	0.25	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Glycera	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Glycinde	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00
Halosydnella	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Hemipodia	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Hermundura	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Hesionides	0.00	1.00	0.00	0.00	0.50	0.00	0.00	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.50	0.50	1.00	0.00	0.00
Heteromastus	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Heterospio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Hydroides	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00
Hypereteone	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Isolda	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.00	0.00	0.50	0.50	1.00	0.00	0.00	0.00	1.00	0.00
Kinbergonuphis	0.00	0.00	0.00	1.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Laeonereis	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Leiocapitella	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Leitoscoloplos	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Lumbrineris	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	1.00	0.00	0.00
Magelona	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00
Manayunkia	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.50	0.00	0.50	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Marphysa	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Mediomastus	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Myrianida	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.00	0.50	1.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Naineris	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00
Nereis	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Nicolea	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Notomastus	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Onuphis	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Owenia	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00
Paradoneis	0.00	1.00	0.00	0.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Paraonides	0.00	1.00	0.00	0.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Pectinaria	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00
Pettiboneia	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.33	0.33	0.33	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Pholoe	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Phyllodoce	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Poecilochaetus	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Protomystides	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Pseudobranchiomma	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
Sabellaria	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00
Scolelepis	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Scoloplos	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Sigambra	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Spio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Spiochaetopterus	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00
Spionidae	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Spiophanes	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Sternaspis	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Sthenelanella	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Streblospio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.00	0.50	0.00	1.00	0.00	1.00	0.00	0.00	0.50	0.50	0.00
Syllis	0.00	1.00	0.00	0.00	0.50	0.00	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.50	0.00	1.00	0.00
Synelmis	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Timarete	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Travisia	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Trochochaeta	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00

Table A.2. Matrix taxa-by-trait for the polychaetes in the Mamanguape. The 21 trait categories for the 64 different taxa are standardised so that their sum for a given taxa and a given trait equalled 1 (or 100 %). See Table 1 for the definition of the labels.

Taxa	B_VS	B_S	B_M	B_L	F_PRE	F_SCA	F_GRA	F_DEP	F_SUS	LD_DIR	LD_LEC	LD_PLA	LS_S	LS_M	LS_L	H_T	H_B	H_S	FE_L	FE_M	FE_H
Alitta	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Aonides	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.50	0.00	0.50	1.00	0.00	0.00
Aricidea	0.00	1.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Armandia	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Capitella	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Ceratonereis	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Chaetozone	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Chone	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
Cirratulidae	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Clymenella	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.50	0.00	0.50	0.50	1.00	0.00	0.00	1.00	0.00	0.00
Diopatra	0.00	0.00	0.00	1.00	0.33	0.33	0.00	0.33	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Dipolydora	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00
Dorvillea	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Eteone	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Eumida	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Eunoe	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Exogone	0.00	1.00	0.00	0.00	0.25	0.25	0.25	0.25	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Glycera	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Glycinde	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00
Hemipodia	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Hesionides	0.00	1.00	0.00	0.00	0.50	0.00	0.00	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.50	0.50	1.00	0.00	0.00
Heteromastus	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Heteropodarke	1.00	0.00	0.00	0.00	0.50	0.00	0.00	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Heterospio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Hydroides	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00
Hypereteone	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Inermonephtys	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Isolda	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.00	0.00	0.50	0.50	1.00	0.00	0.00	0.00	1.00	0.00
Kinbergonuphis	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Laeonereis	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Laonice	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00
Leiocapitella	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Leitoscoloplos	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Lumbrineris	0.00	0.00	1.00	0.00	0.33	0.33	0.00	0.33	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	1.00	0.00	0.00
Magelona	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00
Manayunkia	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.50	0.00	0.50	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Mediomastus	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Myrianida	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.50	0.00	0.50	1.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Nereis	0.00	1.00	0.00	0.00	0.33	0.33	0.00	0.33	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Nicolea	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Onuphis	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Paradoneis	0.00	1.00	0.00	0.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Paraonides	0.00	0.00	0.00	1.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Paraonis	1.00	0.00	0.00	0.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Paraprionospio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00
Pectinaria	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00
Pettiboneia	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.33	0.33	0.33	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Phyllodoce	1.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Prionospio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00
Protomystides	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Pseudobranchiomma	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
Sabellaria	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00
Scolelepis	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Scoloplos	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Sigambra	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Spio	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Spiochaetopterus	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.00
Spiophanes	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00
Sternaspis	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00
Syllidae	0.00	1.00	0.00	0.00	0.50	0.00	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Syllis	0.00	1.00	0.00	0.00	0.50	0.00	0.50	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.50	0.00	1.00	0.00
Synelmis	0.00	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Timarete	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Trochochaeta	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00

Table A.3. Matrix taxa-by-trait for the molluscs in the Paraíba. The 20 trait categories for the 24 different taxa are standardised so that their sum for a given taxa and a given trait equalled 1 (or 100 %). See Table 1 for the definition of the labels.

Taxa	B_VS	B_S	B_M	B_L	F_P	F_S	F_G	F_D	F_F	LD_D	LD_L	LD_P	LS_S	LS_M	LS_L	H_B	H_S	FE_L	FE_M	FE_H
Acteocina	1.00	0.00	0.00	0.00	0.00	0.50	0.33	0.17	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Anadara	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Anomalocardia	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.25	0.75	0.00	1.00	0.00	0.00	1.00	0.00	0.75	0.25	0.00	0.00	1.00
Bittiolum	1.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Brachidontes	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Caryocorbula	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.75	0.25	0.00	1.00	0.00
Cerithium	0.00	0.00	1.00	0.00	0.00	0.17	0.50	0.33	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.33	0.33	0.33
Chione	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Cyclostremiscus	1.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Cylichnella	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Leukoma	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Macoma	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.25	0.75	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Mytella	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Nassarius	0.00	0.00	1.00	0.00	0.75	0.00	0.25	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Neritina	0.00	0.00	1.00	0.00	0.00	0.50	0.17	0.33	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Olivella	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Parvanachis	1.00	0.00	0.00	0.00	0.75	0.25	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Pyramidellidae	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Scaphopoda	1.00	0.00	0.00	0.00	0.75	0.00	0.00	0.25	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00
Strigilla	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.75	0.25	0.00	1.00	0.00
Teinostoma	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Tellina	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Transennella	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Turbonilla	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00

Table A.4. Matrix taxa-by-trait for the molluscs in the Mamanguape. The 20 trait categories for the 22 different taxa are standardised so that their sum for a given taxa and a given trait equalled 1 (or 100 %). See Table 1 for the definition of the labels.

Taxa	B_VS	B_S	B_M	B_L	F_P	F_S	F_G	F_D	F_F	LD_D	LD_L	LD_P	LS_S	LS_M	LS_L	H_B	H_S	FE_L	FE_M	FE_H
Anomalocardia	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.25	0.75	0.00	1.00	0.00	0.00	1.00	0.00	0.75	0.25	0.00	0.00	1.00
Bittiolum	1.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Brachidontes	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Caecum	1.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Caryocorbula	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.75	0.25	0.00	1.00	0.00
Cerithium	1.00	0.00	0.00	0.00	0.00	0.17	0.50	0.33	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.33	0.33	0.33
Cylichnella	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Echinolittorina	0.00	1.00	0.00	0.00	0.00	0.50	0.33	0.17	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Ervilia	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.75	0.25	1.00	0.00	0.00
Eulithidium	1.00	0.00	0.00	0.00	0.00	0.20	0.60	0.20	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Haminoea	1.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Leukoma	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Macoma	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.25	0.75	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Neritina	0.00	1.00	0.00	0.00	0.00	0.50	0.17	0.33	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Olivella	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Parvanachis	1.00	0.00	0.00	0.00	0.75	0.25	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Parviturboides	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Pyramidellidae	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Schwartziella	1.00	0.00	0.00	0.00	0.00	0.60	0.20	0.20	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
Tellina	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Transennella	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.75	0.25	0.00	1.00	0.00
Turbonilla	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00