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Marine macroinvertebrates as indicators to assess the effects of Marine Protected Areas on temperate rocky reefs

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2021

Agradecimentos (PT)

Para a concretização deste trabalho, e face aos tempos difíceis que se passaram durante a sua realização, foi necessária a ajuda de algumas pessoas, e sem as quais nada teria sido possível. Como tal, não poderia deixar de expressar o meu profundo e sincero agradecimento a todas estas pessoas que de alguma forma contribuíram para esta tese, em especial:

À Doutora Sofia Henriques, pela supervisão desta tese e por me ter proporcionado todas as condições necessárias para o seu desenvolvimento, mas mais importante por todo o apoio e camaradagem ao longo deste último ano. Por me ter ensinado o que é ser um membro integrante de grupo de investigação, e por me ter ensinado a ser um “Homem” durante os dias agrestes das campanhas de amostragem.

À Doutora Marisa Batista, pela sua colaboração no desenvolvimento da tese, pelo apoio e camaradagem ao longo deste último ano; pelos conselhos e respostas rápidas às minhas dúvidas; pelas boleias de carro, e pelas generosas paragens para abastecimento de farinha torrada, durante as campanhas de amostragem.

Ao Doutor Miguel Pais, que embora não sendo oficialmente meu orientador, me ajudou como tal; pela sua colaboração no desenvolvimento da tese; por todo apoio e camaradagem ao longo deste último ano; pelos conselhos que me deu relativos a softwares e aplicações que eu desconhecia e que me facilitaram bastante o trabalho;

A todas as pessoas que nos ajudaram no laboratório e nas campanhas de amostragem, nomeadamente:

À Catarina e à Marta, pelo apoio fundamental que deram no laboratório, e nas identificações das espécies.

À Mariana pela ajuda que deu tanto nas identificações no laboratório como nos mergulhos das amostragens.

Aos “nossos” espanhóis, a Maria do ISPA que nos “safou” bastantes dias durante a primeira campanha de amostragem, e ao Carlos o meu *compañero* de invertebrados pela sua ajuda fundamental nos mergulhos da segunda campanha e no laboratório.

Ao Ronan, pela sua ajuda nos mergulhos da segunda campanha de amostragem; pelos chocolates que sempre trazia para partilhar no barco; pelo trabalho de equipa bem-sucedido na nossa primeira e única missão de mergulho de busca e recuperação da hélice do barco que se soltou; por todos os momentos divertidos que proporcionou, tanto durante a campanha como nos passeios a Sintra. *Thank you mate!*

A todos os meus amigos, que durante a fase de escrita e durante o confinamento, me ligaram para socializar, ajudando a ultrapassar esta fase difícil.

E por fim, à minha família, pelo amor e amizade incondicional; por todo o apoio e incentivo que me deram ao longo de toda a minha vida escolar e académica, principalmente aos meus pais pelo apoio moral e financeiro nesta fase da minha vida.

Os trabalhos desta tese foram financiados pelo projecto ReefNets "Using ecological networks to predict marine ecosystem responses to human threats" (PTDC/BIA-ECO/28687/2017), da Fundação para a Ciência e a Tecnologia (FCT).

Abstract

The increasing degradation of marine coastal habitats due to many types of anthropogenic pressures (e.g. overfishing, water pollution, underwater tourism, etc.), led governments and intergovernmental organizations to create directives and regulations for the protection of marine ecosystems (e.g. Marine Strategy Framework Directive) and establish Marine Protected Areas (MPA), which are now widely accepted as adequate tools to protect, maintain, and restore marine ecosystems. However, in order to well manage their effectiveness, periodic monitoring is required. Biological indicators are typically used to access the protection effect of a MPA. Nevertheless, for temperate marine subtidal rocky reefs, most of these indicators are based on fish and commercial invertebrates' traits, whereas for the overall invertebrates' assemblages, information is lacking. Therefore, the present study assessed the taxonomic and functional response of invertebrate assemblages to the protection effect of the Arrábida Marine Protected Area (Portugal), in order to define specific traits, useful to be used as biological indicators. The combined results of PERMANOVA and discriminant analyses showed an overall strong environmental gradient along the MPA, revealing an already expected situation of multiple stressors (natural and anthropogenic) influencing the local invertebrate communities. This environmental gradient is evident and seemed capable of weakening protection effects. Nevertheless, some potentially good biological indicators of protection effects were detected, namely "density of high value species", "density of bycatch species with high value", "densities of scavengers and omnivores" and "densities of grazers and herbivores" showed responsive trends to fishing pressure; Bryozoa and Gorgoniidae taxa showed responsive trends to diving pressure, even though no functional trait responded significantly to this pressure. Although most of these trends could not provide clear insights, this study improves the understanding of rocky reefs invertebrate assemblage's response to protection effect and their potential use as biological indicators of anthropogenic pressures, contributing to the future development of environmental assessment tools.

Key words: Marine invertebrates; functional traits; MPA assessment; temperate rocky reefs; biological indicators; anthropogenic pressures

Resumo

O aumento da degradação dos habitats marinhos costeiros, como consequência de diversos tipos de pressões antropogénicas (ex.: excesso de pesca, poluição da água, turismo subaquático, etc.), levou a que os governos e organizações intergovernamentais criassem diretivas e regulamentos para proteger os ecossistemas marinhos (ex.: Diretiva Quadro Estratégia Marinha) e estabelecessem Áreas Marinhas Protegidas (AMP), que são atualmente consideradas uma ferramenta adequada para a manutenção e recuperação de ecossistemas marinhos. Contudo, para uma gestão eficiente, são necessárias monitorizações periódicas, nas quais são frequentemente utilizados indicadores biológicos, que suportem a avaliação do efeito de proteção de uma AMP. No entanto, para os recifes rochosos de zonas temperadas, a maior parte destes indicadores são atualmente baseados em grupos funcionais de peixes e invertebrados com valor comercial, enquanto que os restantes grupos de invertebrados são pouco representados devido à falta de informações. Por isso, neste estudo foi avaliada a resposta destes grupos ao efeito de proteção da Área Marítima Protegida da Arrábida (Portugal), de modo a definir grupos funcionais específicos, úteis como indicadores biológicos. Os resultados da PERMANOVA e das análises discriminantes mostraram um forte gradiente ambiental ao longo da AMP, revelando uma situação já esperada de vários fatores de stress (naturais e antropogénicos) que influenciam as comunidades de invertebrados locais. Este gradiente ambiental tornou difícil a identificação clara de eventuais efeitos da proteção nas comunidades de invertebrados. No entanto, foram detetadas algumas tendências que sinalizam potenciais indicadores biológicos, nomeadamente a “densidade de espécies com alto valor comercial”, a “densidade de espécies acessórias com elevado valor comercial” , as “densidades de necrófagos e omnívoros”, e ainda as “densidades de raspadores e herbívoros” revelaram tendências de resposta à pressão de pesca, e apesar de nenhum grupo funcional ter respondido significativamente à pressão da atividade de mergulho recreativo, os taxa Bryozoa e Gorgoniidae mostraram capacidade de resposta a esta pressão. Embora, a maioria destas tendências sejam fracas e não providenciem uma resposta clara, (pelo que estudos adicionais sobre a sensibilidade destes indicadores são necessários), este estudo contribui para uma melhor compreensão da resposta das comunidades de invertebrados de recifes rochosos ao efeito de proteção, e o seu potencial uso como indicador biológico dos efeitos antropogénicos, contribuindo para o futuro desenvolvimento de ferramentas de avaliação ambiental.

Palavras-chave: Invertebrados marinhos; grupos funcionais; efeito de proteção; recifes rochosos temperados; indicadores biológicos; pressões antropogénicas.

Resumo alargado

Os ecossistemas marinhos costeiros estão entre os locais mais produtivos e diversos do planeta. No entanto, ao longo das últimas décadas do século passado, a industrialização das regiões costeiras e a má gestão dos recursos marinhos, consequências do elevado crescimento populacional, têm levado ao crescente impacto sobre estes ecossistemas. Isto originou problemas como a exploração excessiva de recursos, poluição, introdução de espécies invasoras, que degradam habitats costeiros, afetando não só os seres vivos residentes a um nível individual, mas também as complexas relações tróficas a que estes pertencem. De modo a minimizar e reverter tais impactos, os governos e organizações intergovernamentais criaram regulamentos e diretivas para proteger os ecossistemas marinhos (ex.: Diretiva Quadro Estratégia Marinha) e adotaram medidas de conservação (ex.: estabelecimento de Áreas Marinhas Protegidas). Atualmente, vários estudos já demonstraram a capacidade de recuperação de ecossistemas em AMPs. Contudo, para uma boa gestão da sua eficácia, são necessárias monitorizações periódicas do seu efeito de proteção, e para tal são frequentemente utilizados indicadores biológicos. Nas últimas décadas foram desenvolvidos vários indicadores para avaliar o estado dos ecossistemas aquáticos (e.g. estuários, rios), e mais recentemente para ao meio marinho (fundo de areia e rochoso), no entanto a maioria dos indicadores biológicos desenvolvidos para o meio marinho foram baseados em grupos funcionais de peixes, enquanto que as comunidades de invertebrados estão normalmente pouco representadas (maioritariamente invertebrados de interesse comercial). Embora já existam estudos de indicadores baseados em espécies de invertebrados marinhos em zonas intertidais e zonas subtidais arenosas, capazes de detectar distúrbios nestes habitats, poucos estudos o fazem para espécies de invertebrados no subtidal rochoso, e menos ainda utilizam grupos funcionais destas espécies, possivelmente devido à falta de informações para várias espécies.

O presente estudo teve como principal objetivo avaliar a resposta taxonómica e funcional de grupos de invertebrados marinhos associados a recifes subtidais rochosos, ao efeito de proteção da Área Marinha Protegida da Arrábida (Portugal), de modo a definir grupos funcionais específicos que possam ser úteis como indicadores biológicos.

A AMP da Arrábida foi implementada entre 2005 e 2009, contando por isso com mais de uma década de proteção. No entanto, a pouca disponibilidade de dados biológicos de pré-implementação (nomeadamente para invertebrados), torna difícil a avaliação do efeito de proteção da AMP. De modo a contornar este problema, o presente estudo recorreu à comparação de resultados entre zonas sob 3 níveis diferentes de proteção da AMP, nomeadamente: zona de proteção complementar (ZPC), zona de proteção parcial (ZPP) e zona de proteção total (ZPT), que funcionou como controlo. Por outro lado, as variáveis ambientais e as características do habitat, podem ter uma forte influência nas comunidades de invertebrados, criando situações em que múltiplos fatores de stress podem enfraquecer e mascarar potenciais efeitos de proteção. Assim, neste estudo, foram ainda recolhidos e posteriormente incluídos nas análises, variáveis ambientais com maior potencial para influenciar as comunidades de invertebrados. As amostragens decorreram em dois outonos (2019 e 2020), tendo sido recolhidas as abundâncias de invertebrados (<1cm) em 270 quadrats de 50x50cm (90 por zona de proteção) e 54 transectos (18 por zona de proteção), com duas passagens: uma passagem para espécies pelágicas (25x4m) e outra para espécies crípticas (25x1m). Os resultados obtidos foram analisados com recurso a análises multivariadas, nomeadamente PERMANOVA e análises discriminantes, nomeadamente análise de coordenadas principais (PCO) e análise canónica de coordenadas principais (CAP). Adicionalmente, uma análise SIMPER foi realizada para identificar os taxa que mais contribuem para a estas diferenças entre zonas de proteção.

Os resultados da abordagem taxonómica mostraram uma maior separação entre a zona de proteção complementar em relação às zonas mais protegidas (parcial e total) que, embora semelhantes, apresentaram também pequenas diferenças entre si. Por outro lado, os resultados dos grupos funcionais mostraram uma separação distinta entre as zonas de proteção complementar e a total, enquanto a parcial revelou pouca distinção com ambas as zonas, sugerindo a existência de um gradiente ao longo da AMP. De facto, as análises focadas nas variáveis ambientais revelaram um já esperado forte gradiente natural que dominou os padrões observados relativamente ao fator proteção, sendo provavelmente responsável pelos padrões mais fortes detetados (ex.: densidade de espécies solitárias vs. coloniais). No entanto, também foram detetadas algumas tendências possivelmente ligadas ao efeito de proteção da AMP, revelando potenciais bons indicadores biológicos. A "densidade de espécies com elevado valor comercial", a "densidade de espécies acessórias com elevado valor comercial", as "densidades de necrófagos e omnívoros", e ainda as "densidades de raspadores e herbívoros" revelaram tendências de resposta à pressão de pesca, e apesar de nenhum grupo funcional ter respondido significativamente à pressão da actividade de mergulho recreativo, os taxa Bryozoa e Gorgoniidae mostraram capacidade de resposta a esta pressão. Os grupos ecológicos AMBI, sugeriram que a qualidade da água é boa em todo o parque, no entanto existe uma possibilidade remota dos emissários de Sesimbra terem alguma influência na zona de proteção parcial.

A maioria das tendências identificadas parecem levantar mais hipóteses do que dar respostas claras, sendo por isso necessário realizar mais estudos sobre a sensibilidade destes indicadores. Nomeadamente estudos sobre a resposta funcional dos invertebrados que revelaram uma potencial resposta ao efeito de proteção (ex.: estudos de impacto-resposta nos grupos de invertebrados com interesse comercial, ou espécies de invertebrados raspadores, nomeadamente ouriços). Além disso, mais estudos deveriam ser feitos para reduzir lacunas de conhecimento acerca de alguns grupos funcionais de invertebrados. Adicionalmente, um estudo baseado nas biomassas dos grupos funcionais deveria ser feito para verificar se estas tendências se mantinham, no entanto, a falta de curvas tamanho-peso para algumas espécies de invertebrados dificulta o estudo, visto que a alternativa seria a recolha de espécies para o cálculo de biomassa. Por fim, tendo em conta que este estudo foi baseado na comparação entre zonas de proteção, fatores como o tamanho da AMP em relação à distribuição natural das espécies e a proibição de algumas atividades humanas em toda a AMP, que podem influenciar os padrões de efeito de proteção, não foram avaliados.

No entanto, este estudo dá um passo no sentido de uma melhor compreensão da resposta das comunidades de invertebrados de recifes rochosos ao efeito de proteção, e o seu potencial uso como indicador biológico de efeitos antropogénicos, contribuindo para o futuro desenvolvimento de novas ferramentas de avaliação ambiental, baseadas em grupos funcionais de invertebrados, que poderão ser utilizadas não só na gestão de AMP como também cumprir os requisitos de diretivas internacionais (ex.: Diretiva Quadro Estratégia Marinha) permitindo o estabelecimento de medidas de conservação que tenham em conta as comunidades de invertebrados.

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General Introduction

Marine ecosystems are some of the most productive and diverse of all ecosystems on the planet. Covering 71% of the Earth's surface and containing 90% of the biosphere (EU, 2005), marine ecosystems, a source of goods (e.g. food and raw material) and services (*i.e* several ecosystem processes that are essential to the proper functioning of the Earth), such as climatic regulation, absorption of the carbon dioxide, nutrient cycling, prevention of the erosion and maintenance of the biological control (Barbier, 2017; Beaumont et al., 2007; Costanza et al., 1997). In addition, marine ecosystems also provide cultural services such as leisure and recreational activities, which contribute to economic prosperity, social well-being and quality of life. However, the increasing population growth caused increasing anthropogenic pressures due to overfishing, eutrophication, introduction of invasive species, water pollution and habitat loss, which allied to the effects of climate change, have significantly contributed to biodiversity loss and degradation of marine ecosystems (Crain et al., 2009; Halpern, 2003; Sala et al., 2012). Marine coastal zones are some of the most impacted areas by these anthropogenic pressures, as although they only make up 4% of the earth's total land area and 11% of the world's oceans, they contain more than a third of the world's population and account for 90% of the catch from marine fisheries (Barbier, 2017).

In an effort to conserve and protect the marine environment, several national and international initiatives and policies have arisen. The most remarkable of such policies are, at a global level, the Convention on Biological Diversity (CBD) and, at a European level, the Convention for the protection of the marine environment of the North-East Atlantic ("OSPAR Convention"), the Birds and Habitats Directives (i.e. Natura 2000 network) and the Marine Strategy Framework Directive (MSFD). The latter establishes a framework of measures to achieve and maintain a good environmental status of the marine environment, being one of those measures the establishment of marine protected areas (MPAs). MPAs have been established for a wide range of purposes, but the main objectives include to: preserve and restore marine habitats, prevent and reverse the widespread declines in biodiversity and exploited marine populations, maintain ecosystem services, restore fisheries stocks, manage other economic activities, and minimize conflicts among resource users and decrease poverty (Abdulla et al., 2009; Gerber et al., 2003). However to efficiently achieve their objectives MPAs needs to have an adequate design associated with specific objectives (Batista et al., 2015; Claudet et al., 2008; Halpern, 2003; McLeod et al., 2009) and also an adequate management, based on periodic monitoring, that allows the identification and correction of major gaps in the implemented regulations and design. Include local stakeholders within the process and provide adequate funding and enforcement since the beginning is also mandatory for MPA success (Batista et al., 2015; Claudet et al., 2008; McCarthy and Possingham, 2007). When efficiently managed MPAs have shown overall positive results, such as general increases in density, biomass, individual size, and diversity in all functional groups within its limits, thus often contributing to the increase in fish stocks through spillover effects (Gell and Roberts, 2003; Halpern, 2003; Lester et al., 2009).

As referred above, periodic monitoring is essential to evaluate MPAs performance. In fact, established adequate monitoring is of the utmost importance for overall marine ecosystems as recognized, for instance, by the MSFD, which sets several indicators aiming to guide the progress towards achieving good environmental status of the marine environment (see annex III in Directive 2008/56/CE). Those include several biological features such as plankton, algae, macroinvertebrates, fish, marine mammals, reptiles and seabirds, together with physical, chemical and habitat features. Naturally occurring ecological indicators are commonly used to monitor and assess the conservational effects of MPAs, since they are also an important tool for detecting changes in the biological communities due to environmental and anthropogenic pressures, either positive or negative, and their subsequent effects on human society (Parmar et al., 2016). Initially, studies to find biological organisms able to respond to anthropogenic pressures were focused on taxonomic-based approaches. Many taxa of marine living creatures have shown to be good biological indicators, for instance, some algae and mussels species are known to be excellent indicators for marine pollutants (Kureishy et al., 1995; Ostapczuk et al., 1997), as well as some fish species (Azzurro et al., 2010; Chen, 2002; Chovanec et al., 2003). Nevertheless, since some species are not ecologically identical, and considering the inevitable presence of biotic and abiotic interactions in the marine environment, the complexity of anthropogenic-induced changes cannot be solely as species differences in terms of tolerance to disturbance, as the stress-response relationships are far from be unimodal (Hughes et al., 2005; Mouillot et al., 2013). Thereby the studies gradually became more ecological, species traits approaches started to be used to assess the functional structure of communities, as they respond rapidly and consistently across taxa and ecosystems to multiple disturbances (Mouillot et al., 2013). For instance, metrics based on fish assemblages in coastal temperate reefs proved to be good ecological indicators for anthropogenic pressures, such as overharvesting, pollution and habitat degradation (Henriques et al., 2014, 2013b, 2013a). Since these trait-based functional approaches are able to detect multiple disturbances, are more resilient to natural variation and respond more predictably to stress (Elliott et al., 2007; Henriques et al., 2013b; Pais et al., 2012), they are very useful biological indicators in the assessment of MPA effectiveness. Furthermore, since traits represent functional groups rather than specific species, they can be applied in MPAs from different regions (Elliott et al., 2007; Henriques et al., 2013b).

In fact, one way to assess the protection effect of an MPA is by comparing present assemblage data, with data from before its implementation, since by doing this we are able to perceive shifting patterns on local communities over time due to the MPA's presence. However, pre-implementation data is rare (Batista et al., 2015), therefore, most studies on MPA's protection effect use a control-effect design where the control sites are inside the reserve/ no-take zone (the most intrinsic zone), whereas the “impacted” sites are either sites outside the MPA or sites inside the MPA, but in lower protection regimes (e.g. buffer zones). Nevertheless, it is important to note that misleading estimates of the effect of protection may arise when control-effect designs do not consider intrinsic habitat or environmental variability, which may vary among nearby sites. Therefore, a complete understanding of the local environmental variables is imperative, in order to separate the influences of environmental features (both spatial and temporal variability) from other sources of variation, such as the effect of protection measures (García-Charton and Pérez-Ruzafa, 2001; Pais et al., 2013). This can be achieved by collecting data from the environment variables with greater potential to influence the local communities and later include them in the analyses (García-Charton and Pérez-Ruzafa, 2001).

As already mentioned, fish assemblages in coastal temperate reefs provide good metric-based ecological indicators. On the other hand, non-commercial marine invertebrate assemblages are rarely used as environmental indicators, but their higher site-attachment and sessile life-cycle of many organisms make them promising indicators due to potentially higher exposure times to impact sources. Therefore, the present dissertation aims to assess the taxonomic and functional response of marine invertebrate communities associated with rocky subtidal reefs to protection effects, in order to identify functional groups that may be useful as ecological indicators.

This study is presented in a scientific article that will be submitted to an international journal quoted in the “Science Citation Index”.

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1. Introduction

In the last few decades, marine coastal habitats like temperate rocky reefs are subject to many threats as a result of different anthropogenic activities in both, terrestrial and maritime domains. The continuous increase of coastal human populations promotes industrialization of human activities in the region and increases the exploitation of local marine resources (Crain et al., 2009; Halpern et al., 2008). This causes issues such as resource overharvesting, pollution or the settlement of invasive species. The direct and cumulative impacts caused by anthropogenic pressures can lead to homogenization of ecosystems due to reductions in food-web complexity, biogenic habitat structure, diversity within functional groups, distribution range, and size of organisms (Batista et al., 2014; Claudet and Fraschetti, 2010). Therefore, it is very important to identify sensitive community components to monitor these communities in both local (e.g. Marine Protected Areas (MPAs)) and regional (e.g. Marine Strategy Framework Directive (MSFD)) contexts.

Temperate rocky reef ecosystems are affected by the intense exploitation of commercial species, pollution, introduced species and impacts of climate change (Sala et al., 2012). In fact, overharvesting mostly associated with fishing activities, is one of the worst threats to the structure and diversity of marine rocky reefs ecosystems, due to its capability to reduce living resources, both target and by-catch species, which can have serious effects on trophic webs, namely when species caught play a keystone role in the ecosystem (Crain et al., 2009; Henriques et al., 2013b). Another great threat to these ecosystems is marine pollution that can be of many different kinds, such as agricultural waste (e.g. fertilizers, pesticides and agrochemicals), domestic and municipal wastes and sewage sludge (e.g. pathogens, organic compounds, plastics, trace elements and heavy metals), shipping (e.g. oil spills) aquaculture (e.g. organic compound), etc. (see Islam and Tanaka, 2004 for more details). Many of these pollutants are a major issue to marine ecosystems, not only because they can negatively affect the survival and growth of marine organisms by causing deformations, cancers and reproductive failure due to their interference in metabolic processes, but also because they accumulate and biomagnify through the trophic web, getting more concentrated at higher trophic levels (Crain et al., 2009; Islam and Tanaka, 2004). Invasive species are also a growing problem to rocky reefs due to the many human activities such as shipping, fisheries and aquaculture. Even though only a small percentage of the introduced species can survive and invade new habitats outside of their native range, their impacts can be as severe as displacing native species, changing community structure and trophic webs, and even change fundamental natural processes (e.g. nutrient cycling) (Crain et al., 2009; Molnar et al., 2008). In most current studies, the several impacts of global climate change are also being considered (Crain et al., 2009; Russell et al., 2009). Global stressors such as the increase of ocean temperatures, ocean acidification, sea-level rise and UV exposure have been shown capable of impact marine systems from the individual level (e.g. changes in morphology, physiology, and behaviour), to population level (e.g. changes in dispersal and recruitment processes, and shifts in biogeographic distributions), and to community level (e.g. changes in species interaction) (Crain et al., 2009; Harley et al., 2006). It is also important to mention the drastic synergistic effects that result from the combination of global stressors with a minor local stressor, for instance, the combination of global CO_2 rise and local nutrients perturbations has the potential to accelerate the currently change of temperate reefs from perennial canopies of kelp and their associated understorey to mats of turf which inhibit kelp (Russell et al., 2009).

In order to minimize and when possible reverse the current increasing degradation of marine rocky reef ecosystems, it is imperative to classify these ecosystem vulnerabilities and key threats, with the purpose of prioritize conservation efforts and direct management measures to reduce these impacts (Crain et al., 2009; Henriques et al., 2013b). In this context, numerous Marine Protected Areas (MPAs) were established in the last decades as tools to monitor and protect marine biodiversity from several anthropogenic pressures. In order to maximize their efficiency and protect ecosystems resilience, MPAs should follow certain recommendations regarding their size, shape, connectivity, location, management practices, among others, which are usually strongly associated with their specific objectives and characteristics of each MPA (see McLeod et al., 2009 for details). For instance, one way to increase the MPAs efficiency is through adaptive management (i.e. a structured, iterative process of robust decision making, that allows managers to identify the major gaps regarding the reserve design, and implement management measures, mostly based in adequate monitoring, correcting them if necessary over time, in order to the reserve successfully achieve the proposed goals), based in efficient monitoring that usually includes local stakeholder within the process. These also contribute to minimize future conflicts and consequently enables the MPA to better fulfil its goals (Batista et al., 2015; Claudet et al., 2008; McCarthy and Possingham, 2007).

Marine Protected Areas can have different purposes, namely protecting endangered species, pristine areas and other areas of ecological importance (e.g. nursery areas). MPAs can also help ensure a sustainable provision of multiple ecosystem services that are fundamental for human well-being, such as food source (through fishing activities) and leisure (e.g. tourism activities). For that, most MPA's use a zonation of two or three levels: one central (no take) zone where all uses and human activities are generally prohibited, except for management interventions; one intermediate (buffer) zone where certain uses are forbidden or are subject to limitations; and a peripheral zone, that when exists, the regulation of activities there is not very restrictive, unzoned MPA's are mostly integral reserves (no take areas) whose purpose is only conservational (Francour et al., 2001). No take zones (or marine reserves) over time can become control areas for the evaluation of population and ecosystem effects caused by anthropogenic impacts on the marine environment. By comparing data from before the establishment of the MPA with data from monitoring studies after the MPA implementation, it is possible to understand the sources of ecological variability at different scales, and better perceive the protection effect of the MPA (Horta e Costa et al., 2013b; Pais et al., 2013a).

Many effects of protection of rocky reefs MPA's are already known, such as the increases in diversity, biomass, density and individual size in all functional groups (Gell and Roberts, 2003; Halpern, 2003; Lester et al., 2009) inside the MPA's, and sometimes spillover to the nearby areas. For instance, top predators like groupers, which experienced dramatic population declines in the Mediterranean due to overfishing, had their individual size and biomass increased in MPA's and surrounding areas (Hackradt et al., 2014). It is also important to take into account the trophic cascade effect (i.e. predatory interactions involving at least three trophic levels, whereby primary carnivores, by suppressing herbivores, increase producers' abundance) that results from MPA's protection effect. For instance the recovery of predators' populations like *Diplodus* spp. reduces the abundance of the grazer sea urchins, which in their turn, increases the abundance of erect macroalgae (Guidetti, 2006). Several studies have shown these protection effects in species with commercial value (e.g. Guidetti, 2006; Hackradt et al., 2014; Pederson and Johnson, 2006 among others). Comparatively, studies on non-target species are limited and much less focused on whole communities, often focusing only on specific groups of species such as algae, molluscs or fish rather than on biodiversity at the whole community level, probably due to the inherent difficulty to assign causality to changes in diversity and identity of species (Villamor and Becerro, 2012).

Besides anthropogenic impacts, natural variability (e.g. climate, hydrodynamics, etc.) and reefs structural complexity can strongly influence marine communities. In fact, structural complexity of rocky reefs can be a key factor that shapes these communities (Rees et al., 2014; Trebilco et al., 2015). For instance, abiotic factors as physical structure, water currents and luminosity are especially important to demersal and benthic communities (e.g. invertebrates); however, it also makes measuring anthropogenic impacts more difficult, since it adds more variables capable of influencing the structure of these communities. Therefore, these variables must be completely understood and controlled in order to detect the changes due to anthropogenic pressures (Pais et al., 2013a), as well as, several specific indicators are needed to detect and provide us with information on the anthropogenic changes that we intend to evaluate.

Marine benthic invertebrate communities have relatively low mobility (and so cannot avoid pollutants in the water and sediments) besides they have relatively long life spans (thus allowing to integrate this pollutants with time), and consist of several different species (thus having different tolerances to stress) (Borja et al., 2000). Therefore, they are often used as ecological indicators in marine coastal ecosystem, such as soft-bottom habitats (e.g. Borja et al., 2000; Muniz et al., 2005), and rocky reef intertidal (Smith, 2005; Vinagre et al., 2016), showing a high capability to detect anthropogenic alterations in natural system (e.g. engineering works, sewerage plans and the dumping of polluted waters, organic enrichment) and providing a more accurate view of the evolution of the ecological status in particular locations. However, there is a considerable knowledge gap regarding the effects of MPAs on rocky reef subtidal invertebrate communities, and the potential of these communities as indicators of anthropogenic impacts.

Therefore, based on subtidal rocky reef invertebrates assemblages representing the three different levels of protection (no-take, partial and complementary protection) of Arrábida MPA, which have habitats with many different levels of exposure and structural complexity (therefore ideal for studying which components of benthic communities best help to distinguish anthropogenic impacts from impacts of natural variability), the purpose of this study is to assess the taxonomic and functional response of these assemblages to the protection in order to define specific metrics, useful to be used as indicators of MPA effects.

2. Material and Methods

2.1 Study area

The Professor Luiz Saldanha Marine Park is the marine area of Arrábida Natural Park, located in the central region of Portugal (hereafter referred as Arrábida MPA). It was created in 1998 by DR. Nº 23/98 of 14 October, and has an area of approximately 53 km^2, corresponding to 38 km of rocky coast between Figueirinha beach, at the exit from the Sado estuary and Foz beach, at north of Espichel Cape (Fig. 2.1). However, the management plan was only published in 2005 (Portuguese legislation, Council of Ministers Resolution 141/2005) and the final regulations, namely total no-take area, entered into force four years later (regulations were gradually implemented for fishing activities between 2005 and 2009). In this MPA three types of protection zones were established: one no take zone (NTZ) covering 4 km^2, where no human activities are allowed (except scientific research); four partial protection zones (PPZ) covering 21 km^2, where fishing with some specific gears (octopus traps, jigging, handlines) are allowed if farther than 200 m from shore line; and three complementary protection zones (CPZ) covering 28 km^2, where fishing activities with traps, gill and trammel nets, jigging, longlines and handlines are allowed, following fisheries general regulations, whereas nets are permitted only farther than 1/4 Nm from shore line. Besides that, only vessels under 7 m length are allowed to fish, and trawling, dredging, purse seining and hand harvesting are forbidden in the whole MPA. Finally, recreational angling is only permitted in the CPZ while spearfishing is forbidden in the entire MPA.

Regarding the geography, this MPA faces south, therefore is protected from the prevailing north and northwest winds by the adjacent mountain chain of Arrábida. The rocky coast is in general very steep and the intertidal zone includes mainly rocky cliffs, small beaches and several areas covered by boulders (Gonçalves et al., 2002b). The subtidal zone is mainly rocky and structurally complex, with large boulders resulting from the erosion of calcareous cliffs that border the coastline (Gonçalves et al., 2002b; Henriques et al., 2013b). The nearshore rocky subtidal extends for some tens of meters usually to depths of less than 15 m, from where sandy bottom habitats begin to dominate (except at the Espichel Cape area where rocky habitats reach more than 40m in depths). This MPA has very particular characteristics that contribute to an unusually high biodiversity, for instance is near the northern limit of the main north-east Atlantic upwelling events (Wooster et al., 1976), which means that, during the summer, water temperature nearshore is frequently lower than offshore waters at the same latitude, but also more rich in nutrients. The Sado estuary is another factor that can influence the water quality of eastern MPA zones', because, despites having a relatively low annual flow rate, 5 $m^3.s^{-1}$ (Brogueira et al., 1994) to 19 $m^3.s^{-1}$ (Gonçalves et al., 2002a), its proximity to the study area is sufficient to give a strong influence on local tidal currents and the chemical composition of water (Brogueira et al., 1994). It is also relevant to mention that there are two active wastewater treatment plant (WWTP) emissaries from Sesimbra village located in California beach area: main one (38º 25,745 'N 009º 06,978' W) and a secondary one (38º 25,780 'N 009º 06,941' W) that may have a minor local influence in the water quality (Rodrigues, 2008).

2.2 Sampling methods

In the present study, a total of nine sampling sites were selected (three per protection level) as shown in figure 2.1. Sites were selected in order to represent structurally similar habitats between 5 and 10 m depth. Three replicates per site were performed for each biological group in two autumns (2019 and 2020). Autumn was the chosen season for the sampling as it is included in the warm sea conditions after the spawning period (July–November), that was shown to probably give better assemblage results (Henriques et al., 2013b).

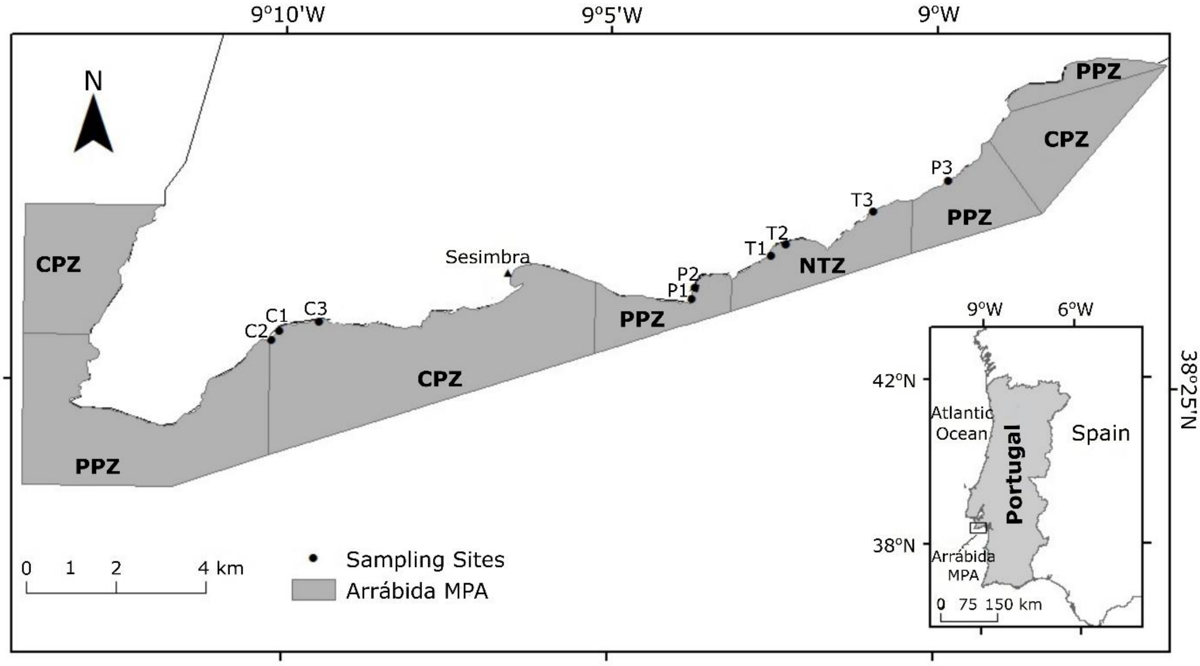

Figure 2.1- Map of the Arrábida Marine Protected Area (Portugal), showing the different protection zones: CPZ — Complementary Protection Zones, PPZ — Partial Protection Zones, and NTZ — No-take Zone. Sampling sites locations are shown by the black squares (C1, C2, C3; P1, P2, P3; T1, T2, T3).

2.2.1 Invertebrates Sampling

Benthic and demersal species of invertebrates (>1cm) were sampled using two different underwater visual census methods depending on species mobility and size: quadrats were used for small and low mobile species, while large and high mobile species were sampled using strip transects. In both methods, species were identified to the lowest level possible and their size and abundance estimated in order to characterize the invertebrate community associated with rocky reefs. Within each site, three replicates of five quadrats of 50 x 50 cm were placed randomly over the rocky reefs. A total of 135 quadrats (45 per protection level) were sampled per season, making a total of 270 quadrats. Each sampling team consisted of two divers, where one diver was responsible for measuring and registering the invertebrate fauna (>1cm) observed and the other diver was responsible for assisting with the species identification using an underwater species pictures guide (developed by the team), and taking photos of the quadrats for further estimations of algae cover. In addition, a few specimens were collected for future identification at the laboratory (only if photo-identification was considered not enough). Specimens collected were frozen at -18°C with seawater until identification. All invertebrates were identified to the species or genus level with exception of Porifera and Bryozoa species that were not identified to a lower level given the difficulty of underwater identification. Some small-scale environmental variables were also registered for each quadrat in order to further assess if the differences found could be due to quadrat sampling variability. For high mobility and larger species, data was obtained through strip transects travelled twice: one passage for pelagic/demersal species (25 x 4m) and another for cryptic species (25 x 1m). Three replicates per site were performed in a total of 27 transects per season. Abundance and size of predatory fish was also sampled during the transects to estimate the predators' biomass.

With the purpose of characterizing functional and structural changes in the invertebrates communities caused by MPA protection, all invertebrate taxa were classified according to their functional traits, based on available literature and online databases such as SeaLifeBase and BIOTIC (Table 2.1; Table A1). These traits were previously selected from a list of candidate traits compiled from an extensive review of existing studies about fish and marine invertebrates response to anthropogenic pressures (Bremner et al., 2006; Costello et al., 2015; Degen and Faulwetter, 2019; Leitão et al., 2020; Tyler et al., 2012). The final list was reduced by removing not only those traits lacking information for many species, but also redundant traits based on Pearson's correlations (i.e. $r \geq |0,90|$; Fig. B1).

2.2.2 Habitat characterization

A total of 19 environmental variables and five anthropogenic pressure were defined based in previous published studies (Alexander et al., 2009; Batista et al., 2015; Horta e Costa et al., 2013b; Pais et al., 2013b; Ruitton et al., 2000; among others) in order to characterize the rocky reef habitat and assess natural effects among different protected areas on the invertebrate assemblages. These variables that shown potential to influence the structure and function of invertebrate communities on rocky temperate reefs, and were grouped in two different dimensional scales: large-scale - those that influence and characterize habitats at a site or protection level; small-scale - include those that characterize microhabitats at a quadrat level (Table 2.2). Redundant variables based on Pearson´s correlations (i.e. $r \geq |0,90|$) were removed from the analysis.

Table 2.1- List of selected functional traits: maximum body size, feeding habits, diet, adult habitat, adult movement, sociability, sexual differentiation, resilience, and commercial value. Trait' categories were used as candidate indicators to characterize the invertebrate assemblage response to anthropogenic pressures. Invertebrate taxa were classified based on available literature and online database (Table A1). (*) Trait categories removed from the analysis because of redundancy. (**) Trait categories with no taxa represented in the assemblage.

Trait	Trait categories	Abbreviation	Description	Relevance
Maximum Body Size	Very small species	XS	<1cm	One of the most fundamental traits as it correlates with other traits, for example, enabling conversion of length and abundance to biomass. Reflects the position of the species in the food web, species abundance and metabolic rates and response to disturbance (Costello et al., 2015; Leitão et al., 2020; Tyler et al., 2012)
	Small species	S	1-5cm	
	Small-medium species	SM	5-10cm	
	Medium species*	M	10-15cm	
	Medium-large species	ML	15-30cm	
	Large species*	L	30-50cm	
	Very large species	XL	>50cm	
Feeding habits	Grazers	Graz	An organism that feeds by active removal of organic material from the substratum surface. Includes species, which scrape and/or graze algal matter from surfaces	Influences inter-specific interactions, nutrient and energy cycling (affecting the depth of oxygen and detritus penetration and can enhance organic matter decomposition and nutrient recycling/regeneration) and predicts response to disturbance. Can indicate hydrodynamic conditions (suspension feeders in turbulent, deposit feeders in calmer water). Impacts resource utilization and facilitation (e.g., deposit feeders facilitate microbes that further decompose organic carbon) (Degen and Faulwetter, 2019; Tyler et al., 2012)
	Predators*	Pred	An organism that feeds by preying on other organisms.	
	Deposit-feeders	DepFeed	An organism that feeds on detritus that have settled on the bottom	
	Filter/suspension-feeders *	FiltFeed	An organism that feeds by straing suspended matter and food particles from water	
	Scavengers/opportunistic	Scv	An organism that can use different types of food sources	
	Parasites**	Para	An organism that lives in or on another living organism (the host), from which it obtains food and other requirements.	
Diet	Omnivores*	Omn	Feeds on detritus, macroalgae, and epi/infauna	Determines trophic structure, energy flow and nutrient cycling within the assemblages (Tyler et al., 2012)
	Herbivores*	Herb	Feeds on macroalgae	
	Macrocarnivores	Ma_carn	Feeds predominantly on macro invertebrates and fish	
	Microcarnivores	Mi_carn	Feeds predominantly on micro invertebrates	
	Detritivores	Detri	Feeds predominantly on detritus	
	Planktonivores	Plank	Feeds on plankton	
	Sessile invertebrate feeders	Sess_inv_feed	Feeds on sessile invertebrates (including colonial species)	

Table 2.1 (continued)

Trait	Trait categories	Abbreviation	Description	Relevance
Adult life habit	Free living	Free	Adults are able to move freely within and/or on the sediments	Attached species are more vulnerable to predation and perturbations. Burrowing, crevice and tube dwelling taxa affect sediment biogeochemistry, carbon transport and elemental cycling and are less affected by strong hydrodynamic disturbance, anoxic conditions and water pollution. Tube building can add to local storage of chemicals and waste materials. Microbial processes are facilitated, and microbial biomass is promoted by deep-dwelling fauna. Burrowing and irrigation generally facilitate life of associations. Burrowing or attached living can be related to habitat creation and facilitation. (Degen and Faulwetter, 2019)
	Crevice dweller	Crev	Adults are typically cryptic, inhabiting spaces made available by coarse/rock substrate and/or biogenic species or algae holdfasts.	
	Tube dweller	Tube	Adults live inside and can withdraw into tubes	
	Burrow dweller **	Burr_Dw	Adults inhabiting permanent or temporary burrows in the sediment or are just burrowing in the sediment	
	Epi-/endozoic or epi-/endophytic	Epi_Endo	Adults living on or in other organisms	
	Attached*	Attach	Adults are adherent to a substratum	
Adult movement	Sessile	Sessile	No movement as adult	Indicates the dispersal and recolonization potential and the invasiveness of an organism. Related to nutrient cycling (burrowing taxa contribute most to nutrient cycling and regeneration; burrows increase the total sediment surface area available for exchange with the water column), carbon deposition (sessile calcifying taxa), facilitation of microbial and other fauna (either via burrowing or via constructing biogenic habitats) and habitat stability. Swimmers may escape predators and local disturbances (Degen and Faulwetter, 2019)
	Swimmers	Swim	Movement above the substratum	
	Crawlers	Crawl	Moves along on the substratum via movements of its legs, appendages or muscles	
	Burrowers**	Burr	Movement in the sediment (e.g., annelids, bivalves).	
Sociability	Solitary species	Sol	Single individual	Determines sensitivity to physical disturbance and can indicate if a species can increase habitat heterogeneity or is habitat forming. If yes, then it affects habitat creation, nursery, refuge, facilitation and sediment oxygenation (Degen and Faulwetter, 2019)
	Gregarious species	Greg	Single individuals forming groups; growing in clusters	
	Colonial	Col	Living in permanent colonies	
Sexual differentiation	Gonochoristic	Gon	Organisms with just one sex	May relate to the ability of a population to recover from reduced abundance due to human induced disturbance (Bremner et al., 2006; Costello et al., 2015)
	Hermaphrodite	Hmph	Organisms with presence of both male and female reproductive organs	

Table 2.1 (continued)

Trait	Trait categories	Abbreviation	Description	Relevance
Resilience	Ecological group I species	AMBI I	Sensitive to organic matter, present in unpolluted conditions	Indicates vulnerability or resistance/resilience of a species towards pollution or man-induced changes in water biogeochemistry (Degen and Faulwetter, 2019)
	Ecological group II species	AMBI II	Indifferent to organic enrichment	
	Ecological group III species	AMBI III	Tolerant to excess organic matter	
	Ecological group IV species**	AMBI IV	First-order opportunistic species (small-size, short-life cycle)	
	Ecological group V species**	AMBI V	First-order opportunistic species (deposit-feeders- close to anoxic)	
Commercial or bycatch value	Highly valued commercial species	€€€	Targeted by fishing activities in MPA or nearby its limits	Reflects the value of the species for human society and evaluates the importance of each commercial species to fisheries (Leitão et al., 2020)
	Highly valued bycatch species	€€	Important bycatch; or very valuable and caught in sporadic illegal fishing events	
	Low valued bycatch species	€	Bycatch species with low commercial value	
	No valued species *	Ø	Species with no commercial value	

Table 2.2- List of environmental variables (habitat and biotic cover) and anthropogenic pressures (Human) measured for each site, categorized as large (those affecting large areas at a site level; macrohabitat) and small scale (affecting small areas at a quadrat level; microhabitat). Methods used to characterize variables are also described.

Scale	Category	Variables	Measuring Method
Large	Habitat	Structural complexity	Combined topography index (CTI) (Pais et al., 2013b) - In underwater 25m transect (3 replicates per site); one diver place the lead rope and counts the number of upwards (N_u) and downwards (N_d), while a second diver stretch a measuring tape up to the 25m. The linear distance (Ld) given by the measuring tape from the anchor point to the end of the leaded rope, and Lc is the stretched length of the leaded rope (25 m). The first diver also records the depth (in metres) at the deepest (D_d) and shallowest (D_s) points. CTI final form is: $CTI = (1 - SR) + NC/25 + MVR/25$, where $SR = Ld/Lc$, $NC = (N_u + N_d)/2$ and $MVC = D_d - D_s$
Large	Habitat	Substratum composition: Bedrock (%) Sand (%) Cobbles (%) Small boulders (%) Medium boulders (%) Large boulders (%)	In underwater 25m transect (3 replicates per site), one diver stretches the measuring tape up to the 25m and estimate the substratum composition, i.e. number of meters cover by each type: bedrock; sand; cobbles (pebbles and rocks with less than 0.2m); small boulders (0.2-0.5m); medium boulders (0.5-1.5m); and large boulders (>1.5m). Adapted (Alexander et al., 2009)

Table 2.2 (continued)

Scale	Category	Variables	Measuring Method
Large	Habitat	Nº Refuges per size category: (5-15cm) (15-50cm) (>50cm)	In the last 5m of the 25m transect, one diver estimates the number of refuges (holes) per size category: (5-15 cm); (15-50cm); (>50 cm). Adapted from (Alexander et al., 2009)
Large	Habitat	Mean depth (m)	Measured by the diving computer in each site
Large	Habitat	Coast exposure	Total wave fetch (Burrows et al., 2008)
Large	Habitat	Particulate organic matter (POM) (g/ L)	Collect 6L of water (3 replicates of 2L per site) with a Van Dorn Bottle at 3 to 4 meters depth (in the morning at same tide); filtered with a Whatman GF/C filters (47 mm; 1.2 µm nominal pore size), burned at 550ºC at muffle at the laboratory
Large	Habitat	Water temperature (◦C)	Measured by the diving computer in each site
Large	Human	Distance to the nearest city (Km)	Measured using QGIS mapping tools
Large	Habitat	Distance to the Sado estuary (Km)	Measured using QGIS mapping tools
Large	Human	Distance to the sewage outfall 1 (Km)*	Measured using QGIS mapping tools
Large	Human	Distance to the sewage outfall 2 (Km)*	Measured using QGIS mapping tools
Large	Human	Fishing pressure	Estimated for each site on a numerical scale (0 to 3, where 0 is no pressure and 3 is high pressure) based on published bibliography on the study area (see Batista et al., 2015)
Large	Human	Diving pressure	Estimated for each site on a numerical scale (0 to 3, where 0 is no pressure and 3 is high pressure) based on published bibliography on the study area (see Cabral et al., 2008)
Large	Biotic cover	Biomass of predators per category (g): Predators of small benthos (g) Predators of medium benthos (g) Scrapers of large benthos (g) Predators of large benthos (g)	Visual Census in each site; Measured during the underwater 25m transect for high mobility species (3 replicates per site), with two passages: one pelagic/demersal (25x4m) and other cryptic (25x1m)
Large	Biotic cover	Density of dominant macroalgae: *Saccorhiza polyschides* (N/m^2) *Cystoseira usneiodes* (N/m^2)	Visual Census in each site (three 1 x1 m quadrats were sampled in each site); Number of holdfasts per 1m^2
Large	Habitat	Visibility (m)	Observation *in situ* for each site, measured during visual census sampling (3 replicates)

(*) Distances to the sewage outfall were not used in further in the analysis as they showed to be proxies of the distance to the nearest city

Table 2.2 (continued)

Scale	Category	Variables	Measuring Method
Small	Habitat	Mean Slope (°)	Observation in situ for each 50x50 cm sampled quadrats (see methods section 2.2. for details), grouped into five categories: 0°; < 45°; 45°; > 45°; 90° (categories < 45° and > 45°, were later converted to 22,5° and 67,5° respectively)
Small	Habitat	Exposure: North South East West	Measured for each 50x50 cm sampled quadrat (see methods section 2.2. for details) with a compass, grouped into eight categories (N, NE, E, SE, S, SW, W, NW). If the compass pointed near to a collateral point (NE, SE, SW, NW) it was assumed that it was exposed to both nearest cardinal points (e.g. if the compass pointed NW the quadrat was exposed to both N and W). If the quadrat slope was equal to 0° (and therefore horizontal), the quadrat was exposed from all cardinal points (N, E, S, W)
Small	Habitat	Quadrat luminosity	Observation in situ for each 50x50 cm sampled quadrats (see methods section 2.2. for details), grouped into three categories: light; light and shadow; shadow.
Small	Habitat	Microhabitat features: Boulders over rock Crevices Pebbles Boulders over sand Cave Vertical wall	Presence/absence observed in 50x50 cm sampled quadrats (see methods section 2.2. for details). Categories were adapted from (Horta e Costa et al., 2013b)
Small	Habitat	Quadrat depth (m)	Measured by the diving computer for each 50x 50 cm sampled quadrats (see methods section 2.2. for details)
Small	Biotic cover	Algal cover (%): Green Algae Red Algae Brown Algae	Photo quadrats analyses for each 50x50 cm sampled quadrat, using PhotoQuad software to estimate the percentage of algal coverage in the three main algae phyla: Green (Chlorophyta), Red (Rhodophyta), Brown (Ochrophyta)
Small	Biotic cover	Algae morphological categories: Thick Leathery Algal cover (%) Jointed Calcareous Algal cover (%) Coarsely-Branched Algal cover (%) Encrusting Algal cover (%) Sheet Algal cover (%) Filamentous Algal cover (%)	Photo analyses for each 50x50 cm sampled quadrat (see methods section 2.2. for details), using PhotoQuad software to estimate the percentage of algal coverage in five morphological categories adapted from (Littler and Littler, 1984; Ruitton et al., 2000): thick leathery (thick blades and branches and leathery-rubbery texture); jointed calcareous (articulated, calcareous upright algae with stony texture); coarsely-branched (coarsely branched, upright and morphologically complex algae with fleshy-wiry texture); encrusting (epilithic encrusting algae with mostly stony but fewer fleshy algae; sheet algal (sheet like algae - with or without rib -, thin tubular and bubble shaped algae with soft texture; and filamentous (filamentous, delicately branched and simple branched algae with soft texture). Table A2 shows a complete list of species identified and their respective classification in these functional groups

2.3 Data analyses

The similarity of habitat complexity among protection zones was tested using PERMANOVA analyses (factors: year, protection and site, where sites are nested in protection) for environmental variables (habitat and biotic cover), and for anthropogenic pressures (Human) (see Table 2.2 for detailed information on specific variables**.** Although multivariate analysis of variance using permutations (PERMANOVA) tests the effect of one or more factors on one or more variables based on any measure of distance or dissimilarity of choice, it does not assume the homoscedasticity nor the normality of errors since p-values are obtained by permutations (Anderson et al., 2008). However, PERMANOVA is sensitive to differences in dispersion between groups and, therefore, the homogeneity of multivariate dispersions was tested using a PERMDISP routine before running the PERMANOVA tests (Anderson et al., 2008). Then the effects of different protection levels and annual variation on invertebrate taxa and functional traits densities, were analysed through a multivariate perspective using PERMANOVA with the same design explained above (Anderson et al., 2008). When significant differences were found, factors were investigated through post-hoc pair-wise comparisons. In addition, a Similarity Percentage Analysis (SIMPER) was used to help the interpretation of such differences.

For a better visualization of the multivariate patterns, unconstrained Principal Coordinates Analysis (PCO) were done to assess species abundance and invertebrate-based traits patterns (Anderson and Willis, 2003). Furthermore, Canonical Analysis of Principal Coordinates (CAP) were also performed with the purpose of uncovering patterns that could be masked by unconstrained analysis, by finding axes through the multivariate cloud that best discriminate between different protection zones and years (Anderson and Willis, 2003). Spearman correlation coefficients with PCO and CAP axes were calculated and the vectors of the most correlated ($r_s > |0.4|$ and $r_s > |0.5|$) supported the discussion of the observed patterns. For functional traits, vectors with all categories of the same trait were overlaid in CAP plots to search for any trait-specific patterns.

All the analyses performed with taxa abundance were fourth-root transformed in order to increase the importance of rare species, while functional trait data was not transformed. In both cases, resemblance matrices were constructed based on Bray–Curtis similarities. Lastly, the environmental variables and anthropogenic pressures were based on Euclidean distance matrices, constructed after normalizing each variable by subtracting the mean and dividing by the standard deviation, in order to place all variables on a comparable scale. P-values were calculated using 9999 permutations and the level of statistical significance adopted was 0.05. In PERMANOVA analyses, whenever the number of unique permutations available did not reach 100 due to lack of replicates, P-values were based on the Monte Carlo method (Anderson et al., 2008). All analyses were done using PRIMER software with the PERMANOVA package.

3. Results

3.1 Habitat characterization

There were some environmental variability among protection zones, with a presence of a clear environmental gradient along the MPA, namely regarding biotic cover, where CPZ showed higher percentage of jointed calcareous algae species as well as dominance of kelp *Saccorhiza polyschides* (Batters, 1902), while PPZ and NTZ showed dominance of *Cystoseira usneoides* ([L.] M.Roberts, 1968) algae forests (Table 3.1). Besides, differences among protection zones in habitat features were also found, for instance, PPZ was the zone with higher mean depths, while NTZ had the shallower sites. Regarding substratum composition, habitats in CPZ and PPZ showed an overall higher percentage of medium and large boulders respectively, and presence of bedrock, while NTZ showed higher percentage of cobbles and considerably more sand.

Despite this environmental variability, no significant differences were found in these zones for habitat features (Pseudo-F = 0.99697 $P>0.05$), but significant differences occurred for factors “year”, “site” as well as their interaction (Pseudo-F = 3.3921, $P<0.05$; Pseudo-F = 10.589, $P<0.05$ and Pseudo-F = 2.193, $P<0.05$, respectively). Results obtained from the unconstrained PCO analysis (Fig. 3.1A) showed an influence of habitat variables at site level, with a tendency for closer sites (geographically) inside a protection zone to be similar (e.g. C1 and C2 are closer with each other than with C3), which reveals a geographic influence on habitat structure inside each protection zone (Fig.3.1A). Vectors representing Spearman correlations with PCO axes ($r_s > |0.5|$) showed that these geographic influence could be mainly due to different substratum composition (e.g. difference between NTZ sites). Nevertheless other variables showed some effect as well, for instance, coast exposure and refugee size seemed to contribute to the differentiation of sites inside the CPZ and PPZ. In addition, the discriminant CAP clearly separated the three protection zones (Fig.3.1B), with a squared canonical correlation of $\delta^2 = 0.95645$ ($P < 0.05$). The first canonical axis (CAP1) separated the habitat variables of Complementary (CPZ) from both the No-take (NTZ) and Partial (PPZ) zones, while the second canonical axis (CAP2) separated the NTZ from the PPZ. Vectors representing Spearman correlations with CAP axes ($r_s > |0.5|$) showed that the NTZ had an overall higher percentage of substratum composed by cobbles, lower percentage of medium sized boulders and shallower mean depths, while PPZ had an overall higher percentage of small boulders and higher particulate organic matter (POM), and CPZ has less influence from the Sado River (Fig.3.1B).

Furthermore, regarding the biotic cover, the PERMANOVA analysis showed significant differences among years and protection zones (Pseudo-F = 1.9396 $P < 0.05$, and Pseudo-F = 2.2233 $P<0.05$, respectively) but with no significant differences for their interaction (Pseudo-F= 4.6397 $P> 0.05$) were found, meaning that although biotic covers were slightly different among years, their effect on protection zones were similar. Pair-wise comparisons showed significant differences between the CPZ and NTZ (P(MC) < 0.05), but no significant differences between PPZ and the other zones, once more evidencing the natural biotic cover gradient along the MPA. The first axis of the unconstrained PCO analysis was able to differentiate (with few overlaying) two major sampling groups (CPZ and PPZ+NTZ). PPZ and NTZ showed some level of similarity being partially separated by the second axis (Fig.3.1C). Spearman correlations with PCO axes ($r_s > |0.5|$) showed that CPZ is dominated by *S. polyschides*, while NTZ and PPZ are dominated by *C. usneoides.* CPZ had higher percentages of red algal cover, as well as higher percentages of jointed-calcareous and encrusting algae cover, than those observed in NTZ and PPZ that showed higher percentages of brown and thick leathery algae cover. NTZ also showed to be the area with less cover of green and coarsely branched algae. This effect was more evident in the discriminant CAP analysis, which clearly separated the three protection zones (Fig.3.1D), with a squared canonical correlation of $\delta^2 = 0.88133$ ($P < 0.05$). Similar patterns were observed through the correlation vectors, but in addition, CPZ also showed higher biomasses of small predator species than NTZ and PPZ.

Finally, significant differences in anthropogenic pressures among protection zones were also identified (Pseudo-F = 4.6168< 0.05). Both the unconstrained PCO and CAP analyses differentiated the three protection zones (squared canonical correlation of $\delta^2 = 0.99978$ ($P < 0.05$) (Fig.3.1E, 3.1F). A high level of fishing pressure characterizes the CPZ, while PPZ is characterized by the higher diving pressure. PPZ sites also showed differences due to the “distance to the nearest city” (P3 is further away from the city than P1 and P2). As expected, NTZ is clearly differentiated due to the very low level of anthropogenic pressures observed.

Table 3.1-Biotic variables (algal cover, dominant algae taxa and biomass of predators) per protection zone. Present results are the mean of the results measured in each site within a protection zone and respective standard deviation. Results for all environmental variables and anthropogenic pressures measured available in the appendix A (Table A3).

Biotic Variable	**Mean value (± standard deviation)**		
	CPZ	**PPZ**	**NTZ**
Biomass of small predators (g)	1904.71 (1908.74)	3307.65 (2132.80)	54519.04 (109499.10)
Biomass of medium predators (g)	14454.08 (11422.02)	8270.84 (4429.02)	9869.75 (6568.42)
Biomass of large scraper predators (g)	2599.09 (2490.28)	3705.84 (1516.63)	1987.48 (1806.59)
Biomass of large predators (g)	1629.78 (1985.50)	1975.51 (1589.49)	963.26 (846.27)
Sacchoriza polyschides (N/m^2)	9.89 (4.99)	0.00 (0.00)	0.00 (0.00)
Cystoseira usneoides (N/m^2)	0.00 (0.00)	10.58 (7.92)	22.72 (10.28)
Green Algal cover (%)	3.02 (3.29)	7.56 (9.26)	0.93 (1.94)
Red Algal cover (%)	40.94 (14.84)	26.93 (9.30)	31.56 (14.48)
Brown Algal cover (%)	18.04 (13.86)	38.27 (35.40)	35.45 (14.35)
Thick Leathery Algal cover (%)	16.76 (14.16)	35.05 (36.15)	31.65 (13.36)
Jointed Calcareous Algal cover (%)	6.70 (10.27)	0.51 (1.04)	0.88 (2.35)
Coarsely-Branched Algal cover (%)	6.56 (4.81)	9.15 (9.48)	1.83 (2.09)
Encrusting Algal cover (%)	29.98 (10.56)	20.66 (9.31)	24.58 (13.97)
Sheet Algal cover (%)	0.56 (0.96)	2.57 (5.67)	3.77 (6.08)
Filamentous Algal cover (%)	1.44 (1.85)	4.82 (6.50)	5.22 (6.53)

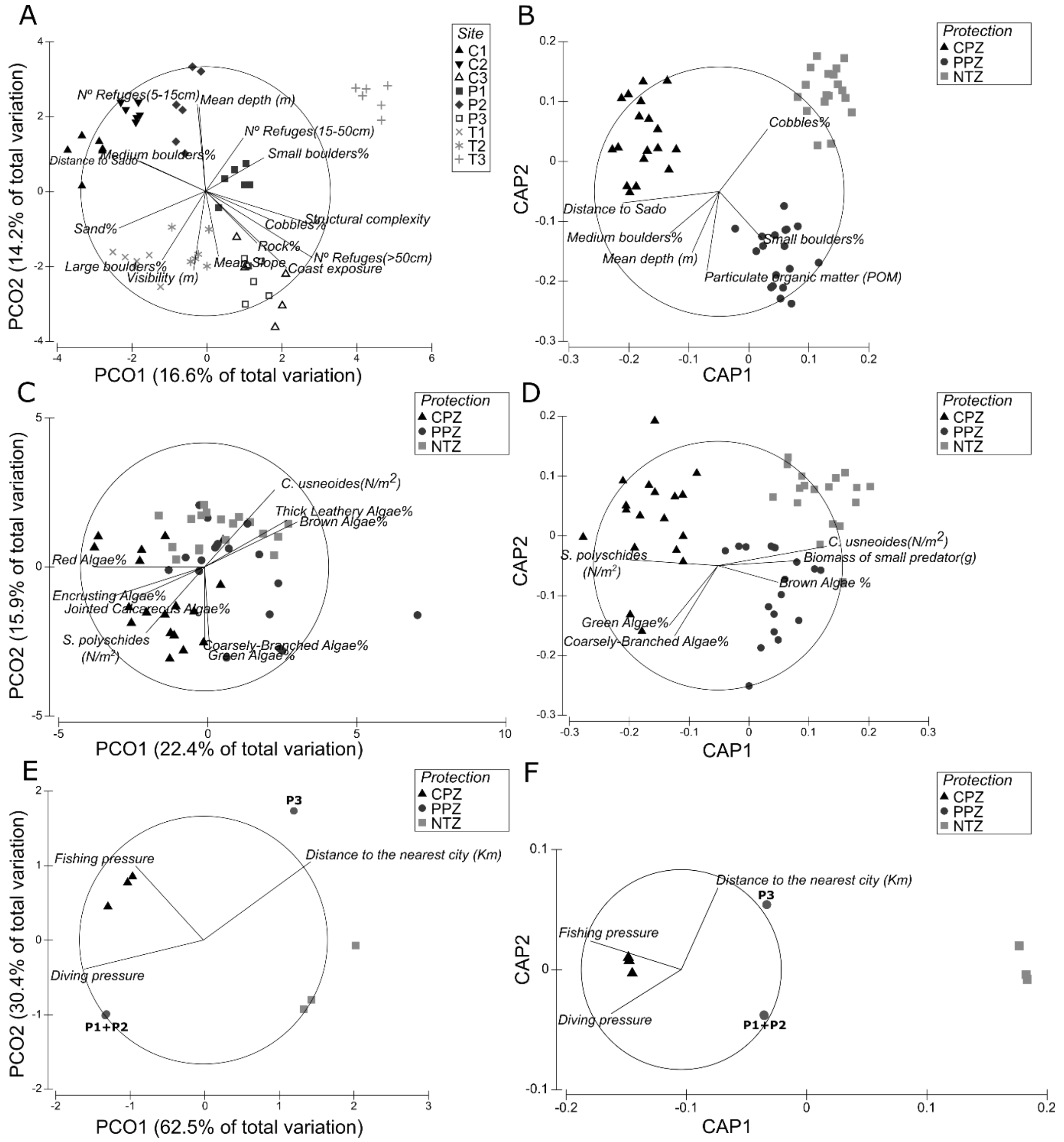

Figure 3.1- Ordination plots of Principal Coordinates Analysis (PCO) and Canonical Analysis of Principal Coordinates (CAP) comparing habitat characterization variables (A and B, respectively), biotic variables (C and D, respectively) and anthropogenic pressures (E and F, respectively) among sites (A) and protection zones (B,C,D,E and F). Correlations with canonical axes are only shown when Spearman's $r_s > |0.5|$ (circles represent vector correlations of 1).

3.2 Invertebrate assemblages' response to protection and habitat variability

In this study a total of 86 different invertebrate taxa were identified, belonging to nine distinct Phyla (Table A4). *Anemonia sulcata* (Pennant, 1777), Porifera (Grant, 1836), *Holothuria forskali* (Delle Chiaje, 1823), *Aiptasia mutabilis* (Gravenhorst, 1831), *Ophioderma longicaudum* (Bruzelius, 1805), *Sphaerechinus granularis* (Lamarck, 1816) and *Inachus* spp. (Weber, 1795) were the most abundant taxa, although with some variability between protection zones (Table 3.2). Invertebrate assemblages observed were significantly affected by factors "year" and "protection" (Pseudo-F = 2.3666 $P < 0.05$ and Pseudo-F = 1.7751 $P < 0.05$, respectively) but no significant differences for the interaction of both factors, (Pseudo-F = 0.73813 $P > 0.05$) were observed. In addition, no significant differences in multivariate dispersions were found by the PERMDISP routine (F = 2.1063 $P > 0.05$). Pair-wise comparisons showed significant differences among years ($P < 0.05$), as well as significant differences between the CPZ and PPZ ($P(MC) > 0.05$). In agreement, the unconstrained PCO analysis (for taxa abundance) differentiated the CPZ from the remaining protection zones (PPZ and NTZ), which seemed to have some proximity in the multivariate data cloud (Fig.3.2A). Vectors representing Spearman correlations with PCO axes ($r_s > |0.5|$) showed that *A. mutabilis* and Bryozoa. (Ehremberg, 1813) were more associated with NTZ. *Holothuria forskali* and *O. longicaudum* seemed associated with all protection zones but of higher importance to PPZ, while Porifera species showed an overall importance to CPZ. These patterns among protection zones were more evident in the CAP analysis (Fig.3.2B): CAP1 axis clearly differentiated invertebrate assemblages of CPZ from the NTZ and PPZ, and the CAP2 axis distinguished NTZ from PPZ; squared canonical correlation of $\delta^2 = 0.62491$ ($P < 0.05$). The Spearman correlations with CAP axes ($r_s > |0.4|$) showed that some taxa have particular influence in the different protection zones. *Ophioderma longicaudum* and *A. sulcata* seemed to be particularly important in assemblages from PPZ, while *A. mutabilis* was more associated with NTZ. *Sepia officinalis* (Linnaeus, 1758) and *Inachus phalangium* (J.C. Fabricius, 1775) showed particular importance in invertebrate assemblages of lower fishing pressure zones (i.e. PPZ and NTZ) while *S. granularis, Psammechinus miliaris* (P.L.S. Müller, 1771) *and Felimare tricolor* (Cantraine, 1835) seemed to have special importance in CPZ, where fishing pressure is higher. Lastly, SIMPER (similarity analysis results, cut-off restriction = 90%) allowed the better identification of species most contributing for the dissimilarities among protection zones, showing that in fact *A. sulcata* and Porifera were the top two taxa contributing to the observed dissimilarities (approximately 50% in all protection zones, Table 3.2). *Anemonia sulcata* had a high contribution to the patterns observed in the PPZ and NTZ, while Porifera species contribution was higher in the CPZ. *Holothuria forskali* also had high contribution in all zones (9 to 11%, approximately), being in the third position in the CPZ and PPZ, and fourth in NTZ. *Aiptasia mutabilis* was also important in PPZ and NTZ. Besides these, some taxa showed a significantly higher contribution in one specific protection zone over the others (e.g. *O. longicaudum* in the PPZ; *S. granularis* in the CPZ), other taxa showed significant contribution to only one protection zone (e.g. *F. tricolor* in the CPZ).

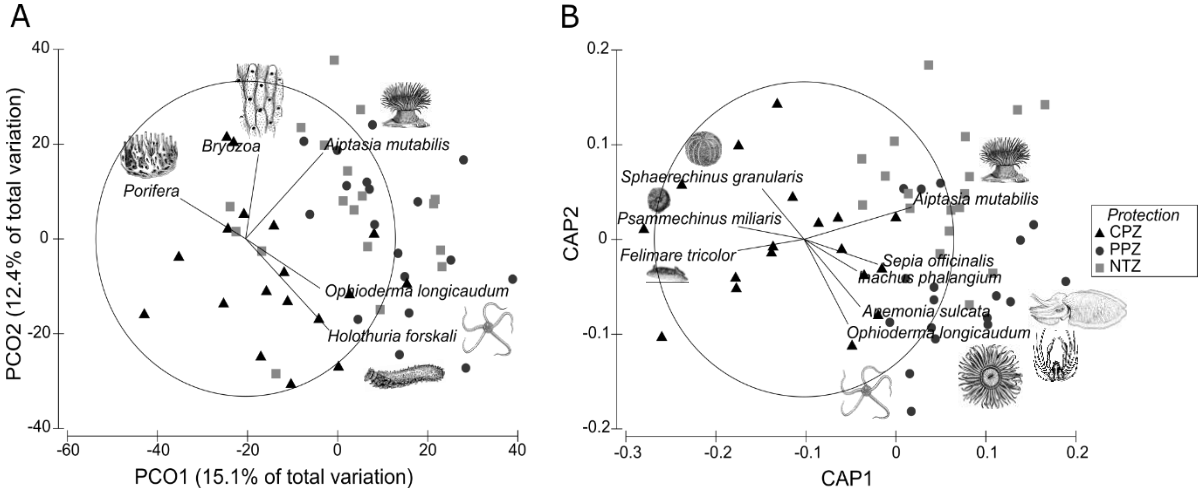

Figure 3.2 - Ordination plots of Principal Coordinates Analysis (PCO) and Canonical Analysis of Principal Coordinates (CAP) comparing taxa abundances among protection zones (A and B, respectively). Correlations with canonical axes are only shown when Spearman's $r_s > |0.5|$ (A), and $r_s > |0.4|$ (B) (circles represent vector correlations of 1). Taxa are also illustrated and images are from Clipart courtesy FCIT (http://etc.usf.edu/clipart/).

Table 3.2-. SIMPER analysis results for species abundance (fourth root transformed; cut-off at 90% cumulative dissimilarity). Average similarity: 36.68, 42.75 and 38.05 for CPZ, PPZ and NTZ, respectively). (a) Species with contribution in more than two zones, but higher contribution in one specific zone; (b) Species with contribution in more than two zones, but lower contribution in one specific zone; (c) Species with contribution for only one zone. Abundances without transformation for all taxa, available in the appendix A (Table A4).

CPZ Species	**Av.Abundance**	**Av.Similarity**	**Sim/SD**	**Contribution%**	**Cumulative%**
Porifera [a]	1.51	11.97	3.11	32.64	32.64
Anemonia sulcata [b]	1.35	9.22	1.57	25.14	57.77
Holothuria forskali [b]	0.72	3.37	0.72	9.18	66.95
Sphaerechinus granularis [a]	0.64	3.12	0.73	8.51	75.46
Felimare tricolor [c]	0.48	1.90	0.46	5.18	80.64
Ophioderma longicaudum [b]	0.38	0.89	0.32	2.43	83.07
Psammechinus miliaris [c]	0.37	0.81	0.33	2.22	85.29
Marthasterias glacialis [c]	0.27	0.56	0.26	1.54	86.83
Ophiothrix fragilis [c]	0.26	0.54	0.26	1.48	88.31
Inachus phalangium [b]	0.27	0.53	0.26	1.45	89.77
Paracentrotus lividus [c]	0.28	0.52	0.26	1.43	91.19
PPZ Species	**Av.Abundance**	**Av.Similarity**	**Sim/SD**	**Contribution%**	**Cumulative%**
Anemonia sulcata [a]	1.88	14.15	4.96	33.10	33.10
Porifera [b]	1.23	6.53	1.34	15.28	48.38
Holothuria forskali [a]	0.88	4.69	0.92	10.96	59.34
Ophioderma longicaudum [a]	0.81	4.47	0.96	10.46	69.80
Aiptasia mutabilis [b]	0.85	3.62	0.84	8.47	78.27
Inachus phalangium [a]	0.62	2.20	0.62	5.14	83.41
Necora puber [c]	0.20	0.90	0.54	2.10	85.51
Octopus vulgaris [a]	0.17	0.73	0.54	1.72	87.23
Bryozoa [b]	0.37	0.72	0.32	1.69	88.92
Eunicella verrucosa [c]	0.33	0.70	0.33	1.63	90.55
NTZ Species	**Av.Abundance**	**Av.Similarity**	**Sim/SD**	**Contribution%**	**Cumulative%**
Anemonia sulcata [b]	1.49	9.89	1.63	25.99	25.99
Porifera [b]	1.30	8.49	1.71	22.32	48.31
Aiptasia mutabilis [a]	1.03	4.71	0.93	12.39	60.70
Holothuria forskali [b]	0.78	3.38	0.71	8.89	69.59
Sphaerechinus granularis [b]	0.57	2.34	0.62	6.16	75.74
Inachus leptochirus [c]	0.42	1.10	0.38	2.89	78.63
Bryozoa [a]	0.43	1.04	0.32	2.72	81.36
Inachus phalangium [b]	0.39	0.91	0.39	2.38	83.74
Octopus vulgaris [a]	0.18	0.64	0.53	1.69	85.44
Periclimenes sagittifer [c]	0.35	0.64	0.32	1.69	87.13
Calliostoma zizyphinum [c]	0.29	0.52	0.25	1.38	88.51
Tunicata sp. [c]	0.28	0.47	0.26	1.24	89.75
Leptogorgia sarmentosa [c]	0.24	0.33	0.20	0.87	90.62

3.3 Functional trait approach

Nine invertebrate traits were chosen for the trait analysis, accounting with a total of 39 trait categories (Table A5). Most trait categories showed noteworthy higher average densities in the PPZ (e.g. density of medium sized species, density of predators, density of solitary species), followed by CPZ (e.g. density of grazer, density of herbivores, density of gregarious) and NTZ (e.g. density of scavengers, density of AMBI III species, densities of highly valued commercial and highly valued bycatch species) (Table 3.3). However, PERMANOVA showed, significant differences for the factor “year” (Pseudo-F = 4.5717 $P < 0.05$) but not for the factor “protection” (Pseudo-F = 0.80829 $P > 0.05$). In addition, no significant differences in multivariate dispersions were found by the PERMDISP routine ($F = 0.81053$ $P > 0.05$). In line with these findings, the unconstrained PCO analysis for invertebrate functional traits didn’t show clear patterns neither for “year” (Fig.3.3A) nor for “protection” factors (Fig.3.3B). Nevertheless, the discriminant CAP analysis differentiated the functional traits between CPZ and NTZ (CAP1 axis), but not for PPZ (Fig. 3.3C), with a squared canonical correlation of $\delta^2 = 0.29173$ ($P < 0.05$). The Spearman correlation with CAP axes ($r_s > |0.5|$) showed that the density of epi-/endozoic or epi-/endophytic species had a positive correlation with the NTZ, and a negative correlation with the CPZ. The densities of microcarnivores, solitary and gonochoristic species also showed a negative correlation with the CPZ. In addition, trait specific density patterns found in the pre-analyses that were masked in the multivariate analyses were better visualized, if vectors with all categories of the same trait were overlaid in CAP plots (Table 3.3). Among the feeding habit categories, density of grazers and deposit feeders showed a low positive correlation with CPZ, while density of scavengers had high correlation with NTZ. Similarly, diet categories density of herbivores and sessile invertebrate feeders appeared to have a low positive correlation with CPZ, while density omnivores had higher correlation with NTZ. Resilience categories showed high density of AMBI I and AMBI III species in the NTZ. Although the PPZ was spread across the multivariate data cloud, most of its points were located in the negative part of the CAP2 axis, therefore traits with negative correlations to the axis (e.g. densities of predators, microcarnivores, among others) had an overall weight in this zone.

Table 3.3- Traits and respective trait categories with a noteworthy difference in average density (ind/m^2) per protection zone. Differences in average densities between protection zones were considered noteworthy when at least one of the protection zones had an average abundance difference with another zone, greater than one sixth of the total abundance. Abundances for all trait categories analysed, available in the appendix A (Table A5). (+) vectors with positive correlation to a protection zone based in the CAP analyses where vectors with all categories of the same trait were overlaid (Fig.B2).

Trait	**Trait category**	**Average Density (± standard deviation)**			**Trait categories CAP vector's correlation**		
		CPZ	**PPZ**	**NTZ**	**CPZ**	**PPZ**	**NTZ**
Max body size	Very small	0.444 (0.877)	1.822 (2.940)	0.578 (1.026)		(+)	(+)
	Small medium	5.698 (11.936)	1.664 (8.604)	1.660 (2.168)	(+)	(+)	
	Medium	8.360 (7.890)	16.627 (11.086)	10.362 (7.918)		(+)	
	Large	0.400 (0.566)	1.692 (2.099)	0.716 (0.749)		(+)	(+)
Feeding habits	Grazer	1.556 (1.588)	1.289 (2.098)	0.669 (0.717)	(+)	(+)	
	Predator	11.649 (8.269)	18.365 (11.079)	11.256 (8.337)		(+)	
	Scavengers/Opportunistic	0.538 (0.549)	1.480 (2.126)	1.800 (1.937)		(+)	(+)
Diet	Omnivores	0.538 (0.549)	1.524 (2.116)	1.844 (1.922)		(+)	(+)
	Herbivores	1.556 (1.588)	1.289 (2.098)	0.669 (0.717)	(+)	(+)	
	Macrocarnivores	0.316 (0.620)	0.009 (0.459)	0.101 (0.370)			
	Microcarnivores	10.267 (8.178)	17.822 (11.111)	10.844 (8.442)		(+)	
	Sessile Invertebrate feeders	1.067 (1.131)	0.489 (0.616)	0.311 (0.543)	(+)		
Adult life habit	Tube dweller	0.267 (0.475)	1.333 (1.959)	0.489 (0.761)		(+)	(+)
	Epi-/endozoic or epi-/endophytic	0.933 (1.265)	1.733 (1.424)	1.600 (2.100)		(+)	(+)
Adult movement	Swimmer	0.004 (0.006)	0.721 (1.415)	0.367 (1.273)		(+)	(+)
Sociability	Solitary	13.973 (8.324)	26.167 (12.958)	20.181 (10.171)		(+)	(+)
	Gregarious	4.444 (5.201)	3.244 (4.469)	2.533 (3.742)	(+)	(+)	
Resilience	Ecological group II	0.978 (1.360)	2.667 (2.234)	1.422 (2.447)		(+)	
	Ecological group III	0.311 (0.680)	2.311 (4.039)	4.533 (5.600)		(+)	(+)
Commercial or bycatch value	Highly valued commercial	0.004 (0.006)	0.009 (0.009)	0.012 (0.013)		(+)	(+)
	Highly valued bycatch	0.004 (0.006)	0.013 (0.012)	0.022 (0.033)		(+)	(+)
	Low valued bycatch	0.044 (0.189)	0.133 (0.255)	0.089 (0.251)	(+)	(+)	

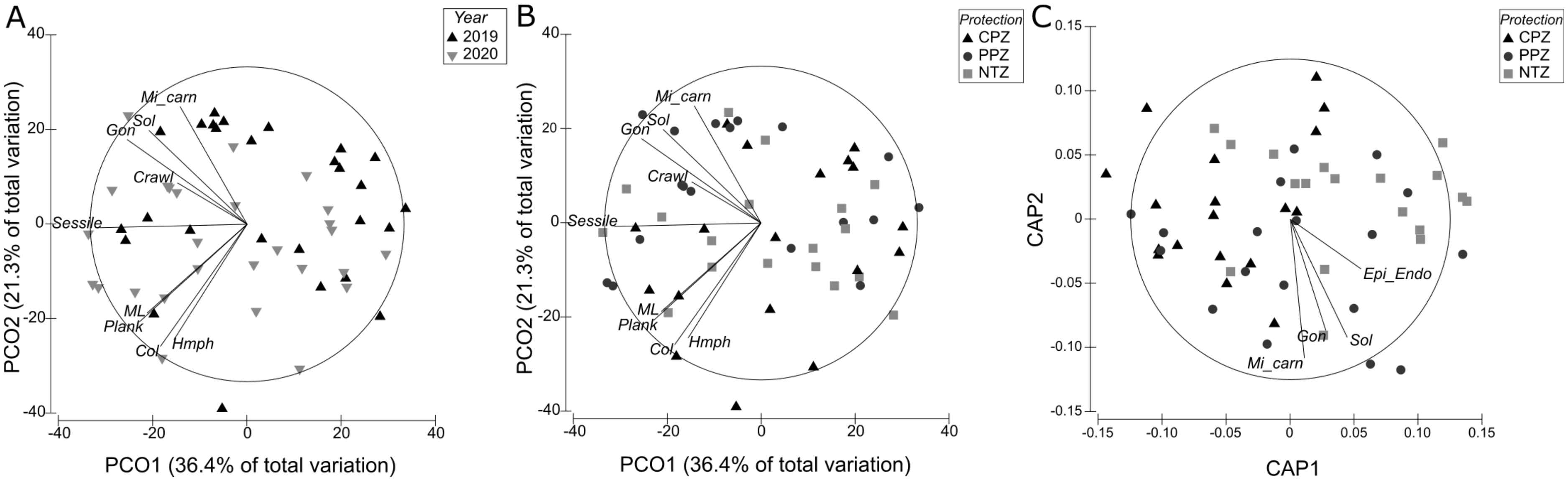

Figure 3.3- Ordination plots of Principal Coordinates Analysis (PCO) and Canonical Analysis of Principal Coordinates (CAP) comparing invertebrate traits densities among years (A) and protection zones (B and C). Correlations with canonical axes are only shown when Spearman's $r_s > |0.5|$ (circles represent vector correlations of 1). Mi_carn- Density of microcarnivores; Sol- Density of Solitary species; Gon- Density of gonochoristic species; Crawl- Density of crawler species; Sessile- Density of sessile species; ML- Density of medium-large size species; Plank- Density of planktonivores species; Col- Density of colonial species; Hmph- Density of hermaphroditic species; Epi_Endo- Density of epi-/endozoic or epi-/endophytic species.

4. Discussion

In order to properly assess the protection effect of a MPA, it is useful to have assemblage data from before its implementation, which is a rare scenario (Batista et al., 2015). Although this MPA has studies from pre-implementation (Almada et al., 1999; Gonçalves et al., 2002b; Henriques et al., 2007), these studies were focused on fish assemblages, and therefore their results are not completely comparable. Thus, in this study, a comparison between subtidal rocky reef invertebrate assemblages from different protection zones was made, using the no-take area (NTZ) as control, and using both taxa and functional traits. The taxa analysis approach showed that the highly protected zones (PPZ and NTZ) had some relevant signs on structural difference from the most impacted area (CPZ). Although with lower magnitude, this analysis also allowed the identification of differences between PPZ and NTZ. On the other hand, the functional trait analyses approach showed a distinct separation of CPZ and NTZ, whereas the PPZ showed little distinction from both the other zones, suggesting a presence of some sort of gradient influence along protection zones.

Similar patterns among high protection levels were expected since PPZ sites are geographically closer to NTZ, which is supported by the observed effects of both environmental and habitat variables. In addition, the CPZ is under higher anthropogenic pressure. In fact, although traps and handlines fishing are allowed in PPZ beyond 200m off-shore, the sampling sites were very nearshore (30-100m off-shore), whereas fishing is forbidden. Therefore, these patterns between protection zones might be related with both the protection effect of the different MPA zones, and environmental variation. This situation of multiple stressors influencing an area is rather common in coastal ecosystems (Niemi et al., 2004), and often hinders the protection effect assessment, due to natural variability patterns that many times works as confounding effects (García-Charton and Pérez-Ruzafa, 2001; Niemi et al., 2004; Pais et al., 2013a), especially if data from before the MPA establishment is not available. In such cases, a complete understanding of the local environmental variables is required, in order to know at glance what are the patterns of natural variability capable of influencing local communities, and introducing them into our analytical procedures. This way a better differentiation between patterns from natural and anthropogenic pressures can be made (García-Charton and Pérez-Ruzafa, 2001; Pais et al., 2013a).

Patterns detected in the functional traits multivariate analyses appear to be mainly a consequence of environmental and habitat variability, than a response to the effect of protection. In fact, the dominant environmental gradient found along the MPA, as well as the minor influences of habitat structure, seemed to mask and weaken eventual protection effects in the functional traits. For instance, “sociability” trait differentiated the CPZ from the PPZ and NTZ, as CPZ showed higher correlation to colonial (but also gregarious) species, while the PPZ and NTZ were characterized by more solitary species. This pattern is obvious in the SIMPER analysis (see Table 3.2), which showed that the anemone *A. sulcata* (solitary species) was present in all protection zones, nevertheless more relevant to the PPZ and NTZ. In contrast, sponges (colonial Porifera), which were also present in all protection zones, were more relevant in CPZ. Both are sessile benthic species and competition for substrate occupation between them, as well as with macroalgae is expected. Therefore, this pattern was probably a result of environmental variables, rather than protection effects. In fact, in the temperate rocky reefs substratum, erect algae (e.g. *Cystoseira* spp.) and coralline algae covers almost all available space, in contrast to tropical reefs, where sessile animal cover is often nearly 100% of the substratum (Jackson, 1977; Lewis 1964). Thus, animal colonies like sponges, which usually would have a superior competitive capacity for substratum colonization over solitary sessile animals (e.g. anemones), due to their proliferation by both asexual and sexual reproduction, and exhibition of both simple growth of fundamental units (e.g.

zooids) and varyingly complex budding patterns of these units, (all of which contribute to the colony size increase), in the presence of dense growths of macroalgae diminishes the density and displaces colonial animals to crevices and other cryptic environments (Jackson, 1977). Furthermore, although most CPZ sites were located in a bay, the described higher hydrodynamics in the MPA western zone (Saldanha, 1974), might be the main cause of the difference in algae facies. Therefore, the higher contribution of the solitary species *A. sulcata* over colonial Porifera in the PPZ and NTZ could in part be due to the higher density of algae in the dominant *Cystoseira* spp. forests, which can limit Porifera colonies growth, while the CPZ, dominated by lower densities of *Saccorhiza polyschides* and coralline algae tufts, provide better conditions for Porifera colonies development. In addition, the positive correlation of sessile invertebrate feeders density in the CPZ, namely for the most abundant nudibranch, *Felimare tricolor*, may also be due to trophic relationships. Nudibranchs from the *Felimare*'s Family (Chromodorididae Bergh, 1891) are known to prey on specific Porifera species (Goddard et al., 2013) and thus zones with higher density of Porifera (CPZ) can, by principle, support higher density of their predators, such as *F. tricolor* (sessile invertebrate feeders).

On the other hand, colonial Bryozoa e Gorgoniidae were an exception to the above explained trend, as they showed higher abundances in the NTZ and PPZ. The shelter from physical disturbances, such as recreational scuba diving and traps fishing, was probably the reason for their overall higher abundances in these zones, since both taxa are sensitive to such disturbances. Diving activities (present in both CPZ and PPZ) are a known source of disturbance for fragile epifauna like bryozoans and gorgonians, mainly due to human trampling (Milazzo et al., 2002; Rodrigues, 2008; Sala et al., 1996). Therefore, the absence of diving activities might be the reason for the overall higher abundances of these taxa in NTZ, whereas for the PPZ, even though it has some diving pressure, there is a known gorgonian hotspot near P1 (known as "Garden of gorgonians") which probably influences the nearby area. Additionally, in the CPZ, traps fishery is relatively intense, including very nearshore (Batista et al., 2015; Horta e Costa et al., 2013a). As referred above, these fishing gears can impact fragile epifauna, mostly due to their landing (if on one fragile species) and hauling (by exogenous forces) (Jennings and Kaiser, 1998). However, with the exception of some bryozoa species (e.g. *Pentapora* colonies) such minor impacts seemed to have little or no immediate effect on most of these fragile epifauna species (Coleman et al., 2013; Eno et al., 2001), nevertheless, there might exist a potential long-term cumulative effect after repeated impacts from the fishing gear. Thereby, it is possible that the higher abundances of these taxa in the NTZ and the PPZ, were a result of protection effects.

Invertebrate assemblages showed some similarity in PPZ and NTZ although some significant differences between them arose. For instance, microcarnivores and predators (e.g. *O. longicaudum, A. sulcata, I. phalangium*) density was higher in PPZ. Once more, this was probably related to differences in habitat structure, which according to present results might have influenced communities on a site level. The ophiuroid *O. longicaudum* was more abundant in the PPZ (with some importance to CPZ as well), whose sites had overall more rocks, boulders and fissures, which are where this specie is usually found (Stöhr et al., 2009; Tortonese, 1983), whereas the NTZ had more places covered by sand and cobbles. Similarly, the *A. sulcata* higher importance in the PPZ over NTZ could be also due to environmental variability, as the higher cover of cobbles and sand in the NTZ sites might difficult the attachment of the anemone's pedal disk (González Delgado et al., 2018), plus the higher abundances of the anemone *Aiptasia mutabilis* and macroalgae (*C. usneiodes*) that compete for rocky substratum in the NTZ. In fact, *A. mutabilis* showed higher abundances in NTZ also possibly due to differences in habitat structure, such as differences in hydrodynamics, depth, among others, rather than due to protection, since it has been shown to be tolerant to organic pollution (AMBI III), as well as insensitive to fishing traps (Coleman et al., 2013). Lastly, the spider-crab *I. phalangium* showed a similar pattern with *A. sulcata* (present in all protection zones, PPZ with the highest value). This result was expected, since this species lives in association with *A. sulcata* (i.e. commensalism relationship), as they remain in anemones during the day for protection from predators, and only left at night to feed on the bases, for a moult, when expelled by a stronger animal, and (if males) in search of sexual partners (Diesel, 1988; Wirtz and Diesel, 1983).

Although the protection effects were not as clear as the environmental-habitat patterns, some evidence of such effects were found, which is supported by previous studies on this MPA(e.g.Cunha et al., 2014; Henriques et al., 2013a; Horta e Costa et al., 2013b). These studies pointed out the fishing pressure has the main anthropogenic disturbance expecting to directly or indirectly influence invertebrate communities in this MPA as some trends related to this pressure were already detected on commercial species, where target species showed a trending increase of biomass in the NTZ since MPA implementation (Cunha et al., 2014; Horta e Costa et al., 2013b). Besides, some of these studies also showed higher densities of target species inside the NTZ (Henriques et al., 2013a; Horta e Costa et al., 2013b), in agreement with the patterns found for some species in the present study. For instance, common larger omnivore arthropods like *Necora puber* (valvet crab), showed higher abundances in the zones highly protected from fishing (PPZ and NTZ). Furthermore, the NTZ was the only zone where some rarer large omnivore arthropods like *Pagurus* sp. (hermit crab), *Maja squinado* (spiny spider crab), and *Scyllarus arctus* (slipper lobster) were registered. Large crustacean distribution (e.g. crabs and lobsters) is limited by abiotic factors (e.g. currents, winds, but mostly temperature) in larval stages (Alborés et al., 2019; Cobb and Wahle, 1994; Green et al., 2014), which were similar in all protection zones, whereas habitat features and biotic factors (possibly density-dependent factors such as predation, food availability, competition) are more relevant during the benthic adult stage (Cobb and Wahle, 1994; Green et al., 2014). Shelter-providing habitats such as cobbles and boulders, which are usually preferred by these species in their early life (Cobb and Wahle, 1994), were present in all protection zones (NTZ had more cobles, while PPZ and CPZ had more small and medium boulders), thereby suggesting availability of shelters in all protection zones. Therefore, this pattern might be a consequence of the fishing prohibition in this zone, as it has been shown that the density of large arthropoda like rock lobsters could increase within marine reserves (Edgar and Barrett, 1999). The traits density of scavengers, omnivores and bycatch species with high commercial value, where most of these species were included, were higher in the NTZ as well, therefore supporting this hypothesis. *Octopus vulgaris* and *Sepia officinalis* (high commercial value) showed higher density in the NTZ, followed by the PPZ. The higher densities of these species in the NTZ could be a direct consequence of the fishing prohibition in the no-take zone. In the PPZ, the fishing restrictions in the sampled areas might cause a similar effect, in a relative smaller scale, as it was already proved that highly regulated PPZ's adjacent to fully protected areas are an effective way to protect marine ecosystems, while benefit from socioeconomic advantages (Zupan et al., 2018). Additionally, the fact that both are high mobile species some degree of spillover, might also contribute to the significant abundances in the PPZ, as it was already hypothesized for octopus and seabreams populations in this MPA (Horta e Costa et al., 2013b).

Densities of grazer and herbivore species were higher in the CPZ. Among these species sea urchins *Sphaerechinus granularis* (most abundant urchin species), *Psammechinus miliaris* and *Paracentrotus lividus* showed a particular importance in the CPZ, which is the protection area with higher fishing pressure and lower cover of erect algae. In fact, predators' biomass results showed that the overall biomass of major urchin predators (e.g. *Diplodus sargus, Diplodus vulgaris, Diplodus cervinus*, and larger *Coris julis*), was lower in the CPZ, which suggest that these species are probably being protected by the MPA. Therefore, this pattern might be driven by trophic cascade effects, a consequence of protection already described for other MPAs in the Mediterranean Sea (Guidetti, 2006; Sala, 1997; Sievers and Nebelsick, 2018), where strong fishing pressure reduces the abundance of sea urchin predators, which increases their abundance and, consequently, reduces the erect algae cover. However, note that the reduced erect algae cover in the CPZ, might also be partially due to the higher hydrodynamics found in this area (Saldanha, 1974).

Concerning organic matter pollution pressure, the response of ecological groups (AMBI) is supported by a well-established and accepted scientific knowledge (Borja et al., 2009). AMBI I (sensitive species) showed higher density in NTZ and lower density in PPZ. This could be related to the slightly higher POM concentration measured in the PPZ, however since POM is associated with moving water masses, it is a very difficult link to establish. It is a pattern that should be further studied due to the predominant West-East current (Borges et al., 2007) that might affect the plume of the WWTP emissary in the nearby Sesimbra village, but other biotic factors (predation, competition) may be influencing the observed pattern. This pattern was also detected in the proportion of epi-/endozoic or epi-/endophytic species per anemone, (e.g. *I. phalangium*, *Inachus leptochirus, Periclimenes sagittifer*) all of which are AMBI I species associated to *A. sulcata* (Calado et al., 2007; Diesel, 1988; Wirtz and Diesel, 1983). Nevertheless, even though AMBI I species were more abundant in the NTZ, compared to PPZ, they were also present in PPZ. Besides that, higher densities of AMBI II species (indifferent to organic pollution) were observed in PPZ, while AMBI IV and AMBI V species (First-order opportunistic species) were not observed, suggesting that although the PPZ had more POM, the water quality was generally good in all protection zones sampled. However, it is important to keep in mind that although the AMBI species list is being updated yearly, there was a considerable number of identified invertebrate species not classified in the AMBI system and therefore there might be hidden patterns related to those species.

In summary, in this MPA the effects of environmental variables seemed to be one of the main forces influencing the marine invertebrate communities. In order to have a better control on such variables, future studies should compare the invertebrate assemblages from sites with similar micro-habitat typologies on different protection regimes (e.g. comparison between invertebrate assemblages on vertical walls, with the same depth and coast exposure from different MPA protection zones), taking into account the type of methodologies applied and the sample effort (e.g. if the objective is to assess the effect of protection on commercial valued invertebrates, such as cephalopods and large arthropods, underwater transects are preferable due to their higher mobility, whereas effort can be based on previous studies using this method), so that the variance remains at acceptable levels for detecting differences in the middle of all the normal noise of these data (Pais et al., 2012). In this study, even with the strong effect of environmental variables, the applied approach seemed to be able to detect some trends possibly related to protection effect on local invertebrate communities, namely "the highly valued species", "Scavenger species" and "Grazer species", as well as Bryozoa e Gorgoniidae taxa, therefore being potential biological indicators. However, considering that most identified trends could not provide clear insights, only hypotheses, the need for further research about the sensitivity of these potential indicators is clear. Therefore, further studies focused on both species and specific invertebrate functional traits that showed a potential response to the MPA's protection effect should be done. Namely, studies to assess the effects of natural variability on these groups in time (e.g. in different seasons and years - improve the knowledge on temporal variability) and space (e.g. in several zones with different habitat and environmental features within each protected area- improve the knowledge on spatial variability), as well as specific studies on their impact-response to different anthropogenic pressure effects (e.g.

experimental studies to assess the response of species/traits to specific impacts in order to make the predictions of the community response more clear).

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General Conclusions

The strong environmental gradient found in this study, seems to be the main driver of macroinvertebrate assemblages (those higher than 1cm) in this MPA. In fact, stress on coastal ecosystems, which have no defined boundaries between habitats, is usually a result of the combined effect of natural and anthropogenic disturbances (Niemi et al., 2004). This situation of multiple stressors can have synergistic and additive effects on biological responses, but also antagonistic effects that might be able to weaken eventual patterns of protection effect. In addition, data from before or right after the establishment of MPAs are often nonexistent, which encumbers the assessment of protection effects. The applied approach, i.e. previously identifying the expected pressures (natural and anthropogenic) and then analysing the response of local communities using both taxa and functional traits, together with a comparison of results between protection zones (pressured zones vs. controls), could be a way to assess protection effects, and seemed to be able to detect some cases of protection effect on local invertebrate communities.

Traits are more resilient to natural variation and respond more predictably to stress (Elliott et al., 2007; Henriques et al., 2013; Pais et al., 2012). Nevertheless, due to the strong natural gradient, most trait categories that showed higher correlations to protection zones in the discriminant CAP analysis (e.g. density of solitary species, density of predators and density of microcarnivores), appeared to be a result of environmental variations between protection zones. However, other trends that possibly result from the effect of protection were detected, revealing potential good indicators for the assessment of the MPA effect. Some trait categories (e.g. density of scavengers, density highly valued species, density of grazers) showed responsive trends to fishing pressure. Regarding diving pressure, no functional trait responded significantly. However, populations of bryozoans and gorgonians showed responsive trends to this pressure, therefore being potentially good indicators for diving pressure in invertebrate assemblages. AMBI ecological groups are already known to respond to organic matter pollution, and suggested an overall good water quality in the MPA, with only a remote possibility of WWTP emissaries influence in the PPZ, which should be studied in future research.

However, considering that most identified trends seem to raise hypotheses rather than provide clear insights, the need for further research about the sensitivity of these indicators is clear. Therefore, further studies focused on specific species and invertebrate functional traits that showed a potential response to the MPA protection effect should be done (e.g. a impact-response study on invertebrate target species or grazer species, namely urchins). Additionally, there is a generalized knowledge gap regarding invertebrate species functional traits, compared to fish functional traits. For instance, few invertebrate species had available information regarding their longevity, age of maturity and reproductive frequency, which are known important traits for invertebrate assemblages (Degen and Faulwetter, 2019; Tyler et al., 2012). Furthermore, a future study on these trends focused on invertebrates' biomass should also be done, since many studies on fish assemblages found protection patterns using biomass data (Cunha et al., 2014; Horta e Costa et al., 2013). However, such approach could be hampered once more by the lack of information on length-weight curves for some invertebrate species, whereby, alternatively species with lack of information could be collected for the biomass calculation. Lastly, since this study was based on the comparison between protection zones, and there was few pre-establishment data available, MPA protection effect on factors such as: (1) the size of the protection zones compared species' natural range; (2) the prohibition of destructive fishing activities (trawling and dredging) as well as, the ban of hand harvesting and spearfishing in the whole MPA; that could hinder patterns differences among zones, are difficult to assess. In order to bypass this, studies comparing these protected areas with unprotected areas near the marine park should be done.

In conclusion, although further research is needed, this study improves the understanding of rocky reefs invertebrate assemblages' response to protection effect and their potential use as biological indicators of anthropogenic pressures, contributing to the future development of new invertebrate-based biological indicators and other environmental assessment tools. Furthermore, since functional trait indicators are more easily transposed to other areas (Elliott et al., 2007; Henriques et al., 2013), their use could have strong implications, not only in the success of local management plans (e.g. MPA's), but also to fulfil the requirements of international policies (e.g. Marine Strategy Framework Directive), allowing for more conservation measures to be established based on invertebrates communities.

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Appendix A

Table A1- List of invertebrate taxa found in the assemblage, with their respective assigned functional group for each trait selected. Based on available literature and online databases.

Taxa	Traits									References*
	Max body size	Feeding habits	Diet	Adult life habit	Adult movement	Sociability	Sexual differentiation	Ecological group	Commercial value	
Acanthochitona crinita	Small	Grazer	Herbivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Aiptasia mutabilis	Medium large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Solitary	Gonochoristic	Ecological group III	none	[1],[2],[3],[4]
Alicia mirabilis	Large	Predator	Microcarnivore	Attached	Sessile	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3],[4]
Anemonia sulcata	Medium	Predator	Microcarnivore	Attached	Sessile	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Antedon bifida	Small medium	Filter/Suspension Feeder	Planktonivore	Crevice dweller	Crawler	Gregarious	Gonochoristic	Not assigned	none	[1],[2],[3]
Aplysia punctata	Medium large	Grazer	Herbivore	Free Living	Crawler	Solitary	Hermaphrodite	Ecological group I	none	[1],[2],[3],[5]
Arthropoda sp.	Small	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Ascidia mentula	Medium large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Solitary	Hermaphrodite	Ecological group III	none	[1],[2],[3]
Ascídia sp.	Small medium	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Hermaphrodite	Not assigned	none	[1],[2],[3]
Aslia lefevrii	Medium	Filter/Suspension Feeder	Detritivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3],[6]
Asterina gibbosa	Small medium	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Balanophyllia regia	Small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3],[4]
Bonellia viridis	Medium	Filter/Suspension Feeder	Detritivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Botryllus schlosseri	Very small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Hermaphrodite	Ecological group I	none	[1],[2],[3]
Bryozoa	Small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Hermaphrodite	Not assigned	none	[1],[2],[3]

Table A1 (continued)

Taxa	**Max body size**	**Feeding habits**	**Diet**	**Adult life habit**	**Adult movement**	**Sociability**	**Sexual differentiation**	**Ecological group**	**Commercial value**	**References***
Calliostoma zizyphinum	Small	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Cancer sp.	Medium	Predator	Microcarnivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Cerianthus membranaceus	Very large	Predator	Microcarnivore	Tube dweller	Sessile	Solitary	Hermaphrodite	Ecological group I	none	[1],[2],[3],[4]
Clavelina lepadiformis	Very small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Gregarious	Hermaphrodite	Not assigned	none	[1],[2],[3],[6]
Clavularia sp.	Small medium	Filter/Suspension Feeder	Detritivore	Attached	Sessile	Colonial	Gonochoristic	Not assigned	none	[1],[2],[3]
Corynactis viridis	Small	Predator	Microcarnivore	Attached	Sessile	Gregarious	Gonochoristic	Ecological group I	none	[1],[2],[3],[4]
Decapoda sp.	Small medium	Scavengers/Opportunistic	Omnivore	Free Living	Swimmer	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Didemnum maculosum	Very small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Hermaphrodite	Not assigned	none	[1],[2],[3]
Diogenes pugilator	Very small	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3]
Doris pseudoargus	Medium	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Ecological group I	none	[1],[2],[3]
Echinodea sp.	Very small	Grazer	Herbivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Edwardsia claparedii	Medium	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Solitary	Gonochoristic	Ecological group III	none	[1],[2],[3],[4],[6]
Eunicella gazella	Medium large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Gonochoristic	Ecological group I	none	[1],[2],[3]
Eunicella labiata	Large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Gonochoristic	Ecological group I	none	[1],[2],[3],[6]
Eunicella verrucosa	Large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Gonochoristic	Ecological group I	none	[1],[2],[3],[5]

Table A1 (continued)

Taxa	**Max body size**	**Feeding habits**	**Diet**	**Adult life habit**	**Adult movement**	**Sociability**	**Sexual differentiation**	**Ecological group**	**Commercial value**	**References***
Felimare bilineata	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[4]
Felimare cantabrica	Small medium	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[4]
Felimare sp.	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3]
Felimare tricolor	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[4]
Felimare villafranca	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[6]
Felimida krohni	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[4]
Felimida purpurea	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[4]
Galathea squamifera	Small medium	Deposit-Feeder	Detritivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Galathea strigosa	Medium	Deposit-Feeder	Detritivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Haliotis tuberculata	Medium	Deposit-Feeder	Detritivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group I	Bycatch low	[1],[2],[3]
Holothuria arguinensis	Large	Grazer	Herbivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Holothuria forskali	Medium large	Deposit-Feeder	Detritivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Holothuria mammata	Medium	Deposit-Feeder	Detritivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Holothuria sp.	Medium large	Deposit-Feeder	Detritivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Holothuria tubulosa	Medium large	Deposit-Feeder	Detritivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3],[6]

Table A1 (continued)

Taxa	**Max body size**	**Feeding habits**	**Diet**	**Adult life habit**	**Adult movement**	**Sociability**	**Sexual differentiation**	**Ecological group**	**Commercial value**	**References***
Inachus leptochirus	Small	Predator	Microcarnivore	Epi-/endozoic or epi-/endophytic	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Inachus phalangium	Small	Predator	Microcarnivore	Epi-/endozoic or epi-/endophytic	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3],[4],[5]
Inachus sp.	Very small	Predator	Microcarnivore	Epi-/endozoic or epi-/endophytic	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Inachus thoracicus	Very small	Predator	Microcarnivore	Epi-/endozoic or epi-/endophytic	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Leodice torquata	Medium large	Deposit-Feeder	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3],[6],[7]
Lepidochitona cinerea	Small	Grazer	Herbivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3]
Leptogorgia sarmentosa	Very large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Gonochoristic	Not assigned	none	[1],[2],[3],[4]
Macropodia longirostris	Small	Predator	Omnivore	Epi-/endozoic or epi-/endophytic	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3],[4]
Maja squinado	Large	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	Bycatch high	[1],[2],[3]
Marthasterias glacialis	Very large	Predator	Macrocarnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Microcosmus sp.	Small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3]
Munida Sarsi	Small medium	Predator	Macrocarnivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3]
Mysidacea sp.	Very small	Filter/Suspension Feeder	Planktonivore	Free Living	Swimmer	Gregarious	Gonochoristic	Ecological group II	none	[1],[2],[3]

Table A1 (continued)

Taxa	**Max body size**	**Feeding habits**	**Diet**	**Adult life habit**	**Adult movement**	**Sociability**	**Sexual differentiation**	**Ecological group**	**Commercial value**	**References***
Necora puber	Small medium	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	Bycatch high	[1],[2],[3]
Nudibranchia sp.	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3]
Octopus vulgaris	Very large	Predator	Macrocarnivore	Free Living	Swimmer	Solitary	Gonochoristic	Not assigned	Highly Commercial	[1],[2],[3]
Ophiocomina nigra	Medium	Deposit-Feeder	Detritivore	Crevice dweller	Crawler	Gregarious	Gonochoristic	Ecological group I	none	[1],[2],[3]
Ophioderma longicaudum	Medium	Predator	Microcarnivore	Crevice dweller	Crawler	Gregarious	Gonochoristic	Ecological group II	none	[1],[2],[3],[6]
Ophiothrix fragilis	Small medium	Deposit-Feeder	Detritivore	Crevice dweller	Crawler	Gregarious	Gonochoristic	Ecological group I	none	[1],[2],[3],[5]
Ophiuroidea sp.	Small medium	Deposit-Feeder	Detritivore	Crevice dweller	Crawler	Gregarious	Gonochoristic	Not assigned	none	[1],[2],[3]
Pachygrapsus marmoratus	Small	Scavengers/Opportunistic	Omnivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3]
Paguros sp.	Small	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3]
Palaemon sp.	Small medium	Scavengers/Opportunistic	Omnivore	Crevice dweller	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Paracentrotus lividus	Small medium	Grazer	Herbivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Paralcyonium spinulosum	Small medium	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Gonochoristic	Not assigned	none	[1],[2],[3],[6]
Parazoanthus axinellae	Small	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Gonochoristic	Not assigned	none	[1],[2],[3],[6]
Periclimenes sagittifer	Small	Scavengers/Opportunistic	Omnivore	Epi-/endozoic or epi-/endophytic	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3],[6]
Pisa nodipes	Small	Deposit-Feeder	Detritivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Polycera quadrilineata	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Hermaphrodite	Ecological group I	none	[1],[2],[3]
Porifera	Medium large	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Hermaphrodite	Not assigned	none	[1],[2],[3]

Table A1 (continued)

Taxa	Max body size	Feeding habits	Diet	Adult life habit	Adult movement	Sociability	Sexual differentiation	Ecological group	Commercial value	References*
Psammechinus miliaris	Small	Deposit-Feeder	Detritivore	Free Living	Crawler	Gregarious	Gonochoristic	Ecological group I	none	[1],[2],[3],[5]
Pycnogonida sp.	Very small	Predator	Microcarnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3]
Sabella sp.	Large	Filter/Suspension Feeder	Detritivore	Tube dweller	Sessile	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Sabella spallanzanii	Large	Filter/Suspension Feeder	Detritivore	Tube dweller	Sessile	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3],[7]
Scyllarus arctus	Medium large	Scavengers/Opportunistic	Omnivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	Bycatch high	[1],[2],[3]
Sepia officinalis	Large	Predator	Macrocarnivore	Free Living	Swimmer	Solitary	Gonochoristic	Not assigned	Highly Commercial	[1],[2],[3]
Sphaerechinus granularis	Medium large	Grazer	Herbivore	Free Living	Crawler	Solitary	Gonochoristic	Ecological group I	none	[1],[2],[3]
Thysanozoon brocchii	Small medium	Predator	Sessile Invertebrate Feeders	Free Living	Swimmer	Solitary	Hermaphrodite	Not assigned	none	[1],[2],[3],[5]
Tunicata sp.	Small medium	Filter/Suspension Feeder	Planktonivore	Attached	Sessile	Colonial	Hermaphrodite	Not assigned	none	[1],[2],[3]
Urosalpinx cinerea	Small	Predator	Sessile Invertebrate Feeders	Free Living	Crawler	Solitary	Gonochoristic	Ecological group II	none	[1],[2],[3]
Xanthidae sp.	Small	Predator	Microcarnivore	Free Living	Crawler	Solitary	Gonochoristic	Not assigned	none	[1],[2],[3],[8]

*[1] WoRMS Editorial Board (2019). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2019-11-11. doi:10.14284/170

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*[4] Reeflex team (2019). Reeflex.net online encyclopedia. Accessed on 2019-12-27. Available from https://www.reeflex.net/

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*[6] Doris Team (2019). Données d'Observations pour la Reconnaissance et l'Identification de la faune et la flore Subaquatiques. FFESSM,Fédération Française d’Études et de Sports Sous-Marins. Accessed on 2019-12-27. Available from https://doris.ffessm.fr/

*[7] Polytraits Team (2019). Polytraits: A database on biological traits of polychaetes. LifewatchGreece, Hellenic Centre for Marine Research. Accessed on 2019-12-27. Available from http://polytraits.lifewatchgreece.eu

*[8] Milke, L. M., & Kennedy, V. S. (2005). Mud crabs (Xanthidae) in Chesapeake Bay: claw characteristics and predation on epifaunal bivalves. Invertebrate Biology, 120(1), 67–77. doi:10.1111/j.1744-7410.2001.tb00027.x

Table A2- List of algal taxa present in the Arrábida MPA, with their respective phyla, and assigned adapted functional groups (Litter et al. 1984, Ruitton et al. 2000). Sheet algal group is characterized by sheet like algae (with or without rib), thin tubular and bubble shaped algae with soft texture; Filamentous group are filamentous, delicately branched and simple branched algae with soft texture; Coarsely Branched group are coarsely branched, upright and morphologically complex algae with fleshy-wiry texture; Thick Leathery group have thick blades and branches and leathery-rubbery texture; Jointed Calcareous group are articulated, calcareous upright algae with stony texture; and encrusting group are epilithic encrusting algae with mostly stony but fewer fleshy algae**.**

Algal taxa	**Phylum**	**Functional Groups**
Acrosorium ciliolatum	Rhodophyta	Sheet
Amphiroa beauvoisii	Rhodophyta	Jointed Calcareous
Asparagopsis armata	Rhodophyta	Filamentous
Asparagopsis taxiformis	Rhodophyta	Filamentous
Bonnemaisonia asparagoides	Rhodophyta	Filamentous
Bornetia secundiflora	Rhodophyta	Filamentous
Calliblepharis ciliata	Rhodophyta	Sheet
Callophyllis laciniata	Rhodophyta	Sheet
Caulacanthus ustulatus	Rhodophyta	Coarsely Branched
Ceramium ciliatum	Rhodophyta	Filamentous
Chondracanthus acicularis	Rhodophyta	Coarsely Branched
Chondracanthus teedei	Rhodophyta	Coarsely Branched
Chondria coerulescens	Rhodophyta	Filamentous
Cladophora sp.	Chlorophyta	Filamentous
Cladostephus spongiosus	Ochrophyta	Coarsely Branched
Codium adhaerens	Chlorophyta	Encrusting
Codium effusum	Chlorophyta	Encrusting
Codium tomentosum	Chlorophyta	Coarsely Branched
Codium vermilara	Chlorophyta	Coarsely Branched
Colpomenia sp.	Ochrophyta	Sheet
Cryptonemia seminervis	Rhodophyta	Sheet
Cryptopleura ramosa	Rhodophyta	Sheet
Cutleria adspersa	Ochrophyta	Encrusting
Cystoseira baccata	Ochrophyta	Thick Leathery
Cystoseira compressa	Ochrophyta	Thick Leathery
Cystoseira foeniculacea	Ochrophyta	Thick Leathery
Cystoseira tamariscifolia	Ochrophyta	Thick Leathery
Cystoseira usneoides	Ochrophyta	Thick Leathery
Derbesia tenuissima	Chlorophyta	Filamentous
Dictyopteris membranacea	Ochrophyta	Sheet
Dictyopteris polypodioides	Ochrophyta	Sheet
Dictyota cyanoloma	Ochrophyta	Sheet
Dictyota dichotoma	Ochrophyta	Sheet
Ellisolandia elongata	Rhodophyta	Jointed Calcareous
Erythroglossum lusitanicum	Rhodophyta	Sheet
Fucus vesiculosus	Ochrophyta	Thick Leathery
Gelidium corneum	Rhodophyta	Coarsely Branched
Gelidium spinosum	Rhodophyta	Coarsely Branched
Griffithsia sp.	Rhodophyta	Filamentous
Halopteris filicina	Ochrophyta	Filamentous
Hydroclathrus clathratus	Ochrophyta	Encrusting
Jania longifurca	Rhodophyta	Jointed Calcareous
Jania rubens	Rhodophyta	Jointed Calcareous
Kallymenia reniformis	Rhodophyta	Sheet
Laminaria ochroleuca	Ochrophyta	Thick Leathery
Lithophyllum incrustans	Rhodophyta	Encrusting
Lithophyllum stictiforme	Rhodophyta	Encrusting
Lomentaria sp.	Rhodophyta	Filamentous
Mesophyllum expansum	Rhodophyta	Encrusting
Mesophyllum lichenoides	Rhodophyta	Encrusting
Metacallophyllis laciniata	Rhodophyta	Sheet
Nitophyllum punctatum	Rhodophyta	Sheet
Osmundea hybrida	Rhodophyta	Coarsely Branched

Table A2 (continued)

Algal taxa	**Phylum**	**Functional Groups**
Padina pavonica	Ochrophyta	Encrusting
Pelvetia canaliculata	Ochrophyta	Coarsely Branched
Peyssonnelia squamaria	Rhodophyta	Encrusting
Phyllariopsis brevipes	Ochrophyta	Thick Leathery
Phymatolithon lenormandii	Rhodophyta	Encrusting
Plocamium cartilagineum	Rhodophyta	Coarsely Branched
Plocamium raphelisianum	Rhodophyta	Coarsely Branched
Pterocladiella capillacea	Rhodophyta	Coarsely Branched
Pterosiphonia complanata	Rhodophyta	Coarsely Branched
Rhodothamniella sp.	Rhodophyta	Filamentous
Rhodymenia pseudopalmata	Rhodophyta	Sheet
Saccorhiza polyschides	Ochrophyta	Thick Leathery
Sargassum flavifolium	Ochrophyta	Thick Leathery
Scinaia interrupta	Rhodophyta	Coarsely Branched
Spatoglossum solieri	Ochrophyta	Sheet
Sphaerococcus coronopifolius	Rhodophyta	Coarsely Branched
Stypocaulon scoparium	Ochrophyta	Filamentous
Taonia atomaria	Ochrophyta	Sheet
Ulva lactuca	Chlorophyta	Sheet
Ulva rigida	Chlorophyta	Sheet
Valonia utricularis	Chlorophyta	Sheet
Vertebrata fruticulosa	Rhodophyta	Filamentous
Vertebrata tripinnata	Rhodophyta	Filamentous
Zonaria tournefortii	Ochrophyta	Thick Leathery

Table A3- Environmental variables (habitat and biotic) and anthropogenic pressures measured for each site, categorized as large (those affecting large areas at a site level; macrohabitat) and small scale (affecting small areas at a quadrat level; microhabitat).Present results are the mean of the results measured in each site within a protection zone and respective standard deviation. (*) Distances to the sewage outfall were not used in the analysis as they showed to be proxies of the distance to the nearest city.

Scale	**Category**	**Variables**	**Mean value (± standard deviation)**		
			CPZ	**PPZ**	**NTZ**
Large	Habitat	Structural complexity	0.42 (0.09)	0.41 (0.04)	0.41 (0.06)
Large	Habitat	Substratum composition bedrock (%)	15.11 (21.37)	11.11 (9.88)	0.00 (0.00)
Large	Habitat	Substratum composition sand (%)	2.67 (2.88)	0.44 (0.63)	4.00 (3.27)
Large	Habitat	Substratum composition cobbles (%)	3.56 (5.03)	3.56 (5.03)	34.22 (32.19)
Large	Habitat	Substratum composition small boulders (%)	3.11 (4.40)	19.11 (9.51)	10.67 (9.30)
Large	Habitat	Substratum composition medium boulders (%)	45.78 (22.21)	28.44 (21.01)	23.11 (28.10)
Large	Habitat	Substratum composition large boulders (%)	28.00 (10.39)	37.33 (9.98)	28.00 (21.03)
Large	Habitat	Nº Refuges (5-15cm)	4.56 (0.68)	5.33 (4.78)	6.22 (3.59)
Large	Habitat	Nº Refuges (15-50cm)	1.67 (0.27)	3.89 (2.28)	7.67 (5.79)
Large	Habitat	Nº Refuges (>50cm)	1.00 (1.19)	1.78 (1.03)	1.44 (0.68)
Large	Habitat	Mean depth (m)	6.61 (1.45)	7.24 (1.04)	5.41 (0.97)
Large	Habitat	Coast exposure	851.80 (168.70)	1005.07 (112.22)	1047.34 (113.69)
Large	Habitat	Particulate organic matter (POM) (g $L{-1}$)	0.0016 (0.0002)	0.0018 (0.0001)	0.0015 (0.0001)
Large	Habitat	Water temperature (◦C)	16.87 (1.47)	16.27 (0.97)	17.53 (1.33)
Large	Human	Distance to the nearest city (Km)	5.26 (0.49)	5.86 (2.82)	6.57 (1.07)
Large	Habitat	Distance to the Sado estuary (Km)	22.43 (0.50)	11.65 (2.94)	10.83 (1.09)
Large	Human	Distance to the sewage outfall 1 (Km)*	5.36 (0.49)	5.73 (2.86)	6.47 (1.08)
Large	Human	Distance to the sewage outfall 2 (Km)*	5.85 (0.48)	5.24 (2.88)	5.99 (1.08)
Large	Human	Fishing pressure	3.00 (0.00)	1.33 (0.47)	0.00 (0.00)
Large	Human	Diving pressure	2.00 (0.00)	2.33 (0.94)	0.00 (0.00)
Large	Biotic	Biomass of small predators (g)	1904.71 (1908.74)	3307.65 (2132.80)	54519.04 (109499.1)
Large	Biotic	Biomass of medium predators (g)	14454.08 (11422.02)	8270.84 (4429.02)	9869.75 (6568.42)
Large	Biotic	Biomass of large scraper predators (g)	2599.09 (2490.28)	3705.84 (1516.63)	1987.48 (1806.59)
Large	Biotic	Biomass of large predators (g)	1629.78 (1985.50)	1975.51 (1589.49)	963.26 (846.27)

Table A3 (continued)

Scale	Category	Variables	Mean value (± standard deviation)		
			CPZ	**PPZ**	**NTZ**
Large	Biotic	*Sacchoriza* (N/m^2)	9.89 (4.99)	0.00 (0.00)	0.00 (0.00)
Large	Biotic	*Cystoseira* (N/m^2)	0.00 (0.00)	10.58 (7.92)	22.72 (10.28)
Small	Habitat	Visibility (m)	7.00 (1.41)	8.17 (3.62)	10.67 (4.35)
Small	Habitat	Mean Slope (°)	40.75 (12.32)	38.08 (14.90)	39.25 (15.79)
Small	Habitat	Exposure to North	0.60 (0.30)	0.53 (0.23)	0.52 (0.25)
Small	Habitat	Exposure to South	0.44 (0.29)	0.51 (0.23)	0.47 (0.31)
Small	Habitat	Exposure to East	0.61 (0.23)	0.62 (0.25)	0.54 (0.30)
Small	Habitat	Exposure to West	0.44 (0.22)	0.53 (0.23)	0.48 (0.28)
Small	Habitat	Quadrat luminosity	1.00 (0.00)	1.00 (0.00)	0.94 (0.23)
Small	Habitat	Quadrat substratum boulders above rock	0.89 (0.31)	0.83 (0.37)	0.89 (0.31)
Small	Habitat	Quadrat substratum crevices	0.78 (0.42)	0.78 (0.42)	0.61 (0.49)
Small	Habitat	Quadrat substratum pebbles	0.17 (0.37)	0.17 (0.37)	0.22 (0.42)
Small	Habitat	Quadrat substratum boulders above sand	0.11 (0.31)	0.39 (0.49)	0.33 (0.47)
Small	Habitat	Quadrat substratum cave	0.06 (0.23)	0.00 (0.00)	0.06 (0.23)
Small	Habitat	Quadrat vertical rock	0.56 (0.50)	0.61 (0.49)	0.56 (0.50)
Small	Habitat	Quadrat depth (m)	7.46 (1.88)	7.61 (1.52)	6.73 (1.15)
Small	Biotic	Green Algal cover (%)	3.02 (3.29)	7.56 (9.26)	0.93 (1.94)
Small	Biotic	Red Algal cover (%)	40.94 (14.84)	26.93 (9.30)	31.56 (14.48)
Small	Biotic	Brown Algal cover (%)	18.04 (13.86)	38.27 (35.40)	35.45 (14.35)
Small	Biotic	Thick Leathery Algal cover (%)	16.76 (14.16)	35.05 (36.15)	31.65 (13.36)
Small	Biotic	Jointed Calcareous Algal cover (%)	6.70 (10.27)	0.51 (1.04)	0.88 (2.35)
Small	Biotic	Coarsely-Branched Algal cover (%)	6.56 (4.81)	9.15 (9.48)	1.83 (2.09)
Small	Biotic	Encrusting Algal cover (%)	29.98 (10.56)	20.66 (9.31)	24.58 (13.97)
Small	Biotic	Sheet Algal cover (%)	0.56 (0.96)	2.57 (5.67)	3.77 (6.08)
Small	Biotic	Filamentous Algal cover (%)	1.44 (1.85)	4.82 (6.50)	5.22 (6.53)

Table A4- List of taxa found in the invertebrate assemblage, their respective phyla and average abundance (ind/m^2) and standard deviation per protection zone.

Taxa	**Phyla**	**Average Abundance (± standard deviation)**		
		CPZ	**PPZ**	**NTZ**
Acanthochitona crinita	Mollusca	0.089 (0.259)	0.000 (0.000)	0.000 (0.000)
Aiptasia mutabilis	Cnidaria	0.133 (0.307)	2.311 (2.979)	4.533 (5.762)
Alicia mirabilis	Cnidaria	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Anemonia sulcata	Cnidaria	7.289 (7.943)	14.889 (11.469)	8.578 (7.524)
Antedon bifida	Echinodermata	0.889 (3.771)	0.044 (0.189)	0.311 (1.320)
Aplysia punctata	Mollusca	0.000 (0.000)	0.000 (0.000)	0.002 (0.005)
Arthropoda sp.	Arthropoda	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Ascidia mentula	Chordata	0.133 (0.566)	0.000 (0.000)	0.000 (0.000)
Ascidiacea sp.	Chordata	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Aslia lefevrii	Echinodermata	0.000 (0.000)	0.000 (0.000)	0.089 (0.259)
Asterina gibbosa	Echinodermata	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Balanophyllia regia	Cnidaria	0.133 (0.566)	0.444 (1.203)	0.000 (0.000)
Bonellia viridis	Annelida	0.004 (0.010)	0.004 (0.010)	0.007 (0.010)
Botryllus schlosseri	Chordata	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Bryozoa	Bryozoa	0.267 (1.131)	0.578 (1.056)	1.200 (2.534)
Calliostoma zizyphinum	Mollusca	0.178 (0.342)	0.400 (0.879)	0.356 (0.684)
Cancer sp.	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Cerianthus membranaceus	Cnidaria	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Clavelina lepadiformis	Chordata	0.133 (0.566)	0.889 (3.771)	0.311 (0.995)
Clavularia sp.	Cnidaria	2.222 (9.428)	0.000 (0.000)	0.000 (0.000)
Corynactis viridis	Cnidaria	1.556 (4.299)	0.000 (0.000)	0.000 (0.000)
Decapoda sp.	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.311 (1.320)
Didemnum maculosum	Chordata	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Diogenes pugilator	Arthropoda	0.133 (0.307)	0.133 (0.307)	0.178 (0.342)
Doris pseudoargus	Mollusca	0.089 (0.377)	0.044 (0.189)	0.000 (0.000)

Table A4 (continued)

Taxa	**Phyla**	**Average Abundance (± standard deviation)**		
		CPZ	**PPZ**	**NTZ**
Echinodea sp.	Echinodermata	0.133 (0.412)	0.000 (0.000)	0.000 (0.000)
Edwardsia claparedii	Cnidaria	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Eunicella gazella	Cnidaria	0.000 (0.000)	0.044 (0.189)	0.089 (0.259)
Eunicella labiata	Cnidaria	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Eunicella verrucosa	Cnidaria	0.089 (0.377)	0.311 (0.486)	0.178 (0.342)
Felimare bilineata	Mollusca	0.000 (0.000)	0.000 (0.000)	0.089 (0.259)
Felimare cantabrica	Mollusca	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Felimare sp.	Mollusca	0.044 (0.189)	0.044 (0.189)	0.000 (0.000)
Felimare tricolor	Mollusca	0.667 (0.999)	0.089 (0.259)	0.089 (0.259)
Felimare villafranca	Mollusca	0.089 (0.259)	0.044 (0.189)	0.089 (0.259)
Felimida krohni	Mollusca	0.044 (0.189)	0.089 (0.259)	0.000 (0.000)
Felimida purpurea	Mollusca	0.044 (0.189)	0.044 (0.189)	0.044 (0.189)
Galathea squamifera	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Galathea strigosa	Arthropoda	0.000 (0.000)	0.002 (0.005)	0.007 (0.010)
Haliotis tuberculata	Mollusca	0.044 (0.189)	0.133 (0.307)	0.089 (0.259)
Holothuria arguinensis	Echinodermata	0.044 (0.189)	0.044 (0.189)	0.000 (0.000)
Holothuria forskali	Echinodermata	1.378 (1.523)	1.956 (2.182)	2.178 (3.150)
Holothuria mammata	Echinodermata	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Holothuria sp.	Echinodermata	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Holothuria tubulosa	Echinodermata	0.222 (0.460)	0.178 (0.439)	0.044 (0.189)
Inachus leptochirus	Arthropoda	0.444 (1.105)	0.356 (0.787)	0.667 (1.379)
Inachus phalangium	Arthropoda	0.267 (0.475)	1.022 (1.339)	0.444 (0.684)
Inachus sp.	Arthropoda	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Inachus thoracicus	Arthropoda	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)

Table A4 (continued)

Taxa	**Phyla**	**Average Abundance (± standard deviation)**		
		CPZ	**PPZ**	**NTZ**
Leodice torquata	Annelida	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Lepidochitona cinerea	Mollusca	0.133 (0.566)	0.444 (0.919)	0.000 (0.000)
Leptogorgia sarmentosa	Cnidaria	0.044 (0.189)	0.356 (0.959)	0.356 (0.833)
Macropodia longirostris	Arthropoda	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Maja squinado	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.002 (0.005)
Marthasterias glacialis	Echinodermata	0.267 (0.475)	0.000 (0.000)	0.089 (0.377)
Microcosmus sp.	Chordata	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Munida Sarsi	Arthropoda	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Mysidacea sp.	Arthropoda	0.000 (0.000)	0.667 (1.534)	0.044 (0.102)
Necora puber	Arthropoda	0.004 (0.006)	0.013 (0.014)	0.011 (0.020)
Nudibranchia sp.	Mollusca	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Octopus vulgaris	Mollusca	0.004 (0.006)	0.007 (0.007)	0.009 (0.010)
Ophiocomina nigra	Echinodermata	0.222 (0.535)	0.178 (0.754)	0.489 (1.884)
Ophioderma longicaudum	Echinodermata	0.667 (1.235)	1.333 (1.344)	1.067 (2.376)
Ophiothrix fragilis	Echinodermata	0.222 (0.369)	0.133 (0.307)	0.178 (0.754)
Ophiuroidea sp.	Echinodermata	0.133 (0.566)	0.000 (0.000)	0.000 (0.000)
Pachygrapsus marmoratus	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)
Paguros sp.	Arthropoda	0.000 (0.000)	0.044 (0.189)	0.044 (0.189)
Palaemon sp.	Arthropoda	0.000 (0.000)	0.622 (2.255)	0.311 (0.783)
Paracentrotus lividus	Echinodermata	0.311 (0.558)	0.622 (2.640)	0.000 (0.000)
Paralcyonium spinulosum	Cnidaria	1.778 (7.542)	0.000 (0.000)	0.178 (0.754)
Parazoanthus axinellae	Cnidaria	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Periclimenes sagittifer	Arthropoda	0.178 (0.342)	0.267 (0.614)	0.489 (0.995)
Pisa nodipes	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.044 (0.189)

Table A4 (continued)

Taxa	**Phyla**	**Average Abundance (± standard deviation)**		
		CPZ	**PPZ**	**NTZ**
Polycera quadrilineata	Mollusca	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Porifera	Porifera	7.333 (6.706)	7.333 (12.118)	5.289 (6.075)
Psammechinus miliaris	Echinodermata	0.622 (1.182)	0.000 (0.000)	0.133 (0.307)
Pycnogonida sp.	Arthropoda	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Sabella sp.	Annelida	0.222 (0.460)	1.289 (2.676)	0.356 (0.737)
Sabella spallanzanii	Annelida	0.044 (0.189)	0.000 (0.000)	0.133 (0.412)
Scyllarus arctus	Arthropoda	0.000 (0.000)	0.000 (0.000)	0.009 (0.013)
Sepia officinalis	Mollusca	0.000 (0.000)	0.003 (0.005)	0.003 (0.005)
Sphaerechinus granularis	Echinodermata	0.844 (1.044)	0.178 (0.342)	0.667 (0.739)
Thysanozoon brocchii	Platyhelminthes	0.000 (0.000)	0.044 (0.189)	0.000 (0.000)
Tunicata sp.	Chordata	0.044 (0.189)	0.133 (0.412)	0.311 (0.622)
Urosalpinx cinerea	Mollusca	0.044 (0.189)	0.000 (0.000)	0.000 (0.000)
Xanthidae sp.	Arthropoda	0.000 (0.000)	0.044 (0.189)	0.044 (0.189)

Table A5- List of invertebrate-based traits in their respective trait categories and their average density (ind/m^2) and standard deviation per protection zone.

Trait	Trait category	Average Density (± standard deviation)		
		CPZ	**PPZ**	**NTZ**
Max body size	Very_small (<2cm)	0.444 (0.877)	1.822 (2.940)	0.578 (1.026)
	Small (2-5cm)	4.978 (5.952)	4.000 (4.577)	3.778 (2.499)
	Small_medium (5-10cm)	5.698 (11.936)	1.664 (8.604)	1.660 (2.168)
	Medium (10-15cm)	8.360 (7.890)	16.627 (11.086)	10.362 (7.918)
	Medium_large (15-30cm)	10.089 (6.006)	12.000 (9.951)	12.856 (8.665)
	Large (30-50)	0.400 (0.566)	1.692 (2.099)	0.716 (0.749)
	Very_large (>50cm)	0.316 (0.484)	0.407 (0.906)	0.453 (0.854)
Feeding habits	Grazer	1.556 (1.588)	1.289 (2.098)	0.669 (0.717)
	Predator	11.649 (8.269)	18.365 (11.079)	11.256 (8.337)
	Deposit-feeders	2.893 (2.144)	2.629 (2.912)	3.204 (3.531)
	Filter/Suspension-feeders	13.649 (14.413)	14.449 (15.117)	13.473 (9.435)
	Scavengers/Opportunistic	0.538 (0.549)	1.480 (2.126)	1.800 (1.937)
Diet	Omnivores	0.538 (0.549)	1.524 (2.116)	1.844 (1.922)
	Herbivores	1.556 (1.588)	1.289 (2.098)	0.669 (0.717)
	Macrocarnivores	0.316 (0.620)	0.009 (0.459)	0.101 (0.370)
	Microcarnivores	10.267 (8.178)	17.822 (11.111)	10.844 (8.442)
	Detritivores	5.387 (10.655)	3.922 (8.000)	3.744 (3.690)
	Planktonivores	11.156 (11.428)	13.156 (13.388)	12.889 (9.174)
	Sessile_Invertebrate_feeders	1.067 (1.131)	0.489 (0.616)	0.311 (0.543)
Adult life habit	Free living	5.298 (2.301)	4.867 (4.012)	4.703 (3.381)
	Crevice dweller	2.453 (3.846)	2.900 (2.517)	2.500 (4.049)
	Tube dweller	0.267 (0.475)	1.333 (1.959)	0.489 (0.761)
	Epi-/endozoic or epi-/endophytic	0.933 (1.265)	1.733 (1.424)	1.600 (2.100)
	Attached	21.333 (13.590)	27.378 (15.642)	21.111 (11.427)

Table 5 (continued)

Trait category	Traits	Average Density (± standard deviation)		
		CPZ	**PPZ**	**NTZ**
Adult movement	Sessile	21.600 (13.610)	28.711 (16.628)	21.600 (11.439)
	Swimmer	0.004 (0.006)	0.721 (1.415)	0.367 (1.273)
	Crawler	8.680 (4.226)	8.780 (3.830)	8.436 (6.643)
Sociability	Solitary	13.973 (8.324)	26.167 (12.958)	20.181 (10.171)
	Gregarious	4.444 (5.201)	3.244 (4.469)	2.533 (3.742)
	Colonial	11.867 (13.100)	8.800 (12.415)	7.689 (6.215)
Sexual differentiation	Gonochoristic	19.040 (11.472)	28.701 (13.389)	22.934 (11.731)
	Hermaphrodite	9.022 (7.519)	9.511 (11.623)	7.469 (6.541)
Resilience	Ecological group I (AMBI)	5.031 (6.528)	4.642 (5.703)	6.871 (4.700)
	Ecological group II (AMBI)	0.978 (1.360)	2.667 (2.234)	1.422 (2.447)
	Ecological group III (AMBI)	0.311 (0.680)	2.311 (4.039)	4.533 (5.600)
Commercial or bycatch value	Highly valued commercial	0.004 (0.006)	0.009 (0.009)	0.012 (0.013)
	Highly valued bycatch	0.004 (0.006)	0.013 (0.012)	0.022 (0.033)
	Low valued bycatch	0.044 (0.189)	0.133 (0.255)	0.089 (0.251)
	No value	30.231 (14.717)	38.056 (17.421)	30.280 (14.587)

Appendix B

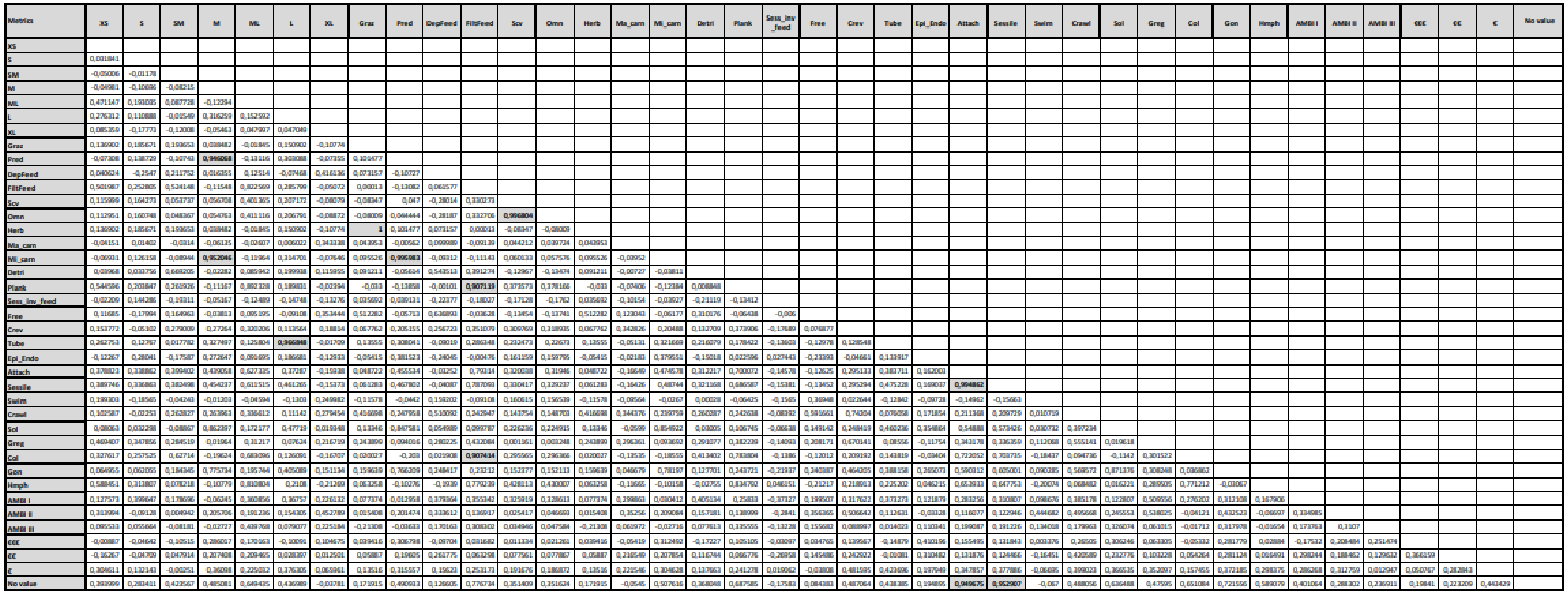

Figure B1- Draftsmen plot with Pearson's correlations between the invertebrate-based traits. Traits with correlations higher than |0,90| were assumed as proxies. Trait categories code: XS- Density of very small species; S- Density of small species; SM-Density of small-medium species; M- Density medium species; ML-Density medium-large species; L- Density large species; XL- Density very large species; Graz-Density of grazers; Pred- Density of predators; DepFeed- Density of deposit-feeders; FiltFeed- Density of filter/suspension-feeders; Scv- Density of scavengers/opportunistic; Omn- Density of omnivores; Herb- Density of herbivores; Ma_carn- Density of macrocarnivores; Mi_carn- Density of microcarnivores; Detri- Density of detritivores; Plank- Density of planktonivores; Sess_inv_feed- Density of sessile invertebrate feeders; Free- Density of free living; Crev- Density of crevice dweller; Tube- Density of tube dweller; Epi_Endo- Density of epi-/endozoic or epi-/endophytic; Attach-Density of attached; Sessile- Density of sessile; Swim-Density of swimmers; Crawl- Density of crawlers; Sol- Density of solitary species; Greg- Density of gregarious species; Col- Density of colonial; Gon- Density of gonochoristic; Hmph- Density of hermaphrodites; AMBI I- Density of ecological group I species; AMBI II- Density of ecological group II species; AMBI III- Density of ecological group III species; €€€- Density of highly valued commercial species; €€- Density of highly valued bycatch species; €- Density of low value bycatch species

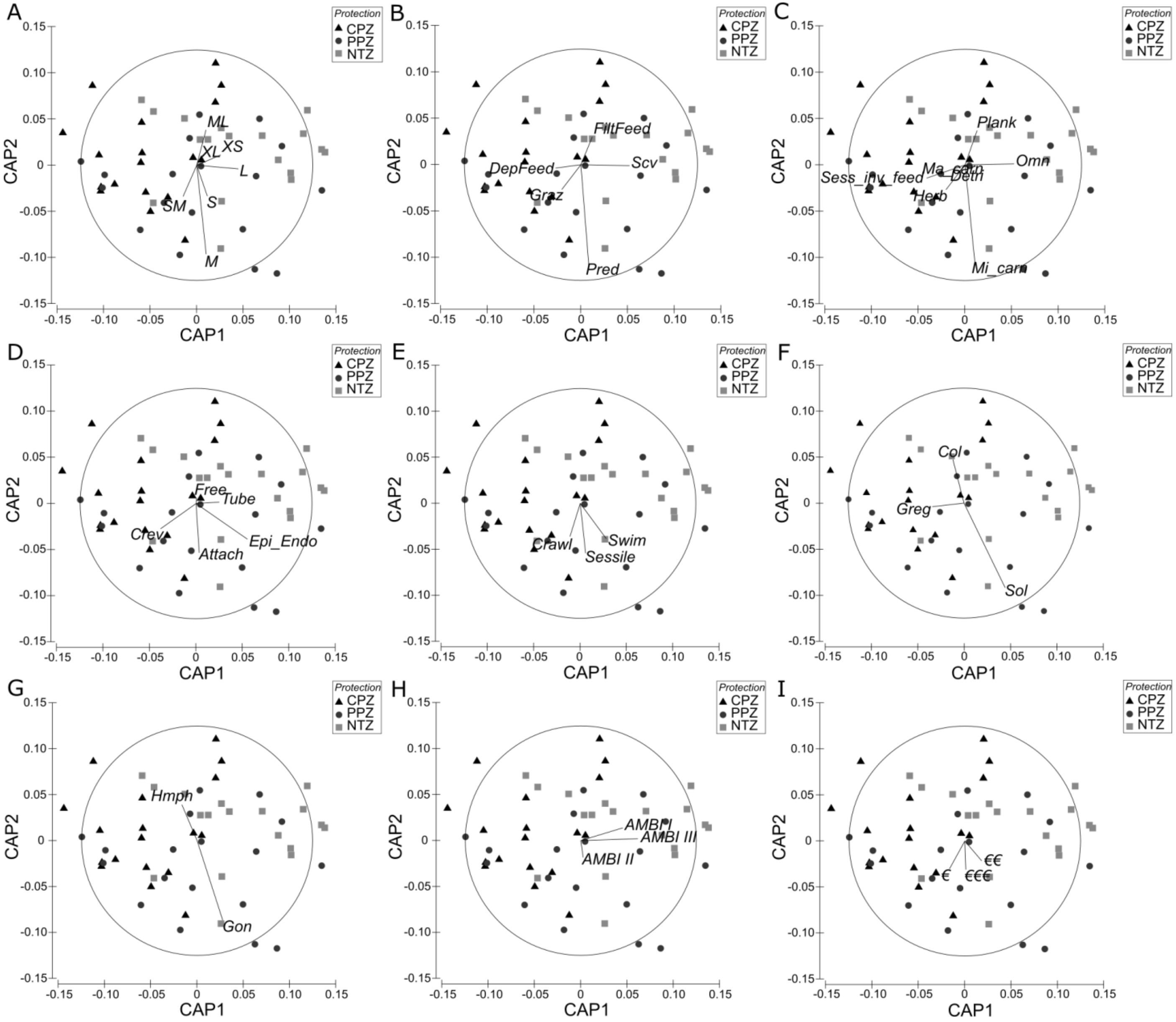

Figure B2- Canonical Analysis of Principal Coordinates (CAP) comparing invertebrate traits densities among protection zones. Spearman's correlations with canonical axes are grouped per trait category: max body size (A), feeding habits (B), diet (C), adult life habit (D), adult movement (E), sociability (F), sexual differentiation (G), resilience (H), and commercial or bycatch value (I). Circles represent vector correlations of 1. Trait categories code: XS- Density of very small species; S- Density of small species; SM- Density of small-medium species; M- Density medium species; ML-Density medium-large species; L- Density large species; XL- Density very large species; Graz-Density of grazers; Pred- Density of predators; DepFeed- Density of deposit-feeders; FiltFeed- Density of filter/suspension-feeders; Scv- Density of scavengers/opportunistic; Omn- Density of omnivores; Herb- Density of herbivores; Ma_carn- Density of macrocarnivores; Mi_carn- Density of microcarnivores; Detri- Density of detritivores; Plank- Density of planktonivores; Sess_inv_feed- Density of sessile invertebrate feeders; Free- Density of free living; Crev- Density of crevice dweller; Tube- Density of tube dweller; Epi_Endo- Density of epi-/endozoic or epi-/endophytic; Attach-Density of attached; Sessile- Density of sessile; Swim-Density of swimmers; Crawl- Density of crawlers; Sol- Density of solitary species; Greg- Density of gregarious species; Col- Density of colonial; Gon- Density of gonochoristic; Hmph- Density of hermaphrodites; AMBI I- Density of ecological group I species; AMBI II- Density of ecological group II species; AMBI III- Density of ecological group III species; €€€- Density of highly valued commercial species; €€- Density of highly valued bycatch species; €- Density of low value bycatch species