Pedro de Almeida Bica Lança Vinagre

Development of tools for the ecological quality status assessment of rocky shore benthic macroinvertebrate communities

Tese de doutoramento em Biociências, ramo de especialização em Ecologia Marinha, orientada pelo Professor Doutor João Carlos de Sousa Marques e co-orientada pelo Dr. João Miguel Magalhães Neto e pelo Dr. Ángel Borja, apresentada à Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Fevereiro 2017

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Universidade de Coimbra

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Doctoral thesis in Biosciences (scientific area of Marine Ecology) presented to the University of Coimbra, supervised by Professor Doctor João Carlos de Sousa Marques and co-supervised by Doctor João Miguel Magalhães Neto and by Doctor Ángel Borja

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DEPARTMENT OF LIFE SCIENCES
UNIVERSITY OF COIMBRA
COIMBRA | 2017

This thesis was supported by:

Fundação para a Ciência e Tecnologia (FCT)
PhD grant attributed to
Pedro de Almeida Bica Lança Vinagre
SFRH/BD/74524/2010

MARE – UC
Marine and Environmental Sciences Centre
Faculty of Sciences and Technology
University of Coimbra

IMAR – CMA
Marine and Environmental Research Centre
Department of Life Sciences
Faculty of Sciences and Technology
University of Coimbra

AZTI – Tecnalia
Marine Research Division

Thesis outline

The thesis is structured into seven sections: a general introduction section which sets the framework for the topics covered in the chapters of the thesis; four chapters that sequentially back the scope for the next chapter; a general discussion section with a contextual analysis of the topics covered and main findings; and a final section with final remarks and recommendations for future research. The chapters are based on published/submitted manuscripts, and are briefly summarized below:

In **Chapter I**, patterns of structural variation of macroinvertebrate and macroalgal communities were analysed, and was also investigated the relation of that variation with the disturbance gradients impacting on the study areas. The potential of macroinvertebrates to be used solely (in parallel to macroalgae) in monitoring activities on rocky shores was highlighted and, accordingly, the development of a benthic macroinvertebrates-based index for rocky shore quality assessment seemed feasible.

Vinagre, P.A., Pais-Costa, A.J., Gaspar, R., Borja, Á., Marques, J.C., Neto, J.M., 2016. Response of macroalgae and macroinvertebrates to anthropogenic disturbance gradients in rocky shores. Ecological Indicators 61 (Part 2), 850-864.
DOI: 10.1016/j.ecolind.2015.10.038

In **Chapter II**, the performance of several ecological indices, widely used in ecological studies (e.g., Margalef index, Shannon-Wiener index) or developed specifically for soft-bottom macroinvertebrate communities (e.g., AZTI Marine Biotic Index – AMBI), was evaluated seasonally along the disturbance gradients. This was owed to the lack of indices available based on rocky shore communities. Several macroinvertebrate indices, including abundance/diversity and taxonomic composition indices, showed good performance along the disturbance gradients, especially during summer, and using biomass data, thus becoming potential candidates to constitute a multimetric index for rocky shore quality assessment.

Vinagre, P.A., Pais-Costa, A.J., Hawkins, S.J., Borja, Á., Marques, J.C., Neto, J.M., 2016. Ability of invertebrate indices to assess ecological condition on intertidal rocky shores. Ecological Indicators 70, 255-268.

DOI: 10.1016/j.ecolind.2016.06.004

In **Chapter III**, the rocky shore macroinvertebrate communities were analysed seasonally using a different, functional trait-based, approach. Functioning was assessed using biological traits analysis (BTA) and functional diversity (FD) indices (Community-Weighted Mean trait values and Rao's Quadratic Entropy). Also, the performance of the trait-based measures was compared to that of more traditional taxonomic-based ones (richness, diversity, composition). The trait-based approach using BTA and FD indices provided useful and complementary results to the taxonomic-based ones, and showed potential to be used, together with other metrics, in monitoring activities in rocky shores.

Vinagre, P.A., Veríssimo, H., Pais-Costa, A.J., Hawkins, S.J., Borja, Á., Marques, J.C., Neto, J.M., 2017. Do structural and functional attributes show concordant responses to disturbance? Evidence from rocky shore macroinvertebrate communities. Ecological Indicators 75, 57-72.

DOI: 10.1016/j.ecolind.2016.12.023

In **Chapter IV**, a multimetric index – *Rocky shore Macroinvertebrates Assessment Tool* (*RMAT*) was derived through the combination of metrics (=indices) which better reflected the disturbance gradients, during the most consistent season (Chapter II). The model was built on the relation between the selected macroinvertebrate indices and the Marine Macroalgae Assessment Tool. The RMAT was validated using independent data. The RMAT is compliant with the WFD requirements, showing great potential to be used for quality assessments in the scope of the directive.

Vinagre, P.A., Pais-Costa, A.J., Hawkins, S.J., Borja, Á., Marques, J.C., Neto, J.M., 2017. Addressing a gap in the WFD implementation: Rocky shores assessment based on benthic macroinvertebrates.

Accepted in Ecological Indicators

This thesis should be cited as:

Vinagre, P.A., 2017. Development of tools for rocky shore macrobenthic communities' ecological quality status assessment. PhD Thesis, University of Coimbra, Coimbra, Portugal.

Contents

Acknowledgements

I would like to express my sincere gratitude to my supervisor Professor Doctor João Carlos de Sousa Marques for allowing me to dedicate to this area which fascinates me since I was a child. Thank you for sharing your immense scientific and personal knowledge, for all suggestions, critiques, motivation and friendship.

I vehemently thank my super co-supervisor Doctor João Magalhães Neto, who was too a mentor throughout this study. Thank you for your countless advices concerning not only scientific but also personal issues, and all the energy you have inputted to the fulfilment of this work.

I am very grateful to my co-supervisor Doctor Ángel Borja for the shared expertise on marine ecosystems, and for all of your critiques and suggestions which contributed to the improvement of the manuscripts, and the study as a whole.

I would like to give special thanks to the work collaborator Professor Doctor Stephen John Hawkins. Thank you for sharing your knowledge, namely with regard to the rocky shore habitat and the rocky intertidal assemblages, and for all your comments which allowed improving this thesis.

I thank all colleagues who have directly or indirectly contributed to this study. Thank you Alexandra Guerra, Ana Carriço, Alexandra Baeta, Carla Garcia, Cristina Docal, Dimitri Vilhena, 'Dona' Teresa, Filipa Bessa, Filipe Ceia, Gabi, João Franco, Luisa Marques, Pablo Barría, Pieter van der Linden, Rute Pinto, Sónia Serra and Tiago Verdelhos for any help, advice or motivation given during the last years. Also, I apologise to the ones who woke up so very early for field sampling. Thanks Joana Cabral Oliveira for your

suggestions and motivation. I also thank any colleague I forgot to mention (☺), and to the trainees who have helped in the laboratory.

Special thanks to Helena Veríssimo and Rui Gaspar for their contribution to this work, and for all their suggestions and general help during the last years.

Thank you to my best friends Hugo and João for just being yourselves. Although I was constantly away, you were there always when I needed. I thank also to my colleagues and friends from prior to University which, to present-day, still remember me despite my long-term absence.

I am thankful to Fundação para a Ciência e Tecnologia (FCT) for the financial support through the grant SFRH/BD/74524/2010. I also thank the logistic support given by MARE – Marine and Environmental Sciences Centre, IMAR – Institute of Marine Research and AZTI-Tecnalia.

Finally, I would like to thank my family. I am very thankful to my wife (to be) Juliana, for making me a better person each day for the last 12 years. Thank you for your patience, advices and help. I thank all of my family members, but I must give special thanks to my sister Teresa for being my ‘little mother’, you have taught me so much and given me so much love I can never repay. And thank you to my little star Runa, for making dark days much brighter. I also give special thanks to my cousin Ana Osório for motivating me throughout this journey, and to my cousin Luis Osório for taking care of my aquarium while I was absent and for letting me shower when my heater wasn’t working.

The gratitude I have to my parents can hardly be described by words. I am immeasurably grateful for the scholar education you were able to provide, for instructing me to be an honest and humble person, for your friendship and love, for letting me pursuit knowledge and make my own choices.

Dedico esta tese à minha mãe. Sei que era seu desejo estar aqui presente.

Summary

The aim of the research work presented in this thesis was to provide further knowledge of the effects of anthropogenic disturbance (organic enrichment) on intertidal rocky shore macroinvertebrate communities.

The main objective was to address a gap in the Water Framework Directive (WFD) implementation with regard to rocky shores, namely with the development and validation of a multimetric index for rocky shore quality assessments based exclusively on the benthic macroinvertebrates quality element.

First was analysed the change in structure of benthic macroinvertebrate communities as a response to anthropogenic disturbance (organic enrichment) gradients, and compared that with the response provided by the macroalgal component. Considering that the macroinvertebrate communities were responding to the disturbance gradients, the performance of several ecological indices (abundance, diversity and taxonomic composition indices) based on the macroinvertebrate communities was evaluated along the disturbance gradients. After, a complementary approach was followed, using functional trait-based methods (biological traits analysis and functional diversity indices), and comparing that approach with the more traditionally taxonomic one. Ultimately, the multimetric index proposed – *Rocky shore Macroinvertebrates Assessment Tool* (*RMAT*) – was developed integrating the macroinvertebrate metrics which showed the best efficiency (performance and consistency) along the disturbance gradients, and was validated using independent data.

The assessment tool proposed in this thesis is compliant with the WFD concerning the evaluation of coastal waters' ecological quality. Therefore, it presents great potential to be used in the assessment of rocky shores in the scope of the Directive.

Keywords: Ecological index; marine intertidal; structural indicators; functional indicators; anthropogenic disturbance; Water Framework Directive.

Resumo

A investigação feita no âmbito desta tese teve a intenção de fornecer maior conhecimento sobre efeitos de perturbação antropogénica (enriquecimento orgânico) em comunidades de macroinvertebrados de intertidal rochoso.

O objetivo principal foi colmatar uma falha na implementação da Diretiva Quadro de Água (DQA) respeitante à costa rochosa, nomeadamente com o desenvolvimento e validação de um índice multimétrico para a avaliação de qualidade de costa rochosa, baseado exclusivamente no elemento de qualidade macroinvertebrados bentónicos,

Primeiro, foram analisadas alterações na estrutura das comunidades de macroinvertebrados bentónicos em resposta a gradientes de perturbação antropogénica (enriquecimento orgânico), e comparadas com alterações identificadas nas macroalgas. Devido à boa resposta mostrada pelos macroinvertebrados à perturbação, foi avaliada a performance de vários índices ecológicos (baseados em abundância, diversidade e composição taxonómica), baseados em macroinvertebrados, ao longo dos gradientes de perturbação. De seguida foi utilizada uma abordagem complementar, através de métodos funcionais baseados em características dos organismos (*biological traits analysis* e índices de diversidade funcional), comparando esses métodos com índices baseados em diversidade/composição. No final, foi desenvolvido um índice multimétrico – *Rocky shore Macroinvertebrates Assessment Tool* (*RMAT*) – pela integração das métricas (baseadas exclusivamente em macroinvertebrados) que mostraram melhor eficiência (*performance* e consistência) ao longo dos gradientes de perturbação, sendo o RMAT também validado com dados independentes.

A ferramenta de avaliação aqui proposta cumpre com os requisitos da DQA para a avaliação qualidade de sistemas costeiros, e apresenta grande potencial para a avaliação de costa rochosa no âmbito da Diretiva.

Palavras-chave: Índice ecológico; intertidal marinho; indicadores estruturais; indicadores funcionais; perturbação antropogénica; Diretiva Quadro de Água.

General introduction

1. The rocky shores

Rocky shores are an important system constituting over 80% of the coastline worldwide (Emery and Kuhn, 1982; Granja, 2004). These habitats depend on local geology, and may range from steep cliffs to gently shelving platforms, from uniform platforms to highly dissected irregular masses, or mainly constituted by boulders (Lewis, 1977) (Fig. 1). Most often, rocky shores are crossed with cracks, crevices, gullies and pools which provide multiple microhabitats, with inherent limitations and advantages, which support great biodiversity (Raffaelli and Hawkins, 1999). In common with other coastal habitats, they offer valuable ecosystem services, namely provisioning (e.g. seaweed and shellfish collection and aquaculture, fish nursery grounds), regulating (e.g. water quality by biofiltration, sea defence), and cultural services (e.g., aesthetics leading to amenity use and tourism) (e.g., Liquete et al., 2013; Galparsoro et al., 2014).

The structural patterns of rocky shore communities have been long-studied. Widely accepted ‘universal’ zonation schemes for rocky shores (Stephenson and Stephenson, 1949, 1972; Lewis, 1961, 1964; Pérès and Picard, 1964), despite differences in terminology given, differentiate three main zones: the supralittoral zone, the littoral zone (intertidal zone), and the sublittoral zone (including the infralittoral and circalittoral zones). The supralittoral zone, placed up in the shore, is permanently exposed but subject to wave splash and marine water spray, and is characterized by the presence of encrusting lichens, cyanobacteria, small littorinid gastropods and few isopods. The next one, the littoral zone is constituted by three main intertidal areas: a high shore zone – ‘supralittoral fringe’ or ‘littoral fringe’, is placed between daily high tide and high water spring levels; a mid-shore zone – ‘midlittoral zone’ or ‘eulittoral zone’ (= true intertidal), restrained by intense tidal influence (between daily high and low tide levels); and a lower shore zone in the intertidal – ‘infralittoral fringe’ or ‘sublittoral fringe’, exposed only during low water spring tides. The intertidal zone is dominated downwards by filter-feeders such as barnacles and mussels associated with a great number of algal, gastropod, polychaete and crustacean species, among many other groups. In the infralittoral fringe various associations of turf-forming red algae and large brown kelps emerge slightly during extremely low water spring tides, which have great expression on the next lower zone, the infralittoral zone. This zone is permanently submerged, and where kelps and turf-forming macroalgae experience greatest development (Fig. 2). Below the infralittoral zone is the circalittoral zone, where macroalgae loose expression and dominance is on sessile organisms, placed under the euphotic boundary.

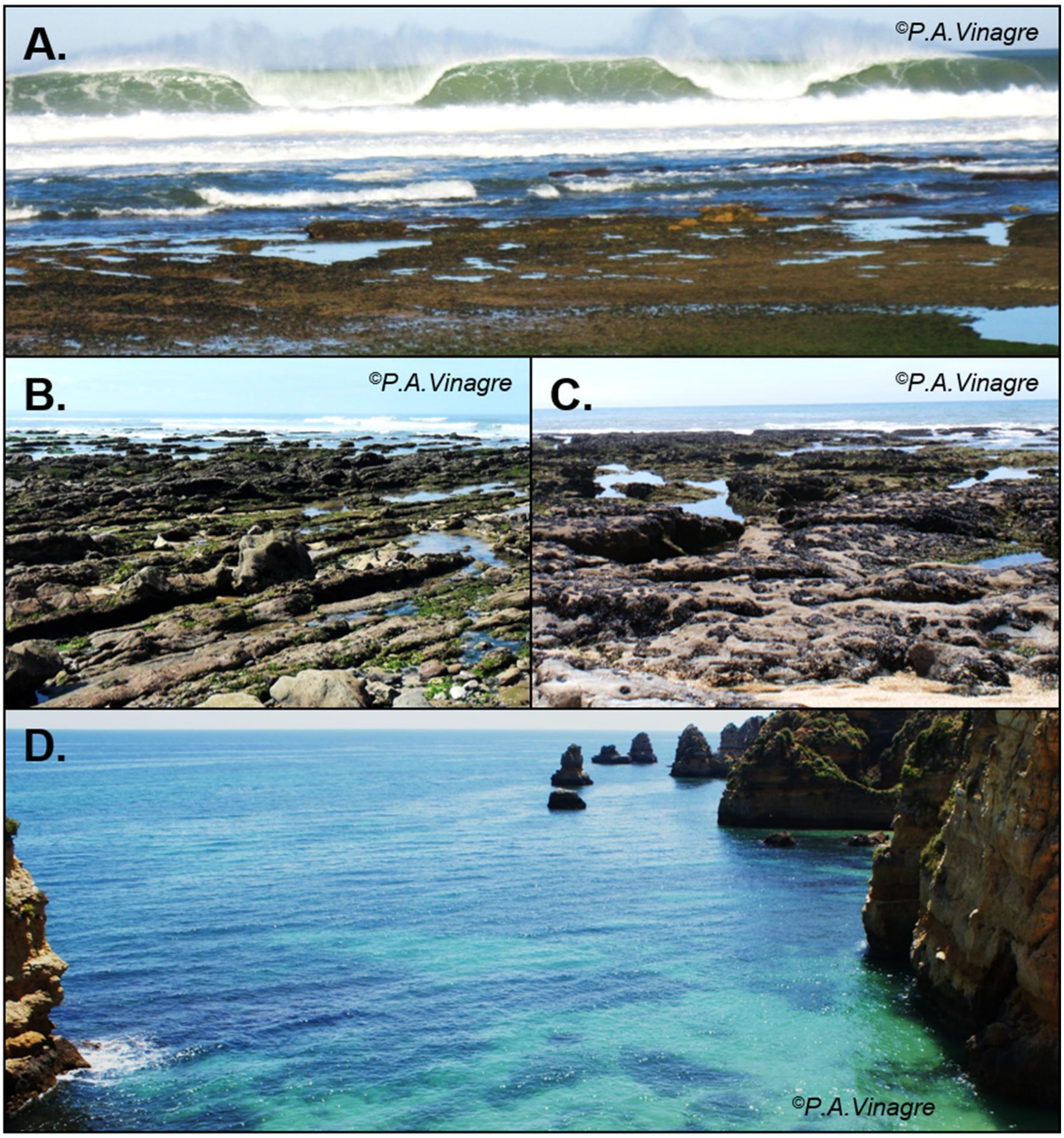


Figure I. *Different rocky shore types: gently sloping platforms with a more regular (A.) or heterogeneous (B. and C.) profile, or constituted mainly by cliffs (D.).*

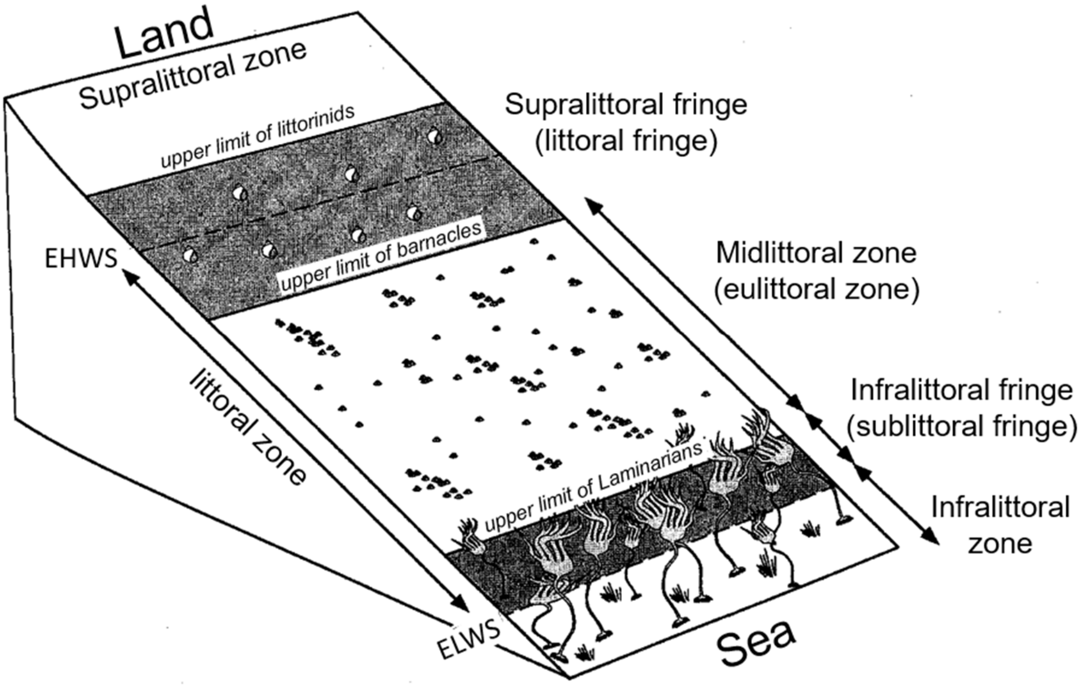


Figure II. *Example of distribution of organisms relative to sea water level at extreme high and low waters of spring tides (EHWS and ELWS, respectively) (from Raffaelli and Hawkins, 1999).*

1.1. Factors influencing distribution of organisms along the intertidal

The intertidal zone makes the transition from fully terrestrial to fully marine conditions, being subject to a highly variable environment. The upper and lower tidal limits are dependent on factors such as wave exposure (e.g., exposed vs sheltered) and the shores' slope (e.g., even vs inclined), profile (e.g., smooth vs heterogeneous) and orientation (e.g., shaded vs sunlit). For example, smooth rocky substrata with even slope present much clearer zonation patterns than more heterogeneous substrata (e.g., of boulders, pools or crevices).

The tidal regime is one of the most important environmental factors in determining the distribution of organisms across the rocky intertidal (Raffaelli and Hawkins, 1999). The physical alternating flood and exposure to air create the conditions of emersion and exposure to light, heat and rain that intertidal organisms are subject to at different positions on any shore. From low water to the spray zone the environmental gradient is of increasingly harsh physical conditions, and because different organisms have different tolerance levels to stress they replace each other along the intertidal gradient, usually being observed bands of organisms – vertical zonation – with abundance and diversity decreasing in that direction (Stephenson and Stephenson, 1972).

Wave exposure is also a determinant physical factor, because wave action may increase the extension of the intertidal zone with marine water extending higher on the shore, usually favouring sessile filter-feeders such as barnacles and mussels which profit from increased available space for colonization (e.g. space created by dislodgement of organisms such as macroalgae) and suspended food particles. Conversely, on sheltered shores the diminished wave action may allow for higher siltation of sand particles, causing erosion and restricting the supply of oxygen and dissolved nutrients for the organisms, and reducing their larval supply (Little and Kitching, 1996; Raffaelli and Hawkins, 1999).

To endure in such harsh environments, the rocky intertidal organisms depend on several biological interactions (e.g. commensalism, mutualism, predator/prey and plant/herbivore relationships) between fauna and flora (Connell, 1972; Stephenson and Stephenson, 1972; Menge et al, 1997; Schiel, 2004). On rocky shores, space (or the lack of it), being a common resource for all intertidal organisms (e.g., offering substratum for living, protection and food), is usually a factor determining the complexity of biotic interactions in the rocky intertidal (Dayton, 1971).

Competition, predation and grazing are important in regulating the distribution of organisms along the intertidal, especially at lower sections on the shore (Raffaelli and Hawkins, 1999). Generally, predation is the dominant interaction structuring communities in less variable conditions (at low shore levels and on sheltered shores). As environments become harsher, predation becomes less important and competition is a major structuring force (at higher shore levels and on more exposed shores). In even harsher environment, competition becomes less important and physical factors assume major importance (at high shore and on very exposed shores) (Raffaelli and Hawkins, 1999). All forms of biological interaction act on the communities' structure. They may cause exclusion of species from certain areas or, contrarily, promote increase in diversity. It will depend on how diverse, and therefore how stable, the communities present.

1.2. Benthic invertebrates as indicator of anthropogenic disturbance

Natural physical disturbance is a very important factor affecting the structure and dynamics of coastal and marine communities, especially of rocky shores. However, as result of increasing human population and coastal development, coastal and marine ecosystems are more frequently impacted from habitat modification or destruction (e.g., land claim, dredging), pollution (e.g., industrial and/or domestic discharges), overexploitation of biological resources and species introduction (Barnes and Hughes, 1988; Carter, 1988). The increased tourist activity also represents a significant source of impact (e.g., increased trampling) to rocky shore communities (Murray et al., 1999; Schiel and Taylor, 1999).

Within the coastal ecosystems, rocky intertidal areas worldwide are subject to considerable and increasing anthropogenic impacts (Schiel and Taylor, 1999) with origin either in land or at sea, more frequently than any other marine system (Schramm, 1991). The effects of anthropogenic impact on rocky intertidal communities are now better understood, both coming from acute (e.g., oil spills: Southward and Southward, 1978; Hawkins and Southward, 1992) and chronic (e.g., harvesting: Addessi, 1994; runoff pollution: Kinsella and Crowe, 2015; sewage pollution: Littler and Murray, 1975; Bishop et al., 2002; O’Connor, 2013; Zubikarai et al., 2014; Tributyl tin pollution from anti-fouling paints: Bryan et al 1986) anthropogenic impacts (reviews in Hill et. al, 1998; Crowe et al., 2000; Thompson et al., 2002; Mearns et al., 2014).

Benthic invertebrates may persist over seasonal time scales, integrating the effects of long-term exposure to natural and anthropogenic disturbances. Therefore, they may reflect not only conditions at the time of sampling but also conditions to which the community was previously exposed (Reish, 1987) and are considered good indicators of pollution level in a given area (e.g., Pearson and Rosenberg, 1978; Elliott, 1994; Diaz et al., 2004; Marques et al., 2009; Pinto et al., 2009; Borja et al., 2015). Rocky intertidal benthic invertebrates are especially suitable for monitoring and for experimental research. They are mainly sessile or have low mobility, and are usually conspicuous, hence, being generally easy to collect. Also, they can be analysed *in situ* using non-destructive techniques (e.g., with abundance estimated as percentage cover), with minimal disturbance to the communities.

Those features, after the already long interest shown by scientists, also received attention from policy authorities for the potential role that benthic communities (including rocky shore ones) may have in the monitoring of marine environments. The scientific knowledge on those communities constituted a great decision support, and legislation to protect the coastal and marine environments has been progressively implemented worldwide in recent years [e.g., European Water Framework Directive (WFD, 2000) and Marine Strategy Framework Directive (MSFD, 2008); Australia Oceans Policy (Commonwealth of Australia, 1998a, b); South Africa Integrated Coastal Management Act (South Africa Government, 2013); US Clean Water Act (US Environmental Protection Agency, 2002) and Oceans Act (US Congress, 2002); People's Republic of China laws on Water (1988/01/21) and Environmental Protection (1989/12/26)].

In the EU, the WFD and MSFD are key directives addressing the coastal and marine ecosystems. Many assessment tools have been developed in the scope of these directives (Birk et al., 2012), aiming to synthetize the complex response of the biological communities to anthropogenic pressure. Among those tools are multimetric indices which combine several single metrics providing complementary information on a system (Salas et al., 2006; Marques et al., 2009). Such indices usually report changes in the communities (e.g., recovery, degradation) in a single value (e.g., ranging from 0-1). This allows the general public, especially the managers and policy makers, to better understand if measures taken are being effective (e.g., environmental conditions have been ameliorating/deteriorating).

1.3. Portuguese rocky shores as a case study

The Portuguese coast is quite appropriate to study biogeographical patterns of species distribution as well as the effects of pollution and other impacts (e.g., climate change). Namely, the coastline presents a contact region between cold- and warm-water species, where both northern and southern boundaries of several organisms can be found (Ardré, 1970, 1971; Santos, 2000; Boaventura et al., 2002; Pereira et al., 2006).

Along the Portuguese coastline, rocky shores mostly present the ‘universal’ zonation patterns mentioned earlier (Lewis, 1964; Raffaelli and Hawkins, 1999; Boaventura et al., 2002; Cabral-Oliveira, 2013). At the intertidal area (=eulittoral, midlittoral) dominant species are mainly sessile filter feeders such as barnacles (e.g., *Chthamalus montagui*) and mussels (e.g., *Mytilus galloprovincialis*), which are gradually replaced towards the sea by many other animal and algal species. This pattern is mostly owed to strong exposure to Atlantic swell. Great part of the coastline is included in the ‘Moderately Exposed’ (4200 km^2) and ‘Exposed’ (3200 km^2) typologies, and only a small extension (1000 km^2) classified into the ‘Sheltered’ typology (Bettencourt et al., 2004). Main differences from the zonation patterns referred could be related to Portugal geographical position which, as mentioned above, sets the transition between cold- and warm-water organisms.

Studies along the Portuguese coastline (including both continental and island coasts) are vast and not recent (e.g., MacAndrew, 1851; Hidalgo, 1870, 1917; Nobre, 1931, 1936, 1938; Cúmano, 1939; Palminha, 1951; Kensler, 1965; Palminha 1971; Andrade and Cancela da Fonseca, 1979; Saldanha, 1979; Monteiro Marques et al., 1982; Santos and Melo, 1984). Despite the rocky shores have historically received less attention comparing to the soft-sediment ecosystems, intertidal communities from Portuguese rocky shores are now quite well characterised, and their ecology, structure, biogeography, and response to anthropogenic pollution and climate change are widely understood (e.g., Boaventura, 2000; Santos, 2000; Lima, 2007; Wallenstein, 2011; Cabral-Oliveira, 2013; Vale 2015).

2. Water Framework Directive (WFD)

Among the EU legislation (e.g., Integrated Coastal Zone Management – ICZM, 2002; Integrated Maritime Policy – IMP, 2007; Common Fisheries Policy – CFP, 2013), the Water Framework Directive (WFD, 2000) (encompassing the rocky shores under the coastal waters category) and the Marine Strategy Framework Directive (MSFD, 2008) (which may overlap with the WFD in the rocky shore assessment) constitute key directives addressing the coastal and marine environments. Both especially recognize the great environmental, economic, social, cultural and recreational importance of those aquatic components.

The WFD was implemented aiming to prevent deterioration or to improve the quality of water resources (namely in what concerns reduction of water pollution), and also to promote a sustainable and fair cost of water to use (in terms of water quality and quantity for human consumption) (WFD, 2000). Its main objective was for Member States to achieve and maintain a 'good' ecological status for all waters (including groundwater, inland surface water, and coastal and transitional waters) by 2015 (WFD, 2000). The process was new and not easy to establish, but it should be able to ensure a reliable and comparable assessment between Member States along EU territory.

For its implementation, the WFD requires Member States to develop/adopt assessment tools, able to report in a common scale, which were also able to translate similar environmental degradation levels into the same quality classes. For coastal waters assessment (and for other surface water categories), the WFD requires several steps, including:

a) Definition of water typologies: Member States should identify the location and boundaries of water bodies and classify them as rivers, lakes, transitional waters or coastal waters, or as artificial or heavily modified surface water bodies (classification systems in Annex II of the WFD);

b) Establishment of type-specific reference conditions: Type-specific quality conditions must be established representing the values of the biological quality elements (BQE), and supporting (hydromorphological and physicochemical) quality elements (Annex V of the WFD), suffering from any, or very minor, disturbance from human activities (reference conditions) (Borja et al., 2012). The reference conditions may be either spatially based (from a sufficient number of sites of ‘high’ status to provide a sufficient level of confidence) or based on modelling (either predictive models or hindcasting methods), or may be derived using a combination of these methods. Member States can also recourse to expert judgement (required with all the above methods), to establish such conditions, something which has been widely adopted (e.g., Muxika et al., 2007; Teixeira et al., 2010) owing to the global inexistence of sufficient or adequate historical data;

c) Identification of pressures: Member States must gather information on the type and magnitude of the significant anthropogenic pressures to which the surface water bodies in each river basin district are liable to be subject (e.g., estimation and identification of significant point source and diffusion source pollution, estimation of land use patterns) (Heiskanen and Solimini, 2005);

d) Assessment of impacts: Member States should carry out an assessment of the susceptibility of the surface water status of bodies to the pressures identified, and evaluate the likelihood of failing to meet the objectives. The assessment of water quality is commonly carried out with use of mathematical/statistical methods (such as the multimetric indices), which numerically evaluate and classify water quality depending on reference conditions and boundaries setting (especially between the classes of high and good status, and of good and moderate status). Each Member State should develop methods that produce similar results wherever they are implemented, i.e., good status is consistent with the WFD requirements and comparable among countries. At the moment, many methods have been developed to assess the status (Birk et al., 2012). In the Portuguese context, two assessment tools are available for the quality evaluation of coastal waters and were already intercalibrated (Carletti and Heiskanen, 2009; European Commission, 2013): (i) the Benthic Assessment Tool (Teixeira et al., 2009) based on soft-bottom macroinvertebrate communities, and (ii) the Marine Macroalgae Assessment Tool (Neto et al., 2012) based on rocky shore macroalgae.

Depending on the outcome of the assessment, it may be necessary to create mitigation measures plans to ensure the ecological quality is maintained, or improved (if the good status is not achieved).

2.1. Coastal waters – Rocky shore assessment

For the WFD coastal waters assessment the phytoplankton, other aquatic flora and benthic invertebrates are the required BQE that Member States should assess. More than having assessment tools, Member States must reach an agreement on quality standards for monitoring and analysis (e.g., set reference conditions and establish boundaries between quality classes), so that the different methods produce comparable classifications for each BQE (Birk et al., 2013). This is a core achievement to ensure transparency and confidence on the assessment results.

Several biological elements have been already intercalibrated among Member States during the first and second intercalibration phases (Carletti and Heiskanen, 2009; European Commission, 2013; Poikane et al., 2014). Benthic macroinvertebrates was one of those BQE; however, intercalibration exercises have been undertaken only for the soft sediment habitat, while for hard substratum (i.e., rocky shores) that was not the case. This is because, despite considering macroalgae and benthic macroinvertebrates as being the most suitable BQEs for rocky shore assessment, the indices available and compliant with the WFD were exclusively (Ballesteros et al., 2007; Juanes et al., 2008; Neto et al., 2012; Ar Gall and Le Duff, 2014) or in part (Hiscock et al., 2005; Díez et al., 2012; O'Connor, 2013) based on the macroalgae. And, among the latter, the WFD compliant index (Díez et al., 2012) was intercalibrated for the macroalgae BQE only (European Commission, 2013).

Therefore, a gap in the WFD implementation could be identified for benthic macroinvertebrates as assessing BQE on rocky shores.

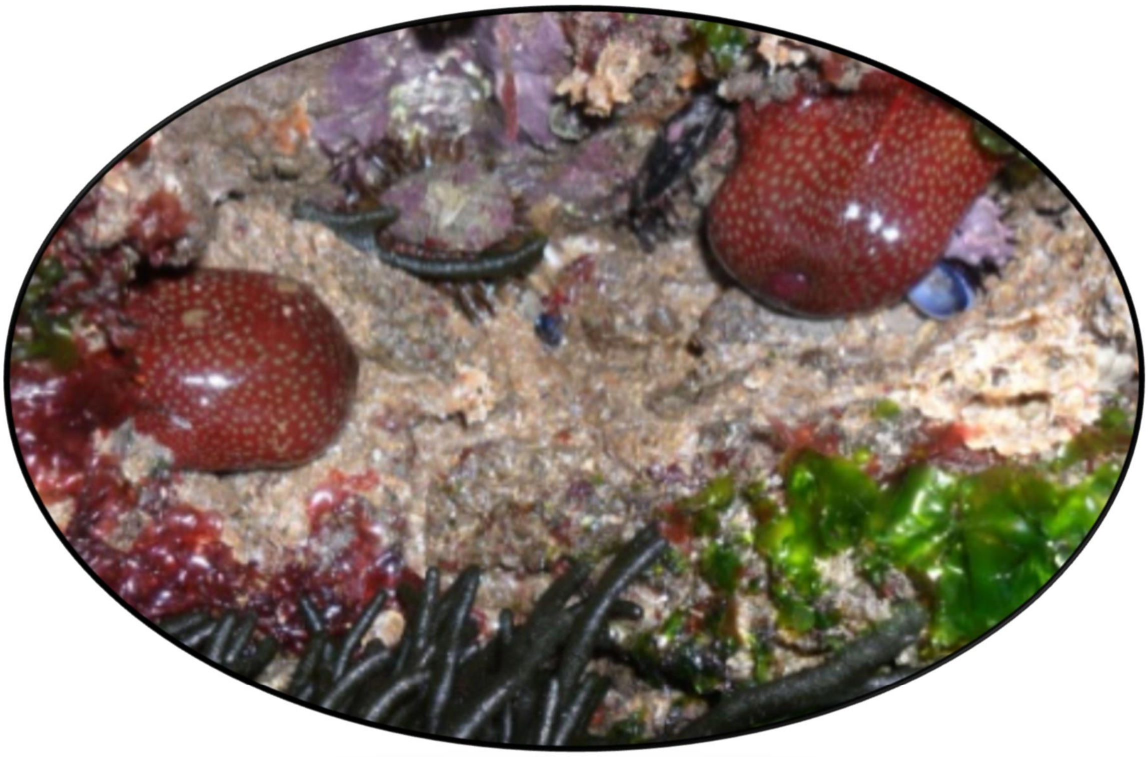
3. General objective

The main objective (=hypothesis) of this thesis was to propose a multimetric ecological index for rocky shore quality assessment, based exclusively on the benthic macroinvertebrates quality element, thereby fulfilling the gap in the WFD implementation with regard to rocky shore assessment. Several steps were carried out to accomplish that objective, namely:

- Assessment of the response of rocky shore macroinvertebrate communities' parameters (e.g., structure, abundance, number of species) along anthropogenic disturbance (organic enrichment) gradients;
- Evaluation of the performance of ecological indicators based on the macroinvertebrate communities (structural and functional indices) along the disturbance gradients;
- Integration of the most efficient indices into a multimetric index based exclusively on the benthic macroinvertebrates biological quality element, and validation of the index using independent data.

Chapter I

Response of macroalgae and macroinvertebrates to anthropogenic disturbance gradients in rocky shores



Vinagre, P.A., Pais-Costa, A.J., Gaspar, R., Borja, Á., Marques, J.C., Neto, J.M., 2016. Response of macroalgae and macroinvertebrates to anthropogenic disturbance gradients in rocky shores. Ecological Indicators 61 (Part 2), 850-864.

DOI: 10.1016/j.ecolind.2015.10.038

Abstract

The compliance of macroalgal and macroinvertebrate assemblages to anthropogenic disturbance gradients (e.g., nutrient enrichment) was investigated at intertidal rocky shores. Macroalgae and macroinvertebrates presented parallel behaviour, both showing shifts in the communities' structural variation along the gradients, in which a higher number of opportunistic species (and higher abundances) were found in more stressful sites (close to the disturbance source), in contrast to less disturbed sites (far from the disturbance source), which showed higher presence of more sensitive species (and higher abundance of several of them).

The macroinvertebrate abundance and taxonomic composition, which are parameters required by the Water Framework Directive (WFD) to be included in tools for the ecological quality status assessment, responded to the disturbance gradient. Results suggest that the macroinvertebrate biological element might be considered an indicator of disturbance in intertidal rocky shores as good as the macroalgae, and therefore the development of a specific methodology based solely on benthic macroinvertebrates of rocky shores, presently a gap in the ecological quality status assessment for the WFD, seems feasible.

Keywords: Marine; Intertidal; Nutrient enrichment; Biological quality elements; Comparative response; Opportunist/sensitive taxa.

I.1. Introduction

Rocky shores, similarly to many coastal systems, have been historically exposed to human pressures (e.g. wastewater discharges) (Mearns et al., 2014). Despite that, rocky shores are considered of great importance in marine ecosystems, providing valuable ecosystem services in terms of biological diversity, contribution to primary productivity, fisheries and tourism (e.g., Liquete et al., 2013; Galparsoro et al., 2014).

In the last decade, the interest on coastal marine biological communities has increased, mainly concerning the classification of water bodies' quality status, which is an essential requirement in terms of the implementation of directives such as the Water Framework Directive (WFD, Directive 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/ 56/EC) in Europe, or the Clean Water Act (CWA, 2002) in the United States, among others (Borja et al., 2008). The use of benthic communities in marine pollution assessments is based on the concept that they reflect not only conditions at the time of sampling but also conditions to which the community was previously exposed (Reish, 1987). Due to their permanence over seasonal time scales, benthic communities may integrate the effects of long-term exposure to natural and anthropogenic disturbances (Borowitzka, 1972). Moreover, there is extensive literature about their taxonomy, ecology and distribution, and about responses to disturbance (e.g. Boaventura et al., 2002; Schiel, 2004; O'Hara et al., 2010; O'Connor, 2013; Cabral-Oliveira et al., 2014a, 2014b).

In coastal waters (CW), where are included the rocky shores, the macroinvertebrates and macroalgae are some of the biological elements encompassed in such quality assessments. However, despite of the readiness of information on rocky shore intertidal communities, the use of both elements in assessment studies is many times hampered by the different methodologies usually applied to each element (e.g. sampling technique, data processing). Consequently, many studies have generally been based in visual census, or have been focused on relations between particular groups of macroinvertebrates and/or macroalgae (e.g. Bishop et al., 2002; Terlizzi et al., 2005;

Pereira et al., 2006; De-la-Ossa-Carretero et al., 2010; Atalah and Crowe, 2012), and not using quantitative data and encompassing entire communities, as in the present work. Furthermore, despite the many historical papers analysing such communities (e.g., Littler and Murray, 1975; López Gappa et al., 1993; Archambault et al., 2001), studies on the structural variation of intertidal rocky shore macroalgae and macroinvertebrate communities, simultaneously, and inside the same disturbance gradient are not common.

The present work aimed to analyse the shifts shown by macroalgal and macroinvertebrate communities exposed to the same anthropogenic disturbance gradient (e.g. small nutrient enriched water discharges). More specifically: a) to verify if the structural variation of macroinvertebrate and macroalgal communities follows similar patterns, and b) to check if the variation in structure of each biological element is related to the disturbance level affecting the study areas.

I.2. Material and methods

I.2.1. Study area

Two rocky shores located in the western Portuguese coast, Buarcos and Matadouro (Fig. I.1A), were studied in this work. They are included in the Exposed (Buarcos) and Moderately Exposed (Matadouro) Atlantic Coast typologies (TICOR project, Bettencourt et al., 2004; available at http://www.ecowin.org/ticor/). Along this coast the prevailing current direction is from West-Northwest (Portuguese Coastal Current) with occurrences from Southwest (Portuguese Coastal Counter-Current) (Bettencourt et al., 2004). In Buarcos area, the Boa Viagem hill may lead to a current turnover from North-South to South-North orientation (Pais-Costa, 2011; personal observation). The most frequent wave period and wave height are in the range of 8-12 s and of 1-3 m, respectively. Tide is semidiurnal and the extreme spring tide ranges from 3.5-4 m (Boaventura et al., 2002, Bettencourt et al., 2004). Surface sea temperature ranges between 13-15 ºC during winter and 20-22 °C during summer, and surface salinity varies between 35 and 36 (Boaventura et al., 2002).

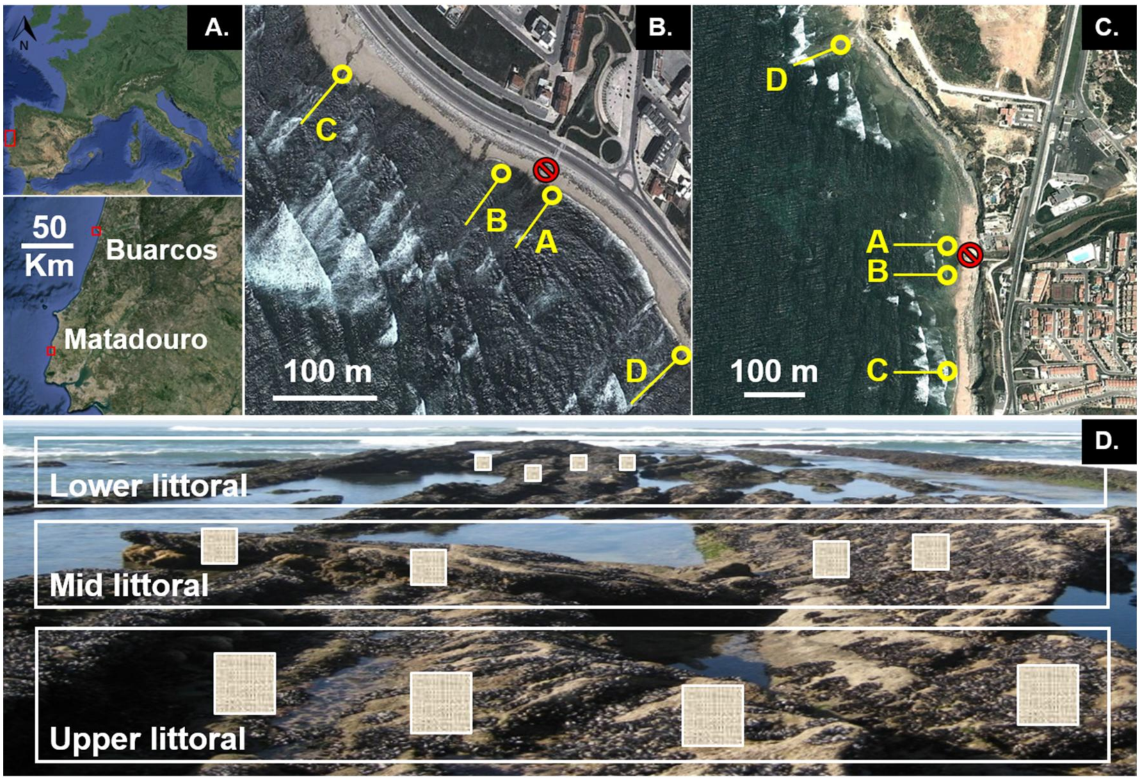


Figure I.1. *Study sites location: A. Europe and Portugal. B. Buarcos. C. Matadouro. D. Sampling design. In both Buarcos and Matadouro sites (A-D) are placed gradually away from the source of pollution (SOP;* 🚫 *sign), with site D positioned as control.*

Both rocky shores are situated in narrow sandy shores and limited landward by urban infrastructures, namely coastline protection adjacent to seaside avenues. Buarcos is approximately 150 km north to Matadouro and is more exposed to the Atlantic influence. There, the rocky surface is composed of slightly sloped shelving platforms. In Matadouro, wave energy is more attenuated, and the rocky surface is less irregular and more horizontal along the platforms.

The sampling areas at both shores were selected due to the presence of a point source of pollution (SOP) discharging almost directly on the upper intertidal zone (Fig. I.2B, C). At each shore, although with a very low run-off, the discharge is continuous throughout the year, crossing urban centres (Buarcos – Figueira da Foz, and Matadouro

– Mafra, with 62,125 and 76,685 residing inhabitants in 2011, respectively) and agricultural land before reaching the shore.

I.2.2. Sampling design

Four sites were selected at Buarcos and Matadouro to characterize the disturbance gradients, sites A, B and C distancing gradually from the SOP (about 30-40 m, 50-60 m and 250-300 m, respectively), and site D situated opposite to the prevailing coastal current direction, used as control (300-500 m, respectively) (Figs. I.2B, I.2C). At each site three horizontal zones were selected, naturally defined by tides – upper intertidal (submersed for ~25% of the tide period, ~6h/day), mid intertidal (submersed for ~50% of the tide period, ~12h/day) and lower intertidal (submersed for ~75% of the tide period, ~18h/day). At each zone four 12 cm x 12 cm replicates were sampled (Fig. I.1D).

This size has been used with success to study anthropogenic disturbance scenarios impacting rocky shore macroinvertebrate communities (e.g., Pais-Costa, 2011; Cabral-Oliveira 2014). Previous to the current work, the authors used Pais-Costa (2011) data to assess the number of replicate samples necessary for stabilization of variability of community parameters (density), and from the six replicates used by Pais-Costa (2011), four were deemed sufficient

Sampling was done twice in summer (August and September 2011), during low-water of spring tides, and pools and crevices were avoided. The samples (96 from each shore) were immediately preserved after sampling in neutralized 4% formalin solution (prepared with sea water). In the laboratory, the samples were sieved through a 1 mm mesh and the organisms sorted out. Macroinvertebrates were preserved in 80% ethanol for posterior count and identification, while macroalgae were frozen. Taxonomy for macroinvertebrates and macroalgae (done to species level whenever possible) was standardized in accordance to the World Register of Marine Species (WoRMS, http://www.marinespecies.org) and the AlgaeBase (http://www.algaebase.org), respectively. Macroinvertebrates' biomass was determined as ash-free dry weight

(AFDW) (loss of ignition after 8 h of incineration at 450 °C of specimens previously dried at 60 °C until weight stabilization). Macroalgae biomass was determined as dry weight (DW) (specimens dried at 60 °C until weight stabilization). Prior to data analysis, macroinvertebrate density and biomass data were standardized to ind m^{-2} and g AFDW m^{-2}, respectively, and macroalgae biomass was standardized to g DW m^{-2}.

In parallel to biological sampling, water samples (~20 L) were collected at each site and at the SOP, for quantification of chlorophyll a (Chl.a) (μg L^{-1}) (Strickland and Parsons, 1972), total suspended solids (TSS) (g L^{-1}), particulate organic matter (POM) (g L^{-1}), and determination of dissolved nutrients concentration (mg L^{-1}) [N-NO_3, N-NO_2, N-NH_4, P-PO_4 (DIP) and silica]. For TSS, a pre-weighed filter (Whatman GF/C glass fibre filter – 47 mm diameter, 1.2 μm pore) was dried (60 ºC until constant weight), re-weighed, and the suspended material content estimated as the weight difference (dry weight). POM was determined weighing the same filter after combustion (450 ºC, 8 h) (ash weight). Nutrients were analysed by colorimetric reaction using a Skalar San++® Continuous Flow Autoanalyser (Skalar, 2010). Dissolved inorganic nitrogen (DIN) was determined as the sum of N-NO_3, N-NO_2 and N-NH_4. Simultaneously to sampling, water temperature (ºC), conductivity (μS cm^{-1}), oxidation-reduction potential (ORP) (mV), salinity, dissolved oxygen (DO) (%) and pH parameters were measured *in situ* (using an YSI Professional Plus handheld multiparameter probe).

I.2.3. Data analysis

All statistical analyses were performed with PRIMER 6 + PERMANOVA© software (Clarke and Gorley, 2006; Anderson et al., 2008).

I.2.3.1. Environmental data

The environmental parameters (Env.) were used to visualize the sites' distribution inside the disturbance gradients by performing Principal Coordinate (PCO) analyses. It was firstly applied to Buarcos and Matadouro data conjunctly, to confirm that the gradients were similar at both shores. After this, PCO was applied to each shore data individually, to check the influence of the parameters inside the gradient within each shore. The Euclidean similarity measure was used in the calculation of similarity matrices, after square root transformation of data (except DIN, Chl.*a* and Silica, 1/X was used in these cases) to approach normality, followed by normalization.

Statistically significant differences between shores and between sampling sites within shore were tested with Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). It was firstly applied to Buarcos and Matadouro data conjunctly, to test for differences between shores. It included two fixed factors, ‘Shore’ (two levels: Buarcos and Matadouro) and ‘Site’ (nested in Shore; five levels: SOP and sites A-D). After this, PERMANOVA was applied to each shore data individually, with only the factor ‘Site’. The above mentioned similarity matrices were used. The statistical significance of variance components was tested using 9999 permutations and unrestricted permutation of raw data, with a significance level of $\alpha = 0.05$.

I.2.3.2. Biological data

Statistically significant differences were tested between shores, between sites within shore, between zones within site, and between zones across sites. Seven assemblage descriptors were used, five as multivariate data [macroalgae’ biomass (B_{alg}) and presence/absence (P/A_{alg}) and macroinvertebrates’ density (D_{inv}), biomass (B_{inv}), presence/absence (P/A_{inv})], and two as univariate data [macroinvertebrates’ and macroalgae’ richness (as number of species, S_{inv} and S_{alg}, respectively)]. For each descriptor, PERMANOVA was applied including three fixed factors, ‘Shore’ (two levels: Buarcos and Matadouro), ‘Site’ (nested in Shore; four levels: sites A-D) and ‘Zone’ (nested or non-nested in ‘Site’ to test within or across sites, respectively; three levels: upper, mid and lower intertidal). For the multivariate descriptors Bray Curtis similarity measure was used in the calculation of similarity matrices, after fourth root transformation of data (to reduce natural dominance of species); for B_{alg} and P/A_{alg} it was included one *dummy variable* since there were samples without algae present, acceptable for their stressful nature (upper intertidal). For the univariate descriptors, Euclidean distance was used, without transformation of data. The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model for multivariate descriptors, and unrestricted permutation of raw data for univariate descriptors, with a significance level of $\alpha = 0.05$.

To infer about the agreement between assemblages, the relationships between the descriptors were analysed with RELATE (comparative Mantel-type tests on similarity matrices), using the similarity matrices calculated earlier for PERMANOVA. Similarly, relationships were analysed between each descriptor and Env. Accordingly, the data matrices were adjusted before calculating the similarity matrices: biotic data matrices were calculated as mean values per site of macroalgae biomass and macroinvertebrates density and biomass, and for Env. matrices the SOP was removed. Spearman correlation and 9999 permutations were used, with a significance level of $\alpha = 0.05$.

To identify the taxa (macroalgae and macroinvertebrates) which contribute mostly to the communities' structural variation between sites, Similarity Percentage Analysis (SIMPER) was applied to each B_{alg} and D_{inv} descriptors of Buarcos and Matadouro shores. Dissimilarities between groups were assessed using two-way crossed designs with factors 'Site' and 'Zone' (as for PERMANOVA) (with a 95% and 85% cut off for macroalgae and macroinvertebrates, respectively, and without transformation of data). After this, taxa showing higher contribution for dissimilarities between sites at each shore (first 10 macroalgae and first 20 macroinvertebrates) were selected from all comparisons (e.g., site A vs site B, site A vs site C), to show the structural variation among sites, by means of their abundances and sensitivity/tolerance to pollution. The sensitivity/tolerance to pollution was described for all taxa found, using the most recent literature. The macroalgae were assigned from a 'Reduced Taxa List' (RTL) (Gaspar et al., 2012; Neto et al., 2012), in which macroalgae are grouped according to morphological and functional characteristics into one of two Ecological Status Groups

(ESG), where ESG I includes late successional or perennial to annual taxa and ESG II includes opportunists or annual taxa.

For the macroinvertebrates, the AZTI Marine Biotic Index – AMBI (Borja et al., 2000) list of November 2014 was used (available at http://ambi.azti.es). In such list, taxa are classified with consensus expert judgment into one of five Ecological Groups (EG I-V) regarding their responses to natural and man-induced changes in water quality, where increasing tolerance to pollution (organic enrichment) is expected from EG I to EG V.

Table I.1. *Environmental parameters measured/estimated monthly (August and September) at the Buarcos and Matadouro source of pollution (SOP) and sites A-D.*

	August					September				
Buarcos	SOP	A	B	C	D	SOP	A	B	C	D
Conductivity (µS cm^{-1})	1659	49705	48834	48472	49557	1323	48442	48080	47307	47981
Chlorophyll a (mg m^{-3})	7.380	0.608	0.675	0.371	0.367	8.670	0.628	0.920	0.737	0.561
DIN (mg L^{-1})	0.600	0.446	0.303	0.265	0.382	0.773	0.632	0.386	0.219	0.303
DIP (mg L^{-1})	0.086	0.051	0.064	0.024	0.020	0.075	0.079	0.00006	0.067	0.024
DO (%)	74.6	149.0	94.6	125.5	125.3	77.7	114.2	109.1	124.1	107.4
ORP (mV)	125.0	114.9	123.8	149.5	211.5	94.3	151.8	148.8	134.6	168.8
pH	7.12	7.11	6.89	6.93	7.02	8.47	8.26	8.24	8.23	8.15
POM (g L^{-1})	0.0037	0.0019	0.0010	0.0010	0.0015	0.0045	0.0017	0.0018	0.0015	0.0016
Salinity	0.94	35.23	35.29	35.37	35.34	0.74	36.12	35.88	36.34	36.26
Silica (mg L^{-1})	16.776	0.626	0.546	0.542	0.574	19.202	0.389	0.415	0.296	0.357
Temperature (ºC)	22.0	21.5	20.5	20.1	21.2	19.9	19.2	19.1	17.8	18.7
TSS (g L^{-1})	0.019	0.016	0.009	0.009	0.010	0.020	0.013	0.017	0.011	0.013
Matadouro										
Conductivity (µS cm^{-1})	1530	6899	48250	48615	47940	1470	45535	46911	47227	47022
Chlorophyll a (mg m^{-3})	0.896	1.999	0.543	0.646	0.688	0.298	1.538	1.399	1.600	1.750
DIN (mg L^{-1})	0.282	0.433	0.164	0.240	0.586	6.422	0.586	0.370	0.281	0.319
DIP (mg L^{-1})	0.031	0.007	0.038	0.000	0.033	0.104	0.046	0.032	0.069	0.001
DO (%)	75.5	143.2	101.4	92.0	92.0	72.1	99.0	87.5	85.6	81.3
ORP (mV)	20.0	127.3	55.0	87.8	149.7	212.6	239.6	258.3	256.7	240.6
pH	7.51	8.14	7.65	7.55	7.50	8.43	8.05	8.15	8.03	7.94
POM (g L^{-1})	0.0011	0.0021	0.0019	0.0020	0.0019	0.0012	0.0031	0.0030	0.0041	0.0032
Salinity	0.90	4.04	34.64	35.96	36.37	0.83	33.80	36.66	36.42	36.45
Silica (mg L^{-1})	15.966	0.370	0.260	0.195	0.369	15.804	0.463	0.336	0.354	0.371
Temperature (ºC)	20.2	22.1	20.7	19.4	18.8	19.9	18.9	17.2	17.6	17.4
TSS (g L^{-1})	0.004	0.010	0.013	0.013	0.014	0.002	0.024	0.020	0.032	0.033

I.2.3.3. Biological and environmental data

Distance-based linear modelling analyses (DistLM) (Legendre and Anderson, 1999) were performed to Buarcos and Matadouro data separately, to check the variation of Dinv and Balg explained by Env. BEST was used as selection procedure and BIC (Bayesian Information Criterion) criterion, with 9999 permutations. Prior to DistLM, abiotic correlated variables (>0.9) were removed. Distance-based Redundancy Analysis (dbRDA) was performed to visualize the fitted models in the multi-dimensional space (McArdle and Anderson, 2001).

I.3. Results

I.3.1. Environmental data

At both shores, the environmental parameters salinity and conductivity showed similar behaviour (Table I.1); low salinity values were registered at the SOP (<1) and were 34-36 at all remaining sites (except at site A of Matadouro in August – 4.04). Water temperature (20-22 ºC) and pH (6.9-8.5) were usually higher at the SOP, while DO (72-77%) was lower there. Chl.a did not vary much between sites at Matadouro (0.3-2 mg L^{-1}), but at Buarcos the SOP (7.4-8.7 mg L^{-1}) registered much higher values than all the other sites (0.37-0.92 mg L^{-1}). POM and TSS behaved similarly, and showed different trends at Buarcos and Matadouro, with the SOP registering for both parameters higher values than the sites in Buarcos, contrary to Matadouro. Silica was much higher at the SOP (16-19 mg L^{-1}), followed by site A (<0.63 mg L^{-1}). DIN and DIP were generally higher at the SOP (maximum of 6.42 mg L^{-1} and 0.10 mg L^{-1}, respectively). The ORP (20-258 mV) was usually lower at the SOP.

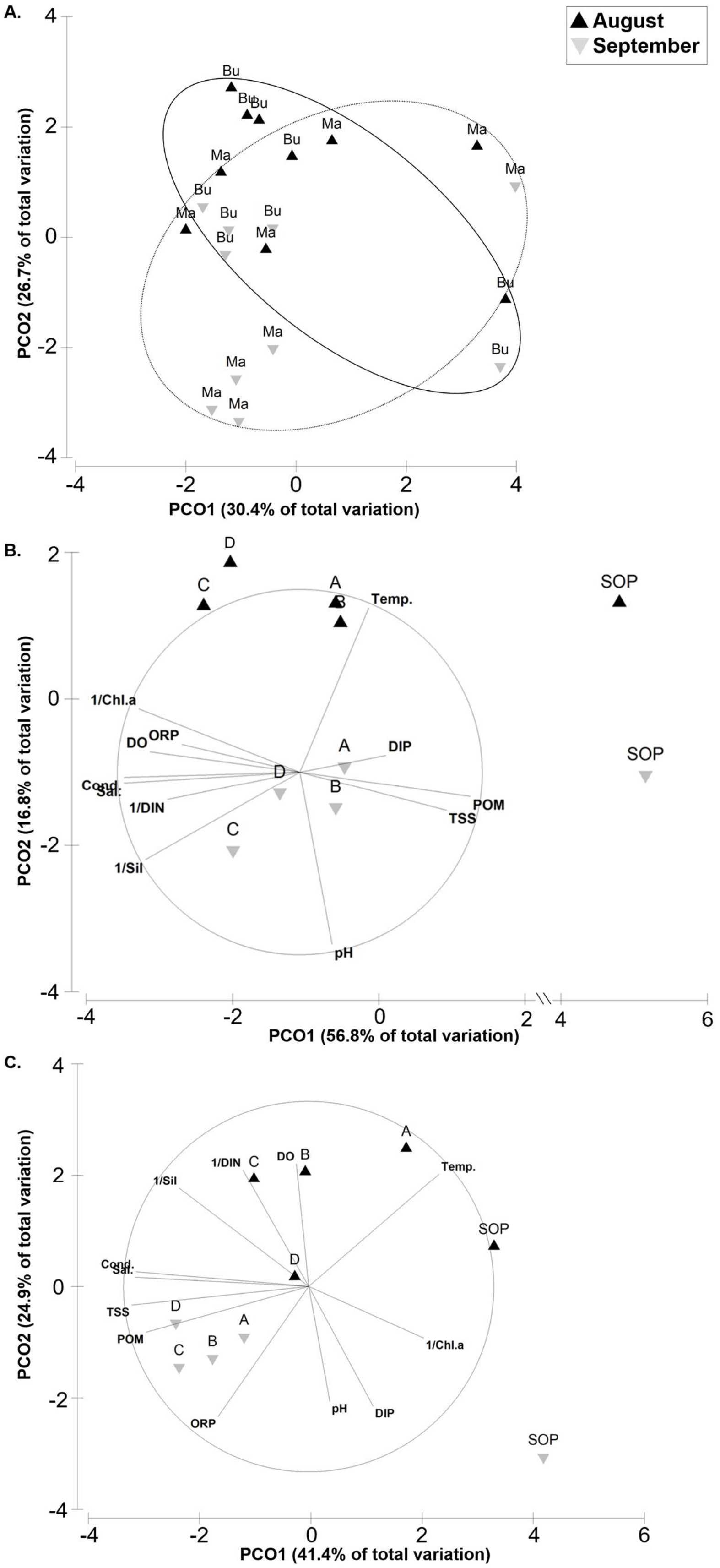


Figure I.2. *Principal Coordinates Ordination (PCO) plots of the environmental parameters: A. Buarcos versus Matadouro shores, B. Buarcos, and C. Matadouro. Key: SOP = source of pollution; A-D = sites A-D. Black upward triangles = August, grey downward triangles = September.*

I.3.2. Assemblage composition

A total of 59 macroalgae (Appendix I.1) and 179 macroinvertebrate (Appendix I.2) taxa were identified. In both Buarcos (42 macroalgae and 143 macroinvertebrate taxa) and Matadouro (49 macroalgae and 147 macroinvertebrate taxa) the macroalgae Rhodophyta showed higher species richness (35 and 34 taxa for Buarcos and Matadouro, respectively), followed by Chlorophyta (4 taxa) and Phaeophyceae (3 taxa) in Buarcos, and by Phaeophyceae (9 taxa) and Chlorophyta (6 taxa) in Matadouro. Higher biomass of Chlorophyta was registered at sites A and B, while higher biomass of Phaeophyceae and Rhodophyta was observed at sites C and D. The opportunist taxa (e.g., *Ulva* spp., filamentous Phaeophyceae, filamentous Rhodophyta) biomass was higher at sites A and B and lower at sites C and D, at both shores. On the other hand, the perennial species *Lithophyllum incrustans* and *Fucus spiralis* showed higher biomass at sites C and D (the latter being present only in Matadouro) and *Corallina* spp. showed higher biomass at site D. Regarding the macroinvertebrates, in both shores six groups constituted to about 90% of the total density and 95% of the total biomass. They were Cirripedia (essentially *Chthamalus montagui*), Bivalvia, Gastropoda, Polychaeta, Tanaidacea (essentially *Tanais dulongii,* contributed to density) and Polyplacophora (mainly *Acanthochitona* spp.*,* contributed to biomass). It was found a higher species richness of Polychaeta (47 and 53 taxa for Buarcos and Matadouro, respectively), followed sequentially by Gastropoda, Amphipoda, Isopoda, Bivalvia, Decapoda, Insecta, Pycnogonida, Polyplacophora and Cirripedia. The density of opportunist taxa (assigned to EG IV-V in AMBI), namely of Oligochaeta, the polychaetes *Boccardia polybranchia, Cirriformia tentaculata*, *Malacoceros fuliginosa* and *Tharyx* sp., and the insects Tipulidae, decreased away from the site (A) closest to the SOP. On the other hand, sensitive taxa (belonging to EG I, excluding *C. montagui*), namely the polychaetes *Ampharete* sp., *Euclymene palermitana, Serpula vermicularis* and *Travisia* sp., the amphipods *Ampelisca rubella* and *Maera grossimana*, the isopod *Paranthura nigropunctata*, the bivalves *Cardita calyculata, Hiatella arctica*, *Musculus costulatus*, Psammobiidae and

Striarca lactea, and the gastropods *Crisilla semistriata*, *Patella* spp. (especially *P. ulyssiponensis)*, *Rissoa parva, Skeneopsis planorbis* and *Tricolia pullus,* showed higher density in site D (sometimes in site C). Nevertheless, some sensitive taxa showed higher abundance at sites (A and B) closest to the SOP, compared to the sites (C and D) furthest from the SOP, namely the amphipods *Apherusa cirrus* and *Photis longicaudata*, and the gastropods *Gibbula* spp. and *Runcina coronata*.

Statistically significant differences (PERMANOVA; Supplementary material A1) were found between Buarcos and Matadouro for all tested descriptors, except S_{inv} [Pseudo-F = 0.0375, p(perm) = 0.8470] and S_{alg} [Pseudo-F = 0.0769, p(perm) = 0.7833]. Within both shores the pattern of descriptors B_{alg}, P/A_{alg}, D_{inv}, B_{inv} and P/A_{inv} was quite similar (Figs. I.3A, B, D, E, F). Differences between sites, between zones within site and between zones across sites were found using these five descriptors. Generally, sites were different from each other, and so were zones within and across sites. Nevertheless, fewer differences were identified between sites A and B, which were closest to the SOP, between inner sites (B and C) inside the disturbance gradient, and between sites C and D, which were furthest from the SOP. Also, fewer differences exist between zones within sites (A and B) closest to the SOP, and between zones across those sites. Fewer differences were also found between zones across sites (C and D) furthest from the SOP. The S_{alg} and S_{inv} were the descriptors with most distinct pattern showing, among descriptors, the fewest differences for all tested terms (Figs. I.3C, G, respectively). Generally, the biggest differences were found between the site (D) furthest from the SOP and the other sites, and in zones between that site and the other sites. Simultaneously, fewer differences exist between zones within sites (A and B) closest to the SOP.

There were significant relationships with high correlation (RELATE) both at Buarcos and Matadouro, not only within the macroalgae and macroinvertebrates descriptors (>0.9 between B_{alg}-P/A_{alg}, D_{inv}-B_{inv}, D_{inv}-P/A_{inv}, and B_{inv}-P/A_{inv}), but also between descriptors of both biological elements, namely B_{alg}-D_{inv} (0.69 and 0.78 in Buarcos and Matadouro, respectively) (Table I.2). Also, there are significant relationships between biotic descriptors and Env. in Buarcos, namely between Env.-B_{alg}, Env.-B_{inv}, Env.-D_{inv} and Env.-P/A_{alg}.

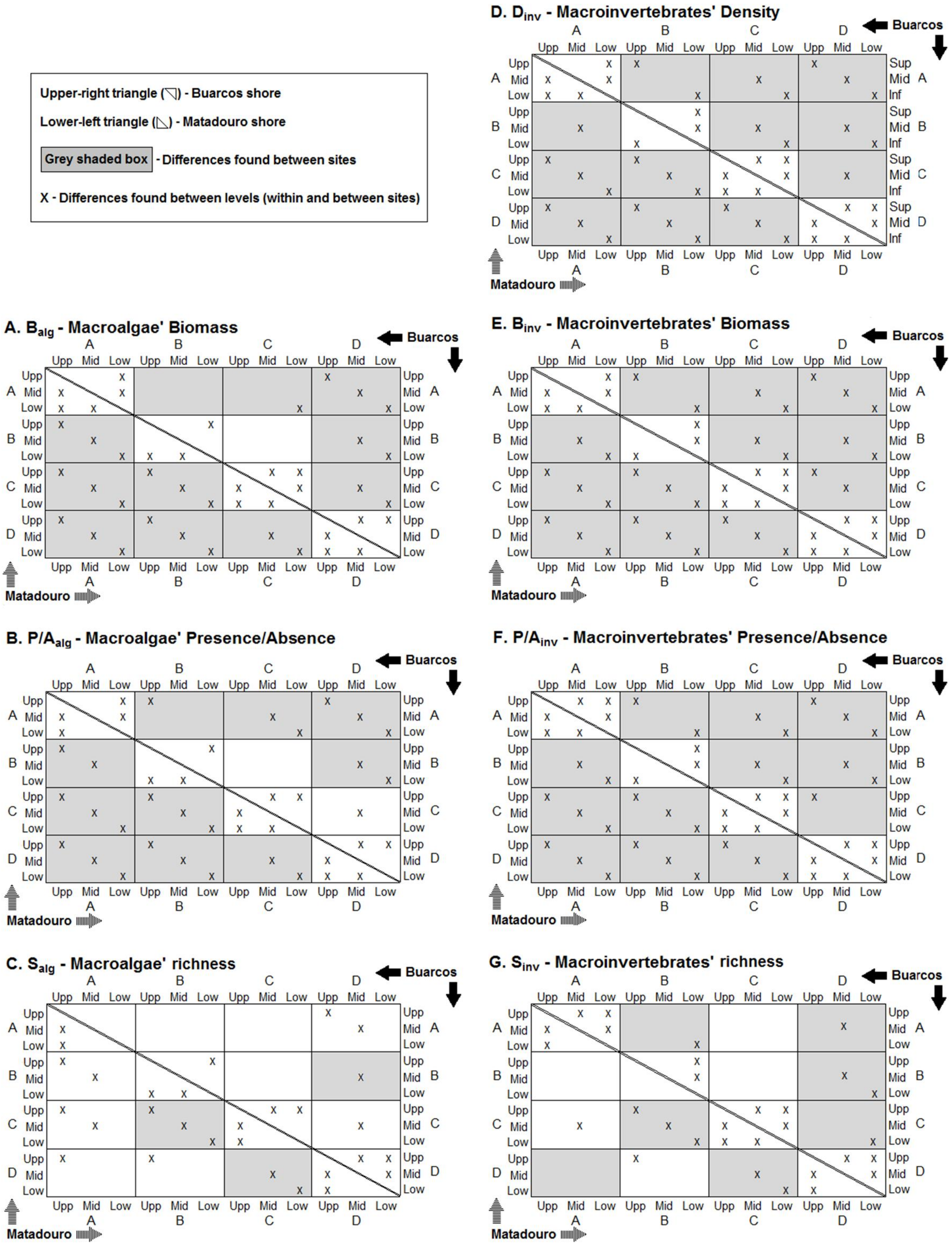


Figure I.3. *Summarized PERMANOVA results for the assemblage descriptors: A. B_{alg}; B. P/A_{alg}; C. S_{alg}; D. D_{inv}; E. B_{inv}; F. P/A_{inv}; and G. S_{inv} in Buarcos (upper-right triangles) and Matadouro (lower-left triangles) shores. Key: A-D = sites A-D. Upp, Mid and Low = upper, mid and lower intertidal zones.*

Table I.2. *RELATE results for environmental (Env.) and biotic descriptors of Buarcos and Matadouro rocky shores: B_{alg}, P/A_{alg} and S_{alg} are the macroalgae' biomass, presence/absence and number of taxa, and D_{inv}, B_{inv}, P/A_{inv} and S_{inv} are the macroinvertebrates' density, biomass, presence/absence and number of taxa. Higher relations (>0.9) are presented in bold and underlined. An asterisk (*) means the analyses were not statistically significant (significance level of α = 0.05).*

		P/A_{alg}	S_{alg}	D_{inv}	B_{inv}	P/A_{inv}	S_{inv}	Env.
Buarcos	B_{alg}	**0.951**	0.782	0.690	0.673	0.657	0.624	0.408
	P/A_{alg}		0.807	0.639	0.623	0.613	0.577	0.408
	S_{alg}			0.498	0.472	0.471	0.500	*
	D_{inv}				**0.972**	**0.976**	0.701	0.503
	B_{inv}					**0.953**	0.687	0.523
	P/A_{inv}						0.701	*
	S_{inv}							*
Matadouro	B_{alg}	**0.927**	0.650	0.778	0.770	0.774	0.646	*
	P/A_{alg}		0.715	0.711	0.703	0.694	0.653	*
	S_{alg}			0.477	0.486	0.462	0.564	*
	D_{inv}				**0.984**	**0.977**	0.680	*
	B_{inv}					**0.957**	0.688	*
	P/A_{inv}						0.674	*
	S_{inv}							*

The patterns in the communities' structural variation (SIMPER dissimilarities between sites and zones) support previous results for B_{alg} and D_{inv} in Buarcos (Supplementary material A2) and Matadouro (Supplementary material A3). Regarding B_{alg} (Figs. I.4A, D; Appendix I.1), in Buarcos dissimilarity was higher between sites B and D (85%) and lower between sites B and C (76%). Dissimilarity was also higher (94%) between the upper and mid zones, and between the upper and lower zones. In Matadouro, dissimilarity was higher between sites B and C (78%) and lower between sites C and D (65%), sites B and D (66%) and sites A and B (67%). Dissimilarity was also higher (89%) between the upper and mid zones, and between the upper and lower zones.

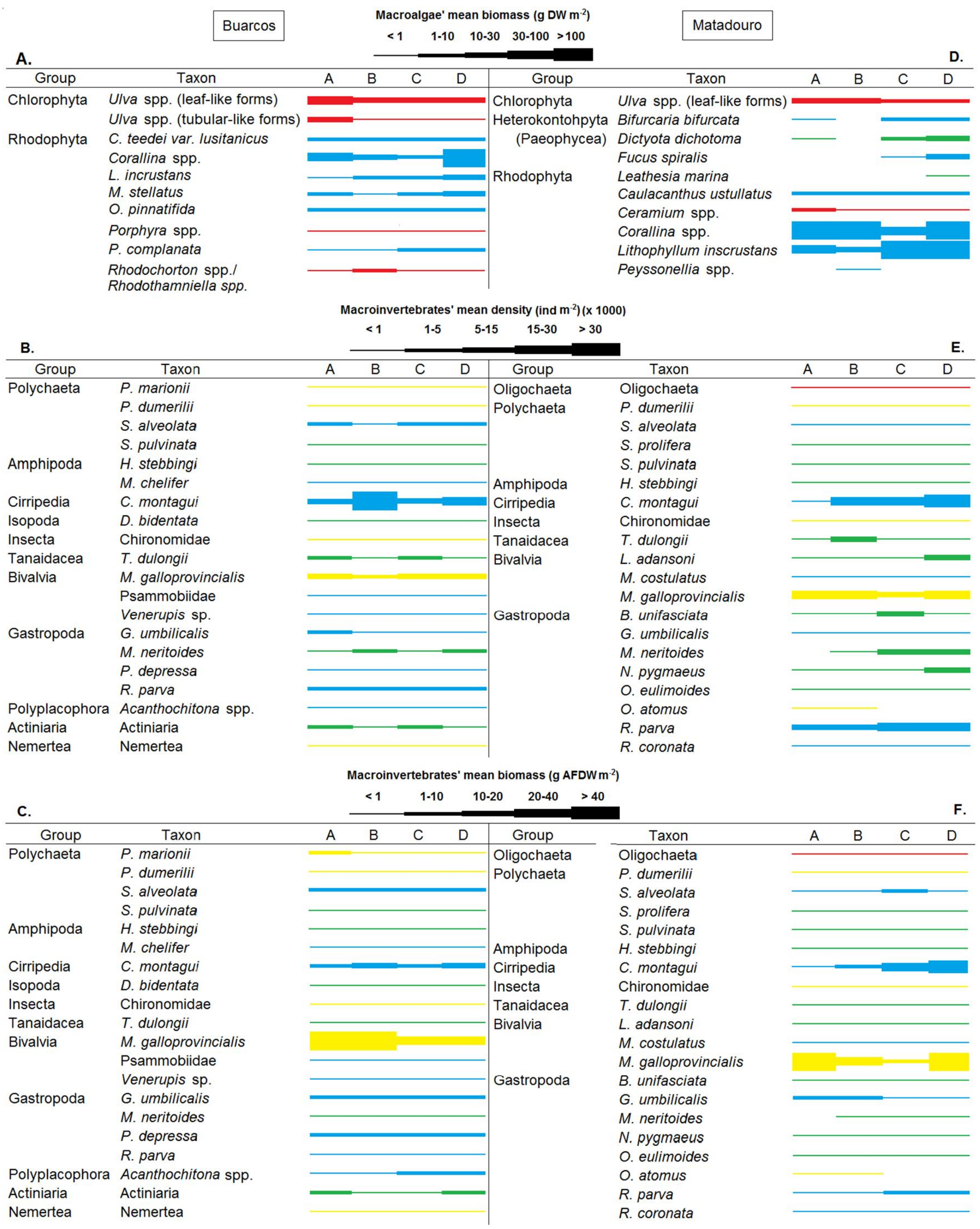


Figure I.4. Taxa contributing to the communities' structural variation across sites A-D, and their abundances (macroalgae' biomass, and macroinvertebrates' density and biomass), in Buarcos (A., B. and C., respectively) and Matadouro (D., E. and F., respectively). [Taxa are alphabetically ordered within higher groups. Key: Thickness of bars represents different abundance classes. Colour of bars represent opportunism of taxa: Macroalgae – red: opportunist taxa from EG II; green: non-opportunist taxa from EG II; and blue: taxa from EG I (Gaspar et al., 2012; Neto et al., 2012); Macroinvertebrates – red: taxa from EG V; yellow: taxa from EG III; green: taxa from EG II; and blue: taxa from EG I (AMBI species list of November 2014)].

Five taxa contributed mainly to the dissimilarities between sites – *Ulva* spp. (leaf-like forms), *Corallina* spp. and *Porphyra* spp. in Buarcos, and the former two plus *L. incrustans* and *F. spiralis* in Matadouro. In both shores *Corallina* spp. (EG I) was much variable in biomass across sites, although registering higher values in the site (D) furthest from the SOP. Sensitive taxa increased in biomass from the site (A) closest to, to the site (D) furthest from, the SOP (or showed higher biomass further away – sites C and D – than closer – sites A and B – to the SOP), namely *Lithophyllum incrustans* (EG I)*, F. spiralis* (EG I), *Mastocarpus stellatus* (EG I)*, Pterosiphonia complanata* (EG I)*, Bifurcaria bifurcata* (EG I)*, Dictyota dichotoma* (EG II) and *Leathesia marina* (EG II). In contrast, tolerant taxa (EG II opportunists) decreased in biomass in that direction (or presented higher biomass in sites A and B comparing to sites C and D), namely *Ulva* spp. (leaf-like forms), *Ulva* spp. (tubular-like forms), *Porphyra* spp., *Rhodochorton* spp./*Rhodothamniella* spp. and *Ceramium* spp.. Four taxa contributed mainly to the dissimilarities between zones - *Ulva* spp. (leaf-like forms), *Corallina* spp. and *Osmundea pinnatifida* in Buarcos, and the former two plus *L. incrustans* in Matadouro.

Regarding D_{inv} (Figs. I.4B, E; Appendix I.2), in Buarcos, dissimilarity was higher between sites A and B (78%) and lower between sites C and D (53%). Dissimilarity was also higher between the upper and lower zones (85%). In Matadouro, dissimilarity was higher between sites A and C (81%) and lower between sites C and D (63%). Dissimilarity was also higher between the upper and lower zones (90%).

Three species were top contributors to dissimilarities between sites in both shores – *C. montagui*, *Mytilus galloprovinciali*s and *Rissoa parva*. *Chthamalus montagui* (EG I) and *M. galloprovincialis* (EG III) showed high variability in density across sites, despite *M. galloprovincialis* registering higher biomass at sites (A and B) more proximate to the SOP (especially in Buarcos). *Rissoa parva* (EG I) increased in density from the site (A) closest to, to the site (D) furthest from, the SOP (or showed higher density further away – sites C and D – than closer – A and B – to the SOP). The same was observed for other (less) contributing taxa such as the sensitive *Dynamene bidentata* (EG II), *Melarhaphe* *neritoides* (EG II) (which was not found in in Matadouro site A), Psammobiidae (EG I), *Patella depressa* (EG I) and *Acanthochitona* spp. (EG I) (which also showed higher biomass in sites C and D). In contrast, the opportunists Oligochaeta (EG V), *Omalogyra atomus* (EG III) (which only occurred in Matadouro), Chironomidae (EG III) and Nemertea (EG III) decreased in density in that direction (or showed higher density in sites A and B comparing to sites C and D). Six taxa contributed mainly to the dissimilarities between zones – *C. montagui, M. galloprovincialis, R. parva and S. alveolata* in Buarcos, and these four plus *M. neritoides* and *Barleeia unifasciata* in Matadouro.

I.3.3. Environmental and biological relationships

Prior to the DistLM, correlated variables (>0.9) from each shore were removed: in Buarcos, pH (correlated with salinity and silica), silica (correlated with conductivity, pH, salinity and temperature), temperature (correlated with conductivity, salinity and silica) and TSS (correlated with POM), and in Matadouro, conductivity (correlated with DO and salinity), DO (correlated with conductivity and salinity), pH (correlated with Chl.a) and TSS (correlated with POM).

The DistLM (BEST) models were constituted by six abiotic variables for B_{alg} and D_{inv} of both shores (BIC = 38.49, R^2 = 0.933 and BIC = 44.56, R^2 = 0.943, respectively, for Buarcos; BIC = 45.54, R^2 = 0.931 and BIC = 44.88, R^2 = 0.917, respectively, for Matadouro). For Buarcos, using B_{alg}, the first two axes captured 56.2% of the fitted variation, which is 53.0% of the total variation. Sites C and D were associated to higher ORP, and lower conductivity, DIN (as higher 1/DIN in dbRDA) and Chl.a (as higher 1/Chl.a in dbRDA), contrary to sites A and B (Fig. I.5A). Using D_{inv}, the first two axes captured 52.5% of the fitted variation, representing 48.9% of the total variation. Sites C and D were associated to higher ORP, and lower DIN (as higher 1/DIN in dbRDA) and Chl.a (as higher 1/Chl.a), contrary to sites A and B (Fig. I.5B). For Matadouro, using B_{alg}, the first two axes captured 68.0% of the fitted variation, corresponding to 63.3% of the total variation. Sites C and D were essentially associated to lower ORP and temperature, contrary to sites A and B (Fig. I.5C). Using D_{inv}, the first two axes captured 60.4% of the fitted variation, which is 55.4% of the total variation. Sites C and D were mainly associated to lower ORP, and associated in minor degree to higher POM and DIN (as lower 1/DIN in dbRDA), and lower silica (as higher 1/silica in dbRDA) (Fig. I.5D).

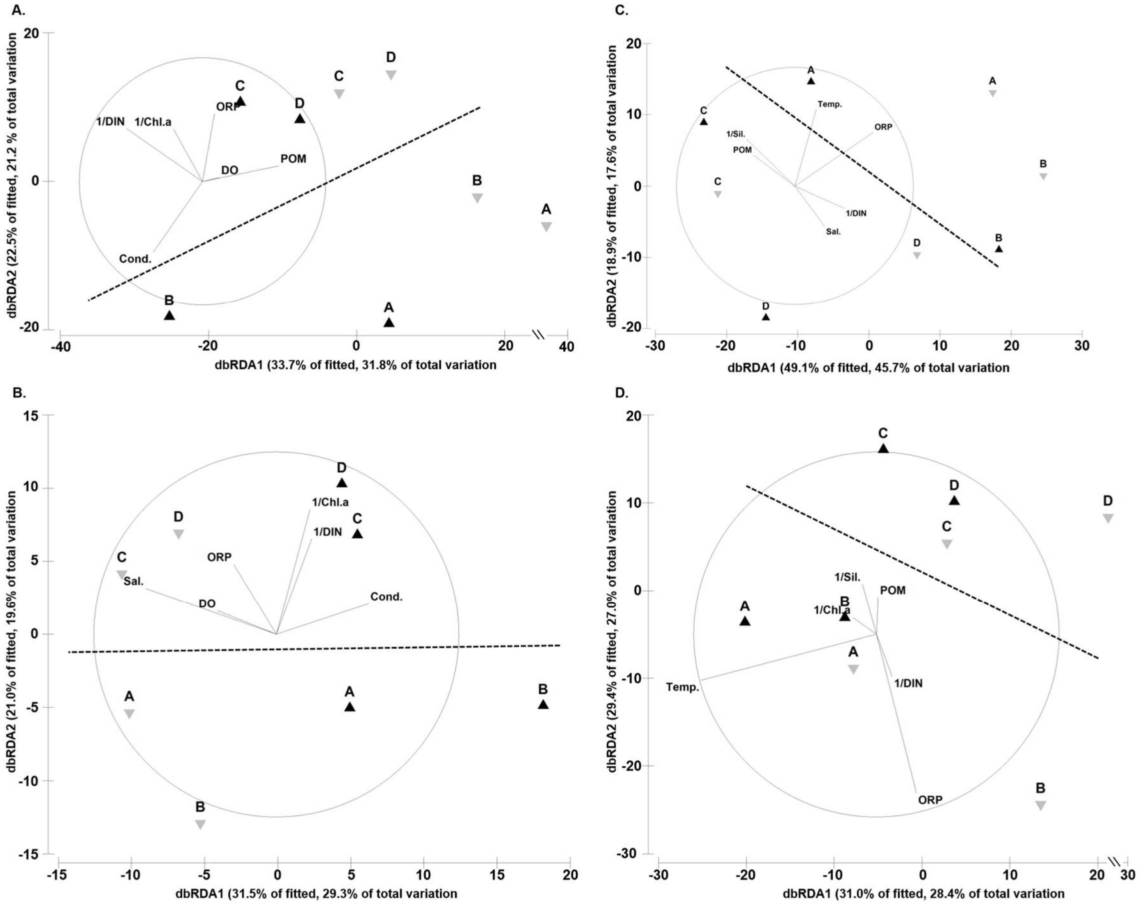


Figure I.5. *Two-dimensional distance-based redundancy analysis (dbRDA) ordination based on the best set of environmental variables (using BEST as selection procedure and BIC as selection criterion) and macroalgal and macroinvertebrate data from Buarcos (A. and B., respectively) and Matadouro (C. and D., respectively). Key: Black upward triangles = August, grey downward triangles = September. A dashed line is aiding to visualize the separation of sites A and B from sites C and D.*

I.4. Discussion and Conclusions

I.4.1. Macroalgal and macroinvertebrate communities' response to disturbance

In rocky shores macroalgae constitute habitats for macroinvertebrates seeking for food, shelter from predators, or avoiding desiccation and thermal stress (Bustamante et al., 2014). Therefore, the response of both macroalgae and macroinvertebrates communities' structure (abundance or richness) under the same environmental influences should, at least in a certain extent, be equivalent. Although in the present work we could only consider prompt measures of environmental factors (Env.), discarding variations through time, the statistical analysis performed showed that, at least in the periods considered, those parameters were generally distinct between the sampling sites, namely regarding the source of pollution (SOP). Levels of disturbance appeared to be similar in both shores, with stronger influence of the SOP at proximate sites (A and B).

The macroalgal assemblages are known to respond to human induced pressures (e.g., organic enrichment), and species may respond positively or negatively according to their tolerance to the disturbance, which may result in pronounced shifts from pristine reference conditions to degraded quality states (Odum, 1985; Orfanidis et al., 2011; Gaspar et al., 2012). In the present work, when comparing sampling sites in each shore (Buarcos and Matadouro shores), the predicted gradient of disturbance was visible. Site A, nearest to the SOP, was more stressful than the one located further away from the SOP, site D. Despite that there was not great variation in richness between sites inside the gradient; such stressful environment has resulted in differences in species composition and abundance. Higher biomass of opportunist taxa (considering all the opportunists: e.g., *Ulva* spp., filamentous Phaeophyceae, filamentous Rhodophyta) was found closer to the SOP (and decreasing to the furthest site from the SOP). On the other hand, higher biomass of perennial species like *Corallina* spp., *F. spiralis* and *L. incrustans*, which have been regarded as sensitive and indicative of good environmental health (Gaspar et al., 2012; Neto et al., 2012), was found further from the SOP. These results were expected since Chlorophyta opportunists are usually dominating species in degraded areas and may cause the diminishing of other less competitive species, while Phaeophyceae and Rhodophyta species usually show a decline in richness and abundance in those areas (Gaspar et al., 2012). These shifts in the communities were verified by performing several statistical routines (e.g., SIMPER, PERMANOVA), which showed several macroalgal community descriptors, namely the macroalgal biomass (B_{alg}), were indicative of more similar structure either between the first two sites, between the inner sites, or between the last two sites in the gradient.

The overall findings for the macroalgal communities in the present work are indicative of their already acknowledged potential to respond to anthropogenic disturbance (e.g., Borowitzka, 1972; Juanes et al., 2008, Neto et al., 2012), being useful for detecting such anthropogenic disturbances by presenting changes in species composition and abundance, which are required by the WFD to be included in tools for the ecological quality status assessment (WFD, 2000).

Regarding the macroinvertebrates, similarly to the macroalgae, certain groups are known to generally respond positively to pollution, being tolerant to more stressful environments and therefore increasing in abundance, such as nemerteans (e.g., Fraschetti et al., 2006; Cabral-Oliveira et al., 2014b) and polychaetes (e.g., Dauer and Conner, 1980; Cabral-Oliveira et al., 2014b), while other more sensitive groups usually decrease in abundance, such as gastropods (e.g., Airoldi, 2003; Terlizzi et al., 2005) and crustaceans (e.g., De-la-Ossa-Carretero et al., 2010). In the present work the macroinvertebrate communities were also responding to the disturbance gradient found, with some of those groups, and also many individual taxa, showing increased abundance in sites closer to the SOP (e.g., Oligochaeta, Insecta and Nemertea), while other (e.g., Gastropoda) showed higher density in sites further to the disturbance. The species *M. galloprovincialis* and *C. montagui* were dominant in the communities and were highly variable in abundance (density and biomass) between sites, which has been already referred by other authors (e.g., Boaventura et al., 2002, Pereira et al., 2006). Nevertheless, *M. galloprovincialis* (EG III in AMBI) was usually more abundant in sites near the SOP (especially regarding biomass), while *C. montagui* (EG I in AMBI) was usually more abundant in sites further from the disturbance, namely in Matadouro. There, the decreased abundance of *C. montagui* at sites closer to the SOP may be explained by the increase of algae richness and biomass, which may have altered the substratum type and the surface topography (important in the settlement and recruitment of sessile marine invertebrates) (e.g., Knox, 2000). Such increase in macroalgal and macroinvertebrate richness has already been reported near impacted areas, owing to intermediate disturbance which may increase availability of resources, promoting less competitive exclusion and therefore the co-existence of species (Connell, 1978; Magurran and McGill, 2010, Cabral-Oliveira et al., 2014b; Díez et al. 2014). Within the macroinvertebrate communities there were also taxa that did not relate to the disturbance gradients. Some amphipods (and few other crustaceans), usually regarded as sensitive, occurred in high densities near the disturbance, but those could be related to the presence of *Corallina* spp., acting as refuge and increasing the heterogeneity of the substrate (Fish and Fish, 2001; Cabral-Oliveira et al., 2014b). Also, some herbivore gastropods (namely *Gibbula* spp., EG I in AMBI) may be benefiting from the increase of primary producers (e.g., macroalgae) richness and abundance (Knox, 2000).

Similarly to the macroalgae, the overall findings for the macroinvertebrate communities indicate changes in the communities relating to a disturbance gradient. Nevertheless, the community descriptors based in macroinvertebrates, namely the macroinvertebrate density (D_{inv}), seem to have better captured the changes in structure between sites in the gradient. Not only they pointed either for higher differences between the exterior sites in the gradient, or for higher relation between the first two, and the last two sites in the gradient, but also showed higher variability (regarding density data) within sites closer to the disturbance, decreasing to sites further from the disturbance, as described by O'Connor (2013) for other rocky shore benthic assemblages when impacted. These findings indicate that benthic invertebrates are a good indicator of stress and pollution in rocky shores, and corroborate what has been described for other coastal ecosystems (e.g., Borja et al., 2000; Marques et al., 2009).

I.4.2. Comparison of the macroalgal and macroinvertebrate biological elements

The present work, to the authors' knowledge, is one of the few that studied quantitatively (number of individuals, biomass, richness and taxonomic composition) the structural variation of the communities of two biological elements – macroalgae and macroinvertebrates – at intertidal rocky shores, with data gathered from the same set of samples. A strong parallelism was found between both macroalgal and macroinvertebrate biological elements in response to disturbance. Several descriptors based on each biological element showed identical behaviour, namely the macroalgae' biomass (B_{alg}) and the macroinvertebrates' density (D_{inv}), which were the ones that better captured the disturbance gradients analysed in the study (especially D_{inv}). This suggests that either one or the other biological element could be selected when studying the quality condition of intertidal rocky shores. The descriptor D_{inv} was able to capture the disturbance gradients and, therefore, indicators based on this descriptor should cover the requirement of the WFD for CW when it demands the use of biological parameters such as abundance and taxonomic composition on the assessment of benthic macrofauna.

In the scope of European Directives, namely of the WFD, several are the ecological indicators based only on the biological quality element macroalgae to assess quality of rocky shores (e.g., Ballesteros et al., 2007; Juanes et al., 2008; Neto et al. 2012; Gall and Duff, 2014). Although the combined use of macroinvertebrates and macroalgae in rocky shore assessments may not be in disagreement with WFD requirements (Díez et al., 2012), according to the present findings the exclusive use of macroinvertebrates seems very much possible. Furthermore, using each biological element separately could allow conducting more dedicated surveys, with appropriate sampling methods, in the most feasible season (e.g., June to late September for rocky shore macroalgae; Juanes et al., 2008; Gaspar et al., 2012; Neto et al., 2012; and winter for soft-bottom macroinvertebrates in open coastal waters; Muxika et al., 2007; Teixeira et al., 2009), which in turn could produce more appropriate data from each element.

If an ecologically sound and consistent pattern of variation can be observed for indicators associated individually to macroalgae and macroinvertebrate communities, then, the use of measures relying on features from these communities should be possible to use and to combine in order to assess separately the quality status of each one of these biological elements. For the studied macroinvertebrate communities, after the preliminary application of sensitivity classes from AMBI to species identified, is highly promising that such kind of indicator has the potential to be developed (after convenient adaptation to rocky shore species) and to integrate a future assessment tool. It was also observed a variation of the species present on sites under different disturbance levels (sensitive species and pollution tolerant species presented opposite tendencies along the disturbance gradient) and between shores (SIMPER results; Fig. I.4), which suggests the use of an indicator based on the parameter taxonomic composition is also promising and possible to be developed aiming to integrate future assessment methods. Similarly to other methods developed for the assessment of soft bottoms, such as the Benthic Assessment Tool – BAT (Teixeira et al., 2009) and the multivariate method AMBI – M-AMBI (Muxika et al., 2007), it would be possible to develop a specific methodology to rocky shores but based exclusively on macrobenthic fauna of this intertidal habitat.

The present work suggests the use of indicators (e.g., diversity and functional) based on macroinvertebrate communities to integrate specific assessment tools for intertidal rocky shores. A further challenge will be eventually to develop and test a suitable multi-metric assessment tool (presently a gap) compliant with the European WFD requirement.

Appendix

Appendix I.1. *List of macroalgal taxa found in Buarcos and Matadouro sites A-D. Presence of species at each site is shown with a cross (x). Biomass of higher groups, and total number of species (S) at each site are shown and highlighted in grey. Each species' Ecological Group (EG) and opportunism are shown.*

Group	Taxon	Buarcos				Matadouro				EG	Opportunist
		A	B	C	D	A	B	C	D		
Chlorophyta		63.24	26.02	26.00	23.62	35.26	39.01	9.50	10.03		
	Bryopsis plumosa							x		II	yes
	Cladophora spp.	x	x	x	x		x		x	II	yes
	Codium spp.	x			x	x		x		I	
	Ulva clathrata						x			II	yes
	Ulva spp. (Leaf-like forms)	x	x	x	x	x	x	x	x	II	yes
	Ulva spp. (Tubular-like forms)	x	x	x	x	x				II	yes
Heterokontophyta (Phaeophyceae)		0.33	0.85	0.81	2.82	6.54	1.09	13.90	39.59		
	Bifurcaria bifurcata					x		x	x	I	
	Cladostephus spongiosus					x				I	
	Colpomenia peregrina					x	x	x		II	
	Cystoseira tamariscifolia					x		x	x	I	
	Dictyota dichotoma	x	x	x	x	x		x	x	II	
	Ectocarpales/*Sphacelaria* spp.	x	x		x	x	x	x	x	II	yes
	Fucus spiralis							x	x	I	
	Leathesia marina								x	II	
	Halopteris scoparia	x		x	x	x		x	x	I	

Appendix I.1. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG	Opportunist
		A	B	C	D	A	B	C	D		
Rhodophyta		79.92	43.95	39.82	152.86	175.45	273.08	283.52	430.72		
	Acrosorium ciliolatum			x		x				I	
	Ahnfeltiopsis devoniensis	x	x	x	x					I	
	Asparagopsis armata					x	x	x	x	II	
	Boergeseniella spp.	x		x	x	x	x	x	x	II	yes
	Callithamnion tetricum	x		x	x			x		II	yes
	Caulacanthus ustullatus	x				x	x	x	x	I	
	Ceramium spp.	x	x	x	x	x	x	x	x	II	yes
	Champia parvula					x	x	x		I	
	Chondracanthus acicularis	x	x	x				x		I	
	Chondracanthus teedei					x	x	x	x	I	
	Chondracanthus teedei var. lusitanicus	x	x	x	x					I	
	Chondria coerulescens	x	x	x	x		x	x	x	I	
	Chondrus crispus	x	x							I	
	Corallina spp.	x	x	x	x	x	x	x	x	I	
	Cryptopleura ramosa	x	x	x	x			x	x	I	
	G1*	x		x	x	x	x	x	x	II	yes
	G2**	x	x	x	x			x	x	II	yes
	G3***					x	x			II	yes
	Gastroclonium reflexum	x	x		x	x	x	x	x	II	
	Gellidium corneum								x	I	
	Gellidium pulchellum	x	x		x					I	
	Gellidium pusillum						x	x		I	
	Gigartina pistillata			x	x			x		I	
	Gracilaria gracilis	x						x		II	
	Grateloupia filicina									I	
	Gymnogongrus griffithsiae	x				x				I	

Appendix I.1. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG	Opportunist
		A	B	C	D	A	B	C	D		
Rhodophyta											
	Halurus equisetifolius			x						I	
	Hypnea musciformis						x			II	
	Hypoglossum hypoglossoides		x	x	x	x		x	x	I	
	Jania rubens	x		x		x		x	x	I	
	Lomentaria articulata	x	x		x					II	
	Lythophyllum incrustans	x	x	x	x	x	x	x	x	I	
	Mastocarpus stellatus	x	x	x	x					I	
	Nitophylum punctatum								x	I	
	Ophidocladus simpliciusculus	x	x	x	x	x		x	x	II	yes
	Osmundea hibrida					x	x	x	x	I	
	Osmundea pinnatifida	x	x	x	x					I	
	Peyssonnelia spp.		x				x			I	
	Plocamium cartilaginium			x	x	x	x	x	x	II	
	Polysiphonia spp.	x	x	x	x	x	x	x	x	II	yes
	Porphyra spp.	x	x	x	x					II	yes
	Pterosiphonia complanata	x	x	x	x	x		x	x	I	
	Pterosiphonia pennata	x	x	x	x	x	x	x	x	II	yes
	Rhodochorton spp./*Rhodothamniella* spp.	x	x	x	x			x		II	yes
Opportunist taxa		65.41	30.75	27.67	26.55	41.86	41.48	6.94	12.94		
Total S		35	28	31	32	31	24	37	31		

[a] G1: Rhodomelaceae except *Chondria* spp., *Osmundea* spp., *Pterosiphonia* spp. and *Boergeseniella* spp. (e.g., *Aphanocladia* spp., *Ctenosiphonia* spp., *Herposiphonia* spp., *Heterosiphonia* spp., *Leptosiphonia* spp., *Lophosiphonia* spp.).

[b] G2: Red Unisseriate filamentous forms except *Rhodochorton* spp. and *Rhodothamniella* spp. (e.g., *Acrochaetium* spp., *Audouinella* spp., *Colaconema* spp., *Bangia* spp., *Stylonema* spp.).

[c] G3: Ceramiaceae except *Ceramium* spp., *Callithamnion tetricum* and *Halurus equisetifolius* (e.g., *Aglaothamnion* spp., *Anotrichium* spp., *Anthithamnion* spp., *other Callithamnion* spp.*, Compsothamnion thuyoides, Halurus flosculosus, Pleonosporium borreri*, *Dasya* spp.).

Appendix I.2. *List of macroinvertebrate taxa found in sites A, B, C and D in Buarcos and Matadouro shores. Presence of species in each site is shown with a cross (x). Density of higher groups, and total number of species (S) in each site are shown and highlighted in grey. Each species' Ecological Group (EG) is shown.*

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Oligochaeta	Oligochaeta	144.7	92.6	26.0	11.6	358.8	538.2	55.0	23.1	V
Polychaeta		6157.4	2106.5	4751.2	3414.4	1736.1	1252.9	4233.2	1446.8	
	Ampharete sp.			x	x			x	x	I
	Aonides oxycephala	x	x	x	x	x	x	x	x	III
	Arabella iricolor							x	x	I
	Boccardia polybranchia					x	x	x	x	IV
	Brania pusilla			x		x	x			II
	Cirriformia tentaculata	x	x		x	x	x	x	x	IV
	Euclymene palermitana							x		I
	Eulalia viridis	x	x	x	x	x	x	x	x	II
	Fabricia stellaris			x		x	x	x	x	II
	Glycera sp.	x	x	x				x		II
	Harmothoe sp.	x	x		x					II
	Lepidonotus clava	x	x	x	x	x	x	x	x	II
	Lumbrineris latreilli					x	x	x		II
	Lysidice ninetta							x		II
	Malacoceros fuliginosa	x	x	x		x	x		x	V
	Maldane sp.						x			I
	Microspio atlantica				x					III
	Myrianida sp.	x		x	x					II
	Mysta picta	x	x		x			x	x	III
	Mysta sp.2	x		x	x					III
	Naineris laevigata				x	x	x	x	x	I
	Naineris quadricuspida	x				x	x	x	x	I
	Neanthes nubila		x	x	x	x		x		III
	Nereidinae	x	x	x	x					III
	Odontosyllis ctenostoma	x	x	x	x		x	x	x	II
	Oriopsis armandi				x					II

Appendix I.2. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Polychaeta										
	Perinereis cultrifera	x	x	x	x	x	x	x	x	III
	Perinereis marionii	x	x	x	x	x	x	x	x	III
	Perinereis oliveirae	x	x		x				x	III
	Perinereis sp.			x	x					III
	Pholoe cf. *synophthalmica*	x	x	x	x	x		x		II
	Platynereis dumerilii	x	x	x	x	x	x	x	x	III
	Polycirrus sp.	x	x	x	x	x	x	x	x	IV
	Protoaricia oerstedi					x				III
	Pseudopotamilla reniformis								x	II
	Sabellaria alveolata	x	x	x	x	x	x	x	x	I
	Sabellaria spinulosa	x	x	x	x	x	x	x	x	I
	Scolelepis (Scolelepis) cantabra								x	II
	Scoletoma impatiens	x	x	x	x	x	x	x	x	II
	Serpula vermicularis							x		I
	Sphaerosyllis sp.	x	x	x	x	x		x	x	II
	Sphaerosyllis taylori								x	II
	Spio filicornis							x		III
	Spirobranchus lamarcki	x	x	x	x	x	x	x	x	II
	Spirobranchus triqueter	x		x				x		II
	Sthenelais boa	x	x		x					II
	Syllides edentatus							x		II
	Syllis amica	x	x	x	x	x	x	x	x	II
	Syllis armillaris	x		x	x					II
	Syllis corallicola		x	x				x		III
	Syllis garciai	x	x	x	x	x	x	x	x	II
	Syllis gerlachi	x	x	x		x	x	x	x	II
	Syllis gracilis	x	x	x	x	x		x	x	III

Appendix I.2. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Polychaeta										
	Syllis hyalina	x	x	x	x			x	x	II
	Syllis prolifera	x	x	x	x	x	x	x	x	II
	Syllis pulvinata	x	x	x	x	x	x	x	x	II
	Syllis rosea	x	x	x	x	x		x		II
	Syllis sp.							x		II
	Syllis vivipara	x	x	x	x		x		x	II
	Tharyx sp.	x	x	x	x	x	x	x	x	IV
	Travisia sp.	x	x	x	x	x		x	x	I
	Vermiliopsis sp.							x		II
Amphipoda		749.4	824.7	445.6	914.4	680.0	850.7	1053.2	448.5	
	Abludomelita gladiosa		x							III
	Abludomelita obtusata			x						III
	Ampelisca rubella				x	x	x	x		I
	Ampelisca sp.					x	x	x	x	I
	Amphilochus spencebatei					x	x	x	x	II
	Ampithoe gammaroides								x	II
	Aora typica							x		I
	Apherusa cirrus					x	x		x	I
	Apherusa jurinei		x	x	x	x	x	x	x	I
	Apohyale prevostii		x	x	x					II
	Caprella acanthifera	x	x	x	x	x		x		II
	Caprella equilibra	x	x	x	x	x		x	x	II
	Dexamine spinosa		x	x		x		x	x	III
	Elasmopus rapax	x	x	x	x	x	x	x	x	III
	Gammaropsis maculata		x		x			x		I
	Hyale perieri	x	x	x	x	x		x	x	II
	Hyale pontica	x								II

Appendix I.2. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Amphipoda										
	Hyale sp.			x						II
	Hyale stebbingi	x	x	x	x	x	x	x	x	II
	Jassa ocia		x	x	x					V
	Jassa pusilla							x		V
	Lysianassidae			x						I
	Maera grossimana	x		x	x					I
	Melita palmata	x	x	x	x					I
	Microdeutopus chelifer	x	x	x	x	x	x	x		I
	Microdeutopus damnoniensis (nomen nudum)	x	x	x	x					I
	Parajassa pelagica	x			x					II
	Photis longicaudata	x	x							I
	Podocerus variegatus		x						x	III
	Protomedeia fasciata	x								II
	Stenothoe monoculoides			x		x	x	x	x	II
Cirripedia		14236.1	51342.6	11571.2	24508.1	2.9	8822.3	14351.9	18906.3	
	Chthamalus montagui	x	x	x	x	x	x	x	x	I
	Balanus sp.	x			x				x	I
Decapoda		83.9	43.4	31.8	43.4	150.5	52.1	292.2	34.7	
	Cancer sp.				x					II
	Carcinus maenas	x				x				III
	Liocarcinus navigator					x	x			I
	Pachygrapsus marmoratus	x			x				x	II
	Pilumnus hirtellus	x	x	x	x	x	x	x		I
	Pirimela denticulata	x	x	x	x	x	x	x	x	I
	Xantho pilipes	x			x	x		x		I

Appendix I.2. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Isopoda		491.9	439.8	448.5	587.4	199.7	338.5	260.4	289.4	
	Campecopea hirsuta	x	x	x	x		x	x	x	II
	Cymodoce truncata		x	x	x		x	x	x	I
	Dynamene bidentata	x	x	x	x				x	II
	Dynamene edwardsi	x	x	x	x		x		x	II
	Dynamene magnitorata		x	x	x	x	x	x	x	II
	Eurydice pulchra					x	x		x	I
	Gnathia maxillaris	x	x	x	x	x		x		I
	Idotea granulosa	x			x					II
	Idotea pelagica	x	x	x	x	x	x	x	x	II
	Ischyromene lacazei	x	x	x	x		x	x	x	II
	Lekanesphaera levii					x	x	x	x	III
	Paranthura nigropunctata			x	x	x		x		I
	Cleantis prismatica					x				II
Tanaidacea		2387.2	575.8	1522.0	607.6	546.9	2083.3	865.2	104.2	
	Apseudes talpa	x	x	x	x					II
	Tanais dulongii	x	x	x	x	x	x	x	x	II
Insecta		225.7	367.5	110.0	188.1	1061.9	636.6	98.4	95.5	
	Chironomidae	x	x	x	x	x	x	x	x	III
	Tipulidae	x	x	x	x	x	x	x	x	IV
Bivalvia		11999.4	3339.1	7624.4	11519.1	7106.5	12916.7	5934.6	11033.0	
	Cardita calyculata					x	x	x	x	I
	Hiatella arctica	x	x	x	x	x	x	x	x	I
	Irus irus	x				x	x	x	x	I
	Lasaea adansoni					x	x	x	x	II
	Musculus costulatus	x	x	x	x	x	x	x	x	I
	Mytilus galloprovincialis	x	x	x	x	x	x	x	x	III
	Parvicardium pinnulatum					x	x	x	x	I

Appendix I.2. *(continued)*

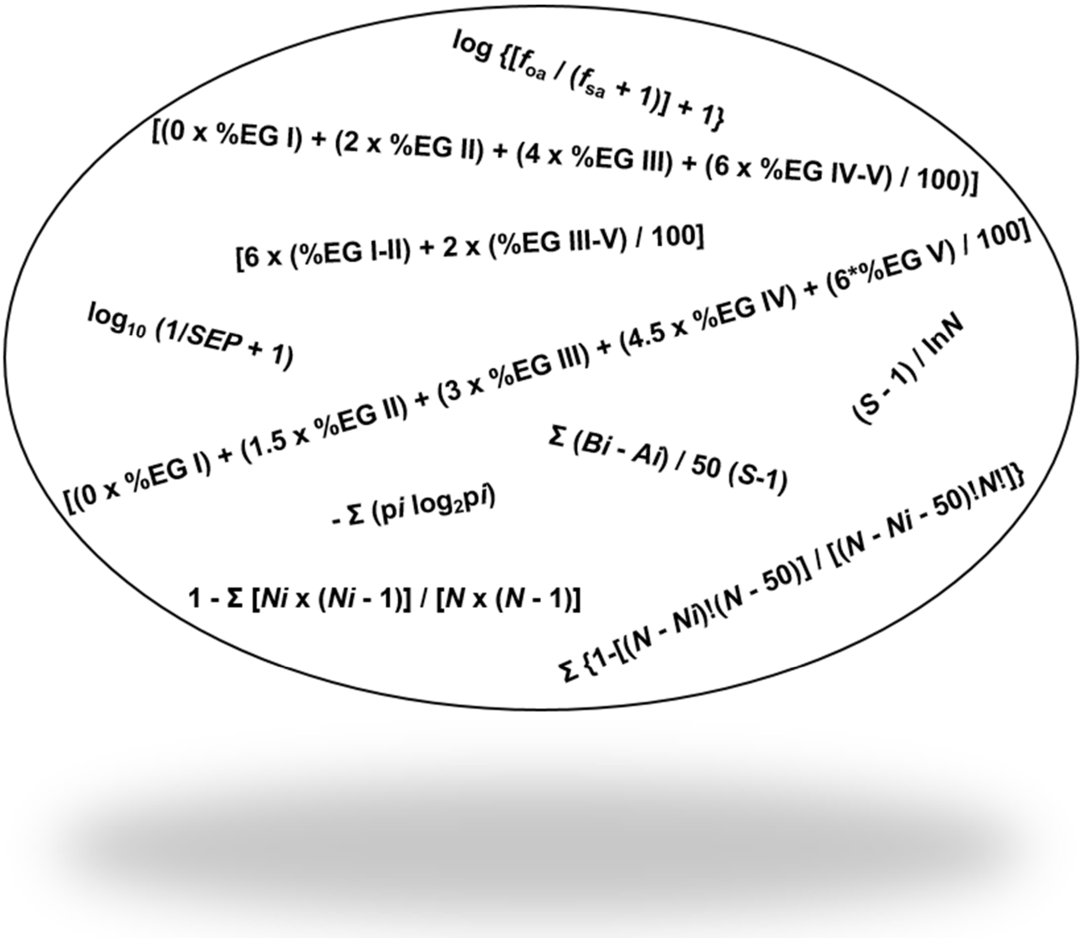
Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Bivalvia										
	Psammobiidae	x	x	x	x	x	x	x	x	I
	Striarca lactea			x						I
	Tellina sp.	x	x							I
	Venerupis sp.	x	x	x	x	x	x	x	x	I
Gastropoda		4239.0	8046.9	5795.7	9207.2	8579.3	5049.2	16886.6	16316.6	
	Crisilla semistriata			x	x	x		x		I
	Aplysia punctata	x		x	x	x		x	x	I
	Barleeia unifasciata					x	x	x	x	II
	Bittium reticulatum							x		I
	Buccinum humphreysianum				x	x			x	II
	Cerithiopsis tubercularis	x	x		x		x	x	x	I
	Epitonium clathratulum	x	x		x		x		x	I
	Gibbula cineraria					x	x	x	x	I
	Gibbula pennanti	x	x	x	x	x	x	x	x	I
	Gibbula umbilicalis	x	x	x	x	x	x	x	x	I
	Melarhaphe neritoides	x	x	x	x		x	x	x	II
	Nassarius incrassatus		x		x	x				II
	Nassarius pygmaeus	x	x	x	x	x	x	x	x	II
	Nassarius reticulatus					x			x	II
	Nucella lapillus		x	x	x	x	x		x	II
	Nudibranchia		x	x	x		x	x		II
	Ocenebra erinaceus	x	x	x	x					II
	Ocinebrina aciculata				x	x	x	x	x	II
	Odostomia eulimoides	x	x	x	x	x	x	x	x	II
	Omalogyra atomus	x				x	x			III
	Onchidella celtica								x	I
	Opalia crenata	x	x	x						I
	Phorcus lineatus	x	x	x	x				x	I
	Patella depressa	x	x	x	x	x	x	x	x	I
	Patella sp.	x			x					I

Appendix I.2. *(continued)*

Group	Taxon	Buarcos				Matadouro				EG
		A	B	C	D	A	B	C	D	
Gastropoda										
	Patella ulyssiponensis	x	x	x	x	x	x	x	x	I
	Patella vulgata	x	x	x	x			x		I
	Rissoa parva	x	x	x	x	x	x	x	x	I
	Runcina coronata	x	x	x	x	x	x	x	x	I
	Siphonaria pectinata						x	x		I
	Skeneopsis planorbis				x			x	x	I
	Testudinalia testudinalis	x			x					I
	Tricolia pullus	x		x	x	x	x	x	x	I
	Urosalpinx cinerea	x	x		x	x	x	x	x	II
Polyplacophora		202.5	133.1	347.2	546.9	269.1	460.1	321.2	303.8	
	Lepidochitona (Lepidochitona) cinerea	x	x	x	x	x	x	x	x	II
	Acanthochitona spp. (*A. crinita* and *A. fascicularis*)	x	x	x	x	x	x	x	x	I
Ophiuroidea	Ophiuroidea	173.6	156.3	144.7	188.1	8.7	23.1	14.5		II
Echinoidea	Echinoidea		23.1		5.8	2.9	2.9	23.1	11.6	I
Anthozoa	Actiniaria	1443.9	735.0	1452.5	613.4	124.4	115.7	312.5	243.1	II
Ascidiacea	Ascidiacea			2.9				2.9		III
Echiura	Echiura				2.9					II
Nemertea	Nemertea	818.9	596.1	269.1	254.6	147.6	315.4	115.7	182.3	III
Picnogonida		211.2	81.0	37.6	81.0	26.0	17.4	11.6	11.6	
	Achelia echinata	x	x	x	x			x	x	I
	Anoplodactylus pygmaeus	x	x			x			x	II
	Anoplodactylus virescens	x	x	x	x	x	x	x		II
	Callipallene brevirostris								x	II
Sipuncula	*Golfingia* sp.	x	x	x	x	x	x	x		I
Turbellaria	Leptoplanidae (*Notoplana* sp. and *Leptoplana* sp.)	x	x	x	x	x		x	x	II
Opportunist taxa		358.8	298.0	147.6	130.2	775.5	758.1	133.1	81.0	IV and V
Sensitive taxa (except *C. montagui*)		8857.1	8023.7	10477.4	10069.4	8521.4	6004.1	17008.1	12323.5	I
Total S		105	101	103	115	99	88	114	103	

Chapter II

Ability of invertebrate indices to assess ecological condition on intertidal rocky shores*



Vinagre, P.A., Pais-Costa, A.J., Hawkins, S.J., Borja, Á., Marques, J.C., Neto, J.M., 2016. Ability of invertebrate indices to assess ecological condition on intertidal rocky shores. Ecological Indicators 70, 255-268.

DOI: 10.1016/j.ecolind.2016.06.004

Abstract

The implementation of directives such as the European Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) has promoted the development of several tools and methods for assessing the ecological health of marine ecosystems. Within the scope of the WFD and in terms of rocky shores, several multimetric tools were developed based on the macroalgae biological quality element (BQE), in addition to those based on macroinvertebrates.

The WFD requires Member States to assess each BQE separately. The present work aimed to test the ability of ecological indices to distinguish sites within anthropogenic disturbance gradients caused by organic enrichment, using macroinvertebrate communities on intertidal rocky shores. Owing to the lack of more specific indices (for rocky shore), indices based on abundance, diversity and/or taxonomic composition were selected from several widely used indices in ecological studies and/or developed for soft-bottom macroinvertebrate communities.

Present findings reveal several indices based on diversity and/or taxonomic composition able to distinguish sites within the disturbance gradients, showing increasing quality from the site nearest the source of organic enrichment to that farthest from it, especially indices calculated using biomass data, and in the summer season. Such results open good perspectives for the use of intertidal macroinvertebrate communities from rocky shores, and also help add the perspective of this biological quality element in the ecological quality assessment of coastal waters.

Keywords: EU Water Framework Directive; Diversity; Taxonomic composition; Ecological indices; Coastal; Benthic macroinvertebrates.

II.1. Introduction

Over the last 15 years, the implementation of directives such as the Water Framework Directive (WFD, Directive 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/ 56/EC) in Europe has triggered the development of a great number of tools and methods for assessing the health of marine ecosystems (Birk et al., 2012; Borja et al., 2016).

In coastal waters, subject to the WFD, rocky shores are considered of vast importance. They support high biological diversity and supply a wide variety of ecosystem goods and services including primary productivity, biofiltration, fish nursery grounds, shellfisheries, recreation and tourism (e.g., Liquete et al., 2013; Galparsoro et al., 2014). Coastal rocky shores are subject to multiple pressures, ranging from global environmental change to regional and local scale impacts (Thompson et al., 2002) requiring assessment monitoring and management actions in order to improve quality.

Macroalgae and benthic macroinvertebrates seem to be the most suitable Biological Quality Elements (BQEs) required by the WFD to be used, separately, in quality assessments on rocky shores (WFD, Directive 2000/60/EC). Accordingly, several multimetric indices, combining simpler metrics which provide complementary information on a system (Salas et al., 2006; Marques et al., 2009), have been developed based on macroalgal communities (Ballesteros et al., 2007; Juanes et al., 2008; Neto et al., 2012; Ar Gall and Le Duff, 2014), although such is not the case of benthic macroinvertebrates (however see Hiscock et al., 2005; Orlando-Bonaca et al., 2012; and O'Connor, 2013).

To date, few attempts have been made to develop a multimetric tool compliant with the WFD requirements based solely on rocky shore macroinvertebrate communities. For example, Díez et al. (2012) argued that few invertebrate taxa would correlate with a disturbance gradient and, thus, a multimetric tool for rocky shores should be more reliable with attributes of both macroinvertebrates and algae being taken into account. In contrast, Vinagre et al., (2016a) showed that benthic macroinvertebrate communities responded similarly to benthic macroalgal communities along disturbance gradients (organic enrichment) with several opportunistic species being more abundant in sites near the source of disturbance and decreasing farther away from it, with the converse occurring for sensitive species. Therefore, multimetric tools for rocky shores based solely on macroinvertebrate communities were not developed yet, but this is a gap in fulfilling WFD requirements.

The present work tested the ability of several ecological indices, using macroinvertebrate communities on intertidal rocky shores, to distinguish sites within anthropogenic disturbance gradients caused by organic enrichment. For this purpose, available indices based on abundance (density and biomass), diversity and/or composition, were applied to rocky intertidal data. Some had been widely used before in ecological studies while others were developed specifically for soft-bottom macroinvertebrate communities (e.g., Salas et al., 2006; ICES, 2008; Marques et al., 2009; Pinto et al., 2009; Martínez-Crego et al., 2010). Indices were used to measure seasonal and spatial variations within disturbance gradients, and results compared with that of an established multimetric tool, developed for use with macroalgae – the MarMAT (Marine Macroalgae Assessment Tool) (Gaspar et al., 2012; Neto et al., 2012). To validate the response of these indices, those showing the best performance were applied to an independent dataset (gathered from different years and sites) and compared with the response provided by the MarMAT. The comparison against physical-chemical parameters was not considered here due to the lack of a robust time series of the study areas, and also hydro-morphological modifications cannot be considered significant on open shores where the study site is located. The verification against the BQE macroalgae was selected due to the accumulated effects they can record over long periods from the recent past, which is not possible with putative measuring of physical-chemical parameters.

In parallel fashion and as a secondary objective, the suitability of response of a particular index along each shore and in each season was analysed, using data from particular intertidal zones (e.g., upper, mid, lower) separately, to check the concordance of results between the whole intertidal zone and certain sampling levels. This is because rocky shores may not provide the three sampling levels encompassed at this time and some levels may show better response to disturbance than others, which should be taken into account for the assessments.

II.2. Material and methods

II.2.1. Study area

Two shores on the western Portuguese coast (Fig. II.1A), Buarcos (40°10'14.2"N, 8°53'26.7"W) and Matadouro (38°58'31.5"N, 9°25'14.4"W) (exposed and moderately exposed Atlantic Coast typologies, respectively; TICOR project, Bettencourt et al., 2004, available at http://www.ecowin.org/ticor), were studied. The sampling areas were subject to a point source of pollution (SOP) discharging almost directly into the upper intertidal zone (Figs. II.1B, C). On each shore the discharge is low but continuous throughout the year, crossing urban centres and agricultural land before reaching the shore (Vinagre et al., 2016a).

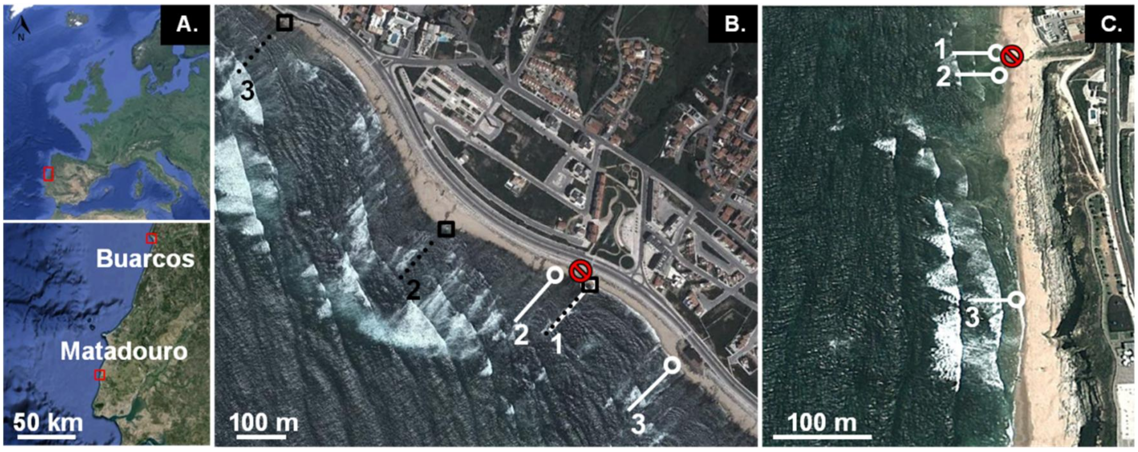


Figure II.1. *Study site locations: A. Europe and Portugal. B. Buarcos (40°10'14.2"N, 8°53'26.7"W). C. Matadouro (38°58'31.5"N, 9°25'14.4"W). Sampling sites = white circles with solid line; Validation sites = black squares with dotted line; Source of pollution (SOP) = ⊘ sign.*

II.2.2. Sampling design

Three sites (sites 1-3) were selected in the intertidal area at Buarcos and at Matadouro to characterise the disturbance gradients at about 30-40, 50-60 and 300m from the SOP (Figs. II.1B, C). At each site three horizontal zones were identified, naturally defined by tides – upper intertidal (submersed for ~25% of the tide period, ~6h day^{-1}), mid intertidal (submersed for ~50% of the tide period, ~12h day^{-1}) and lower intertidal (submersed for ~75% of the tide period, ~18h day^{-1}). Four random replicates (12 cm x 12 cm squares) were collected from each zone. Samples were taken twice in summer (S1 and S2, in August and September 2011, respectively) and twice in winter (W1 and W2, in February and March 2012, respectively), during low water of spring tides. Open, freely draining rock was sampled avoiding pools and crevices. The samples (144 from each shore) were immediately preserved after sampling in neutralised 4% formalin solution (prepared with sea water).

Parallel to biological sampling, water samples were collected at each site and at the SOP for quantification of chlorophyll a (Chl.a) (mg m^{-3}) (Strickland and Parsons, 1972), total suspended solids (TSS) (g L^{-1}), particulate organic matter (POM) (g L^{-1}), and determination of dissolved nutrient concentration (mg L^{-1}) [N-NO_3, N-NO_2, N-NH_4, P-PO_4 (DIP) and silica]. Nutrients were analysed by colorimetric reaction using a Skalar San++® Continuous Flow AutoAnalyzer (Skalar, 2010). Dissolved inorganic nitrogen (DIN) was determined as the sum of N-NO_3, N-NO_2 and N-NH_4. Simultaneously to sampling, water temperature (ºC), conductivity (µS cm^{-1}), oxidation-reduction potential (ORP) (mV), salinity, dissolved oxygen (DO) (%) and pH parameters were measured *in situ* (using a YSI Professional Plus handheld multiparameter probe). For complete details of the laboratory procedures see Vinagre et al. (2016a).

This first dataset was used for all statistical analyses and for the calculation of ecological indices. A second dataset was used to validate indices performance. It included independent data from Buarcos, gathered in September 2009 (henceforth designated as 'summer') and March 2010 (henceforth designated as 'winter') from different sites (except site 1) within the disturbance gradient (using the same methodology as for the first dataset) (Fig. II.1C).

II.2.3. Statistical analysis

All statistical analyses were done with PRIMER 6 + PERMANOVA© software (Clarke and Gorley, 2006; Anderson et al., 2008), with the exception of the principal component analyses (PCA) and corresponding ordinations, which were performed using CANOCO 4.5 for Windows (ter Braak and Šmilauer, 2002).

II.2.3.1. Environmental data

The environmental parameters (Env.) were used to place the sampling sites within the disturbance gradients by performing principal coordinate (PCO) analyses. The Euclidean similarity measure was used in the calculation of similarity matrices, after square root transformation of data (except for DIN, Chl. a and Silica, 1/X was used in these cases) to approach normality, followed by normalisation. Statistically significant differences between shores, between sampling sites within shore and between seasons were tested using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). This included three fixed factors, ‘Shore’ (two levels: Matadouro and Buarcos), ‘Site’ (nested in Shore; four levels: SOP and sites 1, 2, and 3) and ‘Season’ (two levels: summer and winter). The above-mentioned similarity matrices were used. The statistical significance of variance components was tested using 9999 permutations and unrestricted permutation of raw data, with a significance level of $\alpha = 0.05$.

Table II.1. *Ecological indices used to assess the ecological condition of macroinvertebrate communities.*

Ecological indices (density / biomass)		Calculation	Symbols meaning	Reference
Indices based on species abundance and diversity	D_{opp} / B_{opp}	Number of individuals m^{-2} (density)	D_{opp} and B_{opp} are the total density and biomass of species assigned to ecological groups (*EG*) *III-V*	Borja et al., 2000; Vinagre et al., 2016a
	D_{sens} / B_{sens}	g AFDW m^{-2} (biomass)	D_{sens} and B_{sens} are the total density and biomass of species assigned to *EG I-II*	Borja et al., 2000; Vinagre et al., 2016a
	S	Number of species/taxa		
	d / d_B	$(S - 1) / \ln N$	*S* is the number of taxa and *N* is the number of individuals in a sample	Margalef, 1968
	ES_{1000} / ES_{10}	$\Sigma \{1-[(N - Ni)!(N - 50)] / [(N - Ni - 50)!N!]\}$	*N* is the number of individuals in a sample and *Ni* is the number of individuals of the *i*-th species	Hurlbert, 1971
	H' / H'_B	$1 - \Sigma (pi \log_2 pi)$	p*i* is the proportion of individuals belonging to species *i* in the sample	Shannon and Weaver, 1963
	1-λ' / 1-λ'_B	$1 - \Sigma [Ni \times (Ni - 1)] / [N \times (N - 1)]$	*N* is the number of individuals in a sample and *Ni* is the number of individuals of the *i*-th species	Simpson, 1949
	W-Statistic	$\Sigma (Bi - Ai) / 50 (S-1)$	*Bi* is the biomass of species *i*, *Ai* is the density of species *i* and *S* is the number of species in a sample	Warwick and Clarke, 1994
	ISEP	$\log_{10} (1/SEP + 1)$	*SEP* is the Shannon–Wiener evenness proportion index (ratio between H'_B and H')	McManus and Pauly, 1990; Yoo et al., 2010
Indices based on species composition	AMBI / AMBI_B	[(0 x %*EG I*) + (1.5 x %*EG II*) + (3 x %*EG III*) + (4.5 x %*EG IV*) + (6 x %*EG V*) / 100]	*EG I* are species that are sensitive to organic enrichment, *EG II* are species indifferent to enrichment, *EG III* are species tolerant to excess organic matter enrichment, *EG IV* are 2nd order opportunistic species and *EG V* are 1st order opportunistic species	Borja et al., 2000; Borja and Muxika, 2005
	MEDOCC / MEDOCC_B	[(0 x %*EG I*) + (2 x %*EG II*) + (4 x %*EG III*) + (6 x %EG *IV-V*) / 100)]		Pinedo and Jordana, 2008
	BENTIX / BENTIX_B	[6 x (%*EG I-II*) + 2 x (%*EG III-V*) / 100]		Simboura and Zenetos, 2002
	BO2A / BO2A_B	$\log \{[f_{oa} / (f_{sa} + 1)] + 1\}$	f_{oa} is the opportunistic annelida (Clitellata and Polychaeta) frequency (i.e. the ratio of the total number of opportunistic annelid individuals to the total number of individuals) and f_{sa} the sensitive Amphipoda frequency (i.e. the ratio of the total number of sensitive amphipod individuals, excluding the opportunistic Jassa spp., to the total number of individuals in the sample	Dauvin and Ruellet, 2009

II.2.3.2. Biological community composition

Prior to data analysis, macroinvertebrate density and biomass data were standardised to ind m^{-2} and g ash free dry weight (AFDW) m^{-2}, respectively.

Statistically significant differences between shores, between sites within shore and between seasons were tested in terms of macroinvertebrate density (D_{inv}) and biomass (B_{inv}) using PERMANOVA. It included four fixed factors, ‘Shore’ (as above), ‘Site’ (three levels: sites 1, 2, and 3), ‘Zone’ (nested in ‘Site’; three levels: upper intertidal, mid intertidal and lower intertidal) and ‘Season’ (as above). The Bray-Curtis similarity measure was used in the calculation of similarity matrices, after the fourth root transformation of data (to reduce natural species dominance). The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model with a significance level of $\alpha = 0.05$.

II.2.4. Ecological indices

II.2.4.1. Candidate indices

To illustrate the ecological condition of hard substratum communities, 23 ecological indices were calculated (Table II.1). Owing to the lack of indices based on rocky shore benthic macroinvertebrates, the indices were selected from among many traditionally used in ecology studies (e.g., Salas et al., 2006; ICES, 2008; Marques et al., 2009; Pinto et al., 2009; Martínez-Crego et al., 2010) and/or developed based on soft bottom communities: (i) basic descriptors: total density and biomass of opportunistic taxa [from AZTI’s Marine Biotic Index (AMBI, Borja et al., 2000) ecological groups (EG) III-V] (Dopp and Bopp, respectively), total density and biomass of sensitive taxa (from AMBI EG I-II) (D_{sens} and B_{sens}, respectively) and number of taxa (S); (ii) diversity indices: Margalef index (Margalef, 1968), Hurlbert index (Hurlbert, 1971), Shannon-Wiener index ($\log_2$) (Shannon and Weaver, 1963) and (complement of) Simpson index (Simpson, 1949), calculated with density data – d, ES_{1000}, H’ and 1-λ’, respectively – and biomass data– d_B, ES_{10}, H’_B and 1-λ’_B, respectively; (iii) indices based on species biomass and density: W-statistic index (Warwick and Clarke, 1994) and Inverse Shannon-Wiener Evenness Proportion (ISEP, McManus and Pauly, 1990; Yoo et al., 2010); and (iv) indices based on indicator species/ecological strategies: AMBI, MEDiterranean OCCidental index (MEDOCC, Pinedo and Jordana, 2008), Bentix biotic index (BENTIX, Simboura and Zenetos, 2002) and Benthic Opportunistic Annelida Amphipod index (BO2A, Dauvin and Ruellet, 2009) calculated with density data, and AMBI_B, MEDOCC_B, BENTIX_B and BO2A_B calculated with biomass data. All taxa found at this time were assigned to one EG; taxa which were not present on the AMBI list (of November 2014; available at http://ambi.azti.es) were assigned using expert judgement (Appendix II.1). All indices were calculated per replicate.

II.2.4.2 Performance of candidate indices

To check the ability of the indices to distinguish sites across the disturbance gradients, they were compared with the assessment provided by the MarMAT – Marine Macroalgae Assessment Tool (Neto et al., 2012) for those sites (1, 2 and 3) within the gradient observed on each shore (Buarcos and Matadouro). This is a multimetric methodology compliant with the WFD requirements, based on the 'Composition' (Chlorophyta, Phaeophyceae and Rhodophyta) and 'Abundance' (coverage of opportunists) of rocky shore macroalgae. For methodological reasons, the assessment with the MarMAT, as defined by the authors (see Gaspar et al., 2012 and Neto et al., 2012), was performed only in summer at the site level within shore.

The results achieved by the selected indices were compared with Ecological Quality Ratio (EQR) values obtained by the MarMAT for the same sites in summer 2011. Specifically, it was assessed whether the relative classification obtained by each index for sites within the gradient followed, as expected, the same order as the MarMAT results (e.g., the ecological condition presented by the indices followed the same order as the MarMAT: EQR of site 1 < site 2 < site 3). These indices results, based on macroinvertebrates, could agree with the MarMAT (summer assessment) for every sampling event during the season (S1 and S2 in summer, and W1 and W2 in winter), and also with the average value of a season (summer as the average of S1 and S2, and winter as the average of W1 and W2). Depending on this agreement level with the MarMAT assessment results, the indices tested were included in one of the following classes:

Class 1 – the index results in every sampling event within the season and in the average value of the respective season (e.g., S1, S2 and summer average) agreed with the MarMAT (summer) assessment;

Class 2 – the index results in one of the sampling events within the season and in the average value of the respective season (e.g., S1 and summer average) agreed with the MarMAT assessment;

Class 3 – the index either in one of the sampling events within the season or in the average value of the respective season agreed with the MarMAT assessment;

Class 4 – all remaining scenarios, i.e., in any sampling event or seasonal average, the index showed inverse behaviour compared to the MarMAT.

II.2.4.3 Agreement between environmental parameters and indices results

To evaluate the relationships between the indices and environmental data principal component analyses (PCA) of the values of indices within Classes 1 and 2 (individually for each shore and season) were performed with the Env. as supplementary data. Summer and winter trends were presented for each shore. Prior to the analyses, mean values were calculated per month (August, September, February and March) for the indices data. The Env. data were transformed (as above for PCO), and the indices were centred and standardised.

II.2.4.4 Indices validation

A second dataset (data collected in ‘summer’ 2009 and ‘winter’ 2010) was used to validate the ability of indices to distinguish the sites across the disturbance gradients.

Indices classified as Class 1 and/or Class 2 (using the first dataset) were applied to the second dataset and their performance was evaluated similarly to that previously described.

II.2.4.5 Variation of indices between intertidal zones

In parallel and as a secondary objective, the variation of indices among the four defined classes was analysed (within each shore and season), selecting only particular data from: (i) the upper intertidal; (ii) the mid intertidal; (iii) the lower intertidal; and (iv) the mid and lower intertidal together, instead of using all intertidal data.

II.3. Results

II.3.1. Environmental parameters

Salinity and conductivity showed similar behaviour along both shores (Appendix II.2); salinity was lower at the SOP (<1) and was 33-37 at all remaining sites (except for one occasion, at site 1 of Matadouro in August – 4.04). Generally speaking, water temperature (9.9-22.1 ºC) was higher at the SOP in summer, and lower there in winter. Silica (0.16-19.2 mg L^{-1}), pH (6.9-8.8), Chl. a (0.3-8.7 mg m^{-3}), DIN (0.16-6.4 mg L^{-1}) and DIP (<0.2 mg L^{-1}) were all higher at the SOP, while DO (72-149%) and ORP (20-415 mV) were lower there. TSS (0.001-0.03 g L^{-1}) and POM (<0.004 g L^{-1}) behaved similarly and showed different trends at Buarcos and Matadouro. In summer, both showed higher values at the SOP at Buarcos, but the converse occurred at Matadouro. In winter, the trends were more variable: at Buarcos, the SOP had higher TSS values than the other sites in February and March, and at Matadouro the SOP had higher POM and TSS values than the other sites in February.

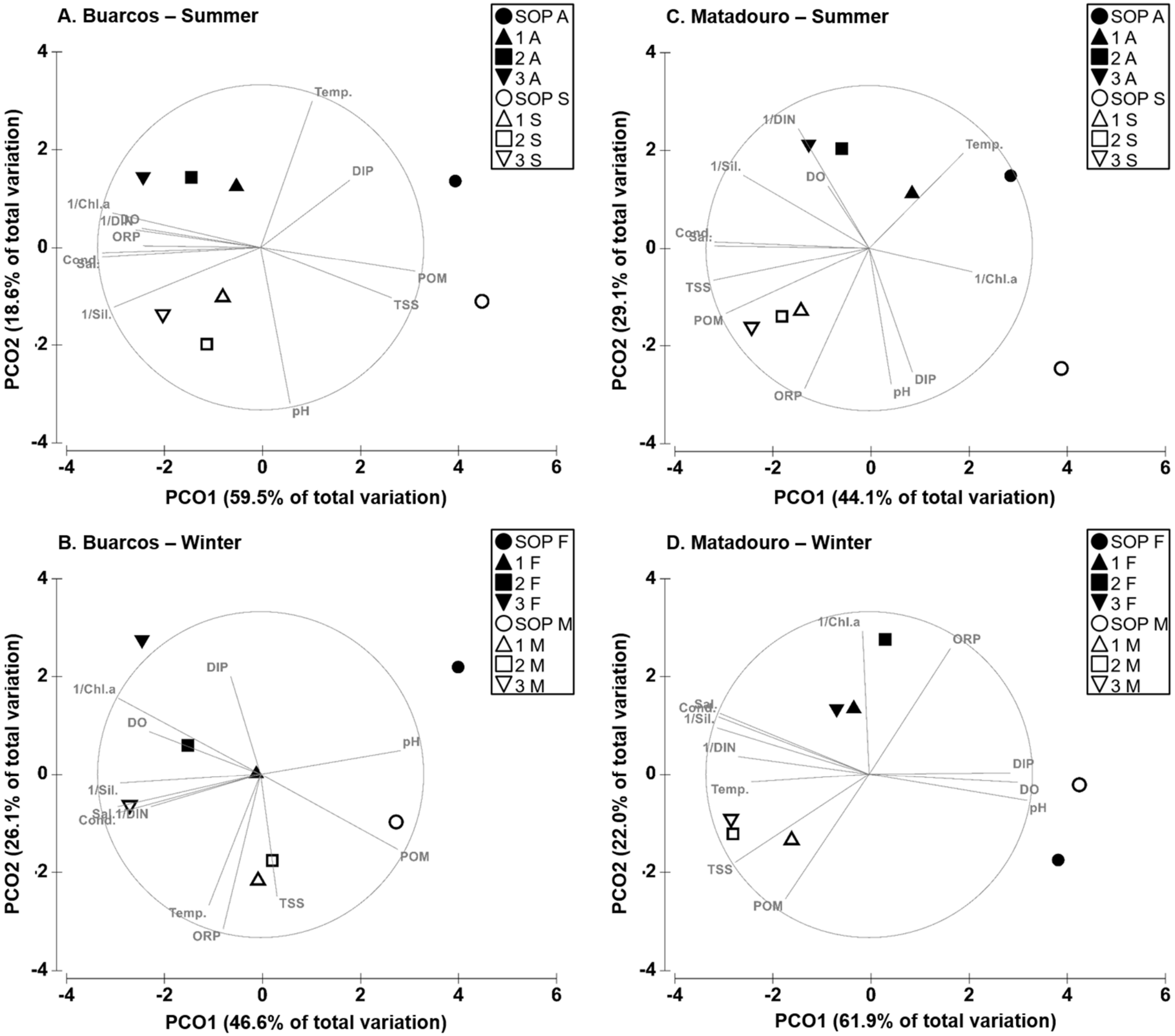


Figure II.2. Principal coordinate (PCO) analysis plots of the environmental parameters for Buarcos (A. summer and B. winter) and Matadouro (C. summer and D. winter). SOP = source of pollution; 1-3 = sites 1-3. A, S, F and M = August, September, February and March.

Using the environmental parameters, the PCO ordination showed, for both shores and in both summer and winter, a separation between the SOP and the remaining sites, generally followed by a gradual separation of site 1, site 2 and site 3. At Buarcos (Figs. II.2A, B), the SOP had higher POM, Chl. a, DIN and silica (respectively as lower 1/Chl. a, 1/DIN and 1/silica in the PCO), and lower conductivity, salinity and DO. The SOP in summer had higher TSS and DIP, and lower ORP, and in winter higher pH. At Matadouro (Figs. II.2C, D) the pattern was similar, except that the SOP had lower TSS and POM. Moreover, in summer the SOP experienced higher water temperature, and lower Chl. a (as higher 1/Chl. a in PCO), and, in winter, higher DIP, DO and pH, and lower water temperature.

Statistically significant differences were found between summer and winter (PERMANOVA; Table II.2). Differences were not found between Buarcos and Matadouro, but were found within those shores between the SOP and all other sites.

II.3.2. Biological community composition

In the present work 171 macroinvertebrate taxa were identified (Appendix II.1). Considering both Buarcos (141 taxa) and Matadouro (139 taxa), the majority of taxa in the communities were assigned to EG II (73 taxa), followed by EG I (63 taxa), EG III (26 taxa), EG IV (five taxa) and EG V (four taxa). More specifically, at the site level (taking both shores into consideration), EG I and EG II accounted for 31.0-45.2% and 35.5-45.9% of taxa found, followed EG III (11.1-17.2%), EG IV (1.0-6.3%) and EG V (0.0-3.4%) (Table II.3).

Using D_{inv} and $B_{inv,}$ statistically significant differences (PERMANOVA; Table II.2) were found between Buarcos and Matadouro in both summer and winter, and each shore was also different between seasons. Differences were found within shore between all sites, except for Buarcos in winter between sites 1 and 2 (using D_{inv} and B_{inv}) and between sites 2 and 3 (using B_{inv}). At the same time, differences were found in all sites (within shore) between summer and winter (except site 1 at Buarcos, using B_{inv}).

Table II.2. *PERMANOVA results using the environmental parameters (Env.), and invertebrate density (D_{inv}) and biomass (B_{inv}) data: A. Main tests; B. Pairwise tests (significant comparisons are presented).*

A. Main tests

Env.

	Pseudo-F	P (perm)
Shore	1.495	0.1753
Season	11.924	0.0001
Site (Shore)	4.231	0.0001
Shore x Season	0.707	0.6411
Site (Shore) x Season	0.991	0.4916

D_{inv}

	Pseudo-F	P (perm)
Shore	51.312	0.0001
Season	11.902	0.0001
Site (Shore)	8.779	0.0001
Shore x Season	5.775	0.0001
Zone [Site (Shore)]	16.763	0.0001
Site (Shore) x Season	2.554	0.0002
Zone [Site (Shore)] x Season	1.988	0.0001

B_{inv}

	Pseudo-F	P (perm)
Shore	39.850	0.0001
Season	7.526	0.0001
Site (Shore)	7.733	0.0001
Shore x Season	5.739	0.0001
Zone [Site (Shore)]	15.063	0.0001
Site (Shore) x Season	2.423	0.0001
Zone [Site (Shore)] x Season	1.996	0.0001

B. Pairwise tests

Env.

			t	P (perm)
Site (Shore)	***Buarcos***			
		SOP, site 1	2.920	0.0292
		SOP, site 2	2.591	0.0304
		SOP, site 3	3.529	0.0301
	Matadouro			
		SOP, site 1	2.248	0.0286
		SOP, site 2	2.365	0.0294
		SOP, site 3	2.788	0.0274

D_{inv}

			t	P (perm)
Shore x Season	Summer			
		Buarcos, Matadouro	5.033	0.0001
	Winter			
		Buarcos, Matadouro	5.678	0.0001
	Buarcos			
		Summer, Winter	2.670	0.0001
	Matadouro			
		Summer, Winter	3.315	0.0001

B_{inv}

			t	P (perm)
Shore x Season	Summer			
		Buarcos, Matadouro	4.497	0.0001
	Winter			
		Buarcos, Matadouro	5.076	0.0001
	Buarcos			
		Summer, Winter	2.212	0.0004
	Matadouro			
		Summer, Winter	2.955	0.0001

Table II.2 *(continued)*

B. Pairwise tests

Env.	**D_{inv}**			
Site (Shore) x Season	***Buarcos***		t	P (perm)
	Summer			
		Sites 1, 2	1.741	0.0106
		Sites 1, 3	1.809	0.0010
		Sites 2, 3	2.145	0.0002
	Winter			
		Sites 1, 3	1.806	0.0123
		Sites 2, 3	1.617	0.0242
	Site 1			
		Summer, Winter	1.660	0.0214
	Site 2			
		Summer, Winter	2.185	0.0017
	Site 3			
		Summer, Winter	2.069	0.0001
	Matadouro		t	P (perm)
	Summer			
		Sites 1, 2	1.883	0.0003
		Sites 1, 3	3.555	0.0001
		Sites 2, 3	3.403	0.0001
	Winter			
		Sites 1, 2	2.005	0.0001
		Sites 1, 3	4.601	0.0001
		Sites 2, 3	2.798	0.0001
	Site 1			
		Summer, Winter	2.602	0.0001
	Site 2			
		Summer, Winter	2.332	0.0002
	Site 3			
		Summer, Winter	2.057	0.0001

Env.	**B_{inv}**			
Site (Shore) x Season	***Buarcos***		t	P (perm)
	Summer			
		Sites 1, 2	1.582	0.0201
		Sites 1, 3	1.661	0.0035
		Sites 2, 3	0.187	0.0007
	Winter			
		Sites 1, 3	1.757	0.0100
	Site 2			
		Summer, Winter	1.902	0.0035
	Site 3			
		Summer, Winter	1.982	0.0001
	Matadouro		t	P (perm)
	Summer			
		Sites 1, 2	2.057	0.0002
		Sites 1, 3	3.382	0.0001
		Sites 2, 3	3.082	0.0001
	Winter			
		Sites 1, 2	1.835	0.0001
		Sites 1, 3	4.137	0.0001
		Sites 2, 3	2.717	0.0001
	Site 1			
		Summer, Winter	2.413	0.0001
	Site 2			
		Summer, Winter	2.158	0.0004
	Site 3			
		Summer, Winter	1.774	0.0003

Table II.3. *Number of taxa (S) and their proportion across ecological groups (EG) at Buarcos and Matadouro shores.*

	Total	Summer			Winter		
Buarcos		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
S	141	105	101	118	87	74	96
EG I	50 (35.5%)	37 (35.2%)	33 (32.7%)	43 (36.4%)	27 (31.0%)	24 (32.4%)	38 (39.6%)
EG II	61 (43.3%)	48 (45.7%)	45 (44.6%)	52 (44.1%)	39 (44.8%)	34 (45.9%)	43 (44.8%)
EG III	23 (16.3%)	15 (14.3%)	17 (16.8%)	16 (13.6%)	15 (17.2%)	12 (16.2%)	14 (14.6%)
EG IV	4 (2.8%)	3 (2.9%)	3 (3.0%)	4 (3.4%)	4 (4.6%)	3 (4.1%)	1 (1.0%)
EG V	3 (2.1%)	2 (1.9%)	3 (3.0%)	3 (2.5%)	2 (2.3%)	1 (1.4%)	0 (0%)
Matadouro							
S	139	101	90	117	79	88	93
EG I	53 (38.1%)	38 (37.6%)	37 (41.1%)	48 (41.0%)	32 (40.5%)	35 (39.8%)	42 (45.2%)
EG II	58 (41.7%)	41 (40.6%)	36 (40.0%)	46 (39.3%)	30 (38.0%)	33 (37.5%)	33 (35.5%)
EG III	20 (14.4%)	15 (14.9%)	10 (11.1%)	16 (13.7%)	11 (13.9%)	12 (13.6%)	12 (12.9%)
EG IV	5 (3.6%)	5 (5.0%)	5 (5.6%)	5 (4.3%)	5 (6.3%)	5 (5.7%)	4 (4.3%)
EG V	3 (2.2%)	2 (2.0%)	2 (2.2%)	2 (1.7%)	1 (1.3%)	3 (3.4%)	2 (2.2%)

II.3.3 Ecological indices

II.3.3.1 Performance of candidate indices

Based on macroalgae, the MarMAT tool showed increasing quality on both shores, from the site nearest the SOP (site 1) to the site farthest from the SOP (site 3). Accordingly, the MarMAT showed increasing EQR values in that direction, achieving the classifications of 'Good' for site 1 (EQR = 0.75) and 'High' status for sites 2 (EQR = 0.83) and 3 (EQR = 0.89) at Buarcos, and 'Moderate', 'Good' and 'High' status for sites 1 (EQR = 0.58), 2 (EQR = 0.78) and 3 (EQR = 0.83) at Matadouro.

Regarding the indices based on the macroinvertebrate communities, certain spatial and seasonal trends were found (Table II.4). For Buarcos, in summer (considering S1, S2 and summer average), all indices which showed good performance (i.e., following, as expected, the MarMAT trend, meaning an increase in quality from site 1 to site 3), were calculated with biomass. On the other hand, in winter (considering W1, W2 and winter average), a higher number of indices with good performance were calculated with density. In both seasons, the indices showing the expected behaviour were mostly abundance/diversity indices. The indices not showing the expected behaviour were all calculated with biomass and were usually composition indices (except the ISEP in summer, and B_{opp} in winter);

For Matadouro, both in summer (considering S1, S2 and summer average) and in winter (considering W1, W2 and winter average), a higher number of indices calculated with biomass showed good performance (although in summer, within the composition indices, the number of indices calculated with density data was always higher – four, three and four indices, against three, zero and three indices calculated with biomass, in S1, S2 and summer average, respectively). In contrast to Buarcos, a higher number of composition indices showed the expected behaviour (except for the winter average). The majority of indices showing unexpected behaviour were abundance/diversity indices (especially ISEP, which showed such behaviour in all events).

Both shores presented a higher number of indices assigned to Class 1 in summer than in winter. On the other hand, a higher number of indices assigned to Class 2 were found in winter (Table II.4).

Regarding both shores' results, concerning summer, nine indices (four at Buarcos, seven at Matadouro) were assigned to Class 1 (Table II.4), six of which were abundance/diversity indices. In Class 1, abundance/diversity indices were mainly calculated using biomass (five out of six), while the composition indices were all calculated using density (three out of three). In Class 2, four out of five were composition indices and four were calculated using biomass. The ES_{10} and H'_B (Class 1) and BENTIX_B (Class 2) were present on both shores. Concerning winter, seven indices (two at Buarcos, six at Matadouro) were assigned to Class 1, four of which were based on composition, and mainly calculated using biomass (six out of seven). In Class 2, five out of nine were composition indices and six out of nine were calculated using density. The D_{sens} (Class 1) and BO2A_B (Class 1 at Buarcos and Class 2 at Matadouro) were present on both shores.

Table II.4. *Ecological indices behaviour using the first (A.) and second (B.) datasets. A.: Dark and light grey cells represent sampling events in which indices were assigned to Class 1 and Class 2 (presented in bold), respectively. B.: Black cells represent indices which were not tested (i.e., previously assigned to Class 3 and Class 4). For both datasets, plus (+) and minus (-) signs represent agreement with the MarMAT and inverse behaviour, respectively, and indices with intermediate behaviour are coloured in white.*

	A.	Buarcos						Matadouro						**B.** Buarcos	
		S1	S2	Summer	W1	W2	Winter	S1	S2	Summer	W1	W2	Winter	'Summer'	'Winter'
Indices based on species abundance and diversity	D_{opp}													■	■
	B_{opp}			+	-			+		+	+	+	+	+	
	D_{sens}				+	+	+	+	+	+	+	+	+		
	B_{sens}		+					+	+	+		+	+		-
	S				+								+	■	■
	d				+								+	■	■
	d_B	+	+	+	+			+						+	
	ES_{1000}				+								+	■	■
	ES_{10}	+	+	+	+			+	+	+	+	+	+	+	
	H'				+		+			-		-			
	H'_B	+	+	+	+			+	+	+	+		+	+	+
	1-λ'				+		+		-	-		-			
	1-λ'_B	+	+	+				+				-		+	+
	W-statistic				+					-			-	■	■
	ISEP		-					-	-	-	-	-	-	■	■
Indices based on species composition	**AMBI**				+			+	+	+	+		+	+	
	AMBI_B		+		-			+		+	+	+	+	+	
	MEDOCC				+			+	+	+	+		+	+	
	MEDOCC_B		+		-			+		+	+	+	+	+	
	BENTIX							+	+	+	+		+	+	
	BENTIX_B		+	+	-			+		+	+	+	+	+	
	BO2A					+	+	+		+					
	BO2A_B		-	-	+	+	+					+	+	-	
	Agreement with the MarMAT	4	8	6	13	3	5	14	7	12	10	8	15		
	Inverse to MarMAT	0	2	1	4	0	0	1	2	4	1	4	2		

II.3.3.2 Agreement between environmental parameters and indices results

Taking into account both the indices (assigned to Class 1 and Class 2) and the Env. data, the first two axes of the PCA explained 95.2% of the total variability found among the indices for Buarcos in summer (Fig. II.3A) and 89.8% in winter (Fig. II.3B). For Matadouro, the first two axes of the PCA explained 93.1% of the total variability found among the indices in summer (Fig. II.3C) and 93.7% in winter (Fig. II.3D). In all cases, the PC1 was related more to the disturbance gradient, in which a gradual separation from site 1 was visible, closer to the SOP, to site 3, while PC2 was more related to variations during the season. Within the gradients, the indices showed an increase in quality from site 1 to site 3, namely B_{opp}, AMBI, AMBI_B, MEDOCC, MEDOCC_B, BO2A and BO2A_B, which presented higher values closer to site 1 (where applicable), and were more correlated to higher values of parameters associated with anthropogenic disturbance/degradation (e.g., DIN, DIP, Chl. a). On the other hand, the indices d_B, H', H'_B, 1-λ', 1-λ'_B, ES_{10}, BENTIX, BENTIX_B, D_{sens} and B_{sens} presented higher values closer to site 3 (where applicable), and were more correlated to lower values of those parameters.

II.3.3.3 Validation of indices

From the first dataset (23 indices), 17 indices (assigned to Class 1 and Class 2) were selected and applied to the second dataset (Table II.4). The excluded indices were all calculated using density (except S, W-statistic and ISEP). Of the 17 indices, 11 indices showed agreement with the MarMAT (the 11 in 'summer', two in 'winter'). Six (out of those 11) were indices based on composition (found only in 'summer'), and eight were calculated using biomass (the eight in 'summer', two in 'winter'). Only the H'_B and 1-λ'_B were assigned to 'Class 1' in both seasons. Considering the two data sets used in this work, only the ES_{10}, H'_B and BENTIX_B were assigned to Class 1 (the first two indices) or Class 2 (the third index), in 'summer'.

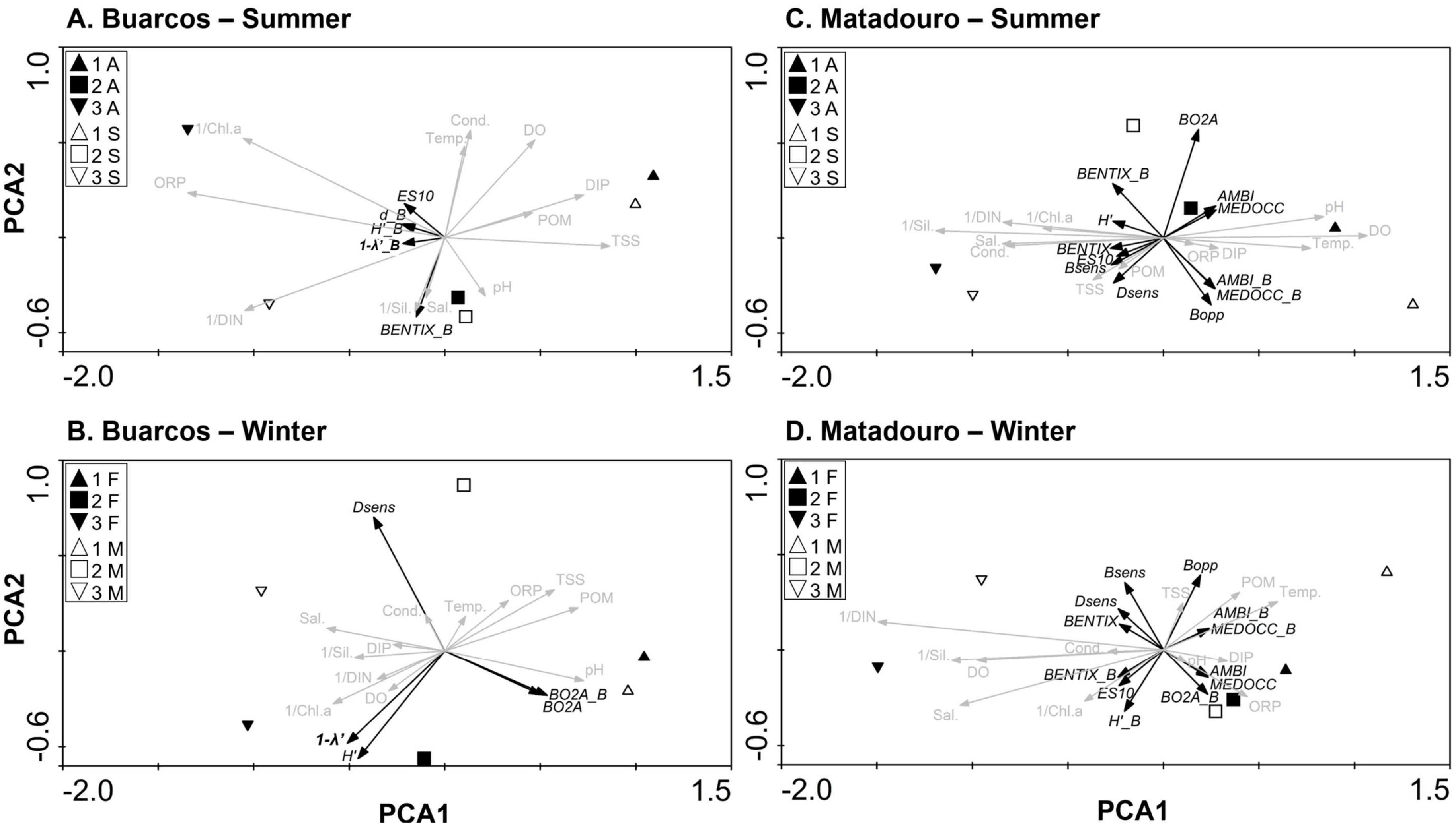


Figure II.3. *Principal component analysis (PCA) plots of ecological indices, plotting environmental parameters as supplementary variables, for Buarcos (A. summer and B. winter) and Matadouro (C. summer and D. winter). 1-3 = sites 1-3. A, S, F and M = August, September, February and March.*

Table II.5. *Indices behaviour between intertidal zones. Dark and light grey cells represent sampling events in which indices were assigned to Class 1 and Class 2 respectively. For all cases, plus (+) and minus (-) signs represent agreement with the MarMAT and inverse behaviour, respectively, and indices with intermediate behaviour are coloured in white.*

		Buarcos						Matadouro					
		S1	S2	Summer	W1	W2	Winter	S1	S2	Summer	W1	W2	Winter
Upper intertidal													
Indices based on species abundance and diversity	B_{opp}	-		+		-		+	-	+	+		+
	D_{sens}			-	+	+		+	+	+	+	+	+
	B_{sens}						+	+	+	+	+	+	+
	d_B		-		-	-	-	-			-	-	-
	ES_{10}							+	+	+			
	H'		-					-		-	-		
	H'_B	+	-	-									
	1-λ'							-	-	-	-	-	
	1-λ'_B	+						+					-
Indices based on species composition	AMBI					+	+	+	+	+	+	+	+
	AMBI_B				-	-	-	+	+	+	+	+	+
	MEDOCC					+		+	+	+	+	+	+
	MEDOCC_B				-	-	-	+	+	+	+	+	+
	BENTIX		+			+		+	+	+	+	+	+
	BENTIX_B				-	-	-	+	+	+	+	+	+
	BO2A		+	+		+	+	+					
	BO2A_B		+	+	+	+	+	+		+			
Mid intertidal													
Indices based on species abundance and diversity	B_{opp}	+				+	+	+		+		+	
	D_{sens}	+	+	+	+			+	+		+		
	B_{sens}			+									-
	d_B			+	+		+	+		+	+		
	ES_{10}	+	+	+		+		+	+	+		+	
	H'		-	-					-				
	H'_B	+	+	+		+		+	+	+		+	
	1-λ'			-									
	1-λ'_B	+		+		+		+		+		+	
Indices based on species composition	AMBI		+	+					+				
	AMBI_B	+				+		+				+	+
	MEDOCC		+	+					+				
	MEDOCC_B	+				+		+				+	+
	BENTIX	+	+	+				+	+				-
	BENTIX_B	+				+		+				+	+
	BO2A		+	+	+				+		+		+
	BO2A_B	-		-		+	+	-		-		+	+

***Table II.5** (continued)*

		Buarcos						Matadouro					
		S1	S2	Summer	W1	W2	Winter	S1	S2	Summer	W1	W2	Winter
Lower intertidal													
Indices based on species abundance and diversity	B_{opp}	+	+	+					+			+	+
	D_{sens}				+					-	-	+	-
	B_{sens}							+		+			
	d_B		+	+					+				
	ES_{10}								+	+		+	
	H'			+	+			+		+	+		+
	H'_B	+		+				+	+	+	+		
	1-λ'			+	+			+	+	+	+		+
	1-λ'_B			+				-	+	+			
Indices based on species composition	AMBI	+			+			-	-	-			
	AMBI_B								+	+	+	+	+
	MEDOCC	+			+			-		-			
	MEDOCC_B								+	+	+	+	+
	BENTIX				+			-					
	BENTIX_B								+		+	+	
	BO2A		-		+	+	+	+		+		+	+
	BO2A_B				+	+	+					+	+
Mid + lower intertidal													
Indices based on species abundance and diversity	B_{opp}	+	+	+				+				+	+
	D_{sens}		-		+	-		+		+			
	B_{sens}	+		-			-	+		+			
	d_B	+	+	+	+			+	+	+	+	+	+
	ES10	+	+	+				+	+	+		+	
	H'				+			+		+	+		
	H'_B	+	+	+	+			+	+	+	+	+	+
	1-λ'				+			+	+	+			
	1-λ'_B	+	+	+				+	+	+			+
Indices based on species composition	AMBI								+				
	AMBI_B			+	-			+				+	+
	MEDOCC								+	+			
	MEDOCC_B			+	-			+				+	+
	BENTIX				-				+	+			
	BENTIX_B	+	+	+	-			+				+	+
	BO2A		-			+	+	+	+	+			
	BO2A_B		-	-	+	+	+					+	+

II.3.3.4 Variation of indices between intertidal zones

Regarding both Buarcos and Matadouro, and considering both summer and winter assessments, several changes were observed in the behaviour of different indices (from those previously included in Class 1 and 2) (Table II.5), as follows:

i) including only the upper intertidal, the indices based on abundance/diversity generally performed worse than when using all of the intertidal. On the other hand, the indices based on composition performed better, moving from Class 4 and Class 3 to Class 2, and in most cases from Class 2 to Class 1. Within these indices, generally those calculated with density improved in winter, while the ones calculated with biomass improved in summer. Overall, four abundance/diversity indices (three in summer, three in winter) and eight composition indices (the eight in each season) showed good performance (assigned to Class 1 or Class 2).

ii) including only the mid intertidal, both the abundance/diversity and the composition indices performed worse. Nevertheless, four abundance/diversity indices (at Buarcos: one in summer and two in winter; at Matadouro: one in summer) and four composition indices (in summer, three at Buarcos and one at Matadouro) showed improved performance, the first indices moving from Class 4 and Class 3 to Class 2 and from Class 4 to Class 1, and the second indices from Class 4 to Class 2 and Class 1 and from Class 3 to Class 2. Overall, six abundance/diversity indices (five in summer, two in winter) and eight composition indices (four in each season) showed good performance.

iii) including only the lower intertidal, as when including only the mid intertidal, both the abundance/diversity and the composition indices performed less well. Despite that, three abundance/diversity indices (at Buarcos: one in summer; at Matadouro: two in summer and the three in winter) improved, moving from Class 4 to Class 2 and Class 1. One composition index improved (in winter), moving from Class 2 to Class 1 (at Buarcos) and from Class 4 to Class 2 (at Matadouro). Overall, seven abundance/diversity indices (the seven in summer, three in winter) and five composition indices (three in summer, the five in winter) showed good performance;

iv) including the mid and lower intertidal together, results were more variable. In summer, the abundance/diversity indices and the composition indices performed better at Buarcos (one index of each type moving from Class 3 and Class 2 to Class 1). At Matadouro the abundance/diversity indices generally improved (four indices, against two indices which worsened), but the composition indices generally worsened (five indices, against two which improved). Five indices improved, moving from Class 4 to Class 2 and from Class 4 and Class 3 to Class 1. In winter, the performance of abundance/diversity indices worsened on both shores, and the composition indices were maintained (at Buarcos) or worsened (at Matadouro). Overall, nine abundance/diversity indices (the nine in summer, three in winter) and seven composition indices (four in summer, five in winter) showed good performance.

II.4. Discussion

II.4.1. Assessment of the disturbance gradients

The disturbance gradients within the surveyed shores had already been pointed out for summer (Vinagre et al., 2016a), with regard to not only several environmental parameters (e.g., DIN, DIP), but also the benthic macroinvertebrate and macroalgal communities. Here, these findings were reinforced for summer and extended for the winter period. The gradients were similar in both seasons on both shores (Fig. II.2; Table II.2), when the values of parameters usually related to anthropogenic disturbance (e.g., organic enrichment) decreased gradually from the source of pollution (SOP) towards the site (3) farthest away from the SOP. Differences in the macroinvertebrate communities corroborate these results, since statistical differences were found between sites within each shore, and fewer differences were found (at Buarcos) between sites (1 and 2) closer to the SOP, and between sites (2 and 3) farther away from the SOP (Table II.2). Furthermore, the MarMAT presented increasing quality from site 1 to site 3 (i.e., increasing EQR values in that direction), highlighting the existence of disturbance gradients on both shores.

II.4.2 Indices performance

II.4.2.1 Within the disturbance gradients

Several of the candidate indices showed good performance when assessing the studied sites (i.e., following, as expected, the trend of the MarMAT). At Buarcos, a higher number of abundance/diversity indices showed good performance in both seasons, in contrast to Matadouro where a higher number of indices based on composition showed that result. Moreover, at Buarcos a higher number of indices calculated with biomass and density, respectively for summer and winter, showed good performance, while at Matadouro, this was shown in both seasons by indices calculated using biomass data (which was more evident in summer, and less in winter).

Despite the existing differences, on both shores and in both seasons, several indices based either on abundance/diversity or composition were able to capture the pressure gradients across sites. With the first approach, 17 metrics (assigned to Class 1 and Class 2 on different occasions), of a total of 23, were selected for validation. It was confirmed that several of the indices tested were able to distinguish sites within the disturbance gradients, namely in summer, which showed far more indices (11) in agreement to the MarMAT, compared to winter (two indices). This could be related to different aspects that eventually mask the organic enrichment effects. The harsh winter conditions (e.g., wave energy and currents) and simultaneously the less suitable light and temperature environment may cause disruptions in the rocky shore community and reduced development (e.g., reproduction and growth) of its species. Difficulties in sampling and the lower abundances naturally found in winter may cause the indices to show worse performance (namely the ones based on composition) (Borja and Muxika, 2005; Dauvin and Ruellet, 2007; Keeley et al., 2012), when compared to summer. And too, the majority of indices in summer (and all in winter) with such performance were those calculated using biomass (including both abundance/diversity and composition indices).

It is widely assumed that, at least in the case of soft-bottom ecosystems, macroinvertebrate communities subject to organic enrichment may exhibit, among other trends, a decrease in species richness, accompanied by an increase in density (owing to a higher number of opportunistic individuals, in small size and in short life cycle species) and a decrease in individual average biomass (Pearson and Rosenberg, 1978). However, on rocky shores, such disturbance may lead to an increase of filter-feeder species with high biomass, such as mussels, which are normally assigned to EG III. Also, this dominance of filter-feeders, which are ecological engineers, can increase the habitat availability and some increased richness could take place. This was reflected in the communities found at this time which, like other European intertidal rocky shores, are composed of a high number of species and richness (in number of species) was in some cases positively influenced by intermediate disturbance (e.g., Archambault et al., 2001; Magurran and McGill, 2010; Bustamante et al. 2012; Díez et al., 2012; O'Connor 2013; Cabral-Oliveira et al., 2014b; Vinagre et al., 2016a). Despite such high richness, communities along such rocky shores are usually dominated by few species, namely barnacles and mussels (both filter-feeders and space occupiers), which appear naturally in dense assemblages and account mostly for the densities and biomass found there (e.g., Boaventura et al., 2002, Araújo et al., 2005; Pereira et al., 2006; Vinagre et al., 2016a).These might be the reasons for the higher agreement observed between composition indices and the MarMAT, when compared to indices based on abundance/diversity data, and the better performance shown by indices calculated using biomass data, compared to those calculated using density.

The effectiveness of the indices tested in evaluating anthropogenic impact (namely organic enrichment) has been reported worldwide, not only when using macroinvertebrate density in the calculations, but also when using biomass (e.g., Warwick et al., 2010; Muxika et al., 2012; Cai et al., 2014; Mistri and Munari, 2015). Among these indices, the top three consist of two abundance/diversity indices – H'_B and ES_{10} – and one composition index – BENTIX_B, which were calculated using biomass and showed the greatest consistency in their performance, presenting the best correspondence between the two datasets used (the first two were assigned to Class 1 and the third assigned to Class 2 in both cases). The Shannon-Wiener (H') and Hurlbert (ES_n) diversity indices are commonly used in benthic ecology and have widely shown good sensitivity to and correlation with anthropogenic impacts (e.g., Boon et al., 2011; Borja et al., 2011; Spagnolo et al., 2014). The BENTIX index, like the AMBI and MEDOCC, is based on the relative individual percentages of species classified into ecological groups according to their sensitivity or tolerance to disturbance (organic enrichment). However, compared to AMBI and MEDOCC, BENTIX simplifies the assessment of taxa to two ecological groups – sensitive (EG I-II) and tolerant (EG III-V) and, thus it could offer better resolution to the disturbance gradient than the others, namely on organic enriched rocky shores where EG III tends to dominate. Furthermore, the higher performance of BENTIX compared to AMBI and MEDOCC could be due to the different structures of the indices, since the communities surveyed are mainly composed of species from EG I-II which in AMBI and MEDOCC have a small weighting compared to BENTIX (e.g., AMBI gives a weighting coefficient of 0 and 1.5 to % EG I and % EG II, respectively, while BENTIX gives a weighting coefficient of 1.5 to % EG I-II).

Of these three composition indices, AMBI (from which the other two have been adapted) is commonly included in multimetric tools based on soft-bottom macroinvertebrate communities (together with H', among other) (Muxika et al., 2007; Teixeira et al., 2009). Nevertheless, BENTIX now showed better performance than the other two indices (for the reasons mentioned above), which had been found elsewhere (e.g., Kalkan et al., 2007; Pranovi et al., 2007; Simboura and Argyrou, 2010; Spagnolo et al., 2014; Basatnia et al., 2015).

In the present work several 'universal' ecological indices (e.g. H') and others, which were developed based on soft-bottom communities (e.g., AMBI), were tested. On the Portuguese (and worldwide) coasts, rocky shores are commonly surrounded by sandy beaches, with intermixing of sand with the biota attached to hard substratum being common (Littler, 1980; Littler et al., 1983). This could explain the great number of taxa which could be assigned to EG from the AMBI list. Despite the fact that sedimentation rates on the surveyed shores were not measured, the authors acknowledge that the level of natural sedimentation was similar across sites on both shores, and such an impact (i.e. potential increase of diversity and abundance of opportunistic species in sites closer the disturbance) (e.g., Littler et al., 1983; Airoldi et al., 1995; Airoldi, 2003) should not be relevant in influencing the distribution of (sensitive/tolerant) taxa across sites within the disturbance gradients.

Of all indices tested, the H'_B, ES_{10} and BENTIX_B presented the best overall performance in distinguishing sites within the disturbance gradients, and they fulfilled the requirements of the WFD to include the communities' abundance, diversity and composition attributes in quality assessments, thus becoming potential candidate indices for integration into multimetric tools based solely on rocky shore macroinvertebrate communities. Even though it should be pointed out that higher disturbance levels were lacking, the MarMAT was never able to identify lower levels of quality (such as poor and bad) among study sites, meaning that more situations (with a complete disturbance gradient) should be analysed.

On rocky shores there is an intrinsic relation between algae and sessile invertebrates, i.e. i) all compete for the same limited resource space (Raffaelli and Hawkins, 1999); ii) many algae provide habitat for invertebrates (especially turf-forming species that trap sediments); iii) top-down control of algal biomass through grazing primarily by patellid limpets occurs on the most exposed and moderately exposed shores (Hawkins, 1981; Jenkins et al, 2005; Coleman et al, 2006). Despite this, the present work has shown that a macroinvertebrate tool can be developed as a separate tool from other BQEs (e.g., macroalgae), as required by the WFD. In this study, a set of potentially useful indices for assessing the macroinvertebrate community from intertidal rocky shores was identified and proved to have suitable response to pressure. This marks the first step in the development of a multimetric assessment tool to assess the benthic ecological status of rocky shores, preceding other steps such as: (i) metric combination; (ii) index validation; (iii) index application to different human pressures; (iv) index interpretation; and (v) index intercalibration (Borja and Dauer, 2008; Borja et al., 2009b).

II.4.2.2 Between intertidal zones

Another objective of this work was to check the trend of candidate indices when assessing only one particular zone of the shore. Rocky shores are very variable worldwide, and may have different lengths of rocky substratum for sampling. They may therefore present not all of the three possible intertidal zones, or sampling the whole intertidal may not be possible due to economic (e.g., extra effort in data treatment) and/or seasonal constraints (e.g., the rise in tourism in summer, strong wave action in winter).

When only the upper zone of intertidal was considered, indices based on composition were those showing improvement in their trends (diversity indices worsened there). Compared to others, this zone presented low diversity and high dominance by the few species present, belonging mostly to EG I (e.g. barnacles), and it was also where EG V species (namely oligochaetes) showed highest abundance, which may give the zone's community a swift response to disturbance. When considering only the mid or the lower zone in the intertidal, the trends of the indices based on composition or diversity worsened, respectively. Despite this fact, more indices improved their performance in summer. Finally, considering the mid and lower zones in the intertidal together, the diversity indices maintained or improved their trends, while the indices based on composition showed poorer relative results when compared to MarMAT assessment. Summer was also the season when a higher number of indices showed agreement with MarMAT results. Of the three intertidal zones assessed (upper, mid and lower intertidal), none has explicitly shown better resolution of the disturbance gradients than the others. Regardless, the mid and lower intertidal zones are characterised by richer communities, unlike the upper zone which is dominated by only a few and therefore these two may be the more appropriate zones for use in assessments when sampling is not possible along the full intertidal region.

II.5. Conclusion

The datasets used at this time made it possible to compare the results of different ecological indices, not only within disturbance gradients, but also between seasons (summer and winter). Summer proved to be the better sampling season for two main reasons: clearer definition of biological properties of rocky shore macroinvertebrate communities, and the favourable sampling conditions found at that time. Among the tested indices, the top three were two abundance/diversity indices – H'_B and ES_{10} – and one composition index – BENTIX_B, which were calculated using biomass, and showed the greatest consistency in their performance.

When the assessment was carried out separately for each intertidal zone (upper, mid and lower intertidal), the results were not completely discrepant with the former ones. Indices such as H'_B, ES_{10} and BENTIX_B showed interesting performance and, in general, the mid/lower intertidal zones (alone) and the mid plus lower intertidal zones (together) proved to be the zones recommended for assessment when hard substratum is not available for sampling along the full intertidal.

Other indices showed good results but not as consistent as those indicated above. These findings open good perspectives on the use of intertidal macroinvertebrate communities from rocky shores to monitor ecological quality of coastal water. Within the scope of WFD, requirements such as the communities' abundance, diversity and composition must be included in the assessment tool, and several of the indices tested at this time may be selected from that perspective. The next step in the development of such a multimetric assessment tool would be to combine metrics providing complementary attributes (namely abundance, diversity and composition) of the communities.

Appendix

Appendix II.1. *List of macroinvertebrate taxa found on Buarcos and Matadouro shores (higher groups are highlighted in grey), and their Ecological Group (EG) from the AMBI list. Taxa assigned a posteriori by expert judgement (EJ) are marked with an X.*

Group	Taxon	EG	
		AMBI	EJ
Oligochaeta	Oligochaeta	V	
Polychaeta			
	Ampharete sp.	I	
	Aonides oxycephala	III	
	Arabella iricolor	I	
	Boccardia polybranchia	IV	
	Brania pusilla	II	
	Cirriformia tentaculata	IV	
	Euclymene palermitana	I	
	Eulalia viridis	II	
	Fabricia stellaris	II	
	Glycera sp.	II	
	Harmothoe sp.	II	
	Lepidonotus clava	II	
	Lumbrineris latreilli	II	
	Lysidice ninetta	II	
	Malacoceros fuliginosus	V	
	Maldane sp.	I	
	Microspio atlantica	III	
	Myrianida sp.	II	
	Mysta picta	III	
	Mysta sp.2	III	X
	Naineris laevigata	I	
	Naineris quadricuspida	I	
	Neanthes nubila	III	
	Nereidinae	III	X
	Odontosyllis ctenostoma	II	
	Oriopsis armandi	II	
	Paraehlersia ferrugina	II	
	Perinereis cultrifera	III	
	Perinereis marionii	III	X
	Perinereis oliveirae	III	X
	Perinereis sp.	III	
	Pholoe inornata (accepted name; identified as *P. synophthalmica* and, being different from the previous one, took its EG instead)	II	
	Platynereis dumerilii	III	
	Polycirrus sp.	IV	
	Protoaricia oerstedii	III	
	Pseudopotamilla reniformis	II	

Appendix II.1. *(continued)*

Group	Taxon	EG	
		AMBI	EJ
Polychaeta			
	Sabellaria alveolata	I	
	Sabellaria spinulosa	I	
	Scoletoma impatiens	II	
	Serpula vermicularis	I	
	Sphaerosyllis sp.	II	X
	Spio filicornis	III	
	Spirobranchus lamarcki	II	
	Spirobranchus triqueter	II	
	Sthenelais boa	II	
	Syllides edentatus	II	
	Syllis amica	II	
	Syllis armillaris	II	
	Syllis corallicola	III	
	Syllis garciai	II	
	Syllis gerlachi	II	
	Syllis gracilis	III	
	Syllis hyalina	II	
	Syllis prolifera	II	
	Syllis pulvinata	II	
	Syllis rosea	II	
	Syllis sp.	II	
	Syllis vivipara	II	
	Tharyx sp.	IV	
	Travisia sp.	I	
	Vermiliopsis sp.	II	
Amphipoda			
	Abludomelita gladiosa	III	
	Ampelisca rubella	I	X
	Ampelisca sp.	I	
	Ampithoe gammaroides	II	X
	Aora typica	I	
	Apherusa cirrus	I	
	Apherusa jurinei	I	X
	Apohyale prevostii	II	
	Caprella acanthifera	II	
	Caprella equilibra	II	
	Dexamine spinosa	III	
	Elasmopus rapax	III	
	Gammaropsis maculata	I	
	Hyale perieri	II	
	Hyale pontica	II	X
	Hyale stebbingi	II	

Appendix II.1. *(continued)*

Group	Taxon	EG	
		AMBI	EJ
Amphipoda			
	Jassa ocia	V	
	Jassa pusilla	V	
	Maera grossimana	I	
	Melita palmata	I	
	Microdeutopus chelifer	I	
	Microdeutopus damnoniensis (*nomen nudum*)	I	
	Parajassa pelagica	II	
	Photis longicaudata	I	
	Podocerus variegatus	III	
	Protomedeia fasciata	II	
	Stenothoe monoculoides	II	
Cirripedia			
	Balanus sp.	I	X
	Chthamalus montagui	I	
Decapoda			
	Cancer sp.	II	X
	Carcinus maenas	III	
	Liocarcinus navigator	I	
	Pachygrapsus marmoratus	II	
	Pilumnus hirtellus	I	
	Pirimela denticulata	I	
	Xantho pilipes	I	
Isopoda			
	Campecopea hirsuta	II	X
	Cymodoce truncata	I	
	Dynamene bidentata	II	X
	Dynamene edwardsi	II	X
	Dynamene magnitorata	II	X
	Eurydice pulchra	I	
	Gnathia maxillaris	I	
	Idotea granulosa	II	
	Idotea pelagica	II	
	Ischyromene lacazei	II	X
	Lekanesphaera levii	III	
	Paranthura nigropunctata	I	
	Cleantis prismatica	II	

Appendix II.1. *(continued)*

Group	Taxon	EG	
		AMBI	EJ
Tanaidacea			
	Apseudes talpa	II	
	Tanais dulongii	II	
Insecta			
	Chironomidae	III	
	Tipulidae	IV	
Bivalvia			
	Cardita calyculata	I	
	Hiatella arctica	I	
	Irus irus	I	
	Lasaea adansoni	II	
	Musculus costulatus	I	
	Mytilus galloprovincialis	III	
	Parvicardium pinnulatum	I	
	Psammobiidae	I	
	Tellina sp.	I	
	Venerupis sp.	I	
Gastropoda			
	Crisilla semistriata	I	
	Aplysia punctata	I	
	Barleeia unifasciata	II	
	Bittium reticulatum	I	
	Buccinum humphreysianum	II	
	Cerithiopsis tubercularis	I	
	Cheirodonta pallescens	I	X
	Epitonium clathratulum	I	
	Gibbula cineraria	I	
	Gibbula pennanti	I	
	Gibbula umbilicalis	I	
	Melarhaphe neritoides	II	
	Nassarius incrassatus	II	
	Nassarius pygmaeus	II	
	Nassarius reticulatus	II	
	Nucella lapillus	II	X

Appendix II.1. *(continued)*

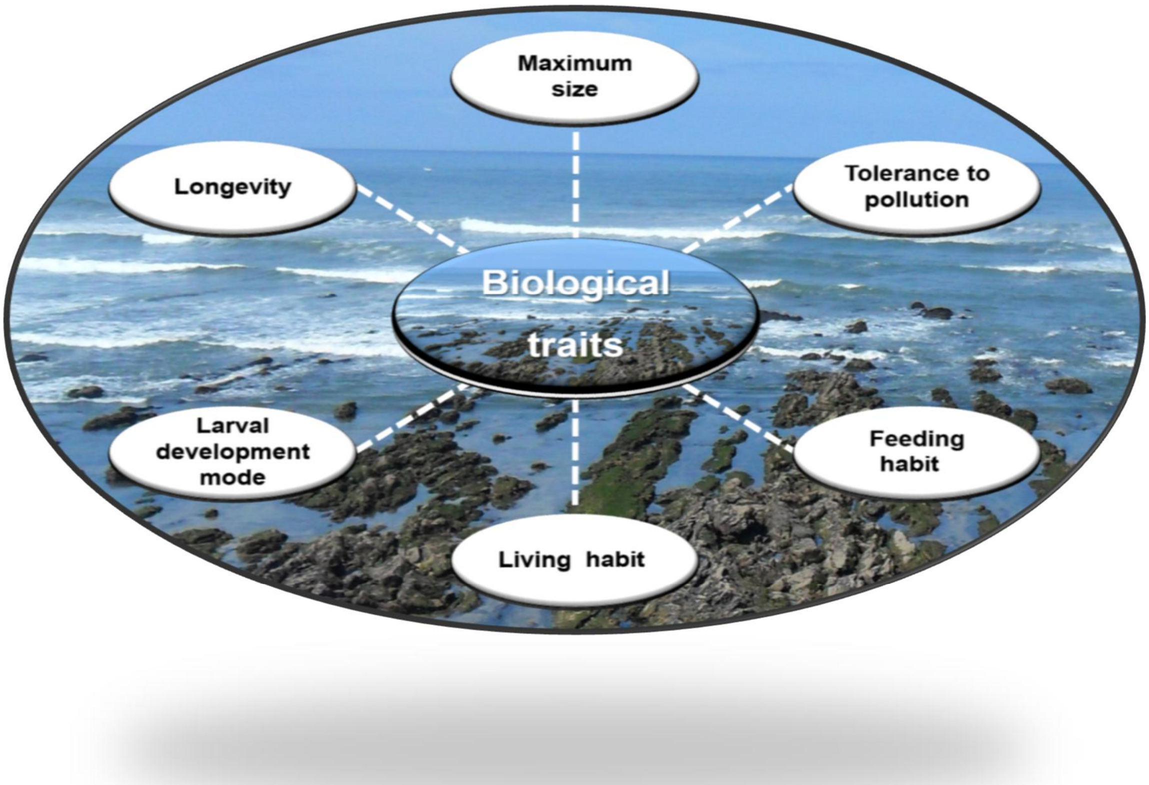
Group	Taxon	EG	
		AMBI	EJ
Gastropoda			
	Nudibranchia	II	X
	Ocenebra erinaceus	II	
	Ocinebrina aciculata	II	
	Odostomia eulimoides	II	
	Omalogyra atomus	III	X
	Patella depressa	I	X
	Patella sp.	I	
	Patella ulyssiponensis	I	X
	Patella vulgata	I	X
	Phorcus lineatus	I	X
	Rissoa parva	I	
	Runcina coronata	I	X
	Siphonaria pectinata	I	
	Skeneopsis planorbis	I	
	Testudinalia testudinalis	I	X
	Tricolia pullus	I	
	Urosalpinx cinerea	II	X
Polyplacophora			
	Lepidochitona (*Lepidochitona*) *cinerea*	II	
	Acanthochitona spp. (*A. crinita* and *A. fascicularis*)	I	
Echinoidea	Echinoidea	I	
Ophiuroidea	Ophiuroidea	II	
Anthozoa	Actiniaria	II	
Ascidiacea	Ascidiacea	III	
Echiura	Echiura	II	
Nemertea	Nemertea	III	
Pycnogonida			
	Achelia echinata	I	
	Anoplodactylus pygmaeus	II	
	Anoplodactylus virescens	II	X
Sipuncula	*Golfingia* sp.	I	
Turbellaria	Leptoplanidae (*Notoplana* sp. and *Leptoplana* sp.)	II	

Appendix II.2. *Environmental parameters measured/estimated monthly (August, September, February and March) at the source of pollution (SOP) and sites 1, 2 and 3 at Buarcos and Matadouro shores.*

	August				September				February				March			
Buarcos	SOP	Site 1	Site 2	Site 3	SOP	Site 1	Site 2	Site 3	SOP	Site 1	Site 2	Site 3	SOP	Site 1	Site 2	Site 3
Chlorophyll a (mg m^{-3})	7.380	0.608	0.675	0.367	8.670	0.628	0.920	0.561	2.345	1.059	0.810	0.664	2.991	1.696	1.748	0.868
Conductivity (μS cm^{-1})	1659	49705	48834	49557	1323	48442	48080	47981	1214	39279	40907	41053	1019	44675	43180	44160
DIN (mg L^{-1})	0.600	0.446	0.303	0.382	0.773	0.632	0.386	0.303	5.537	0.486	0.488	0.260	0.345	0.259	0.959	0.216
DIP (mg L^{-1})	0.086	0.051	0.064	0.020	0.075	0.079	0.00006	0.024	0.058	0.043	0.040	0.085	0.010	0.023	0.067	0.023
DO (%)	74.6	149.0	94.6	125.3	77.7	114.2	109.1	107.4	94.2	129.0	108.8	132.2	101.1	109.4	82.3	141.1
ORP (mV)	125.0	114.9	123.8	211.5	94.3	151.8	148.8	168.8	164.5	245.5	254.2	163.3	263.5	297.8	299.1	295.5
pH	7.12	7.11	6.89	7.02	8.47	8.26	8.24	8.15	8.58	8.33	8.12	8.02	8.35	8.29	8.02	8.03
POM (g L^{-1})	0.0037	0.0019	0.0010	0.0015	0.0045	0.0017	0.0018	0.0016	0.0016	0.0014	0.0009	0.0006	0.0016	0.0015	0.0016	0.0007
Salinity	0.94	35.23	35.29	35.34	0.74	36.12	35.88	36.26	0.95	33.35	34.88	34.81	0.61	34.13	35.10	34.92
Silica (mg L^{-1})	16.776	0.626	0.546	0.574	19.202	0.389	0.415	0.357	16.882	0.345	0.155	0.273	16.437	0.448	0.241	0.188
Temperature (ºC)	22.0	21.5	20.5	21.2	19.9	19.2	19.1	18.7	9.9	13.1	12.8	13.3	16.6	17.7	15.1	16.4
TSS (g L^{-1})	0.0190	0.0164	0.0090	0.0104	0.0200	0.0134	0.0167	0.0126	0.0051	0.0087	0.0052	0.0027	0.0046	0.0093	0.0128	0.0055
Matadouro																
Chlorophyll a (mg m^{-3})	0.896	1.999	0.543	0.646	0.298	1.538	1.399	1.600	3.535	2.082	0.728	1.033	6.152	4.785	4.852	3.877
Conductivity (μS cm^{-1})	1530	6899	48250	48615	1470	45535	46911	47227	1276	40919	40732	40688	1117	40820	43306	42820
DIN (mg L^{-1})	0.282	0.433	0.164	0.240	6.422	0.586	0.370	0.281	5.168	0.435	0.496	0.229	3.221	0.598	0.310	0.167
DIP (mg L^{-1})	0.0310	0.0066	0.0379	0.0002	0.1045	0.0464	0.0322	0.0686	0.1510	0.0430	0.0872	0.0025	0.1975	0.0071	0.0040	0.0381
DO (%)	75.5	143.2	101.4	92.0	72.1	99.0	87.5	85.6	101.9	86.0	89.5	96.6	106.0	87.0	83.2	85.3
ORP (mV)	20.0	127.3	55.0	87.8	212.6	239.6	258.3	256.7	334.8	390.2	414.5	329.6	375.2	311.9	299.7	306.5
pH	7.51	8.14	7.65	7.55	8.43	8.05	8.15	8.03	8.76	8.25	8.27	8.30	8.64	8.24	8.07	8.08
POM (g L^{-1})	0.0011	0.0021	0.0019	0.0020	0.0012	0.0031	0.0030	0.0041	0.0016	0.0008	0.0008	0.0007	0.0006	0.0020	0.0019	0.0019
Salinity	0.90	4.04	34.64	35.96	0.83	33.80	36.66	36.42	0.90	34.69	34.01	34.76	0.72	32.66	34.97	34.73
Silica (mg L^{-1})	15.966	0.370	0.260	0.195	15.804	0.463	0.336	0.354	18.368	0.279	0.322	0.275	15.601	0.406	0.245	0.218
Temperature (ºC)	20.2	22.1	20.7	19.4	19.9	18.9	17.2	17.6	10.5	13.3	13.7	13.0	13.8	15.4	15.3	15.1
TSS (g L^{-1})	0.0040	0.0097	0.0130	0.0135	0.0021	0.0242	0.0199	0.0322	0.0053	0.0050	0.0034	0.0048	0.0014	0.0112	0.0143	0.0137

Chapter III

Do structural and functional attributes show concordant responses to disturbance? Evidence from rocky shore macroinvertebrate communities*



Vinagre, P.A., Veríssimo, H., Pais-Costa, A.J., Hawkins, S.J., Borja, Á., Marques, J.C., Neto, J.M., 2017. Do structural and functional attributes show concordant responses to disturbance? Evidence from rocky shore macroinvertebrate communities. Ecological Indicators 75, 57-72.

DOI: 10.1016/j.ecolind.2016.12.02

Abstract

The utility and concordance of application of taxonomic-based (diversity, richness and composition) and functional-based (biological traits analysis and functional diversity indices) metrics to distinguish anthropogenic disturbance or stress gradients (e.g., nutrient enrichment) on intertidal rocky shores were explored using macroinvertebrate communities. Metrics from both approaches showed similar trends in the variation of communities along the gradients, in which higher ecological health was found in less disturbed sites (farthest from the disturbance source), with the converse at more stressful sites (close to the disturbance source). The functional-based approach, using biological traits analysis and functional diversity indices, showed potential to be included in monitoring programmes at rocky shores alongside taxonomic-based metrics.

Keywords: Benthos; Biological traits analysis; Taxonomic diversity; Functional diversity; Organic enrichment.

III.1. Introduction

How ecosystems respond to natural disturbance and anthropogenic pressures has become a major concern (Piggott et al., 2015). Such understanding is essential for the assessment of the likely resistance and resilience (Tett et al., 2007; Pinto, 2012) of an ecosystem and its subsequent potential for recovery after being impacted (Bremner, 2008; Statzner and Bêche, 2010). Local and regional scale impacts must be considered in the context of natural climate fluctuations and more recent anthropogenically driven climate change (Root and Schneider, 1995; Parmesan, 2006; Firth and Hawkins, 2011; Mieszkowska et al, 2014; Birchenough et al., 2015).

Coastal areas in particular are under the influence of multiple disturbances and stressors, naturally or anthropogenically driven, that impact their biodiversity and functioning (Micheli et al., 2016), thereby compromising their ability to sustain ecosystem services (Worm et al., 2006; Halpern et al., 2008). To manage pressures and impacts, several sets of legislation have been established worldwide over recent decades [e.g., European Water Framework Directive (WFD, 2000) and Marine Strategy Framework Directive (MSFD, 2008); Australia Oceans Policy (Commonwealth of Australia, 1998a, b); South Africa Integrated Coastal Management Act (South Africa Government, 2013); US Clean Water Act (US Environmental Protection Agency, 2002) and Oceans Act (US Congress, 2002); People's Republic of China laws on Water (1988/01/21) and Environmental Protection (1989/12/26)] in order to protect and restore integrity within marine ecosystems, ensuring that human activities are carried out in a sustainable manner (Borja et al., 2008). There is thus a societal demand for robust approaches to evaluate ecosystems status (Borja et al., 2016). This requires in-depth knowledge of the response of communities and ecosystems to anthropogenic impacts (Western, 2001; Hooper et al., 2005).

Traditional approaches to assess anthropogenic disturbance have usually been focused on taxonomically based structural features (e.g., metrics based on species richness, density/biomass, and diversity). Growing awareness that changes in biodiversity may potentially modify ecosystem functioning (Loreau et al., 2001, 2002; Hawkins et al., 2009) led to the recognition of the importance of considering functional attributes when detecting change (e.g., Loreau et al., 2001; Hooper et al., 2005; Elliott and Quintino, 2007). The biological characteristics of organisms (traits - Violle et al., 2007) determine outcomes of interactions with the physical-chemical environment, population, community and ecosystem processes (Snelgrove, 1998). Thus, a trait-based approach offers useful proxies to investigate ecosystem functioning and the effects of disturbance at the ecosystem-functioning level (Bremner et al., 2006a).

In the past two decades, an interest in functional diversity (FD) has emerged: the functional component of biodiversity usually measured through species traits (Tilman, 2001). A suite of metrics and tools has been developed (Bremner, 2008; Mouchet et al., 2010). Recent approaches to address FD have often included Biological Traits Analysis (BTA; Statzner et al., 1994) and the computation of FD indices (Petchey and Gaston, 2006; Schleuter et al., 2010). BTA is a multivariate approach that combines information on species distributions over space and time, with the multiple traits (life-history, morphological, behavioural) they exhibit (Bremner, 2008). This multi-trait method had its genesis in terrestrial and freshwater ecology, but later it was translated to the marine benthic environment (Bremner et al., 2003) where it has been proved useful to: (i) assess fishing effects on benthic fauna (e.g., Bremner et al., 2003; Tillin et al., 2006); (ii) investigate the effects of climate change (e.g., Neumann and Kröncke, 2010); (iii) use for management and conservation purposes (e.g., Bremner, 2008; Frid et al., 2008; Veríssimo et al., 2012); and (iv) assess functional diversity in different species assemblages (e.g., Bremner et al., 2003; Hewitt et al., 2008; van der Linden et al., 2016). Two of the most often used FD indices are the Community-Weighted Mean trait values (CWM; Garnier et al., 2004) and Rao's Quadratic Entropy (RQE; Rao, 1982; Botta-Dukát, 2005). These indices provide complementary information on the changes in the mean trait values (CWM), and on the patterns of trait dispersion (RQE), within the communities (Ricotta and Moretti, 2011). The CWM expresses the trait mean per sample weighted by species relative biomass, and allows investigation of shifting patterns in traits within communities indicating which traits are dominating ecosystems processes (Lepš et al., 2011; Ricotta and Moretti, 2011). The RQE expresses the amount of trait dissimilarity between species pairs in the community (Botta-Dukát, 2005).

Rocky shores are an important system which, in common with other coastal habitats, provide valuable ecosystem provisioning (e.g., seaweed and shellfish collection and aquaculture, fish nursery grounds), regulating (e.g., water quality by biofiltration, sea defence), and cultural services (e.g., aesthetics leading to amenity use and tourism) (e.g., Liquete et al., 2013; Galparsoro et al., 2014). Moreover, rocky shores have been recognized as warning systems for climate change (e.g., Southward et al., 1995; Sagarin et al., 1999; Thompson et al., 2002; Hawkins et al., 2003; Helmuth et al., 2006; Mieszkowska et al., 2014).

The basic descriptive ecology of distribution patterns rocky shores has been long-studied (e.g., Stephenson and Stephenson, 1949; Lewis, 1964). The processes involved in setting distributions, driving population dynamics and structuring communities are well understood from a long history of field experimental studies on the interactions of the physical environment with biota and amongst the organisms themselves, including the role of recruitment in driving fluctuations (Connell, 1961; Menge, 1976; Paine, 1994; Raffaelli and Hawkins, 1999; Menge, 2000; Underwood, 2000).

There has been much attention to the responses of rocky shore organisms and assemblages to acute (e.g., oil spills: Southward and Southward, 1978; Hawkins and Southward, 1992) and/or chronic (harvesting: Addessi, 1994; runoff pollution: Kinsella and Crowe, 2015; Vinagre et al., 2016a, b; sewage pollution: Littler and Murray, 1975; Bishop et al., 2002; O'Connor, 2013; Zubikarai et al., 2014; Tributyl tin pollution from anti-foulants: Bryan et al, 1987) anthropogenic impacts (reviews of several acute and chronic impacts: Hill et. al, 1998; Crowe et al., 2000; Thompson et al., 2002; Mearns et al., 2014). Despite this attention, in contrast to other coastal habitats (soft-bottom), few ecological tools are currently available for the ecological quality assessment of rocky shores, the existing ones being exclusively (Ballesteros et al., 2007; Juanes et al., 2008; Neto et al., 2012; Ar Gall and Le Duff, 2014) or in part (Díez et al. 2012) based on the macroalgae. Furthermore, the use of functional trait approaches on rocky shores has focussed nearly exclusively on macroalgae (e.g., Littler and Littler, 1980, 1984; Orfanidis et al., 2001; Martins et al., 2016), rather than on macroinvertebrates (but see, e.g., Törnroos et al., 2013; Bustamante et al., 2014; Vinagre et al., 2015) or the whole community considered together.

This work is, as far as the authors are aware, the first to assess impacts on rocky shore intertidal macroinvertebrate communities using a functional traits, coupled with a traditional taxonomically based approach. In particular, communities were assessed along anthropogenic disturbance gradients (organic enrichment) on two shores using trait-based descriptors (BTA and FD indices) as well as taxonomically based analyses (e.g., species composition, richness and diversity indices). For this purpose, (i) differences in the expression of biological traits across sites within the disturbance gradients were analysed; (ii) changes in FD over those gradients were investigated; and (iii) results obtained using trait-based descriptors were compared against those of taxonomic-based ones.

This study will contribute to a better understanding of the structure and functioning of intertidal rocky shore communities in the context of the future design of assessment tools. Such approaches will aid development of suitable management and conservation actions preventing further degradation, and where necessary enabling restoration.

III.2. Materials and methods

III.2.1. Study sites

Two rocky shores were monitored, Buarcos (40°10'14.2"N, 8°53'26.7"W) and Matadouro (38°58'31.5"N, 9°25'14.4"W), located on the western Portuguese coast (Fig. III.1A) and, respectively, classified as Exposed and Moderately Exposed Atlantic Coast typologies (TICOR project, Bettencourt et al., 2004; available at http://www.ecowin.org/ticor/). Along this coast the prevailing current and wave direction are from West-Northwest with episodic occurrence from the Southwest (Ambar and Fiúza, 1994; Bettencourt et al., 2004). The most frequent wave period and wave height are in the range of 8-12 s and of 1-3 m, respectively. The tides are semi-diurnal and may reach 3.5-4 m during extreme spring tides (Boaventura et al., 2002, Bettencourt et al., 2004). Surface sea temperature ranges between 13-15 ºC during winter and 20-22 °C during summer, with surface salinity varying between 35 and 36 (Boaventura et al., 2002).

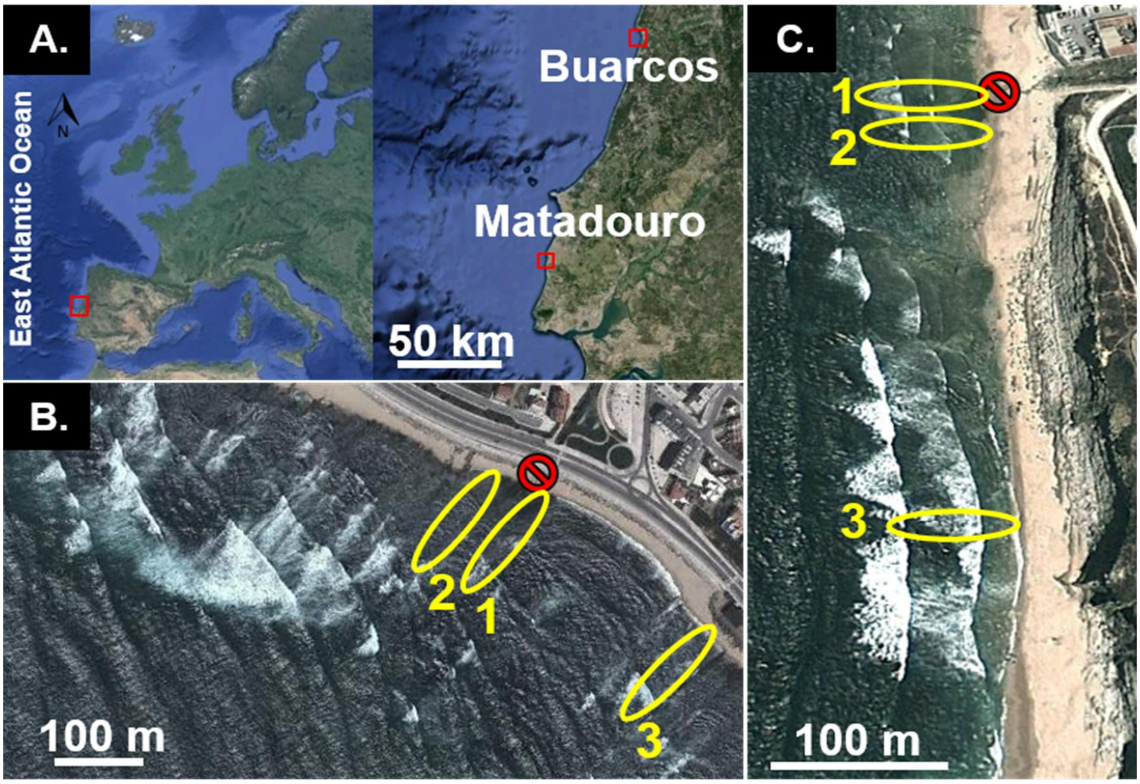


Figure III.1. *Study area (from Google Earth): A. Europe and Portugal; B. Buarcos (40°10'14.2"N, 8°53'26.7"W); C. Matadouro (38°58'31.5"N, 9°25'14.4"W). Sampling sites (1-3) are represented in yellow (ovals); source of pollution (SOP) is represented by 🚫 sign.*

On both shores the rocky surface is situated among narrow sandy areas limited landward by seawalls fronting promenades. The sampling areas are moderately impacted by continuous run-off throughout the year of water (crossing urban centres and agricultural land before reaching the shore) close to the upper intertidal zone creating disturbance gradients across the shores. These gradients on both shores were characterised by Vinagre et al., (2016a, b) showing differences in several physical-chemical parameters among sites [e.g., higher nutrient concentrations – dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP), chlorophyll a and particulate organic matter (POM), closer to the source of pollution (SOP)], thus confirming the hypothesized gradients away from the SOP. Within these gradients, higher numbers of opportunistic macroalgal and macroinvertebrate species were found at the more stressed sites (close to SOP), and more sensitive species (and higher abundance of several of them) in less disturbed sites (farthest from SOP) (Vinagre et al., 2016a). Moreover, several macroinvertebrate (e.g., Hurlbert index, Shannon-Wiener index, Bentix) and one macroalgal (MarMAT) ecological indices have been shown to reflect these disturbance gradients (Vinagre et al., 2016b).

III.2.2. Biological data collection

Biological samples were collected at sites 1-3, concurrently with physical-chemical measurements (reported in Vinagre et al 2016a, b). At each site three intertidal sampling zones were defined: upper intertidal (submersed for ~6h day^{-1}), mid intertidal (submersed for ~12h day^{-1}) and lower intertidal (submersed for ~18h day^{-1}). In each zone four random replicates (12 cm x 12 cm squares) were collected avoiding pools and crevices. The samples (144 from each shore) were immediately preserved after sampling in neutralized 4% formalin solution (prepared with sea water).

Taxonomy of macroinvertebrates (identified to species level whenever possible) was standardized in accordance to the World Register of Marine Species (WoRMS, http://www.marinespecies.org). Biomass was preferred over density as it better reproduces the amount of energy and resources assimilated within a species (Brey et al., 1988; Brey, 2012). It was determined as ash-free dry weight (AFDW) and standardized to g AFDW m^{-2} prior to data analysis. Methodology for data processing and trait attribution and analysis are summarized in the work flow chart (Fig. III.2).

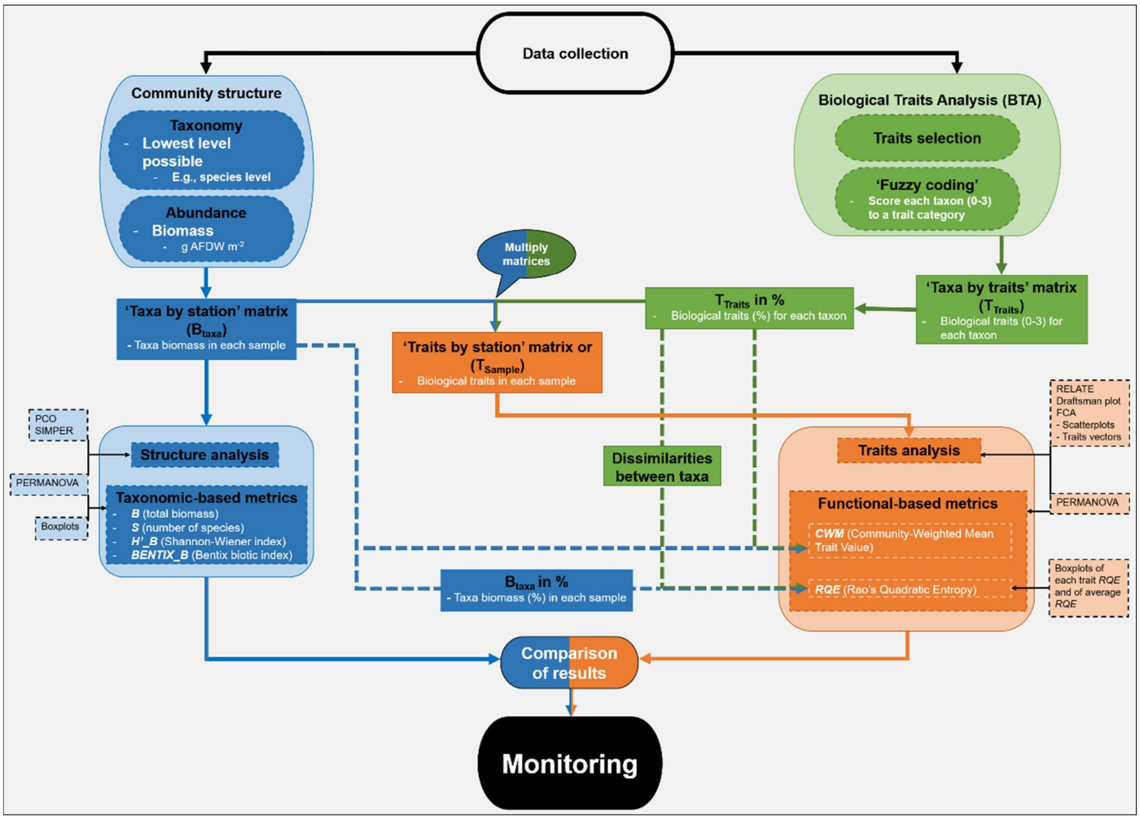


Figure III.2. *Flow chart for work methodology.*

III.2.3. Biological traits analysis (BTA)

To conduct BTA three different data matrices were built: (a) 'taxa by station' (taxa biomass in each sample; B_{Taxa}); (b) 'taxa by traits' (biological traits for each taxon; T_{Traits}); and (c) 'traits by station' (biological traits in each sample; T_{Sample}), which results from the cross-product between the previous two matrices (Bremner et al., 2003; Bremner, 2008).

Information on biological traits was gathered from various sources including identification guides (e.g., Hayward and Ryland, 1995; Marine Species Identification Portal (http://species-identification.org/)), scientific journals and online databases (e.g., MarLIN BIOTIC (http://www.marlin.ac.uk/biotic/); polytraits (polytraits.lifewatchgreece.eu/); Encyclopedia Of Life (eol.org/); Marine Macrofauna Genus Trait Handbook (www.genustraithandbook.org.uk/)). When reliable information was missing, expert judgment and/or data from the nearest phylogenetic neighbour were considered. Six biological traits were selected and divided into 24 categories, covering different aspects of the life history, morphology and behaviour of each species: maximum size, longevity, larval development mode, living habit, feeding habit and tolerance to pollution (Table III.1). Rationale for choosing traits was based on previous studies using BTA coupled with the potential of particular traits to illustrate changes in ecosystem functioning and responses of benthos to and recovery from disturbance (e.g., Bremner, 2005; Tyler et al., 2012; Veríssimo et al., 2012; van der Linden et al., 2016). Maximum size and longevity are representative of the movement of organic matter within the benthic system (long-lived and large organisms hold matter within the system and short-lived small species contribute to higher turnover). These traits could also be indicative of disturbance within the system, with small-sized and short-lived species increasing in both abundance and species number as disturbance increases (Pearson and Rosenberg, 1978). Larval development mode captures energy/materials transfer pathways and the nature of connections between benthic and pelagic habitats. As larval development is associated with different modes of recruitment, it may also give insights on potential recovery patterns (with different types of recruitment conferring different recovery potentials, e.g., communities recruiting mainly by pelagic dispersal are theoretically likely to recover from disturbance more quickly than those with predominantly direct benthic recruitment (Thrush and Whitlatch, 2001). Living habit outlines a species ability to survive different environmental conditions and may also indicate a particular mode of living (e.g., tube-dweller). Feeding habit describes the movement of energy and matter through the food web and determines abilities of species to utilize/tolerate different hydrodynamic conditions, e.g., with a switch from predominantly suspension feeders to surface deposit feeders indicating a potential reduction in water movement and siltation (Rosenberg, 1995). Tolerance to pollution (ecological groups: EG) reflects the response of communities to environmental stress (organic enrichment), and may also be related to other traits such as size and feeding habit. For example, species sensitive or indifferent to organic enrichment [EG I-II; sensu AZTI's Marine Biotic Index (*AMBI*, Borja et al., 2000)] are usually present in unpolluted conditions and account for, among other, selective carnivores, deposit-feeding tubiculous polychaetes, suspension feeders and scavengers. On the other hand, opportunist species (EG IV-V) are stimulated by the excess organic matter, such as small-sized subsurface deposit-feeding polychaetes (Grall and Glémarec, 1997; Borja et al., 2000).

The ‘fuzzy coding’ procedure (Chevenet et al., 1994) on a scale 0-3 was used to categorize species by their biological traits, in which an affinity score of ‘0’ indicates no affinity of a taxon to a trait category, whereas a score of ‘3’ indicates a high affinity to the trait category. The scores for each trait (except for larval development mode) were assigned considering the species adult form on the shore.

Table III.1. *List of biological traits and respective categories, and number of species (S) coded for each category (some taxa were coded for more than one category).*

Biological traits	Trait categories	Labels	S
Maximum size	Very small (<1 cm)	S-1	52
	Small (1-3 cm)	S1-3	46
	Medium (3-10 cm)	S3-10	53
	Large (>10 cm)	S10+	27
Longevity	Very short (<1 year)	L-1	31
	Small (1-3 years)	L1-3	75
	Medium (3-10 years)	L3-10	69
	Long (>10 years)	L10+	17
Larval development mode	Planktotrophic	Plan	62
	Lecitotrophic	Lec	61
	Direct	Dir	68
Living habit	Burrow dweller	Bd	38
	Attached	Att	17
	Tube dweller	Td	30
	Free living	Fl	121
Feeding habit	Deposit feeder	Dep	49
	Filter/suspension feeder	F/S	48
	Herbivore/opportunist/scavenger	H/O/S	101
	Predator	Pred	67
Tolerance to pollution (ecological group*)	1st order opportunistic	EG V	4
	2nd order opportunistic	EG IV	5
	Tolerant	EG III	26
	Indifferent	EG II	73
	Sensitive	EG I	63

*Borja et al., 2000; Vinagre et al., 2016a, b

The T_{Sample} matrix was produced by the combination of the previous B_{Taxa} and T_{Traits} matrices, by multiplying trait categories for each taxon present at a sample by their biomass at that sample, and then summing over all taxa present at each sample to obtain a single value for each trait category in each sample (Bremner et al., 2006b; Hewitt et al., 2008). Prior to statistical analysis, T_{Sample} data were fourth-root transformed to reduce natural dominance of species.

III.2.4. Taxonomic and functional diversity metrics

For comparison with the FD indices, four taxonomic-based metrics were selected for the present study: total biomass (B), richness (as number of species; S), Shannon-Wiener index ($\log_2$; H'_B) (Shannon and Weaver, 1963) and Bentix biotic index (BENTIX_B) (Simboura and Zenetos, 2002) (both calculated using biomass data). The H'_B accounts for the abundance and evenness of species and a higher index value indicates higher diversity. The BENTIX_B accounts for abundance of species and taxonomic composition (based on the relative percentages of ecological groups of species grouped according to their sensitivity or tolerance to disturbance) and higher values indicate higher ecological quality states. These two ecological indices have previously been tested within the disturbance gradients on Buarcos and Matadouro shores and showed, among several indices, the best overall performance in the assessment of ecological condition (Vinagre et al., 2016b).

Two of the most often used FD indices were selected, the CWM and RQE. Both indices should respond to environmental disturbance, the first by showing a higher proportion of disturbance sensitive trait-categories, and the second by presenting a decrease in its values after disturbance. Both indices were computed using an Excel macro from Lepš et al. (2006) (available at http://botanika.bf.jcu.cz/suspa/FunctDiv.php). The *RQE* was calculated for each of the six traits, and also as an overall *RQE* (as the mean value of all traits *RQE*).

All taxonomic- and functional-based metrics were calculated per replicate.

III.2.5. Data analysis

Univariate and multivariate statistical analyses were performed with PRIMER 6 + PERMANOVA© software (Clarke and Gorley, 2006; Anderson et al., 2008). For the computation of the T_{Sample} matrix and the Fuzzy Correspondence Analysis (FCA) was used the R-3.2.3 (R Development Core Team) open-source software, with the 'ade4' library (Thioulouse et al., 1997). The boxplots were drawn using Minitab® V.17 (Minitab Inc.) statistical software.

III.2.5.1. Taxonomic-based metrics

The B_{Taxa} matrix was used to visualize differences in the structure of the communities inside the disturbance gradients, by performing PCO analyses on the basis of Bray-Curtis similarities, after fourth-root transformation of data. PCO was firstly applied using data from both Buarcos and Matadouro pooled. Afterwards it was applied to data from each shore separately, using centroids from crossed factors 'Site' (three levels: sites 1-3) and 'Season' (two levels: summer and winter) to simplify visualization. Significant differences were tested between shores, among sampling sites within shore and between seasons, using PERMANOVA (Anderson, 2001). The design included four fixed factors, 'Shore' (two levels: Buarcos and Matadouro), 'Site' (nested in 'Shore'; as above), 'Zone' (nested in 'Site'; three levels: upper, mid and lower intertidal) and 'Season' (as above). Permutation of residuals under a reduced model (9999 permutations) was selected, with a significance level of $\alpha = 0.05$. To identify the macroinvertebrate taxa that contribute mostly to the communities' structural variation, Similarity Percentage Analysis (SIMPER) was applied to the B_{Taxa} data matrices of Buarcos and Matadouro shores. For each shore, similarities were assessed within and dissimilarities between groups using two-way crossed designs with factors 'Site' and 'Season' (as above) (with 85% cut off, and without transformation of data). Those taxa were then selected to show differences in biomass among sites and between seasons at each shore.

For each taxonomic-based metric – B, S, H'_B and BENTIX_B – were drawn boxplots to visualize patterns within each shore. Each metric was used to test for significant differences (univariate PERMANOVA) in the communities, using the same design and options as previously for the B_{Taxa} matrix, except without transformation of data for S, H'_B and BENTIX_B. For all metrics was used the Euclidean distance as a similarity measure, and unrestricted permutation of raw data.

III.2.5.2. Trait-based metrics

III.2.5.2.1. Biological traits analysis

The T_{Sample} matrix was used in several analyses to test differences in the expression of biological traits among sites within the disturbance gradients.

First, relationships between biological traits were analysed by performing the RELATE routine (comparative Mantel-type tests on similarity matrices) on the basis of the Euclidean similarity measure (Spearman correlation and 9999 permutations were used). RELATE was firstly applied using data from both Buarcos and Matadouro pooled, and afterwards it was applied to data from each shore separately for higher detail. Second, Pearson correlations between categories across traits were examined for each shore separately, using Draftsman plot analysis. Third, T_{Sample} was used to identify the traits associated with differences in the distribution of species. Differences were analysed between seasons, between shores, and among sites within shore through FCA. This is a correspondence analysis method appropriate for fuzzy coded data, providing the variability contained in every axis and the correlation ratios of each biological trait along the principal axes. It also allows plotting of the scores of trait category on two-dimensional factor maps (Chevenet et al., 1994). FCA was firstly applied considering data from both Buarcos and Matadouro pooled, and afterwards was applied to data from each shore separately. Fourth, T_{Sample} was used to assess spatial (between shores and among sites within shore) and seasonal (between summer and winter) significant differences (PERMANOVA). The previous similarity matrix (RELATE) was used, with the same design and options as for the B_{Taxa} matrix.

III.2.5.2.2. Functional diversity indices

Functional diversity patterns were explored using CWM and RQE. Boxplots were drawn for each trait RQE and for the overall RQE (mean value of all traits RQE) to visualize patterns within each shore. Significant differences were tested between shores, among sites within shore and between seasons (PERMANOVA), with the same design and options used previously for H'_B and BENTIX_B.

III.3. Results

III.3.1. Macroinvertebrate community structure

III.3.1.1. Multivariate data analysis

The PCO analysis using the macroinvertebrate biomass (B_{Taxa}) data highlighted differences between Buarcos and Matadouro (Fig. III.3A). At both Buarcos and Matadouro (Fig. III.3B and III.3C) there was a clear separation between summer and winter, and a gradual separation of sites 1-2-3. Significant differences (PERMANOVA; Supplementary material B1.A) were found between shores during both seasons. Simultaneously, each shore was different between seasons. On Buarcos, differences occurred during summer between all sites, and during winter between sites 1 and 3. Simultaneously, sites 2 and 3 were each different between seasons. On Matadouro, differences were found between all sites in both seasons, and each site was different between seasons.

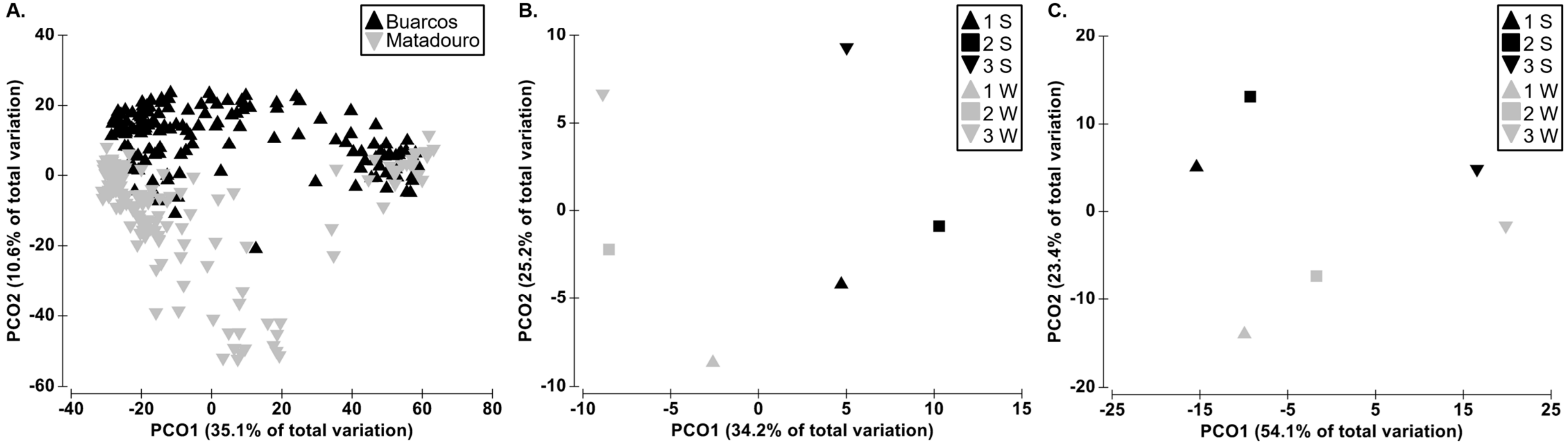


Figure III.3. *Principal coordinates (PCO) analysis plots of the taxa biomass (A.). Detail is given for Buarcos (B.) and Matadouro (C.) separately, represented by centroids from crossed factors 'Site' (sites 1-3) and 'Season' (summer: S; winter: W).*

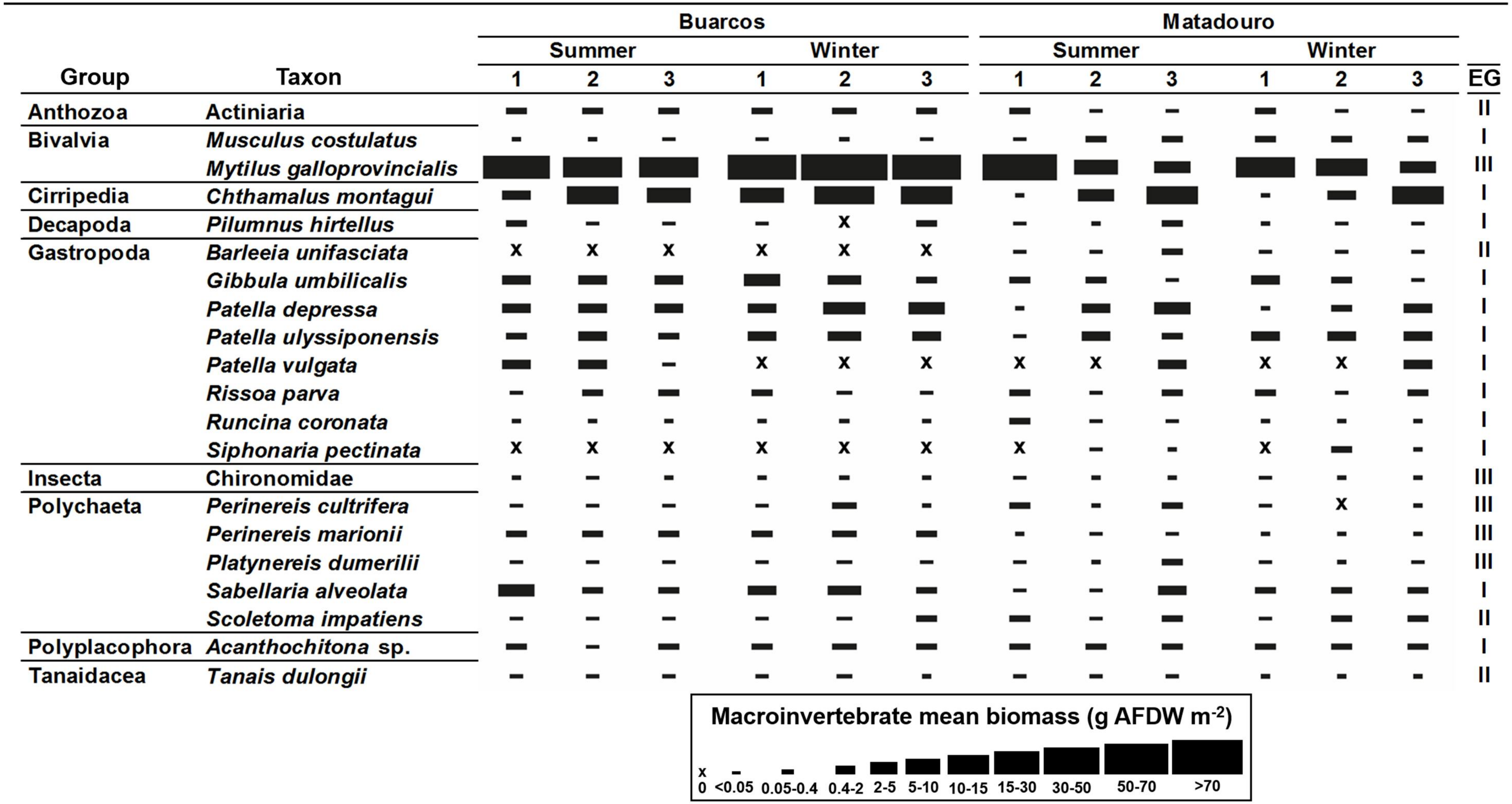


Figure III.4. *Macroinvertebrate mean biomass (g AFDW m^{-2}) in each sampling site (1-3) at Buarcos and Matadouro.*

At both Buarcos and Matadouro, similarity was highest within the less disturbed site (3) (28% and 22%, respectively) (SIMPER; Supplementary material B2). Dissimilarity was lowest between sites 2 and 3 (76% and 83%, respectively for Buarcos and Matadouro). Twenty-one taxa (85% cut-off) contributed to the similarities/dissimilarities among sites and between seasons found on both Buarcos (encompassing 10/21 taxa) and Matadouro (accounting for 20/21 taxa). On both shores, contribution was shared by more species further from disturbance (site 3). *Mytilus galloprovincialis* showed highest biomass, which was lowest at site 3, and was top contributor (Fig. III.4). In contrast, *Chthamalus montagui*, which was the second most abundant top contributor, showed higher biomass usually at site 3. The remaining species made lower contributions but many showed generally linear trends, either decreasing in mean biomass from site 1 to site 3 (e.g., Actiniaria, *Gibbula umbilicalis, Runcina coronata* and Chironomidae) or increasing from site 1 to site 3 (e.g., *Musculus costulatus*, *Pilumnus hirtellus*, *Patella depressa*, *Patella ulyssiponensis*, *Patella vulgata*, *Siphonaria pectinata* – found only on Matadouro, *Perinereis cultrifera*, *Platynereis dumerilii* and *Acanthochitona* spp.). Other species showed variable patterns between shores, seasons or among sites (*Barleeia unifasciata* – found only on Matadouro, *Rissoa parva*, *Perinereis marionii, Sabellaria alveolata*, *Scoletoma impatiens* and *Tanais dulongii*) (Fig. III.4).

III.3.1.2. Univariate data analysis

The trends in total biomass (*B*) were not consistent between shores. Higher *B* was observed during winter at Buarcos and during summer at Matadouro. Site 1 had higher values, except at Buarcos during winter (Fig. III.5A). Significant differences (PERMANOVA; Supplementary material B1.B) were observed during winter between shores, and between Matadouro sites 2 and 3 during both seasons.

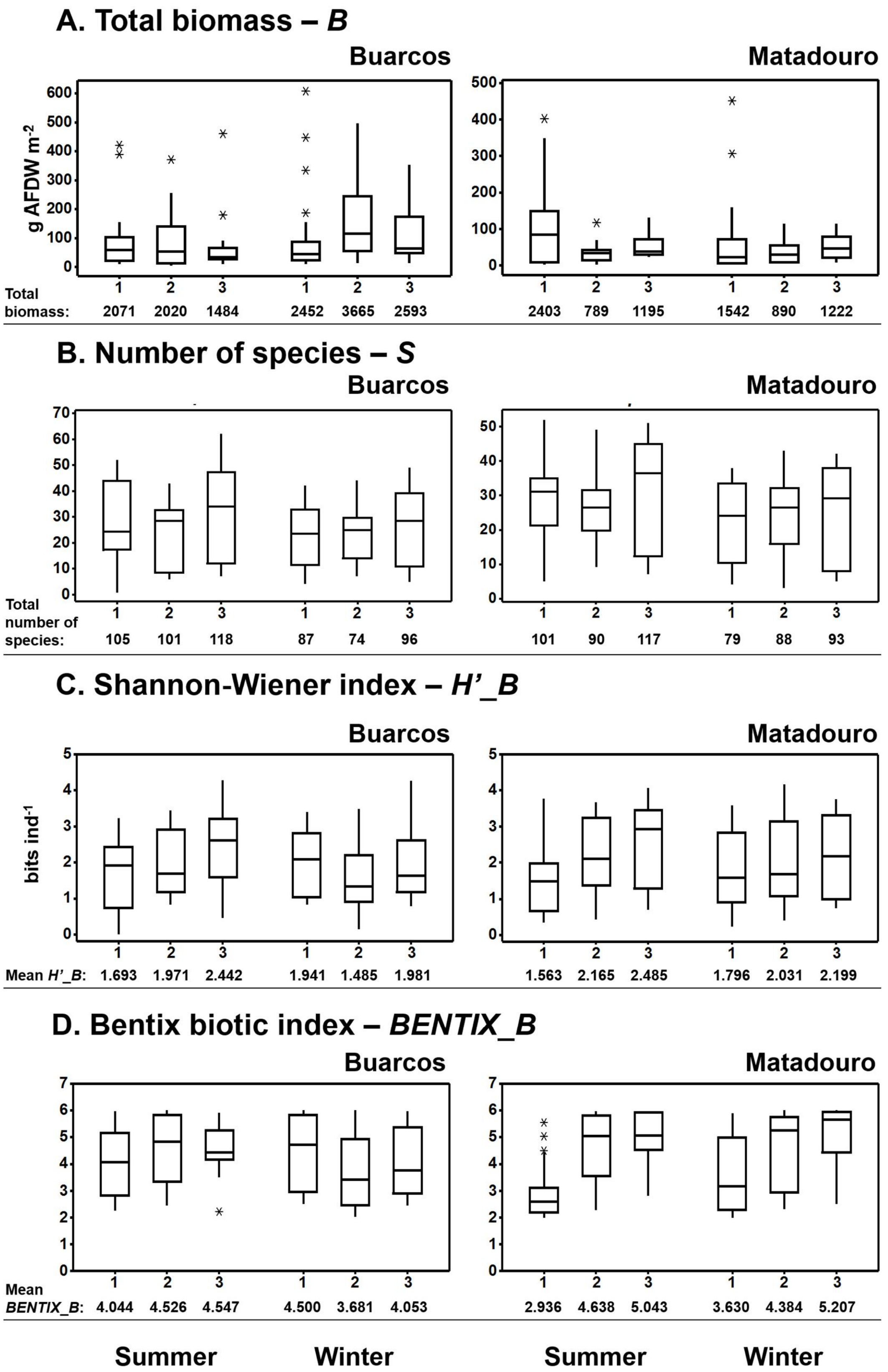


Figure III.5. *Summary results of A. macroinvertebrate total biomass (B), B. number of species (S), C. Shannon-Wiener index (H'_B) and D. Bentix biotic index (BENTIX_B), seasonally on Buarcos and Matadouro sites 1, 2 and 3. Metrics values shown at site level in the graphs bottom. Box: mid line = 50th percentile (median); bottom and top of the box = 25th (Q1) and 75th (Q3) percentiles, respectively; lower and upper whiskers = [Q_1-1.5(Q_3-Q_1)] and [Q_3+1.5(Q_3-Q_1)], respectively; outliers = values outside whiskers limits (*).*

A total of 171 macroinvertebrate taxa were identified. At both Buarcos (141 taxa) and Matadouro (139 taxa), higher number of species (S) was observed during summer and at site 3 (followed by site 1) (Fig. III.5B). Significant differences were observed between summer and winter (PERMANOVA; Supplementary material B1.C), and also observed at each shore between site 3 and the other sites.

The taxonomic diversity assessed with H'_B and BENTIX_B showed generally increasing values from site 1 to site 3, especially at Matadouro, suggesting increasing quality in that direction (Figs. III.5C and III.5D, respectively). Using H'_B, significant differences were not found both between shores and between seasons (PERMANOVA; Supplementary material B1.D). At Buarcos, differences existed mainly between site 3 and the remaining sites (and site 1 being the only site not different between seasons). At Matadouro, site 1 was the most different from the other sites (and any site differed between seasons). Using BENTIX_B, differences were observed between shores during winter (PERMANOVA; Supplementary material B1.E). At Buarcos, differences existed between sites 1 and 2 during winter (being the latter the only site different between seasons). At Matadouro, differences existed between site 1 and the other sites during summer, and between all sites during winter (site 1 was the only site different between seasons).

III.3.2. Multivariate analysis of biological traits

Using the T_{Sample} matrix, statistically significant relations (ranging between 0.433-0.798, $p = 0.0001$ for all tests) were found for pairs of traits at both Buarcos and Matadouro (RELATE analysis on similarity matrices; Table III.2). At both shores, the strongest relationships (>0.7) were between maximum size and tolerance to pollution, and between living habit and feeding habit. Weakest relations (<0.5) were between maximum size and larval development mode. High Pearson correlations (>+0.85 or <-0.85) were observed among categories within and across traits (Supplementary material B3), namely between the planktotrophic (Plan) and lecithotrophic (Lec) larval development modes (negative correlations), free living (Fl) habit and herbivore/opportunistic/scavenger (H/O/S) feeding habit, and attached (Att) living habit and filter/suspension (F/S) feeding habit (both positive correlations). Other high correlations were also found at Buarcos, between small size (S1-3) and medium longevity (L3-10), large size (S10+) and pollution tolerant (EG III) (both positive correlations), and predator (Pred) feeding habit and Plan (negative correlation). Also, at Matadouro, between Pred and Lec (positive correlation).

Table III.2. *RELATE results between biological traits on Buarcos and Matadouro shores. Size = maximum size, Long = longevity, LDM = larval development mode, Liv = living habit, Feed = feeding habit, Tol = tolerance. Stronger relations (>0.7) in bold. p = 0.0001 for all tests.*

		Long	LDM	Liv	Feed	Tol
	Size	0.603	0.433	0.542	0.588	**0.798**
	Long		0.619	0.657	0.650	0.612
Buarcos	LDM			0.677	0.672	0.546
	Liv				**0.777**	0.541
	Feed					0.628
	Size	0.630	0.472	0.560	0.592	**0.764**
	Long		0.657	0.675	0.603	0.622
Matadouro	LDM			0.664	0.679	0.515
	Liv				**0.737**	0.551
	Feed					0.586

Table III.3. *Contribution (%) of the first two FCA axes (FC1 and FC2), and correlation ratios between each axis and biological trait, for both shores together, and for Buarcos and Matadouro separately. Higher contributions in bold.*

Biological Traits	Both shores (63%)		Buarcos (65%)		Matadouro (66%)	
	FC1 (43%)	FC2 (20%)	FC1 (45%)	FC2 (20%)	FC1 (42%)	FC2 (24%)
Maximum size	0.03	**0.05**	0.04	**0.05**	0.02	**0.05**
Longevity	**0.07**	0.02	0.07	0.01	0.06	0.04
Larval development mode	**0.08**	0.00	**0.08**	0.00	**0.07**	0.00
Living habit	**0.08**	0.03	**0.09**	0.04	**0.09**	0.04
Feeding habit	0.05	0.02	0.06	0.03	0.05	0.01
Tolerance to pollution	0.06	**0.06**	0.06	**0.06**	**0.06**	**0.08**

In the FCA using data from Buarcos and Matadouro together, the first two axes accounted for 63% (43% in FC1) of the total variability (Table III.3; Fig. III.6A). A clear separation between shores was observed (FC1), influenced mostly by living habit, larval development mechanism and longevity. On one hand, Buarcos was more related to organisms which reach small size (S1-3) to medium size (S3-10), have medium longevity (L3-10) to long longevity (L10+), are planktotrophic (Plan) developers, show free living (Fl) and attached (Att) living habits, and filter/suspension (F/S) and herbivore/opportunistic/scavenger (H/O/S) feeding habit, and are sensitive (EG I) to pollution. On the other hand, Matadouro was more related to organisms which reach very small size (S-1), have very short life (L-1) to short life (L1-3), are lecithotrophic (Lec) and direct (Dir) developers, show tube-dwelling (Td) and burrow-dwelling (Bd) living habits, and deposit (Dep) and predatory (Pred) feeding habit, and are indifferent and opportunistic (1st and 2nd order) regarding tolerance to pollution (Fig. III.6B).

The FCA using each shore data separately accounted in the first two axes for 66% of the total variability (45% and 42% in FC1, respectively for Buarcos and Matadouro). At Buarcos, a linear gradient in distribution of sites was not observed, but a separation was visible (stronger during winter) of site 1 from the other sites (FC2; Fig. III.6B). Significant differences were found between sites 1 and 3 (PERMANOVA; Supplementary material B1.F). In turn, a seasonal pattern (less obvious for site 1) was visible (also in FC2). These spatial and seasonal trends were influenced mostly by tolerance to pollution and maximum size traits (Table III.3). In particular, sites during summer, and also site 1 during winter, were more related with organisms of small size (S1-3) to medium size (S3-10), free-living (Fl), herbivore/opportunistic/scavenger feeders (H/O/S), sensitive to pollution (EG I) and 1st order opportunists (EG V); whereas during winter sites 2 and 3 were more related with large sized (S10+) organisms, living attached (Att), filter/suspension feeders (F/S) and tolerant to pollution (EG III) (Fig. III.6B).

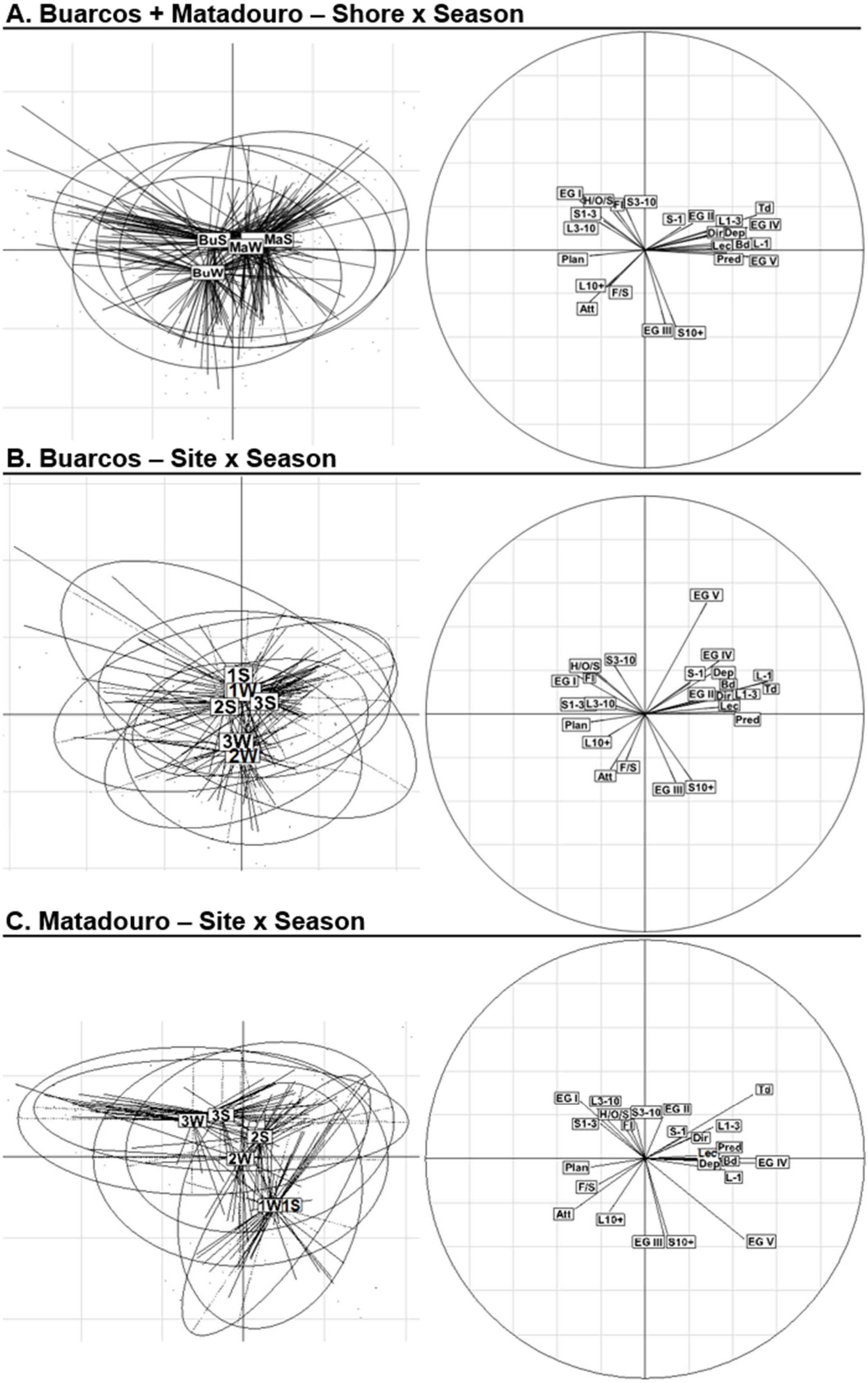


Figure III.6. *FCA plots for T_{Sample} matrices of A. Buarcos + Matadouro; B. Buarcos; and C. Matadouro. To the left and right are the distribution of sites and of trait categories, respectively. Bu = Buarcos; Ma = Matadouro; S = summer; W = winter; 1, 2 and 3 = sites 1, 2 and 3.*

At Matadouro (Fig. III.6C), a linear separation of sites was observed mostly influenced by living habit and larval development mode (PC1), and by tolerance to pollution and maximum size (PC2) traits. Site 1 was mainly related with S10+, EG III and EG V, while site 3 was related with S1-3, S3-10, Fl, H/O/S and EG I-II. Significant differences were observed between all sites (PERMANOVA; Supplementary material B1.F). Also, each site (especially sites 2 and 3) separated between summer and winter (particularly in FC1). Sites during summer were related with S-1, L-1, L1-3, Lec, Dir, Bd, Td, Pred, Dep and EG IV-V; whereas sites during winter were more related with S1-3, L3-10, L10+, Plan, Att, F/S and EG I (Fig. III.6C).

III.3.3. Functional diversity indices

III.3.3.1. Community-Weighted Mean trait values (CWM)

The CWM reflected trends in the proportion of biomass of species in the communities (Fig. III.7) (extensive description in Supplementary material B4). Generally, the communities were composed of large sized (S10+) organisms (followed by medium sized, S3-10), long-lived (L10+) organisms (followed by medium-lived, L3-10), with planktotrophic (Plan) larval development, living attached (Att) to the substratum (followed by free living, Fl), filter/suspension feeders (F/S) (followed by herbivores/opportunists/scavengers, H/O/S), and organisms sensitive to pollution (EG I) (followed by tolerant organisms, EG III).

In particular, considering the sites closest (1) and farthest (3) from the SOP, site 1 showed a higher proportion of S10+, L10+, Plan, Att, F/S (all at both shores), EG I (at Buarcos) and EG III (at Matadouro). In contrast, site 3 showed a higher proportion of S10+ and L10+ (at Buarcos), S3-10, L3-10 (both at Matadouro), Att (at Buarcos and at Matadouro during winter), Fl (at Matadouro during summer), F/S (at both shores), EG I (at Buarcos during summer and at Matadouro during both seasons), and EG III (at Buarcos during winter).

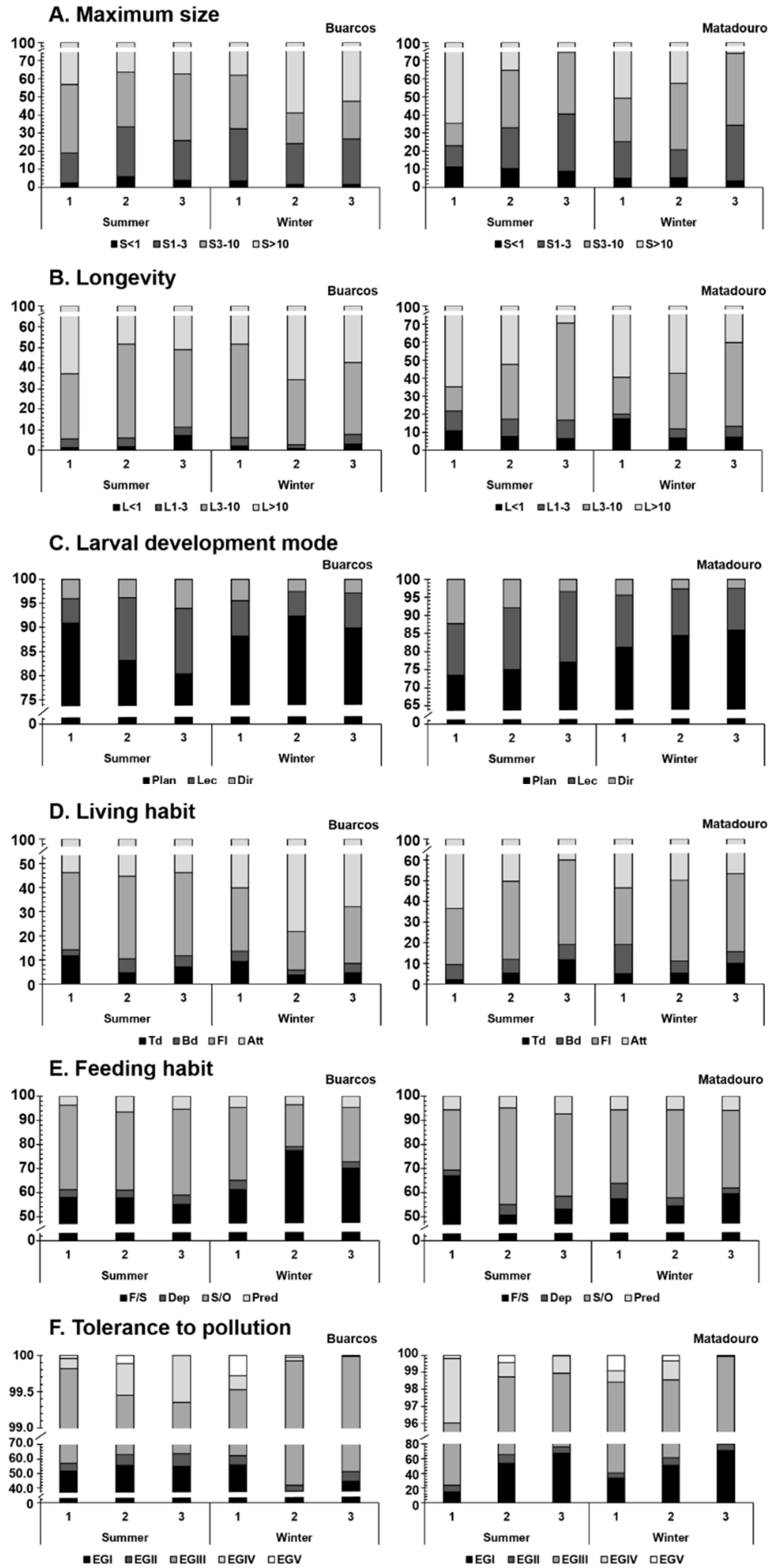


Figure III.7. *Seasonal and spatial variation of each category within traits representativeness (%) at Buarcos and Matadouro. Vertical axes are cut for better visualization of categories with lower values.*

Significant differences (PERMANOVA; Supplementary material B5) were observed in all traits. (i) Maximum size – differences were found between shores during both seasons with regard to very small size (S-1) and medium size (S3-10), among sites mainly with regard to the small size (S1-3), S3-10 and large size (S10+) (especially at Matadouro), and on each shore between summer and winter with regard to S3-10. (ii) Longevity – differences were found between shores during both seasons for all categories except long life (L10+), and among sites regarding very short life (L-1) and L10+, (especially at Matadouro). (iii) Larval development mode – differences were found between shores for all categories except direct development (Dir), among sites (at Matadouro) regarding Dir, and between seasons regarding all categories. (iv) Living habit – differences were found between shores with regard to burrow-dwelling (Bd), free living (Fl) and attached (Att) living (for the latter two this was only during winter), and among sites regarding all categories (for Fl and Att this was observed only at Matadouro). (v) Feeding habit – differences were found between shores regarding deposit feeding (Dep), herbivory/opportunism/scavenging (H/O/S) and filter/suspension feeding (F/S) (the latter two during winter only), among sites regarding Dep and H/O/S (both at Matadouro), and between seasons regarding F/S and H/O/S (both at Buarcos). Differences were not observed for predation (Pred). (vi) Tolerance to pollution – differences were found between shores regarding sensitive organisms (EG I, during summer), indifferent organisms (EG II), tolerant organisms (EG III, during winter), 2nd order opportunists (EG IV) and 1st order opportunists (EG V), among sites regarding all categories except EG II (especially EG III, EG IV and EG V and at Matadouro), and between seasons regarding EG IV.

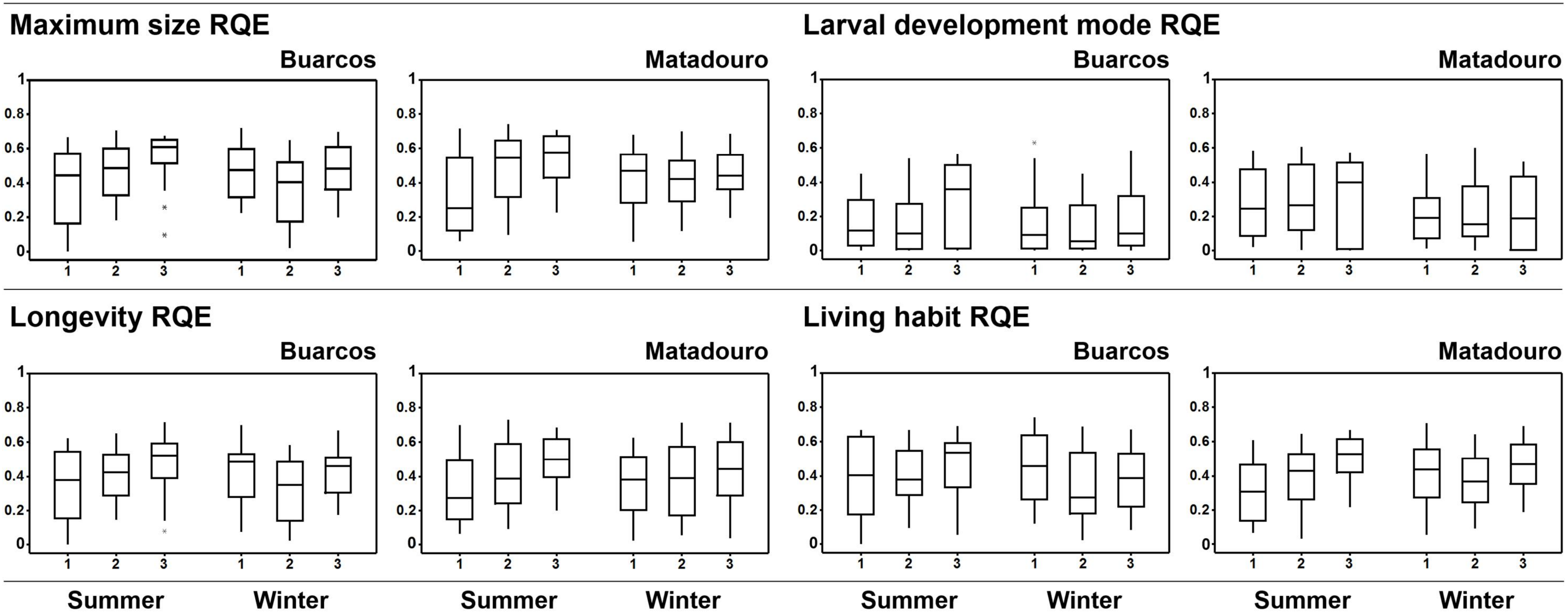
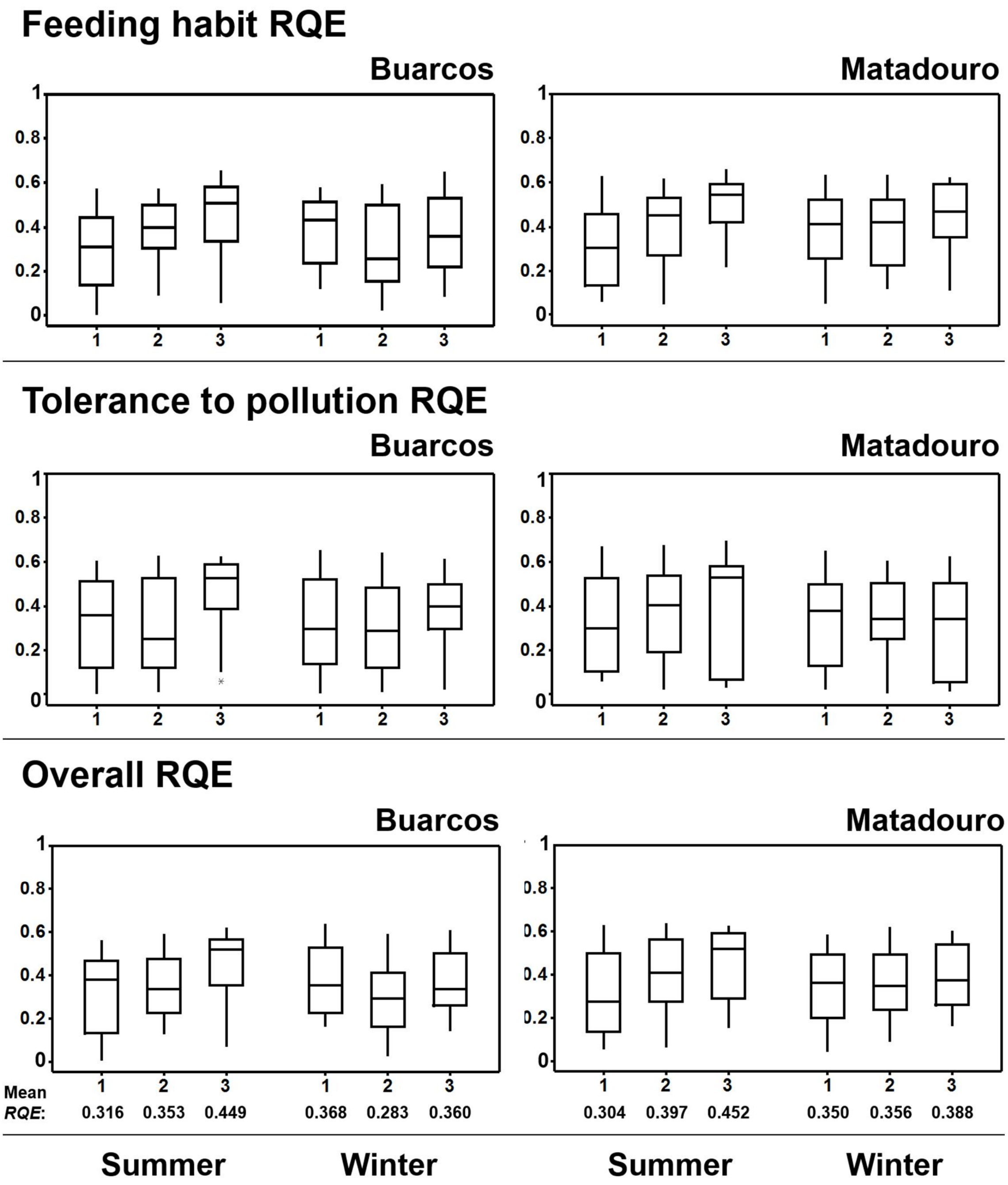


Figure III.8. *Boxplots of each trait RQE and the overall RQE (as the mean value of all traits RQE), seasonally on Buarcos and Matadouro sites 1, 2 and 3. Overall RQE value shown at site level in the graph bottom. Box: mid line = 50th percentile (median); bottom and top of the box = 25th (Q1) and 75th (Q3) percentiles, respectively; lower and upper whiskers = [Q_1-1.5(Q_3-Q_1)] and [Q_3+1.5(Q_3-Q_1)], respectively; outliers = values outside whiskers limits (*).*

Figure III.8. *(continued)*



III.3.3.2. Rao’s quadratic entropy (RQE)

Functional diversity measured as RQE for each biological trait showed patterns quite similar to H’_B and BENTIX_B. Values generally increased at both shores from site 1 to site 3, especially during summer, which means the dissimilarity in traits between two random individuals in a community, a measure of functional diversity, increased in that direction (Fig. III.8). During winter, only longevity, living habit (both at Matadouro) and tolerance to pollution (at Buarcos) showed that trend. The overall RQE (mean value of all traits RQE) behaved accordingly, showing also increasing functional diversity from sites 1 to 3 during summer (at both shores) and during winter (at Matadouro).

For each trait RQE (except larval development mode) and the overall RQE significant differences were not observed either between shores or between seasons (PERMANOVA; Supplementary material B6). Generally, more differences were found between site 1 or 3 and the remaining sites, and also during summer. More seasonal differences were observed at site 3.

III.4. Discussion

To date, the use of functional-based approaches, namely biological traits analysis and functional diversity indices have not been considered for rocky shores, in contrast to their widespread historical application in terrestrial and fresh-water ecology (e.g., Grime, 1974; Southwood, 1977; Statzner et al., 1994; Charvet et al., 2000; Menezes et al., 2010), and more recently to soft-bottom benthos of transitional and coastal waters (e.g., Bremner, 2003, 2005, 2008; Veríssimo et al., 2012, van der Linden et al., 2016). Macroinvertebrates should be considered along with macroalgae for quality assessments on rocky shores (WFD, 2000). This work sought further insights on functional patterns in rocky shore macroinvertebrate communities, using functional-based descriptors together with more traditional taxonomic approaches. This is a novelty for such ecosystems.

III.4.1. Macroinvertebrate communities’ structure

At Buarcos and Matadouro, the macroinvertebrate shore communities differed mainly in species composition and dominance associated with it. For example, at Buarcos (exposed shore) there was higher dominance by fewer species (10/141 taxa from SIMPER), whereas at Matadouro dominance was shared among more species (20/139 taxa). Despite this, patterns were clear at Matadouro. Moreover, both shores were influenced mainly by the same species, and these species showed higher biomass either closer to disturbance (site 1) – *M. galloprovincialis,* Actiniaria*,* Chironomidae, *R. coronata* and *G. umbilicalis* – or farthest from disturbance (site 3) – *Acanthochitona* spp., *C. montagui, M. costulatus*, nereids, patellids, *Pilumnus hirtellus* and *S. pectinata*. Regardless of structural differences in the communities, on both shores a gradual separation of sites 1-2-3 (PCO centroids) was observed and higher differences (SIMPER, PERMANOVA) were found between site 1, or site 3, and the remaining sites. Also, site 3 showed least variation in data (SIMPER) at both shores, which could be associated with less disturbance (Warwick and Clarke, 1993).

All the above reinforces the existence of disturbance gradients on both shores, which could be recognized from changes in the community structure, namely using biomass. Nevertheless, traditional assumptions regarding biomass and richness (e.g., Pearson and Rosenberg, 1978; Odum, 1985; McManus and Pauly, 1990; Warwick and Clarke, 1994) seem not to hold as valid on the rocky shores assessed as they do for coastal soft-bottom ecosystems. This is because on both shores intermediate disturbance (by low but continuous organic enrichment) is probably promoting increased biomass (mainly related to *M. galloprovincialis*) and number of species (S) closer to disturbance (at site 1, although site 3 registered the highest *S*). At site 1, the increased abundance and number of macroalgal species, and also the presence of *M. galloprovincialis*, may provide more diversified habitats to accommodate more macroinvertebrate species (besides the opportunists) and enable less competitive exclusion of species (Fish and Fish, 2001; Magurran and McGill, 2010).

III.4.2. Trait selection for BTA

Applying functional-based approaches to coastal ecosystems is recognized as a complex task (e.g., Veríssimo et al., 2012; van der Linden et al., 2016).

Trait selection (e.g., how many and which categories should be used) and standardization of trait information from various sources, need to be done before data analysis. Gathering information on each trait category for each organism can be difficult, requiring time and financial resources that are often not available. In our study, traits were selected not only owing to their ecological relevance (Bremner, 2008; Veríssimo et al., 2012), but also because they could be obtained from more easily available data (e.g., identification keys, online databases, scientific publications).

Some traits may correlate with each other in a particular species, often due to phylogenetic constraints, or be ecologically linked across species that occupy similar habitats (Statzner and Bêche, 2010; Verberk et al., 2013). Despite high correlations being observed between categories across different traits (e.g., attached living habit and filter/suspension feeding habit), the analysis of trait structure (RELATE) suggested traits were generally not very strongly related. In fact, all (namely living habit, larval development mode, tolerance to pollution and maximum size) were important in distinguishing (in different cases) between shores, sites within shore and seasons (FCA, PERMANOVA). Moreover, since the communities were composed of many species, many did not conform to expected correlations and exhibited theoretically opposing traits (Bremner et al., 2006b). As an example, deposit feeding species are often tolerant or opportunistic regarding organic enrichment; however, several amphipods and polychaetes were coded (fuzzy coding score of 3) both as ‘deposit feeding’ and ‘sensitive to pollution’. Overall, the variety of traits used do not seem to be redundant in the assessment (Lepš et al., 2006; Bremner, 2008).

Besides trait selection, BTA relies on a proper ‘fuzzy coding’. This procedure is very useful for the analysis as it not only allows describing the affinity of species for different categories, it enables analysis of biological information derived from diverse sources (e.g., literature, personal observations) (Chevenet et al., 1994). Together with other limitations of BTA, fuzzy coding can be arguable since different experts may have different points of view (e.g., deciding on whether to score a taxon between 1-2 to certain category). Nevertheless, the advantages are far larger for the reasons mentioned above: it aids reducing uncertainty in the information gathered or when information is missing;

takes account of intraspecific variability in trait expression (Bremner, 2008); and enables optimized statistical analysis of more homogenized data.

Correct identification of species is essential for trait allocation and the fundamental basis of analysis. Time and financial resources, as well as uncertainty, could be reduced using higher taxonomic levels (e.g., genus, family) (e.g., Pagola-Carte et al., 2002; Bevilacqua et al., 2009; De-La-Ossa-Carretero et al., 2012). However, plasticity in the expression of biological traits (which can be accounted in the fuzzy coding) is much greater when using lower taxonomic resolution (e.g., different species within Gammaridae were coded for all feeding habit categories, and from sensitive to 1st order opportunists regarding tolerance to pollution). Traits information was gathered mostly at species level enabling greater discrimination.

Despite all the above limitations, overall, BTA allowed the recognition of spatial (between and within two shores) and seasonal (between summer and winter) changes in functioning on rocky shore macroinvertebrate communities within disturbance gradients.

III.4.3. Community-weighted mean trait values (*CWM*)

The CWM index is directly related to the biomass ratio hypothesis, which considers traits of the most abundant species in a community to greatly determine ecosystem processes (Garnier et al. 2004; Ricotta and Moretti, 2011). In many cases (e.g., maximum size and longevity) CWM results seemed to be affected by the frequency and biomass of dominant species (such as *M. galloprovincialis*, *C. montagui* and *Patella* spp.). Such results were independent of the number of species coded for a certain category. However, other cases (e.g., tolerance to pollution) were indicative that higher number of species may have reduced the influence of biomass dominating species. Another limitation of this index may be its ambiguous results since each trait may be coded to species showing different associations with disturbance. For example, tolerance to pollution includes in EG III (tolerant species) organisms that can reach large size and long life such as *M. galloprovincialis,* which is more often associated with more polluted sites (e.g., Bellan-Santini, 1965; Borja et al., 2000; Díez et al., 2012; Cabral-Oliveira et al., 2014b) compared to less polluted, and amphipods (e.g., *Elasmopus rapax*) which are commonly regarded as sensitive (e.g., Dauvin and Ruellet, 2009). Planktotrophic larval development mode, which may be associated with more disturbed areas (e.g., Levin and Huggett, 1990; Thrush and Whitlatch, 2001), encompasses tolerant-opportunistic (EG III-V, e.g., *M. galloprovincialis*, *Malacoceros fuliginosus*) and sensitive (EG I-II, e.g., *C. montagui*, patellids, aphroditiids) species. Other examples of ambiguous patterns were found among traits.

Overall, the *CWM* performed well within the disturbance gradients on the rocky shores surveyed, both spatially and seasonally, and should not be discarded from monitoring activities despite the limitations identified.

III.4.4. Rao's quadratic entropy (*RQE*) versus other metrics

The RQE offers advantages over the H'_B, BENTIX_B and CWM indices. Comparing to CWM, it summarizes FD into single values thus making it easier to evaluate and interpret changes in FD, despite the CWM allows emphasising each category within trait. With regard to the Shannon-Wiener index (H'_B) and Bentix biotic index (BENTIX_B), although the two indices summarize information about a community, the RQE takes into account also the degree of dissimilarity between species in that community (Botta-Dukát, 2005; Lepš et al., 2006). Both indices showed comparable trends to the *RQE*: taxonomic (H'_B and BENTIX_B) and functional (RQE) diversity generally increased from the site closest (1) to the site furthest (3) from disturbance, meaning an increase in ecosystem health in that direction. However, the RQE better captured such trends, independently of structural differences (richness/diversity and composition) between Buarcos and Matadouro and between summer and winter seasons, comparatively to H'_B and BENTIX_B. Furthermore, intermediate disturbance is most probably enabling increased richness (e.g., with an increase of opportunistic species; Vinagre et al., 2016a, b) closer to disturbance, which can be misleading concerning traditional assumptions of higher richness found in less disturbed areas.

Overall, results for RQE (considering each trait and the average value) showed more consistent patterns in both shores during summer, reinforcing previous results (Vinagre et al., 2016b) which indicated this could be the season (comparing to winter) better reflecting the disturbance gradients and therefore could be the best period to undertake monitoring activities.

III.5. Conclusions

Using macroinvertebrate biomass, the BTA was able to detect spatial and seasonal differences in functioning along the disturbance gradients on rocky shores.

The BTA and the assessment of functional diversity using the CWM (emphasising each biological trait category) and RQE (summarizing FD into single values for each trait) indices, provided detailed, different and complementary information on the functioning of rocky shore communities, and have shown similar performance with regard to traditional approaches (analysis of community structure based on taxonomic-based metrics).

Such findings seemed to be more consistent during summer, suggesting this could be the best season (compared to winter) to perform monitoring programmes (e.g., degradation/recovery) in these ecosystems, in agreement to what was found previously when testing several other macroinvertebrate ecological indices (Vinagre et al., 2016b).

From the above mentioned, there seems to be potential to use BTA and the FD indices tested in the present study, together with more traditional methods, in the implementation of monitoring programmes. It could even be used during evaluation cycles in the scope of the WFD, to assess community trends at rocky shores. Despite not being mandatory in the scope of this directive, functional-based approaches could complement the use of available multimetric tools since they are based on ‘abundance’ and ‘composition’ data, as required by the WFD.

More studies on intertidal rocky shores are needed to confirm the present findings. Although the use of functional-based approaches to assess community functioning in those aquatic ecosystems is promising, confidence should be reinforced by further testing.

Chapter IV

Addressing a gap in the Water Framework Directive implementation: Rocky shores assessment based on benthic macroinvertebrates*

Vinagre, P.A., Pais-Costa, A.J., Hawkins, S.J., Borja, Á., Marques, J.C., Neto, J.M., 2017. Addressing a gap in the WFD implementation: Rocky shores assessment based on benthic macroinvertebrates.

Accepted in Ecological Indicators

Abstract

A gap in the European Water Framework Directive (WFD) is addressed, aiming for the development of an ecological quality status assessment tool based solely on the Biological Quality Element benthic macroinvertebrates from intertidal rocky shores. The proposed Rocky shore Macroinvertebrates Assessment Tool (RMAT) was tested and validated along disturbance gradients (organic enrichment). During the whole process, the response of widely used metrics (e.g. Hurlbert index, Shannon-Wiener index, AZTI's Marine Biotic Index; Bentix biotic index) and models (i.e., metrics combined) was compared to results provided by the Marine Macroalgae Assessment Tool to the same sampling sites.

The RMAT is a multimetric index compliant with the WFD based on the benthic macroinvertebrates community, combining 'abundance' (Hurlbert index) and 'taxonomic composition' (Bentix index using density and biomass data) metrics. It performed well along anthropogenic disturbance gradients, showing ecological quality increasing from close to far away from the disturbance.

The RMAT is a promising tool for rocky shore ecological assessment in the scope of the WFD or other monitoring activities worldwide

Keywords: Benthic communities; Hard bottom; Organic enrichment; Ecological quality assessment; Water Framework Directive.

IV.1. Introduction

The European Water Framework Directive (WFD, 2000) was implemented to 'establish a framework for the protection of inland surface waters, transitional waters, coastal waters and ground waters'. The WFD requires Member States to assess the ecological quality status (EQS) of all water bodies, based on the status of the biological quality elements (BQE) as well as hydromorphological and physical-chemical quality elements. The EQS is determined by the deviation (ecological quality ratio, EQR) that the biological elements exhibit from the expected at undisturbed or nearly undisturbed situations (reference conditions) (WFD, 2000). The WFD specified a five-point scale for water quality, 'Bad', 'Poor', 'Moderate', 'Good' and 'High'; the 'High status' is represented by EQR values close to 1, whilst the 'Bad status' is expressed by values close to 0.

A major issue in the implementation of the WFD is defining reference conditions. This should be done using historical and monitoring data, modelling or, ultimately, resorting to expert judgement (WFD, 2000). This is largely because historical data is scarce on the pressures impacting ecosystems and the consequent long-term changes (Borja et al., 2012). Also, recent monitoring data may not be comparable due to different methodologies (e.g., sampling and processing) and lack of intercalibration among Member States, further slowing the implementation of the WFD (Poikane et al., 2014). In brief, Member States should reach an agreement on quality standards (e.g., set reference conditions and establish boundaries between EQS classes) so that the different methods produce comparable classifications for each BQE (Birk et al., 2013).

Coastal rocky shores extend to over 80% of the coastline worldwide (Emery and Kuhn, 1982; Granja, 2004). They are important marine habitats with great biodiversity, providing valuable ecosystem services, namely provisioning, regulating and cultural services (e.g., Liquete et al., 2013; Galparsoro et al., 2014). The particular environmental conditions (e.g., wave exposure, tidal regime) of rocky shores add challenges to the ecological status assessment. The intertidal rocky shore is a very harsh environment and biotic communities there are naturally highly variable (Thompson et al., 2002). Difficulties in distinguishing natural from anthropogenic disturbance (e.g., organic enrichment) have often been highlighted (e.g., Crowe et al., 2000; Thompson et al., 2002; Elliott and Quintino, 2007). This hampers the WFD implementation with regard to rocky shores, namely in the development of an ecological assessment tool (e.g., defining reference conditions, setting boundaries between EQS classes). Despite that, rocky shore communities have also often shown to respond to different levels of disturbance (e.g., Bishop et al., 2002; Kraufvelin, 2007; O'Connor, 2013; Cabral-Oliveira et al, 2014; Vinagre et al., 2016a).

For assessment of coastal and transitional waters, several multimetric ecological tools have been developed based on the different BQEs (Birk et al., 2012), combining complementary, metrics to summarize the ecosystem health into a single, and comprehensible value. Also, several biological elements (e.g., macroalgae, phytoplankton) have been intercalibrated among Member States (Poikane et al., 2014). For benthic macroinvertebrates, however, the intercalibration exercise has been undertaken only for the soft sediment habitat, while for hard substratum (i.e., rocky shores) that has not been the case (Borja et al., 2009a). This is because, despite macroalgae and benthic macroinvertebrates being the most suitable BQEs for rocky shore assessment, the tools available are exclusively (Ballesteros et al., 2007; Juanes et al., 2008; Neto et al., 2012; Ar Gall and Le Duff, 2014), or in part (Hiscock et al., 2005; Díez et al., 2012; O'Connor, 2013) based on the macroalgae. Although macroinvertebrates are widely recognized as good indicators of water quality and pollution, to date, attempts to develop an index based exclusively on this BQE (Hiscock et al., 2005; Díez et al., 2012; Orlando-Bonaca et al., 2012) were not totally successful. This was possibly because of the approaches widely used by rocky shore ecologists (e.g., using non-destructive percentage cover instead of destructive samples of density or biomass, or using a low taxonomic resolution). Therefore, a method based specifically on the benthic macroinvertebrates from hard substratum constitutes a gap in the WFD implementation (Birk et al., 2012).

The overall aim of this work was to address that gap in the WFD implementation, and to propose a multimetric index based exclusively on rocky shore macroinvertebrates, the *Rocky shore Macroinvertebrates Assessment Tool* (*RMAT*). The RMAT seems promising for WFD rocky shore quality assessments, and may be a valuable indicator in the scope of other European Directives (e.g., Marine Strategy Framework Directive).

In parallel to the RMAT, an alternative index (*alt-RMAT*) is presented; this is not as accurate as the former but is quicker and less expensive to apply when time or resources are limited.

IV.2. Methods

IV.2.1. Study sites

The Buarcos (40°10'14.2"N, 8°53'26.7"W) and Matadouro (38°58'31.5"N, 9°25'14.4"W) rocky shores are located in the western Portuguese coast (Fig. IV.1A) and classified as Exposed and Moderately Exposed Atlantic Coast typologies (TICOR project, Bettencourt et al., 2004; available at http://www.ecowin.org/ticor), respectively.

Along this coast the prevailing current direction is from West-Northwest, and the most frequent wave period and wave height are in the range of 8-12 s and of 1-3 m, respectively. Tide is semidiurnal and the extreme spring tide ranges from 3.5-4 m (Boaventura et al., 2002, Bettencourt et al., 2004).

Both shores are subject to moderate impact from continuous throughout the year runoff of waters crossing urban centres and agricultural land before reaching the shore (Vinagre et al., 2016a, b, 2017).

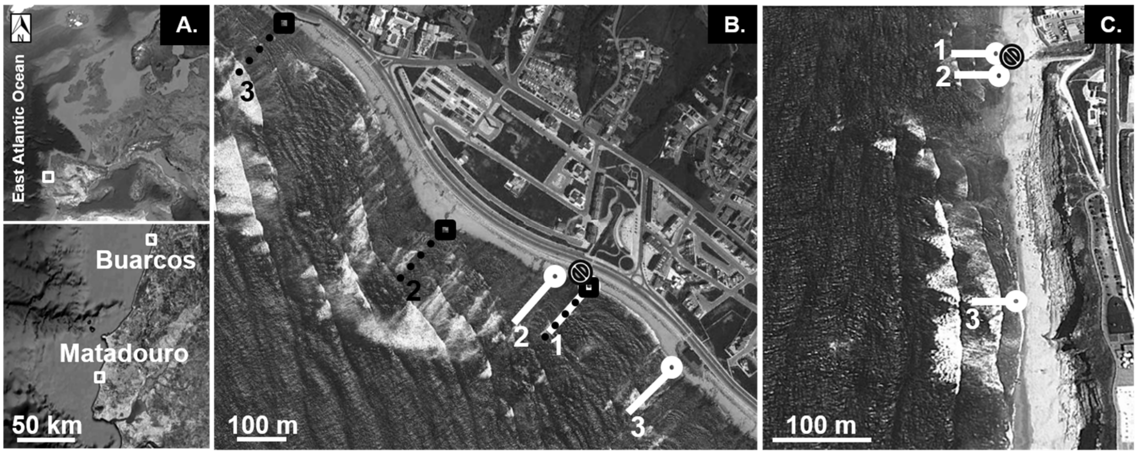


Figure IV.1. *Study sites location: A. Europe and Portugal. B. Buarcos (40°10'14.2"N, 8°53'26.7"W). C. Matadouro (38°58'31.5"N, 9°25'14.4"W). Sampling sites = white circles full line; Validation sites = black squares dotted line; Source of disturbance = ⊘ sign.*

IV.2.2. Data collection

Eleven ecological indices based on macroinvertebrates were selected from Vinagre et al. (2016b). These were those that performed best along the disturbance gradients at both shores, especially during summer. Summer data (collected during August and September 2011) was used as it was previously found as the better season (comparing to winter) for monitoring activities on rocky shores (Vinagre et al., 2016b, 2017). The indices were calculated using macroinvertebrates' density (ind m^{-2}) and biomass (g AFDW m^{-2}) data, estimated from samples collected at three sites distancing gradually along the disturbance gradient (site 1 closest to the disturbance, site 3 farthest from the disturbance) (Figs. IV.1B, IV.1C). Each site was divided into three intertidal zones: upper intertidal (submersed for ~25% of the tide period, ~6h/day); mid intertidal (submersed for ~50% of the tide period, ~12h/day); and lower intertidal (submersed for ~75% of the tide period, ~18h/day). Four random 12 x 12 cm samples were collected at each intertidal zone. Hence, a total of 144 samples (2 sampling events during summer * 2 shores * 3 sites* 3 zones per site * 4 replicates per zone) were used for each index.

The data set included abundance/diversity metrics and indices based on taxonomic composition. In the former group were listed the total biomass of opportunistic taxa [ecological groups (EG) III-V from AZTI's Marine Biotic Index (AMBI, Borja et al., 2000), after update of missing EG (Vinagre et al., 2016a, b)], Margalef index (Margalef, 1968), Hurlbert index (Hurlbert, 1971), Shannon-Wiener index ($\log_2$) (Shannon and Weaver, 1963) and (complement of) Simpson index (Simpson, 1949) (all calculated using biomass data, B_{opp}, d_B, ES_{10}, H'_B and 1-λ'_B, respectively). The last group was composed of the AMBI, MEDiterranean OCCidental index (MEDOCC, Pinedo and Jordana, 2008) and Bentix biotic index (BENTIX, Simboura and Zenetos, 2002), calculated using density: AMBI, MEDOCC and BENTIX, and using biomass: AMBI_B, MEDOCC_B and BENTIX_B, respectively. All indices were calculated per replicate.

IV.2.3. Data analysis

The construction of the proposed multimetric index RMAT was based on two different methods. Method 1 was essential to find the most suitable metrics (i.e., correlating stronger with the disturbance gradient) to integrate the multimetric index (e.g. using multivariate analysis of data). Method 2 was followed in parallel to reinforce the results of Method 1 (e.g., using multiple linear regressions to aid in the selection of the metrics more correlated to the disturbance).

Some analyses may seem redundant (e.g., Method 1 step B. vs step C., Method 1 step G. versus Method 2). However, owing to the novelty of the work, it was necessary to reduce any uncertainty from the results obtained, to assure the indices selected were the most appropriate.

In both methods, the relationship between macroinvertebrate indices and pressure is indirect being tested against the *MarMAT* (*Marine Macroalgae Assessment Tool*) assessment, a WFD compliant tool based on rocky shore macroalgae that has been shown to respond to pressure (Neto et al., 2012).

IV.2.3.1. Developing Method 1

All analyses (except boxplots) were performed with PRIMER 6 + PERMANOVA© software (Clarke and Gorley, 2006; Anderson et al., 2008). Boxplots were drawn using Minitab® V.17 (Minitab Inc.) statistical software.

IV.2.3.1.1. Preliminary data set

The data set including the 11 ecological indices was used to visualize the distribution of sampling sites along disturbance gradients, by performing Principal coordinate (PCO) analyses. The Euclidean similarity measure was used in the calculation of similarity matrices, after normalisation of data. The main indices related to that distribution were found looking at principal component analysis (PCA) eigenvalues for the first two axes.

Subsequently, data from each index were analysed separately to assess which were the best candidates to be included in the multimetric index:

A. Significant differences were investigated using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001), including one fixed factor, ‘Site’ (three levels: sites 1-3). Similarity matrices were calculated as for the PCO analysis. The statistical significance of variance components was tested using 9999 unrestricted permutations of raw data, with a significance level of $\alpha = 0.05$. This was done to verify the indices that could better distinguish among sites along disturbance gradients (together with PCO and PCA analyses);

B. The resemblance in data structure between each macroinvertebrate index and MarMAT was analysed using the RELATE routine (comparative Mantel-type tests on similarity matrices) on the basis of the Euclidean similarity measure (Spearman correlation and 9999 permutations were used). For the invertebrate indices were used values per replicate in the calculation of similarity matrices. For the MarMAT were used values calculated at site level for each shore (for methodological reasons; see Gaspar et al., 2012 and Neto et al., 2012);

C. Relations among macroinvertebrate indices were examined using the Draftsman plot routine with calculation of Pearson correlations). Highly correlated (>0.9) indices were removed from the dataset. In this step correlations were also calculated between macroinvertebrate indices and the MarMAT, to reinforce the results from RELATE (step B. in this section) (using raw data instead of similarity matrices). Hence, to make the correspondence between the invertebrate indices and MarMAT possible, the same MarMAT value from a site was compared to all samples from that site for each invertebrate index (e.g., MarMAT value of Buarcos site 1 was given to 24 samples of Buarcos site 1 from each invertebrate index; 24 samples from site 1 = 4 replicates * 3 intertidal zones * 2 sampling events during summer).

This was the final data set used in further analysis of Method 1. All variables in this data set were used in the remaining analyses of Method 1, to aid the comparison with results from Method 2.

IV.2.3.1.2. Final data set

After correlated (>0.9) variables been removed, the final data set was used to:

D. Re-analyse the sampling sites' distribution and the indices responsible for it, by performing a second PCO and PCA;

E. Show the indices trends across sites along the gradients, by drawing box-and-whiskers plots (with median values) and mean values (with respective standard deviation) and comparing them with MarMAT;

F. Analyse indices' contribution to differences within and among sites, by performing (i) multivariate Similarity Percentage Analysis (SIMPER) and (ii) univariate SIMPER. Average square distances (ASD) were assessed looking for the indices which showed lower distance within, and higher distance among, sampling sites;

G. Assess the 'best' indices to integrate RMAT, using Distance-based Linear Modelling (DistLM; Anderson, 2004): i) entering all variables, and using ii) backward selection, iii) forward selection, iv) step-wise selection and v) BEST selection – best variable, best two variables and best three variables. The BIC (Bayesian Information Criterion) selection criterion was selected, and 9999 permutations were used;

H. Select the best indices to be included in the multimetric index. The index which performed worst at each analysis (steps A.-C. in section 2.3.1.1, and steps D. and F. in this section), showing poorer relation with the disturbance gradients, received lowest score (of 1), the second worse index got the second lowest score (of 2), and so forth. From the PCA (step D. in this section), the scores given were calculated as [((2 * PCA1) + PCA2) / 3], to give additional weighing to PCA1. From the multivariate SIMPER (step F.(i) in this section) were used the mean value of all sites scores, and the score from the comparison between sites 1 and 3. From the univariate SIMPER (F.(ii) in this section) was used the mean value [((2*Between sites 1,3) + Within sites 1,2,3) / 3].

The indices with highest final scores (sum of all analyses scores), reflecting best overall performance, were selected to integrate the RMAT (considering also the indices selected in step G. in this section).

IV.2.3.2. Developing Method 2

In parallel to Method 1, the data set with 11 indices was used together with MarMAT in multiple linear regressions (MLR), using the Brodgar© V2.7.1 (Highland Statistics Ltd.) software, linked to the R-2.9.1 (R Development Core Team) open-source software. This was done to determine which indices (explanatory variables, EVs) were best related to MarMAT (response variable, RV), to complement Method 1 in the selection of ‘best’ indices to integrate the proposed RMAT. Similarly to what was done in Method 1 (step C. in section 2.3.1.1), to make the correspondence between RMAT and MarMAT possible, all RMAT Buarcos site 1 samples were given the MarMAT value for Buarcos site 1, all RMAT Matadouro site 1 samples were given the MarMAT value for Matadouro site 1, and so forth for the remaining sites at both shores.

To conduct the MLRs, collinearity between EVs was first analysed, looking at variance inflation factors (VIFs). After each test, the metric showing highest VIF was removed from the data set before the next test, until all EV in the data set presented acceptable VIFs (≤5). Simultaneously, interactions between EVs were inferred using conditional scatterplots (Coplots) for the RV against one EV conditioned on another EV. This checked for the interactions to be included in the models, and to aid in deciding which collinear variables (VIF >5) to remove from the dataset.

The MLRs were performed using the AIC (Akaike Information Criteria) selection criteria with i) forward selection; ii) backward selection; and iii) forward and backward selection. For each model obtained, the residuals were checked for: i) normality; ii) homoscedasticity; iii) independence; and iv) if ‘x’ is fixed. Also, it was verified the existence of influential points. After, the models were tested using ANOVA (drop 1 variable; F), and residuals from each model were compared. The ‘best’ model was after applied to the independent data set used for validation in Method 1.

IV.2.4. Multimetric index development: The RMAT – Rocky shore Macroinvertebrates Assessment Tool

The RMAT was designed considering the results from Method 1 (steps G. and H. in section 2.3.1.2) and Method 2. First, reference conditions (RC) were searched among the literature for each of the selected indices. When RC were not available in the literature, the maximum value was selected (corresponding to highest quality) obtainable by the metric, or calculated a mean value between the maximum and the 95th percentile values (obtained at present). Second, EQR values were calculated (as the ratio of the sample value and the RC, and ranging from 0-1) for each of the 144 samples. The EQR >1 (for samples with values higher than the RC) were truncated to 1. Third, the RMAT was calculated per sample as the mean value of the indices’ EQR values, and after was calculated and presented per sampling site.

In this step different models were tested, in which different weightings were given to the indices included in RMAT (combination rule). The purpose was to select the RMAT model with results best matching to the MarMAT’, emphasizing the indices that showed best response to the disturbance (regarding their final scores).

The model with the most similar behaviour to MarMAT, by showing at sampling sites 1, 2 and 3 the mean EQR values closest to MarMAT EQR values, was selected for further validation.

IV.2.5. Validation of the RMAT

The ‘best’ model (=RMAT) was applied to independent data, gathered from Buarcos during summer 2009 (using the same methodology as for the first dataset) at different sites (except site 1) along the disturbance gradient (Fig. IV.1C). The results were compared to the MarMAT EQR values calculated for that period, and also compared to the previous response provided by the model (section 2.4).

IV.3. Results

IV.3.1. Method 1

IV.3.1.1. Preliminary data set

The PCO ordination using the 11 macroinvertebrate indices (explaining 77.0% of total variability, 48.4% in PCO1) showed a separation between sites 1, 2 and 3 (Fig. IV.2A), mainly related to BENTIX_B, AMBI_B and MEDOCC_B, followed by BENTIX, MEDOCC and AMBI (PCA eigenvalues; Table IV.1A). Also, site 1 was more related to higher values of AMBI_B, MEDOCC_B, MEDOCC, AMBI and B_{opp}, while on the other hand site 3 was more related to higher values of BENTIX_B, BENTIX, H’_B, d_B, 1-λ’_B and ES_{10}.

A. The ES_{10} showed significant differences between site 3 and the other two sites (PERMANOVA; Table IV.2). The remaining indices (except H’_B and d_B) showed differences between site 1 and the other two sites. The H’_B found differences between sites 1 and 3. For d_B the separation of sites was not statistically significant.

B. The RELATE routine showed significant relations between MarMAT and all macroinvertebrate indices except d_B (Table IV.3). Relationships were stronger (higher rho) between similarity matrices of MarMAT and BENTIX, followed by B_{opp}, MEDOCC, AMBI, BENTIX_B, and AMBI_B and MEDOCC_B.

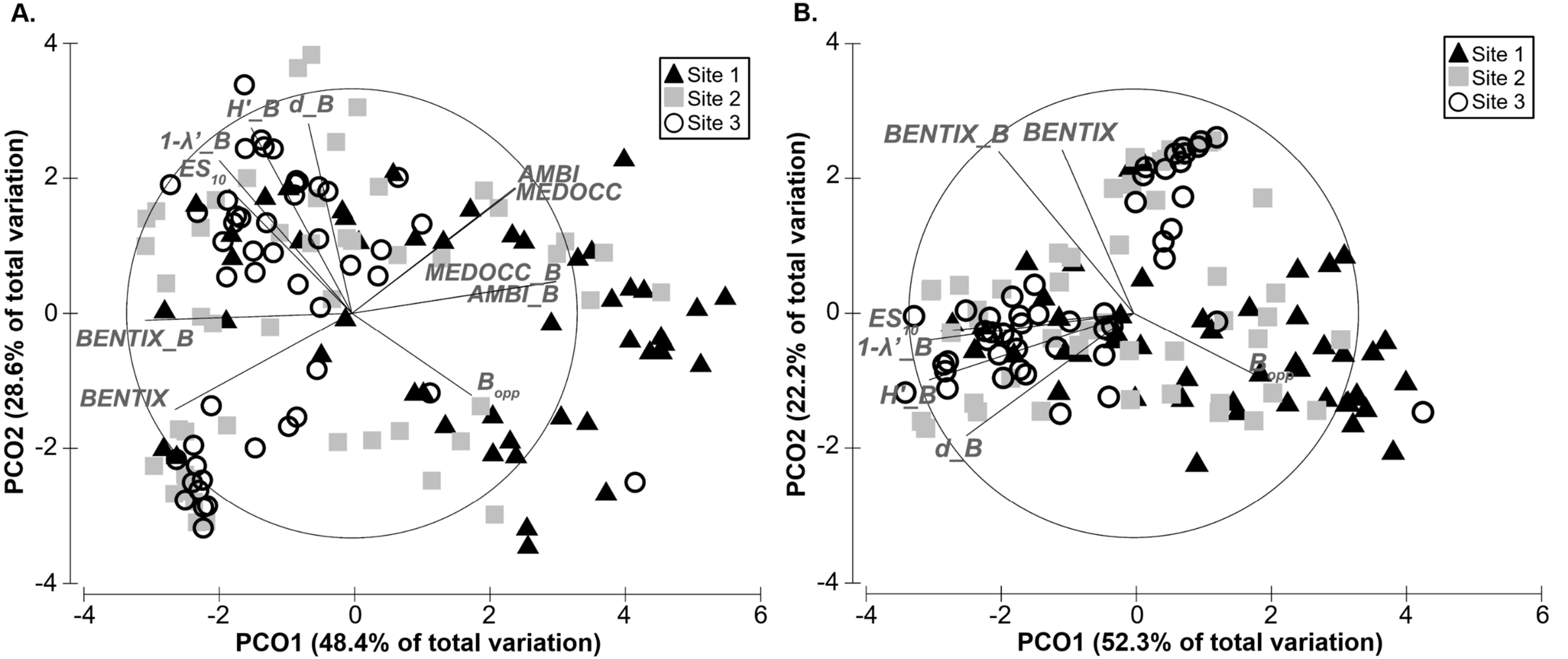


Figure IV.2. *Principal coordinates (PCO) analysis plots considering eleven invertebrate indices (A.) and after removing high correlated (>0.9) indices (B).*

Table IV.1. *PCA eigenvalues (first two axes) for the eleven invertebrate indices (A.) and after removing high correlated (>0.9) indices (B.). Higher scores in bold*

		A.	PCA1	PCA2	B.	PCA1	PCA2
Biomass	Abundance/Diversity	d_B	0.084	-0.476	d_B	0.389	0.438
		ES_{10}	0.237	-0.312	ES_{10}	**0.421**	0.061
		H'_B	0.194	-0.466	H'_B	**0.476**	0.239
		1-λ'_B	0.255	-0.383	1-λ'_B	**0.472**	0.095
		B_{opp}	-0.231	0.206	B_{opp}	-0.310	0.242
	Taxonomic composition	AMBI_B	**-0.393**	-0.080			
		MEDOCC_B	**-0.393**	-0.079			
		BENTIX_B	**0.398**	0.017	BENTIX_B	0.314	**-0.580**
Density		AMBI	**-0.314**	-0.315			
		MEDOCC	**-0.316**	-0.313			
		BENTIX	**0.340**	0.242	BENTIX	0.166	**-0.586**

Table IV.2. *PERMANOVA results for the eleven macroinvertebrate indices. A. main tests. B. Pairwise tests (significant tests are presented).*

		A. Main tests	Pseudo-F	P(perm)
Biomass	Abundance/Diversity	d_B	2.357	0.0969
		ES_{10}	15.285	0.0001
		H'_B	8.320	0.0005
		1-λ'_B	9.073	0.0004
		B_{opp}	6.341	0.0014
	Taxonomic composition	AMBI_B	18.455	0.0001
		MEDOCC_B	18.479	0.0001
		BENTIX_B	19.058	0.0001
Density		AMBI	10.706	0.0002
		MEDOCC	10.730	0.0001
		BENTIX	12.925	0.0001

		B. Pairwise tests		t	P(perm)
Biomass	Abundance/Diversity	ES_{10}	Site 1, Site 3	5.254	0.0001
			Site 2, Site 3	3.748	0.0005
		H'_B	Site 1, Site 3	3.992	0.0002
		1-λ'_B	Site 1, Site 2	3.059	0.0026
			Site 1, Site 3	4.244	0.0001
		B_{opp}	Site 1, Site 2	2.590	0.0092
			Site 1, Site 3	3.008	0.0010
	Taxonomic composition	AMBI_B	Site 1, Site 2	4.291	0.0001
			Site 1, Site 3	6.151	0.0001
		MEDOCC_B	Site 1, Site 2	4.308	0.0001
			Site 1, Site 3	6.143	0.0001
		BENTIX_B	Site 1, Site 2	4.387	0.0001
			Site 1, Site 3	6.219	0.0001
Density		AMBI	Site 1, Site 2	2.918	0.0050
			Site 1, Site 3	4.854	0.0001
		MEDOCC	Site 1, Site 2	3.013	0.0049
			Site 1, Site 3	4.781	0.0001
		BENTIX	Site 1, Site 2	3.296	0.0015
			Site 1, Site 3	5.102	0.0001

C. Pearson correlations were very high between AMBI_B and MEDOCC_B, and AMBI and MEDOCC (~+1), followed by the comparisons of BENTIX_B with the prior two, and BENTIX with the latter two (~-1) (Table IV.4). The indices showing stronger correlations with MarMAT were AMBI_B (-0.487), MEDOCC_B (-0.487), BENTIX_B (+0.476), BENTIX (+0.472), AMBI (-0.463), MEDOCC (-0.460) and ES_{10} (+0.411).

IV.3.1.2. Final data set

The PCO (Fig. IV.2A) and PCA (Table IV.1A) analyses showed BENTIX_B distinguished sampling sites better than AMBI_B and MEDOCC_B. The BENTIX and BENTIX_B where the indices most similar to MarMAT (RELATE, Table IV.3). Considering this and previous findings from Vinagre et al. (2016b) where BENTIX performed better than AMBI and MEDOCC, and BENTIX_B performed better than AMBI_B and MEDOCC_B, in terms of showing high correlations (namely among these six indices) the AMBI_B, MEDOCC_B, AMBI and MEDOCC were removed from the data set previous to further analysis.

Table IV.3. *RELATE relationships between MarMAT and each invertebrate index.*

			Rho	p
Biomass	Abundance/Diversity	d_B	-0.018	0.7532
		ES_{10}	0.129	0.0001
		H'_B	0.047	0.0181
		$1-\lambda'$_B	0.161	0.0001
		B_{opp}	0.213	0.0001
	Taxonomic composition	AMBI_B	0.197	0.0001
		MEDOCC_B	0.197	0.0001
		BENTIX_B	0.205	0.0001
Density		AMBI	0.208	0.0001
		MEDOCC	0.209	0.0001
		BENTIX	0.261	0.0001

Table IV.4. *Pearson correlations between all indices. Highest correlations in bold.*

	Macroinvertebrates										Macroalgae
	Biomass							Density			
	Abundance/Diversity				Taxonomic composition						
	ES_{10}	H'_B	1-λ'_B	B_{opp}	AMBI_B	MEDOCC_B	BENTIX_B	AMBI	MEDOCC	BENTIX	MarMAT
d_B	+0.542	+0.781	+0.775	-0.196	+0.043	+0.041	+0.062	+0.217	+0.207	-0.055	+0.128
ES_{10}		+0.789	+0.589	-0.303	-0.311	-0.310	+0.361	-0.191	-0.190	+0.283	+0.411
H'_B			+0.804	-0.447	-0.211	-0.212	+0.318	+0.063	+0.061	+0.071	+0.273
1-λ'_B				-0.442	-0.387	-0.388	+0.472	-0.108	-0.117	+0.228	+0.329
B_{opp}					+0.557	+0.558	-0.606	+0.013	+0.014	-0.061	-0.258
AMBI_B						**+1.000**	**-0.984**	+0.582	+0.584	-0.611	-0.487
MEDOCC_B							**-0.984**	+0.580	+0.583	-0.611	-0.487
BENTIX_B								-0.531	-0.536	+0.588	+0.476
AMBI									**+0.999**	**-0.965**	-0.463
MEDOCC										**-0.969**	-0.460
BENTIX											+0.472

D. In the second PCO ordination (with seven indices, explaining 74.5% of total variability) separation of sites was again observed (Fig. IV.2B), with higher variation of data being explained by PCO1 (52.3%), which mainly related to H'_B, 1-λ'_B and ES_{10} (PCA eigenvalues; Table IV.1B). This was accompanied by less dispersion of the sites data across PCO2 and a slight rotation in the data cloud, in which could also be seen a slight separation of sites, mainly related to BENTIX and BENTIX_B.

E. The MarMAT showed increasing quality from site 1 < site 2 < site 3, as indicated by the increasing mean values in that direction. All indices presented a response parallel to MarMAT, with mean and median values increasing (decreasing in the case of B_{opp}) from site 1 to site 3 (Fig. IV.3). The indices showed lower variation (lower standard deviation and box and whiskers size) within site 3 (variation of B_{opp}, BENTIX, BENTIX_B and 1-λ'_B decreased from site 1 to site 3; variation of ES_{10} and H'_B was comparable across sites). The exceptions were the d_B showing lower variation within site 1, and the H´_B showing higher variation within site 3 (Fig. IV.3).

F. The multivariate SIMPER (Table IV.5A) calculated average squared distances (ASD) within sites which decrease from site 1 > site 2 > site 3, indicating larger data variation within site 1 contrary to site 3. Within site 1, the least contribution to the ASD was from the d_B, H'_B, ES_{10} and BENTIX_B; at site 2 these were the B_{opp}, ES_{10} and H'_B; and at site 3 they were the BENTIX, 1-λ'_B and BENTIX_B. The ASD was higher between sites 1 and 3, mainly due to the ES_{10}, B_{opp}, H'_B, BENTIX_B and BENTIX. Between sites 1 and 2 the top contributors to the ASD were the BENTIX, BENTIX_B, 1-λ'_B and B_{opp}. Between sites 2 and 3 the ASD was lower, which was related to the d_B, ES_{10} and H'_B. In the univariate SIMPER (Table IV.5B), d_B showed the lowest ASD within site 1 and also between sites 1 and 3. The ES_{10} and H'_B, despite showing the highest ASD within site 3, also showed the highest ASD between sites 1 and 3. The 1-λ'_B, BENTIX_B and BENTIX showed the lowest ASD within site 3 and the highest between sites 1 and 2, followed by sites 1 and 3. The B_{opp} showed the best results, with higher ASD within site 1 and between sites 1 and 3.

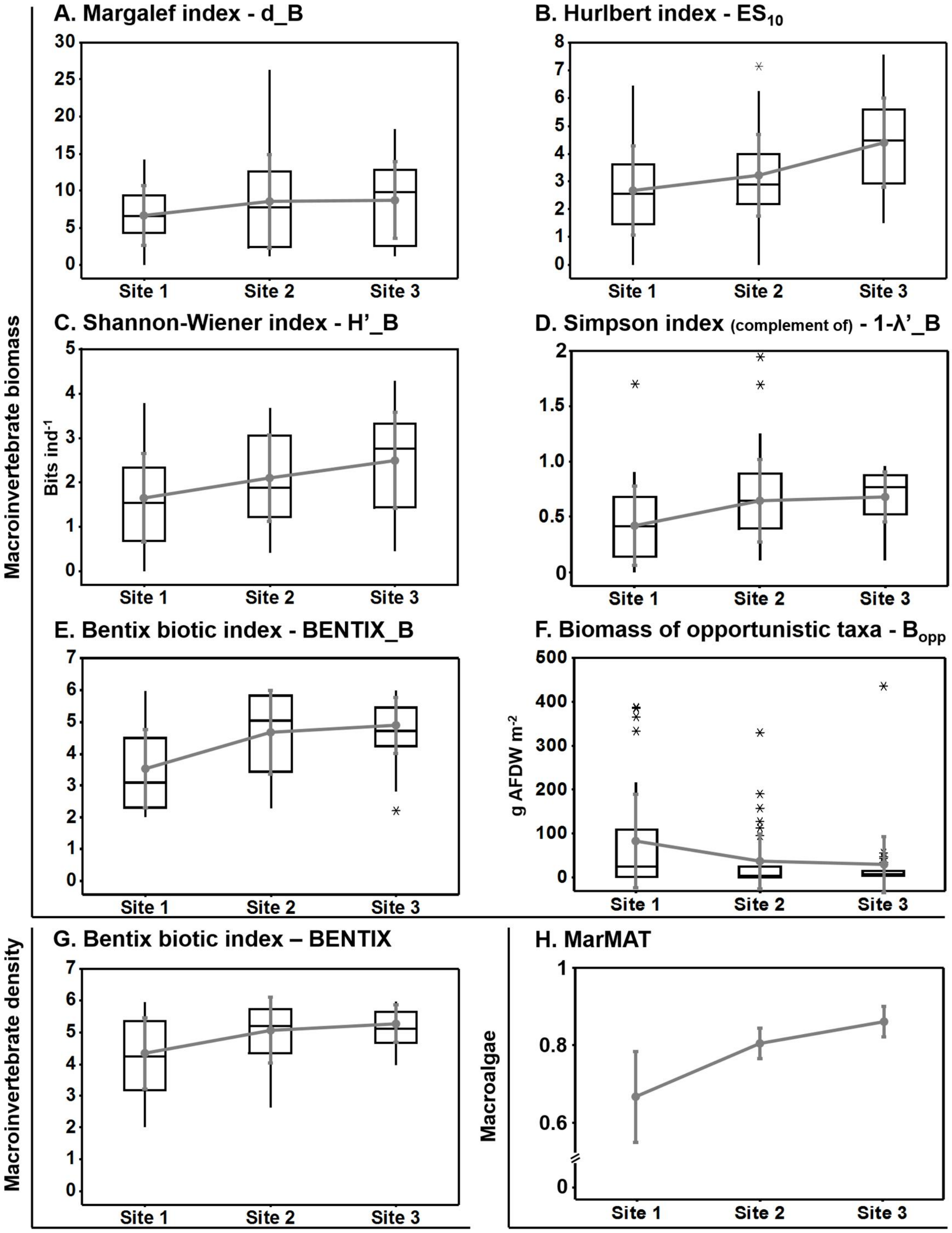


Figure IV.3. *Comparison of invertebrate indices with MarMAT. Mean values (and standard deviation) in grey. Box plots in black – Box: mid line = 50th percentile (median); bottom and top of the box = 25th (Q1) and 75th (Q3) percentiles, respectively; lower and upper whiskers = [Q_1-1.5(Q_3-Q_1)] and [Q_3+1.5(Q_3-Q_1)], respectively; outliers = values outside whiskers limits (*).*

Table IV.5. *SIMPER analysis on factor 'Site'. A. Multivariate analysis, showing the indices contribution (%) to the average squared distances (in bold) within sites (shaded boxes) and between sites (non-shaded boxes). B. Univariate analysis, showing the distances within sites (shaded boxes) and between sites (non-shaded boxes).*

A.

	Site 1		Site 2		Site 3	
Site 1	**7.18**	%				
	d_B	8.08				
	H'_B	12.15				
	ES_{10}	12.24				
	BENTIX_B	12.60				
	1-λ'_B	15.13				
	BENTIX	16.86				
	B_{opp}	22.94				
Site 2	**16.10**	%	**6.77**	%		
	BENTIX	16.76	B_{opp}	8.31		
	BENTIX_B	16.61	ES_{10}	10.84		
	1-λ'_B	16.50	H'_B	12.24		
	B_{opp}	15.36	BENTIX	15.18		
	d_B	12.91	BENTIX_B	15.26		
	H'_B	11.42	1-λ'_B	17.38		
	ES_{10}	10.44	d_B	20.79		
Site 3	**16.33**	%	**11.91**	%	**4.67**	%
	ES_{10}	16.82	d_B	19.45	BENTIX	7.14
	B_{opp}	15.94	ES_{10}	17.32	1-λ'_B	9.27
	H'_B	15.20	H'_B	16.40	BENTIX_B	9.80
	BENTIX_B	14.89	1-λ'_B	13.31	B_{opp}	12.46
	BENTIX	14.38	BENTIX_B	12.49	ES_{10}	19.01
	1-λ'_B	12.60	BENTIX	11.54	d_B	20.47
	d_B	10.17	B_{opp}	9.49	H'_B	21.86

B.

d_B	Site 1	Site 2	Site 3
Site 1	15.96		
Site 2	57.18	38.72	
Site 3	45.66	63.69	26.31

ES_{10}	Site 1	Site 2	Site 3
Site 1	2.49		
Site 2	4.76	2.08	
Site 3	7.78	5.85	2.52

H'_B	Site 1	Site 2	Site 3
Site 1	0.97		
Site 2	2.04	0.92	
Site 3	2.76	2.17	1.13

1-λ'_B	Site 1	Site 2	Site 3
Site 1	0.12		
Site 2	0.30	0.13	
Site 3	0.23	0.18	0.05

BENTIX_B	Site 1	Site 2	Site 3
Site 1	1.48		
Site 2	4.38	1.69	
Site 3	3.98	2.44	0.75

B_{opp}	Site 1	Site 2	Site 3
Site 1	11035.0		
Site 2	16569.1	3772.6	
Site 3	17441.8	7571.9	3901.9

BENTIX	Site 1	Site 2	Site 3
Site 1	1.19		
Site 2	2.66	1.01	
Site 3	2.32	1.36	0.33

Table IV.6. *DistLM analyses showing invertebrate indices which explain most the MarMAT data, and the proportion of data explained (in grey).*

A. Enter all variables

	Pseudo-F	p	Proportion
d_B	2.351	0.1282	0.0163
ES$_{10}$	28.780	0.0001	0.1685
H'_B	11.446	0.0010	0.0746
1-λ'_B	17.274	0.0001	0.1085
BENTIX_B	41.646	0.0001	0.2268
B$_{opp}$	10.148	0.0028	0.0667
BENTIX	40.779	0.0001	0.2231
BIC	R^2		
-691.29	0.3452		

B. Backward selection

Starting solution: All variables					
BIC	R^2				
-691.29	0.3452				
	Pseudo-F	p	Proportion	Cumulative	BIC
-d_B	0.031	0.8585	0.0002	0.3451	-696.23
-B$_{opp}$	0.584	0.4372	0.0028	0.3423	-700.59
-1-λ'_B	0.601	0.4359	0.0029	0.3394	-704.93
-H'_B	0.204	0.6525	0.0010	0.3384	-709.69
Best solution: ES$_{10}$, BENTIX_B and BENTIX					
BIC	R^2				
-709.69	0.3384				

C. Forward and D. Step-wise selection

	Pseudo-F	p	Proportion	Cumulative	BIC
+BENTIX_B	41.646	0.0001	0.2268	0.2268	-697.17
+ES$_{10}$	13.049	0.0005	0.0655	0.2923	-704.95
+BENTIX	9.766	0.0017	0.0461	0.3384	-709.69
Best solution: ES$_{10}$, BENTIX_B and BENTIX					
BIC	R^2				
-709.69	0.3384				

E. BEST selection

Best solutions		
Number of variables	BIC	R^2
1: BENTIX_B	-697.17	0.2268
2: ES$_{10}$ and BENTIX	-707.87	0.3065
3: ES$_{10}$, BENTIX_B and BENTIX	-709.69	0.3384

Table IV.7. *Scores given to invertebrate indices in each step (A.-D. and F.) of Method 1 (highlighted in grey), and their final score (highlighted in black).*

A.

PERMANOVA	d_B	1
	ES$_{10}$	3
	H'_B	2
	1-λ'_B	3
	BENTIX_B	3
	B$_{opp}$	3
	BENTIX	3

B.

RELATE (with MarMAT)	d_B	1
	ES$_{10}$	3
	H'_B	2
	1-λ'_B	4
	BENTIX_B	5
	B$_{opp}$	6
	BENTIX	7

C.

Correlations (with MarMAT)	d_B	1
	ES$_{10}$	5
	H'_B	3
	1-λ'_B	4
	BENTIX_B	7
	B$_{opp}$	2
	BENTIX	6

D.		PCA1	PCA2	Mean*
PCA	d_B	4	5	4.33
	ES$_{10}$	5	1	3.67
	H'_B	7	3	5.67
	1-λ'_B	6	2	4.67
	BENTIX_B	3	6	4.00
	B$_{opp}$	2	4	2.67
	BENTIX	1	7	3.00

F.(i)		Site 1	Site 2	Site 3	Mean	Site 1 Vs Site 3
Multivariate SIMPER	d_B	7	1	2	3.33	1
	ES$_{10}$	5	6	3	4.67	7
	H'_B	6	5	1	4.00	5
	1-λ'_B	3	2	6	3.67	2
	BENTIX_B	4	3	5	4.00	4
	B$_{opp}$	1	7	4	4.00	6
	BENTIX	2	4	7	4.33	3

F.(ii)		Within sites	Site 1 Vs Site 3	Mean**
Univariate SIMPER	d_B	1	1	1.00
	ES$_{10}$	1	3	2.33
	H'_B	1	3	2.33
	1-λ'_B	2	2	2.00
	BENTIX_B	2	2	2.00
	B$_{opp}$	2	3	2.67
	BENTIX	3	2	2.33

Final Score	
d_B	12.67
ES$_{10}$	**28.67**
H'_B	24.00
1-λ'_B	23.33
BENTIX_B	**29.00**
B$_{opp}$	26.33
BENTIX	**28.67**

*calculated as [((2 x 'PCA1') + 'PCA2') / 3]
**calculated as [((2 x 'Site 1 Vs site 3') + 'Within sites') / 3]

G. The DistLM analysis between the MarMAT EQR values and the final seven indices highlighted the variables with most potential to be included in the final model (Table IV.6). Entering all variables in the model, all except d_B were statistically significant ($p<0.05$). The BENTIX_B (0.227), BENTIX (0.226) and ES$_{10}$ (0.16) showed the highest contributions in the model. Using the backward selection criterion, all variables except those three were removed from the model. Using forward, step-wise and BEST selection criteria those three variables were too the ones selected (BIC = -709.7, R^2 = 0.3384) for the model.

H. Ultimately, the seven macroinvertebrate indices were scored considering their performance in each analysis (steps A.-C. in section 2.3.1.1, and steps D. and F. in section 2.3.1.2) (Table IV.7). The indices with highest final scores (sum of all scores), reflecting best overall performance, were the BENTIX_B, BENTIX and ES_{10}. Accordingly, these were the indices selected, together with those from step G. (section 2.3.1.2), to integrate the RMAT.

IV.3.2. Method 2

The successive tests for collinearity between macroinvertebrate indices showed very high variable inflation factors (VIFs), meaning high collinearity between variables. The metrics showing highest VIF were sequentially removed from the data set, namely the AMBI, MEDOCC, AMBI_B and MEDOCC_B.

The best model found (AIC = -730.04; R^2 = 0.3933) included the indices ES_{10}, BENTIX_B and BENTIX, and interactions between BENTIX_B and the other two indices.

The final model was given as:

Y1 ~ 1 + ES_{10} + BENTIX_B + BENTIX + (BENTIX_B:ES_{10}) + (BENTIX_B:BENTIX).

The respective equation was given as:

Y = 0.270249 (±0.102833) + [**0.062444** (±0.021953) *ES_{10}] + [0.093982 (±0.030617) ***BENTIX_B] + [0.055431** (±0.024125) ***BENTIX] - [0.011275** (±0.004623) ***BENTIX_B:ES_{10}] - [0.009355** (±0.006211) ***BENTIX_B:BENTIX]**.

Although the model fulfilled the assumptions for MLR models, influential points were observed, which could be contributing for the low R^2 (<0.5). The model could be improved with transformation of the indices data. However, this was not done owing to the intended purpose which was to identify the most efficient (in this case, most significant) metrics, these being the ES_{10}, BENTIX_B and BENTIX.

IV.3.3. Index development: The RMAT – Rocky shore Macroinvertebrates Assessment Tool

From Method 1 and Method 2, the indices selected to combine in the RMAT were the ES_{10}, BENTIX_B and BENTIX. Reference conditions (RC) for these indices in rocky shores could not be found in the literature. Therefore, for BENTIX_B and BENTIX were used the maximum values obtainable by the metrics, i.e., RC = 6 for both. For ES_{10}, since the metric does not have an upper limit; the RC was calculated as the mean of the maximum value obtained (7.570) and the 95th percentile value (6.385). Hence, RC = 7 for ES_{10}.

All models tested showed parallels with MarMAT, with increasing mean EQR values from site 1 < site 2 <site 3 (Table IV.8A). The model deviating least from MarMAT was calculated as RMAT = $[(ES_{10} + (2 \times \text{BENTIX\_B}) + (2 \times \text{BENTIX})) / 5]$. As an alternative, if using only biomass data is advantageous (e.g., due to time constraints), the best model would be alt-RMAT = $[ES_{10} + (2 \times \text{BENTIX\_B})) / 3]$.

For the MLR model the same trend as above was found, with increasing values from site 1 < site 2 <site 3. The MLR model showed amongst all models the least deviation, and highest correlation, from MarMAT. However, it showed an over-estimation of site 1 mean EQR value with regard to the MarMAT values (Table IV.8A).

Table IV.8. *Models tested using the indices ES$_{10}$ (X), BENTIX_B (Y) and BENTIX (Z): A. Mean ecological quality ratio (EQR) values using the original data set; B. Mean EQR values using independent data for validation. In black: models selected for validation; in dark grey: models with three indices; in light grey: models with two indices; in white: multiple linear regression (MLR) model, and MarMAT assessment results [(EQR and associated ecological quality status (EQS)]. In bold: highest correlations with MarMAT.*

A.		Three indices										Two indices			MLR	MarMAT	
		X+Y+Z /3	*X+2Y+Z* /4	*X+2Y+2Z* /5	*2X+Y+Z* /4	*2X+2Y+Z* /5	*3X+Y+Z* /5	*3X+2Y+Z* /6	*2X+3Y+Z* /6	*3X+3Y+Z* /7	*1.5X+1.5Y+Z* /4	*X+Y* /2	*2X+Y* /3	*X+2Y* /3	AIC = -730.04 R^2 = 0.3933	EQR	EQS
RAT samples	Site 1	0.551	0.557	0.586	0.507	0.521	0.480	0.496	0.530	0.508	0.532	0.475	0.442	0.509	0.735	0.667	G
	Site 2	0.678	0.700	0.723	0.622	0.650	0.588	0.617	0.669	0.638	0.661	0.608	0.556	0.660	0.786	0.806	H
	Site 3	0.756	0.767	0.784	0.722	0.737	0.701	0.717	0.748	0.729	0.744	0.708	0.678	0.739	0.812	0.861	H
Deviation from MARMAT	Site 1	0.116	0.110	0.081	0.160	0.146	0.186	0.170	0.137	0.159	0.135	0.191	0.225	0.158	-0.068		
	Site 2	0.127	0.106	0.082	0.184	0.156	0.218	0.189	0.137	0.168	0.145	0.198	0.250	0.146	0.019		
	Site 3	0.105	0.094	0.077	0.139	0.124	0.160	0.144	0.114	0.132	0.117	0.153	0.183	0.122	0.050		
Correlation with MarMAT		**0.579**	0.569	0.576	0.554	0.566	0.528	0.550	0.564	0.558	0.572	0.535	0.505	**0.539**	**0.627**		
B.																	
Validation samples	Site 1	0.433	0.435	0.465	0.394	0.403	0.370	0.381	0.409	0.390	0.414	0.357	0.329	0.384	0.679	0.470	M
	Site 2	0.465	0.472	0.503	0.418	0.433	0.389	0.407	0.443	0.419	0.445	0.385	0.348	0.421	0.700	0.630	G
	Site 3	0.571	0.572	0.611	0.520	0.531	0.490	0.504	0.539	0.515	0.546	0.472	0.438	0.507	0.749	0.750	G
Deviation from MARMAT	Site 1	0.037	0.035	0.005	0.076	0.067	0.100	0.089	0.061	0.080	0.056	0.113	0.141	0.086	-0.209		
	Site 2	0.165	0.158	0.127	0.212	0.197	0.241	0.223	0.187	0.211	0.185	0.245	0.282	0.209	-0.070		
	Site 3	0.179	0.178	0.139	0.230	0.219	0.260	0.246	0.211	0.235	0.204	0.278	0.312	0.243	0.001		
Correlation with MarMAT		0.406	0.376	**0.409**	0.396	0.377	0.387	0.375	0.361	0.363	0.388	0.325	**0.334**	0.310	**0.397**		

IV.3.4. Validation of the *RMAT*

After being applied to the independent data set, RMAT and alt-RMAT results were concordant with the previous ones, both showing mean EQR values increasing from site 1 < site 2 < site 3, and deviating the least (after the MLR) from MarMAT EQR values (Table IV.8B).

For comparative reasons only, the MLR model was also applied to the independent data. As previously, despite presenting the desired trend, it showed over-estimation of mean EQR values for sites 1 and 2.

IV.4. Discussion

The WFD demands a sectoral approach for water quality assessments, undertaken individually for each water body and using each mandatory BQE. In the WFD context, macroalgae have been the biological quality element receiving most of the attention of researchers and monitoring teams in rocky shores. Quality assessment tools developed for rocky shores in the scope of WFD were mainly based on macroalgae, and only a few have explored macroinvertebrate communities alone, or their features, to produce any assessment tool, or part of it (Birk et al., 2012).

Taking the findings from Vinagre et al. (2016b) into account, the response of the 11 macroinvertebrate indices tested in the present work was not surprising. Using data from the summer, which was found as the best season (compared to winter) for monitoring activities, the indices were able to capture the disturbance gradients by showing increasing values (decreasing in the case of total biomass of opportunists – B_{opp}) from site 1 < site 2 < site 3. The different analyses performed within Method 1 and Method 2 allowed for the selection of the 'best' indices (i.e., better related to the disturbance gradients) to integrate the proposed multimetric index (RMAT). Therefore, the combination of the mean EQR values of the 'best' indices should also provide a response similar to the indices alone, which was confirmed, and afterwards validated using independent data. The best model found (i.e., showing EQR values increasing from site 1< site 2 < site 3, and deviating least from MarMAT) was RMAT = [(ES_{10} + (2 x BENTIX_B) + (2 x BENTIX)) / 5]. Within the RMAT, results from Bentix index seem duplicated. However, the metrics used are different since each (BENTIX and BENTIX_B) use different parameters (density and biomass, respectively) of the communities, which have shown to be not redundant, but complementary (Vinagre et al., 2016a). In fact, their combined use in another metric (W-statistic) did not show, by far, such good performance (Vinagre et al., 2016b). Also, other metrics (e.g., AMBI, multivariate AMBI – M-AMBI) have been tested with success, showing different results when using biomass or density data, or when applying different data transformation to those parameters, in soft-bottoms (Warwick et al., 2010; Muxika et al., 2012; Cai et al., 2014).

The proposed RMAT is compliant with WFD requirements, because: i) the selected indices cover the abundance and taxonomic composition parameters from rocky shore benthic macroinvertebrate communities and ii) the indices respond well to disturbance. The indices are easy to apply and their responses to disturbance, namely organic enrichment, are well known. This allows for a higher confidence when interpreting the results of assessments (Borja and Dauer, 2008); iii) the indices were used in combination, constituting a multimetric assessment tool; iv) the output of the RMAT is provided as an ecological quality ratio (EQR), varying from zero to one (0 to 1), as the deviation from the reference condition point; and v) the EQR scale is divisible by adjustable boundary thresholds into 5 quality classes (Bad, Poor, Moderate, Good, High) to report the EQS of the system. The RMAT has therefore shown potential to be applied to rocky shore benthic macroinvertebrates for quality assessments in the scope of the WFD.

As was previously suggested (Vinagre et al., 2016a, b, 2017), the rocky shores surveyed were under moderate (intermediate) disturbance. This was shown by the MarMAT which, considering each shore, presented EQR values covering only from moderate to high status. A similar trend was also shown by the RMAT (mean EQR at site level). However, several individual samples showed a low RMAT value, an EQR close to 0 (zero), which means the multimetric index should be able to report to all of the five quality classes. Hence, the RMAT should be able to show a low EQS (Bad status) when disturbance is significant. The RMAT will need further validation (and also setting of boundaries between all quality classes), testing on different rocky shores with greater disturbance, a crucial step in refining the accuracy and precision of a developing index (Borja et al., 2009b). In the WFD context, there is also the need to calibrate its use across the long-known geographic gradients in assemblage composition on European rocky shores (e.g., Southward et al, 1995).

Concerning other marine Directives and Regional Conventions in Europe (e.g., MSFD, OSPAR, HELCOM), even though the use of rocky shore macroinvertebrate indicators could be effective and easy to use, environmental assessment approaches rarely have seen that possibility implemented. For example, in the MSFD list of Descriptors (European Commission, 2010) most of the indicators are based on water column and sedimentary habitat organisms; those from communities of rocky shores not frequently considered. It is true the former habitats have greater extent in European Shelf Seas, but the development of indicators based on hard substratum, able to respond to anthropogenic disturbance, may be of importance for several of the above mentioned Directives (van Hoey et al., 2010; Rice et al., 2012). The use of more complex macroinvertebrate indicators has not occurred, then, RMAT is a valid proposal to integrate the assessment for MSFD Descriptors (e.g., D1 – Biological Diversity and D6 – Seafloor Integrity). Here, the RMAT can be considered as a promising indicator, contributing to improve the holistic perspective of the study area. With the RMAT, instead of using basic measures from benthic rocky shore community, a more complex type of indicator, combining different perspectives of the community, will be available.

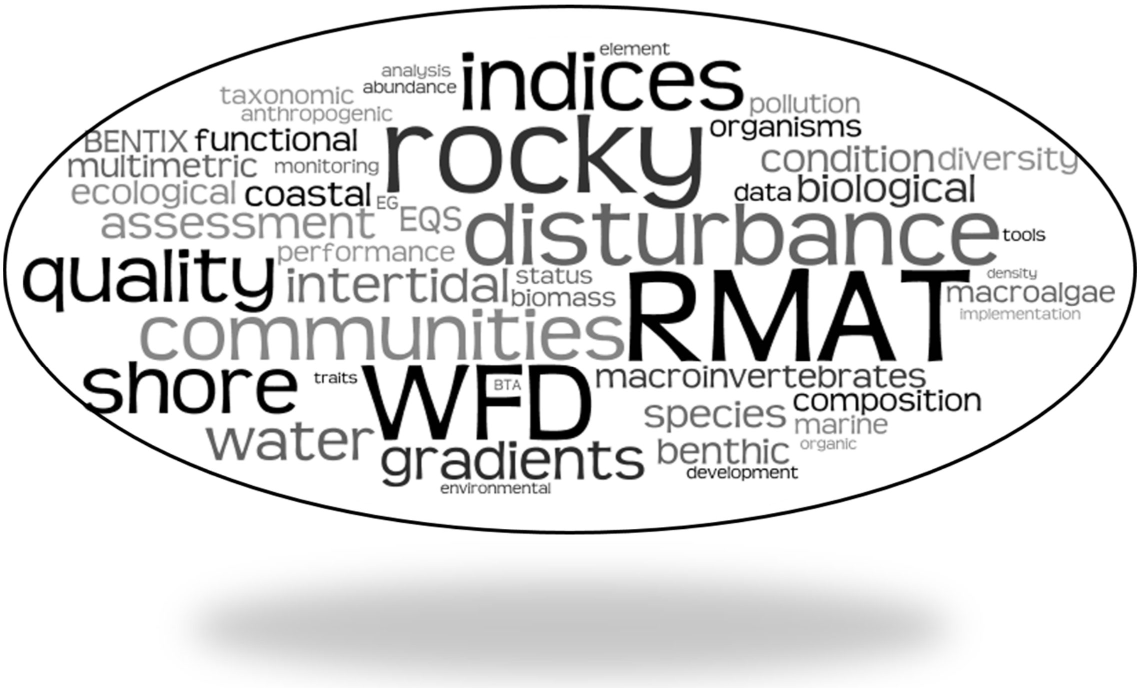
In parallel to the RMAT, a second best alternative model was determined, including only indices calculated using biomass: alt-RMAT = $[(ES_{10} + (2 \times BENTIX\_B)) / 3]$. Although being not as accurate as the RMAT, the alt-RMAT could be helpful when time is a constraint, since there is no need to count every organism. For a quick analysis (rough estimations per sample) were taken Vinagre et al. (2016b) summer and winter data as an example: i) sample processing: 2.5 h (includes removing all formalin solution used for preservation, removing any debris such as empty shells hardening the sorting, checking macroalgae and debris for organisms, sort all organisms); ii) counting all individual organisms: summer data – 1.8 h (636.3 mean organisms per sample, multiplied by 10 sec to count each organism), and winter data – 1.6 h (were counted 561.8 mean organisms) (includes preparing/using equipment, counting the organisms, and preparing the data matrix); iii) estimating biomass: the organisms of each species from each sample were separately placed in containers, dried and weighed, burnt and re-weighed (biomass was calculated as ash-free dry weight). Summer data – 0.15 h (mean 27.7 containers per sample, multiplied by 10 sec for processing each container), and winter data: 0.13 h (mean 23.9 containers) (includes preparing/using equipment, weighing twice each container, and preparing the data matrix). This is disregarding the time needed to dry (at least 72 h) and burn (8 h) the organisms. The time spent counting all organisms would correspond to about 40% and 37% for summer and winter data, respectively, of sample processing, whilst to calculate their biomass would constitute only 3.5% and 3.2%. The most difficult task in the whole process is taxonomy, and would require a minimum 50% of the total processing time of a sample. Therefore, considering all estimates, if time is a constraint, using alt-RMAT instead of RMAT could save between 18.5-20% of processing time per sample.

IV.5. Conclusions

The present work addressed a gap in the implementation of the WFD, where no tool was available to exclusively assess macroinvertebrates on rocky shores. The RMAT is a multimetric index compliant with the WFD, integrates widely known ecological indices (Hurlbert and Bentix indices) which shown, during summer season, best performance in distinguishing sites along disturbance gradients (moderate organic enrichment).

As it has shown potential to be used in quality assessments in the scope of the WFD and other statutory requirements, its applicability needs further validation, using data covering other geographical areas and stronger environmental degradation, to adequately define the five EQS classes and adjust boundaries between them.

General discussion



This thesis focussed specifically on the development and validation of a new multimetric ecological index for the quality assessment of rocky shores. The index – Rocky shore Macroinvertebrates Assessment Tool (RMAT) – performance was evaluated depending on its ability to reflect anthropogenic disturbance gradients (organic enrichment) on rocky shore macroinvertebrate communities. At the same time, it fulfils WFD requirements and contributes to cover the present gap in its implementation with regard to those habitats. To do so, some steps were followed aiming to achieve a robust final product. It was first analysed the response of macroinvertebrate communities to the disturbance gradients identified; then, several (twenty-one) ecological indices (well known and described in the literature) were tested based on the same macroinvertebrate data; and at last, the most promising ones were selected and mathematically combined into a WFD compliant assessment tool.

Benthic macroinvertebrates response to anthropogenic disturbance (organic enrichment) gradients

Previous work (Pais-Costa, 2011), together with current results (e.g., from the environmental parameters and the MarMAT results), showed generally an increase in ecological condition along the disturbance gradients, namely with regard to the macroalgae. Then, it seemed possible that the macroinvertebrate communities, subject to the same pressure as the macroalgae, would show similar behaviour. In fact, on both rocky shores surveyed (Buarcos, A5 – CW exposed type; Matadouro, A6 – CW moderately exposed type), it was found a strong parallelism between macroalgal (namely biomass) and macroinvertebrate (especially density and biomass) descriptors in response to the disturbance gradients. Both biological elements showed water quality increasing from close to far away from the source of disturbance, as suggested by community changes registered along the gradients (presence of more ‘tolerant’/‘opportunistic’ species, and greater abundance of those species, close to the source of disturbance; more ‘sensitive’ species, and with higher abundance, generally found farther away from the disturbance).

It is well acknowledged the intrinsic relation between algae and invertebrates, namely all compete for the same limited resource space, and many algae provide habitat and food for invertebrates (especially turf-forming species that trap sediments) (Hawkins, 1981; Raffaelli and Hawkins, 1999; Jenkins et al, 2005; Coleman et al, 2006). In previous works was mentioned that few macroinvertebrate species were correlating to the anthropogenic disturbance (Hiscock et al., 2005; Díez et al., 2012), so that the development of an ecological index for rocky shore monitoring should be empowered with use of macroalgal parameters. However, the methodology in the present work could probably allow for a more thorough analysis of the macroinvertebrate communities. First, the used macroinvertebrate parameters such as density and biomass provide a more realistic idea of the communities’ structure than coverage (%), since many organisms live hiding beneath other organisms (e.g., barnacles and mussels, macroalgae) and which may not be detected and accounted. This third dimension is surely important for distinguishing macroinvertebrate communities under different natural conditions and disturbance levels; second, it was used highest taxonomic resolution possible, preferentially to species level, to capture the maximum variation of the communities. Indeed, some species (e.g., amphipods, gastropods) didn’t show positive or negative response to the pressure. And the presence of such species close to the source of disturbance could sometimes be influenced positively by the increased macroalgae coverage/biomass (e.g., Magurran and McGill, 2010, Cabral-Oliveira et al., 2014b; Díez et al. 2014). But as a whole, the communities did show a response to the pressure. This indicates that, despite the lack of time and financial resources which usually hampers the assessment/monitoring of ecosystems, complex communities such as from rocky shores should be thoroughly analysed gathering as much information as possible.

Ability of invertebrate indices to assess ecological condition along disturbance gradients

With the previous findings, it seemed that indicators based on the benthic macroinvertebrates should cover the WFD requirements for quality assessments, namely in rocky shores. It was highly promising, if WFD requirements of 'abundance' and 'taxonomic composition' attributes were covered by the assessment metrics, that the development of a multimetric index based exclusively on the benthic macroinvertebrates was feasible. The lack of indices developed for the assessment of rocky shore macroinvertebrate communities made it necessary to test, for the first time, several ecological indices already existent, with universal use (e.g., Margalef index, Shannon-Wiener index) or based on soft-bottom communities (e.g., AMBI, BENTIX).

The application of ecological indices to macroinvertebrate data allowed to find differences in the structure/composition of macroinvertebrate communities, both between shores (Buarcos and Matadouro) and seasons (winter and summer). But this was not unexpected, since the two rocky shores are characterised by different wave exposure conditions which, together with their geographical position, enable the communities to be different from each other. For example, even with the same species dominating in the intertidal of both shores, at Buarcos ('exposed') those species registered much higher abundance comparing to Matadouro ('moderately exposed'), a pattern also found on other rocky shores with similar exposure levels (Raffaelli and Hawkins, 1999; Boaventura 2002). Despite that, several indices based either on abundance/diversity or taxonomic composition were able to capture differences in communities affected by different disturbance levels on both shores, but with much higher efficiency during summer and using biomass. These results are most probably linked to aspects that may mask the organic enrichment effects during winter. Communities are affected there by harsher conditions (e.g., strong wave energy and currents, and less suitable light and temperature regimes), which may cause disruptions in the rocky shore community and a seasonal reduced development of species (e.g., reproduction and growth). The lower species abundances naturally found during winter may cause the indices to perform worse (namely the ones based on composition) (Borja and Muxika, 2005; Dauvin and Ruellet, 2007; Keeley et al., 2012), when compared to summer.

The Shannon-Wiener, Hurlbert and Bentix indices, calculated using biomass (*H'_B*, ES_{10} and *BENTIX_B*, respectively), showed the best overall performance in distinguishing sites along the disturbance gradients. The first two indices have widely shown good sensitivity to and correlation with anthropogenic impacts (e.g., Boon et al., 2011; Borja et al., 2011; Spagnolo et al., 2014), and are commonly used in benthic ecology. On the other hand, the Bentix index had never been applied to rocky shore communities (to the authors awareness). The Bentix (both using biomass – BENTIX_B, or density – BENTIX) was more efficient than AMBI and MEDOCC, probably for two reasons: first, Bentix simplifies the assessment of taxa to two ecological groups – *sensitive* (EG I-II) and *tolerant* (EG III-V), thus offering better resolution to the disturbance gradients than AMBI and MEDOCC, namely on organic enriched rocky shores where EG III tends to dominate; and second, the communities surveyed are mainly composed of species from EG I-II, and Bentix gives higher weighting coefficient to EG I-II than the other two indices). Within the macroinvertebrate communities surveyed, only a little number of organisms (comparing to the total found) could not be assigned to a EG in the list used for AMBI(_B), MEDOCC(_B) and BENTIX(_B). Also, assigning such organisms to one of the five EG was consensual among experts. For use in rocky shores, species' EG in that list could still be subject to a more refined adjustment. However, this was not part of the work scope. Moreover, an important finding of the work was the behaviour of the tested indices along the vertical gradient of sampled sites; when the assessment was carried out separately for each intertidal zone (upper, mid and lower intertidal), the mid/lower intertidal zones (alone) and the mid plus the lower intertidal zones (together) were showing generally greater consistency in the indices results. Accordingly, those zones were the recommended for the assessment when the hard substratum available for sampling doesn't fully cover the intertidal zone.

Several indices that showed the best performance along the disturbance gradients are based on the communities' abundance, diversity and/or composition attributes, therefore fulfilling the WFD requirements for quality assessments. Thus, they seemed potential candidate metrics to integrate the multimetric index based solely on data from rocky shore macroinvertebrate communities.

Concordant response to disturbance between structural and functional attributes

This work sought further insights on structural and functional patterns in rocky shore macroinvertebrate communities. For the first time were applied functional trait-based methods (biological traits analysis – BTA, and functional diversity – FD – indices) to assess the ecological condition of rocky shore macroinvertebrate communities, which to date had only been considered in terrestrial and fresh-water ecology (e.g., Grime, 1974; Southwood, 1977; Statzner et al., 1994; Charvet et al., 2000; Menezes et al., 2010), and more recently applied to soft-bottom benthos of transitional and coastal waters (e.g., Bremner, 2003, 2005, 2008; Veríssimo et al., 2012, van der Linden et al., 2016).

Overall, the BTA and the FD indices (Community-Weighted Mean trait values – CWM, and Rao's quadratic entropy – RQE) could recognize spatial (between and within two shores) and seasonal (between summer and winter) changes in functioning on rocky shore macroinvertebrate communities within disturbance gradients. And, similarly to the taxonomic-based metrics tested (Shannon-Wiener – H'_B and Bentix – BENTIX_B indices), results were much more consistent during summer, compared to winter.

The RQE offers advantages over the other three indices. Comparing to CWM, the RQE is much easier to interpret. First, the CWM results are much more affected by the organisms' abundances (Garnier et al. 2004; Ricotta and Moretti, 2011); second, the CWM may show more ambiguous results since each trait may be coded to species showing different associations with disturbance [e.g., Tolerance to pollution: EG III (tolerant species) includes amphipod species (e.g., *Elasmopus rapax*) which are commonly regarded as sensitive (e.g., Dauvin and Ruellet, 2009)]; and third, despite the CWM allows emphasising each category within trait, the RQE summarizes functional diversity into single values thus making it much easier to evaluate and interpret changes in functional diversity. With regard to H'_B, BENTIX_B, although the indices summarize information about a community, the RQE takes into account also the degree of dissimilarity between species in that community (Botta-Dukát, 2005; Lepš et al., 2006). Furthermore, despite the taxonomic-based metrics showed comparable trends to the RQE (all showing diversity generally increasing from the site closest to the source of disturbance to the site farthest from it), the RQE could better capture such trends, more independently of structural differences (richness/diversity and composition) between shores and between seasons, comparatively to those metrics.

However, applying functional trait-based approaches to marine communities (in particular) is a complex task (e.g., Veríssimo et al., 2012; van der Linden et al., 2016), and particularly time consuming. This is true not only with regard to taxonomy, which is a task for experts, but especially gathering information on each trait category for each organism, which must be done *a priori* to any analysis and requires an immense variety of literature sources (e.g., online databases, identification keys, journals).

Considering the good performance shown by the functional trait-based approach (BTA and FD indices), and despite not being mandatory in the scope of the WFD, functional trait-based approaches could complement other more traditional methods (e.g., diversity and taxonomic composition indices) in assessments/monitoring activities. However, its applicability in such activities seems to remain quite hampered by several limitations, such as the lack or scarcity of information, which makes it heavily time consuming when compared to more traditional methodologies.

The Rocky shore Macroinvertebrates Assessment Tool – RMAT

The WFD demands the water quality assessments to be undertaken by water body and using each mandatory BQE. To date, quality assessment tools developed for WFD rocky shore assessment were mainly based on macroalgae. Only a few have explored macroinvertebrate communities alone, or their features, to produce any assessment tool, or part of it (Birk et al., 2012).

The multimetric tool proposed here – Rocky shore assessment tool (RMAT) – was developed based on macroinvertebrate communities from two different rocky shores (under different levels of exposure to Atlantic conditions), with different community structure/composition. Summer data was used since it was found as the best season (compared to winter) for monitoring activities (Chapters II and III), so that the response of the RMAT could be as precise and comprehensible (owing to the greater variation in the communities during winter) as possible.

This index [RMAT = (ES_{10} + (2 x BENTIX_B) + (2 x BENTIX) / 5)] was built as the combination of the mean EQR values of the most promising single metrics (=indices). The selection picked the metrics showing the best efficiency along the disturbance gradients (good response against pressure) and, at the same time, that could fulfil WFD requirements to cover the abundance and taxonomic composition parameters from rocky shore’ benthic macroinvertebrate communities.

Since the response of those metrics to pressure is well known, the interpretation of results of assessments is easier and the confidence on results is higher too (Borja and Dauer, 2008). The RMAT was built under a combination rule, articulating those metrics. Its output is provided as an ecological quality ratio (EQR; deviation from the reference condition point) varying from zero to one (0-1). That scale is divisible by adjustable boundary thresholds into 5 quality classes (Bad, Poor, Moderate, Good, High) to report the ecological quality status of the system (water body).

Throughout the work was acknowledged that the two rocky shores surveyed were under moderate to light disturbance (Chapter I, II and III). This was shown by the

MarMAT, which, considering each shore alone, presented EQR values covering only from moderate to high status. Despite the RMAT showed trends in a similar fashion to the MarMAT, i.e., mean EQRs at site level didn't cover the five ecological states, several individual samples presented a low RMAT value (EQR close to zero), which means that RMAT should be able to report to all of the five quality classes. Hence, the RMAT should be able to show a low EQS (Bad status) when disturbance is significant.

The RMAT has therefore shown potential to be used in quality assessments in the scope of the WFD (and other monitoring activities in general). However, its applicability needs further validation, testing at different rocky shores and covering more extensive disturbance gradients, a crucial step to refine the accuracy and precision of a developing index (Borja et al., 2009b). This would allow for a broader geographical applicability of RMAT, and eventually to its proposal as a WFD compliant ecological assessment tool.

After proper validation, the RMAT could be used by Portuguese authorities on monitoring activities as well as a support for management decisions. Owing to its WFD compliance, the RMAT could be officially accepted and submitted to the European Council. In this case, the RMAT will eventually need to be compared and have its boundaries harmonised with other assessment tools in a next intercalibration exercise. Depending on the assessment concept, one of the three possible options should then be used on harmonization of boundaries: a) using same data acquisition and same numerical evaluation – Option 1; b) using different data acquisition and numerical evaluation – Option 2; and c) using similar data acquisition, but different numerical evaluation – Option 3). Despite option 1 is the most straightforward and apparently preferential to avoid difficulties and uncertainties involved in comparing the results of different assessments (Member States can then focus on the definition of reference conditions and harmonization of class boundaries), it is often not feasible since different assessment methods are usually used by different countries (Birk et al., 2012). Regardless of the option chosen, it will always require a quantitative evaluation of the impacting pressure, for a better calibration of the RMAT, namely to adjust thresholds between EQS classes. Regardless, intercalibration of methods for rocky shores is not possible at present, since there are no other tools being used in other countries.

In a parallel analysis, an alternative multimetric index was defined (alt-RMAT) which, comparing to the RMAT, combines only two (from the three) metrics based on biomass data (Hurlbert and Bentix indices). The alt-RMAT wasn't as efficient as the RMAT, showing a lesser correlation to the disturbance gradients than the RMAT. Nonetheless, that difference could be compensated by a quicker assessment of the communities, considering that a great number of samples must be processed (taking much longer time) to build a more robust data set. Therefore, if time is a constraint (depending on the amount of samples to process), alt-RMAT could be a feasible alternative for a more rapid assessment of rocky shore benthic macroinvertebrate communities, keeping still a high correlation with the pressure. The alt-RMAT, as well as the RMAT, is WFD compliant as it also includes the macroinvertebrate communities parameters of 'abundance' and 'composition'.

Final remarks and recommendations

The research work developed throughout this thesis allow for a better understanding of structural and functional changes in the macroinvertebrate communities under moderate anthropogenic disturbance gradients. The macroinvertebrate communities surveyed were able to respond to the organic enrichment pressure and, based on those communities, several taxonomic-based indices (especially Shannon-Wiener and Bentix indices) and functional trait-based indices (particularly the Rao's quadratic entropy) were able to report those changes (especially using summer and biomass data), and identified generally an increase in ecological quality along the disturbance gradients (more effectively during summer). Ultimately, the multimetric index (developed from the combination of the most efficient indices) followed similar trend, being able to report, during summer, the expected increase in quality along the disturbance gradients.

The applicability of the RMAT on other rocky shores will reside on the methodology selected, which should be as proximate as possible to that presented here, namely with regard to sample acquisition (e.g., during summer, covering the most of the intertidal area, and using appropriate number and size of quadrat replicates) and processing (e.g., taxonomy done at the lowest level possible, preferentially to species level), and using macroinvertebrate parameters such as biomass (g AFDW m^{-2}) and density (ind m^{-2}). That applicability is yet dependent on a more robust validation of the RMAT, and on its eventual intercalibration. Also, it will require in parallel a quantitative evaluation of the impacting pressure.

In the case of greater restrictions concerning time, using the alternative multimetric index – alt-RMAT – would allow for a more rapid (although with lower efficiency) assessment. Also, if the whole intertidal is not available for sampling, the mid to lower zones should be the ones considered (and excluding the upper, more variable, zone).

Both the RMAT and the alt-RMAT include the Bentix biotic index, which relies on the highest taxonomic resolution possible and on the species list used (developed for soft bottom). Possibly, the list of species used in the present work could be subject to a more advanced adjustment. For example, not all organisms were identified to species level, which could cause any minimal change in the results from taxonomic composition indices. Despite that, this doesn’t seem much problematic since i) the number of organisms identified to species level was very high, and ii) the Bentix index (used in both the RMAT and the alt-RMAT) classifies the organisms in the communities into two groups, *sensitive* (EG I-II) and *tolerant* (EG III-V), which indirectly allows reducing more substantial misclassification of species into ecological groups.

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Supplementary material

Supplementary material A1. PERMANOVA results for Buarcos and Matadouro assemblage descriptors: B_{alg}, P/A_{alg} and S_{alg} are the macroalgae biomass, presence/absence and number of species; D_{inv}, B_{inv}, P/A_{inv} S_{inv} are the macroinvertebrates density, biomass, presence/absence and number of species.

	Macroalgae								
	B_{alg}			P/A_{alg}			S_{alg}		
Main tests		Pseudo-F	P(perm)		Pseudo-F	P(perm)		Pseudo-F	P(perm)
	Shore	31.281	0.0001	Shore	28.292	0.0001	Shore	0.166	0.6895
	Site(Shore)	5.983	0.0001	Site(Shore)	6.001	0.0001	Site(Shore)	3.092	0.0088
	Zone[Site(Shore)]	9.480	0.0001	Zone[Site(Shore)]	9.085	0.0001	Zone[Site(Shore)]	14.040	0.0001
	Zone x Site(Shore)]	3.450	0.0001	Zone x Site(Shore)]	3.558	0.0001	Zone x Site(Shore)]	3.325	0.0001
Pairwise Tests	Buarcos	t	P(perm)	Buarcos	t	P(perm)	Buarcos	t	P(perm)
Site(Shore)	A, B	1.814	0.0160	A, B	2.029	0.0082	A, B	1.629	0.1140
	A, C	1.692	0.0156	A, C	1.863	0.0083	A, C	0.666	0.5064
	A, D	2.218	0.0001	A, D	2.407	0.0001	A, D	0.789	0.4482
	B, C	1.458	0.0846	B, C	1.525	0.0698	B, C	1.006	0.3241
	B, D	2.435	0.0001	B, D	2.455	0.0002	B, D	2.791	0.0072
	C, D	1.635	0.0178	C, D	1.261	0.1493	C, D	1.665	0.0977
	Matadouro	t	P(perm)	Matadouro	t	P(perm)	Matadouro	t	P(perm)
	A, B	2.449	0.0002	A, B	2.699	0.0001	A, B	1.588	0.1248
	A, C	3.775	0.0001	A, C	3.333	0.0001	A, C	1.206	0.2321
	A, D	3.356	0.0001	A, D	3.179	0.0001	A, D	1.037	0.3124
	B, C	3.641	0.0001	B, C	3.482	0.0001	B, C	4.038	0.0002
	B, D	2.780	0.0001	B, D	2.803	0.0001	B, D	0.794	0.4231
	C, D	2.255	0.0001	C, D	2.663	0.0001	C, D	3.261	0.0016

Supplementary material A1. *(continued)*

			Macroalgae					
			B_{alg}		P/A_{alg}		S_{alg}	
Pairwise Tests	Buarcos							
Zone[Site(Shore)]	Site A		t	P(perm)	t	P(perm)	t	P(perm)
		Upper, Mid	1.450	0.0768	1.396	0.1172	0.518	0.6649
		Upper, Lower	2.439	0.0022	2.490	0.0017	2.049	0.0641
		Mid, Lower	2.007	0.0005	1.951	0.0017	2.122	0.0657
	Site B							
		Upper, Mid	1.785	0.0803	1.845	0.0589	1.729	0.1212
		Upper, Lower	2.753	0.0060	2.724	0.0126	3.567	0.0036
		Mid, Lower	1.267	0.1612	1.199	0.2072	2.218	0.0521
	Site C							
		Upper, Mid	2.309	0.0209	1.960	0.0442	2.273	0.0474
		Upper, Lower	3.191	0.0019	3.032	0.0013	4.458	0.0005
		Mid, Lower	1.550	0.0344	1.447	0.0895	2.085	0.0575
	Site D							
		Upper, Mid	5.971	0.0002	5.719	0.0003	12.348	0.0003
		Upper, Lower	8.579	0.0005	7.382	0.0003	12.647	0.0003
		Mid, Lower	1.360	0.0754	1.305	0.1415	2.478	0.0276
	Matadouro							
	Site A		t	P(perm)	t	P(perm)	t	P(perm)
		Upper, Mid	4.314	0.0002	3.546	0.0003	5.147	0.0009
		Upper, Lower	2.814	0.0007	2.672	0.0028	2.680	0.0141
		Mid, Lower	1.510	0.0128	1.250	0.1421	0.524	0.6409
	Site B							
		Upper, Mid	1.301	0.2046	1.090	0.2521	0.102	0.9180
		Upper, Lower	1.811	0.0224	1.864	0.0171	2.391	0.0412
		Mid, Lower	1.555	0.0443	1.820	0.0197	2.687	0.0266
	Site C							
		Upper, Mid	5.624	0.0002	6.231	0.0002	9.856	0.0004
		Upper, Lower	6.118	0.0002	6.919	0.0003	11.472	0.0006
		Mid, Lower	3.446	0.0002	3.821	0.0002	0.511	0.6828
	Site D							
		Upper, Mid	5.016	0.0001	4.773	0.0002	4.977	0.0001
		Upper, Lower	5.280	0.0003	5.685	0.0004	9.800	0.0003
		Mid, Lower	2.322	0.0002	1.657	0.0110	1.468	0.1943

Supplementary material A1. *(continued)*

		Macroalgae					
		B_{alg}		P/A_{alg}		S_{alg}	
Pairwise Tests	Buarcos						
Zone[Site(Shore)]	Upper						
	A, B	1.635	0.0615	1.951	0.0256	1.827	0.0860
	A, C	1.261	0.1600	1.243	0.1930	1.469	0.1786
	A, D	2.266	0.0013	2.175	0.0081	2.531	0.0114
	B, C	0.694	0.5794	0.940	0.3005	0.551	0.6975
	B, D	1.059	0.3192	0.954	0.5164	0.745	0.9455
	C, D	1.357	0.2689	1.135	0.3684	1.576	0.2449
	Mid						
	A, B	1.186	0.2243	1.407	0.1125	1.229	0.2427
	A, C	1.428	0.0575	1.633	0.0143	0.074	0.9434
	A, D	1.913	0.0002	2.099	0.0002	3.211	0.0071
	B, C	1.176	0.2030	1.195	0.2004	0.915	0.3666
	B, D	2.379	0.0003	2.583	0.0002	3.842	0.0020
	C, D	1.735	0.0028	1.653	0.0291	2.325	0.0352
	Lower						
	A, B	1.113	0.2443	1.208	0.1934	0.100	0.9179
	A, C	1.491	0.0223	1.531	0.0206	0.346	0.7330
	A, D	1.971	0.0002	2.221	0.0002	1.663	0.1194
	B, C	1.218	0.1729	1.213	0.2020	0.389	0.7087
	B, D	1.834	0.0007	1.761	0.0005	1.412	0.1767
	C, D	1.589	0.0037	1.301	0.0794	1.153	0.2729
	Matadouro	t	P(perm)	t	P(perm)	t	P(perm)
	Upper						
	A, B	1.756	0.0192	1.792	0.0296	1.240	0.2639
	A, C	4.570	0.0004	3.853	0.0002	2.655	0.0142
	A, D	4.248	0.0008	4.056	0.0002	2.411	0.0202
	B, C	2.939	0.0051	3.083	0.0038	3.696	0.0044
	B, D	2.972	0.0022	3.285	0.0018	3.507	0.0074
	C, D	1.416	0.1433	1.310	0.2823	0.780	0.7057
	Mid						
	A, B	2.413	0.0005	2.951	0.0009	3.808	0.0074
	A, C	2.266	0.0003	2.319	0.0006	2.423	0.0321
	A, D	2.113	0.0011	2.526	0.0002	0.753	0.4615
	B, C	2.890	0.0006	3.210	0.0004	5.592	0.0005
	B, D	1.878	0.0004	2.064	0.0001	2.093	0.0519
	C, D	2.295	0.0002	2.063	0.0009	2.622	0.0197
	Lower						
	A, B	1.546	0.0282	1.428	0.0644	0.838	0.4127
	A, C	2.362	0.0004	2.409	0.0009	0.976	0.3474
	A, D	1.939	0.0002	1.563	0.0254	0.095	0.9243
	B, C	3.218	0.0001	3.229	0.0006	3.504	0.0036
	B, D	2.126	0.0002	1.833	0.0075	1.536	0.1469
	C, D	3.225	0.0002	3.873	0.0002	2.192	0.0449

Supplementary material A1. *(continued)*

		Macroinvertebrates							
		D_{inv}		B_{inv}		P/A_{inv}		S_{inv}	
Main tests		Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)
	Shore	30.019	0.0001	111.380	0.0001	106.480	0.0001	0.133	0.7155
	Site(Shore)	6.304	0.0001	5.528	0.0001	5.626	0.0001	59.843	0.0002
	Zone[Site(Shore)]	9.738	0.0001	8.714	0.0001	8.940	0.0001	29.280	0.0001
	Zone x Site(Shore)]	4.263	0.0001	3.944	0.0001	3.721	0.0001	3.237	0.0003
Pairwise Tests	Buarcos	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Site(Shore)	A, B	1.741	0.0128	1.586	0.0216	1.523	0.0362	2.174	0.0364
	A, C	1.544	0.0239	1.616	0.0139	1.558	0.0222	0.363	0.7153
	A, D	1.815	0.0014	1.671	0.0046	1.705	0.0028	1.937	0.0241
	B, C	2.187	0.0009	2.051	0.0009	1.971	0.0022	1.793	0.0813
	B, D	2.154	0.0003	1.878	0.0004	2.031	0.0006	3.870	0.0008
	C, D	1.558	0.0052	1.405	0.0173	1.573	0.0033	2.232	0.0342
	Matadouro	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
	A, B	1.901	0.0005	2.074	0.0001	1.958	0.0006	1.074	0.2866
	A, C	3.532	0.0001	3.345	0.0001	3.357	0.0001	1.303	0.1929
	A, D	3.392	0.0001	3.052	0.0001	3.196	0.0001	2.292	0.0272
	B, C	3.403	0.0001	3.084	0.0001	3.379	0.0001	2.881	0.0056
	B, D	3.346	0.0001	2.980	0.0001	3.145	0.0001	1.170	0.2415
	C, D	2.868	0.0001	2.772	0.0001	2.602	0.0001	5.288	0.0001

Supplementary material A1. *(continued)*

Pairwise Tests		Macroinvertebrates							
		D_{inv}		B_{inv}		P/A_{inv}		S_{inv}	
Zone[Site(Shore)]	Buarcos								
	Site A	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
	Upper, Mid	1.573	0.0530	1.617	0.0513	1.599	0.0445	3.084	0.0090
	Upper, Lower	2.260	0.0003	2.389	0.0004	2.307	0.0002	8.984	0.0001
	Mid, Lower	2.852	0.0004	2.749	0.0002	3.081	0.0003	9.223	0.0001
	Site B								
	Upper, Mid	1.760	0.0555	1.575	0.0652	1.629	0.0701	1.536	0.1444
	Upper, Lower	4.121	0.0001	3.681	0.0004	3.929	0.0001	6.516	0.0001
	Mid, Lower	2.268	0.0011	2.122	0.0020	2.394	0.0007	3.617	0.0028
	Site C								
	Upper, Mid	3.638	0.0002	3.635	0.0006	3.478	0.0005	4.048	0.0009
	Upper, Lower	3.759	0.0003	3.826	0.0002	3.896	0.0001	7.371	0.0002
	Mid, Lower	2.039	0.0003	1.853	0.0009	2.126	0.0003	4.537	0.0008
	Site D								
	Upper, Mid	4.736	0.0004	4.375	0.0004	3.853	0.0003	7.116	0.0001
	Upper, Lower	5.150	0.0002	4.644	0.0004	4.634	0.0004	9.436	0.0001
	Mid, Lower	1.981	0.0003	1.939	0.0002	1.913	0.0003	4.274	0.0007
	Matadouro								
	Site A	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
	Upper, Mid	2.519	0.0008	2.488	0.0003	2.598	0.0003	4.243	0.0004
	Upper, Lower	2.621	0.0003	2.414	0.0005	2.707	0.0005	3.428	0.0040
	Mid, Lower	1.828	0.0004	1.685	0.0003	1.687	0.0014	1.045	0.3182
	Site B								
	Upper, Mid	1.084	0.2476	1.009	0.3356	1.161	0.2280	1.907	0.0776
	Upper, Lower	1.754	0.0059	1.660	0.0122	1.760	0.0070	2.130	0.0511
	Mid, Lower	1.220	0.2020	1.268	0.1407	1.177	0.2756	0.722	0.4848
	Site C								
	Upper, Mid	6.770	0.0003	6.244	0.0003	5.604	0.0003	12.413	0.0001
	Upper, Lower	6.932	0.0002	6.504	0.0002	6.157	0.0002	27.444	0.0001
	Mid, Lower	2.879	0.0001	2.780	0.0003	2.341	0.0002	4.027	0.0012
	Site D								
	Upper, Mid	4.789	0.0003	4.352	0.0002	4.314	0.0001	6.615	0.0001
	Upper, Lower	5.171	0.0003	4.826	0.0003	4.524	0.0002	6.060	0.0001
	Mid, Lower	1.517	0.0069	1.342	0.0241	1.478	0.0114	1.138	0.2708

Supplementary material A1. *(continued)*

			Macroinvertebrates							
			D_{inv}		B_{inv}		P/A_{inv}		S_{inv}	
Pairwise Tests	Buarcos		t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Zone[Site(Shore)]	Upper									
		A, B	1.936	0.0119	1.790	0.0186	1.648	0.0318	0.053	0.9808
		A, C	1.484	0.0906	1.386	0.1201	1.484	0.0778	0.079	0.9604
		A, D	2.147	0.0015	1.895	0.0106	1.939	0.0061	0.123	0.9265
		B, C	1.096	0.2318	1.227	0.1987	0.979	0.3665	0.139	0.9317
		B, D	1.193	0.1867	1.026	0.3612	1.132	0.2564	0.066	0.9681
		C, D	1.549	0.0532	1.620	0.0177	1.568	0.0271	0.230	0.8918
	Mid									
		A, B	1.027	0.3293	1.087	0.2857	1.082	0.2972	1.001	0.3433
		A, C	2.429	0.0002	2.307	0.0002	2.221	0.0003	0.943	0.3817
		A, D	2.327	0.0003	2.320	0.0003	2.212	0.0003	3.013	0.0119
		B, C	2.240	0.0015	2.092	0.0030	2.029	0.0013	1.582	0.1402
		B, D	2.173	0.0009	2.041	0.0016	2.029	0.0009	2.872	0.0152
		C, D	1.273	0.0285	1.430	0.0004	1.088	0.2614	1.994	0.0718
	Lower									
		A, B	1.795	0.0049	1.854	0.0019	1.839	0.0023	5.030	0.0004
		A, C	1.466	0.0157	1.590	0.0012	1.482	0.0121	1.595	0.1443
		A, D	1.563	0.0162	1.601	0.0063	1.613	0.0090	1.607	0.1444
		B, C	1.800	0.0022	1.842	0.0044	1.852	0.0027	2.075	0.0679
		B, D	1.808	0.0025	1.683	0.0066	1.910	0.0004	4.698	0.0018
		C, D	1.220	0.1202	1.309	0.0552	1.119	0.2366	2.524	0.0305
	Matadouro		t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
	Upper									
		A, B	1.394	0.0743	1.479	0.0656	1.400	0.0706	0.725	0.4826
		A, C	4.328	0.0001	4.071	0.0006	4.072	0.0001	1.927	0.0839
		A, D	3.920	0.0003	3.584	0.0004	3.735	0.0003	1.765	0.1103
		B, C	3.858	0.0002	3.493	0.0006	3.725	0.0001	3.502	0.0039
		B, D	3.365	0.0018	3.076	0.0009	3.265	0.0013	3.075	0.0089
		C, D	2.290	0.0002	2.035	0.0003	2.172	0.0002	7.5E-09	1.0000
	Mid									
		A, B	1.888	0.0003	2.040	0.0002	1.957	0.0005	1.905	0.0772
		A, C	2.702	0.0001	2.442	0.0002	2.377	0.0005	2.494	0.0281
		A, D	2.248	0.0002	2.019	0.0002	1.976	0.0003	2.004	0.0750
		B, C	2.461	0.0002	2.368	0.0002	2.234	0.0003	3.375	0.0022
		B, D	2.267	0.0002	2.063	0.0004	2.051	0.0002	0.202	0.8849
		C, D	1.860	0.0012	1.750	0.0017	1.593	0.0100	3.585	0.0041
	Lower									
		A, B	1.462	0.0575	1.464	0.0039	1.579	0.0003	1.081	0.3126
		A, C	1.868	0.0001	1.981	0.0002	1.584	0.0016	1.942	0.0680
		A, D	1.871	0.0003	1.833	0.0004	1.782	0.0002	0.944	0.3801
		B, C	2.449	0.0004	2.414	0.0003	2.426	0.0003	3.946	0.0013
		B, D	1.814	0.0002	1.691	0.0002	1.688	0.0009	0.273	0.8141
		C, D	3.106	0.0003	3.033	0.0001	2.555	0.0003	4.621	0.0014

Supplementary material A2. *Two-way SIMPER analysis for Buarcos' macroalgae and macroinvertebrates, showing the taxa responsible for similarity/dissimilarity (cut-off percentage: 95% for macroalgae and 85% for macroinvertebrates) within/between sampling sites (A. and C., respectively) and zones (B. and D., respectively). Shaded boxes: taxa contributing for similarities within each group. Non-shaded box, taxa contributing for dissimilarities between groups. Key: AB, AD, Ct, Opp and EG are the taxa average biomass (for macroalgae), average density (for macroinvertebrates), contribution for the similarities/dissimilarities, opportunism (for macroalgae) and Ecological Group (for macroinvertebrates).*

A.	**Site A**				**Site B**				**Site C**				**Site D**			
Site A	**22.92%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**												
	Ulva spp. (Leaf-like forms)	43.19	52.95	X												
	Corallina spp.	57.98	20.17													
	Porphyra spp.	0.10	10.12	X												
	O. pinnatifida	7.34	9.81													
	Ulva spp. (Tubular-like forms)	19.18	3.81	X												
Site B	**81.14%**				**18.86%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**								
	Ulva spp. (Leaf-like forms)		35.16	X	*Ulva* spp. (Leaf-like forms)	25.8	82.1	X								
	Corallina spp.		15.15		*O. pinnatifida*	3.3	5.8									
	Porphyra spp.		12.36	X	*Corallina* spp.	21.5	5.2									
	Ulva spp. (Tubular-like forms)		10.19	X	*C. teedei* var. *lusitanicus*	5.4	4.3									
	O. pinnatifida		9.11													
	Rhodochorton spp. / *Rhodothamniella* spp.		2.47	X												
	G. griffithsiae		2.26													
	C. acicularis		2.15													
	C. teedei var. *lusitanicus*		2.11													
	M. stellatus		1.80													
	Ceramium spp.		1.53	X												
	L. incrustans		1.45													
Site C	**79.53%**				**76.36%**				**29.83%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**				
	Ulva spp. (Leaf-like forms)		31.07	X	*Ulva* spp. (Leaf-like forms)		37.7	X	*Ulva* spp. (Leaf-like forms)	26.0	77.2	X				
	Corallina spp.		13.78		*Porphyra* spp.		13.8	X	*O. pinnatifida*	7.0	7.8					
	Porphyra spp.		12.30	X	*Corallina* spp.		9.5		*Porphyra* spp.	0.0	4.2	X				
	Ulva spp. (Tubular-like forms)		9.96	X	*O. pinnatifida*		9.1		*Corallina* spp.	3.0	3.7					
	O. pinnatifida		8.31		*M. stellatus*		8.1		*L. incrustans*	7.6	2.7					
	M. stellatus		7.19		*L. incrustans*		5.0									
	L. incrustans		2.67		*C. teedei* var. *lusitanicus*		3.8									
	G. griffithsiae		2.18		*Rhodochorton* spp. / *Rhodothamniella* spp.		2.1	X								
	C. acicularis		2.02		*G. pistilata*		1.8									
	C. teedei var. *lusitanicus*		1.79		*C. crispus*		1.3									
	G. pistillata		1.21		*A. devoniensis*		1.1									
	Ceramium spp.		1.16	X	G1		1.1	X								
Site D	**82.51%**				**84.69%**				**81.90%**				**26.84%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**
	Corallina spp.		26.85		*Corallina* spp.		33.5	X	*Corallina* spp.		30.9		*Corallina* spp.	108.5	61.7	
	Ulva spp. (Leaf-like forms)		21.07	X	*Ulva* spp. (Leaf-like forms)		27.4	X	*Ulva* spp. (Leaf-like forms)		18.4	X	*Ulva* spp. (Leaf-like forms)	23.4	18.0	X
	Porphyra spp.		15.67	X	*Porphyra* spp.		8.8	X	*Porphyra* spp.		16.2	X	*O. pinnatifida*	8.4	5.5	
	Ulva spp. (Tubular-like forms)		10.37	X	*M. stellatus*		6.0		*M. stellatus*		10.0		*L. incrustans*	14.0	5.3	
	M. stellatus		7.03		*O. pinnatifida*		5.7		*O. pinnatifida*		7.8		*Porphyra* spp.	6E-05	5.0	X
	O. pinnatifida		5.58		*L. incrustans*		5.2		*L. incrustans*		5.6					
	L. incrustans		2.40		*P. complanata*		2.5		*P complanata*		2.3					
	G. griffithsiae		1.89		*H. scoparia*		2.1		*H scoparia*		1.4					
	P. complanta		1.36		*C. teedei* var. *lusitanicus*		1.5		*C. teedei* var. *lusitanicus*		1.2					
	H. scoparia		1.35		G1		1.3	X	G1		1.2	X				

Supplementary material A2. *(continued)*

B.	**Upper littoral**				**Mid littoral**				**Lower littoral**			
Upper littoral	**6.35%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**								
	Porphyra spp.	0.1	72.6	X								
	Ulva spp. (Tubular-like forms)	14.4	13.7	X								
	Ulva spp. (Leaf-like forms)	7.4	5.6	X								
	O. pinnatifida	1.4	4.6									
Mid littoral	**93.70%**				**27.46%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**				
	Ulva spp. (Leaf-like forms)		48.3	X	*Ulva* spp. (Leaf-like forms)	33.5	77.4	X				
	Corallina spp.		11.2		*Corallina* spp.	19.3	8.6					
	O. pinnatifida		10.2		*O. pinnatifida*	6.3	7.9					
	M. stellatus		9.0		*M. stellatus*	15.6	2.5					
	Ulva spp. (Tubular-like forms)		5.9	X								
	P. complanata		2.0									
	Rhodochorton spp. / *Rhodothamniella* spp.		1.9	X								
	L. incrustans		1.9									
	Porphyra spp.		1.8	X								
	S. scoparium		1.4									
	G. griffithsiae		1.2									
	Ceramium spp.		1.1	X								
Lower littoral	**94.26%**				**73.98%**				**35.96%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp**
	Corallina spp.		32.7		*Corallina* spp.		32.1		*Ulva* spp. (Leaf-like forms)	47.8	47.3	X
	Ulva spp. (Leaf-like forms)		29.1	X	*Ulva* spp. (Leaf-like forms)		25.9	X	*Corallina* spp.	123.0	37.3	
	O. pinnatifida		7.8		*O. pinnatifida*		8.0		*O. pinnatifida*	11.9	7.3	
	L. incrustans		7.5		*L. incrustans*		7.3		*L. incrustans*	16.19	3.18	
	C. teedei var. *lusitanicus*		4.2		*M. stellatus*		5.3					
	Ulva spp. (Tubular-like forms)		3.4	X	*C. teedei* var. *lusitanicus*		4.8					
	C. acicularis		2.0		*C. acicularis*		2.5					
	G. pistillata		1.5		*G. pistillata*		1.6					
	P. complanata		1.5		*P. complanata*		1.3					
	C. crispus		1.1		*Rhodochorton* spp. / *Rhodothamniella* spp.		1.3	X				
	C. coerulescens		1.0		*C. crispus*		1.2					
	Ceramium spp.		1.0	X	*C. coerulescens*		1.0					
	M. stellatus		0.8		*Ceramium* spp.		1.0	X				
	S. scoparium		0.7		*A. devoniensis*		0.9					
	A. devoniensis		0.7		*S. scoparium*		0.9					
	D. dichotoma		0.6									

Supplementary material A2. *(continued)*

C.	**Site A**				**Site B**				**Site C**				**Site D**			
Site A	**33.96%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**												
	M. galloprovincialis	11171.9	35.4	III												
	C. montagui	14236.1	19.0	I												
	S. alveolata	3559.0	12.0	I												
	T. dulongii	2381.4	5.4	II												
	Actiniaria	1443.9	4.4	II												
	G. umbilicalis	1030.1	3.4	I												
	Nemertea	818.9	3.0	III												
	S. pulvinata	653.9	2.3	II												
	R. parva	1736.1	1.9	I												
Site B	**78.12%**				**39.68%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**								
	C. montagui		49.0	I												
	M. galloprovincialis		14.7	III	*C. montagui*	51342.6	65.2	I								
	R. parva		8.1	I	*R. parva*	4403.9	8.7	I								
	S. alveolata		5.6	I	*M. galloprovincialis*	2890.6	6.3	III								
	T. dulongii		3.0	II	Nemertea	596.1	2.4	III								
	M. neritoides		2.1	II	*Venerupis* sp.	381.9	2.0	I								
	Actiniaria		2.0	II	Actiniaria	735.0	1.7	II								
	G. umbilicalis		1.6	I												
Site C	**72.37%**				**68.15%**				**48.79%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**				
	C. montagui		36.9	I	*C. montagui*		44.1	I								
	M. galloprovincialis		19.7	III	*R. parva*		11.9	I	*M. galloprovincialis*	6406.3	27.5	III				
	R. parva		8.8	I	*M. galloprovincialis*		10.5	III	*C. montagui*	11571.2	22.4	I				
	S. alveolata		7.1	I	*S. alveolata*		7.3	I	*S. alveolata*	3391.2	11.3	I				
	T. dulongii		4.0	II	*T. dulongii*		2.9	II	*R. parva*	3608.2	10.3	I				
	Actiniaria		2.6	II	Actiniaria		2.8	II	*T. dulongii*	1519.1	5.6	III				
	G. umbilicalis		2.1	I	*M. neritoides*		1.7	II	Actiniaria	1452.6	4.7	II				
	M. neritoides		2.0	II	*G. umbilicalis*		1.6	I	*G. umbilicalis*	697.3	2.5	I				
	Venerupis sp.		1.5	I	Psammobiidae		1.5	I	Psammobiidae	622.1	2.4	I				
	Nemertea		1.2	III	*Venerupis* sp.		1.1	I								
Site D	**72.24%**				**62.23%**				**52.64%**				**54.01%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**
	C. montagui		42.2	I	*C. montagui*		41.3	I	*C. montagui*		25.2	I				
	M. galloprovincialis		17.1	III	*M. galloprovincialis*		15.0	III	*M. galloprovincialis*		19.4	III	*C. montagui*	24508.1	32.0	I
	R. parva		8.4	I	*R. parva*		12.3	I	*R. parva*		12.6	I	*M. galloprovincialis*	10497.7	23.7	III
	S. alveolata		5.5	I	*S. alveolata*		2.9	I	*S. alveolata*		7.7	I	*R. parva*	4548.6	14.7	I
	M. neritoides		3.6	II	*M. neritoides*		2.0	II	*T. dulongii*		3.4	II	*M. neritoides*	2601.3	2.9	II
	T. dulongii		2.9	II	*G. umbilicalis*		2.0	I	Actiniaria		3.1	II	*G. umbilicalis*	963.5	2.7	I
	Actiniaria		1.8	II	*T. dulongii*		1.8	II	*M. neritoides*		3.0	II	*S. alveolata*	1009.8	2.6	I
	G. umbilicalis		1.6	I	*S. pulvinata*		1.7	II	*G. umbilicalis*		2.3	I	*Venerupis* sp.	561.3	2.1	I
	Nemertea		1.1	III	Actiniaria		1.4	II	*S. pulvinata*		1.7	II	*S. pulvinata*	763.9	2.1	II
	Venerupis sp.		1.1	I	*Venerupis* sp.		1.2	I	*Venerupis* sp.		1.6	I	*T. dulongii*	604.8	2.0	II
					M. chelifer		1.0	I	Psammobiidae		1.4	I	Actiniaria	613.4	1.9	II
					Nemertea		1.0	III	*P. dumerilii*		1.1	III				
					Acanthochitona spp.		1.0	I	*Acanthochitona* spp.		1.1	I				
					P. dumerilii		0.8	III	*D. bidentata*		0.8	II				
									H. stebbingi		0.8	II				

Supplementary material A2. *(continued)*

D.	**Upper littoral**				**Mid littoral**				**Lower littoral**			
Upper littoral	**43.25%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**								
	C. montagui	52152.8	82.2	I								
	M. galloprovincialis	5438.4	7.9	III								
Mid littoral	**78.07%**				**46.16%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**				
	C. montagui		55.2	I								
	M. galloprovincialis		10.6	III	*M. galloprovincialis*	6152.3	28.5	III				
	R. parva		8.3	I	*C. montagui*	21911.9	20.5	I				
	S. alveolata		4.9	I	*R. parva*	5191.0	17.8	I				
	M. neritoides		4.1	II	*S. alveolata*	2278.7	6.3	I				
	T. dulongii		2.3	II	*G. umbilicalis*	1265.2	4.6	I				
					Actiniaria	926.7	3.7	II				
					T. dulongii	824.7	3.5	II				
					S. pulvinata	579.4	2.3	II				
Lower littoral	**85.27%**				**66.25%**				**42.92%**	**AD (ind m^{-2})**	**Ct (%)**	**EG**
	C. montagui		47.1	I	*C. montagui*		27.1	I				
	M. galloprovincialis		13.7	III	*M. galloprovincialis*		18.3	III	*M. galloprovincialis*	11634.1	32.5	III
	R. parva		5.7	I	*R. parva*		13.8	I	*S. alveolata*	3834.6	12.6	I
	S. alveolata		5.6	I	*S. alveolata*		6.7	II	*R. parva*	4819.9	10.2	I
	M. neritoides		3.4	II	*T. dulongii*		3.5	II	*T. dulongii*	2200.5	6.4	II
	T. dulongii		3.3	II	Actiniaria		3.2	II	Actiniaria	2031.3	5.4	II
	Actiniaria		2.6	II	*G. umbilicalis*		2.2	I	*Venerupis* sp.	842.0	3.5	I
	G. umbilicalis		1.3	I	*Venerupis* sp.		1.6	I	Nemertea	831.2	3.3	III
	Venerupis sp.		1.2	I	*S. pulvinata*		1.4	II	*S. pulvinata*	850.7	2.1	II
	S. pulvinata		1.2	II	*M. chelifer*		1.3	I	*A. squamata*	410.2	1.8	I
					Nemertea		1.2	III	*Acanthochitona* spp.	347.2	1.5	I
					Psammobiidae		0.9	I	Psammobiidae	447.1	1.4	I
					H. stebbingi		0.9	II	*M. chelifer*	536.0	1.4	I
					P. dumerilii		0.8	III	*G. umbilicalis*	640.2	1.3	I
					A. squamata		0.8	I	*P. dumerilii*	301.7	1.2	III
					D. bidentata		0.8	II	*D. bidentata*	336.4	1.2	II
					Acanthochitona spp.		0.6	I				
					Chironomidae		0.6	III				

Supplementary material A3. *Two-way SIMPER analysis for Matadouro' macroalgae and macroinvertebrates, showing the taxa responsible for similarity/dissimilarity (cut-off percentage: 95% for macroalgae and 85% for macroinvertebrates) within/between sampling sites (A. and C., respectively) and zones (B. and D., respectively). Shaded boxes: taxa contributing for similarities within each group. Non-shaded box, taxa contributing for dissimilarities between groups. Key: AB, AD, Ct, Opp and EG are the taxa average biomass (for macroalgae), average density (for macroinvertebrates), contribution for the similarities/dissimilarities, opportunism (for macroalgae) and Ecological Group (for macroinvertebrates).*

A.	**Site A**				**Site B**				**Site C**				**Site D**			
Site A	**45.04%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**												
	Ulva spp. (Leaf-like forms)	33.8	50.9	X												
	Corallina spp.	106.0	30.1													
	L. inscrustans	55.2	16.1													
Site B	**67.40%**				**45.37%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**								
	Corallina spp.		53.5		*Corallina* spp.	243.1	75.8									
	Ulva spp. (Leaf-like forms)		22.3	X	*Ulva* spp. (Leaf-like forms)	38.8	19.0	X								
	L. inscrustans		13.6		*L. inscrustans*	18.1	2.7									
	C. ustullatus		3.0													
	Ceramium spp.		1.8	X												
	C. tamariscifolia		1.0													
Site C	**76.38%**				**78.48%**				**39.91%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**				
	Ulva spp. (Leaf-like forms)		38.0	X	*Corallina* spp.		41.0		*L. inscrustans*	178.1	52.7					
	L. inscrustans		26.5		*L. inscrustans*		23.3		*Corallina* spp.	87.9	36.6					
	Corallina spp.		19.5		*Ulva* spp. (Leaf-like forms)		19.2	X	*D. dichotoma*	7.2	3.7					
	Ceramium spp.		2.1	X	*Peyssonellia* spp.		4.2		*P. complanata*	3.7	1.5					
	D. dichotoma		1.6		*C. ustullatus*		2.1		*Ulva* spp. (Leaf-like forms)	3.8	1.4	X				
	C. ustullatus		1.5		*F. spiralis*		1.7									
	F. spiralis		1.3		*D. dichotoma*		1.7									
	C. tamariscifolia		1.2		*B. bifurcata*		1.0									
	Codium spp.		1.1		Ectocarpales/*Sphacelaria* spp.		0.9	X								
	P. complanata		1.0													
	B. bifurcata		0.9													
	A. armata		0.9													
Site D	**73.93%**				**66.24%**				**65.08%**				**46.46%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**
	Ulva spp. (Leaf-like forms)		33.3	X	*Corallina* spp.		38.8		*Corallina* spp.		34.5		*Corallina* spp.	264.9	62.7	
	Corallina spp.		27.0		*Ulva* spp. (Leaf-like forms)		19.6	X	*F. spiralis*		27.6		*L. inscrustans*	156.2	26.9	
	L. inscrustans		18.5		*L. inscrustans*		17.1		*L. inscrustans*		19.8		*F. spiralis*	12.2	6.2	
	F. spiralis		9.3		*F. spiralis*		10.9		*L. marina*		5.5					
	Ceramium spp.		1.9	X	*Peyssonellia* spp.		2.9		*D. dichotoma*		3.2					
	D. dichotoma		1.9		*C. ustullatus*		2.1		*B. bifurcata*		1.9					
	C. tamariscifolia		1.5		*D. dichotoma*		1.6		*Ulva* spp. (Leaf-like forms)	X	1.6					
	C. ustullatus		1.4		*L. marina*		1.4		*C. tamariscifolia*		1.1					
	B. bifurcata		1.1		*B. bifurcata*		1.4									

Supplementary material A3. *(continued)*

B.	**Upper littoral**				**Mid littoral**				**Lower littoral**			
Upper littoral	**28.20%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**								
	Ulva spp. (Leaf-like forms)	30.6	81.3	X								
	F. spiralis	9.7	8.8									
	Corallina spp.	20.3	7.9									
Mid littoral	**89.22%**				**56.51%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**				
	Corallina spp.		60.8									
	L. inscrustans		17.8		*Corallina* spp.	287.5	78.5					
	Ulva spp. (Leaf-like forms)		9.8	X	*L. incrustans*	68.1	12.4					
	B. bifurcata		2.5		*Ulva* spp. (Leaf-like forms)	18.6	5.1	X				
	D. dichotoma		2.0									
	F. spiralis		1.8									
	C. ustullatus		1.6									
Lower littoral	**89.23%**				**60.39%**				**45.82%**	**AB (g DW m^{-2})**	**Ct (%)**	**Opp.**
	L. inscrustans		40.4		*Corallina* spp.		47.3					
	Corallina spp.		32.0		*L. incrustans*		33.2		*L. incrustans*	237.5	46.5	
	Ulva spp. (Leaf-like forms)		13.4	X	*Ulva* spp. (Leaf-like forms)		4.9	X	*Corallina* spp.	218.6	43.2	
	C. tamariscifolia		3.0		*D. dichotoma*		2.6		*Ulva* spp. (Leaf-like forms)	16.2	6.0	X
	D. dichotoma		1.6		*C. tamariscifolia*		1.7					
	F. spiralis		1.4		*C. ustullatus*		1.7	X				
	P. complanata		1.3		*B. bifurcata*		1.4					
	Codium spp.		1.1		*Codium* spp.		1.0					
	C. ustullatus		1.1		*Ceramium* spp.		0.9	X				
	Ceramium spp.		0.9	X	*P. complanata*		0.9					

Supplementary material A3. *(continued)*

C.	**Site A**				**Site B**				**Site C**				**Site D**			
Site A	**44.49%**	**AD**	**Ct**	**EG**												
		(ind m^{-2})	**(%)**													
	M. galloprovincialis	6417.8	42.7	III												
	R. parva	4997.1	14.0	I												
	R. coronata	920.1	6.0	I												
	Chironomidae	998.3	5.8	III												
	O. atomus	191.0	4.8	III												
	B. unifasciata	978.0	3.8	II												
	T. dulongii	546.9	2.9	II												
	L. levii	92.6	2.0	III												
	S. pulvinata	387.7	1.6	II												
	G. umbilicalis	410.9	1.3	I												
	Oligochaeta	358.8	1.2	V												
Site B	**69.93%**				**31.03%**	**AD**	**Ct**	**EG**								
	M. galloprovincialis		29.7	III		**(ind m^{-2})**	**(%)**									
	C. montagui		14.6	I	*M. galloprovincialis*	11614.6	30.4	III								
	R. parva		10.5	I	*T. dulongii*	2083.3	16.6	II								
	T. dulongii		6.8	II	*R. parva*	1707.2	6.7	I								
	Chironomidae		3.1	III	*R. coronata*	995.4	6.4	I								
	B. unifasciata		2.7	II	*O. atomus*	680.0	4.4	III								
	R. coronata		2.7	I	*B. unifasciata*	787.0	3.4	II								
	O. atomus		1.9	III	*M. costulatus*	648.2	3.4	I								
	Oligochaeta		1.8	V	Oligochaeta	538.2	3.2	V								
	H. stebingi		1.8	II	*C. montagui*	8822.3	3.0	I								
	M. costulatus		1.7	I	Chironomidae	474.5	2.7	III								
	G. umbilicalis		1.6	I	*Acanthochitona* spp.	350.1	2.4	I								
	O. eulimoides		1.5	II	*P. ovale*	350.1	2.2	I								
	S. pulvinata		1.2	II	*G. umbilicalis*	286.5	1.9	I								
	Nemertea		1.0	III												
	P. ovale		0.8	I												
	Acanthochitona spp.		0.8	I												
	S. impatiens		0.8	II												
	B. polybranchia		0.7	IV												
Site C	**81.00%**				**78.38%**				**62.24%**	**AD**	**Ct**	**EG**				
	C. montagui		28.2	I	*C. montagui*		23.8	I		**(ind m^{-2})**	**(%)**					
	R. parva		26.2	I	*R. parva*		19.7	I	*C. montagui*	14351.9	32.6	I				
	M. galloprovincialis		8.4	III	*M. galloprovincialis*		17.3	III	*R. parva*	11958.9	21.3	I				
	B. unifasciata		3.3	II	*T. dulongii*		3.4	II	*M. galloprovincialis*	4537.0	7.4	III				
	M. neritoides		2.7	II	*B. unifasciata*		3.1	II	*B. unifasciata*	1909.7	6.3	II				
	S. alveolata		2.2	I	*M. neritoides*		2.3	II	*P. dumerilii*	706.0	3.8	III				
	Chironomidae		1.8	III	*S. alveolata*		2.2	I	*T. dulongii*	865.2	3.5	II				
	P. dumerilii		1.6	III	*P. dumerilii*		2.1	III	*S. alveolata*	966.4	3.3	I				
	T. dulongii		1.6	II	*R. coronata*		1.7	I	*M. neritoides*	1524.9	2.7	II				
	R. coronata		1.3	I	*M. costulatus*		1.5	I	*M. costulatus*	604.8	2.1	I				
	O. eulimoides		1.1	II	*O. atomus*		1.1	III	*S. prolifera*	376.2	1.2	II				
	M. costulatus		1.1	I	*H. stebingi*		1.0	II	*S. impatiens*	303.8	1.2	II				
	S. prolifera		0.9	II	*S. prolifera*		1.0	II								
	H. stebingi		0.8	II	*P. cultrifera*		1.0	III								
	P. cultrifera		0.8	III	Oligochaeta		0.9	V								
	G. umbilicalis		0.8	I	*P. ovale*		0.9	I								
	P. depressa		0.7	I	Chironomidae		0.8	III								
	P. ulyssiponensis		0.7	I	*P. depressa*		0.8	I								
	S. pulvinata		0.6	II	*Acanthochitona* spp.		0.7	I								
	Oligochaeta		0.6	V												
Site D	**72.05%**				**73.91%**				**63.27%**				**53.02%**	**AD**	**Ct**	**EG**
	C. montagui		25.9	I	*C. montagui*		24.0	I	*R. parva*		33.9	I		**(ind m^{-2})**	**(%)**	
	M. galloprovincialis		19.9	III	*M. galloprovincialis*		22.4	II	*M. galloprovincialis*		18.5	III	*R. parva*	10136.0	34.1	I
	R. parva		19.3	I	*R. parva*		20.0	I	*C. montagui*		11.8	I	*C. montagui*	18906.3	25.6	I
	M. neritoides		5.1	II	*T. dulongii*		4.2	II	*B. unifasciata*		3.4	II	*M. galloprovincialis*	8833.9	21.0	III
	R. coronata		2.1	II	*M. neritoides*		4.1	II	*M. neritoides*		3.2	II	*M. neritoides*	3738.4	4.0	II
	Chironomidae		2.0	III	*R. coronata*		2.1	I	*S. alveolata*		2.4	I	*B. unifasciata*	610.5	2.6	II
	B. unifasciata		1.8	II	*B. unifasciata*		1.7	II	*T. dulongii*		1.9	II				
	L. adansoni		1.6	II	*L. adansoni*		1.2	II	*P. dumerilii*		1.9	III				
	N. pygmaeus		1.4	II	*M. costulatus*		1.2	I	*L. adansoni*		1.3	II				
	T. dulongii		1.3	II	*O. atomus*		1.2	III	*N. pygmaeus*		1.2	II				
	O. eulimoides		1.1	II	Oligochaeta		1.2	V	*R. coronata*		1.1	I				
	M. costulatus		1.0	I	*N. pygmaeus*		1.1	II	*M. costulatus*		0.9	I				
	S. pulvinata		1.0	II	*H. stebingi*		1.0	II	*P. cultrifera*		0.9	III				
	G. umbilicalis		0.9	I					*S. prolifera*		0.9	II				
	Oligochaeta		0.9	V					*P. ovale*		0.7	I				
									Actiniaria		0.6	II				
									S. impatiens		0.6	II				

Supplementary material A3. *(continued)*

D.	Upper littoral				Mid littoral				Lower littoral			
Upper littoral	**44.62%**	**AD**	**Ct**	**EG**								
		(ind m^{-2})	**(%)**									
	C. montagui	29233.9	57.9	I								
	M. galloprovincialis	8684.9	13.8	III								
	M. neritoides	4019.1	6.3	II								
	O. atomus	462.2	4.8	III								
	T. dulongii	724.8	3.8	II								
Mid littoral	**81.38%**				**52.03%**	**AD**	**Ct**	**EG**				
	C. montagui		34.1	I		**(ind m^{-2})**	**(%)**					
	M. galloprovincialis		22.8	III	*M. galloprovincialis*	10178.0	38.7	III				
	R. parva		12.5	I	*R. parva*	11234.8	27.0	I				
	M. neritoides		4.3	II	*T. dulongii*	1020.0	5.0	II				
	R. coronata		2.1	I	*R. coronata*	1020.0	4.4	I				
	Chironomidae		1.9	III	*B. unifasciata*	1243.5	4.3	II				
	B. unifasciata		1.8	II	*Acanthochitona*	377.6	1.8	I				
	T. dulongii		1.7	II	*S. pulvinata*	397.1	1.6	II				
	O. atomus		1.0	III	Chironomidae	377.6	1.5	III				
	L. adansoni		1.0	II	*M. costulatus*	405.8	1.4	I				
	S. pulvinata		0.9	II								
	Oligochaeta		0.9	V								
Lower littoral	**89.80%**				**69.69%**				**46.51%**	**AD**	**Ct**	**EG**
	C. montagui		32.2	I	*R. parva*		34.7	I		**(ind m^{-2})**	**(%)**	
	R. parva		15.6	I	*M. galloprovincialis*		23.6	III	*R. parva*	10353.7	33.8	I
	M. galloprovincialis		13.2	III	*C. montagui*		4.0	I	*M. galloprovincialis*	4689.7	14.8	III
	M. neritoides		4.1	II	*B. unifasciata*		3.8	II	*B. unifasciata*	1961.8	8.2	II
	B. unifasciata		3.7	II	*T. dulongii*		2.3	II	*T. dulongii*	954.9	4.7	II
	T. dulongii		2.2	II	*R. coronata*		2.0	I	*P. dumerilii*	627.2	4.0	III
	M. costulatus		1.6	I	*M. costulatus*		1.9	I	*M. costulatus*	987.4	4.0	I
	Chironomidae		1.5	III	*H. stebingi*		1.3	II	*S. alveolata*	722.7	3.3	I
	H. stebingi		1.4	II	*P. dumerilii*		1.2	III	*R. coronata*	607.6	2.6	I
	R. coronata		1.3	I	*S. alveolata*		1.2	I	*Scoletoma impatiens*	403.7	1.8	II
	O. atomus		1.2	III	Chironomidae		1.0	III	*S. prolifera*	377.6	1.5	II
	S. alveolata		1.2	I	*G. umbilicalis*		1.0	I	*S. pulvinata*	323.4	1.5	II
	P. dumerilii		1.2	III	Oligochaeta		1.0	V	*Acanthochitona*	397.1	1.5	I
	O. eulimoides		0.8	II	*S. pulvinata*		0.9	II	*P. ovale*	347.2	1.4	I
	P. ovale		0.7	I	*Scoletoma impatiens*		0.9	II	*P. cultrifera*	284.3	1.2	III
	L. adansoni		0.7	II	*P. ovale*		0.9	I	*Pilumnus hirtellus*	162.8	1.0	I
	Scoletoma impatiens		0.7	II	*O. eulimoides*		0.9	II				
	N. pygmaeus		0.7	II	*Acanthochitona*		0.8	I				
	S. pulvinata		0.7	II	*S. prolifera*		0.8	II				
	S. prolifera		0.7	II	Actiniaria		0.7	II				
					Nemertea		0.7	III				

Supplementary material B1. *PERMANOVA results using A. invertebrate biomass (B_{Taxa}), B. total biomass (B), C. number of species (S), D. Shannon-Wiener index (H'_B), E. Bentix biotic index (BENTIX_B) and F. 'traits by station' ($T_{Station}$). For pairwise tests, significant comparisons are presented.*

Main tests

A. B_{Taxa}

	Pseudo-F	P(perm)
Shore	39.850	0.0001
Season	7.526	0.0001
Site (Shore)	7.733	0.0001
Shore x Season	5.739	0.0001
Zone [Site (Shore)]	15.063	0.0001
Site (Shore) x Season	2.423	0.0001
Zone [Site (Shore)] x Season	1.996	0.0001

B. B

	Pseudo-F	P(perm)
Shore	30.303	0.0001
Season	3.156	0.0739
Site (Shore)	2.467	0.0460
Shore x Season	9.748	0.0022
Zone [Site (Shore)]	8.012	0.0001
Site (Shore) x Season	1.825	0.1219
Zone [Site (Shore)] x Season	2.278	0.0097

C. S

	Pseudo-F	P(perm)
Shore	0.034	0.8577
Season	19.316	0.0001
Site (Shore)	6.233	0.0001
Shore x Season	0.448	0.5110
Zone [Site (Shore)]	54.400	0.0001
Site (Shore) x Season	2.185	0.0714
Zone [Site (Shore)] x Season	1.592	0.0992

Pairwise tests

Shore x Season

		t	P(perm)
Summer			
	Buarcos, Matadouro	4.497	0.0001
Winter			
	Buarcos, Matadouro	5.076	0.0001
Buarcos			
	Summer, Winter	2.212	0.0004
Matadouro			
	Summer, Winter	2.955	0.0001

Site (Shore) x Season

Buarcos		t	P(perm)
Summer			
	Sites 1, 2	1.582	0.0201
	Sites 1, 3	1.661	0.0035
	Sites 2, 3	0.187	0.0007
Winter			
	Sites 1, 3	1.757	0.0100
Site 2			
	Summer, Winter	1.902	0.0035
Site 3			
	Summer, Winter	1.982	0.0001

Matadouro		t	P(perm)
Summer			
	Sites 1, 2	2.057	0.0002
	Sites 1, 3	3.382	0.0001
	Sites 2, 3	3.082	0.0001
Winter			
	Sites 1, 2	1.835	0.0001
	Sites 1, 3	4.137	0.0001
	Sites 2, 3	2.717	0.0001
Site 1			
	Summer, Winter	2.413	0.0001
Site 2			
	Summer, Winter	2.158	0.0004
Site 3			
	Summer, Winter	1.774	0.0003

Shore x Season

Winter		t	P(perm)
	Buarcos, Matadouro	6.2927	0.0001
Buarcos			
	Summer, Winter	3.226	0.0015

Site (Shore)

Matadouro		t	P(perm)
	Sites 2, 3	4.024	0.0001

Site (Shore)

Buarcos		t	P(perm)
	Sites 1, 3	2.579	0.0125
	Sites 2, 3	4.119	0.0002
Matadouro			
	Sites 1, 3	2.283	0.0278
	Sites 2, 3	2.376	0.0198

Supplementary material B1. *(continued)*

Main tests

D. H'_B

	Pseudo-F	P(perm)
Shore	1.950	0.1681
Season	2.902	0.0905
Site (Shore)	7.902	0.0001
Shore x Season	0.969	0.3332
Zone [Site (Shore)]	18.920	0.0001
Site (Shore) x Season	2.707	0.0313
Zone [Site (Shore)] x Season	3.773	0.0001

E. BENTIX_B

	Pseudo-F	P(perm)
Shore	0.398	0.5232
Season	0.165	0.6876
Site (Shore)	20.147	0.0001
Shore x Season	4.945	0.0241
Zone [Site (Shore)]	8.228	0.0001
Site (Shore) x Season	3.156	0.0157
Zone [Site (Shore)] x Season	2.299	0.0082

F. $T_{Station}$

	Pseudo-F	P(perm)
Shore	13.804	0.0001
Season	3.989	0.0049
Site (Shore)	7.367	0.0001
Shore x Season	2.699	0.0327
Zone [Site (Shore)]	11.668	0.0001
Site (Shore) x Season	1.348	0.1566
Zone [Site (Shore)] x Season	2.259	0.0001

Pairwise tests

Site (Shore) x Season

		t	P(perm)
Buarcos			
Summer		t	P(perm)
	Sites 1, 3	4.267	0.0004
	Sites 2, 3	2.927	0.0078
Winter			
	Sites 2, 3	2.122	0.0414
Site 2			
	Summer, Winter	2.235	0.0316
Site 3			
	Summer, Winter	2.529	0.0159
Matadouro			
Summer		t	P(perm)
	Sites 1, 2	2.038	0.0484
	Sites 1, 3	3.910	0.0005
Winter			
	Sites 1, 3	2.473	0.0177

Shore x Season

	t	P(perm)
Winter	t	P(perm)
Buarcos, Matadouro	2.062	0.0423

Site (Shore) x Season

		t	P(perm)
Buarcos			
Winter		t	P(perm)
	Sites 1, 2	2.261	0.0287
Site 2			
	Summer, Winter	2.272	0.0266
Matadouro			
Summer			
	Sites 1, 2	5.206	0.0002
	Sites 1, 3	10.174	0.0001
Winter			
	Sites 1, 2	3.207	0.0028
	Sites 1, 3	6.766	0.0001
	Sites 2, 3	4.292	0.0002
Site 1			
	Summer, Winter	2.481	0.0186

Shore x Season

		t	P(perm)
Summer		t	P(perm)
	Buarcos, Matadouro	2.707	0.0001
Winter			
	Buarcos, Matadouro	3.069	0.0001
Buarcos			
	Summer, Winter	1.980	0.0089
Matadouro			
	Summer, Winter	1.597	0.0385

Site (Shore)

		t	P(perm)
Buarcos		t	P(perm)
	Sites 1, 3	1.666	0.0349
Matadouro			
	Sites 1, 2	2.818	0.0001
	Sites 1, 3	5.645	0.0001
	Sites 2, 3	2.616	0.0003

Supplementary material B2. *Two-way SIMPER analysis with factors 'Site' and 'Season' for Buarcos (A. and B.) and Matadouro (C. and D.) shores, showing the taxa responsible for similarities/dissimilarities (cut-off 85%). Shaded boxes: taxa contributing for similarities within each group. Non-shaded boxes, taxa contributing for dissimilarities between groups. AB and Ct are the taxa average biomass (g AFDW m^{-2}) and contribution (%).*

A.	**Site 1**			**Site 2**			**Site 3**		
Site 1	**20.05%**	**AB**	**Ct**						
		(g AFDW m^{-2})	**(%)**						
	M. galloprovincialis	60.41	51.30						
	S. alveolata	5.31	12.89						
	P. depressa	3.22	7.65						
	C. montagui	7.27	6.99						
	G. umbilicalis	4.22	6.76						
Site 2	**81.62%**			**23.19%**	**AB**	**Ct**			
	M. galloprovincialis		46.27		**(g AFDW m^{-2})**	**(%)**			
	C. montagui		16.76	*M. galloprovincialis*	76.67	58.46			
	P. depressa		6.31	*C. montagui*	19.52	19.52			
	S. alveolata		5.42	*P. depressa*	4.90	5.50			
	G. umbilicalis		4.57	*S. alveolata*	2.44	2.73			
	P. ulyssiponensis		4.47						
	P. vulgata		2.71						
Site 3	**78.49%**			**76.41%**			**27.75%**	**AB**	**Ct**
	M. galloprovincialis		41.99	*M. galloprovincialis*		42.91		**(g AFDW m^{-2})**	**(%)**
	C. montagui		13.84	*C. montagui*		20.06	*M. galloprovincialis*	48.67	58.21
	P. depressa		7.36	*P. depressa*		7.30	*C. montagui*	14.79	8.78
	S. alveolata		6.02	*P. ulyssiponensis*		4.08	*P. depressa*	4.83	5.88
	G. umbilicalis		5.15	*S. alveolata*		3.63	*G. umbilicalis*	2.11	5.70
	P. ulyssiponensis		4.01	*G. umbilicalis*		3.37	*Acanthochitona* sp.	1.37	5.14
	Acanthochitona sp.		2.02	*Acanthochitona* sp.		2.09	*S. alveolata*	1.97	4.75
	Actiniaria		1.92	*P. vulgata*		2.08			
	P. marionii		1.55						
	P. vulgata		1.50						

B.	**Summer**			**Winter**		
Summer	**21.73%**	**AB**	**Ct**			
		(g AFDW m^{-2})	**(%)**			
	M. galloprovincialis	43.34	48.31			
	C. montagui	10.96	11.03			
	S. alveolata	3.69	7.60			
	P. depressa	3.75	6.98			
	G. umbilicalis	2.38	6.32			
	Acanthochitona sp.	0.94	4.36			
	P. ulyssiponensis	2.03	2.62			
Winter	**79.03%**			**25.60%**	**AB**	**Ct**
	M. galloprovincialis		45.39		**(g AFDW m^{-2})**	**(%)**
	C. montagui		16.53	*M. galloprovincialis*	80.50	63.16
	P. depressa		6.78	*C. montagui*	16.76	12.43
	S. alveolata		4.82	*P. depressa*	4.88	5.64
	G. umbilicalis		4.49	*S. alveolata*	2.79	5.37
	P. ulyssiponensis		4.06			
	P. vulgata		1.81			
	Actiniaria		1.58			

Supplementary material B2. *(continued)*

C.	**Site 1**			**Site 2**			**Site 3**		
Site 1	**21.70%**	**AB (g AFDW m^{-2})**	**Ct (%)**						
	M. galloprovincialis	67.45	74.67						
	P. ulyssiponensis	1.92	4.29						
	Chironomidae	0.21	4.10						
	Acanthochitona sp.	1.41	3.35						
Site 2	**84.30%**			**20.39%**	**AB (g AFDW m^{-2})**	**Ct (%)**			
	M. galloprovincialis		51.59						
	P. ulyssiponensis		6.70	*M. galloprovincialis*	15.83	49.55			
	C. montagui		6.01	*P. ulyssiponensis*	3.56	10.07			
	G. umbilicalis		4.27	*Acanthochitona* sp.	1.00	8.00			
	Acanthochitona sp.		3.17	*G. umbilicalis*	1.08	3.89			
	P. depressa		2.84	*M. costulatus*	0.88	3.50			
	S. alveolata		2.51	*S. impatiens*	0.57	3.17			
	M. costulatus		2.27	*T. dulongii*	0.18	2.58			
	S. impatiens		1.90	*C. montagui*	5.77	2.33			
	S. pectinata		1.46	*P. depressa*	2.34	2.20			
	Actiniaria		1.32						
	R. parva		1.30						
Site 3	**88.19%**			**82.98%**			**22.01%**	**AB (g AFDW m^{-2})**	**Ct (%)**
	M. galloprovincialis		39.28	*C. montagui*		24.09			
	C. montagui		17.42	*M. galloprovincialis*		23.81	*C. montagui*	18.53	25.95
	S. alveolata		5.22	*P. depressa*		7.43	*M. galloprovincialis*	7.78	18.31
	P. depressa		4.57	*P. ulyssiponensis*		6.73	*S. alveolata*	2.58	12.92
	P. ulyssiponensis		3.88	*S. alveolata*		5.85	*Acanthochitona* sp.	1.38	6.80
	P. vulgata		2.92	*P. vulgata*		3.36	*P. depressa*	4.87	5.79
	Acanthochitona sp.		2.72	*Acanthochitona* sp.		2.82	*P. ulyssiponensis*	2.64	3.87
	G. umbilicalis		1.96	*M. costulatus*		2.20	*S. impatiens*	0.76	3.82
	R. parva		1.79	*R. parva*		2.00	*M. costulatus*	0.80	3.49
	S. impatiens		1.64	*P. hirtellus*		1.94	*R. parva*	0.99	2.98
	P. hirtellus		1.49	*G. umbilicalis*		1.90	*B. unifasciata*	0.30	2.06
	M. costulatus		1.28	*S. impatiens*		1.74			
	P. cultrifera		1.28	*P. dumerilii*		1.15			

D.	**Summer**			**winter**		
Summer	**22.32%**	**AB (g AFDW m^{-2})**	**Ct (%)**			
	M. galloprovincialis	34.26	51.66			
	C. montagui	8.53	8.72			
	Acanthochitona sp.	1.39	6.17			
	S. alveolata	1.29	4.86			
	P. depressa	3.03	2.74			
	M. costulatus	0.51	2.40			
	S. impatiens	0.91	2.39			
	P. ulyssiponensis	1.78	2.19			
	G. umbilicalis	0.84	2.14			
	R. coronata	0.35	2.00			
Winter	**80.60%**			**20.41%**	**AB (g AFDW m^{-2})**	**Ct (%)**
	M. galloprovincialis		38.59			
	C. montagui		14.17	*M. galloprovincialis*	26.45	42.59
	P. ulyssiponensis		6.73	*C. montagui*	7.68	10.67
	P. depressa		4.98	*P. ulyssiponensis*	3.63	10.14
	S. alveolata		3.63	*S. alveolata*	1.26	6.40
	Acanthochitona sp.		2.89	*Acanthochitona* sp.	1.14	5.84
	G. umbilicalis		2.77	*M. costulatus*	0.88	3.34
	M. costulatus		2.02	*S. impatiens*	0.57	3.01
	P. vulgata		1.94	Chironomidae	0.12	2.67
	R. parva		1.77	*P. depressa*	1.79	2.62
	S. impatiens		1.68			
	P. cultrifera		1.14			
	P. hirtellus		1.11			
	Chironomidae		1.05			
	Actiniaria		0.94			

Supplementary material B3. *Draftsman plot (Pearson) correlations between categories across traits. Higher correlations (>+0.85 and <-0.85) are underlined and in bold.*

A. Buarcos	**Maximum size**				**Longevity**				**Larval development mode**			**Living habit**				**Feeding habit**				**Tolerance to pollution**				
	S-1	**S1-3**	**S3-10**	**S10+**	**L-1**	**L1-3**	**L3-10**	**L10+**	**Plan**	**Lec**	**Dir**	**Td**	**Bd**	**Fl**	**Att**	**F/S**	**Dep**	**H/O/S**	**Pred**	**EG I**	**EG II**	**EG III**	**EG IV**	**EG V**
S-1		-0.252	-0.208	-0.103	0.397	0.588	0.006	-0.608	-0.559	0.379	0.607	0.352	0.531	-0.188	-0.415	-0.337	0.347	-0.191	0.427	-0.288	0.711	-0.226	0.255	0.217
S1-3			-0.063	-0.578	-0.446	-0.425	**0.857**	-0.163	0.509	-0.388	-0.503	-0.504	-0.495	0.249	0.467	0.249	-0.139	0.243	-0.570	0.580	-0.292	-0.431	-0.103	-0.131
S3-10				-0.657	-0.148	-0.290	-0.100	0.346	0.338	-0.316	-0.266	-0.130	-0.218	0.799	-0.515	-0.515	-0.144	0.831	-0.311	0.681	-0.328	-0.654	0.008	0.109
S10+					0.268	0.279	-0.560	0.122	-0.393	0.368	0.309	0.317	0.299	-0.737	0.253	0.380	0.060	-0.756	0.477	-0.843	0.154	**0.945**	-0.046	-0.088
L-1						0.513	-0.491	-0.579	-0.752	0.687	0.612	0.696	0.620	-0.400	-0.558	-0.456	0.537	-0.372	0.677	-0.487	0.574	0.163	0.200	0.031
L1-3							-0.338	-0.630	-0.836	0.667	0.792	0.644	0.793	-0.487	-0.528	-0.404	0.505	-0.500	0.813	-0.621	0.686	0.133	0.315	0.270
L3-10								-0.298	0.410	-0.302	-0.418	-0.407	-0.384	0.194	0.375	0.255	-0.141	0.171	-0.474	0.482	-0.156	-0.466	0.010	-0.042
L10+									0.649	-0.600	-0.520	-0.507	-0.554	0.388	0.372	0.331	-0.538	0.395	-0.530	0.284	-0.652	0.199	-0.322	-0.135
Plan										**-0.886**	-0.846	-0.728	-0.840	0.571	0.547	0.447	-0.599	0.573	**-0.892**	0.728	-0.708	-0.241	-0.377	-0.209
Lec											0.502	0.607	0.753	-0.508	-0.454	-0.385	0.532	-0.518	0.787	-0.656	0.520	0.287	0.421	0.073
Dir												0.660	0.702	-0.481	-0.497	-0.391	0.504	-0.472	0.758	-0.604	0.723	0.119	0.219	0.305
Td													0.578	-0.593	-0.612	-0.412	0.635	-0.492	0.692	-0.530	0.524	0.163	0.255	0.197
Bd														-0.477	-0.595	-0.486	0.567	-0.476	0.839	-0.621	0.587	0.168	0.360	0.272
Fl															-0.166	-0.292	-0.383	**0.957**	-0.586	0.811	-0.394	-0.667	-0.133	-0.027
Att																**0.887**	-0.493	-0.213	-0.502	0.035	-0.400	0.391	-0.290	-0.291
F/S																	-0.622	-0.340	-0.361	-0.098	-0.292	0.484	-0.255	-0.325
Dep																		-0.336	0.428	-0.317	0.340	-0.026	0.232	0.348
H/O/S																			-0.623	0.843	-0.425	-0.686	-0.148	-0.012
Pred																				-0.762	0.703	0.309	0.357	0.154
EG I																					-0.506	-0.728	-0.315	-0.100
EG II																						-0.002	0.170	-0.062
EG III																							-0.175	-0.242
EG IV																								0.144

B. Matadouro	**Maximum size**				**Longevity**				**Larval development mode**			**Living habit**				**Feeding habit**				**Tolerance to pollution**				
	S-1	**S1-3**	**S3-10**	**S10+**	**L-1**	**L1-3**	**L3-10**	**L10+**	**Plan**	**Lec**	**Dir**	**Td**	**Bd**	**Fl**	**Att**	**F/S**	**Dep**	**H/O/S**	**Pred**	**EG I**	**EG II**	**EG III**	**EG IV**	**EG V**
S-1		-0.092	-0.193	-0.256	0.550	0.663	-0.183	-0.621	-0.639	0.338	0.778	0.393	0.506	0.037	-0.568	-0.470	0.432	-0.005	0.318	-0.254	0.356	-0.100	0.292	0.098
S1-3			-0.137	-0.709	-0.315	-0.171	0.637	-0.268	0.433	-0.526	-0.093	-0.434	-0.267	0.416	0.241	0.178	-0.309	0.467	-0.461	0.509	-0.229	-0.326	-0.172	-0.105
S3-10				-0.443	-0.286	-0.030	0.330	-0.087	0.079	0.011	-0.173	0.266	-0.271	0.534	-0.294	-0.229	-0.220	0.552	-0.008	0.582	0.335	-0.656	-0.085	-0.345
S10+					0.197	-0.141	-0.665	0.566	-0.122	0.280	-0.167	0.013	0.167	-0.711	0.248	0.215	0.203	-0.747	0.247	-0.688	-0.188	0.744	0.065	0.266
L-1						0.539	-0.680	-0.401	-0.797	0.727	0.522	0.433	0.825	-0.213	-0.651	-0.659	0.683	-0.242	0.615	-0.673	-0.029	0.381	0.416	0.428
L1-3							-0.268	-0.738	-0.812	0.604	0.733	0.690	0.611	-0.076	-0.774	-0.647	0.525	-0.138	0.639	-0.388	0.406	-0.104	0.481	0.170
L3-10								-0.288	0.502	-0.496	-0.274	-0.167	-0.559	0.430	0.214	0.262	-0.458	0.424	-0.405	0.831	0.274	-0.742	-0.349	-0.409
L10+									0.588	-0.418	-0.559	-0.569	-0.433	-0.204	0.730	0.615	-0.375	-0.140	-0.445	-0.064	-0.480	0.485	-0.260	-0.025
Plan										**-0.876**	-0.709	-0.724	-0.812	0.245	0.826	0.802	-0.728	0.289	-0.840	0.606	-0.374	-0.087	-0.488	-0.335
Lec											0.280	0.691	0.750	-0.338	-0.724	-0.716	0.647	-0.393	**0.892**	-0.600	0.293	0.144	0.434	0.355
Dir												0.430	0.518	0.007	-0.586	-0.549	0.502	0.001	0.367	-0.327	0.315	-0.038	0.337	0.147
Td													0.390	-0.241	-0.782	-0.601	0.475	-0.223	0.703	-0.207	0.629	-0.242	0.269	-0.008
Bd														-0.281	-0.685	-0.683	0.708	-0.305	0.693	-0.633	0.061	0.276	0.400	0.467
Fl															-0.171	-0.171	-0.325	**0.895**	-0.352	0.594	-0.047	-0.491	-0.130	-0.225
Att																**0.875**	-0.559	-0.118	-0.693	0.202	-0.441	0.253	-0.342	-0.144
F/S																	-0.742	-0.178	-0.651	0.262	-0.263	0.176	-0.380	-0.298
Dep																		-0.305	0.507	-0.529	0.128	0.157	0.338	0.460
H/O/S																			-0.467	0.663	-0.102	-0.514	-0.154	-0.256
Pred																				-0.583	0.388	0.110	0.422	0.286
EG I																					0.202	-0.747	-0.524	-0.537
EG II																						-0.594	-0.090	-0.305
EG III																							0.095	0.304
EG IV																								0.065

Supplementary material B4. *Traits trends outlined by the CWM, reflecting the proportion of species' biomass in the communities within a given trait category.*

(i) Maximum size

General trends: an both Buarcos and Matadouro, proportion generally increased from very small size (S-1) (1.6-11.1 %) to large size (S10+) (25.6-64.6 %) organisms. Exceptions occurred: Buarcos sites 2 and 3 (during winter) showed small size (S1-3) > medium size (S3-10) organisms; Matadouro site 3 (during both seasons) registered S3-10 > S1-3 > S10+.

Spatial and seasonal trends: at Buarcos, site 1 registered during summer the lowest proportions of S-1 and S1-3 and highest proportions of S3-10 and S10+. In contrast, site 1 registered during winter the highest proportions of S-1 and S1-3 and lowest proportion of S10+. At Matadouro, linear trends were observed during summer for all categories: S-1 and S10+ decreased from site 1 to site 3; S1-3 and S3-10 increased in that direction. Same trends were found during winter for S3-10 and S10+.

Was visible a closer seasonal relation between sites 2 and 3. During summer, compared to winter, both sites registered at Buarcos higher S-1 and lower S10+, with the opposite shown by site 1; all sites showed higher S3-10. At Matadouro, sites 2 and 3 showed higher S1-3, with the converse at site 1; all sites showed higher S-1, and lower S3-10 and S10+.

(ii) Longevity

General trends: at both shores, proportion generally increased from very short life (L-1) (1.1-17.4 %) to long life (L10+) (29.6-65.7 %) organisms. Several exceptions occurred: Buarcos site 3 showed during summer L-1 > short life (L1-3) organisms; Matadouro site 3 registered during both seasons medium life (L3-10) > L10+ organisms and during winter L-1 > L1-3; Matadouro sites 1 and 2 showed during winter L-1 > L1-3 (especially site 1: 17.4% for L-1 and 2.6% for L1-3).

Spatial and seasonal trends: at Buarcos, L-1 and L1-3 showed linear trends during summer, the first increasing and the second decreasing from site 1 to site 3. Site 1 registered during summer the lowest proportion of L3-10 and highest of L10+, with the opposite during winter. At Matadouro, all categories showed linear trends: L-1 (during summer) and L10+ (during both seasons) decreased from site 1 to site 3, while L1-3 (during winter) and L3-10 (during both seasons) increased in that direction.

Was observed closer seasonal relation between sites 2 and 3 (both shores), and between sites 1 and 2 (Matadouro). During summer, compared to winter, sites 2 and 3 registered on Buarcos higher L-1 and L3-10, and lower L10+. On Matadouro, those sites showed lower L10+, and sites 1 and 2 showed lower L3-10; all sites showed higher L1-3.

(iii) Larval development mode

General trends: at both Buarcos and Matadouro, proportions were always much higher for planktotrophic (Plan) organisms (>80% and >73%, respectively), contrary to direct (Dir) developers (<7% and <13%, respectively).

Spatial and seasonal trends: at Buarcos, linear trends were observed for Plan and Lec (both during summer), the first decreasing and the second increasing from site 1 to site 3. Site 1 showed during summer the highest Plan and lowest Lec, but the opposite during winter. Site 3 registered during summer the highest Dir, but the lowest during winter. At Matadouro, all categories showed linear trends: Plan increased, and Dir decreased, from site 1 to site 3 during both seasons; Lec showed contrary trends between seasons, increasing in that direction during summer and decreasing in winter.

Was visible a closer seasonal relation between sites 2 and 3. During summer, compared to winter, both sites registered at Buarcos lower Plan, and higher Lec and Dir. At Matadouro those sites presented higher Lec; all sites showed higher Plan.

(iv) Living habit

General trends: at both shores, proportion was higher for attached (Att) (>45% and >39% for Buarcos an Matadouro, respectively), followed by free-living (Fl) (21-46%), organisms. On Buarcos, those two were followed D3:D5by tube-dwellers (Td) and burrow-dwellers (Bd) (only site 2 registered during summer Bd > Td). On Matadouro, the sequence was more variable. Generally, Att > Fl > Bd > Td, except at site 3: Td > Bd during both seasons, and at sites 2 and 3: Fl > Att during summer.

Spatial and seasonal trends: at Buarcos, site 1 registered the highest proportion of Td (during both seasons), and showed contrary trends between seasons for Bd, registering the lowest and highest proportions during summer and winter, respectively; site 2 registered the lowest Fl and highest Att. At Matadouro were observed linear trends for Td, increasing from site 1 to site 3 during both seasons; contrary seasonal trends of Bd were observed at site 3, which registered the highest and lowest proportions during summer and winter, respectively. Fl was lowest and Att was highest at site 1.

Was observed a closer seasonal relation between sites 2 and 3. During summer, compared to winter, both sites registered at both shores higher Bd, and at Matadouro higher Td, Fl and Att. At Buarcos, all sites showed higher Td, Fl and Att.

(v) Feeding habit

General trends: on both shores, proportion increased from deposit (Dep) (1.6-6.2%) < predator (Pred) < herbivore/opportunistic/scavenger (H/O/S) < filter/suspension (F/S) (50.6-77.5%) feeders. The only exception was on Matadouro site 1 (during winter) which registered Dep > Pred. The Dep and Pred showed always low proportions within the communities, whereas H/O/S and F/S accounted for most of the proportions.

Spatial and seasonal trends: F/S decreased from site 1 to site 3 at Buarcos during summer). F/S was during summer highest at both shores site 1, and was lowest during winter at site 1 (Buarcos) and site 3 (Matadouro); Dep increased at Matadouro from site 1 to site 3 during summer but decreased during winter, and at Buarcos was highest at sites 1 during summer and at site 3 during winter; H/S/O was lowest at Matadouro site 1 during both seasons, and was highest at Buarcos sites 3 during summer and site 1 during winter; Pred increased at Matadouro from site 1 to site 3 during winter and was highest at site 3 during both seasons. At Buarcos, Pred was lowest and highest at site 1 during summer and winter, respectively.

Was visible a closer seasonal relation between sites 2 and 3. During summer, compared to winter, both sites registered at both shores higher Dep, at Buarcos higher Pred, and at Matadouro higher H/O/S and lower F/S. At Buarcos, all sites presented higher H/O/S and lower F/S.

(vi) Tolerance

General trends: on both shores, proportions were generally higher of species sensitive to organic enrichment (EG I) (14.7-71.4 %), followed by tolerant (EG III), indifferent (EG II), second-order opportunist (EG IV) and first-order opportunist (EG V) (0.0-0.3 %) species. Several exceptions occurred: at Buarcos, site 1 registered EG IV > EG V, and sites 2-3 presented EG III > EG I (all during winter); at Matadouro, site 1 showed EG III > EG I (during both seasons).

Spatial and seasonal trends: EG I increased from site 1 to site 3 at Matadouro during both seasons (and was lowest at Buarcos site 1 during summer), but site 1 showed at Buarcos the highest EG I during winter; EG II increased at Buarcos from site 1 to site 3 during summer and was highest at site 3 during both seasons, and at Matadouro was highest at site 2 during both seasons; EG III decreased from site 1 to site 3 at Buarcos during summer and at Matadouro during both seasons, but was lowest at Buarcos site 1 during winter; EG IV increased from site 1 to site 3 at Buarcos during summer, but decreased during winter, and at Matadouro, EG IV was highest on site 1 during summer and lowest at site 3 during winter; and EG V decreased at both shores during winter, and was lowest at site 3 at both shores during summer.

Was visible a closer seasonal relation between sites 2 and 3, and between sites 1-2. During summer, compared to winter, sites 2 and 3 registered at both shores higher EG V, and at Buarcos higher EG I, EG II and EG IV. At Matadouro, sites 1 and 2 showed during summer, compared to winter, higher EG II .

Supplementary material B5. *PERMANOVA results using CWM index, showing main and pairwise tests for the traits categories A. Maximum size, B. Longevity, C. Larval development mode, D. Living habit, E. Feeding habit and F. Tolerance to pollution. For pairwise tests, significant comparisons are presented.*

A.Maximum size

Main tests

S<1

	Pseudo-F	P(perm)
Shore	24.562	0.0001
Season	17.308	0.0001
Site (Shore)	0.495	0.7441
Shore x Season	4.983	0.0244
Zone [Site (Shore)]	4.347	0.0001
Site (Shore) x Season	0.907	0.4691
Zone [Site (Shore)] x Season	2.257	0.0075

S1-3

	Pseudo-F	P(perm)
Shore	0.526	0.4742
Season	0.454	0.4994
Site (Shore)	3.686	0.0063
Shore x Season	0.424	0.5196
Zone [Site (Shore)]	14.328	0.0001
Site (Shore) x Season	1.862	0.1204
Zone [Site (Shore)] x Season	1.312	0.2106

S3-10

	Pseudo-F	P(perm)
Shore	0.173	0.6759
Season	1.150	0.2897
Site (Shore)	8.002	0.0002
Shore x Season	18.432	0.0002
Zone [Site (Shore)]	5.719	0.0001
Site (Shore) x Season	0.481	0.7457
Zone [Site (Shore)] x Season	1.946	0.0318

S>10

	Pseudo-F	P(perm)
Shore	1.209	0.2726
Season	1.966	0.1642
Site (Shore)	9.050	0.0001
Shore x Season	4.126	0.0409
Zone [Site (Shore)]	7.694	0.0001
Site (Shore) x Season	2.718	0.0333
Zone [Site (Shore)] x Season	1.753	0.0536

Pairwise tests

S<1

Shore x Season

		t	P(perm)
Summer			
	Buarcos, Matadouro	4.136	0.0004
Winter			
	Buarcos, Matadouro	2.752	0.0062
Matadouro			
	Summer, Winter	3.731	0.0004

S1-3

Site (Shore)

		t	P(perm)
Matadouro			
	Sites 1, 3	4.450	0.0001
	Sites 2, 3	3.268	0.0021

S3-10

Shore x Season

		t	P(perm)
Summer			
	Buarcos, Matadouro	2.550	0.0131
Winter			
	Buarcos, Matadouro	3.624	0.0005
Buarcos			
	Summer, Winter	3.566	0.0006
Matadouro			
	Summer, Winter	2.445	0.0156

Site (Shore)

		t	P(perm)
Buarcos			
	Sites 1, 2	2.274	0.0242
Matadouro			
	Sites 1, 2	4.000	0.0001
	Sites 1, 3	5.709	0.0001

S>10

Shore x Season

		t	P(perm)
Winter			
	Buarcos, Matadouro	2.387	0.0169
Buarcos			
	Summer, Winter	2.256	0.0267

Site (Shore) x Season

Buarcos		t	P(perm)
Winter			
	Sites 1, 2	2.376	0.0233
Site 2			
summer, winter		2.4982	0.0159
Site 3			
summer, winter		2.1241	0.0423
Matadouro			
Summer			
	Sites 1, 2	2.970	0.0054
	Sites 1, 3	5.112	0.0001
Winter			
	Sites 1, 3	4.468	0.0004
	Sites 2,3	3.466	0.0017

B. Longevity

Main tests

L<1

	Pseudo-F	P(perm)
Shore	44.643	0.0001
Season	0.164	0.6927
Site (Shore)	6.883	0.0001
Shore x Season	3.547	0.0580
Zone [Site (Shore)]	9.082	0.0001
Site (Shore) x Season	1.855	0.1145
Zone [Site (Shore)] x Season	2.364	0.0054

L1-3

	Pseudo-F	P(perm)
Shore	16.809	0.0001
Season	13.314	0.0001
Site (Shore)	0.427	0.8006
Shore x Season	9.438	0.0017
Zone [Site (Shore)]	10.203	0.0001
Site (Shore) x Season	0.886	0.4779
Zone [Site (Shore)] x Season	2.702	0.0013

L3-10

	Pseudo-F	P(perm)
Shore	4.223	0.0395
Season	0.028	0.8667
Site (Shore)	14.567	0.0001
Shore x Season	0.081	0.7749
Zone [Site (Shore)]	6.859	0.0001
Site (Shore) x Season	3.257	0.0109
Zone [Site (Shore)] x Season	2.147	0.0155

L>10

	Pseudo-F	P(perm)
Shore	2.635	0.1035
Season	1.068	0.2946
Site (Shore)	6.801	0.0002
Shore x Season	0.001	0.9711
Zone [Site (Shore)]	5.644	0.0001
Site (Shore) x Season	2.805	0.0256
Zone [Site (Shore)] x Season	2.257	0.0113

Pairwise tests

L<1

Site (Shore)

		t	P(perm)
Buarcos			
	Sites 1, 3	3.640	0.0002
	Sites 2, 3	3.790	0.0002
Matadouro			
	Sites 1, 2	2.597	0.0092
	Sites 1, 3	2.724	0.0072

L1-3

Shore x Seasor

		t	P(perm)
Summer			
	Buarcos, Matadouro	3.952	0.0002
Matadouro			
	Summer, Winter	3.719	0.0003

L3-10

Site (Shore) x Season

Matadouro		t	P(perm)
Summer			
	Sites 1, 2	2.683	0.0103
	Sites 1, 3	9.147	0.0001
	Sites 2, 3	3.523	0.0012
Winter			
	Sites 1, 2	2.126	0.0393
	Sites 1, 3	5.772	0.0001
	Sites 2, 3	3.618	0.0010

L>10

Site (Shore) x Season

Buarcos		t	P(perm)
Winter			
	Sites 1, 2	2.267	0.0292
Site 2			
	Summer, Winter	2.269	0.0282
Matadouro			
Summer			
	Sites 1, 3	4.329	0.0002
	Sites 2, 3	2.778	0.0088
Winter			
	Sites 1, 3	2.888	0.0062
	Sites 2, 3	3.317	0.0025
Site 3			
	Summer, Winter	2.035	0.0474

Supplementary material B5. *(continued)*

C. Larval development mode

Main tests

	Plan		Lec		Dir	
	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Shore	17.753	0.0001	19.498	0.0001	2.729	0.0986
Season	13.714	0.0003	7.567	0.0046	10.066	0.0007
Site (Shore)	0.869	0.4884	0.800	0.5322	2.809	0.0191
Shore x Season	0.762	0.3823	0.000	0.9835	3.119	0.0817
Zone [Site (Shore)]	8.312	0.0001	9.171	0.0001	2.770	0.0018
Site (Shore) x Season	1.152	0.3360	1.853	0.1173	1.483	0.2088
Zone [Site (Shore)] x Season	2.432	0.0056	2.469	0.0047	2.806	0.0014

Pairwise tests

Dir

			t	P(perm)
Site (Shore)	Matadouro			
		Sites 1, 3	2.361	0.0099
		Sites 2, 3	1.970	0.0421

D. Living habit

Main tests

	Td		Bd		Fl		Att	
	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Shore	0.194	0.6668	20.479	0.0001	8.970	0.0036	14.876	0.0003
Season	0.737	0.3981	0.075	0.7884	6.073	0.0145	5.312	0.0226
Site (Shore)	9.896	0.0001	2.548	0.0384	2.793	0.0265	3.358	0.0094
Shore x Season	1.646	0.2039	1.656	0.1977	5.200	0.0253	7.703	0.0064
Zone [Site (Shore)]	17.745	0.0001	7.885	0.0001	5.597	0.0001	13.693	0.0001
Site (Shore) x Season	0.639	0.6334	2.768	0.0267	0.644	0.6296	1.426	0.2222
Zone [Site (Shore)] x Season	1.641	0.0801	1.952	0.0269	1.815	0.0441	1.905	0.0320

Pairwise tests

Td

			t	P(perm)
Site (Shore)	Buarcos			
		Sites 1, 2	3.198	0.0016
		Sites 1, 3	2.461	0.0143
	Matadouro			
		Sites 1, 3	5.284	0.0001
		Sites 2, 3	4.010	0.0001

Bd

			t	P(perm)
Site (Shore) x Season	**Buarcos**			
	Summer			
		Sites 1, 3	2.126	0.0353
	Matadouro			
	Winter			
		Sites 1, 2	2.358	0.0211
		Sites 1, 3	2.364	0.0199

Fl

			t	P(perm)
Shore x Season	Winter			
		Buarcos, Matadouro	4.468	0.0002
	Buarcos			
		Summer, Winter	3.302	0.0014

			t	P(perm)
Site (Shore)	Matadouro			
		Sites 1, 2	2.427	0.0191
		Sites 1, 3	3.039	0.0021

Att

			t	P(perm)
Shore x Season	Winter			
		Buarcos, Matadouro	5.395	0.0001
	Buarcos			
		Summer, Winter	3.607	0.0005

			t	P(perm)
Site (Shore)	Matadouro			
		Sites 1, 3	3.161	0.0023

Supplementary material B5. *(continued)*

E. Feeding habit

Main tests

	F/S Pseudo-F	F/S P(perm)	Dep Pseudo-F	Dep P(perm)	S/O Pseudo-F	S/O P(perm)	Pred Pseudo-F	Pred P(perm)
Shore	5.497	0.0165	5.633	0.0197	3.083	0.0800	2.820	0.0887
Season	5.708	0.0206	0.705	0.3990	5.216	0.0241	0.878	0.3504
Site (Shore)	1.827	0.1269	0.734	0.5819	2.438	0.0449	0.521	0.7264
Shore x Season	5.319	0.0207	0.769	0.3813	5.282	0.0219	0.270	0.6033
Zone [Site (Shore)]	6.212	0.0001	5.091	0.0001	4.166	0.0001	10.842	0.0001
Site (Shore) x Season	1.623	0.1652	5.498	0.0007	0.801	0.5300	1.082	0.3687
Zone [Site (Shore)] x Season	2.745	0.0025	2.451	0.0040	2.128	0.0165	2.331	0.0072

Pairwise tests

F/S

Shore x Season			t	P(perm)
	Winter			
		Buarcos, Matadouro	3.653	0.0005
	Buarcos			
		Summer, Winter	3.449	0.0009

Dep

Site (Shore) x Season			t	P(perm)
	Buarcos			
	Site 2			
		Summer, Winter	2.0202	0.0474
	Site 3			
		Summer, Winter	2.182	0.0346
	Matadouro			
	Summer			
		Sites 1, 3	2.984	0.0037
	Winter			
		Sites 1, 2	2.183	0.0360
		Sites 1, 3	3.353	0.0012
	Site 1			
		Summer, Winter	3.411	0.0018
	Site 3			
		Summer, Winter	2.908	0.0045

S/O

Shore x Season			t	P(perm)
	Winter			
		Buarcos, Matadouro	3.207	0.0019
	Buarcos			
		Summer, Winter	3.318	0.0014

Site (Shore)			t	P(perm)
	Matadouro			
		Sites 1, 2	2.184	0.0312

F. Tolerance to pollution

Main tests

	EG I Pseudo-F	EG I P(perm)	EG II Pseudo-F	EG II P(perm)	EG III Pseudo-F	EG III P(perm)	EG IV Pseudo-F	EG IV P(perm)	EG V Pseudo-F	EG V P(perm)
Shore	0.235	0.6188	7.478	0.0079	0.666	0.4182	9.567	0.0001	12.056	0.0001
Season	0.034	0.8513	1.782	0.1822	0.694	0.3988	6.184	0.0024	3.379	0.0656
Site (Shore)	19.747	0.0001	1.272	0.2787	18.056	0.0001	2.440	0.0261	5.511	0.0001
Shore x Season	5.698	0.0172	0.092	0.7592	5.279	0.0227	2.233	0.1449	1.240	0.2877
Zone [Site (Shore)]	8.739	0.0001	7.504	0.0001	8.143	0.0001	3.230	0.0006	4.197	0.0001
Site (Shore) x Season	2.249	0.0635	0.545	0.7058	2.521	0.0426	2.471	0.0203	4.409	0.0005
Zone [Site (Shore)] x Season	1.791	0.0507	2.287	0.0091	2.423	0.0060	3.549	0.0002	5.084	0.0001

Pairwise tests

EG I

Shore x Season			t	P(perm)
	Summer			
		Buarcos, Matadouro	2.018	0.0475

Site (Shore)			t	P(perm)
	Matadouro			
		Sites 1, 2	5.849	0.0001
		Sites 1, 3	13.777	0.0001
		Sites 2, 3	3.920	0.0001

EG III

Shore x Season			t	P(perm)
	Winter			
		Buarcos, Matadouro	2.259	0.0257

Site (Shore) x Season			t	P(perm)
	Buarcos			
	Winter			
		Sites 1, 2	2.306	0.0268
	Site 2			
		Summer, Winter	2.325	0.0238
	Matadouro			
	Summer			
		Sites 1, 2	4.610	0.0003
		Sites 1, 3	8.492	0.0001
	Winter			
		Sites 1, 2	3.183	0.0033
		Sites 1, 3	6.328	0.0001
		Sites 2, 3	4.191	0.0005

EG IV

Site (Shore) x Season			t	P(perm)
	Buarcos			
	Summer			
		Sites 1, 3	2.835	0.0018
	Winter			
		Sites 1, 3	2.294	0.0037
	Site 2			
		Summer, Winter	2.013	0.0017
	Site 3			
		Summer, Winter	3.686	0.0001
	Matadouro			
	Winter			
		Sites 1, 3	2.346	0.0027
		Sites 2, 3	2.108	0.0005
	Site 1			
		Summer, Winter	1.847	0.0326

EG V

Site (Shore) x Season			t	P(perm)
	Buarcos			
	Summer			
		Sites 1, 3	1.829	0.0294
		Sites 2, 3	1.824	0.0046
	Winter			
		Sites 1, 2	2.702	0.0045
		Sites 1, 3	3.049	0.0006
		Sites 2, 3	2.653	0.0011
	Site 1			
		Summer, Winter	2.580	0.0077
	Site 3			
		Summer, Winter	1.471	0.0202
	Matadouro			
	Summer			
		Sites 2, 3	2.219	0.0117
	Winter			
		Sites 1, 3	2.864	0.0001
		Sites 2, 3	2.784	0.0003
	Site 1			
		Summer, Winter	2.193	0.0119
	Site 3			
		Summer, Winter	5.296	0.0001

Supplementary material B6. *PERMANOVA results using Rao's quadratic entropy (RQE) average value, and using each biological trait RQE. For pairwise tests, significant comparisons are presented.*

Main tests

RQE

	Pseudo-F	P(perm)
Shore	1.570	0.2058
Season	3.188	0.0774
Site (Shore)	5.671	0.0002
Shore x Season	0.255	0.6100
Zone [Site (Shore)]	9.354	0.0001
Site (Shore) x Season	3.122	0.0159
Zone [Site (Shore)] x Season	2.865	0.0010

Maximum size

	Pseudo-F	P(perm)
Shore	0.162	0.6914
Season	2.332	0.1300
Site (Shore)	6.351	0.0001
Shore x Season	0.295	0.5841
Zone [Site (Shore)]	4.819	0.0001
Site (Shore) x Season	4.738	0.0010
Zone [Site (Shore)] x Season	2.323	0.0071

Longevity

	Pseudo-F	P(perm)
Shore	0.016	0.9015
Season	1.709	0.1864
Site (Shore)	5.615	0.0003
Shore x Season	0.073	0.7949
Zone [Site (Shore)]	5.894	0.0001
Site (Shore) x Season	1.961	0.1055
Zone [Site (Shore)] x Season	3.909	0.0001

Larval development mode

	Pseudo-F	P(perm)
Shore	16.654	0.0002
Season	9.638	0.0020
Site (Shore)	1.683	0.1517
Shore x Season	0.218	0.6443
Zone [Site (Shore)]	13.429	0.0001
Site (Shore) x Season	1.411	0.2244
Zone [Site (Shore)] x Season	2.921	0.0010

Pairwise tests

RQE — **Site (Shore) x Season**

		t	P(perm)
Buarcos			
Summer		t	P(perm)
	Sites 1, 3	3.747	0.0010
	Sites 2, 3	3.322	0.0022
Site 3			
	Summer, Winter	2.970	0.0050
Matadouro			
Summer		t	P(perm)
	Sites 1, 3	3.527	0.0011
Site 3			
	Summer, Winter	2.469	0.0176

Maximum size — **Site (Shore) x Season**

		t	P(perm)
Buarcos			
Summer		t	P(perm)
	Sites 1, 3	3.203	0.0031
	Sites 2, 3	2.050	0.0471
Winter			
	Sites 1, 2	2.100	0.0412
	Sites 2, 3	2.644	0.0118
Site 2			
	Summer, Winter	2.395	0.0224
Matadouro			
Summer		t	P(perm)
	Sites 1, 2	2.528	0.0161
	Sites 1, 3	4.3141	0.0002
Site 3			
	Summer, Winter	2.571	0.0150

Longevity — **Site (Shore)**

		t	P(perm)
Buarcos			
Summer		t	P(perm)
	Sites 1, 3	2.001	0.0486
	Sites 2, 3	3.031	0.0037
Matadouro			
Summer		t	P(perm)
	Sites 1, 3	3.834	0.0005
	Sites 2, 3	2.0119	0.0436

Main tests

Living habit

	Pseudo-F	P(perm)
Shore	0.125	0.7215
Season	0.553	0.4602
Site (Shore)	5.628	0.0002
Shore x Season	1.738	0.1870
Zone [Site (Shore)]	6.288	0.0001
Site (Shore) x Season	2.928	0.0215
Zone [Site (Shore)] x Season	1.290	0.2229

Feeding habit

	Pseudo-F	P(perm)
Shore	5.829	0.0179
Season	0.487	0.4810
Site (Shore)	6.700	0.0002
Shore x Season	1.142	0.2834
Zone [Site (Shore)]	6.276	0.0001
Site (Shore) x Season	4.552	0.0017
Zone [Site (Shore)] x Season	2.315	0.0084

Tolerance to pollution

	Pseudo-F	P(perm)
Shore	0.034	0.8602
Season	2.325	0.1291
Site (Shore)	4.540	0.0018
Shore x Season	0.003	0.9581
Zone [Site (Shore)]	11.266	0.0001
Site (Shore) x Season	0.714	0.5870
Zone [Site (Shore)] x Season	2.041	0.0232

Pairwise tests

Living habit — **Site (Shore) x Season**

		t	P(perm)
Buarcos			
Site 3		t	P(perm)
	Summer, Winter	2.530	0.0177
Matadouro			
Summer		t	P(perm)
	Sites 1, 3	5.048	0.0003
	Sites 2, 3	2.7993	0.0089
Winter			
	Sites 2, 3	2.386	0.0223
Site 1			
	Summer, Winter	2.1266	0.0412

Feeding habit — **Site (Shore) x Season**

		t	P(perm)
Buarcos			
Summer		t	P(perm)
	Sites 1, 2	2.053	0.0457
	Sites 1, 3	3.792	0.0005
Winter			
	Sites 1, 2	2.016	0.0462
Site 1			
	Summer, Winter	2.167	0.0331
Site 3			
	Summer, Winter	2.229	0.0341
Matadouro			
Summer		t	P(perm)
	1, 3	5.136	0.0001
	2, 3	2.6066	0.0151

Tolerance to pollution — **Site (Shore)**

		t	P(perm)
Buarcos		t	P(perm)
	Sites 1, 3	2.996	0.0040
	Sites 2, 3	4.346	0.0001