



Comparison of exposed and protected soft-bottom macrobenthic assemblages of the shallow sublittoral of Northern Portugal

Ana Carolina Castro Costa

UMinho | 2023



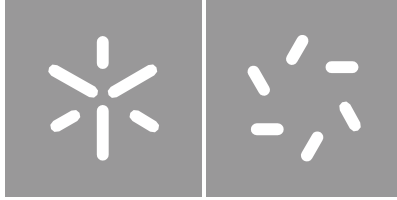
Universidade do Minho

Escola de Ciências

Ana Carolina Castro Costa

Comparison of exposed and protected soft-bottom macrobenthic assemblages of the shallow sublittoral of Northern Portugal

outubro 2023



Universidade do Minho

Escola de Ciências

Ana Carolina Castro Costa

Comparison of exposed and protected soft-bottom macrobenthic assemblages of the shallow sublittoral of Northern Portugal

Dissertação de Mestrado

Mestrado em Biodiversidade, Ecologia e Alterações Globais

Trabalho efetuado sob a orientação do(a)

Investigador Diego Carreira Flores

Investigador Marcos Rubal Garcia

outubro de 2023

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

Este é um trabalho académico que pode ser utilizado por terceiros desde que respeitadas as regras e boas práticas internacionalmente aceites, no que concerne aos direitos de autor e direitos conexos.

Assim, o presente trabalho pode ser utilizado nos termos previstos na licença abaixo indicada.

Caso o utilizador necessite de permissão para poder fazer um uso do trabalho em condições não previstas no licenciamento indicado, deverá contactar o autor, através do RepositóriUM da Universidade do Minho.



**Atribuição
CC BY**

<https://creativecommons.org/licenses/by/4.0/>

Agradecimentos

Aos meus orientadores, Diego Carreira Flores e Marcos Rubal Garcia, pela oportunidade de realizar este trabalho sob a sua orientação, pelo conhecimento transmitido, por toda a ajuda prestada ao longo dos meses de trabalho de laboratório e na escrita desta dissertação, pela leveza com que me ajudaram a encarar os aspetos mais difíceis na realização deste trabalho, e por tornarem todo este processo mais divertido.

A todos os que fizeram parte do meu percurso académico e sempre me motivaram a fazer melhor, por todos os momentos de alegria que vivemos e por estarem sempre disponíveis.

À minha amiga Inês, pelos almoços no jardim e no vegetariano, e pela companhia nos dias mais difíceis e solitários no laboratório.

Ao Samuel, pela calma, paciência e confiança que me transmitiu nos momentos de dúvida ao longo da execução deste trabalho.

À minha irmã, por toda a ajuda e por ter sempre a palavra certa nos momentos certos. Por nunca me deixar desistir e por me motivar sempre a seguir os meus sonhos.

Aos meus pais, por todo o esforço, carinho, dedicação e apoio incondicional que demonstraram durante todo este percurso. Sem vocês nada disto seria possível, obrigada por me incentivarem sempre a lutar pelo que gosto e a nunca desistir, por muito difícil que as coisas se tornem.

A todos vós, o meu mais sincero obrigado!

DECLARAÇÃO DE INTEGRIDADE

Declaro ter atuado com integridade na elaboração do presente trabalho académico e confirmo que não recorri à prática de plágio nem a qualquer forma de utilização indevida ou falsificação de informações ou resultados em nenhuma das etapas conducente à sua elaboração.

Mais declaro que conheço e que respeitei o Código de Conduta Ética da Universidade do Minho.

Resumo

Os fundos sedimentares são o maior ecossistema do planeta, em área de cobertura, e albergam uma grande diversidade de organismos macrobentônicos. As comunidades macrobentônicas apresentam um importante papel nos ecossistemas marinhos, na reciclagem de nutrientes, decomposição de matéria orgânica, e nas condições do sedimento. Apesar da sua extensão e importância, apenas uma pequena percentagem da sua comunidade macrobentônica foi estudada, e muitas espécies continuam por descrever. Os principais fatores físicos na determinação da distribuição da fauna macrobentônica são: a ação das ondas, e as características do sedimento, podendo estes ser afetados pela presença de estruturas artificiais de proteção costeira.

Este estudo foi realizado na zona costeira do Norte de Portugal, em Viana do Castelo, em duas áreas: Baía de Coral, uma área protegida pela presença de um molhe, e em Cabedelo, uma área exposta à ação das ondas. Em cada área, foram amostrados três locais escolhidos aleatoriamente, em três datas diferentes. A fauna macrobentônica recolhida nestas amostragens foi identificada, foram medidos parâmetros ambientais, e calculados parâmetros como a diversidade, riqueza de espécies e a abundância de indivíduos. Os resultados mostraram diferenças significativas entre os dois habitats: na salinidade, riqueza de espécies e número de indivíduos. A análise PERMANOVA mostrou diferenças significativas entre os habitats, demonstrando que as comunidades macrobentônicas são diferentes, e a análise AMBI mostrou que a área protegida apresenta um maior grau de perturbação do que a área exposta. Os resultados obtidos relativamente à abundância e riqueza de macroinvertebrados sugere que a estrutura das comunidades pode estar relacionada com os diferentes recursos disponíveis e com as condições hidrodinâmicas e do sedimento, sendo as últimas alteradas pela presença do molhe.

Palavras-chave: fundos sedimentares, estruturas artificiais, molhe, comunidades macrobentônicas, protegido, exposto.

Abstract

Soft-bottoms are the largest ecosystem on Earth in areal coverage and harbor a wide diversity of macrobenthic organisms. Macrobenthic fauna plays a very important role in marine ecosystems in nutrient cycling, decomposing organic matter, and on the conditions of the sediment. Although soft-bottoms are the largest ecosystem on earth, only a small percentage of their macrobenthic fauna has been studied and most of its species are still undescribed. Two of the main physical factors affecting macrobenthic fauna distribution are wave action and sediment characteristics, and these can be changed by the presence of artificial protection structures.

This study was conducted on the Northern coast of Portugal, in Viana do Castelo, in two areas: Coral Bay, protected by a breakwater, and Cabedelo, which is exposed to wave action. In each area, three random sites were sampled on three different dates. The macrobenthic fauna was later identified and environmental parameters, richness, diversity and abundance were measured.

The results showed significant differences in salinity, species richness and number of individuals between the two habitats. PERMANOVA analysis showed significant differences between the habitats, showing the assemblages are different, and AMBI analysis showed that the protected habitat was more disturbed than the exposed one.

The patterns of abundance and richness of macroinvertebrates suggest that assemblage's structure may be related to different resources available, sediment and hydrodynamic characteristics, that are altered by the presence of the breakwater.

Keywords: soft-bottoms, artificial structures, breakwater, macrobenthic assemblages, protected, exposed.

Index

Agradecimentos	iii
Resumo	v
Abstract	vi
List of Figures	ix
List of tables	x
1. Introduction	2
1.1. Coastal zones	2
1.2. Portuguese coast characteristics	4
1.3. Soft-bottoms	5
1.4. Macrobenthic fauna	6
1.5. Anthropogenic pressures	8
1.6. Goals	9
2. Material and Methods	12
2.1. Study area	12
2.2. Sampling design	13
2.3. Sample processing	15
2.4. Statistical analysis	16
2.5. AMBI (AZTI's Marine Biotic Index)	18
3. Results	20
3.1. Environmental parameters	20
3.1.1. Salinity	20
3.1.2. Granulometry	21
3.2. Richness, diversity and abundance	21
3.3. Multivariate analysis	25

3.3.1.	Permanova, PERMDISP and nMDS.....	25
3.3.2.	Simper analysis	27
3.4.	AMBI (AZTI's Marine Biotic Index)	33
4.	Discussion	36
5.	Conclusion	42
6.	References	44
Appendix 1	49

List of Figures

Figure 1- Biogeographic subdivisions in the Atlantic Ocean, from Afonso et al. (2000);	3
Figure 2- Seafloor lithology, available in: http://portal.gplates.org/cesium/?view=seabed	6
Figure 3- Map from 1913 representing Viana do Castelo coastal area, overlaid with a satellite image of Viana in 2022;	13
Figure 4- Sampling sites in Coral Bay and Cabedelo, and surrounding habitat type. Blue dots: sampling sites in Coral Bay; Red dots: sampling sites in Cabedelo area. Adapted from Carreira-Flores et al. (2023);	14
Figure 5- Collection of sediment samples. A: Retrieving the grab from the water; B: Opening the grab and depositing the sample in the bucket; C: Buckets with the labeled samples inside; Erro! Marcador não definido.	
Figure 6- Sample processing. A: Plastic container with one sample; B: Petri dish with small quantity of sediment ready for sorting; C: Example of organisms already identified and placed in proper container; .	16
Figure 7- Sampling design used for univariate and multivariate analyses of this study;.....	17
Figure 8- Salinity of the two habitats on the three different dates.....	20
Figure 9- Total number of individuals (A) and percentage (B) of each Phylum in the two habitats throughout the different dates;.....	22
Figure 10- Shannon diversity index of the two habitats on the three different dates;	23
Figure 11- Number of individuals of the two habitats on the three different dates.* indicates the significant differences.....	24
Figure 12- Species richness in the two habitats on the three different dates.....	25
Figure 13- nMDS plot of centroids Coral (black) and Cabedelo (white) at three dates of sampling;.....	27
Figure 14- Exclusive and common species in the habitats in July 2021;	29
Figure 15- Exclusive and common species in the habitats in December 2021;.....	31
Figure 16- Exclusive and common species in the habitats in May 2022;	33
Figure 17- Ecological status of each site in the two habitats. Stations: 1,2,3: Coral July 2021/ 4,5,6: Cabedelo July 2021/ 7,8,9: Coral December 2021/ 10,11,12: Cabedelo December 2021/ 13, 14, 15: Coral May 2022/ 16, 17, 18: Cabedelo May 2022;	34
Figure 18- Ecological groups in each replica, in each site, on the two habitats. Stations: 1,2,3: Coral July 2021/ 4,5,6: Cabedelo July 2021/ 7,8,9: Coral December 2021/ 10,11,12: Cabedelo December 2021/ 13, 14, 15: Coral May 2022/ 16, 17, 18: Cabedelo May 2022;	34

List of tables

Table 1- Results of ANOVA salinity. *p<0.05, significant differences indicated in bold;	20
Table 2- Granulometry of the collected sediment samples. Fine gravel (2-4 mm), very coarse sand (1-2 mm), coarse sand (0.5-1 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.063-0.125 mm) and silt/clay (<0.063 mm).	21
Table 3- Number of individuals and species richness in each habitat on the three different dates;	21
Table 4- Results of ANOVAs of Shannon diversity index (H), Number of individuals (N) and Species richness (S). *p<0.05, significant differences indicated in bold;	23
Table 5- Results of PERMANOVA testing differences in macrobenthic assemblage structure across the studied habitats. Analyses based on Bray-Curtis dissimilarity matrix from square-root transformed data. All tests used 999 random permutations. Results of PERMDISP analysis. *p<0.05, significant differences indicated in bold;	26
Table 6- Post hoc analyses of PERMANOVA. *p<0.05, significant differences indicated in bold;	26
Table 7- PERMDISP pairwise comparison results;	26
Table 8- Average abundance, contribution of individual taxa and cumulate percentage (Cum %) from Coral and Cabedelo in July 2021;	28
Table 9- Average abundance, contribution of individual taxa and cumulate percentage (Cum %) from Coral and Cabedelo in December 2021;	30
Table 10- Average abundance, contribution of individual taxa and cumulate percentage (Cum %) from Coral and Cabedelo in May 2022;	32
Table 11- List of species present in Coral and Cabedelo in the three dates;	49

1. Introduction

1. Introduction

One of the main goals of ecology is understanding and detecting the patterns underlying the changes in the structure of organism assemblages, which may occur over space and time (Horner-Devine et al., 2007). Ecologists have devoted substantial attention to the role of the factors that shape and can alter the richness, structure, distribution and abundance of such assemblages (Dunson & Travis, 1991). These factors can be biotic, (predation, competition) or abiotic (habitat structure, food availability and hydrodynamic characteristics) (Hoffman & Blows, 1994). The underlying challenge, however, is to quantify and understand which combination of factors has the greatest influence on the structure of ecological communities (Dunson & Travis, 1991)

Planet Earth is covered by oceans, which represent 71% of the Earth's surface. These are life-filled ecosystems, harboring wide biodiversity, and offer a wide range of essential goods and services with economic and ecological value (Hoegh-guldberg & Bruno, 2010; Scavia et al., 2002).

However, with the rapid growth of population in coastal areas, these ecosystems are under several anthropogenic pressures, with human activities driving rapid changes in the structure of organism assemblages (Hoegh-guldberg & Bruno, 2010). The near-shore development leads to the construction of many structures to facilitate resource exploitation and protection of human activities (Todd et al., 2019). The history of coastal occupancy and coastal development around the globe shows a pattern of depletion of coastal resources and loss of biodiversity (Clark, 1995). The problem in detecting resource damage and lowered biodiversity is that many critical marine habitats are often not visible or evident to most observers (Clark, 1995), therefore the process of understanding the effects of urbanization on marine ecosystems and ecological processes is growing.

1.1. Coastal zones

Oceans cover the majority of the Earth's surface and play a fundamental role in the functioning of the planet through their biomass, productivity, and diversity, providing an extensive area of resources. Its biosphere is dominated by marine organisms with high ecological and socioeconomic importance, concentrated mainly in coastal ecosystems (Harley et al., 2006).

Coastal zones are unique. They are defined as the interface between the terrestrial and marine domains and develop between the edge of the continental shelf (depth of 200m) and the limit of

the land that emerged from the last marine transgression (CMIA, 2012). These zones include the intertidal and supratidal zones of the water's edge, which include coastal floodplains, mangroves, marshes, and tide flats, as well as beaches and dunes and fringing coral reefs (Clark, 1995). This transitional strip of land and sea that straddles the coastline, encompasses some of the most valuable habitats of the biosphere, including estuaries, coastal wetlands, and lagoons, with high biological productivity. It is also a place of natural dynamism where huge amounts of natural energy are released and a great abundance of life is nurtured (Clark, 1995).

Biogeographic areas and subareas, with common biotic and abiotic features, can be defined according to the flora and fauna present in the context of the general ecosystem structure, which results from the major climatic, topographical, and evolutionary characteristics of the oceans and continental land masses (Afonso et al., 2000).

There is a significant north-south temperature gradient on the European continental border of the Atlantic Ocean, limiting the distribution of many species and leading to a biogeographic subdivision of the eastern Atlantic into two provinces: the boreal Atlantic province and the subtropical Lusitanian province (Fig.1). The climatic conditions and communities of animal and plant species differ significantly across the Lusitanian province, which stretches from the western coastlines of the Iberian Peninsula to Brittany. The Lusitanian province has been subdivided into the subtropical subprovince, Strait of Gibraltar to Finisterre, and the subtropical/boreal transition subprovince, Finisterre to Brittany (Afonso et al., 2000).



Figure 1- Biogeographic subdivisions in the Atlantic Ocean, from Afonso et al. (2000);

1.2. Portuguese coast characteristics

Portugal is located in the subtropical Lusitanic zone (Fig.1), in south-west Europe, occupying most of the western part of the Iberian Peninsula. Continental Portugal's coastline is particularly characterized by intertidal and shallow subtidal, rocky and sandy beaches. The shores are completely wave-exposed from the North to the Center of the country, from where they become moderately wave-exposed until the South of Portugal (Gaspar et al., 2019).

The sea surface temperature pattern is different in the north and south of Portugal, in the winter it fluctuates between 12°C in the north, to 16.5°C in the south. In the summer, the temperature varies from 18°C in the north, to 22°C in the south. From June to October occurs a very important phenomenon near the northern coast that brings cold, mineral-rich, deep water to the surface, thereby decreasing coastal water temperature to 16°C. This phenomenon is called “Upwelling” responsible for enriching this region with nutrients and therefore increasing productivity (CMIA, 2012; Gaspar et al., 2019).

In the summer, the two main currents affecting the Portuguese coast are: the Canary Current, originating from the north and with a strong southward flow (12 cm s^{-1}), and the Azores Current, that enters the region in the south, establishing a west to east circulation. In winter, the Azores Current presents twice the velocity and there is little circulation of seawater in the region. The North coast of Portugal is affected by a semi-diurnal tidal regime, with the largest spring tides reaching 4m. With a dominant swell from the west and northwest, this straight coast is highly exposed to a particularly intense wave action. The most common wave heights are 1.5 to 2m, with an interval period of 11–13s, and during winter wave extreme events, wave height values can reach about 7–11 m. In terms of the salinity of seawater, it can vary from 35‰ to 26‰ along the Portuguese mainland, depending on the proximity of fresh water sources (Gaspar et al., 2019).

In terms of lithology, the north Portuguese coast is a very fragmented habitat. This area is dominated by rocky reefs, pebbled bottoms, and soft-bottoms resulting from marine erosion (Carreira-Flores et al., 2023). Soft-bottoms at shallow sublittoral are restricted to patches surrounded by large extensions of boulders and rocky substrates, the latter being the predominant habitat (Veiga et al., 2017).

These are some of the unique characteristics of the Portuguese coast, which is strongly influenced by a great climatic diversity, allowing for great biological richness (both flora and fauna) along its approximately 830 km strip (Braun-Blanquet et al., 1973).

1.3. Soft-bottoms

Soft-bottom substrates cover most of the world's ocean bottom, and only a relatively small proportion is formed of primary or secondary hard bottoms (Castelli, A. Lardicci et al., 2004). Covering about 70% of the Earth's surface, this is the largest ecosystem on our planet in areal coverage (Snelgrove, 1997). Soft-bottom subtidal habitats include all of the unconsolidated substrate areas on the ocean bottom (clay, sand, granule pebbles), ranging from gravel to fine muds (Fig.2). Some sediments are uniform in grain size, some are mixed, some are biological in origin and others are geological (Snelgrove, 1997)

These habitats provide an essential substrate for the dominant biomass of organisms present to live, thrive and maintain a substantial part of the world's biodiversity (Snelgrove, 1998). This way, in view of their widespread distribution and the importance and diversity of benthic infaunal assemblages, the soft-bottoms contain several systems that are of ecological interest (Castelli, A. Lardicci et al., 2004).

Although soft-bottoms are a large ecosystem, only a small percentage of their macrobenthic fauna has been studied and most of its species are still undescribed (Snelgrove, 1998). As such, it is important to improve our knowledge of its biodiversity (Boutoumit et al., 2021).

In the North of Portugal, there are few studies regarding soft-bottoms and their macrobenthic assemblages, like, for example, Veiga et al. (2017). There are studies carried out in Viana do Castelo concerning soft-bottoms, Sousa et al. (2006,2007), but these were carried out in the Lima estuary and not exactly in the coastal area. Therefore, this is a poorly studied habitat and there is an increase in the need for these types of studies, particularly in the face of increasing anthropogenic pressures and environmental changes.

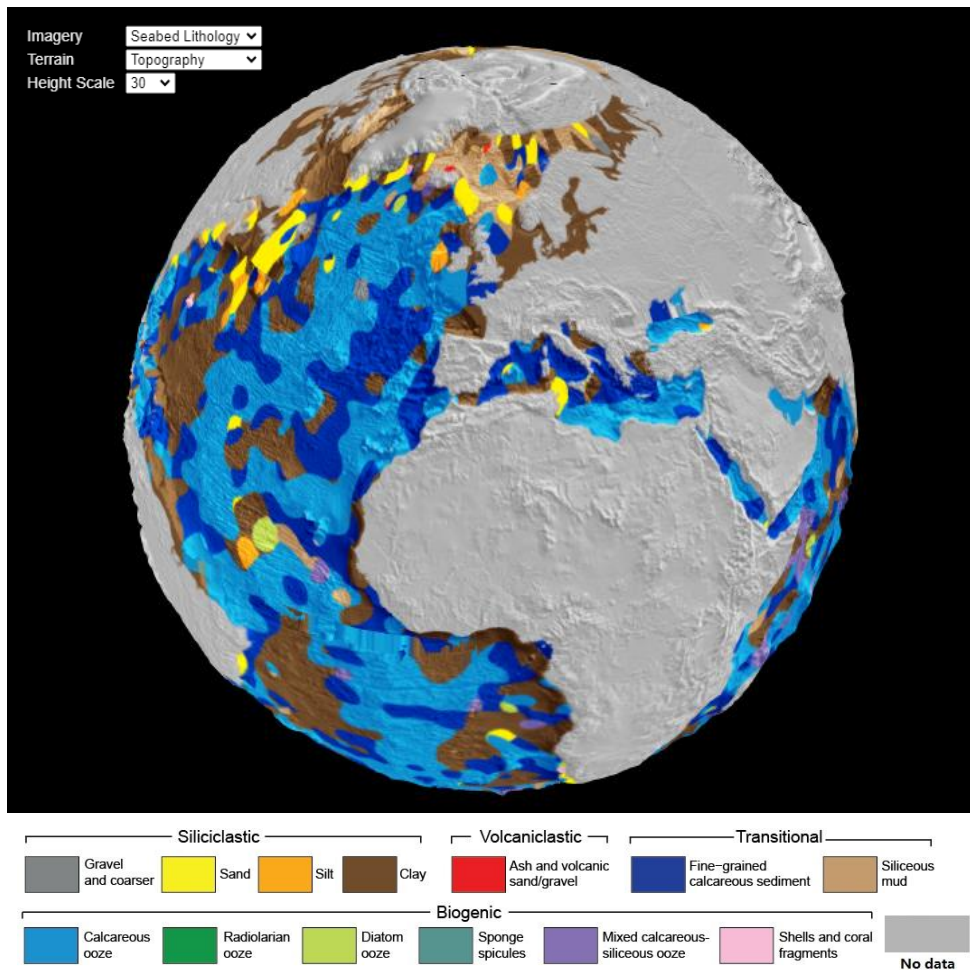


Figure 2- Seafloor lithology, available in: <http://portal.gplates.org/cesium/?view=seabed;>

1.4. Macrobenthic fauna

Macrofauna constitutes a group of animals characterized by their size, that may be abundant in soft-bottoms. This habitat is defined by a combination of sediments types, including sand, mud, and gravel, which serves as an essential substrate for the development and existence of this dominant biomass of organisms (Snelgrove, 1998). The size of these organisms is determined by the mesh of a sieve on which they can be found after the removal of some or all of the sediment particles. For macrofauna, that means sieves with mesh sizes between 500 μ m (0.5 mm) and 5 cm (Watling, 2019). The macrobenthic community is complex and encompasses a huge array of life with many phyla involved (Saurabh, 2014) such as: annelids, molluscs (e.g. bivalves and gastropods), and crustaceans (e.g. amphipods and decapods). Benthic invertebrates can be differentiated by the position they occupy on or in bottom sediments. There are two groups: infauna, characterized by animals that live in sediments, almost all annelids and bivalves belong to this

category; and epifauna, which are the organisms that live on the surface of bottom sediments, many crustaceans and gastropods (Tagliapietra & Sigovini, 2010).

Macrobenthic invertebrates in soft-bottoms are highly diverse in taxonomy, morphology, functionality, mobility and life history characteristics (Ólafsson, 2021). Most macrofaunal species distributions appear to be related to differences in sediment type, temperature, salinity and physical disturbance. All of these variables act together to create physical and ecological constraints on species' distributions (Snelgrove, 1998).

Macrobenthic assemblages play very important roles in the ecology and functioning of soft-bottoms. They structure and oxygenate the bottom by reworking sediments, through burrowing and tunneling activities, they “stir” the sediment and the porewater, moving particles around that might come into contact with other animals who can use them for food. A related activity is the creation of new habitats for smaller meiofaunal species, by the construction of large burrows through which oxygenated water is pumped (Watling, 2019).

Since macrofauna is so abundant and capable of producing large numbers of offspring, and in some cases of rapid reproduction, such organisms are frequent food items for a variety of larger animals, including crabs, fish, birds, and whales (Watling, 2019). Therefore, benthic invertebrates play this important link between primary production with higher trophic levels, by filtering phytoplankton and then acting as a food source for larger organisms (Tagliapietra & Sigovini, 2010). Due to their abundance and “intermediate” position in the aquatic food chain, benthic organisms play an essential role in the natural flow of energy and nutrients. (Saurabh, 2014). Benthic invertebrates also form a major component of the overall animal biodiversity of aquatic ecosystems. The total taxonomic richness of invertebrates may exceed the richness of all vertebrate groups combined by two to three times (Saurabh, 2014).

In addition, several benthic invertebrates, particularly bivalves and decapods, are consumed by humans, and others, such as annelids, are used for recreational purposes as fishing bait (Tagliapietra & Sigovini, 2010).

Benthic assemblages are often used as biological indicators and are effective and useful for the assessment of coastal system quality because they can provide information on environmental conditions, either due to the sensitivity of single species or because of some general feature that makes them integrate environmental signals over a long period (Tagliapietra & Sigovini, 2010). A few of these features are, for instance: a display of a sedentary lifestyle or limited mobility, restricting their ability to avoid adverse conditions, intermediate trophic level positions, relatively

long life-span, taxonomic and functional diversity, varying responses to changes in environmental stress and their easily quantitative sampling (Martin et al., 2005).

Within soft-bottom ecosystems, sublittoral macrobenthic assemblages have been less studied than those from the intertidal and remain largely unknown (Veiga et al., 2017). Since shallow marine areas are among the most endangered ecosystems worldwide, and the impact of use and abuse will no doubt increase, evaluating and understanding macrobenthic biodiversity in these habitats is very important (Gray & Elliot, 2009).

1.5. Anthropogenic pressures

The world's population is undergoing a rapid urbanization, marked by a significant migration trend towards coastal regions. Population density at the coast is continuously increasing, leading to a greater concentration in coastal cities. These cities are hubs for transport and trade, given that human density is strongly related to resource exploitation, and inevitably leads towards nearshore development (Todd et al., 2019).

This development usually starts with a harbor, to facilitate navigation and fishing activities, but eventually leads to the construction of other structures. "Ocean sprawl" is a term used to describe the proliferation of human-made hard structures in the marine environment. This comprises coastal infrastructures, such as artificial shore defenses (e.g., breakwaters, groins), as well as facilities associated with harbors and docks (Todd et al., 2019). Such engineering structures, like artificial coastal protection structures, are very common along European coasts. They can shelter or expose a shoreline to waves from specific directions, altering processes and sediment interaction, and producing landforms that would not evolve in the absence of the structure (Jackson et al., 2015). Breakwaters are built to mitigate coastal erosion by reducing wave action, and this happens through a combination of reflection and dissipation of incoming wave energy. When used for harbors, breakwaters are constructed to create sufficiently calm waters for navigation, protection of harbor facilities, and other human activities (Lemos et al., 2017). Therefore, these structures alter hydrodynamics, by interrupting wave action and modifying the water circulation, and thus sedimentary characteristics, by affecting its transport and leading to changes in bottom topography, sediment grain size, and organic content of the surrounding habitats (Bertasi et al., 2007; Martin et al., 2005). Furthermore, they create a barrier to movement along the shore, leading to altered patterns of larval supply and food availability to the present macrofauna (Walker et al., 2008).

In some heavily urbanized regions, entire habitats have been lost as artificial structures proliferate over vast distances, since these are replaced by concrete and granite. All these changes alter ecological connectivity, and have significant effects on marine assemblages (Bishop et al. 2017). Macrobenthic community structure strongly depends on a range of biotic and abiotic factors, that seem to control directly or indirectly its abundance and distribution (Castelli et al., 2004) by influencing food availability, bottom-water oxygenation, and larval dispersion (Veiga et al., 2017). Wave action, which controls the physical environment, and sediment characteristics are considered one of the main physical factors affecting the distribution of shallow soft-bottoms benthic fauna (Bertasi et al., 2007), two factors widely altered due to the presence of artificial structures, as referred to above. Thus, all these changes and disturbances affect the composition and structure of macrobenthic assemblages and could result in different colonizing assemblages (Todd et al., 2019).

Ocean sprawl is a fundamental and prominent aspect of urbanized marine environments, with man-made structures forming the majority of shorelines in many coastal cities and altering habitats deep into the subtidal zone (Todd et al., 2019). Hence, while these structures provide valuable protection to human infrastructure, they also have significant impacts on soft-bottoms benthic assemblages, as they influence species abundances, distribution patterns, and community structure (Bertasi et al., 2007).

1.6. Goals

In Europe, there are numerous studies regarding the effects of artificial structures on macrobenthic fauna. Martin et al. (2005) is one of many research studies integrated in an European-wide study to identify, describe and quantify the impacts of artificial structures on the biodiversity and functioning of soft-bottoms macrobenthic assemblages. This study included different kinds of artificial structures in different countries, such as Spain, Italy and the United Kingdom.

Unfortunately, in the North of Portugal, there are no studies regarding the effects of artificial protection structure on soft-bottoms macrobenthic fauna. Although there are studies carried out in Viana do Castelo regarding soft-bottoms, Sousa et al. (2006,2007), these were carried out in the Lima estuary, assessing the river gradient, and thus being a study under different hydrodynamic conditions and different environmental parameters from the ones in this study.

Thus, since the macrobenthic assemblages are widely undescribed in soft-bottoms, and the effects of artificial protection structures on such assemblages is yet highly unknown, and considering the high presence of artificial structures along the Portuguese coast, there is a need for these types of studies to increase for a better understanding of our fauna and to help improve management decisions.

This study aims to compare and explore the differences between macrobenthic assemblages inhabiting the shallow soft-bottoms in a natural wave exposed area and an area protected by an artificial structure. Understanding how the presence or absence of this artificial protection influences the species diversity and assemblage structure in soft-bottoms is a very important goal in managing the impact of these hard structures. For that, this study was conducted on the northern coast of Portugal, in Viana do Castelo, in two areas: Coral Bay (41.6836° N, 8.8457° W) and Cabedelo (41.6791° N, 8.8326° W). Coral is a shallow bay protected by one breakwater and Cabedelo is exposed to wave action, and it is expected to observe differences in the macrobenthic assemblages between these two areas. The proposed hypothesis is “that the breakwater will modify water and sediment characteristics and thus affect the structure of macrobenthic assemblages”.

2. Material and Methods

2. Material and Methods

2.1. Study area

This study was carried out in the North of Portugal in Viana do Castelo. This city has developed and occupied the right margin of the Lima River estuary and the adjacent coastal area, the latter being occupied by commercial harbor facilities.

In the last 400 year, Viana do Castelo harbor has suffered countless transformations with the construction of different structures to improve its protection and its navigation (Fig.3) (Geoparque Litoral Viana do Castelo, 2020). Viana's harbor waters are protected by two breakwaters: the outer and main one, developing on a 170° bearing and with 2170 meters ("Molhe Exterior"), and the inner one, with about 700 meters on a South/southwesterly orientation ("Molhe do Cabedelo") (Fig.4). These two heavy constructions were built between 1977 and 1981 and induced profound changes in alluvial transport, essential for the existence of the harbor area, namely the fishing and commercial harbors (Vale, 2008). The disturbance of the predominant swell direction, from the northwest, at the head of the main breakwater, generates a wave pattern from southwest to the interior of the mouth of the Lima River, responsible for the creation of Praia do Coral (Geoparque Litoral Viana do Castelo, 2020). These two areas are the ones chosen for the sampling, Cabedelo (41.6791° N, 8.8326° W) which is exposed to wave action and Coral (41.6836° N, 8.8457° W), which is protected by the main breakwater, the artificial protection structure we focused on for this study (Fig.4).



Figure 3- Map from 1913 representing Viana do Castelo coastal area, overlaid with a satellite image of Viana in 2022;

2.2. Sampling design

Sampling was conducted on three dates: July 2021, December 2021, and May 2022. A three-factor sampling design was used to explore the macrobenthic assemblages present in protected and exposed shallow subtidal soft-bottoms. At each locality, three sites were randomly chosen (Fig.4). Sediment samples within each site were randomly collected, using a Van Veen grab (sampling surface of 0.12 m²) to a maximum depth of 10m. At each site, a total of three grabs were collected for macrofaunal identification.

Once on board the boat, the grab was opened above a plastic bucket and the sample was gently removed and then labeled (Fig.5).

A sample of 100ml of sediment of each habitat was retrieved for posterior granulometric composition. The salinity of the water was measured at just one site in each habitat, on each date, making a total of 6 measurements.

Macrobenthic samples were then sieved with a 0.5 mm mesh. This process consists of sieving the sediment sample in seawater, in order to eliminate fine sediments. The retained macrofauna and sediment in the mesh were then placed in labeled plastic containers and fixated with 4% neutralized formaldehyde solution with Rose Bengal, until its posterior study (Fig.6A). Fixation of a biological sample prevents post-mortem degeneration of tissue, thus preserving the structure as unchanged as possible so the individuals can be more easily identified by their morphological traits.

The material contained in the samples was colored to help the sorting procedures, by making identification of the organisms easier during this phase. Although it may alter the color pattern of some organisms, for example, annelids, making identification more challenging.

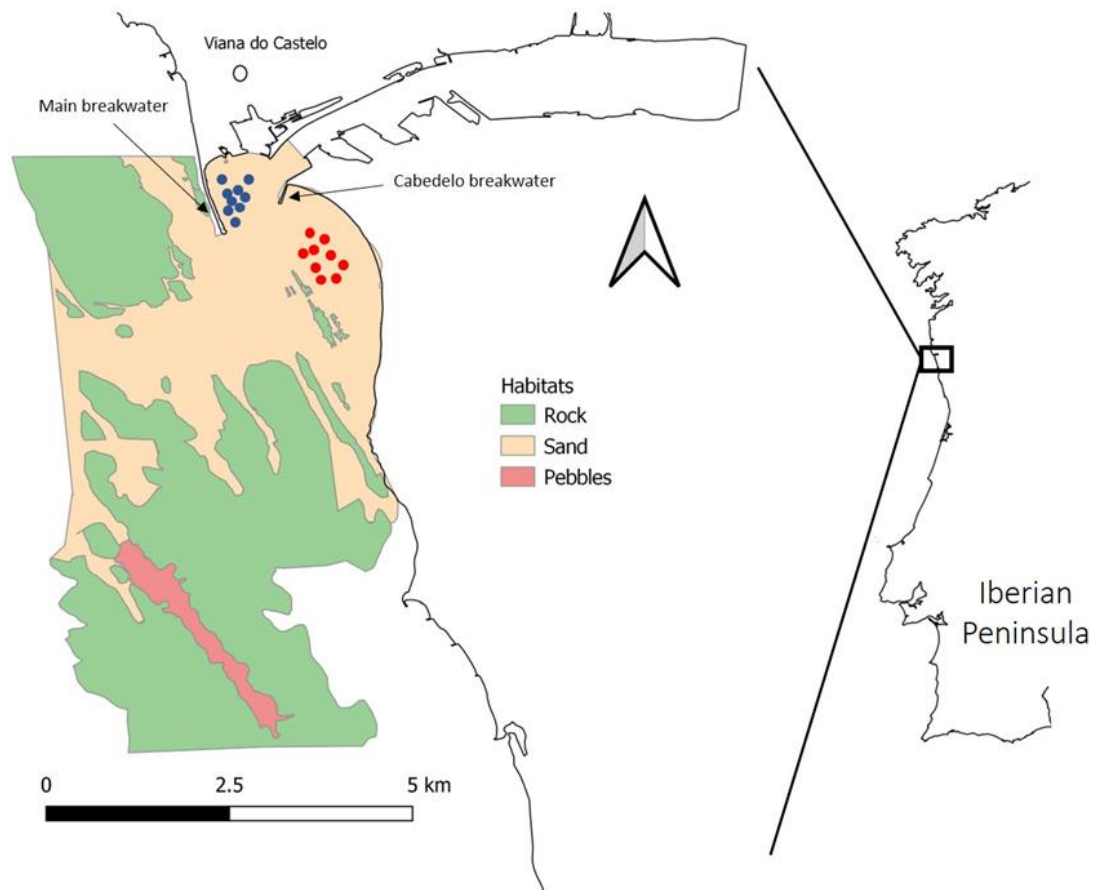


Figure 4- Sampling sites in Coral Bay and Cabedelo, and surrounding habitat type. Blue dots: sampling sites in Coral Bay; Red dots: sampling sites in Cabedelo area. Adapted from Carreira-Flores et al. (2023);

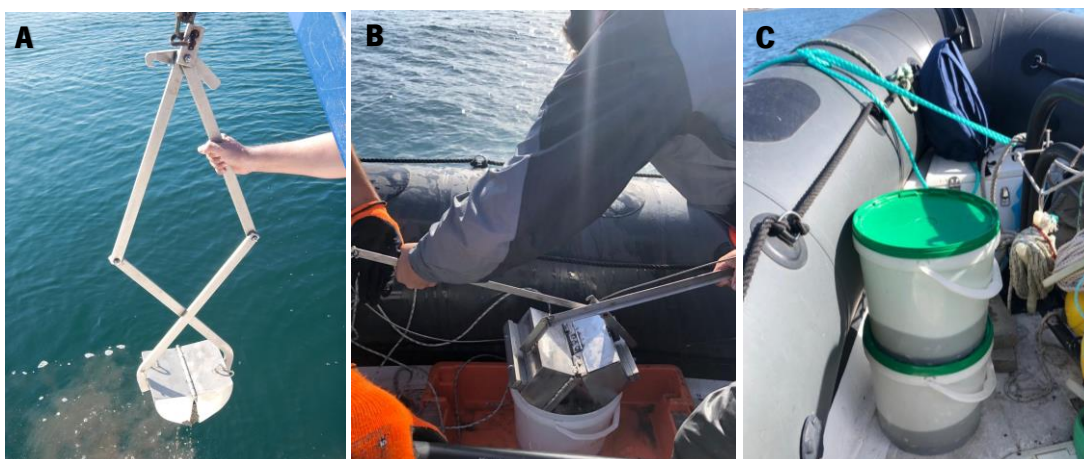


Figure 5- Collection of sediment samples. **A:** Retrieving the grab from the water; **B:** Opening the grab and depositing the sample in the bucket; **C:** Buckets with the labeled samples inside;

2.3. Sample processing

In the lab, the macrobenthic fauna from each replica was sorted. Sorting consists of picking up from the sieved material all the animals that were alive at the moment of the sampling. So, during this phase, a small quantity of the sample was spread onto a Petri dish and carefully examined to identify the organisms (Fig.6B). These organisms were picked up and placed in different containers according to the main taxonomic groups: annelids, molluscs, crustaceans, and “others”. The larger organisms were stored in appropriate containers, making sure that no other smaller animals were attached to their bodies (Fig.6C).

When animals with shells, like bivalves or molluscs, or animals living in calcareous tubes were present, it was necessary to prove the presence of soft parts, which were usually colored pink.

Then, these organisms were identified to the lowest possible taxon (usually species level) with visual help using a stereomicroscope and identification guides, such as “British Fauna”, “Handbook of the Marine Fauna of North-West Europe”, “Fauna Iberica”, species description papers and consulting the database World Register of Marine Species (WoRMS) (WoRMS Editorial Board, 2023) for the most updated species names and taxonomic classification.

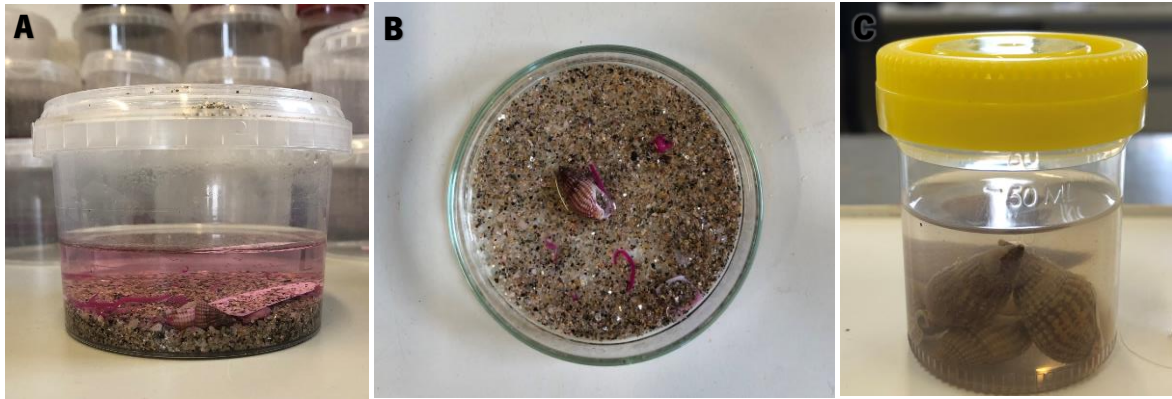


Figure 6- Sample processing. **A:** Plastic container with one sample; **B:** Petri dish with small quantity of sediment ready for sorting; **C:** Example of organisms already identified and placed in proper container;

2.4. Statistical analysis

Multivariate and univariate techniques were used to analyze data in order to test the hypothesis: “the breakwater will modify water and sediment characteristics and thus affect the structure of macrobenthic assemblages”.

The sampling design for the parameter of salinity included two factors: Date (random, three levels, time1 vs. time 2 vs. time 3) and Habitat (fixed, two levels, exposed vs. protected). The sampling design for the rest of the analysis included three factors: Habitat (fixed, two levels, exposed vs. protected), Site (random, three levels, site1 vs. site 2 vs. site 3, nested in “area”), and Date (random, three levels, time1 vs. time 2 vs. time 3), with three replicates sampled in each site at each date (Fig.7).

ANOVA analysis were done to evaluate salinity, species richness, number of individuals, and the Shannon diversity index of macrobenthic assemblages, and results were plotted in SigmaPlot 10.0 (Systat Software Inc., 2006).

A study of the granulometric composition of the samples was also carried out, considering the following sedimentary fractions: coarse gravel (>4 mm), fine gravel (2-4 mm), very coarse sand (1-2 mm), coarse sand (0.5-1 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.063-0.125 mm) and silt/clay (<0.063 mm).

Permutational multivariate analysis of variance (PERMANOVA) (M. J. Anderson, 2001) based on Bray-Curtis untransformed and square-root transformed dissimilarities was used to analyze the multivariate assemblage data. Analysis of untransformed data aimed to detect changes in dominant species but, square root transformed data analysis was done to downweigh the influence of

dominant taxa and consider all the present species (Rubal et al., 2014). In order to test whether differences in assemblages between sites were due to different multivariate dispersion between groups rather than in the location of centroids, the PERMDISP procedure was done (Anderson et al., 2008). Multivariate patterns were illustrated by non-metric multidimensional scaling (nMDS) ordination of sampled sites for each date, which were done with square-root transformation of the data and Bray Curtis distance. The SIMPER procedure was used to determine the percentage contribution of each taxon to the dissimilarity between assemblages sampled in sheltered and exposed habitats.

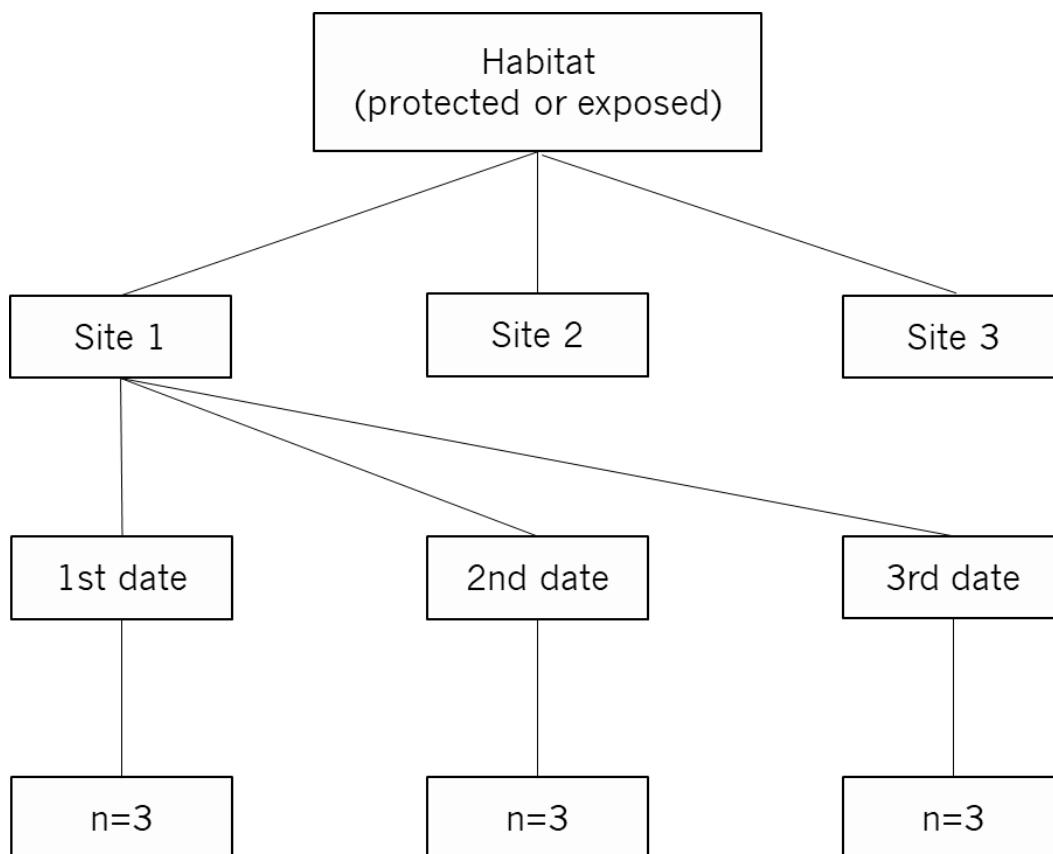


Figure 7- Sampling design used for univariate and multivariate analyses of this study;

2.5. AMBI (AZTI's Marine Biotic Index)

To assess the ecological status of the protected and exposed sites, the AMBI index (AZTI's Marine Biotic Index) was used. AMBI values were calculated using the software available at AZTI's webpage (<http://ambi.azti.es>). This index was designed to analyze the response of macrobenthic assemblages to changes in environmental quality. The species are classified into five ecological groups, and the index is based on the percentage of their abundance according to their sensitivity to environmental stress and organic pollution, already listed in the software (Garaffo et al., 2017; Warwick et al., 2010). According to the species list included in the software package AMBI, most of the species present at Viana do Castelo were assigned to a group, except for *Tanaissus bamberi* (Esquete, 2015) because of its recent discovery.

3. Results

3. Results

3.1. Environmental parameters

3.1.1. Salinity

The ANOVA analysis showed that there were significant differences in the Date and Date x Habitat factors (Table 1). The post hoc analysis showed there are significant differences in the July 2021 and December 2021. The mean value of salinity in July was ≈ 32 and ≈ 35 for Coral and Cabedelo, respectively, and in December this value was ≈ 25 and ≈ 33 for Coral and Cabedelo, respectively (Fig.8).

Table 1- Results of ANOVA salinity. * $p < 0.05$, significant differences indicated in bold;

Source	DF	MS	F	P	F versus
Da	2	40.6739	130.74	0*	RES
Ha	1	67.2800	3.21	0.2152	DaxHa
DaxHa	2	20.9817	67.44	0*	RES
RES	12	0.3111			
Total	17				

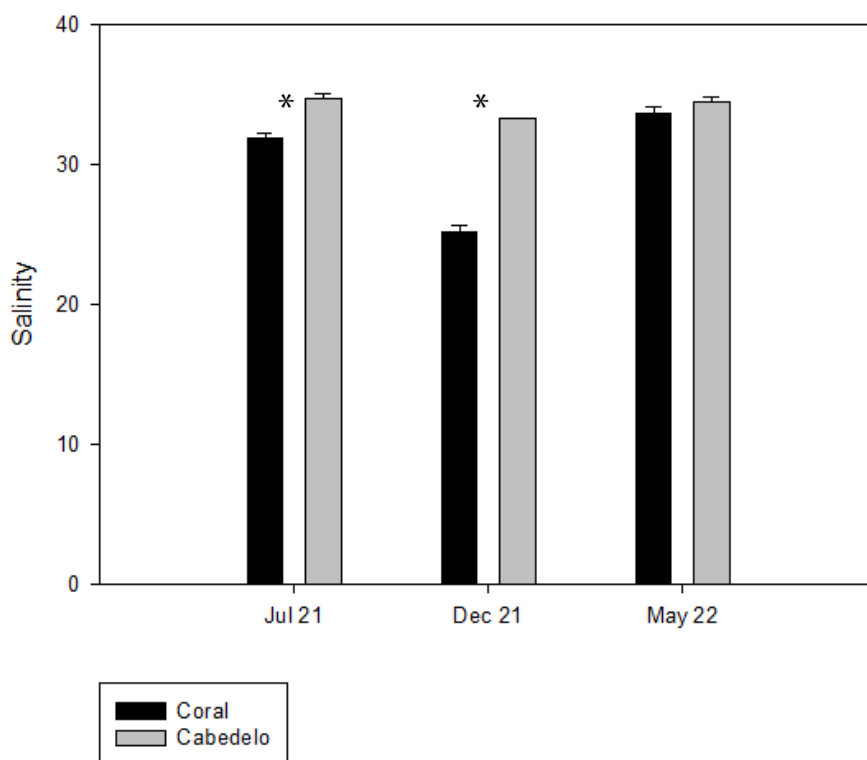


Figure 8- Salinity of the two habitats on the three different dates. *indicates the significant differences;

3.1.2. Granulometry

This analysis showed that Coral sediments had a higher content of organic matter, with a value of 3.1%, whereas Cabedelo sediments had a value of 0.9%. In terms of granulometry, Coral had higher percentages of fine sediments: very fine sand (20.4%) and fine sand (58.5%); and Cabedelo had higher percentages of medium sand (34.2%), coarse sand (42.8%), very coarse sand (3.9%) and fine gravel (3.2%) (Table 2).

Table 2- Granulometry of the collected sediment samples. Fine gravel (2-4 mm), very coarse sand (1-2 mm), coarse sand (0.5-1 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.063-0.125 mm) and silt/clay (<0.063 mm).

Habitat	MO (%)	%(<63um)	%63um	%125um	%250um	%500um	%1mm	%(>2mm)
Coral	3.1	3.5	20.4	58.5	15.3	1.82	0.32	0.08
Cabedelo	0.9	3.8	4.8	7.3	34.2	42.8	3.9	3.2

3.2. Richness, diversity and abundance

In this study, a total of 18 samples were collected in two different habitats, in which 6537 individuals were identified belonging to 97 species (Appendix 1). Coral July 2021 presented the highest abundance of individuals and Cabedelo December 2021 the lowest, with 2488 and 244 individuals respectively. In terms of species richness, Coral July 2021 presented the highest number of species, and the lowest number of species recorded was 33, in Coral in December 2021 as well as Cabedelo in May 2022 (Table 3).

Table 3- Number of individuals and species richness in each habitat on the three different dates;

	July 2021	December 2021	May 2022
Number of individuals			
Coral	2488	783	2010
Number of species			
Coral	70	33	44
Number of individuals			
Cabedelo	481	244	531
Number of species			
Cabedelo	34	36	33

The identified macrofauna were distributed by 5 Phylum: Mollusca, Annelida, Nematoda, Echinodermata and Arthropoda. The phylum with the highest number of individuals was Annelida (3929 in a total of 6536), as well as species (44 in a total of 98) in all dates and habitats. The phylum with highest abundance following Annelida, was Mollusca (1256 individuals) and Arthropoda (971 individuals) (Fig.9).

The most abundant taxa in Coral July 2021 was *Capitella capitata* (Fabricius, 1780), in Cabedelo July 2021 was *Nephtys longosetosa* (Örsted, 1842), in Coral December 2021 was *Chaetozone sp.* (Malmgren, 1867), and in Cabedelo December 2021 was *Donax trunculus* (Linnaeus, 1758). Finally, in Coral May 2022 the most abundant taxa was Oligochaeta, and in Cabedelo May 2022 was *Spio sp.* (Fabricius, 1785) (Appendix 1).

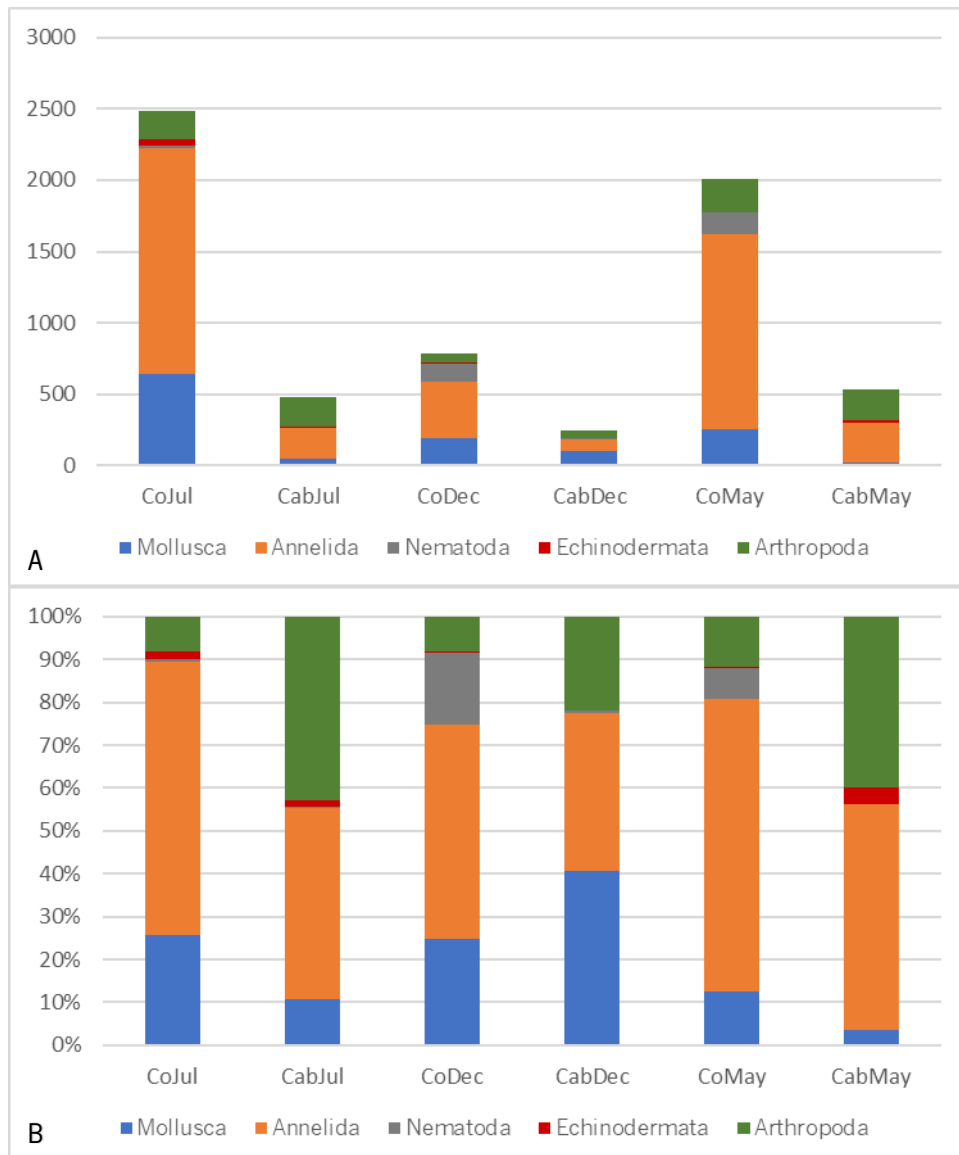


Figure 9- Total number of individuals (A) and percentage (B) of each Phylum in the two habitats throughout the different dates;

The Shannon diversity index ANOVA displayed significant differences in the factor Date (Da) ($p < 0.05$) (Table 4). The post hoc analysis didn't show any significant differences and the graph (Fig.10) shows that this index is very similar in the two habitats in the three different dates, being July 2021 the one with higher values and a slightly bigger difference, with Coral presenting a mean value of 2.41 and Cabedelo a mean value of 2.11.

Table 4- Results of ANOVAs of Shannon diversity index (H), Number of individuals (N) and Species richness (S). * $p < 0.05$, significant differences indicated in bold;

SOURCE	df	H		N		S	
		MS	F	MS	F	MS	F
Da	2	1.1175	9.64	114.3389	3.10	466.7963	6.94
Ha	1	0.2186	2.23	539.2886	21.07	755.6296	2.68
Si (DaxHa)	12	0.1159	0.92	36.9032	10.06	67.2222	13.7
DaxHa	2	0.0978	0.84	25.5975	0.69	282.0185	4.2
Residual	36	0.1257		3.6682		4.9074	
Total	53						
Transf.		None		Sqrt (X+1)		None	
Cochran's test		0.3167	ns	0.3016	ns	0.2415	ns

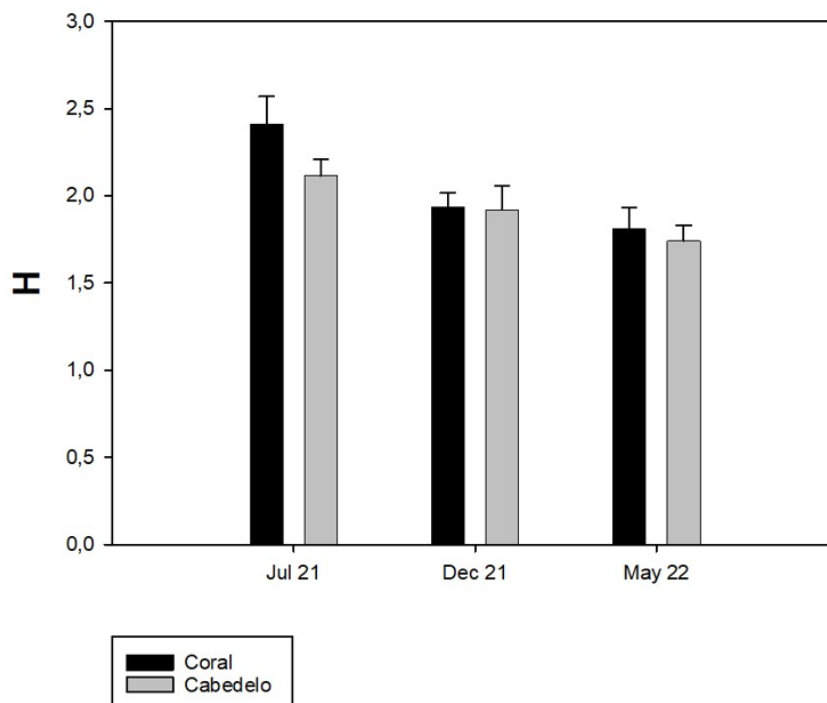


Figure 10- Shannon diversity index of the two habitats on the three different dates;

The number of individuals ANOVA displayed significant differences ($p < 0.05$) (Table 4). The post hoc analysis showed significant differences in the factor Date, Habitat, and Si (DaxHa), and there were significant differences in the number of individuals in the two habitats (Fig.11). The mean of individuals in Coral was ≈ 13 and the mean in Cabedelo was ≈ 7 .

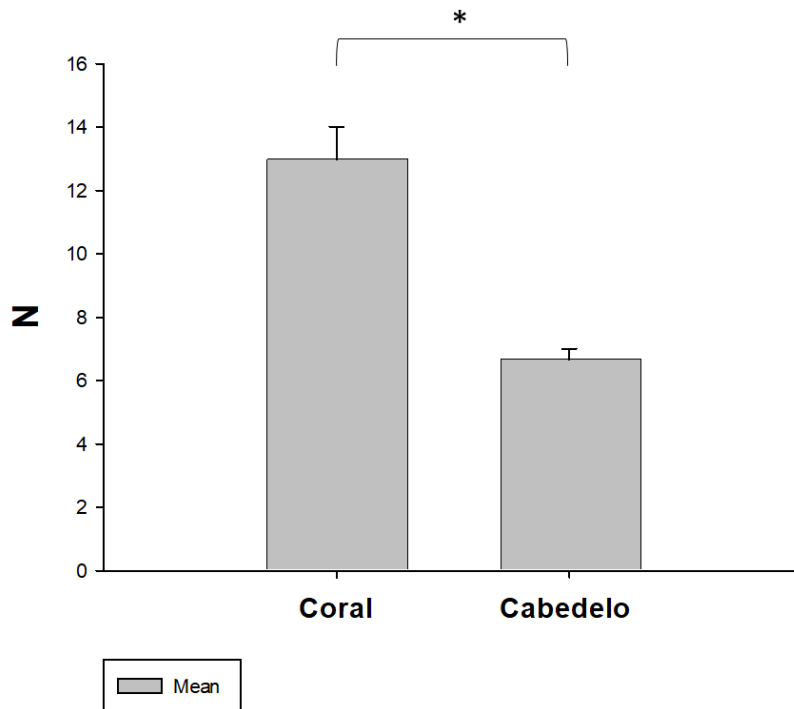


Figure 11-Number of individuals of the two habitats on the three different dates. * indicates the significant differences;

The species richness ANOVA displayed significant differences ($p < 0.05$) (Table 4). The post hoc analysis showed significant differences between the factor Habitat and Date, and there were significant differences in the first date, July 2021, between the two habitats (Fig.12). In Coral the mean number of species was close to 30 and in Cabedelo was almost 13.

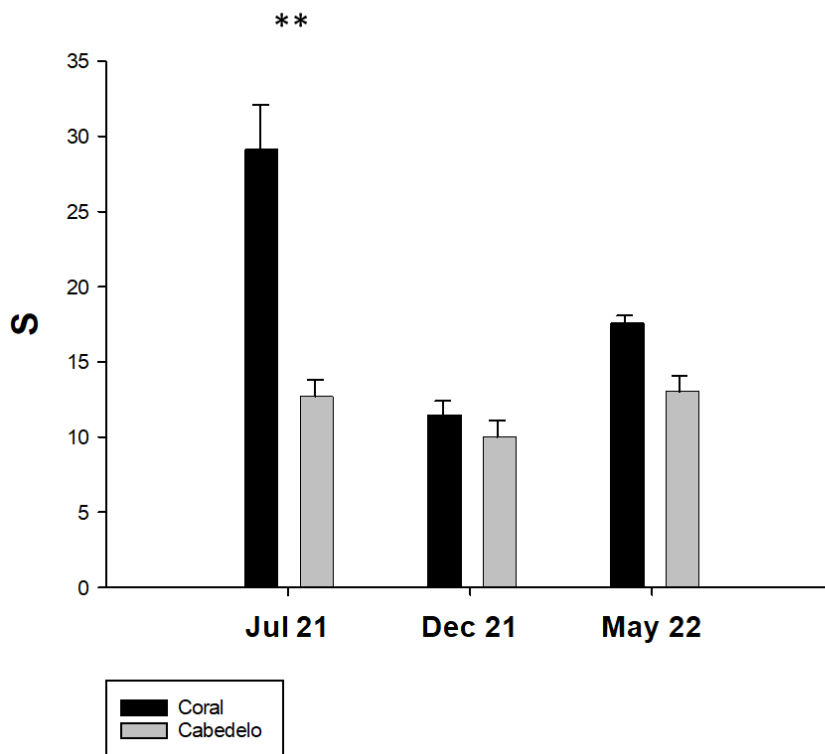


Figure 12- Species richness in the two habitats on the three different dates. * indicates the significant differences;

3.3. Multivariate analysis

3.3.1. Permanova, PERMDISP and nMDS

Results of PERMANOVA analysis for the composition of assemblages showed a significant interaction between Habitat and Date (Table 5). Post hoc analysis revealed significant differences in macrofaunal assemblages between habitats at each date (Table 6), showing that the assemblages in the two habitats were different.

Table 5- Results of PERMANOVA testing differences in macrobenthic assemblage structure across the studied habitats. Analyses based on Bray-Curtis dissimilarity matrix from square-root transformed data. All tests used 999 random permutations. Results of PERMDISP analysis. * $p < 0.05$, significant differences indicated in bold;

Source	df	MS	Pseudo-F	p	Unique perms
Ha	1	36086	5.6851	0.09	38
Da	2	14544	5.045	0.001*	999
HaxDa	2	6347.5	2.2019	0.002*	998
Si (HaxDa)	12	2882.8	2.569	0.001*	998
Residual	36	1122.1			
Total	53				
PERMDISP	F: 4.793		P(perm): 0.006		

Table 6- Post hoc analyses of PERMANOVA. * $p < 0.05$, significant differences indicated in bold;

Groups CoralxCabedelo	t	p (perm)	Unique perms	p (MC)
July	2.279	0.1018	10	0.0093*
December	1.69	0.0983	10	0.0333*
May	4.0295	0.1009	10	0.0002*

Despite the PERMDISP analysis indicating that the dispersion of replicates was significant among habitats (Table 5), the PERMDISP pairwise comparisons showed that the dispersion of replicates did not provide a significant contribution to the observed differences between Coral and Cabedelo in every data (Table 7), which were clearly separated in the nMDS plot (Figure 13).

Table 7 - PERMDISP pairwise comparison results;

Groups	t	p(perm)
Coral July, Cabedelo July	1.4982	0.241
Coral Dec., Cabedelo Dec.	1.7506	0.113
Coral May, Cabedelo May	1.5273	0.193

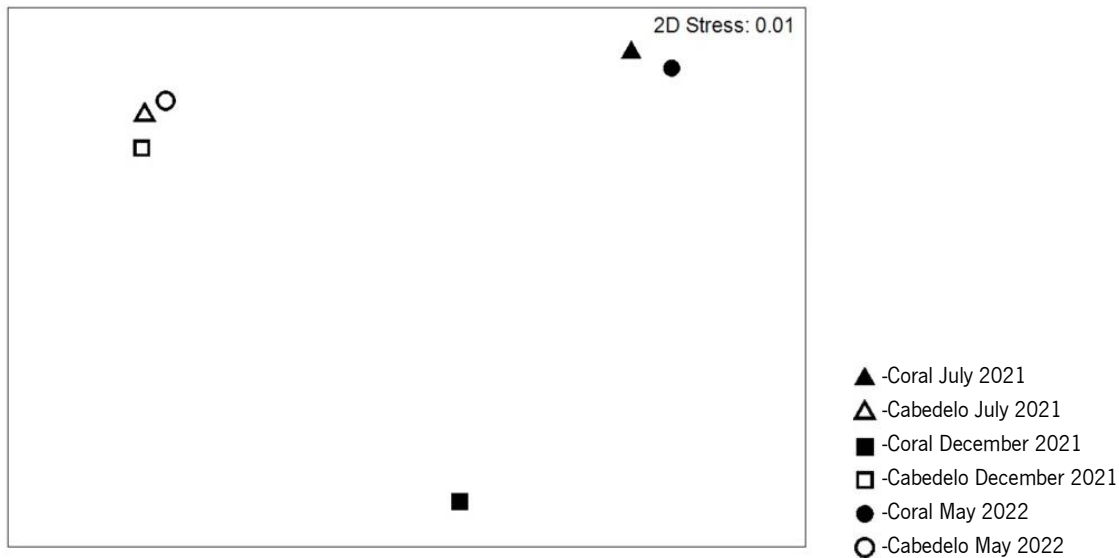


Figure 13- nMDS plot of centroids Coral (black) and Cabedelo (white) at three dates of sampling;

3.3.2. Simper analysis

The dissimilarity between Coral July 2021 and Cabedelo July 2021 was 79.41%, with 44 taxa being responsible for 90.37% of this dissimilarity. It is possible to verify that the species with the highest contribution belonged to Bivalvia and Polychaeta. The highest contribution belonged to *Abra alba* (W. Wood, 1802), with a percentage of 7.82, followed by *Capitella capitata* with a percentage of 6.83. The species with the least contribution in this date was *Glycera tridactyla* (Schmarda, 1861), with a percentage of 0.76%. The most abundant species in Coral is *Abra alba* and in Cabedelo was *Nephtys longosetosa* (Table 8).

In July 2021, 18 taxa were exclusive to Coral and only one taxa was exclusive to Cabedelo. There were 25 species associated with both habitats (Fig.14).

Of all the taxa present in this analysis, 12 belonged to *Polychaeta*, mostly belonging to the following Orders: Phyllodocida, Spionida and Terebellida. The rest of the taxa was distributed across Bivalvia, Amphipoda, Cumacea, Gastropoda, Oligochaeta, Nematoda, Decapoda, Copepoda, Echinodermata, Isopoda and Malacostraca.

Table 8- Average abundance, contribution of individual taxa and cumulate percentage (Cum %) from Coral and Cabedelo in July 2021;

Taxa	Coral	Cabedelo		
	Av. Abund	Av. Abund	Contrib. %	Cum. %
<i>Abra alba</i>	5.97	0.19	7.82	7.82
<i>Capitella capitata</i>	5.5	0.3	6.83	14.65
<i>Mediomastus fragilis</i>	4	0.36	4.7	19.35
<i>Ensis siliqua</i>	2.21	0	3.2	22.56
<i>Ampelisca brevicornis</i>	2.23	0	3.19	25.74
<i>Thracia phaseolina</i>	1.83	0	3.17	28.92
Oligochaeta	2.77	0	3.12	32.04
<i>Bathyporeia sp.</i>	0.16	1.92	2.86	34.9
<i>Spisula subtruncata</i>	2.12	0.3	2.74	37.64
<i>Owenia fusiformis</i>	2.1	0.3	2.58	40.22
<i>Donax trunculus</i>	0.11	1.7	2.56	42.78
<i>Tritia reticulata</i>	1.8	0	2.54	45.32
<i>Diogenes pugilator</i>	0.41	1.76	2.48	47.8
<i>Cumopsis longipes</i>	0.54	1.94	2.46	50.26
<i>Chaetozone sp.</i>	1.98	0	2.4	52.66
<i>Scolelepis cantabra</i>	0.16	1.37	2.25	54.91
<i>Spio sp.</i>	3.37	2.62	2.21	57.12
<i>Nephtys longosetosa</i>	1.59	2.85	1.93	59.05
<i>Corophium sp.</i>	1.43	0.22	1.81	60.86
<i>Eurydice</i>	0.3	1.02	1.72	62.58
<i>Parvicardium minimum</i>	1.07	0	1.64	64.22
<i>Prionospio sp.</i>	1.41	0	1.62	65.84
<i>Notrotopis falcatus</i>	1.74	1.07	1.55	67.38
<i>Cylichna cylindracea</i>	1.29	0	1.54	68.92
Calanoida	0.74	0.75	1.45	70.37
<i>Caprella sp.</i>	1.11	0.11	1.43	71.8
Echinodermata larva 2	1.05	0	1.41	73.21
<i>Ophiura sp.</i> (juvenile)	0.79	0.11	1.29	74.5
<i>Sthenelais boa</i>	1.07	0	1.28	75.78
<i>Eteone picta</i>	0.83	0.22	1.22	77.01
<i>Echinocardium cordatum</i>	0.52	0	1.15	78.16
<i>Lagis koreni</i>	0.94	0	1.11	79.27
Nematoda	0.92	0.11	1.11	80.39
Echinodermata larva 1	0.57	0.51	1.1	81.49
<i>Tanaissus bamberi</i>	0.46	0	1.05	82.54
<i>Pontocrates arenarius</i>	0.22	0.66	1.04	83.58
<i>Tritia sp.</i> (juvenile)	0.33	0.19	0.96	84.53
<i>Macroclymene santanderensis</i>	0.84	0	0.95	85.48
<i>Eocuma dollfusi</i>	0	0.57	0.92	86.4
<i>Lanice conchilega</i>	0.71	0	0.85	87.25

<i>Tapes rhomboides</i>	0.64	0	0.8	88.05
<i>Eumida so.</i>	0.65	0	0.78	88.83
<i>Magelona filiformis</i>	0.11	0.5	0.77	89.6
<i>Glycera tridactyla</i>	0.49	0.22	0.76	90.37

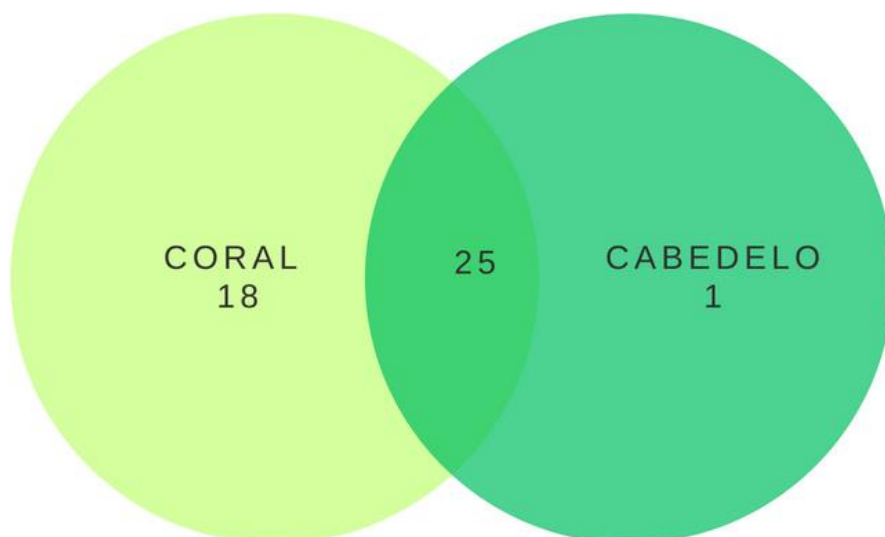


Figure 14- Exclusive and common species in the habitats in July 2021;

The dissimilarity between Coral December 2021 and Cabedelo December 2021 was 78.79%, with 30 taxa being responsible for 90.77% of this dissimilarity. The taxa with the highest contribution to the dissimilarity belonged to Spionida and Nematoda. The highest contribution belonged to *Prionospio sp.* (Malmgren, 1867), with a percentage of 7.86, followed by Nematoda with a contribution of 7.69%. The species with the lowest contribution in this date was *Rissoa parva* (da Costa, 1778), with a contribution of 0.99%. In both habitats the most abundant species was *Donax trunculus* (Table 9).

In December 2021, there were 6 taxa exclusive to Coral and 9 were exclusive to Cabedelo. In total, there were 15 taxa associated to both sites (Fig.15).

Among all the species, 12 belonged to Polychaeta, with the majority belonging to the following Orders: Phyllodocida, Spionida and Terebellida. The rest of the taxa was distributed through Bivalvia, Amphipoda, Gastropoda, Nematoda, Decapoda, Copepoda and Malacostraca.

Table 9- Average abundance, contribution of individual taxa and cumulate percentage (Cum %) from Coral and Cabedelo in December 2021;

Taxa	Coral	Cabedelo	Contrib. %	Cum. %
	Av. Abund	Av. Abund		
<i>Prionospio sp.</i>	2.86	0	7.86	7.86
Nematoda	2.9	0.16	7.69	15.55
<i>Chaetozone sp.</i>	2.86	0	7.42	22.97
<i>Donax trunculus</i>	3.55	1.83	7.32	30.29
<i>Tanaissus bamberi</i>	1.58	0.22	5.83	36.12
<i>Spisula subtruncata</i>	1.47	0.22	5.22	41.35
<i>Capitella capitata</i>	1.75	0.11	4.58	45.92
<i>Scolelepis cantabra</i>	0.58	1.24	4.12	50.04
<i>Tritia reticulata</i>	1.3	0	3.87	53.92
<i>Diogenes pugilator</i>	0.11	1.09	3.44	57.36
<i>Pontocrates arenarius</i>	0.33	0.97	2.94	60.3
<i>Bathyporeia sp.</i>	0	0.82	2.82	63.12
<i>Hiatella arctica</i>	0	0.97	2.76	65.88
<i>Nephtys longosetosa</i>	1.35	1.22	2.25	68.14
<i>Owenia fusiformis</i>	0.6	0.22	2.07	70.2
<i>Mediomastus fragilis</i>	0.76	0.11	2.02	72.22
Calanoida	0.43	0.38	2	74.23
<i>Abra alba</i>	0.59	0	1.87	76.1
<i>Microphthalmus sp.</i>	0	0.64	1.74	77.84
<i>Bittium reticulatum</i>	0.11	0.47	1.57	79.41
<i>Urothoe brevicornis</i>	0.27	0.11	1.5	80.9
<i>Steneopsis planorbis</i>	0	0.38	1.28	82.18
<i>Cylichna cylindracea</i>	0.38	0	1.17	83.35
<i>Spio sp.</i>	0	0.38	1.15	84.5
Nemertea	0	0.41	1.1	85.6
<i>Glycera tridactyla</i>	0.16	0.27	1.09	86.69
<i>Tricolia pullus</i>	0	0.38	1.07	87.76
<i>Aonides oxy</i>	0	0.3	1.02	88.77
<i>Ampelisca brevicornis</i>	0.33	0	1	89.77
<i>Rissoa parva</i>	0	0.3	0.99	90.77

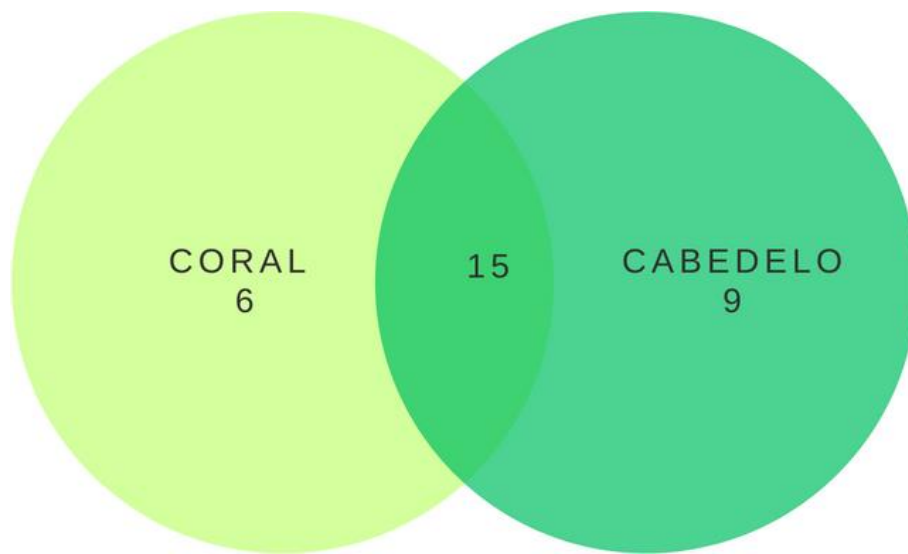


Figure 15- Exclusive and common species in the habitats in December 2021;

The dissimilarity between Coral May 2022 and Cabedelo May 2022 was 80.35%, being 29 taxa responsible for 90.46% of this dissimilarity. The taxa with the highest contribution in the dissimilarity belonged to Oligochaeta, with a percentage of 18.71%, followed by Nematoda with a contribution of 6.52%. The species with lowest contribution on this date was *Lagis koreni* (Malmgren, 1866), with a contribution of 0.88%. The most abundant taxa in Coral was Oligochaeta, and in Cabedelo was *Spio sp.* (Table 10).

In May 2022, 8 taxa were exclusive to Coral, and in the case of Cabedelo, there was only one exclusive taxa. In total, there were 20 taxa associated to both sites (Fig.16).

Once more, among all taxa, 10 belonged to Polychaeta, with the majority belonging to the following Orders: Phyllodocida, Spionida and Terebellida. The rest of the taxa was distributed through Bivalvia, Amphipoda, Oligochaeta, Gastropoda, Cumacea, Nematoda, Decapoda, Copepoda, Ostracoda and Echinodermata.

Table 10- Average abundance, contribution of individual taxa and cumulate percentage (Cum %) from Coral and Cabedelo in May 2022;

Taxa	Coral		Cabedelo	
	Av. Abund	Av. Abund	Contrib.%	Cum.%
Oligochaeta	10.44	0	18.71	18.71
Nematoda	3.48	0	6.52	25.23
<i>Spio sp.</i>	1.44	4.17	6.4	31.63
<i>Abra alba</i>	3.27	0.11	5.71	37.34
<i>Tritia reticulata</i>	2.2	0	4.13	41.47
<i>Prionospio sp.</i>	2.47	0.38	3.94	45.4
<i>Diogenes pugilator</i>	0.54	2.24	3.91	49.31
<i>Thracia phaseolina</i>	1.94	0	3.75	53.06
<i>Ampelisca brevicornis</i>	2.48	0.66	3.51	56.57
<i>Owenia fusiformis</i>	2.46	1.03	3.45	60.02
<i>Cumopsis longipes</i>	0.69	2.21	3.19	63.21
<i>Pontocrates arenarius</i>	2.52	1.16	3.13	66.34
<i>Bathyporeia sp.</i>	0.61	0.96	2.41	68.75
Ostracoda	1.2	0	2.14	70.89
Echinodermata larva 1	0.19	1.18	2.09	72.98
<i>Magelona filiformis</i>	0	1	1.79	74.76
<i>Nephtys longosetosa</i>	0.65	1.09	1.68	76.44
<i>Notrotopsis falcatus</i>	0.66	0.69	1.48	77.92
<i>Spisula subtruncata</i>	0.93	0.88	1.4	79.32
<i>Mediomastus fragilis</i>	0.7	0.11	1.4	80.72
<i>Scolelepis cantabra</i>	0.11	0.74	1.37	82.09
<i>Capitella capitata</i>	0.58	0.16	1.25	83.34
<i>Tritia sp.</i> (juvenile)	0.65	0	1.19	84.54
<i>Ensis siliqua</i>	0.65	0	1.11	85.65
<i>Donax trunculus</i>	0.49	0.22	1.04	86.69
<i>Liocarcinus pusillus</i>	0.56	0.22	1	87.7
<i>Eocuma dollfusi</i>	0.11	0.54	0.96	88.66
<i>Glycera tridactyla</i>	0.22	0.49	0.92	89.58
<i>Lagis koreni</i>	0.49	0	0.88	90.46

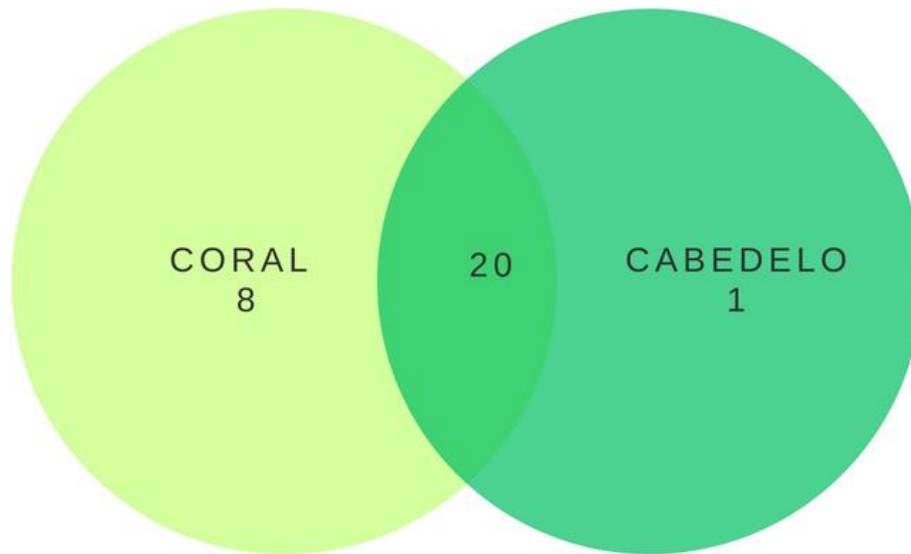


Figure 16- Exclusive and common species in the habitats in May 2022;

3.4. AMBI (AZTI's Marine Biotic Index)

The AMBI analysis showed that the ecological status of Coral stations fluctuated and varied from “Slightly disturbed” to “Moderately disturbed”, whilst Cabedelo stations were more stable and were considered “Undisturbed” and “Sightly undisturbed” (Fig.17).

Coral stations clearly had more species indicating a poorer quality ecological status, many of the species present in these stations were ranked in the IV and V ecological groups. The date with more species ranked in these ecological groups was May 2022. In Cabedelo the species were mainly ranked in the I, II and III ecological groups (Fig.18).

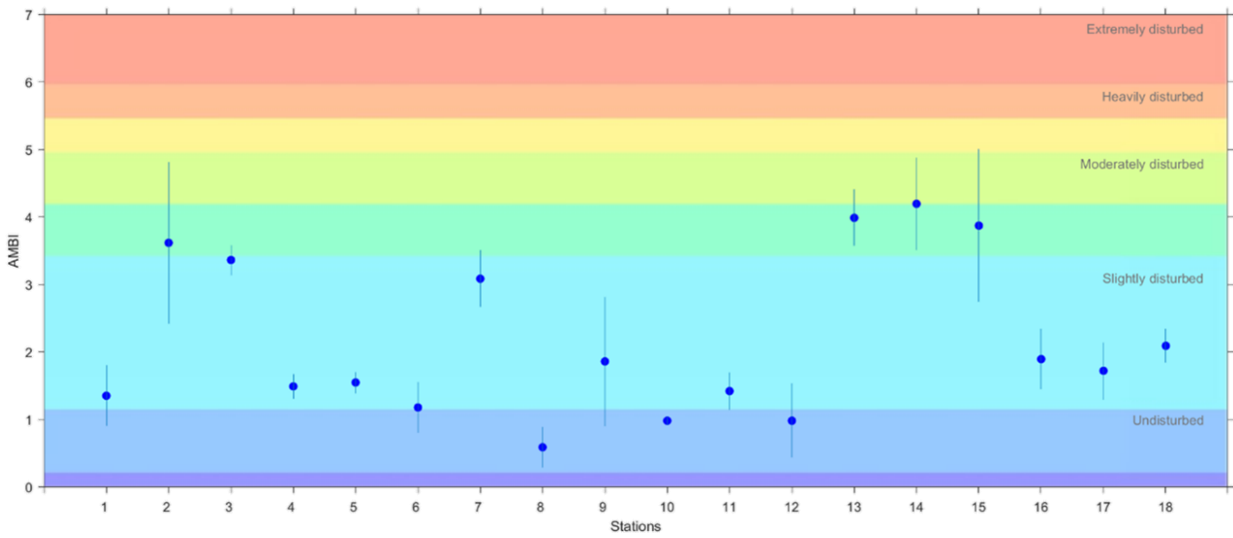


Figure 17- Ecological status of each site in the two habitats. **Stations: 1,2,3:** Coral July 2021/ **4,5,6:** Cabedelo July 2021/ **7,8,9:** Coral December 2021/ **10,11,12:** Cabedelo December 2021/ **13, 14, 15:** Coral May 2022/ **16, 17, 18:** Cabedelo May 2022;

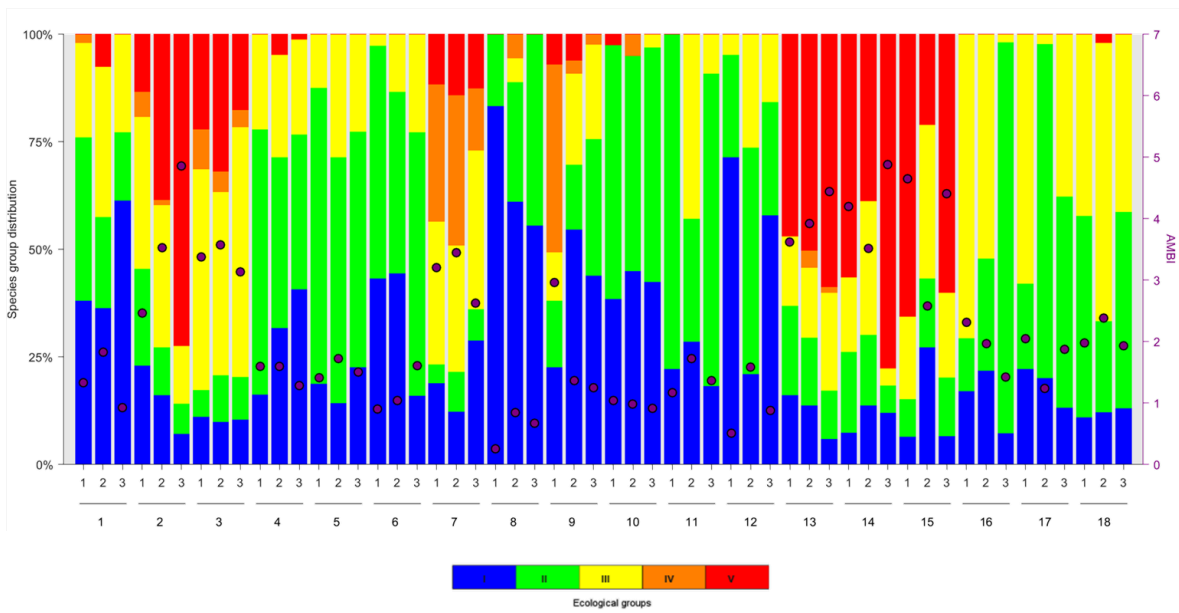


Figure 18- Ecological groups in each replica, in each site, on the two habitats. **Stations: 1,2,3:** Coral July 2021/ **4,5,6:** Cabedelo July 2021/ **7,8,9:** Coral December 2021/ **10,11,12:** Cabedelo December 2021/ **13, 14, 15:** Coral May 2022/ **16, 17, 18:** Cabedelo May 2022;

4. Discussion

4. Discussion

Hydrodynamic and sediment characteristics are key drivers in shaping macrobenthic assemblages on near-shore ecosystems, therefore any modification that may alter these processes may have an unavoidable impact on the ecology of macrobenthic assemblages. Any structure placed within a coastal environment changes the wave regime and sedimentary processes, affecting the species composition, abundance and trophic structure of invertebrate assemblages that inhabit soft-bottoms environments (Martin et al., 2005).

Coral bay is situated right next to the mouth of the Lima River and is protected by the 2170-meter breakwater. When the river flows into the coastal area and the two different habitats come in contact, organisms, organic matter and nutrients can be transferred from one habitat to another (Barros et al., 2001). When the freshwater flows into the coastal area, there is an immediate input of pollutants, nutrients, and organic matter into the ocean basin (Snelgrove, 1998). Artificial structures, such as the Viana's main breakwater, modify local currents, entrapping the organic matter and the existing organic material, such as algae and debris, which creates an organic build-up in the adjacent sediments (Bertasi et al., 2007). Since this area is richer in organic matter than Cabedelo area (Table 2), there is a great quantity of potential food resources, and thus deposit feeders dominate the community, as previously reported by other authors (Martin et al., 2005; Sousa et al., 2006).

The most abundant taxa in the protected area, Coral, in all dates are mostly Annelida (Fig.9): *C. capitata*, *Mediomastus fragilis* (Rasmussen, 1973), *Chaetozone sp.* and Oligochaeta; but there is also a high presence of the bivalve *A. alba* (Appendix 1).

Our results agree with Gary and Elliot (2009) and Sousa et al. (2007), that observed high abundance of *C. capitata* and *Chaetozone sp.* in areas with: fine sediments rich in organic matter and increased organic matter loading areas. Our results also agree with Bertasi et al. (2007) that reported that the area protected by an artificial protection structure hosted an infaunal community characterized by deposit feeders and sub-surface deposit feeders, such as *C. capitata*. The presence of *C. capitata* populations, in areas where artificial protection structures are present, is an index for assessing the disturbance effects on assemblages, particularly as regards organic enrichment (Martin et al., 2005). In fact, Martin et al. (2005) concluded that this species was more abundant in areas protected from water movement by an artificial protection structure.

Oligochaeta, *Chaetozone sp.*, *M. fragilis* and *C. capitata* are considered opportunistic, which is defined as a species who adapts to life in a fleeting and unpredictable habitat by relying on a high growth rate to make use of momentary resources (Grassle & Grassle, 1974). These types of species have the capability to respond rapidly to the sudden appearance of increased organic matter that can be used as food (Watling, 2019), reproduce rapidly to use up the resources before other competing species can exploit the habitat, and then disperse in search of other new habitats as the existing becomes unsuitable (Grassle & Grassle, 1974). Oligochaeta and *Chaetozone sp.* are exclusive to Coral, not being found in Cabedelo, and *C. capitata* and *M. fragilis* are disproportionately more abundant in Coral when compared to the numbers found in Cabedelo (Appendix 1). This reinforces once more that the presence of the breakwater leads to a buildup of organic matter and the appearance of opportunistic species, such as these ones.

According to Sousa et al. (2006, 2007), the bivalve *A. alba* is very abundant in areas with high organic loads, and Bertasi et al. (2007) concluded that *A. alba* prefers hydrodynamically less stressed areas and organically enriched bottoms, and that this bivalve grows quickly when food is available. Our results showed that the number of individuals of *A. alba* found in Coral were much higher than those found in Cabedelo (Appendix 1) confirming the preferences of this bivalve for organic rich habitats.

Cabedelo is exposed to wave action and is under very different conditions from those of Coral. In Cabedelo, the most abundant taxa are: *Nephtys longosetosa* and *Cumopsis longipes* (Dohrn, 1869) in July 2021, *D. trunculus* in December 2021 and *Spio sp.* in May 2022 (Appendix 1). The community in this habitat is different from Coral, being composed by suspension feeders and predators.

Species from the genus *Donax* typically occupy the shallow sublittoral of exposed beaches, high energy environments with strong wave action or high current speed. Despite the frequent disturbances on the sediment, caused by hydrodynamic conditions, these organisms are well adapted to maintain their positions in such conditions *Donax* species are particularly characteristic of highly productive areas and rely on the collection of suspended particles from the water (Ansell, 1983).

The polychaete *N. longosetosa* is a carnivorous predator that can be found in the lower intertidal zone, and most commonly in well-sorted fine or medium sands (Ravara et al., 2010). *C. longipes* is a species adapted to life in dynamic ecosystems, being both rapid and strong enough in order to cope with wave conditions, and feeds by filtrating the particles in the water column (Quillien et

al., 2018). Species of the genus *Spio* have different feeding habits. Although they can be deposit feeders and feed on organic matter present, they are not necessarily an indicator of organic matter enrichment. These species can also feed by filtering the water column, such as *D. trunculus* and *C. longipes*. Furthermore, in the AMBI analysis this species is ranked in the III ecological group, whereas Oligochaeta, *C. capitata* and *Chaetozone sp.* are ranked as IV and IV ecological groups. The presence of these species indicates that the conditions and food resources in Cabedelo are very different than the ones in the breakwater protected habitat. Since there is no accumulation of organic matter in this habitat, the species here present must find other ways to subsist, for example predation and filtration.

The freshwater input from the Lima River directly affects the salinity in the protected area of Coral. According to Snelgrove (1998), this is an important variable in coastal environments, due to its role in defining the distributions of coastal species. This causes the two types of water to mix, the freshwater stays at the surface, and the saltwater, which is denser, stays closer to the bottom, creating a “food trap”, where the nutrients get trapped in this mixture of water types (Mendes, 2009). Additionally, this influx causes the salinity levels to lower in Coral. Whilst in Cabedelo, a habitat affected by high hydrodynamics, and a totally marine habitat (apparently unaffected by the river), these salinity values are higher and stable throughout the year. This could explain the difference between the values obtained when comparing the two habitats of this study (Fig.8).

The community structure in both habitats had temporal oscillations in abundance and diversity, presenting lower values in the winter, in December 2021, and higher values in May 2022, and in the summer time, in July 2021 (Table 3). The assemblages maintained some stability through such temporal fluctuations, with the dominant populations always present (Fig.9).

The results of this study, about the differences in the assemblages in the different habitats, are in line with other studies. Bertasi et al. (2007) detected significant differences in macrobenthic assemblages between locations protected by artificial structures and locations exposed to wave action. The locations sheltered by artificial structures demonstrated an increase in macrobenthic species number and, in contrast, the exposed zones presented a poor level of species and less diversification. They also concluded that the relatively large macrobenthic fauna occupied the most exposed and shallowest location, while the small ones settled in the most sheltered zones since the wave agitation tends to inhibit their deposition as well as the deposition of fine particles. Our studies also agree with Martin et al. (2005), a study that evaluated various artificial protection structures all over Europe, concluding that “the presence of artificial protection structures induced

an overall increase in diversity at all the studied areas". These results help support and explain the significant differences in the values of number of individuals and species richness (Table 3; Fig11,12).

The sediment characteristics is an important factor in structuring macrobenthic assemblages and also determining the trophic status of these assemblages (Martin et al., 2005). This variable can be altered by the presence of the breakwater, and therefore, alter de community present in such habitat. For instance, fine organically rich muds tend to contain more burrowing deposit feeders, such as *C. capitata*, whereas coarser sediments usually harbor more mobile animals and suspension feeders, for example *D. trunculus* (Table 2) (Martin et al., 2005).

There is an evident difference between the assemblages in the two habitats. The two habitats are under different hydrodynamic conditions due to the breakwater, which ultimately affects the type of assemblages that form there. Coral is protected from wave action by the breakwater, that seems to have high influence in sediment characteristics and in the organic matter buildup (Table 2). The fact that Coral bay is located right next to the river mouth also increases the differences between the two habitats, since the river can have a marked influence on the sediment dynamics and organic load and, thus, on the infaunal structure and functioning (Martin et al., 2005). In this habitat, where there can be a certain level of stress and pollution, the most sensitive species become rare or perish and are replaced by opportunistic species (Sousa et al., 2007). This means that, species present under natural conditions might disappear due to the present of the breakwater, but at the same time, different species are able to colonize the new habitat as a result of these new conditions (Martin et al., 2005).

In the other hand, Cabedelo is subject to intense wave action, high currents, and sediment disturbance, meaning that these assemblages have different resources available and different characteristics that allow them to inhabit such conditions. Although there is evidence in another study, (Veiga et al., 2014), with different environmental parameters measured, that the river stills as effects in some coastal areas variables a few kilometers of the river mouth, in this study that does not happen. Cabedelo doesn't seem to be affected by the Lima River, being only affected by the conditions of Viana sea.

These differences are also evident in the AMBI analysis, that shows the levels of disturbance in each habitat. Coral is more disturbed than Cabedelo, fluctuating between "Slightly disturbed" and "Moderately disturbed" and having species attributed to ecological groups with high rankings, meaning that this could be a stressed and organic polluted habitat (Fig.17,18).

As expected, the macrobenthic assemblage are different in response to the presence of the breakwater. Throughout this study is possible to see the clear effects of the breakwater on the sediment and hydrodynamic characteristics and, consequently, on the structure of macrobenthic assemblages of the soft-bottoms of Viana do Castelo. Therefore, the proposed hypothesis “the breakwater will modify water and sediment characteristics and thus affect the structure of macrobenthic assemblages” is accepted.

There is little knowledge of the consequences of ocean sprawl on large spatial areas, but given their prominence and scale they are likely to be significant (Todd et al., 2019). The spatial scale of impacts from artificial structures depends on the type of structure, local hydrodynamic conditions, and a variety of other parameters (Todd et al., 2019). As artificial structures spread across long distances, in some of the most densely urbanized regions, entire habitats have been lost. In order to avoid these large scale effects of habitat loss, fragmentation and community change, the effects of artificial structures should always be minimized and the number of these structures should be reduced to the minimum necessary to protect the coast (Martin et al., 2005).

Artificial protection structures that have already been constructed, cannot realistically be removed. Finding ways to improve the ecological and social value of these coastal areas, whilst maintaining their engineering functions, is crucial given that there are likely to be more human shorelines built in the foreseeable future (Todd et al., 2019). Furthermore, it is also very important to try to obtain further evidence on the ecological impacts of such structures, to help improve decision making processes (Walker et al., 2008).

Since the Portuguese coast is very long, and there are many other artificial structures distributed along the coastal area, more investigations are needed in this country so there's a better understanding of the full extent of the changes caused by these structures and to see if there is a pattern along the coast.

5. Conclusion

5. Conclusion

Overall, the main conclusions of this study are:

- The presence of the breakwater affects and changes the soft-bottom macrobenthic assemblages of the protected area, Coral, when comparing them to the assemblages of the exposed area, Cabedelo;
- The macrobenthic assemblages of the protected area are characterized by deposit-feeders, opportunistic species and, in general, by species with preference for organically enriched bottoms;
- The macrobenthic assemblages of the exposed area are subject to different conditions and resources, so they present different feeding habits, such as filtration and predation;
- The protected area is more diverse and has higher number of individuals than the exposed area;
- The AMBI analysis shows that the protected area is more disturbed, and has more species attributed to ecological groups with high rankings than the exposed area;

6. References

6. References

- Afonso, E., Arnal, I., Arrontes, J., Arzel, P., & Augris, C. (2000). Quality Status Report 2000: Region IV–Bay of Biscay and Iberian Coast. *OSPAR Commission, London*, 82–113.
- Anderson, M., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E, Plymouth, UK. 1.
- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26, 32–46.
- Ansell, A. D. (1983). The Biology of the Genus *Donax*. *Sandy Beaches as Ecosystems*, 3, 607–635. https://doi.org/10.1007/978-94-017-2938-3_46
- Barros, F., Underwood, A. J., & Lindegarth, M. (2001). The influence of rocky reefs on structure of benthic macrofauna in nearby soft-sediments. *Estuarine, Coastal and Shelf Science*, 52, 191–199. <https://doi.org/10.1006/ecss.2000.0734>
- Bertasi, F., Colangelo, M. A., Abbiati, M., & Ceccherelli, V. U. (2007). Effects of an artificial protection structure on the sandy shore macrofaunal community: The special case of Lido di Dante (Northern Adriatic Sea). *Hydrobiologia*, 586, 277–290. <https://doi.org/10.1007/s10750-007-0701-y>
- Boutoumit, S., El Kamcha, R., Bououarour, O., Joulami, L., Boutahar, L., Benhoussa, A., Maanan, M., Godet, L., Bayed, A., & Bazairi, H. (2021). Soft-bottom macrozoobenthos in semi-enclosed coastal systems of Morocco: A latitudinal and biogeographic analysis. *Regional Studies in Marine Science*, 44. <https://doi.org/10.1016/j.rsma.2021.101689>
- Braun-Blanquet, J., Braun-Blanquet, G., Rozeira, A., & da Silva, A. . (1973). Résultats de Trois Excursions Géobotaniques à travers le Portugal Septentrional et Moyen. IV. Esquisse sur la végétation dunale. *Agronomia Lusitana*.
- Carreira-Flores, D., Veiga, P., Fernández-Gutiérrez, J., Gomes, P. T., & Rubal, M. (2023). Exploring Benthic Scavenger Assemblages, a Multi-Habitat Approach in NW Iberian Peninsula. *Journal of Marine Science and Engineering*, 11(1), 184. <https://doi.org/10.3390/jmse11010184>
- Castelli, A. Lardicci, C., Pisa, U., & Tagliapietra, D. (2004). Soft Bottom Macrobenthos. *Biologia Marina Mediterranea*, 11, 99–131.
- Clark, J. (1995). *Coastal Zone Management Handbook*. CRC Press.
- CMIA – Centro de Monitorização e Interpretação Ambiental de Viana do Castelo. (2012). *MARgens com vida*.
- Dunson, W. A., & Travis, J. (1991). The role of abiotic factors in community organization. *American Naturalist*, 138(5), 1067–1091. <https://doi.org/10.1086/285270>
- Garaffo, G. V., Jaubet, M. L., Becherucci, M. E., & Elías, R. (2017). Assessing environmental health using ecological indices for soft bottom in sewage-affected rocky shores: The case of the largest seaside resort of SW Atlantic. *Marine Pollution Bulletin*, 115(1–2), 233–239. <https://doi.org/10.1016/j.marpolbul.2016.12.017>
- Gaspar, R., Pereira, L., & Sousa-Pinto, I. (2019). The seaweed resources of Portugal. *Botanica Marina*. <https://doi.org/10.1515/bot-2019-0012>
- Geoparque Litoral Viana do Castelo. (2020). *A NAVEGAÇÃO NO PORTO DE VIANA*. <https://www.geoparquelitoralviana.pt/explorar/ribeira-de-anha/o-porto-de-mar-na-história/>
- Grassle, J. F., & Grassle, J. P. (1974). Opportunistic Life Histories and Genetic Systems in Marine Benthic

- Polychaetes. *Journal of Marine Research*, 32(2), 253–284.
- Gray, J., & Elliot, M. (2009). *Ecology of Marine Sediments* (Second edi). Oxford University Press.
- Harley, C. D. G., Hughes, A. R., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, 9, 228–241. <https://doi.org/10.1111/j.1461-0248.2005.00871.x>
- Hoegh-guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *American Association for the Advancement of Science*, 328(5985), 1523–1528.
- Hoffman, A., & Blows, M. (1994). Species borders: ecological and evolutionary perspectives. *Trend in Ecology & Evolution*, 9(6), 223–227.
- Horner-Devine, M. C., Silver, J. M., Leibold, M. A., Bohannon, B. J. M., Colwell, R. K., Fuhrman, J. A., Green, J. L., Kuske, C. R., Martiny, J. B. H., Muyzer, G., Øvreås, L., Reysenbach, A. L., & Smith, V. H. (2007). A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology*, 88(6), 1345–1353. <https://doi.org/10.1890/06-0286>
- Jackson, N. L., Harley, M. D., Armaroli, C., & Nordstrom, K. F. (2015). Beach morphologies induced by breakwaters with different orientations. *Geomorphology*, 239, 48–57. <https://doi.org/10.1016/j.geomorph.2015.03.010>
- Lemos, R., Santos, J. A., & Fortes, C. J. (2017). Rubble mound breakwater damage assessment through stereo photogrammetry in physical scale laboratory tests. *Ribagua*, 4(2), 84–98. <https://doi.org/10.1080/23863781.2017.1381455>
- Martin, D., Bertasi, F., Colangelo, M. A., de Vries, M., Frost, M., Hawkins, S. J., Macpherson, E., Moschella, P. S., Satta, M. P., Thompson, R. C., & Ceccherelli, V. U. (2005). Ecological impact of coastal defence structures on sediment and mobile fauna: Evaluating and forecasting consequences of unavoidable modifications of native habitats. *Coastal Engineering*, 52, 1027–1051. <https://doi.org/10.1016/j.coastaleng.2005.09.006>
- Mendes, F. R. . (2009). *Contributos para o Plano do Estuário do Rio Lima*.
- Ólafsson, E. (2021). DO MACROFAUNA STRUCTURE MEIOFAUNA ASSEMBLAGES IN MARINE SOFT-BOTTOMS? A REVIEW OF EXPERIMENTAL STUDIES. *Vie et Milieu / Life & Environment*, 2003, 249–265. <https://hal.sorbonne-universite.fr/hal-03205296>
- Quillien, N., Nordström, M. C., Le Bris, H., Bonsdorff, E., & Grall, J. (2018). Green tides on inter-and subtidal sandy shores: Differential impacts on infauna and flatfish. *Journal of the Marine Biological Association of the United Kingdom*, 98(4), 699–712. <https://doi.org/10.1017/S0025315416002010>
- Ravara, A., Cunha, M. R., & Pleijel, F. (2010). Nephtyidae (annelida, polychaeta) from southern Europe. In *Zootaxa* (Vol. 68, Issue 2682). <https://doi.org/10.11646/zootaxa.2682.1.1>
- Rubal, M., Veiga, P., Reis, P. A., Bertocci, I., & Sousa-Pinto, I. (2014). Effects of subtle pollution at different levels of biological organisation on species-rich assemblages. *Environmental Pollution*, 191, 101–110. <https://doi.org/10.1016/j.envpol.2014.04.019>
- Saurabh, S. (2014). ENVIS Newsletter on wetland ecosystems including inland wetlands. *Environmental Information System*, 10(4).
- Scavia, D., Field, J. C., Boesch, D., Buddemeier, R., Burkett, V., & Cayan, D. (2002). Climate Change Impacts on U.S. Coastal and Marine Ecosystems. *Estuaries*, 25(2), 149–164.
- Snelgrove, P. (1997). The Importance of Marine Sediment Biodiversity in Ecosystem Processes. *Royal Swedish Academy of Sciences*, 26(8), 578–583.

- Snelgrove, P. (1998). The biodiversity of macrofaunal organisms in marine sediments. *Biodiversity and Conservation*, 7, 1123–1132.
- Sousa, R., Dias, S., & Antunes, C. (2007). Subtidal macrobenthic structure in the lower Lima estuary, NW of Iberian Peninsula. *Annales Zoologici Fennici*, 44, 303–313.
- Sousa, R., Dias, S., & Antunes, J. C. (2006). Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima estuary, NW of Portugal. *Hydrobiologia*, 559, 135–148.
<https://doi.org/10.1007/s10750-005-1371-2>
- Systat Software Inc. (2006). *SigmaPlot 10.0 – User's Guide* (10.0).
- Tagliapietra, D., & Sigovini, M. (2010). Benthic Fauna: collection and identification of macrobenthic invertebrates. *NEAR Curriculum in Natural Environmental Science, Terre et Environnement*, 88, 253–261.
- Todd, P. A., Heery, E. C., Loke, L. H. L., Thurstan, R. H., Kotze, D. J., & Swan, C. (2019). Towards an urban marine ecology: characterizing the drivers, patterns and processes of marine ecosystems in coastal cities. *Oikos*, 128, 1215–1242. <https://doi.org/10.1111/oik.05946>
- Vale, L. M. do. (2008). *ESTUDO HIDRODINÂMICO DO PORTO DE VIANA DO CASTELO*.
<http://hdl.handle.net/10773/881>
- Veiga, P., Redondo, W., Sousa-Pinto, I., & Rubal, M. (2017). Relationship between structure of macrobenthic assemblages and environmental variables in shallow sublittoral soft bottoms. *Marine Environmental Research*, 129, 396–407. <https://doi.org/10.1016/j.marenvres.2017.07.002>
- Veiga, P., Rubal, M., Cacabelos, E., Maldonado, C., & Sousa-Pinto, I. (2014). Spatial variability of macrobenthic zonation on exposed sandy beaches. *Journal of Sea Research*, 90, 1–9.
<https://doi.org/10.1016/j.seares.2014.02.009>
- Walker, S. J., Schlacher, T. A., & Thompson, L. M. C. (2008). Habitat modification in a dynamic environment: The influence of a small artificial groyne on macrofaunal assemblages of a sandy beach. *Estuarine, Coastal and Shelf Science*, 79, 24–34.
<https://doi.org/10.1016/j.ecss.2008.03.011>
- Warwick, R. M., Robert Clarke, K., & Somerfield, P. J. (2010). Exploring the marine biotic index (AMBI): Variations on a theme by Ángel Borja. *Marine Pollution Bulletin*, 60, 554–559.
<https://doi.org/10.1016/j.marpolbul.2009.11.009>
- Watling, L. (2019). Macrofauna. *Encyclopedia of Ocean Sciences*, 2, 728–734.
<https://doi.org/10.1016/B978-0-12-409548-9.11069-3>
- WoRMS Editorial Board. (2023). *World Register of Marine Species*. <https://www.marinespecies.org>

Appendix 1

Table 11- List of species present in Coral and Cabedelo in the three dates;

Taxa	CoJul	CabJul	CoDec	CabDec	CoMay	CabMay
<i>Spisula subtruncata</i>	48	4	33	2	12	11
<i>Hiatella arctica</i>	2	0	0	24	6	1
<i>Donax trunculus</i>	1	42	124	51	10	2
<i>Abra alba</i>	379	3	10	0	104	1
<i>Thracia phaseolina</i>	42	0	0	0	45	0
<i>Tapes rhomboides</i>	17	0	0	0	0	0
<i>Ensis siliqua</i>	52	0	1	0	9	0
<i>Parvicardium minimum</i>	22	0	0	0	1	0
<i>Tricolia pullus</i>	0	0	0	6	3	2
<i>Melarhappe neritoides</i>	0	0	0	0	0	1
<i>Rissoa parva</i>	0	0	0	4	0	0
<i>Bittium reticulatum</i>	9	0	1	7	0	1
<i>Tritia reticulata</i>	35	0	21	0	45	0
<i>Tritia sp.</i> (juvenile)	3	3	0	0	7	0
<i>Tritia nitida</i>	0	0	0	0	3	0
<i>Cylichna cylindracea</i>	28	0	4	0	3	0
<i>Euspira nitida</i>	1	0	0	1	5	0
<i>Steneopsis planorbis</i>	0	0	0	4	0	0
<i>Bathyporeia sp.</i>	2	36	0	8	30	12
<i>Megaluropus agilis</i>	0	0	0	1	0	0
<i>Pontocrates arenarius</i>	2	10	3	14	71	16
<i>Urothoe brevicornis</i>	2	0	3	1	0	1
<i>Ampelisca brevicornis</i>	50	0	3	0	67	9
<i>Lysianassidae sp.</i>	0	1	0	1	0	11
<i>Notrotopsis falcatus</i>	35	14	0	0	10	8
<i>Caprella sp.</i>	19	1	1	0	0	0
<i>Corophium sp.</i>	25	2	0	0	1	0
<i>Aora typica</i>	1	0	0	0	0	0
<i>Cumopsis longipes</i>	6	56	1	1	11	57
<i>Eocuma dollfusi</i>	0	7	0	1	1	6
<i>Nannastacidae sp.</i>	0	0	0	1	0	0
<i>Diastylis rathkei</i>	2	5	0	1	1	3
<i>Diogenes pugilator</i>	8	33	1	18	10	82
<i>Liocarcinus pusillus</i>	2	1	0	0	5	2
<i>Liocarcinus depurator</i>	0	1	0	0	0	0
<i>Crangon crangon</i>	0	1	0	0	4	2
<i>Processa canaliculata</i>	6	0	0	0	0	0
<i>Tanaissus bamberi</i>	6	0	41	2	0	2
<i>Zeuxo holdichi</i>	3	1	0	0	0	0
Calanoida	9	13	9	4	1	0

Copepoda	4	0	0	0	0	0
Ostracoda	7	0	1	0	22	0
Decapoda larva	3	2	0	0	1	0
<i>Eurydice sp.</i>	4	23	0	0	0	1
<i>Zenobiana prismatica</i>	2	0	0	0	0	0
<i>Anoplodactylus angulatus</i>	1	0	0	0	0	0
<i>Nephtys longosetosa</i>	27	78	20	16	9	15
<i>Nephtys assimilis</i>	0	0	1	0	0	0
<i>Scolelepis cantabra</i>	2	39	8	27	1	10
<i>Spio sp.</i>	118	69	0	4	58	215
<i>Spio chaetopterus</i>	1	0	0	0	0	0
Spionida	0	0	3	0	0	0
<i>Aonides oxy</i>	0	0	0	4	0	0
<i>Mediomastus fragilis</i>	306	6	18	1	29	1
<i>Microphthalmus</i>	0	0	0	18	0	0
Nemertea	1	0	0	8	0	0
<i>Owenia fusiformis</i>	53	4	8	2	72	15
<i>Levinsenia flava</i>	0	0	0	1	0	0
<i>Glycera oxycephala</i>	0	0	0	0	1	0
<i>Glycera tridactyla</i>	5	2	2	3	2	5
<i>Glycera lapidum</i>	0	0	0	1	0	0
<i>Harmothoe sp.</i>	0	0	1	0	0	0
<i>Capitella capitata</i>	622	4	57	1	10	2
<i>Chaetozone sp.</i>	75	0	150	0	0	0
<i>Prionospio sp.</i>	40	0	118	0	65	4
<i>Phyllodoce lineata</i>	0	0	1	0	0	0
<i>Phyllodoce mucosa</i>	8	0	0	0	0	0
<i>Phyllodoce Aff laminosa</i>	1	0	0	0	0	1
<i>Phyllodoce Aff longipes</i>	1	0	0	0	0	0
Oligochaeta	208	0	3	0	1110	0
Terebellidae	3	0	1	0	0	0
<i>Magelona filiformis</i>	1	7	0	2	0	12
<i>Polydora sp.</i>	0	0	0	1	0	0
<i>Lanice conchilega</i>	11	0	0	0	0	0
<i>Eteone picta</i>	10	2	0	0	0	0
<i>Eumida sp.</i>	10	0	0	0	0	0
<i>Sthenelais boa</i>	25	0	0	0	0	0
<i>Lagis koreni</i>	13	0	0	0	10	0
<i>Syllidia armata</i>	18	0	0	0	0	0
<i>Ampharete sp.</i>	1	0	0	0	0	0
<i>Pholoe inornata</i>	1	0	0	0	0	0
<i>Macroclymene santanderensis</i>	21	0	0	0	0	0
<i>Therochaeta flabellata</i>	0	0	0	0	1	0
Nereididae (juvenile)	1	0	0	0	0	0
<i>Malacoceros sp.</i>	0	2	0	0	0	0

Maldanidae	2	0	0	0	0	0
<i>Tharyx sp.</i>	0	0	1	0	0	0
<i>Diplocirrus glaucus</i>	0	0	0	0	1	0
<i>Lumbrineris sp.</i>	0	0	0	0	1	0
Nematoda	22	1	132	2	147	0
Cnidaria	1	0	0	0	0	0
<i>Ophiura sp.</i> (juvenile)	9	1	2	0	2	2
Echinodermata larva 1	7	7	0	0	3	18
Echinodermata larva 2	16	0	0	0	0	0
<i>Echinocardium cordatum</i>	9	0	0	0	0	0
Chironomidae larva	1	0	0	0	0	0
Indeterminate <i>sp.</i>	0	0	0	1	0	0