

The impact of different anthropogenic disturbances on mussel beds and their associated invertebrate biodiversity

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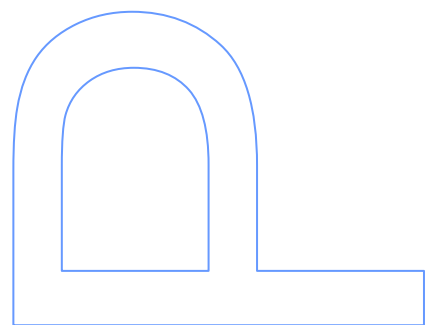
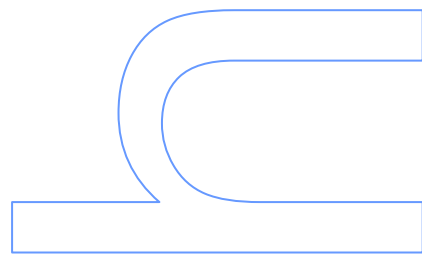
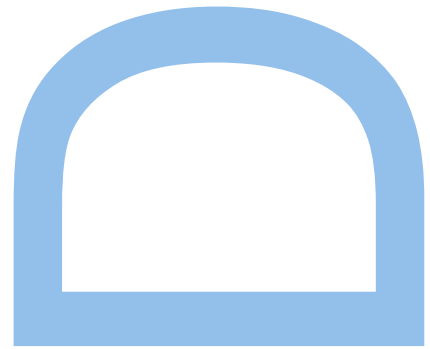
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Brief note

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Resumo

A biodiversidade do meio marinho tem uma importância reconhecida. Em particular as zonas costeiras, albergam uma grande variedade de organismos, mas estes, estão sujeitos a um grande dinamismo natural e estão expostos a diversas atividades humanas. Essas atividades humanas têm aumentado cada vez mais, devido ao aumento da urbanização nas zonas costeiras. Ao aumento da urbanização estão associadas diversas atividades, como as construções, as atividades recreativas, entre outras atividades antropogénicas, que fazem alterar as condições físicas do ambiente, modificando os habitats e por sua vez, afetando diretamente ou indiretamente os padrões de distribuição, abundância e diversidade das comunidades presentes. Devido a todas estas pressões, as comunidades de organismos destes locais apresentam amplas variações espaço-temporais.

Um dos organismos presentes e mais abundantes da zona intertidal rochosa do Norte de Portugal, é o mexilhão mediterrânico *Mytilus galloprovincialis*, Lamarck, 1819. Ele vive em aglomerados, é um animal filtrador e proporciona uma grande variedade de serviços do ecossistema. É também considerado Engenheiro de Ecossistemas, pois proporciona habitat para uma grande diversidade de organismos.

Por todas as suas características, serviços do ecossistema prestados e pela sua função como engenheiro do ecossistema, o mexilhão *M. galloprovincialis* e as comunidades associadas têm especial relevância para trabalhos de monitorização e experimentação e, por isso, foram utilizados como organismos modelo ao longo das diferentes tarefas desta tese.

Na primeira tarefa, o objetivo foi ver se existiam diferenças significativas nos atributos e na fauna associada aos mexilhões entre praias consideradas urbanas e não urbanas.

Os resultados mostraram que os mexilhões de maior tamanho eram mais frequentes nas praias urbanas e os mexilhões de menor tamanho só estavam presentes nas praias não urbanas. Relativamente à macrofauna associada aos mexilhões, o número de taxa foi significativamente maior nas praias não urbanas e a estrutura de toda a comunidade é significativamente diferentes entre praias urbanas e não urbanas.

Na segunda tarefa, foi considerado um tipo de impacto decorrente do aumento da urbanização, a sedimentação. Nesta tarefa exploramos de que forma é que a acumulação de sedimento afeta a abundância, a diversidade e a estrutura da macrofauna associada aos mexilhões.

Os resultados mostraram que existe uma correlação significativa entre a quantidade de sedimento e a abundância, número de taxa, índices de Shannon e Pielou e estrutura de toda a comunidade. E que a abundância, os índices de Shannon e Pielou e a estrutura da comunidade são significativamente diferentes entre mexilhões com alta e baixa quantidade de sedimento, dependendo da praia considerada.

Na terceira tarefa exploramos de que forma é que a presença da alga *Caulacanthus* spp. a crescer sobre as populações de mexilhões, influenciava os atributos e a macrofauna associada aos mexilhões.

Como resultados, obtivemos que a presença da alga sobre os mexilhões afeta significativamente o índice de condição dos mexilhões, dependendo da data de amostragem. Relativamente à fauna associada, o número de taxa, os índices de Shannon e de Pielou e toda a estrutura da comunidade apresenta diferenças significativas entre as amostras de mexilhões com e sem alga, dependendo também da data de amostragem.

Na quarta e última tarefa realizamos um estudo experimental para simular o pisoteio (consideramos 3 intensidades diferentes) sobre as populações de mexilhões, para ver os possíveis efeitos nos atributos dos mexilhões e na sua macrofauna associada.

Os resultados mostraram que tanto os atributos como a fauna associada aos mexilhões não foram afetados significativamente por nenhuma das intensidades de pisoteio testadas.

Com os resultados das quatro tarefas verificamos que de uma forma geral os mexilhões aparentam ter resistência às pressões antropogénicas testadas, mas as suas comunidades associadas são mais sensíveis, dependendo da pressão antropogénica considerada. Devido à extrema importância dos mexilhões para a biodiversidade do intertidal rochoso do Norte de Portugal, é essencial adotar estratégias que minimizem os efeitos das atividades antropogénicas nas zonas costeiras.

Palavras-chave

Biodiversidade; Zonas costeiras; Urbanização; Sedimentação; Alga; Pisoteio; Intertidal Norte de Portugal; *Mytilus galloprovincialis*; Engenheiro do Ecossistema

Abstract

The biodiversity of the marine environment is of recognized importance. In particular, coastal areas are habitat for a wide variety of organisms, but these are subject to great natural oscillation of environmental parameters and are exposed to different human pressures. These pressures have increasing, due to the increase in urbanisation in coastal areas. Various activities are associated with the increase of urbanisation, such as construction, land/marine use change, recreational activities, among others, which alter the physical conditions of the environment, modifying habitats and, in turn, directly or indirectly affecting the patterns of distribution, abundance and diversity of communities. Due to all these oscillations, the rocky intertidal communities show wide spatial-temporal variations.

One of the most abundant organisms in the rocky intertidal zone of northern Portugal is the Mediterranean mussel *Mytilus galloprovincialis*, Lamarck, 1819. It lives in aggregates, is a filter-feeding and provides a wide variety of ecosystem services. It is also considered an Ecosystem Engineer, as it provides habitat for a great diversity of organisms.

Due to all its characteristics, ecosystem services provided and its role as an Ecosystem engineer, the mussel *M. galloprovincialis* and its associated communities have special relevance for monitoring and experimental studies.

So, for this study, this mussel species was used as the focus species, and the different tasks were carried out on rocky shores in the North of Portugal, which were previously selected according to their degree of urbanisation.

In the first chapter, we studied the differences in mussel attributes and associated macrofauna between urban and non-urban shores. The results showed that larger mussels were more frequent on urban shores, while the smallest mussels were only found on non-urban shores. With regards to the associated macrofauna, only the number of species was significantly higher on non-urban shores, although both the number of individuals and the Shannon index showed higher values on non-urban shores. In addition, the whole structure of the associated macrobenthic community was significantly different between urban and non-urban shores.

In the second chapter, we explored how the amount of sediment trapped among mussel beds influenced their associated fauna. Overall, data revealed significant correlations for the Pielou and Shannon indexes and for the abundance and diversity of the associated fauna with sediment content. In particular, the abundance and the diversity showed positive correlations with sediment quantity, while the Shannon index and the Pielou index showed negative correlations. For the entire associated community, a significant high

correlation with the quantity of sediment. We also found and the sediment grain size of 0.500 mm of the one that most relevant in shaping invertebrate assemblages. Particularly, the results of samples with high and low quantity of sediment, showed significant differences in the interaction between shore and quantity of sediment for the number of individuals, with higher values in samples with a high quantity of sediment, but with significant differences only on the shore of Moledo. For the indexes of Shannon and Pielou, a significantly higher values in samples with low quantity of sediment were found and also for the whole structure of the community.

In the third chapter, focus was placed on the effects of the combination of two ecosystem engineers (*M. galloprovincialis* and alga turf of the genus *Caulacanthus* sp.), living among mussels in certain places of the northern of Portugal on their associated fauna throughout the year. In parallel, the effect of algae on the attributes of mussels was also evaluated. For the mussel attributes, the presence of the algae affected only the condition index, though this observation depended on the sampling date. With regard to macrofauna associated with mussels, differences were found between samples with and without algae for number of taxa and for Shannon and Pielou indexes, where the values were always significantly higher in samples with algae. For the entire structure of the community, differences were also found between conditions, but these were also dependent on the sampling date.

In the fourth chapter, we simulate a trampling experiment with different intensities to test the effects of trampling on the attributes of the mussel *M. galloprovincialis* and its associated fauna. The results showed that trampling did not significantly affect the attributes of mussels (density, condition index and size), nor the abundance, diversity and structure of the total assemblage of associated fauna. But significant variability was found among plots for mussel density and for number of taxa, Shannon index and structure of the total assemblage.

With the results of the four tasks, we found that, in general, mussels appear to respond to anthropogenic pressures than their associated communities that are more sensitive, depending on the anthropogenic activity considered. Due to the extreme importance of mussels for biodiversity and human well-being, it is essential to adopt strategies that minimize the effects of anthropogenic activities in coastal areas.

Keywords

Biodiversity; Coastal zones; Urbanisation; Sedimentation; Algae; Trampling; Intertidal North of Portugal, *Mytilus galloprovincialis*; Ecosystem engineer.

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Abbreviations and acronyms

A - Vila Praia de Âncora

ANOVA - Analysis of variance

Co x Si - Condition x Site

Da x Co - Date x Condition

DistLM - Multivariate multiple regression

H' - Shannon index

J' - Pielou index

M - Moledo

N - Number of individuals

ns - not significant

nMDS - non-metric multidimensional scaling

PERMANOVA - Permutational multivariate analysis

PERMDISP – Permutational multivariate analysis of Dispersion

S - Number of taxa

s - significant

SE - Standard error

SIMPER – Similarity percentage analysis

Sh x Se - Shore x Sediment

SNK - Student-Newman-Keuls

CHAPTER I. GENERAL INTRODUCTION

General Introduction

The biodiversity of the marine environment is of recognized importance and more knowledge on biodiversity and how it affects the functioning of ecosystems is of special relevance, not only describing new species, but also exploring their role in maintaining ecosystem functions (Snelgrove, 1999; Gamfeldt and Bracken, 2009). Although this importance is generally acknowledged, several human activities are degrading coastal and marine communities and, in particular, marine ecosystems are being highly exploited and the resources have rapidly been depleted (French, 1997, Lau et al., 2019), in addition to the modification and degradation of natural habitats (Mayer-Pinto et al., 2018). Coastal ecosystems, in particular intertidal rocky shores, play a critical role in the livelihoods and well-being of millions of people around the world (Lau et al., 2019), as they provide a wide range of ecosystem services for humans, from primary production, biofiltration, fish and shellfish nursery, recreational activities, food procurement and tourism (Holmlund and Hammer, 1999; Vinagre et al., 2016). Due to all the services provided, it is essential to maintain the stability and proper functioning of these ecosystems (French, 1997). In addition, rocky intertidal shores contain a wide variety of organisms, being one of the most diverse habitats in the marine environment (Araújo et al., 2005; Gestoso et al., 2010; Bertocci et al., 2012a). Intertidal zone extends over 80% of the world coastline (see in Vinagre et al., 2017) and it is the transition between terrestrial and marine ecosystems and is influenced by natural ecosystems, making it a fragile zone with low stability (see in He and Wang, 2010). The intertidal zone presents great dynamism, as it is subject to different tidal regimes and, in turn, to sudden changes in temperature, radiation, dissection and osmotic pressure (Valdivia et al., 2011), making it a very hostile zone, where biotic communities are naturally highly variable (Thompson et al., 2002). In addition to natural influences, benthic communities in these areas are particularly exposed to human activities, such as urbanisation, recreational and commercial activities, namely fisheries, the harvesting of organisms for bait, trampling, contamination due to the presence of industries and the construction of artificial structures to protect the coast, for example (Thompson et al., 2002; Bertocci et al., 2012a). Due to all these pressures, the communities of organisms in these locations are subject to different stress gradients (Raffaelli and Hawkins, 1996) and, therefore, present wide spatio-temporal variations (Araújo et al., 2005; Rubal et al., 2011; Veiga et al., 2013).

Due to all of this, it is essential to have knowledge of the communities in these places and how they change, and what are the biodiversity trends, carrying out monitoring studies, to understand how the different anthropogenic impacts may affect the composition and

distribution of species, so that measures can be thought for prevention and/or recovery and carry out adequate and sustained management.

1. Anthropogenic Threats

Terrestrial areas adjacent to coastal areas are home to a large and growing proportion of human population, settlements, and socioeconomic activities (Small and Nicholls, 2003; Todd et al., 2019). In fact, coastal areas, up to 100 km from the seacoast, have three times more population per area than the global average (Todd et al., 2019), with predictions pointing that in the coming decades 75% of the population will be concentrated on these locations (Airoldi and Beck, 2007).

Living close to the coast has many economic benefits since there are a number of economic activities. These include tourism, fishing, industry, transport, aquaculture, among others, which may have a major influence on coastal ecosystems (Creel, 2003; Barragán and Andrés, 2015). Coastal ecosystems support many of these services, as well as help prevent erosion, filter pollutants, provide food and shelter, and are breeding and nursery areas and therefore harbour a wide variety of organisms (Creel, 2003). The value of ecosystem services provided by coastal ecosystems is very high and represents about a third of the total for all ecosystems (Barbier et al., 2011; Underwood and Chapman, 2013; Barragán and de Andrés, 2015). While there is a great diversity of advantages for human populations, this population and economic development is causing the decline of the ecosystems that provide those services to the population (Creel, 2003). The anthropogenic impacts are so significant that they make coastal urban expansion one of the strongest, most widespread, and growing threats to coastal ecosystems (Barragán and de Andrés, 2015; Bertocci et al., 2017). With increasing urbanisation, rates of local extinction, alteration of natural habitats and species composition, homogenization of communities and modification of energy flow and nutrient cycling increase (see Mayer-Pinto et al., 2018). Urban development is associated with several problems, such as habitat destruction (see Bertocci et al., 2017). It is currently estimated that half of the salt pans and almost a third of the mangroves, coral reefs and seagrasses worldwide have been destroyed or degraded (Small and Nicholls, 2003). This same habitat destruction occurs due to the proliferation of constructions in the coastal area (see Lai et al., 2015). Consequently, with urban development, problems related to erosion also increase, which makes necessary to build more artificial structures that try to minimize the impacts of the sea-level rise and the increased frequency of storms (see Lai et al., 2015). In addition, these infrastructures will change the geomorphology and ecology of habitats, impacting their biodiversity and functioning, as they provide a different habitat that supports

communities distinct from natural habitats, characterized by reduced diversity and great abundance of opportunistic species (see Mayer-Pinto et al., 2018). Furthermore, construction along the coast, and the erosion caused by it, will cause other types of problems, namely changing the availability of light, water flow, wave energy, transport of resources and sedimentation (Mayer -Pinto et al., 2018). In the case of sedimentation, in addition to the infrastructure to be changed, in more urban places, it tends to increase, due to deforestation, dredging, domestic and industrial discharges, constructions, water replacement and aquaculture (Airoldi, 2003; Ferreira et al. al., 2019). This same sedimentation will interfere at the habitat level, making changes in the ecosystems (Airoldi, 2003). With increasing urbanisation, there is also the problem of the introduction of exotic species (Bertocci et al., 2017) that is considered a threat for marine biodiversity (Carlton and Geller, 1993; Bax et al., 2001; Araújo et al., 2011) since they can change the structure of native communities, reducing the diversity and richness of native species (Freitas et al., 2016), extinction of local populations and reduction of productivity and functioning of ecosystems. Introduction of exotic species can also lead to changes in the food chain (Engelen et al., 2013) and can thus have many economic impacts (Schaffelke et al. 2006; Araújo et al., 2011). In case of macroalgae, they are organisms with high invasive potential (Salvaterra et al. 2013). Worldwide, there are about 277 species of invasive macroalgae (Schaffelke et al., 2006). On the Portuguese coast, 18 invasive algal species are currently found (Araújo et al., 2009a; Invasoras, 2021). The success of invasive macroalgae depends not only on the biological characteristics of the invaders, and their tolerance to stress by different abiotic factors namely, rougher tidal emersion, dramatic osmotic changes, freezing, heat, heavy metal, high irradiance, desiccation (Liu and Pang, 2010) and also depends on different biotic factor that may improve their competition abilities and capabilities for invasion (e.g., reproduction form, growth rate and potential of dispersion (Staeher et al., 2000). Furthermore, also depends on the characteristics of the host communities (Cacabelos et al., 2010).

Another threat for biodiversity is pollution and contamination, which are one of the biggest threats to human health (see in Bertocci et al., 2017). Sewage being the biggest source of environmental pollution in these areas, representing some 80% of marine pollution worldwide (Creel, 2003). With the growth of populations living on the coasts, sources of organic enrichment and pollution also tend to increase, due directly to the leaching of animal manure from farms, fertilizer runoff from agricultural fields, aquaculture waste and also sewage and industrial and domestics discharges who are not properly treated and are directly evicted (Todd et al., 2010). Chemicals and heavy metals that harm both marine organisms and human health, as they tend to accumulate in living tissues and as the food

chain rises, are also found in the sewage and their concentrations also increased (Creel, 2003). In addition to these impacts described, there are still others that result from the increase in urbanisation, which can also significantly affect the populations and communities of the rocky coast of the intertidal, namely trampling, due to visitors' walks over the rocks, the collection of organisms, to bait, for consumption or just for collection and/or the simple manipulation of rocks or organisms (Murray et al., 1999).

All these impacts due to urbanisation cause physical changes to the environment (landscape changes) through modifications in the original habitats (Oliveira et al., 2014; Bertocci et al., 2012b; 2017). Therefore, they can directly or indirectly affect the patterns of distribution, abundance and diversity of communities (Veiga et al., 2020; Torres et al., 2022). Moreover, can cause decline or even extinction of native species, which consequently may have impacts at the level of food chains and biological interactions (Oliveira et al., 2014; Bertocci et al., 2012b; 2017). Also, the anthropogenic impacts can reduce biodiversity due to homogenization in terms of species composition and abundance in which rarer and more sensitive species are replaced by more resistant ones (Oliveira et al., 2014; Bertocci et al., 2017). On the other hand, the impacts of anthropogenic pressures may vary according to the intensity of these pressures, since when the impact is moderate, instead of there being a homogenization of species, there may even be an increase in biodiversity, through the increase of non-native species, for example (Oliveira et al., 2014). All these changes may have deeper implications, which may affect human well-being and for that, it is necessary to monitor and, consequently, adequately manage the coast and key species.

2. *Mytilus galloprovincialis* as an ecosystem engineer

The distribution and abundance of species is mostly determined by the interaction between organisms (Jones, 1994). There are organisms that directly or indirectly control the availability of resources available to other species, modifying, maintaining, or creating habitats (Jones, 1994; 1997). These species are named ecosystem engineers (Jones, 1994; 1997).

In the marine environment, there are many organisms considered to be ecosystem engineers, as they modify the habitat and significantly influence biodiversity. Among these, there are seagrasses and kelps beds, coral reefs and oyster and mussel reefs (Gutiérrez et al., 2003; Norling and Kautsky, 2007). Molluscs are organisms that are present in many

marine habitats and they are considered ecosystem engineers with different characteristics depending of the type of mollusc considered (e.g., suspension feeding, deposit feeding, grazing, predation, bio deposition, bioturbation and production of shells) (Gutiérrez et al., 2003). Among molluscs, bivalves serve as habitat for many organisms, regulate water quality through their filtering capacity, retain carbon and nitrogen, clean the water of particles, leading to increase the transparency of the water column. Moreover, they stimulate the production of phytoplankton by excreting ammonia, which will act as a source of nutrients. Furthermore, they are used for coastal defence and conservation (Wijsman et al., 2019). Bivalves have complex shells, and these shells affects benthic habitats, modifying the complexity and heterogeneity of the habitat (Gutiérrez et al., 2003) by controlling the availability of substrates for encrusting organisms, the availability of free space for protection against predators or stress and the transport of particles and solutes in the benthic environment (Gutiérrez et al., 2003). In addition, some species of bivalves live in aggregates made up of live animals and empty shells, attached to the substrate by byssus, which in the case of mussels. For example, on soft bottoms, provide substrate for sessile organisms to settle, and on rocky bottoms, they provide habitats for sediment organisms, which accumulate in the interstitial spaces between the shells (Gutiérrez et al., 2003; Norling and Kautsky, 2007). The presence of shells increases the complexity of habitats and this, in turn, increases species richness. Despite that, the refuge in the aggregations is not linear as a function of the number of shells, but is also related to the interstices between the shells (Gundersen et al., 2017). In the case of Mediterranean mussels, they inhabit the intertidal zone in aggregations and because they live in this zone, they are well adapted to environmental fluctuations, being considered extremely tolerant to changes in temperature and salinity gradients (Gundersen et al., 2017). Within the aggregations, different sizes of mussels can be found (Gundersen et al., 2017). They are filter-feeding animals that feed on phytoplankton, zooplankton, bacteria and debris from dead plants and animals that float in the water and, because of their type of food, can clean the water, thus increasing its transparency (Gundersen et al., 2017).

There are several threats that could cause changes in mussel populations, namely linked to increased urbanisation or climate change, such as the introduction of exotic species, increased ocean acidification that could cause physical and physiological changes such as a decrease in the growth rate, fertilization and/or increased vulnerability of shells, among other threats (Gundersen et al., 2017; Romero-Freire et al., 2020). Modifications in terms of attributes (e.g., density or size frequency) of mussels may have consequences at the level of the associated fauna, altering their diversity and community structure (Veiga et al., 2020; Torres et al., 2022).

In addition to their importance as a habitat, marine bivalves such as mussels have been cultivated for a long time, being recognized as a sustainable resource with both ecological and economic value (Wijsman et al., 2019; Romero-Freire et al., 2020), providing to local communities a rich source of nutrition for humans and other organisms, but also an economic livelihood (Wijsman et al., 2019). Worldwide, aquaculture is expanding rapidly, with China being the leading country. In Europe, Spain has the largest mussel production (Gundersen et al., 2017).

The Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819, is widely distributed throughout the world (i.e., Atlantic coast of Europe; west coast of North America, Australasia and as an invasive in Southern Africa) (Rius and Cabral, 2004; Vinagre et al., 2015; Ramos-Oliveira et al., 2021) and it is the most abundant mussels on the Portuguese coast (Boaventura et al., 2002). The mussel species *M. galloprovincialis* is traditionally harvested for commercial purposes as bait or food and is therefore severely exploited in some countries such as Italy, Spain, and Portugal (Bertocci et al., 2012b), is the main European species produced in aquaculture and the north-western zone of Spain the main producing area (Romero-Freire et al., 2020). In addition to its commercial importance, *M. galloprovincialis* provides other ecosystem services of great importance, namely the mediation of toxic residues, through the biotransformation of compounds present in the water (Montes et al., 2012), coastal protection through the influence on the flow of tides and wave action and in the modification of patterns of deposition, consolidation and stabilization of sediments, provides habitat for many organisms (Gundersen et al., 2017) and is considered as a sentinel organism and indicator of ecological status with respect to pollution (Romero -Freire et al., 2020).

For all its characteristics and ecosystem services provided, the mussel *M. galloprovincialis* has special relevance for monitoring studies of marine coastal ecosystem's health and consequent protection.

CHAPTER II. MAIN OBJECTIVES

Main goals

Since urbanisation near the coastal zone and all the activities associated with it continue to increase, it is essential to develop monitoring studies of the different activities in order to understand their impacts on key species, such as ecosystem engineers, and on their associated macroinvertebrate communities. For this purpose, two questions underlying this PhD thesis have arise:

1. How do different anthropogenic activities affect the attributes of an ecosystem engineer and their associated macroinvertebrate communities?
2. Are the differences found in the associated communities due to differences in the attributes of the ecosystem engineer or are they directly due to environmental differences caused by certain anthropogenic activity?

In order to achieve the main objectives, different experimental studies were designed focusing on a set of specific goals:

- Explore how urbanisation affects the attributes and biodiversity associated with the mussel;
- Explore whether sediment accumulation correlates with associated macrofauna and whether the quantity of sediment influences the structure of macrofauna assemblages associated with mussel beds;
- Explore how the presence of an alga as co-engineer in mussel beds can affect the attributes of mussels and the associated macrofauna;
- Explore how mussels and its associated fauna react to different trampling intensities.

CHAPTER III. DIFFERENCES IN THE STRUCTURE AND DIVERSITY OF INVERTEBRATE ASSEMBLAGES HARBORED BY AN INTERTIDAL ECOSYSTEM ENGINEER BETWEEN URBAN AND NON-URBAN SHORES



Article

Differences in the Structure and Diversity of Invertebrate Assemblages Harbored by an Intertidal Ecosystem Engineer between Urban and Non-Urban Shores

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Differences in the Structure and Diversity of Invertebrate Assemblages Harbored by an Intertidal Ecosystem Engineer between Urban and Non-Urban Shores

Abstract

Nowadays, coastal urbanisation is one of the most serious and prevalent pressures on marine ecosystems, impacting their biodiversity. The objective of this study was to explore differences in attributes and biodiversity associated with an intertidal ecosystem engineer, the mussel *Mytilus galloprovincialis* Lamarck, 1819 between urban and non-urban shores. For this, mussel attributes and their associated macrofauna were compared between urban and non-urban rocky shores in the north of Portugal. Results showed that the largest sized mussels were more frequent on urban shores, whereas the smallest size class was only present in non-urban shores. Regarding macrofauna associated with mussels, the number of taxa was significantly higher on non-urban shores. Moreover, the structure of the macrobenthic assemblages was significantly different between urban and non-urban shores. Most important taxa responsible for differences were more abundant on non-urban shores except for *Nucella lapillus*, *Idotea pelagica* and *Oligochaeta* that were more abundant on urban shores. Therefore, our results showed that the mussel size frequency and the structure of the associated macrobenthic assemblages changed in urban shores. Considering the relevance of mussel beds for biodiversity and human well-being, our results indicate the need of adopting proper management plans to minimize these effects on urban intertidal ecosystems.

Keywords

Ecosystem engineer; *Mytilus galloprovincialis*; urbanisation; intertidal; rocky shores; mussel attributes; macrobenthic assemblages.

1. INTRODUCTION

Organisms that through their behavior and/or morphology can directly or indirectly control the resources available to other species are considered ecosystem engineers (Jones et al., 1994; 1997). These organisms can modulate, maintain or create habitats, increasing heterogeneity and thus, biodiversity (Crain and Bertness, 2006). Bivalve mollusks that

form aggregates and increase substrate complexity are considered ecosystem engineers (Gutiérrez et al., 2003; Borthagaray and Carranza, 2007; Arribas et al., 2014). Moreover, their filter feeding activity improves the benthic-pelagic coupling and thus, intensifies the input of food (Norling and Kautsky, 2007). Bivalve shells can also serve as secondary substratum for many sessile or sedentary organisms, whereas mobile organisms live in the spaces among them (Gutiérrez et al., 2003; Borthagaray and Carranza, 2007). Bivalves are particularly relevant as ecosystem engineers on intertidal habitats because they are capable of ameliorating the high environmental stress during low tide (e.g., thermal stress, desiccation, heavy rains) or the abiotic and biotic pressures during high tide (e.g., wave action, predation) (Crain and Bertness, 2006). Mussel beds are one of the most frequent bivalve aggregations on intertidal habitats, harboring diverse assemblages of invertebrates (Singh et al., 2013; Arribas et al., 2014; Çınar et al., 2020; Hodgson et al., 2021). The Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 is an intertidal filter-feeding bivalve, widely distributed on the Atlantic rocky shores of the Iberian Peninsula and is one of the most abundant species at intertidal Portuguese rocky shores (Boaventura et al., 2002; Ramos-Oliveira et al., 2021). Furthermore, this species also plays an important role in intertidal food chains (Vinagre et al., 2015) and offers valuable ecosystem services, such as food, coastal protection or elimination of pollutants (Gundersen et al., 2017; Romero-Freire et al., 2020; Veiga et al., 2021). Moreover, *M. galloprovincialis* has a high commercial value because it is a popular shellfish in the human diet that is extensively explored in several European countries (Rius and Cabral, 2004). Moreover, it is the main European bivalve species produced in aquaculture (Avdelas et al., 2021). However, mussel beds, particularly in the intertidal, are subjected to various anthropic disturbances, which may have a great impact on the functioning and stability of their aggregates (Thompson et al., 2002; Carranza et al., 2009). One of these disturbances is increasing urbanisation, which is one of the most widespread and growing threats to coastal ecosystems (Barragan and de Andrés, 2015; Momota and Hosokawa, 2021). Coastal urbanisation is associated with a higher population density; areas up to 100 km from the coastline harbor three times more population than the global average (Todd et al., 2019) and predictions point out that in the coming decades, 75% of the population will be concentrated in coastal localities (Airolidi and Beck, 2007). This phenomenon emerges due to the facilitation of activities such as fishing, industry, tourism and transportation, among others in coastal areas (Barragan and de Andrés, 2015). Despite the benefits of these activities to humankind, they are a relevant source of disturbances, such as chemical contamination of water due to domestic or industrial sewage, trampling, harvesting and the introduction of exotic species and/or habitat fragmentation due to construction of

artificial structures (e.g., sea walls) that can cause ecological impacts (Malerba et al., 2019; Todd et al., 2019; Momota and Hosokawa, 2021). Therefore, it is imperative to study the effects of urbanisation, particularly in the intertidal, because it is a very accessible area to human activities (Thompson et al., 2002). Many studies have explored the different impacts associated with urbanisation on intertidal habitats such as trampling or artificial structures (Brown and Taylor, 1999; Chapman, 2003; Airoidi and Bulleri, 2011; Momota and Hosokawa, 2021). The effect of urbanisation on mussel beds has also received some attention, mainly focused on harvesting and trampling e.g., (Brosnan and Crumrine, 1994; Smith and Murray, 2005; Robinson et al., 2007; Veiga et al., 2020) but these studies were focused on mussel populations. However, studies focused on the urbanisation effect on assemblages associated with mussels are still scarce. Some studies explored the effect of different stressors, mainly invasions and pollution, commonly associated with coastal urbanisation. For example, Gestoso et al. (2013) compared the fauna associated in two species of mussels, one native (*Mytilus galloprovincialis*) and one invasive (*Limnoperna securis*) and Davis et al. (2018) compared the diversity harbored by a native mussel (*Perna canaliculus*) and an invading ascidian (*Pyura doppelganger*). Other studies have dealt with the impacts of pollution, such as Chintiroglou et al. (2004) which investigated the spatial and temporal structure of the fauna associated with *M. galloprovincialis* in the Thermaikos Gulf (northern Aegean Sea) and Çinar et al. (2008) where the diversity associated with *M. galloprovincialis* inside and outside ports was monitored. However, on urban shores different stressors act simultaneously and thus, may result in a complex response of assemblages that cannot be inferred from studies considering the effect of isolated stressors (Crain et al., 2008). As ecosystem engineers increase biodiversity locally, they are considered valuable conservational targets (Crain and Bertness, 2006; Van der Zee et al., 2015). However, to understand how environmental disturbances modify the diversity of assemblages harbored by ecosystem engineers, empirical data are still needed. The main goal of this study is to explore differences in mussel's attributes and its associated macrobenthic assemblage among urban and non-urban shores. For this purpose, differences in density, size frequency and condition index of mussels and the abundance, taxa richness, Shannon index and the structure of macrofaunal assemblages associated with mussel beds were explored on urban and non-urban shores in the north Portuguese coast.

2. MATERIALS AND METHODS

2.1. Study Area, Sampling and Sample Procedure

This study was done in April 2017 at four rocky shores in the north coast of Portugal with different degrees of urbanisation. In order to define the degree of urbanisation in the studied shores, we used population density as a proxy because a higher population density implies a higher degree of trampling, harvesting, more artificial structures and a high input of domestic and industrial sewage (e.g., Creel, 2003; Smith and Murray, 2005; Rius et al., 2006; McPhee, 2017; Todd et al., 2019). Two rocky shores were selected in the metropolitan area of Porto with more than 2800 residents/km²: Leça (41°12'16.490 0 N; 8°42'057.160 0 W) and Cabo do Mundo (41°13'030.960 0 N; 8°43'03.290 0 W), considered as urban shores, whereas two other rocky shores: Moledo (41°50'28.810 0 N; 8°52'032.300 0 W) and Vila Praia de Âncora (41°49'025.930 0 N; 8°52'027.420 0 W), placed in areas with a population density lower than 130 residents/km², were considered as non-urban. A previous study (Rubal et al., 2014) also found that concentrations of heavy metals and nutrients were higher in urban shores (Leça and Cabo do Mundo) than in those considered as non-urban (Vila Praia de Âncora and Moledo). The Portuguese north coast is largely straight and thus all the studied shores have the same orientation and similar environmental conditions. The studied area is characterized by a semidiurnal tidal regime, with the largest spring tides about 4.0 m and most common waves from the west and northwest direction, showing a range of variation from 1.5 to 7 m (Dias et al., 2002). The studied rocky shores are characterized by typically granitic substrate and the area presents a fragmented coastal landscape due to the presence of estuaries and varies from soft to hard substrata. Moreover, there is a seasonal upwelling during the spring and summer months, which provides nutrients for organisms (Lemos and Pires, 2004). At each shore, two sites separated about 10 m apart were randomly selected, and at each site, five quadrats (10 x 10 cm) in *M. galloprovincialis* beds at mid-tide level were collected to explore potential impacts of urbanisation by comparing the attributes of mussels and their associated macrofauna between urban and non-urban shores. For each replicate, samples were collected by scrapping all the quadrat area. All samples were placed in labelled plastic bags. At the laboratory, all samples were frozen ($\pm 20^{\circ}$ C) until their processing. Then, each sample was washed through a sieve of 0.5 mm, in order to separate the macrofauna from the mussels, and all mussels in each sample were counted (density). The residue on the sieve was stored in formaldehyde (4%) stained with Rose of Bengal, until sorting and identification of invertebrates to the lowest possible taxonomic level, usually species. From each replicate, 20 mussels were randomly separated to

measure their shell length and each individual was assigned to a specific size class: Class 1: <5 mm, Class 2: 5–15 mm, Class 3: 15–25 mm, Class 4: 25–35 mm, Class 5: 35–45 mm and Class 6: >45 mm. Moreover, 10 mussels per replicate were used to calculate the condition index (i.e., the ratio between dry weight of soft tissue and dry weight of the shell) by drying the mussels at 60° C for 48h.

2.2. Data Analysis

To explore differences between urban and non-urban shores on mussel attributes (i.e., density and condition index) and on the number of individuals (N), taxa richness (S) and Shannon index values (H') of the invertebrate assemblage associated with mussels, analyses of variance (ANOVAs) were done. For mussel density, N, S and H', a three-way model was considered, including the factors: Condition (Co) fixed, orthogonal with two levels (i.e., urban and non-urban), Shore (Sh) random with two levels, nested in Co and Site (Si) random, with two levels, nested in Co and Sh, with 5 replicates. For condition index, a four-way model was considered including the same factors described above and Quadrat (Qu) as an additional random factor nested in Co, Sh and Si with 5 levels and 10 replicates. Cochran's test was done to check the homogeneity of variances previously by ANOVA tests. Data were log-transformed to remove the heterogeneity of variances, when necessary. When this was not possible, untransformed data were analyzed and the results were considered robust if significant at $p < 0.01$ (Underwood, 1997). In order to explore differences in size structure of mussels between urban and non-urban shores, their size-frequency was compared by means of Kolmogorov–Smirnov tests (KS). Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), based on the Bray–Curtis untransformed dissimilarity matrix, was used to analyze the multivariate assemblage data. The model for this analysis was the same as previously described for the three-way ANOVA, using a maximum of 999 permutations in the reduced model with a defined level of significance, a priori, at $p < 0.05$. When the number of unique permutations for a factor was lower than 30 (or close to 30), Monte Carlo p -values were considered (Anderson et al., 2008). Multivariate patterns were illustrated by non-metric multidimensional scaling (nMDS) ordination of sampled sites. The PERMDISP procedure was done to test whether differences between urban and non-urban conditions were due to different multivariate dispersion in the location of centroids (Anderson, 2006). Moreover, the SIMPER procedure (Clarke, 1993) was used to determine the percentage of contribution (%) of each taxon to the Bray–Curtis dissimilarity between conditions. A taxon was considered important if its contribution to total percentage dissimilarity was $\geq 3\%$. The ratio $\delta / SD(\delta)$ was used to quantify the consistency of the contribution of taxa to the

average dissimilarity in all pair-wise comparisons of samples between conditions. Values ≥ 1 indicated a high degree of consistency.

3. RESULTS

3.1. Mussel' Attributes

No significant differences between conditions were detected for density and condition index of mussels (Tables 1 and 2; Figure 1).

Size–frequency distribution of mussels was significantly different between conditions (KS test, $D_{\max} = 12.75$, $p = 0.004$; Figure 2). On the non-urban shores, mussels belonging to size class 15–25 mm dominated in relation to other size categories, whereas on urban shores a clear dominant size class was not found (Figure 2). Moreover, the smallest size class (<5 mm) was only present in non-urban shores, whereas the largest size class (>45 mm) was only present in urban shores where the three largest size classes (25–35 mm, 35–45 mm and >45 mm) were more frequent than in non-urban shores (Figure 2).

Table 1. ANOVA analysis for density of mussels between conditions. ns: not significant.

Source of variation	Df	Density	
		MS	F
Condition	1	110.24	0.76
Shore	2	145.66	1.01
Site	4	144.04	1.29
Residual	32	111.59	
Total	39		
Cochran's test		0.31 ^(ns)	
Transformation		Sqrt(X+1)	

Table 2. ANOVA analysis for condition index of mussels between conditions. s: significant.

Source of variation	Df	Condition index	
		MS	F
Condition	1	0.0277	70.97
Shore	1	0.0073	8.54
Site	4	0.0009	0.88
Quadrat	32	0.0010	1.64
Co x Sh	1	0.0004	0.46
Residual	360	0.0006	
Total	399		
Cochran's test		0.8620 ^(s)	
Transformation		None	

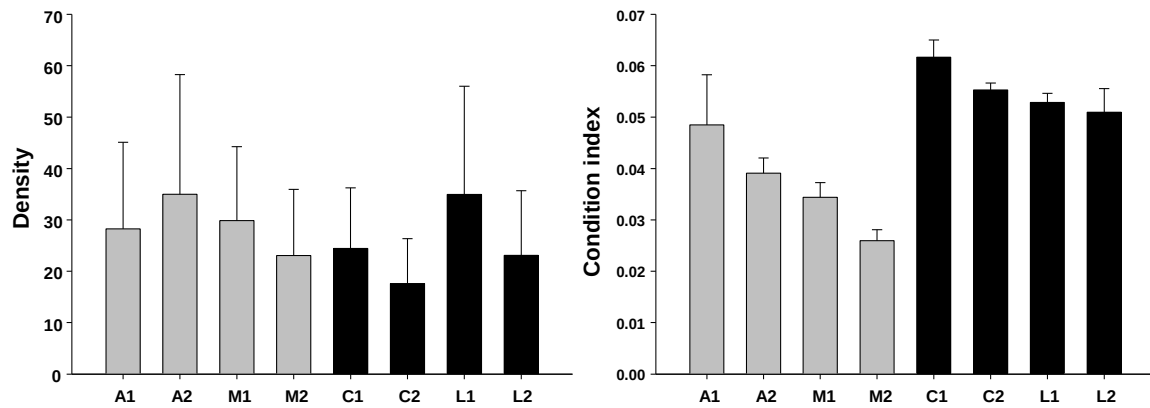


Figure 1. Mean values ($\pm$ SE) of mussel attributes. Density $\sqrt{X+1}$ transformed and Condition index. Grey: non-urban shores (A1/A2: Âncora site 1 and 2, M1/M2: Moledo site 1 and 2); Black: urban shores (C1/C2: Cabo do Mundo site 1 and 2, L1/L2: Leça site 1 and 2).

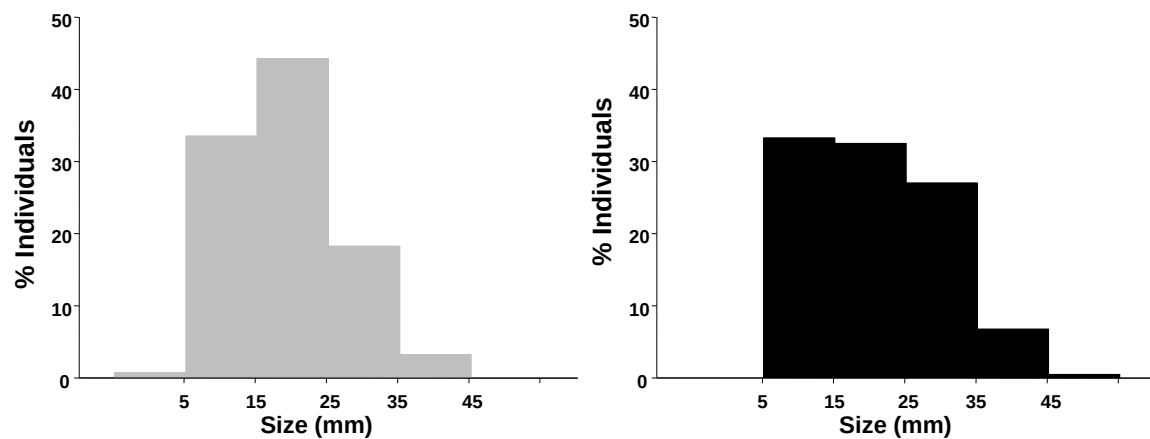


Figure 2. Size-frequency of mussels in the two conditions. Grey: non-urban shores; Black: urban shores.

3.2. Invertebrate Assemblages

A total of 4798 individuals (2650 in non-urban shores and 2148 in urban shores) belonging to 58 different taxa (53 in non-urban shores and 40 in urban shores) were identified. ANOVA indicated significant differences between conditions for S, with significantly higher values on non-urban shores (Table 3; Figure 3). However, no significant differences between conditions were found for N and H' (Table 3, Figure 3).

Table 3. Summary of ANOVAs for the number of taxa (S), number of individuals (N) and Shannon index (H') of faunal assemblage associated with *M. galloprovincialis*. *: $p < 0.05$; **: $p < 0.01$; ns: not significant.

Source variation	of	Df	S		N		H'	
			MS	F	MS	F	MS	F
Condition	1		0.3231	36.83*	12.4339	10.39	0.2738	1.39
Shore	2		0.0088	0.21	1.1972	0.05	0.1966	0.55
Site	4		0.2343	1.27	23.0901	1.15	0.3550	4.17**
Residual	32		0.1845		20.1177		0.0852	
Total	39							
Cochran's test			0.3708 ^(ns)		0.3130 ^(ns)		0.2376 ^(ns)	
Transformation			Ln(X)		Sqrt(X+1)		None	

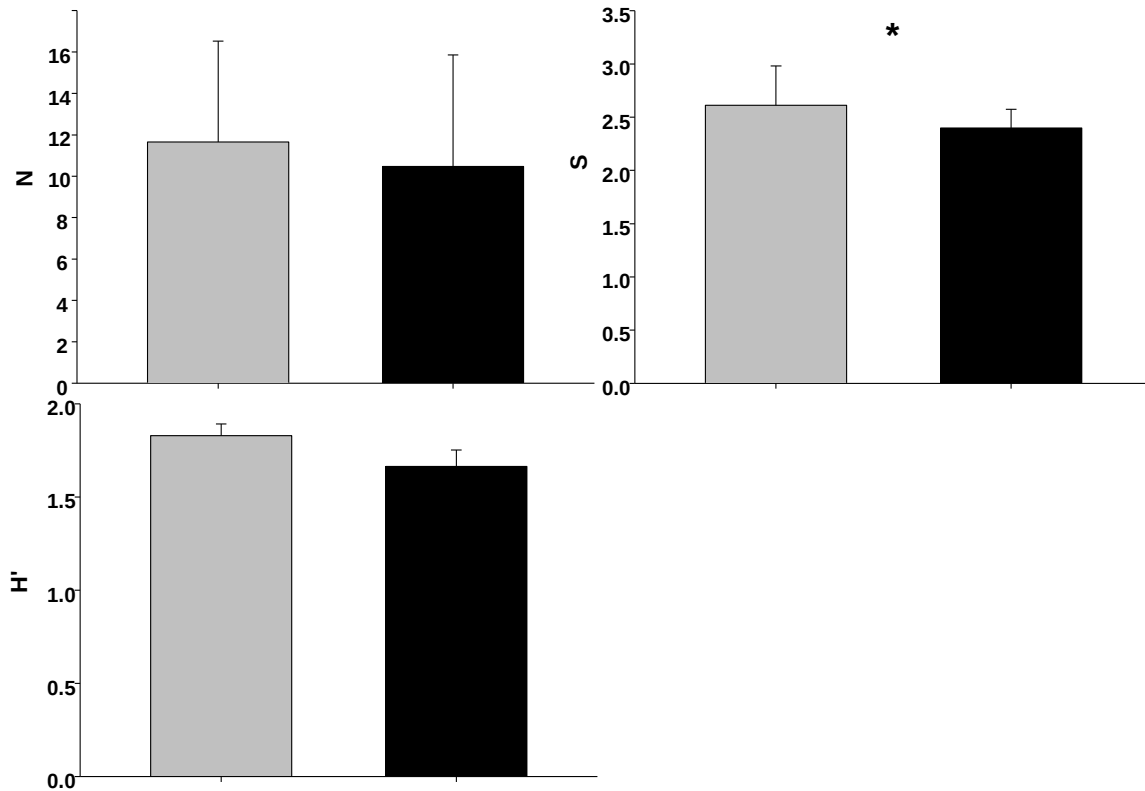


Figure 3. Mean values ($\pm$ SE) of the number of individuals (N) Sqrt (X + 1) transformed, number of taxa (S) Ln (X) transformed and Shannon index (H') of macrobenthic assemblages associated with mussels. *: indicate significant differences between conditions ($p < 0.05$). Grey: non-urban shores; Black: urban shores.

Results of PERMANOVA analysis of the structure of the whole assemblage indicated significant differences between conditions (Table 4). The nMDS ordination showed a clear separation between conditions (Figure 4). The PERMDISP ($F = 2.5487$, $p = 0.149$) indicated that dispersion of samples did not provide a significant contribution to differences detected by PERMANOVA.

Table 4. Summary of PERMANOVAs for total assemblage. *: p (MC) < 0.05 .

Source of variation	Df	Total assemblage		
		MS	Pseudo-F	Unique Perms
Condition	1	13928	3.9564*	3
Shore	2	3520.4	1.0776	296
Site	4	3266.9	1.6877*	998
Residual	32	1935.7		
Total	39			

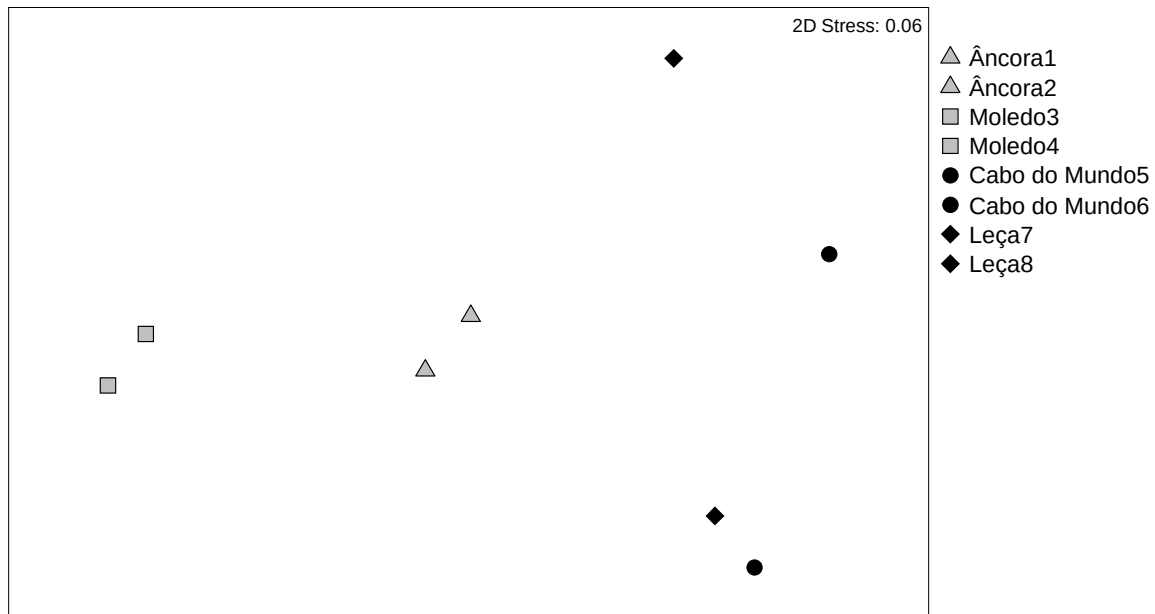


Figure 4. nMDS plots for sites at each urban (black) and non-urban (grey) sites.

The SIMPER analysis identified thirteen taxa as being mainly responsible for differences between conditions. For the total dissimilarity, the percentage of contribution of *Nematoda*, *Nucella lapillus* (Linnaeus, 1758), *Idotea pelagica* Leach, 1816 *Hyale* spp., *Lasaea rubra* (Montagu, 1803), *Oligochaeta*, *Brachystomia scalaris* (MacGillivray, 1843) and *Jaera prae-hirsuta* Forsman, 1949 accounted for almost 80% and the individual contribution of each one was 4% (Table 5). All the species with the exception of *N. lapillus*, *I. pelagica* and *Oligochaeta* were more abundant on non-urban shores (Table 5).

Table 5. Contribution (δ) of individual taxa from faunal assemblages of mussels to the average Bray-Curtis dissimilarity between urban and non-urban shores.

Taxon	Average abundance		δ_i	$\delta_i\%$	$\delta_i/SD(\delta_i)$
	Non-urban	Urban			
<i>Nematoda</i>	48.00	16.15	17.29	23.80	1.34
<i>Nucella lapillus</i>	8.75	36.20	13.21	18.18	1.15
<i>Idotea pelagica</i>	4.85	16.70	7.71	10.61	1.02
<i>Hyale</i> spp.	11.95	3.45	4.65	6.40	1.03
<i>Lasaea rubra</i>	11.75	6.65	4.52	6.22	0.84
<i>Oligochaeta</i>	1.95	12.15	4.40	6.06	0.72
<i>Brachystomia scalaris</i>	9.30	4.05	3.62	4.98	1.02
<i>Jaera prae-hirsuta</i>	6.00	3.80	2.99	4.12	0.86
<i>Steromphala umbilicalis</i>	4.40	2.40	2.31	3.18	0.91
<i>Sabellaria alveolata</i>	6.50	0.05	1.66	2.28	0.34
<i>Apohyale prevostii</i>	2.25	1.10	1.45	2.00	0.75
<i>Syllis pulvinata</i>	2.15	0.35	1.22	1.68	0.36
<i>Patella depressa</i>	2.65	0.75	1.08	1.49	0.80

4. DISCUSSION

Increasing urbanisation has become one of the most serious problems of our time for coastal ecosystems (Barragan and de Andrés, 2015; Todd, 2019). Urbanisation increases stress sources such as contamination, trampling, harvesting and the introduction of exotic species, among others (Mayer-Pinto et al., 2018). Since *M. galloprovincialis* is able to tolerate disturbances, it is present on many impacted urban shores which offer habitat and resources for other invertebrate species (Chintiroglou et al., 2004; Çinar et al., 2008). Therefore, its biomonitoring can provide valuable information about the urbanisation impact on intertidal biodiversity on rocky shores by assessing the effects on mussel attributes and its associated macrofauna. In this study, mussel density and condition index did not show significant differences between urban and non-urban shores. For condition index, Veiga et al. (2020) found equivalent results in the same study area. However, Veiga et al. (2020) found a significant decrease in the density of mussels on urban shores. Similarly, a reduction in the abundance of ecosystem engineers was also found in the same study area for native canopy macroalgae (Rubal et al., 2020). However, a recent study Ramos-Oliveira et al. (2021) about the spatial and temporal variability of *M. galloprovincialis* along the north Portuguese shore showed that differences in its abundance among shores was dependent on sampling dates. Therefore, the inconsistency between results of Veiga et al. (2020) and those of our study may be the result of different sampling dates and natural variability in the abundance of *M. galloprovincialis* on the studied area rather than the effect of urbanisation. Regarding size frequencies of *M. galloprovincialis* in our study, urban shores displayed a higher frequency of larger mussels than non-urban shores, whereas the smallest mussel size was only present in non-urban shores. Similarly, Veiga et al. (2020) also found the same pattern in the north Portuguese coast, suggesting a low recruitment rate on urban shores. Another plausible reason for the largest size of mussels in urban shores could be the wastewater input from domestic sewage, frequent in urban areas, that could increase the availability and quality of food and benefit mussels as filter-feeding organisms (Puccinelli et al., 2016; Conde et al., 2020). The higher nutrient content in the studied urban shores reported by Rubal et al. (2014) suggest a higher input of urban sewage, supporting the previous explanation. Similar results were also found in mussels near fish farms whose organic supply makes mussels reach a greater size than mussels far from the farms (Sarà et al., 2009; Lander et al., 2012). In contrast, Krishnakumar et al. (1994) showed that *M. edulis* in urban sites exposed to chemical pollution presented smaller sizes than in reference sites. Despite the tolerance of mussels to pollution, mixtures of chemical pollution such as metals and PAHs may reduce their fitness and, thus, their size (Beiras et al., 2012).

Regarding the macrofauna associated with mussels, taxa richness and the structure of the total macrobenthic assemblage were significantly different between urban and non-urban shores. Taxa richness showed lower values on urban shores. Previous studies on the same study area, assessing urbanisation effects on marine rocky shore assemblages, also found similar results on the structure of tidepool assemblages (Rubal et al., 2014) and on canopy forming macroalga diversity (Rubal et al., 2020). A recent study comparing the community structure among artificial and natural habitats (eelgrass bed, intertidal flats and subtidal bottom) in an urbanized semi-enclosed coastal sea in Japan, found many sharing species among natural habitats; however, the breakwater showed bit sharing species with natural habitats and the lowest number of species (Momota and Hosokawa, 2021). Moreover, previous studies exploring the effects of pollution on fauna associated with mussels also reported losses of biodiversity and alterations in the structure of benthic assemblages. For example, Elías et al. (2003) found that the distribution of polychaete species associated with the mussel *Brachidontes rodriguezii* was related to the gradient of organic matter associated with a sewage outfall. Çınar et al. (2008) compared the fauna associated with *M. galloprovincialis* inside and outside a port, that has been exposed to numerous pollution discharges since 1960 and is considered as one of the most polluted environments of the Mediterranean Sea. Their results found that the biomass and the number of individuals reached higher values inside the ports, but similar to our urban shores, the number of species was lower in ports. Thus, it appears that the degree of pollution, commonly associated with urban areas, can deeply affect the distribution, composition and abundance of the species harbored by mussels. Therefore, urbanisation may change mussel attributes but also the number of taxa and the structure of invertebrate assemblages associated with mussel beds. Several studies have explored how the attributes of mussels are related to the associated fauna. For example, Chintiroglou et al. (2004) found no relationship between density of mussels and the abundance and diversity of fauna associated with mussels, as in our study, where patterns of mussel density and those of associated fauna were different. However, Hodgson et al. (2021) analyzing the macroinvertebrate communities associated with *M. galloprovincialis* in different regions of a South African estuary reported a negative relationship between the diversity of fauna and the density of mussels, but they found no relationship between mussel size and species richness. On the other hand, O'Connor and Crowe (2007) carried out a manipulative study to assess whether mussel size affected their associated fauna. They found that in one of the studied locations, the fauna associated with larger mussels differed significantly from the fauna associated with smaller mussels. Nevertheless, the size did not affect the species' richness, but rather the abundance and proportion of the organisms

present. When comparing these results with ours, it can be suggested that differences in the mussel size frequency between urban and non-urban shores may affect the structure of the macrobenthic assemblages, showing lower values of species richness in urban shores, where the highest mussel size frequencies were found. However, another potential explanation is that the reduction in diversity on urban shores may be due to changes in the identity and sensitivity of species to anthropic disturbances (Johnston and Roberts, 2009). Similarly, Çınar et al. (2020) also suggests that the differences in fauna composition associated with *M. galloprovincialis* may be due to changes in the water quality. This explanation could be applied to our study, since the studied urban and non-urban shores have different water quality through nutrient enrichment and heavy metal content (Rubal et al., 2014). In view of this, some species could be more sensitive to urbanisation, whereas others could prefer urbanized locations. When analyzing the species that most contributed to the differences in the structure of the community between urban and non-urban shores, only three taxa were more abundant on urban shores (i.e., *Nucella lapillus*, *Idotea pelagica* and *Oligochaeta*), while the remaining taxa were more abundant on non-urban shores. Among the latter, Nematoda, *Hyale* spp. and *Lasaea rubra* were the most relevant in shaping differences between urban and non-urban shores. *Oligochaetes* are considered biological indicators of pollution being more abundant in polluted locations and commonly associated with organic enrichment (Crowe et al., 2004; Parapar et al., 2009). *Oligochaetes*, in our study, were more abundant in urban shores probably as a consequence of the high concentration of heavy metals and nutrient enrichment in urban shores (Rubal et al., 2014). Nematodes are also considered good indicators of the environmental status, since they encompass both sensitive and tolerant species to pollution (Ridall and Ingels, 2021). Our results seem to indicate that the nematode species found in our study should be more sensitive to disturbances, as they were more highly abundant in non-urban shores. Moreover, urban shores, due to wastewater input from domestic sewage, could accumulate a lot of dead organic matter and favor omnivorous scavenger animals such as *Idotea pelagica* (Naylor, 1955), explaining its higher abundance in urban shores. In contrast, amphipods are usually considered sensitive to pollution and, therefore, occur in lower numbers in polluted locations (Suchanek, 1993; Crowe et al., 2004) as we found for *Hyale* spp. in urban shores. In the case of *Nucella lapillus*, this species is known to be very sensitive to tributyltin (TBT) (biocide and anti-fouling paint) (Gibbs et al., 1987), and it is used as a bioindicator of coastal system recovery (Hawkins et al., 2002). However, there is not much available information in relation to other contaminants or disturbances (Leung et al., 2001). Moreover, it is also known that in urban shores, there is more recreational activity and people usually catch

animals for food or bait. *Nucella lapillus* is one of the species subjected to harvest, so it would also be expected that its abundance is lower in urban sites (Murray et al., 1999); however, this was not observed in our study. A possible explanation for its higher abundance in urban shores may be its size, because being a large gastropod, *Nucella* could prefer larger mussels (more frequent on urban shores) as these provide greater interstitial spaces in the mussel aggregates. The same pattern was found for oligochaetes and *Idotea*, which prefer larger mussels (O'Connor and Crowe, 2007). *L. rubra* was found that thrived near a sewage outfall (Conde et al., 2020), so one would expect higher abundances of this species in urban shores, but this was not found in our study. This may be due to its size. Being a small species, it may prefer habitats with smaller interstitial spaces, such as those provided by smaller mussels, more frequent on non-urban shores. Moreover, *Lasaea* is part of the diet of *N. lapillus* juveniles, that were more abundant in urban shores and thus, predation intensity in urban shores may help to reduce *Lasaea* abundance (Wigham and Graham, 2018). Therefore, the differences found in the structure of macrobenthic assemblages between urban and non-urban shores can be linked to changes in the quality of the habitat, through modifications in the mussel size frequency but also in the species' sensitivity or tolerance to anthropic disturbances associated with increased urbanisation (Johnston and Roberts, 2009; Todd et al., 2019). In conclusion, our study showed significant differences between urban and non-urban shores for the mussel size frequency, as well as for taxa richness and the structure of the total macrobenthic assemblages associated with *M. galloprovincialis*. These results support previous studies on other taxa that suggested changes on the traits of ecosystem engineers and a reduction in biodiversity harbored by urban shores. Therefore, the adoption of proper management plans is needed to prevent and minimize the loss of diversity in urban intertidal ecosystems that may reduce human well-being (Díaz et al., 2006; Roe, 2019).

CHAPTER IV. THE EFFECT OF SEDIMENT ACCUMULATION ON MUSSEL BEDS AND ON THE STRUCTURE AND DIVERSITY OF THEIR ASSOCIATE ASSEMBLAGES

The effect of sediment accumulation on mussel beds and on the structure and diversity of their associate assemblages

Abstract

Coastal environments are affected by sedimentation that reaches rocky shores by natural means or by anthropogenic activities. The sediment can accumulate in the environment, changing composition, structure and dynamic of benthic assemblages. The beds of *Mytilus galloprovincialis* due to its morphology and sessile lifestyle can contribute for the accumulation of sediments. Thus, in this study, this species was used as model organism to test the role of sediment in driving the abundance, diversity and structure of its macrofauna assemblage.

Results showed that there was a significant correlation for abundance, number of taxa and structure of associated macrofauna assemblage with sediments. In addition, the abundance, Shannon index, Pielou index and structure of macrofauna assemblage were significantly different between mussel beds with high and low quantity of sediment, but these differences depended on the shore.

In conclusion, the sediment accumulation seems to play an important role in shaping abundance and structure of invertebrate assemblages associated with mussel beds.

Keywords

Sediment accumulation, *Mytilus galloprovincialis*, associated macrofauna, quantity of sediment

1. INTRODUCTION

Benthic communities of the rocky coasts inhabit a dynamic environment prone to several physical variations (Ferreira et al., 2019). An example of this variation is sedimentation, that reaches rocky shores by natural means and periodically due to storms, sediment transport by rivers, floods, cliff erosion and/or in the transition to storm and flood seasons (Airoldi, 2003; Airoldi and Hawkins, 2007; Hutchison et al., 2020). However, the frequency and amount of sedimentation are increasing due to climate change and anthropogenic

activities such as resuspension, deforestation, dredging, domestic and industrial discharges, constructions, water replenishment and aquacultures and currently is one of the biggest problems for marine biodiversity (see in Airoidi, 2003; dos Santos et al., 2018; Ferreira et al., 2019; Hutchison et al., 2020).

Sediment accumulation can be a source of stress and disturbance, as well as a nutrient enrichment supplement, and can affect the composition, structure, and alterations of the rocky coast communities (Airoidi et al., 2003; Airoidi and Hawkins, 2007; dos Santos et al., 2018). In fact, increased sedimentation on rocky shores can result in negative consequences, such as changes in species composition and distribution, inhibition of attachment and recruitment, mortality, species removal, biodiversity reduction, growth or fertility inhibition, among other effects (Irving and Connel., 2002; Airoidi, 2003; dos Santos et al., 2018). For example, in the case of corals, deposition of sediment, especially the finer particles, can cause changes on reproduction, larval recruitment and nesting, and suffocate newly colonized young organisms (see Birrell et al., 2005; Richmond et al., 2018). In addition, sediment derived from land can be an important carrier of harmful and toxic substances, such as petroleum products (polycyclic aromatic hydrocarbons - PAH's), pesticides (insecticides and herbicides), personal care products and heavy metals (Balata et al., 2007; Richmond et al., 2018). Still, aside from its potential negative effects, it also has positive aspects, as the presence of sediment promotes a more heterogeneous habitat, supplemental material for tube-building species and food for detritivores (McQuaid and Dower, 1990; Prathep et al., 2003). For some macroalgae species, increasing quantity of sediment causes a decrease in herbivores (Hughes et al., 2010; Clausing et al., 2016) and it may increase the availability of nutrients (Clausing et al., 2016), thus favouring its growth.

The rocky coasts are potentially one of the most favourable habitats for sediment discharges, which can cause drastic changes in the level of the fauna characteristics that inhabit them (dos Santos et al., 2018). The accumulated sediment in small crevices and depressions or trapped by local assemblages and their distribution is influenced by the characteristics of the sediment itself and the hydrodynamic conditions (Hiscock, 1983; Airoidi, 2003). However, consequences of sediment in each organism will depend on factors such as the type of sediment, life history, habitat, trophic group, motility of the organism and the ability to migrate vertically (see in dos Santos et al., 2018).

There are many organisms that due to their morphology or behaviour favour the accumulation of sediments, such as macroalgae, some species of crabs, mussels, among others. In the case of mussels, these characteristics provide accumulation of sediments due to their sessile lifestyle, which cannot escape disturbed areas, and also due to the

dense aggregates they form (Tsuchiya and Nishihira, 1985; Araújo et al., 2012). This sediment accumulated in mussels can change the type of habitat provided by mussels, because it will allow soft-bottomed species to inhabit rocky shores (dos Santos et al., 2018).

On the rocky coasts of Portugal, the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819, is one of the most abundant species (Boaventura et al., 2002). This species is very important both economically and ecologically, as it offers several ecosystem services (i.e., feeding, coastal protection, mediates toxic wastes, among others (Beaumont et al., 2007; Montes et al., 2012; CICES, 2015; TEEB, 2015). Is also considered an ecosystem engineer (Borthagaray and Carranza, 2007; Buchsbaum et al., 2009), because it provides space and resources, such as habitat and food for other species (Borthagaray and Carranza, 2007; Buschsbaum et al., 2009). In addition, mussel beds increase the complexity of the habitat through their aggregations composed of live individuals, empty shells and byssal threads, which provide substrate for colonization, thus increasing biodiversity and also trap sediments (Borthagaray and Carranza, 2007; Buschsbaum et al., 2009; Arribas et al., 2014). This same accumulation of sediments between mussel matrices has a great influence on the availability of offered habitat (see Llanos et al., 2019), because the sediment that accumulates between the shells of mussels allows for the creation of another type of habitat (Tsuchiya and Nishihira, 1986). With sediment discharge into the marine environment constantly increasing, sediment accumulation becomes one of the biggest problems worldwide (Crain et al., 2009; Doney, 2010; Magris and Ban, 2019). Despite this, sediment accumulation and its influence on organisms has been poorly studied (Ramos-Oliveira et al., 2021). Yet, only few works have explored the different consequences of sediment accumulation and its effect on organisms (e.g., Gibbons, 1988; Birrel et al., 2005; Airoidi and Hawkins, 2007). In the case of rocky coasts, the studies are scarce, and algae were the most widely studied organisms (e.g., Prathep et al., 2003; Bos et al., 2007; Araújo et al., 2012; Clausing et al., 2016). In the case of mussels, the studies that exist focus on sediment effect on the mussel itself (e.g., Zardi et al., 2008; dos Santos et al., 2018; Hutchiton et al., 2020; Ramos-Oliveira et al., 2021), but studies reporting the effects of sedimentation on mussel-associated organisms are until now lacking (e.g., dos Santos et al., 2018). Since mussels are of great ecological importance to other organisms, it is of crucial interest to study the effect of sediment accumulation on their associated organisms.

Therefore, the objective of this work was to explore whether sediment accumulation correlates with associated macrofauna and whether the quantity of sediment influences the abundance, diversity and structure of fauna associated with mussel beds.

2. MATERIALS AND METHODS

2.1. Study area, sampling and sample procedure

This study was done in August 2018. For this study two rocky shores were selected in the north of Portugal, the shore of Moledo (41°50'28.81" N; 8°52'32.30" W) and Vila Praia de Âncora (41°49'25.93" N; 8°52'27.42" W). The Portuguese north coast is characterized by a semi-day tidal regime, with the largest reaching 3.5–4.0 m (Dias et al., 2002). Most common wave are from the west and northwest direction and with a range of variation from 1.5 to 7 m, varying widely between seasons (Rubal et al., 2011). The coast has a variety of substrates, from rocky to sandy, mainly a mixture of both, in which the rock is a mixture of granite and schist. During the spring and summer months there is a seasonal upwelling (Lemos and Pires, 2004).

For sampling, in each shore, six plots (2x2m) were marked, spaced a few meters apart, and in each plot three replicates (10x10cm) of mussel beds were scraped. The collected samples were transported to the laboratory and frozen (-20 °C) until further processing.

For the processing of the samples, each replicate was washed on five sieves of different meshes (1000, 500, 250, 125 and 63 µm), so that the sediment and the fauna were retained. The smaller sediments (retained in the 250, 125 and 63 µm) were dried in an oven at 60° C for 24 h, to quantify their weight separately. While the sediments of the largest sieves (1000 and 500 µm) along with the fauna, were preserved in Formaldehyde (4%), stained with Bengal rose until further fauna sorting. All mussels in each sample were counted (density) and from each replicate, 20 mussels were randomly separated to measure their shell length. After sorting the fauna and identifying it to the lowest possible taxonomic level, generally species, the largest sediment removed was dried for 24 hours at 60° C.

2.2. Data analysis

2.2.1. Relationship between quantity of sediment and invertebrate assemblages

In order to explore the relationship between univariate variables (i.e., number of individuals (N), number of taxa (S), Shannon index (H') and Pielou index (J')) and the quantity of total sediment, rank correlation analyses were done. Due to the non-normal distribution of the data, Spearman's rank correlation was used.

Multivariate multiple regression, using the DISTLM routine, was used to test the significance of the relationship between the quantity of total sediment and the structure of macrofaunal assemblage. DISTLM routine is a linear model based on Bray–Curtis similarity matrix on response variable (i.e. the structure of the macrofaunal assemblage) with predictor variable (i.e. total quantity of sediment). R2 routine was used as a selection criterion and the contribution of each independent variable was described by the quantity of explained variation. All analyses were based on a ‘forward’ selection procedure (Anderson et al., 2008).

2.2.2. Invertebrate assemblages

From the previous mussel bed samples, 5 samples with high quantity of sediment (> 100g) and 5 samples with low quantity of sediment (< 30g) were selected on each rocky shore.

Previously for to analysis the differences on the mussel attributes (i.e., density) between quantity of sediment (high and low) analyses of variance (ANOVAs) were done. For analyses of mussels density a two-way model was considered including the factors: Shore (random with two levels (i.e., Moledo and Âncora)) and quantity of sediment (fixed and orthogonal with 2 levels (i.e., high and low)) and with 5 replicates. Cochran's test was done to check the homogeneity of variances in each ANOVA. Data were transformed to remove the heterogeneity of variances when needed. When this was not possible, untransformed data were analysed and the results were considered robust if significant at $p < 0.01$ (Underwood, 1997).

After Permutational multivariate analysis of variance PERMANOVA (Anderson, 2001), based on Bray–Curtis untransformed dissimilarities, was used for test if the size of mussels can influence the fauna associated with mussel beds. These analyses were done for the dissimilarity matrix based on Bray–Curtis distance calculated for the size of mussels. The model for these analyses was the same as for the case of ANOVA of density of mussels.

Then to test differences in the number of individuals (N), number of taxa (S), Shannon index (H') and Pielou index (J') of the invertebrate assemblage between high and low sediment analyses of variance (ANOVA) were done. For analyses a two-way model was considered, and it's the same as described above.

Permutational multivariate analysis of variance PERMANOVA (Anderson, 2001), based on Bray–Curtis untransformed dissimilarities, was used to analyse the multivariate assemblage data. These analyses were done for the dissimilarity matrix based on Bray-

Curtis distance calculated for the invertebrate assemblage. The model for these analyses was the same as for the case of ANOVA and PERMANOVA.

The Multivariate patterns by non-metric multidimensional scaling (nMDS) ordination illustrates sampled sites at each shore for each sediment condition. PERMDISP procedure was done to test whether differences between high and low sediment were due to different multivariate dispersion in the location of centroids (Anderson, 2006). The SIMPER procedure (Clarke, 1993) was used to determine the percentage of contribution ($\delta\%$) of each taxon to the Bray-Curtis dissimilarity between conditions. A taxon was considered important if its contribution to total percentage dissimilarity was $\geq 3\%$. The ratio $\delta/SD(\delta)$ was used to quantify the consistency of the contribution of a taxon to the average dissimilarity in all pair-wise comparisons of samples between conditions. Values ≥ 1 indicated a high degree of consistency.

3. RESULTS

3.1. Relationship between quantity of sediment and invertebrate assemblages

Spearman's rank correlations pointed out significant positive correlations between sediment and N and S (Figure 1). However, for H' and S' were found to have significant negative correlation with quantity of sediment (Figure 1).

Multivariate multiple regression showed that the proportion of variability in the structure of macrofauna assemblages, explained by sediment measures was significant and higher (almost 75%). The size 0.500 mm was the category that explain more variability, followed by 1 mm, 0.125 mm, 0.063 mm and 0.250 mm (Table 1).

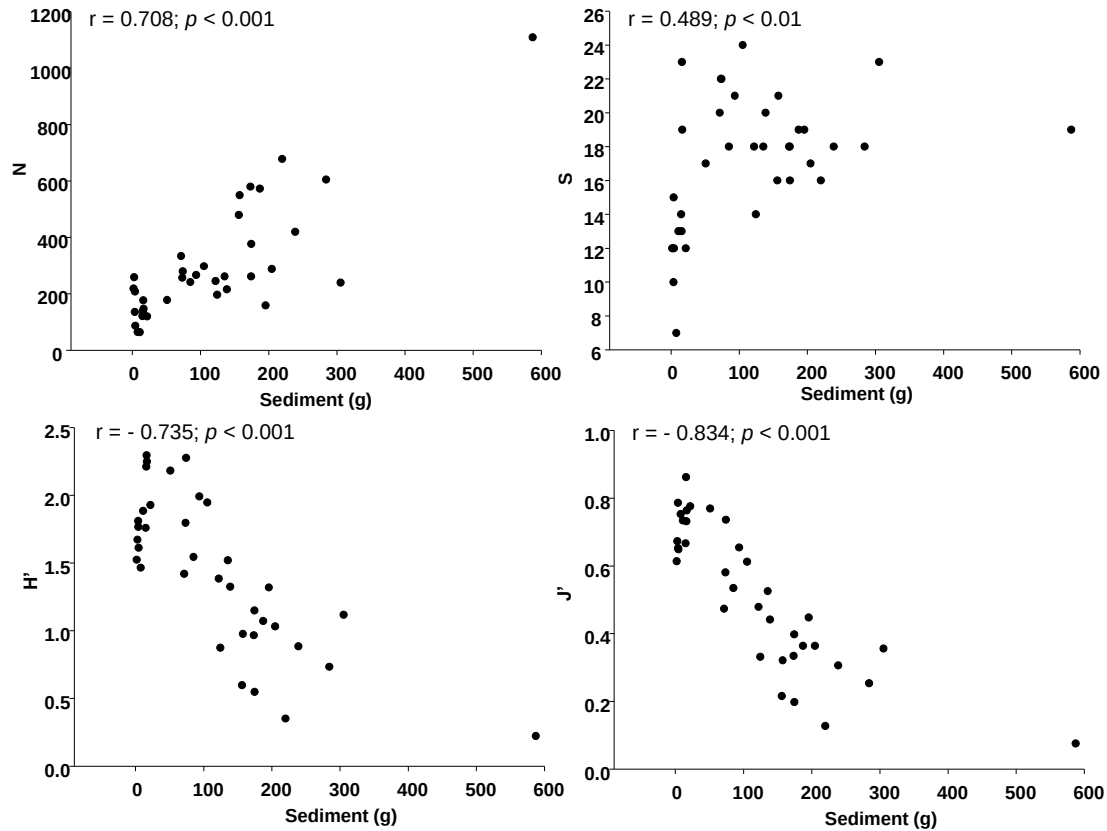


Figure 1. Spearman's rank correlations between total of sediment and number individual (N) number of taxa (S), Shannon index (H') and Pielou index (J') of faunal assemblage of mussels.

Table 1. Results of multivariate multiple regressions testing the effect of grain size of sediment on the fauna assemblages *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Variable	Marginal tests		Sequential tests				
	Pseudo-F	Prop.	R ²	Pseudo-F	Prop.	Cumul.	Res.df
0.500 mm	91.686***	0.729	0.729	91.686***	0.730	0.729	34
1.000 mm	76.540***	0.692	0.774	6.433*	0.040	0.774	33
0.125 mm	1.081	0.030	0.787	2.033	0.013	0.787	32
0.063 mm	8.873**	0.207	0.790	0.392	0.003	0.790	31
0.250 mm	4.283	0.111	0.792	0.321	0.002	0.792	30

3.2. Invertebrate assemblages

For the following analyses the total quantity of sediment was considered. Although the 0.500 mm fraction was the dominant fraction in terms of quantity, so to account for the effect of the other fractions, the total quantity of sediment was used.

Values of density and size of mussels of study samples were the parameters tested to study if they could be confounding factors with effect on the fauna associated with mussels.

Results of ANOVA analyses did not reveal significant differences in the density of mussels between samples with higher quantity of sediment and lower (Table 2). Results of PERMANOVA analyses also did not reveals significant differences on the size of mussels between samples (Table 3).

Table 2. ANOVA analysis for density of mussels between high and low sediment. PERMANOVAs for size between high and low sediment. ns: not significant

Source	Df	ANOVA Density			PERMANOVA Size			
		MS	F	P	MS	Pseudo-F	P(MC)	Unique Perms
Shore	1	1320980.0	5.68	0.0300	1966.3	0.95681	0.491	999
Sediment	1	68445.0	0.18	0.7459	1503.8	0.71057	0.65	3
Sh x Se	1	384753.8	1.65	0.2168	2116.3	1.0298	0.398	999
Residual	16	232754.9			2055			
Total	19							
Cochran's test		0.3175 ^{ns}						
Transformation		None						

A total of 4840 individuals (3352 high quantity of sediment and 1488 low quantity of sediment) belonging to 51 different taxa (42 high quantity of sediment and 37 low quantity of sediment) were identified. Analyses of variance (ANOVA) indicated significant differences on interaction between shore and sediment for N, H' and J' (Table 3). In addition, N reveals a significantly higher values on samples with high quantity of sediment (Figure 2), but H' and J' reveals a significantly higher values on samples with low quantity of sediment (Figure 2).

Table 3. Summary of ANOVAS's for the number of taxa (S), number of individuals (N), Shannon index (H') and Pielou index (J') of faunal assemblage of mussels. **: $p < 0.01$, ***: $p < 0.001$. ns: not significant

Source of variation	Df	Total sediment							
		N		S		H'		J'	
		MS	F	MS	F	MS	F	MS	F
Shore	1	27751.25	4.18	24.20	2.69	0.0006	0.01	0.006	1.16
Sediment	1	173911.25	2.74	51.20	1.78	3.5372	3.41	0.6407	8.96
Sh X Se	1	63506.45	9.57**	28.80	3.20	1.038	20.38***	0.0715	13.78**
Residual	16	6639.00		9.00		0.0509		0.0052	
Total	19								
Cochran's test		0.60 ^{ns}		0.55 ^{ns}		0.35 ^{ns}		0.31 ^{ns}	
Transformation		None		None		None		None	

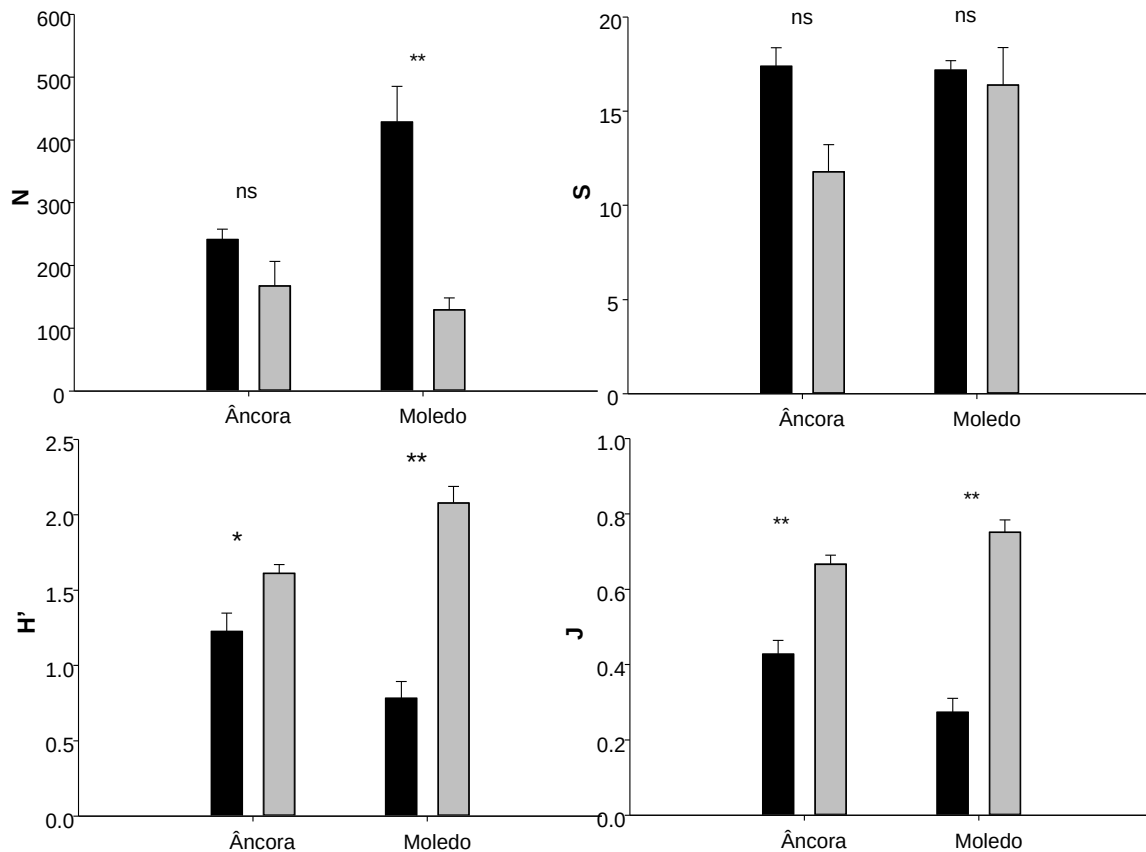


Figure 2. Mean values ($\pm$ SE) of the number individual (N) number of taxa (S), Shannon index (H') and Pielou index (J') of faunal assemblage of mussels. * indicate significant differences between high and low quantity of sediment ($p < 0.05$) as detected by SNK test; ** indicate significant differences between high and low quantity of sediment ($p < 0.01$) as detected by SNK test; ns: not significant. Black: High quantity of sediment; Grey: Low quantity of sediment.

Results of PERMANOVA analyses for the structure of the whole assemblage indicated significant differences for interaction between shore and sediment (Table 4). The nMDS ordinations separated well the replicates assigned to high and low sediment on the two rocky shores (Figure 3). The PERMDISP analyses for differences between high and low sediment ($F: 1.7536, p: 0.281$) indicated that the dispersion of samples did not significantly contribute to differences between conditions.

Table 4. Summary of PERMANOVAs for total assemblage.

Source	df	MS	Pseudo-F	Total sediment		Unique Perms
				P	P (MC)	
Shore	1	3527.8	5.425	0.002	0.003	998
Sediment	1	26126	9.0652	0.2526	0.039	3
Sh x Se	1	2882	4.4319	0.001	0.003	997
Residual	16	650.29				
Total	19					

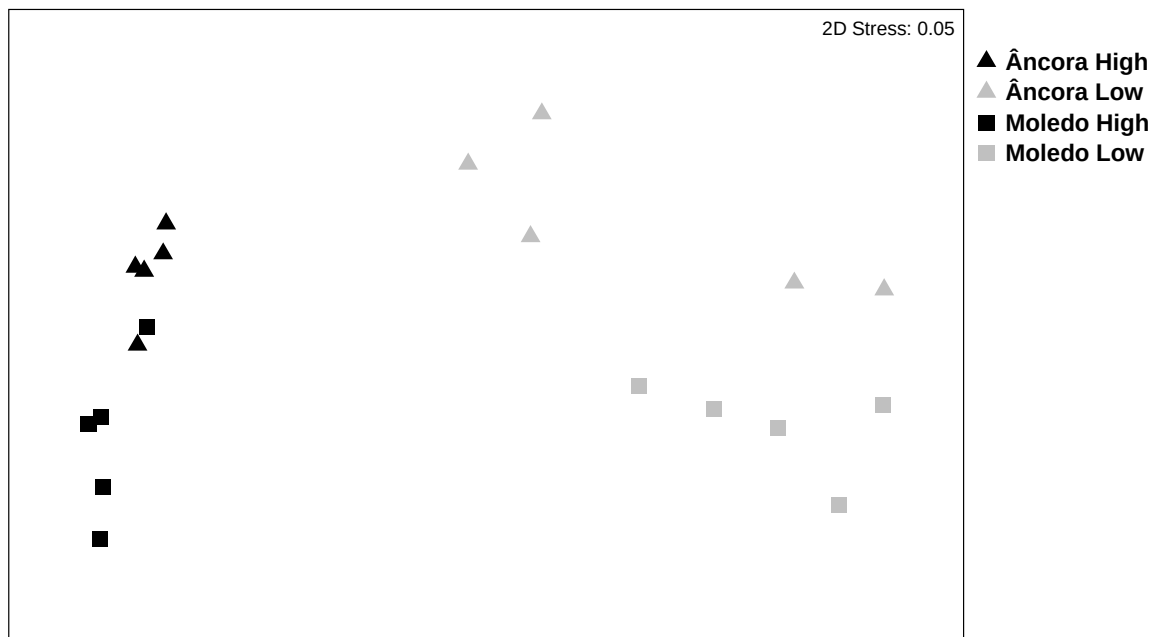


Figure 3. nMDS plots of centroids of replicates assigned to high and low quantity of sediment when significant differences between sediment were detected. Black: High quantity of sediment; Grey: Low quantity of sediment.

The SIMPER analyses identified 27 taxa as the main responsible for dissimilarity between samples (Table 5). The taxa main responsible for dissimilarity were Nematoda and *Idotea neglecta* G. O. Sars, 1897.

Table 5. Contribution (δ) of individual taxa from faunal assemblages of mussels to the average Bray-Curtis dissimilarity between high and low quantity of sediment.

Taxon	Average abundance		δi	$\delta i\%$	$\delta i/SD(\delta i)$
	High	Low			
NEMATODA	15.96	4.97	13.96	19.37	2.07
<i>Idotea neglecta</i>	0.10	6.29	7.74	10.73	4.03
<i>Nucella lapillus</i>	0.14	3.68	4.33	6.00	2.21
<i>Hyale</i> spp.	0.17	3.36	3.99	5.53	2.10
Oligochaeta	2.87	0.95	3.57	4.95	1.52
Copepoda	2.84	0.31	3.26	4.52	1.72
<i>Syllis pulvinata</i>	2.30	0.10	2.85	3.95	1.25
<i>Patella depressa</i>	0.34	2.52	2.78	3.85	2.04
<i>Lasaea rubra</i>	2.11	0.28	2.36	3.27	1.95
<i>Jaera (Jaera) praehirsuta</i>	0.59	1.95	2.23	3.09	1.15
<i>Steromphala umbilicalis</i>	2.18	0.77	2.21	3.07	1.09
<i>Brachystomia</i> sp.	0.57	1.33	1.49	2.07	1.25
<i>Runcina</i> sp.	1.14	0.20	1.33	1.85	1.32
<i>Hiatella arctica</i>	1.64	0.99	1.26	1.75	1.41
Chironomidae	1.20	0.30	1.24	1.73	1.42
<i>Actinia equina</i>	1.56	1.66	1.23	1.70	1.45
<i>Eulalia clavigera</i>	1.38	0.77	1.19	1.65	1.18
<i>Hyale stebbingi</i>	0.00	0.97	1.16	1.61	1.29
<i>Littorina saxatilis</i>	0.14	0.84	1.04	1.44	1.10
Ostracoda	0.84	0.20	0.98	1.36	1.13
<i>Acanthochitona crinita</i>	0.65	0.10	0.82	1.14	0.77
<i>Rissoa parva</i>	0.44	0.68	0.77	1.06	1.12
<i>Tanais dulongii</i>	0.57	0.10	0.75	1.04	0.92
<i>Pollicipes pollicipes</i>	0.00	0.65	0.74	1.03	0.60
Colembola	0.61	0.00	0.74	1.02	0.76
<i>Melarhaphe neritoides</i>	0.44	0.24	0.65	0.90	0.89
<i>Campecopea lusitanica</i>	0.48	0.00	0.60	0.84	0.79

4. DISCUSSION

Sediment discharge into the marine environment has been steadily increasing, becoming one of the biggest problems for benthic communities worldwide (Crain et al., 2009; Doney, 2010; Magris and Ban, 2019).

However, studies exploring the effects of this phenomenon are still scarce, especially with mussels and their associated fauna. Since mussels beds have great ecological importance, it is of fundamental interest to unravel the effects of sediment accumulation on their associated organisms.

Initially, we studied how sediment quantity correlated with abundance, number of taxa and Shannon and Pielou indexes and then explored whether sediment quantity (high or low)

caused differences between associated macrofauna. For the abundance and number of taxa, significant and positive correlations with the quantity of sediment were found. These results showed that an increased number of organisms and taxa are associated with a higher sediment accumulation. The same pattern was corroborated with the ANOVA for the abundance of associated macrofauna between the high and low quantity of sediment, which revealed significant differences, depending on the studied shore. Still, higher values on samples with a high quantity of sediment were recorded. This may be due to increased habitat heterogeneity, since sediment deposition creates a favourable habitat for other types of species and favours the tolerant species, thus increasing the abundance and associated number of taxa (see dos Santos et al., 2018; Ferreira et al., 2019). In the case of number of taxa, although the correlation analysis has shown a positive and significant correlation with the quantity of sediment, the values of this correlation were lower in relation to the other variables. Curiously, this finding is in agreement with ANOVA, that did not reveal significant differences between the high and low quantity of sediment. This result, although might seem contradictory, may be related to the fact that sediment accumulation can reduce recruitment and cause mortality of less tolerant species, but on the other hand, sediment accumulation also provides a different habitat for other species to inhabit mussels (see Ferreira et al., 2019). In accordance to our data, Prathep et al. (2003) also found that animals associated with an alga were increased upon a higher degree of sedimentation, along with the diversity associated with algae. Another study by Magris and Ban (2019), which brought together results from different works, found that, for example, in the case of sponges and polychaetes, they respond positively in terms of abundance to the increase in sediments, but there are other organisms that are more sensitive to sediment. In the case of organisms associated with mussels, dos Santos et al. (2018), who studied the effects of sediment deposition on the fauna associated with mussels, found that the abundance and diversity of the associated fauna benefits from an increase in the quantity of sediment. This agrees with our correlation results. On the other hand, the Shannon and Pielou indexes revealed significant but negative correlations with the quantity of sediment, showing that the greater quantity of sediment, lower values of the indexes. The same pattern was also revealed by the ANOVA results, where significant differences were found for the Shannon and Pielou indexes between high and low quantity of sediment, depending on the shore and with higher values in samples with low quantity of sediment. Curiously, this seems to be contradictory with the results found for abundance and number of taxa. However, the Shannon index consider species richness (number of species) and evenness (number of individuals of each specie) and the Pielou index also the evenness of the species. In addition, some species appear in great abundance, unlike

others that are only represented by a few individuals. This means that there are species that benefit from the large amount of sediment, but there are others that do not.

Furthermore, both the abundance and the studied indexes showed significant differences between the high and low quantity of sediment, but these in turn depended on the studied shore. These results reveal that there are other factors besides the quantity of sediment that may have influenced the associated fauna. The direct effect of sedimentation depends not only on the type of organism, but also on its life stage, the flow and turbulence of the water, and the characteristics of the sediment grains themselves (see Schiel et al., 2006; Smit et al., 2008). So, this could be due to different factors inherent to each rocky shore. Furthermore, the composition in terms of the quantity of each type of sediment is different in each rocky shore, especially in the 0.500 mm fraction, which may explain that the faunal differences between the quantity of sediment depended on the rocky shore. On the rocky shore of Moledo, mussel samples have three times as much sediment of 0.500 mm, than those of Âncora. Moreover, from the multivariate correlation analysis, in which sediment size influenced the associated fauna, we observed that the size 0.500 mm was the category that most influenced the associated fauna. The influence of sediment size on fauna has also been reported by several authors who suggest that sediment size correlates with species abundance and distribution (see Prathep et al., 2003). For example, Airoidi and Hawkins (2007), who studied the effect of sediment deposition on grazing activity and survival of a limpet species, found that the limpet was more affected by fine sediments than by thick sediments. Another study by Vanaverbeke et al. (2011), who explored the differences between macrofauna and nematodes associated with different sediment particle sizes, observed that macrofauna and nematodes reacted differently to the type of sediment: while macrofauna seemed to prefer finer sediments, nematodes vary by species.

Furthermore, the multivariate correlation analysis showed that the entire community structure is also significantly correlated with the quantity of sediment, presenting high correlation values. Likewise, the multivariate analysis found significant differences for the structure of the whole assemblage for the shore x sediment interaction. A previous work performed by Crowe et al. (2004), who studied the influence of some aspects, such as the characteristics of the interstitial sediment, related to the habitat provided by the mussels, reported that the fauna structure associated with the mussels was related to the sediment trapped between them (biomass and composition). These results strongly agree with the ones of the present study, since both the correlation levels and the PERMANOVA analysis, revealed significant differences between high and low quantity of sediment for the structure

of the entire community. Altogether, this indicates that both the quantity of sediment, as well as the granulometry have an influence on mussels-associated fauna.

Also looking at the nMDS, a clear separation between samples with high and low quantity of sediment can be observed. Actually, despite the differences between rocky shores, communities associated with samples with high quantity of sediment were more homogeneous than communities associated with samples with low quantity of sediment. When a habitat is disturbed, there may be a reduction in biodiversity, as rarer and more sensitive species are replaced by more resistant ones, which may lead to homogenization in terms of species composition and abundance (see Oliveira et al., 2014; Bertocci et al., 2017). However, in the case of this study, there was an increase in abundance and number of taxa, which suggest that the presence of more sediment seems to play a positive role in the community. This situation has already been observed by other studies, which discuss that when the disturbance is moderate, it may lead to an increase in heterogeneity (see in Bertocci et al., 2017). In the case of high sediment rates, a proliferation of species with morphological and physical adaptations to this type of habitat, as well as tolerant species adapted to this disturbance, take place (Eriksson and Johansson, 2005; dos Santos et al., 2018). Such results can be confirmed through the composition of the associated community and the species that most contributed to the differences between the quantity of sediment. Of the 27 taxa that contributed to the differences between conditions, more than 50% were more abundant in samples with a high quantity of sediment. In this case, Nematoda and *Idotea neglecta* must be highlighted. Nematoda were the most abundant organisms in samples with a high quantity of sediment, as they benefit from the presence of a large quantity of sediment due to their interstitial lifestyle (see Callaway et al., 2020). On the other hand, the isopod species, *I. neglecta*, was found in greater quantity in samples of mussels with a low quantity of sediment. As it is an omnivorous scavenger species (Kjennerud, 1950; Naylor, 1955) that benefits from the presence of undecomposed organic remnants (Pearson, 1970; O'Connor and Crowe, 2007), it is to be expected that an increased sediment quantity negatively impacts the food source of this species, as less space will be left for undecomposed organic residues. Regarding the other species, their greater abundance in one type of sample or another also meets their preferences, whether in terms of food or lifestyle.

We can conclude that sediment accumulation seems to play an important role in shaping the abundance and structure of invertebrate assemblages associated with mussel beds, where it seems to favour the organisms, but other aspects related to the rocky coast

hydrodynamics (sediment grain composition) and also the biology of the organisms should be considered.

CHAPTER V. Macroalgae turf on mussel beds – Co-engineering effects on associated macrofauna

Macroalgae turf on mussel beds – Co-engineering effects on associated macrofauna

Abstract

On the intertidal rocky coast, there are organisms that live in a close relationship as an epibiont and host, for example mussels and macroalgae. Both are considered ecosystem engineers, as they have a fundamental role in contributing to increasing diversity, not only for their activity, but also for their sessile lifestyle. Specifically, in the intertidal zone of northern Portugal, red algae of the genus *Caulacanthus* Kützinger, 1843 occasionally live as epibionts of mussel beds of *Mytilus galloprovincialis* Lamarck, 1819. Thus, the aim of this study is to explore how the presence of *Caulacanthus* spp. as co-engineer in beds of *M. galloprovincialis* can affect the mussel's attributes and the associated macrofauna. For this purpose, sampling was carried out on four different dates and on each date samples of mussel beds with and without algae were collected and compared. Regarding the mussel attributes, the results available show that the presence of *Caulacanthus* spp., as co-engineer in mussel beds, affected only the condition index, with interaction between date and condition. In relation to the fauna associated with the mussel, the analyses revealed significant differences between the conditions for number of taxa, Shannon index and Pielou index and for the structure of the entire community. An interaction between dates and condition was also observed.

So, *Caulacanthus* spp., acting as co-engineer on *M. galloprovincialis*, plays an important role in the associated fauna of mussel beds.

Keywords

Co-engineer, *Caulacanthus* spp., *Mytilus galloprovincialis*, mussel attributes, associated macrofauna

1. INTRODUCTION

On the intertidal rocky coast, organisms such as macroalgae and mussels coincide in the same habitat (Wangkulangkul et al., 2016). Both have a fundamental role in terms of biodiversity, being able to change the environment, through the habitat and composition of communities, thus contributing to the increase in diversity, being considered ecosystem engineers (Gutiérrez et al, 2003; Christie et al., 2009, Emery-Butcher et al., 2020). Ecosystem engineers are organisms that, due to their morphology, activity and lifestyle,

affect the abiotic and biotic conditions on which other organisms depend (Jones et al., 1994; 1997; Gutiérrez et al., 2019).

In the case of macroalgae, they are key elements in the ecology of the rocky coasts, due to their function as primary producers and as the base of some food chains (Graham, 2004; Macreadie et al., 2017), but also because they offer valuable goods and services such as food and habitat for a large number of species. They increase the heterogeneity of the substrate and offer resources and shelter for other algae and also for sessile and mobile invertebrates (Macreadie et al., 2017; Navarro-Barranco et al., 2019). Mussels, that are also considered ecosystem engineers, often occupy the same spaces as algae in the intertidal (Borthagaray and Carranza, 2007; Buchsbaum et al., 2009). Mussels form a dense three-dimensional layer, which generates interstitial space, where water flow is attenuated, sediments are retained and the impacts of predators, extreme temperatures and desiccation are reduced (see Seed, 1996; Gutiérrez and Iriberne, 1999; Gutiérrez et al., 2003; 2019). For this reason, there is a great biodiversity associated with mussels beds (Buschsbaum et al., 2009). In addition, the surface of mussel shells serves as a fixation support for algae (Tsuchiya and Nishihira, 1985; Laudien and Wahl, 1999; Gutiérrez et al., 2019). Many algae live as epibionts on the mussels, that are the hosts (Laudien and Wals, 1999).

In the case of the rocky coast of northern Portugal, an algae of the genus *Caulacanthus* Kützinger, 1843, a type of small red turf algae, grows on a Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 (Zuccarello et al., 2002; Smith et al., 2014). *M. galloprovincialis* is one of the most abundant organisms in the intertidal zone (Boaventura et al., 2002) and it is an important species as food in the natural food chains (Vinagre et al., 2015), as well as providing ecosystem services such as human food or toxic waste mediation (CICES, 2015; TEEB, 2015) and has high commercial interest (Rius and Cabral, 2004; Bertocci et al., 2012b). The algae turf is constituted by the species *Caulacanthus ustulatus* (Mertens ex Turner) Kützinger, 1843 and *Caulacanthus okamurai* Yamada, 1933, the first native and the second invasive (Zuccarello et al., 2002; Day, 2018). The alga *C. ustulatus* was described by Turner in 1809 in Cadiz, Spain, but its distribution is quite wide throughout the world, namely in several countries on Europe, Asia, Africa, America and Oceania (see in Zuccarello et al., 2002). In the case of *C. okamurai* it is originally from Japan, but it is also distributed over the rocky coasts of Korea and China. Despite this, at this moment, there are already reports of its appearance on the shores of the United Kingdom among other places (see in Day, 2018), namely Portugal.

The introduction of non-native species is considered the second biggest problem for biodiversity and the functioning of ecosystems, second only to habitat destruction (see Smith et al., 2014). In addition to the effects on biological interactions and habitat change, invasions can decrease the abundance of native species, diversity and change in the structure of communities (see Smith et al., 2014). These major invaders macroalgae, can be considered to be a problem for biodiversity and the functioning of coastal ecosystems (see Navarro- Barranco et al., 2019).

In ecosystems, when two engineers are present in the same habitat, it can happen that one facilitates the other, or both compete, or both together improve the abiotic conditions for other associated species (see Gutiérrez et al., 2019).

There are several studies that focused on the effects of invasive algae on fauna (e.g. Janiak and Whitlatch, 2012; Veiga et al., 2018), others on mussels providing habitat for other species (e.g. Seed, 1996; Buschbaum et al., 2009) and also some on the interaction among ecosystem engineers (e.g. Passarelli et al., 2014; Gutiérrez et al., 2019; Flynn et al., 2020). Although we know the relevance for other species of these two ecosystem engineers, our knowledge of the importance of the combined effects between the two is still scarce and there are still many questions about the coexistence of engineers and how this interaction affects the physical habitat and the structure of communities (Donadi et al., 2015). For that, samples of mussel beds with *Caulacanthus* turf and samples of mussel beds without *Caulacanthus* were collected. A comparative study was carried out between these two conditions, where the effect of the algae turf in the attributes of the mussel and in the associated fauna was evaluated.

2. MATERIALS AND METHODS

2.1. Study area, sampling and sample procedure

This study was done in the North of Portugal on rocky shore of Moledo (41°50'28.81" N; 8°52'32.30" W). The north Portuguese coast is characterized by semidiurnal tide regime (Dias et al., 2002) being the most common wave that of west and northwest and with waves variation between 1.5 m and 7 m (Rubal et al., 2011). The rocky shore is made up of granite.

These coasts are meteorologically defined for having four annual Seasons: Spring with 15.5°C and 105mm, Summer with 19°C and 30mm, Autumn with 11°C and 180mm and Winter with 11°C and 168mm (mean values of temperature and precipitation respectively)

(IPMA). In relation to sea water temperature, this varies between 14°C and 18°C, for cold and hot months, respectively (Lemos and Pires, 2004).

Samples were collected in two sites in this rocky shore described above between April of 2018 and March of 2019 during low tide at four different dates: March (Winter), April (Spring), August (Summer) and November (Autumn). In each site, five quadrat (10cm x 10cm) replicates of mussel beds with and without *Caulacanthus* sp. were randomly chosen.

For each replicate, mussels were collected and stored in a plastic bag individually to later process in the laboratory. At the laboratory each sample was washed through a sieve of 0.500 mm, in order to separate the macrofauna from the mussels and from alga. Then, all mussels were counted, and alga were dried at 60 °C for 48h to determinate their biomass.

The invertebrates were stored in formaldehyde (4%) stained with Bengal rose until they were identified down to the lowest possible taxonomic level.

For the size of the mussels, 20 mussels from each sample were randomly separated and measured. For the condition index (i.e., ratio between dry weight soft parts / dry weight of shell) another 10 mussels from each sample were separated.

2.2. Data analysis

To test differences in biomass of algae between dates and differences in mussel attributes (density and condition index) and faunal assemblage (number of individual (N), taxa richness (S), Shannon index (H'), Pielou index (J')) between conditions (with and without *Caulacanthus*), analyses of variance (ANOVA) were done. For condition index a four-way model was considered including the factors: Date (random with four levels), Condition (fixed and orthogonal with two levels (i.e., with and without *Caulacanthus*)), Site (random and nested in Date, with two levels) and Quadrat (random and nested in Date, Condition and Site, with 5 levels) with 10 replicates. For analyses of mussel density, N, S, H' and J', a three-way model was considered including the factors: Date (random with four levels), Condition (fixed, orthogonal with two levels (i.e., with and without *Caulacanthus*)) and Site (random, with two levels, nested in Date) with 5 replicates. Cochran's test was done to check the homogeneity of variances in each ANOVA. Data were transformed to remove the heterogeneity of variances when needed. When this was not possible, untransformed data were analysed and the results were considered robust if significant at $p < 0.01$ (Underwood, 1997).

In order to explore differences in size of mussels and total faunal assemblage between conditions (with and without *Caulacanthus* sp.), Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), based on Bray–Curtis untransformed dissimilarities, was used. These analyses were done for the dissimilarity matrix based on Bray-Curtis distance. The model for these analyses was the same as for the case of ANOVA of density of mussels, N, S, H' and J'.

The samples of each condition on each date were illustrated with Multivariate patterns by non-metric multidimensional scaling (nMDS) ordination. To test whether differences between conditions were due to different multivariate dispersion in the location of centroids, PERMDISP procedure was done (Anderson, 2006). To determine the percentage of contribution ($\delta\%$) of each taxon to the Bray-Curtis dissimilarity between conditions, the SIMPER procedure was used (Clarke, 1993). A taxon was considered important if its contribution to total percentage dissimilarity was $\geq 3\%$. The ratio $\delta/SD(\delta)$ was used to quantify the consistency of the contribution of a taxon to the average dissimilarity in all pair-wise comparisons of samples between conditions. Values ≥ 1 indicated a high degree of consistency. Then, from the SIMPER procedure, the species that most contributed to the differences, an ANOVA was done, to test the differences in abundance of three taxa with more differences between conditions on all dates. The model for these analyses was the same as for the case of ANOVA of density of mussels, N, S, H' and J'.

3. RESULTS

3.1. Mussel' attributes

The parameters of mussels evaluated among the studied conditions (with and without *Caulacanthus*) were density, condition index and size.

Results of ANOVA analyses did not reveal significant differences for the density of mussels between conditions, but for condition index the analyses reveals significant differences for interaction date x condition (on summer and autumn dates) (Table 1). For the size, the PERMANOVA analyses did not reveal significant differences between conditions (Table 1)

Table 1. ANOVA analysis for density and condition index of mussels between mussels with and without algae. PERMANOVAs for size between mussels with and without algae. ns: not significant; s: significant

	DF	ANOVA						PERMANOVA			
		Density			Condition index			Size			
		MS	F	P	MS	F	P	MS	Pseudo-F	P	Unique Perms
Date	3	1601.7805	9.03	0.0297	0.0171	10.39	0.0233	4697.5	1.5043	0.211	105
Condition	1	111.0875	0.46	0.5461	0.0154	4.42	0.1263	2846.2	1.8834	0.164	365
Site	4	177.3083	8.31	0	0.0016	1.30	0.2808	3122.8	1.8416	0.001	998
Quadrat	64				0.0013	1.54	0.0057				
Da x Co	3	241.2679	3.30	0.1393	0.0035	21.46	0.0063	1511.2	0.91803	0.574	998
Co x Si	4	73.0346	3.42	0.0134	0.0002	0.13	0.9717	1646.1	0.97076	0.553	998
Residual	64/720	21.3387			0.0008			1695.7			
Total	79/799										
Cochran's test			0.2306 ^{ns}			0.3347 ^s					
Tranformation			Sqrt(X+1)			None					

3.2. Invertebrate assemblages

A total of 28 733 (15 864 with *Caulacanthus* and 12 869 without *Caulacanthus*) individuals belonging to 68 (58 with *Caulacanthus* and 53 without *Caulacanthus*) different taxa were identified. Analyses of variance (ANOVA) indicated significant differences between conditions for the number of taxa, Shannon index and Pielou index of the total assemblage (table 2; Figure 1).

Table 2. Summary of ANOVAs for the number of taxa (S), number of individuals (N), Shannon index (H') and Pielou index (J') of faunal assemblage of mussels. *: $p < 0.05$; **: $p < 0.01$. ns: not significant

Source of variation		N		S		H'		J'	
		MS	F	MS	F	MS	F	MS	F
Date	3	562219.51	4.66	200.75	27.45**	0.31	1.91	0.07	2.20
Condition	1	112125.31	1.13	437.11	20.01*	1.71	16.35*	0.18	13.95*
Site	4	120609.66	2.47	7.31	0.77	0.16	3.69**	0.03	2.78*
Da x Co	3	98826.05	2.06	21.85	0.65	0.10	0.70	0.01	0.63
Co x Si	4	47884.66	0.98	33.46	3.54*	0.15	3.40*	0.02	1.92
Residual	64	48743.21		9.44		0.04		0.01	
Total	79								
Cochran's test		0.2101 ^{ns}		0.1337 ^{ns}		0.2206 ^{ns}		0.1963 ^{ns}	
Transformation		None		None		Ln (x)		None	

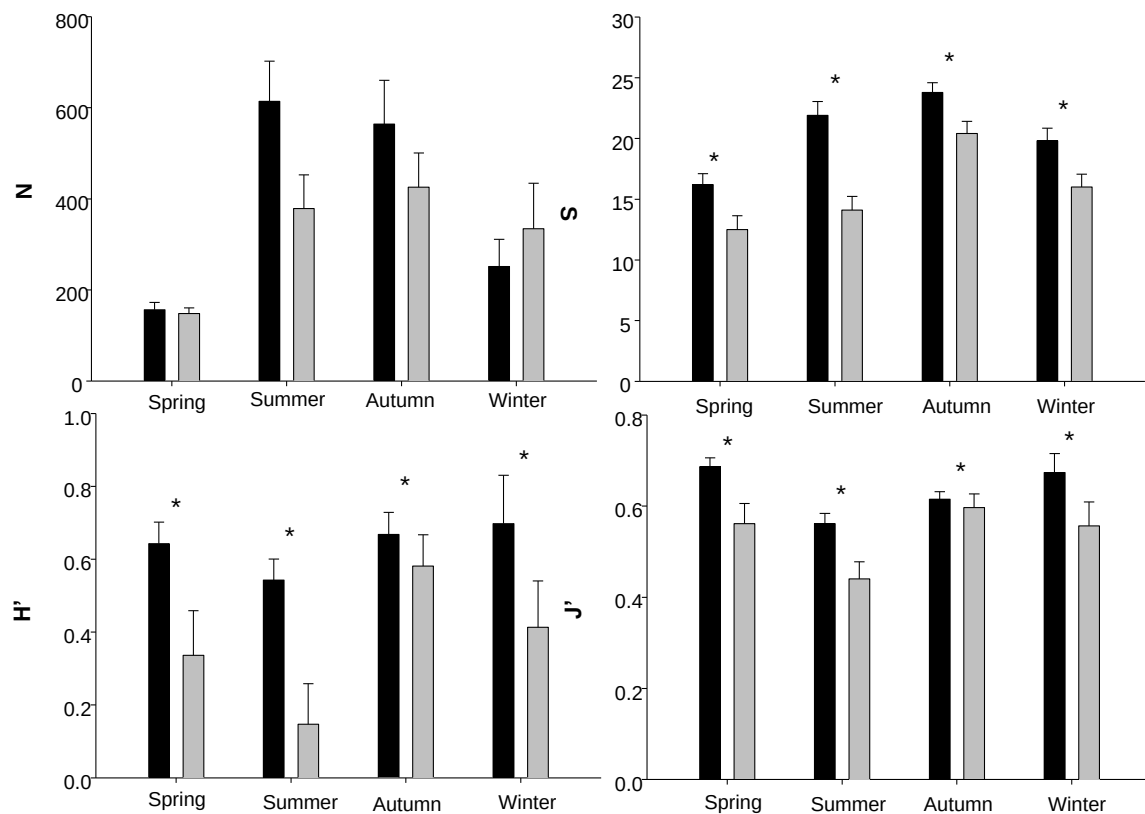


Figure 1. Mean values ($\pm$ SE) of the number individual (N) number of taxa (S), Shannon index (H') and Pielou index (J') of faunal assemblage of mussels. * indicate significant differences between mussels with and without algae on different dates ($p < 0.01$) as detected by SNK test. Black: mussels with *Caulacanthus*; Grey: mussels without *Caulacanthus*

Results of PERMANOVA analysis for the structure of the whole assemblage indicated significant differences for interaction date x condition (Table 3). Results of pair-wise tests revealed that the structure of the assemblage showed significant differences only in spring. The nMDS ordinations showed the separation between conditions and the dates of spring and summer showed a high separation than the autumn and winter (Figure 2).

Table 3. Summary of PERMANOVAs for total assemblage. *: $p < 0.05$; **: $p < 0.01$

Source	Df	Total assemblage		
		MS	Pseudo-F	Unique Perms
Date	3	10237	4.2169*	105
Condition	1	7610.8	3.0743	367
Site	4	2427.5	3.4529**	997
Da x Co	3	2475.6	2.2258*	999
Co x Si	4	1112.2	1.582*	999
Residual	64	703.04		
Total	79			

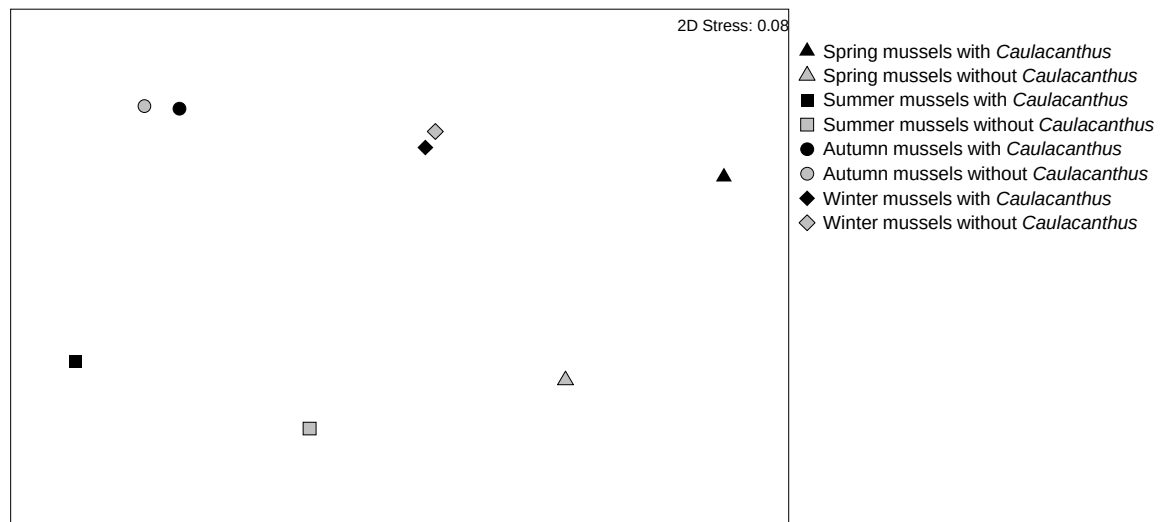


Figure 2: nMDS plots of centroids of faunal assemblages assigned to with and without *Caulacanthus* on different dates.

The SIMPER analysis identified 27 taxa as the main responsible for differences between conditions which showed an average dissimilarity of 51.96%. Moreover, most species are more abundant in replicates with algae, except for *Lasaea rubra* (Montagu, 1803), *Syllis pulvinata* (Langerhans, 1881), *Melarhaphe neritoides* (Linnaeus, 1758), Halacaridae, *Perinereis* spp. and *Cingula trifasciata* (J. Adams, 1800) (Table 4).

Table 4. Contribution (δ) of individual taxa from faunal assemblages of mussels to the average Bray-Curtis dissimilarity between mussels with and without algae.

Taxon	Average abundance		δ_i	$\delta_i\%$	$\delta_i/SD(\delta_i)$
	with	without			
<i>Lasaea rubra</i>	5.65	8.67	6.30	12.12	1.17
Nematoda	10.70	8.55	4.80	9.23	1.39
Oligochaeta	4.59	3.73	4.28	8.24	1.25
<i>Hyale</i> spp.	5.84	2.84	4.00	7.69	1.32
<i>Jaera</i> (<i>Jaera</i>) <i>prae-hirsuta</i>	4.07	2.50	3.06	5.88	1.43
<i>Idotea neglecta</i>	2.57	0.83	2.26	4.35	1.12
Copepoda	2.69	1.20	2.21	4.26	1.08
<i>Syllis pulvinata</i>	1.46	1.51	1.63	3.15	1.12
<i>Melarhaphes neritoides</i>	0.99	1.70	1.48	2.86	1.07
<i>Steromphala umbilicalis</i>	3.10	2.38	1.47	2.82	1.23
<i>Skeneopsis planorbis</i>	1.50	0.26	1.45	2.79	0.90
<i>Brachystomia</i> sp.	1.99	1.19	1.33	2.56	1.33
Chironomidae	1.57	0.99	1.30	2.51	1.30
Halacaridae	0.73	1.39	1.28	2.47	0.65
<i>Runcina</i> sp.	0.95	0.75	1.11	2.14	0.82
<i>Eulalia clavigera</i>	1.08	0.94	0.99	1.91	1.23
<i>Nucella lapillus</i>	1.09	0.46	0.98	1.88	1.12
<i>Musculus costulatus</i>	1.08	0.59	0.94	1.81	1.24
<i>Actina equina</i>	1.00	0.46	0.91	1.75	1.18
<i>Hiatella arctica</i>	0.94	0.38	0.89	1.72	1.06
<i>Patella depressa</i>	0.84	0.55	0.85	1.64	1.13
Ostracoda	0.69	0.58	0.72	1.40	1.08
<i>Acanthochitona crinita</i>	0.65	0.20	0.63	1.22	0.98
<i>Perinereis</i> spp.	0.37	0.53	0.60	1.15	0.87
<i>Tanais dulongii</i>	0.65	0.14	0.59	1.14	0.86
<i>Cingula trifasciata</i>	0.42	0.46	0.58	0.89	1.12
Nudibranchia ind.	0.63	0.08	0.58	1.12	0.57

The ANOVA analyses, for the three taxa that most contributed to the differences, showed that in *L. rubra*, a difference between condition were found. The abundance of *L. rubra* was always higher in samples without algae. Moreover, the analyses found a significant interaction date x condition for Nematoda. The Nematoda showed significant differences in autumn, where they were higher in samples with *Caulacanthus*, but in general they do not show a consistent pattern between with and without *Caulacanthus*. The Oligochaeta did not show the pattern of abundance between samples with and without *Caulacanthus* and only natural variability was found between sites (Table 5 and Figure 3).

Table 5. Summary of ANOVAS's of species that contributed to the differences between conditions on different dates. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. ns: not significant

Source of variation	Df	NEMATODA		<i>L. rubra</i>		Oligochaeta	
		MS	F	MS	F	MS	F
Date	3	4.9781	3.90	34.8833	4.03	24.4288	2.19
Condition	1	4.7744	1.27	16.1253	36.24**	1.5308	0.36
Site	4	1.2754	1.71	8.6605	7.37***	11.1409	5.74***
Da x Co	3	3.7539	8.39*	0.4450	0.22	4.2406	5.29
Co x Si	4	0.4475	0.60	2.0081	1.71	0.8017	0.41
Residual	64	0.7444		1.1759		1.9394	
Total	79						
Cochran's test		0.2288 ^{ns}		0.1925 ^{ns}		0.2069 ^{ns}	
Transformation		Ln (X)		Ln (X+1)		Ln (X+1)	

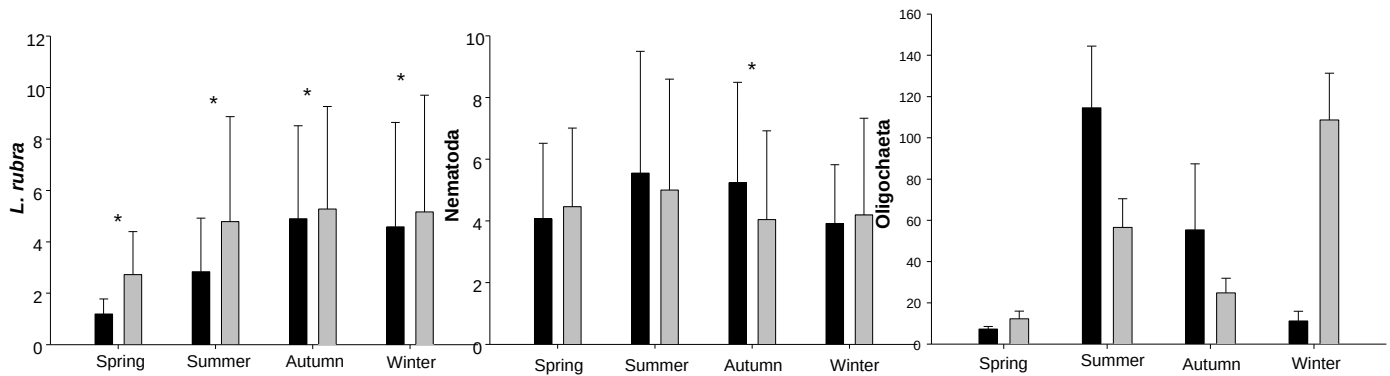


Figure 3: Mean values ($\pm$ SE) of each specie that contributed to the differences between conditions on different dates. * indicate significant differences between conditions ($p < 0.05$) as detected by SNK test. Black: mussels with *Caulacanthus*; Grey: mussels without *Caulacanthus*

4. DISCUSSION

Often different species live in a close relationship as an epibiont and host (Laudien and Wals, 1999). An example of this are mussels and macroalgae. Some studies have been done to explore the effect of algae as an epibiont on mussels (eg Dittman and Robles, 1991; O'Connor et al., 2006;), but very few have seen the combined effect of these two ecosystem engineers on associated fauna (e.g. Gutiérrez et al., 2019; Flynn et al., 2020). Moreover, being partly an invasive alga, it will be even more interesting to explore how the presence of the alga influences both the attributes of the mussels and their associated fauna.

Initially we explore the influence of alga on the attributes of mussels (i.e., density, condition index and size). In the case of mussel density, no significant differences were found between samples with and without algae, although in numerical terms, we found a higher density of mussels in samples with algae. Our results seem to be against some results found in previous studies. Dittman and Robles (1991) who studied the effects of epiphytic algae on a species of mussel of the genus *Mytilus*, saw that there is a negative correlation between epiphyte cover and mussel' survival and productivity. Other study, O 'Connor et al. (2006), put forward the initial hypothesis that epiphytic algae can affect mussel populations by increasing mortality, limiting growth and potentially restricting reproduction, affecting the recruitment rate of mussel beds. However, after the study, they saw that the dense layers of algae that grow half the year on mussel beds seem to have no effect on mussels' productivity and growth. Therefore, it is in accordance with our study. On the other hand, in numerical terms, the density was higher in the samples of mussels with algae and it may have been due to the type of algae (O'Connor et al., 2006). A type of turf alga, is also used for mussels as a recruitment area (Lasiak and Barnard, 1995), which could lead to an increase in the number of smaller mussels. This result was confirmed during the processing of the samples, where among the algae filaments were found several mussels of reduced size.

In the case of the condition index, significant differences were found between samples with and without algae, but these differences depend on the sampling date. According to the post-hoc results, the dates that showed significant differences were the summer and autumn dates. In the case of the summer date, it would be expected, since the alga has a large biomass and the difference between the condition index of mussels with and without algae is significant, being higher in mussels without algae. In the case of the autumn date, it is when the alga has the lowest biomass value and once again it is when the average

value of the condition index is quite different and significant between mussels with and without algae. In terms of absolute values, the condition index was always higher, regardless of dates, in samples without algae. This tells us that the algae seem to have a negative effect on the mussel condition index when it settles in its shell, although in terms of density, this effect has not been seen. This result would be expected, since food seems to be one of the factors that most determines the growth of mussels (Kautsky, 1982; Dolmer, 1998) and there are studies that say that epiphytes in shells limit the ability of suspension feeding of mussels (Dittman and Robles, 1991; O'Connor et al., 2006). In the case of the size of the mussels, this did not reveal any significant differences between the conditions with and without algae, it only revealed natural variability between sites. The condition index is the correlation between the quantity of soft parts of the animal and the shell (Davenport and Chen, 1987), while the size only considers the measurement of the shell length. Both measures can be influenced by several factors, namely the availability of food (Kautsky, 1982; Dolmer, 1998; Zupan and Saric, 2014). When food is scarce, mussels use their reserves, and weight loss in the soft parts begins to be observed (Kautsky, 1982). This effect can then explain the differences in the condition index, whereas in the case of size, the absence of differences may be due to the interaction with other factors, namely the interspecific competition, also involving the density, which makes the mussels have a similar size (Kautsky, 1982). Considering that there is an interaction between size and density, it makes sense that no significant differences were found in both measures.

Moving now to the associated fauna, from the values for abundance obtained we can see that in absolute values, the abundance of fauna associated with samples with algae was higher than that associated with samples without algae, although this value is not significant, as was proved with the statistical analysis. The absence of significant differences in fauna abundance may be due to the type of algae we were studying, as it is a small alga that does not add a large quantity of habitat. In previous studies, such as Anderson et al. (2005) or that of Tuya et al. (2011), they saw that a greater quantity of habitat is associated with a greater abundance of organisms. This agrees with our study, since the alga *Caulacanthus* spp. it is small and therefore offers an extra small space.

Although in the case of abundance, the presence of algae did not have any significant effect, in terms of diversity it was no longer so. Significant differences were found both for number of taxa and for Shannon and Pielou indexes. This result would be expected, because despite *Caulacanthus* spp. being a small-sized alga, it is a turf-type alga (Smith et al., 2014) and therefore offers extra complexity to mussels, which further increases the

complexity of the habitat offered. We know that the greater the complexity of the habitat, the greater the diversity of niches it can offer, and this increases the diversity of the associated fauna (Christie et al., 2009; Torres et al., 2015). An example of favouring complexity with two engineers is Flynn et al. (2020) who studied how a type of crab was favoured by the interaction of an alga (*Chondrus crispus*) with mussel (*Mytilus edulis*), saw that the two engineers together increased complexity, which in turn favoured the crab.

In the same way as in univariate analyses, multivariate analysis also showed that the presence of algae affects the entire structure of the community associated with mussels but depends on the date. In this case, the significant differences were found only at the spring sampling date. A possible explanation for this may be related to the biomass of algae, since it was on this date that the largest biomass of algae was found in the samples. Furthermore, as previously mentioned, the quantity and complexity of the algae can influence the associated fauna. On the other hand, it was on this date that the lowest density of mussels was found both in samples with algae and in samples without algae (although without significant differences). In addition of the largest biomass of algae with the lowest density of mussels may alter the entire structure of the community, as we can see from the analysis. In addition, the presence of algae may not only increase the size and complexity of the habitat, but also can favour the mussels' habitat, by reducing temperature and desiccation. There is a study by Gutiérrez et al. (2019), where they saw that an alga, in this case *Porphyra* sp., when it was present on the mussel beds, reduced the temperature and desiccation. Although *Caulacanthus* sp. is quite different from that of this study, it can still have some effect in that sense, and considering that the differences were in the spring date, which was when the alga had the highest biomass, the greatest capacity had to be able to cover the whole beds of mussels and thus protect against temperature and desiccation during low tide. Moreover, as might be expected, nMDS shows us that there is a greater separation between spring and summer dates than autumn and winter dates. This would be expected, since during the spring and summer months there is a greater recruitment of different fauna, while the biomass of algae differs between spring and summer and autumn and winter dates. In the case of spring and summer dates it is when the alga has the highest quantity, whereas in the case of autumn and winter dates, the algae have the least quantity.

Although there were no significant differences in the abundance of associated invertebrates, there were species that due to their abundance in one or other condition contributed to the significant differences found in the structure of the community. From the analysis we can see that most species are more abundant when algae are present. This

would be to be expected, as, as mentioned above, despite the small algae, it increases the complexity of the algae - mussels complex. In addition, the alga offers a different type of habitat than mussels, which can greatly influence the types of species found. Moreover, the three taxa that explained the higher differences between the samples with and without algae on different dates were *L. rubra*, Nematoda and Oligochaeta. Then, the analysis revealed significant differences between the conditions for *L. rubra* that it was more abundant in samples without algae. *L. rubra* is widely distributed among several habitats, namely among mussels and it looks for places lacking sediment or with little (Crisp and Standen, 1988) and in this case when the algae are present it may cause it to retain a greater amount of sediment that becomes harmful to this organism. In case of Nematoda, showed significant differences between conditions, but these in turn depended on the date. In this case, the significant date was autumn. In absolute terms, in the summer and autumn dates, the abundance of nematoda was higher and alga biomass found was higher and lower, respectively. This result indicates that the biomass of algae does not seem to have any influence. A study by Kito (1982) that studied seasonal variability in the nematode community associated with an alga, found that the highest nematode density was found in the warmer seasons, which agrees with our study, which was in the summer that the largest number of nematodes were found (but not significant). On the other hand, in this study, they also saw that the nematode community depends on the complexity of the habitat and available food resources. In our study, the significant differences found were in the autumn date, which is when the alga had the lowest average biomass value, which is at variance with the study described above. Therefore, it means that there are other factors that influenced the associated nematode, namely food, as described above. Finally in case of oligochaeta, did not show any pattern of abundance between conditions and only natural variability between sites which indicates that this taxon not depend on the presence of the alga, but on the other factors. We know that oligochaetes are considered as a biological indicator organic enrichment (see in O'Connor and Crowe, 2007), so we would expect them to vary more according to this variable.

In conclusion, we can see that the presence of the two engineers together, promotes a more complex and heterogeneous habitat, thus promoting greater associated diversity. Furthermore, it was important to understand how an exotic species interacts in the mussel-algae association. In general, the presence of alga as co-engineers on mussels, even though it may be an invasive species, to play an important role on associated fauna.

CHAPTER VI. EFFECT OF TRAMPLING ON MUSSEL BEDS AND THEIR ASSOCIATED FAUNA – AN EXPERIMENTAL APPROACH

Effect of trampling on mussel beds and their associated fauna – an experimental approach

Abstract

In the last 50 years, trampling on rocky shores has increased, becoming one of the main anthropogenic disturbances. Despite this, its consequences depend on several factors inherent to the species present, the affected habitat and the type of exposure to trampling. To test the effects of trampling on the attributes of the mussel *M. galloprovincialis* and its associated fauna, a field experiment was carried simulating different trampling intensities. The results showed that trampling did not significantly affect the attributes of mussels, nor the abundance, diversity and structure of the total assemblage of their associated fauna. Still, significant variability was found among plots for mussel density and for S, H' and structure of the total assemblage.

Thus, altogether, such observations suggest that other factors have also played a role, somehow mitigating the influence of the trampling experiment. Despite the absence of significant differences among the different trampling intensities for the attributes of mussels and associated fauna, experiments of this type are important in order to predict and minimize the risks for species.

Keywords

Coastal areas; Trampling; *M. galloprovincialis*; invertebrate assemblage; North of Portugal.

1. INTRODUCTION

Marine ecosystems are subjected to large spatial-temporal variations, especially those of coastal zones (Micheli et al., 2016). The communities of different types of habitats in coastal zones are exposed to a set of stresses derived from several variations in physical conditions, namely environmental factors and the frequent concentration of human impacts (Raffaelli and Hawkins, 1996; Araújo et al., 2012; Micheli et al., 2016). Among these variations, nowadays, climate change stands as one of the most critical ones, as it has been causing abrupt changes in temperature, salinity, nutrient concentration and exposure to waves. In parallel, distinct anthropogenic disturbances such as pollution, sedimentation change, changes in the coastline, species introduction, trampling, among

others, derived from increasing urbanisation, can also jeopardize the sustainability of the coastal ecosystems (Thompson et al., 2002; Micheli et al., 2016; Torres et al., 2022).

In the case of trampling, this is one of the main anthropogenic disturbances (Araújo et al., 2009b; Plicanti et al., 2016) and has increased substantially in the last 50 years due to population increase on coastal areas, improvements in access to coastal areas (Thompson et al., 2002) and the development of the tourism industry (Davenport and Davenport, 2006).

The trampling on rocky shores is directly related to commercial and recreational activities, such as bait catching or the collection of live or dead shells (Plicanti et al., 2016). In rocky shores, trampling can directly affect species, through the crushing and death of organisms, or indirectly, through the destruction of habitats offered by habitat-forming organisms, for example (Mendez et al., 2018). Such indirect consequences may then downstream affect biodiversity, altering the distribution, abundance and diversity of communities (Plicanti et al., 2016; Mendez et al., 2018). However, changes upon the disturbance will depend on the ability of communities, which greatly hang on the characteristics of each species present (reproductive capacity, growth rate and dispersal capacity), to resist or recover, in addition to the type of exposure, duration and disturbance intensity, location and habitat type (Araújo et al., 2009b; 2012; Bertocci et al., 2011; Mendez et al., 2018).

Living in a very dynamic and unstable zone, intertidal organisms are almost permanently exposed to natural disturbances, which makes them more adapted and possibly less vulnerable to other disturbances, such as anthropogenic ones (Micheli et al., 2016; Nicastro et al., 2018). In particular, bivalve molluscs show a great capacity to adapt to local conditions, which makes their growth and settlement vary according to the local environment and differ between regions (Ramos-Oliveira et al., 2021).

In the intertidal zone of the Northern Portugal, one of the most abundant species is the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819. It is a sessile, filter-feeding bivalve species that is widely distributed on the Atlantic rocky shores of the Iberian Peninsula (Boaventura et al., 2002; Burnett and Sarà, 2019; Ramos-Oliveira et al., 2021). This species plays an important role at the commercial level, in food chains and also provides valuable ecosystem services (Rius and Cabral, 2004; Vinagre et al., 2015; Gundersen et al., 2017; Romero-Freire et al., 2020; Veiga et al., 2021), in addition of being considered an ecosystem engineer (Borthagaray and Carranza, 2007; Buchsbaum et al., 2009).

In recent years, trampling on rocky shores has received growing attention (Plicanti et al., 2016), with several studies exploring how increasing trampling intensity influences species composition, percent cover, diversity and biomass (Araújo et al., 2009b). Some studies have already explored the effects on algae (e.g. Brown and Taylor, 1999; Araújo et al., 2009b; 2012; Bertocci et al., 2011), animals (e.g. Van de Werfhorst and Pears, 2007; Micheli et al., 2016; Plicanti et al., 2016; Burnett and Sarà, 2019); however, in the case of mussels, in particular *M. galloprovincialis*, in spite of its economic importance and the key role it plays in the coastal marine ecosystem, works devoted to explore the effects of trampling on its role as an ecosystem engineer and how its associated communities are affected, are still scarce (e.g. Smith et al., 2008).

Thus, conducting studies to understand how natural populations of *M. galloprovincialis* and its associated macrofauna respond to anthropogenic factors such as trampling is essential for the conservation of the species.

Therefore, the aim of this study was to explore how *M. galloprovincialis* and its associated fauna react to different trampling intensities. To achieve this aim, an experiment was conducted on two different shores in Northern Portugal.

2. MATERIALS AND METHODS

2.1. Study area, sampling and sample procedure

The study was done at the two rocky shores in the north coast of Portugal (Moledo (41°50'28.81" N; 8°52'32.30" W) and Vila Praia de Âncora (41°49'25.93" N; 8°52'27.42" W)). This area was selected due to the population density to be lower than 130 residents/km², because a higher population density can imply a higher degree of trampling (Creel, 2003; Smith e Murray, 2005; Rius et al., 2006; McPhee, 2017; Todd et al., 2019).

The Portuguese north coast is characterised by semidiurnal tides, with a range of variation of waves from 1.5 to 7 m and most common from west and northwest direction (Dias et al., 2002). The rocky shores substrate is a mix of granite and schist and the coast is fragmented by the presence of estuaries and a range of soft and hard substrates. Additionally, the organisms benefit with a seasonal upwelling of nutrients, during the spring and summer months (Lemos and Pires, 2004).

In each rocky shore, along 10 mts of coastline, 6 plots (2m x 2m), were selected for the experiment, which was done between February and August 2018. Two plots were

randomly assigned to control (without trampling), two assigned to low (one trampling) and two high (three trampling). When plots were marked, the treatment was started and then repeated seven more times (one or two times a month), until the samples were collected. At each plot, three quadrats (10 x 10 cm) of mussel beds were collected by scrapping all the quadrat area, to explore potential impact of trampling on mussel attributes and their associated macrofauna. The samples were placed in plastic bags, transported to the laboratory and frozen (-20° C) until they are treated. Then, using a sieve of 0.5mm, each sample was washed to separate the macrofauna from the larger mussels. The deposit on the sieve were preserved in Formaldehyde (4%), stained with Bengal rose until sorting, identification and counting of macrofauna. Moreover, in each sample, were counted all mussels and then 20 mussels were randomly separated to measure their shell length and 10 mussels to calculate the condition index (i.e., the ratio between dry weight of soft tissue and dry weight of the shell).

2.2. Data analysis

Analysis of variance (ANOVAs) were done to test differences between the treatments (control, low trampling and high trampling) on density and condition index of mussels and on the number of individuals (N), number of taxa (S) and Shannon index (H') of the invertebrate assemblage associated with mussels. In the analysis, a three-way model was considered, including the factors: treatment fixed, orthogonal with three levels (i.e., control, low and high), Shore random with two levels, nested in treatment and plot random, with two levels, nested in treatment and shore, with 3 replicates, for mussel density, N, S and H'. A four-way model was used for the condition index of mussels, with the same factors described above and quadrat, with 3 levels and random nested in treatment, shore and plot and 10 replicates. To check the homogeneity of variances, Cochran's test was done before each ANOVA. When necessary, data were log-transformed to remove the heterogeneity of variances. When this was not possible, untransformed data were analysed and the results were considered robust if significant at $p < 0.01$ (Underwood, 1997).

In order to analyse the multivariate data (size and total assemblage), Permutational multivariate analysis of variance PERMANOVA (Anderson, 2001) based on Bray–Curtis untransformed dissimilarities, was used. The model for these analyses was the same as for three-way ANOVA described above, for density, condition index, N, S and H', using a maximum of 999 permutations in the reduced model with a defined level of significance ($p < 0.05$). When the number of unique permutations for a factor was lower than 30 (or close

to 30), Monte Carlo p-values were considered (Anderson et al., 2008). Multivariate patterns were illustrated by non-metric multidimensional scaling (nMDS) ordination of plots of each treatment on each shore.

3. RESULTS

3.1. Mussel' attributes

No significant differences between treatments were detected for density, condition index and size of mussels (Tables 1, 2 and 3; Figure 1). However, density and condition index showed significant variability at the scale of plot (density) and shore and quadrat (condition index) (Tables 1 and 2; Figure 1).

Table 1. ANOVA analysis for density of mussels between treatments. ***: $p < 0.001$; ns: not significant.

Source of variation	Df	Density	
		MS	F
Treatment	2	588840.7778	0.22
Shore	3	2680984.7500	1.67
Plot	6	1608273.3056	9.59***
Residual	24	167760.3889	
Total	35		
Cochran's test		0.3320 (ns)	
Transformation		None	

Table 2. ANOVA analysis for condition index of mussels between treatments. *: $p < 0.05$; ***: $p < 0.001$; ns: not significant.

Source of variation	Df	Condition index	
		MS	F
Treatment	2	0.0007	0.29
Shore	3	0.0024	9.50*
Plot	6	0.0003	0.78
Quadrat	24	0.0003	3.85***
Residual	324	0.0001	
Total	359		
Cochran's test		0.0805 (ns)	
Transformation		None	

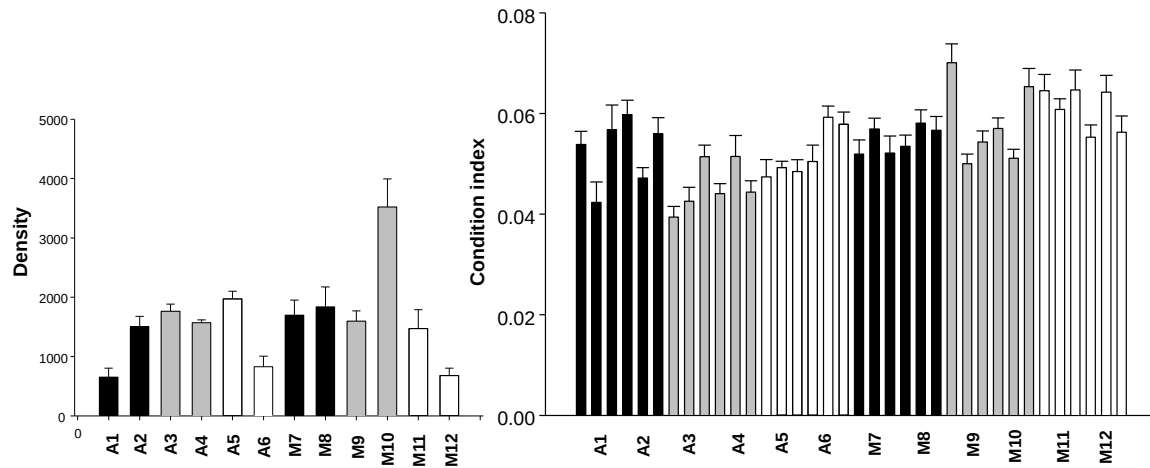


Figure 1. Mean values ($\pm$ SE) of mussel attributes (Density and Condition index). Black: High trampling (A1/A2: Vila Praia de Âncora plot 1 and 2, M7/M8: Moledo plot 7 and 8); Grey: Low trampling (A3/A4: Vila Praia de Âncora plot 3 and 4, M9/M10: Moledo plot 9 and 10); White: control (A5/A6: Vila Praia de Âncora plot 5 and 6, M11/M12: Moledo plot 11 and 12).

Table 3. PERMANOVA analysis for size of mussels between treatments.

Source	Df	Mussel- size		
		MS	Pseudo-F	Unique Perms
Treatment	2	2388.2	0.69195	15
Shore	3	3451.4	1.5006	996
Plot	6	2300	1.2687	997
Residual	24	1812.8		
Total	35			

3.2. Invertebrate assemblages

A total of 10843 individuals were identified (4211 high trampling, 3737 low trampling and 2895 control) belonging to 64 different taxa (45 high trampling, 45 low trampling and 51 control). ANOVA indicated no significant differences between treatments for S, N and H' (Table 4). However, S and H' showed significant variability at the scale of plot (Table 4; Figure 2).

Table 4. Summary of ANOVA's for the number of taxa (S), number of individuals (N) and Shannon index (H') of faunal assemblage associated with *M. galloprovincialis*. *: $p < 0.05$; ***: $p < 0.001$; ns: not significant.

Source of variation	Df	Faunal assemblage					
		S		N		H'	
		MS	F	MS	F	MS	F
Treatment	2	33.2500	0.92	27609.3611	0.30	0.3483	0.27
Shore	3	36.0556	1.39	93331.2500	1.08	1.3037	1.49
Plot	6	25.8889	3.05*	86729.0833	2.97	0.8730	11.42***
Residual	24	8.5000		29226.1111		0.0765	
Total	35						
Cochran's test		0.2974 (ns)		0.7909 (s)		0.3707 (ns)	
Transformation		None		None		None	

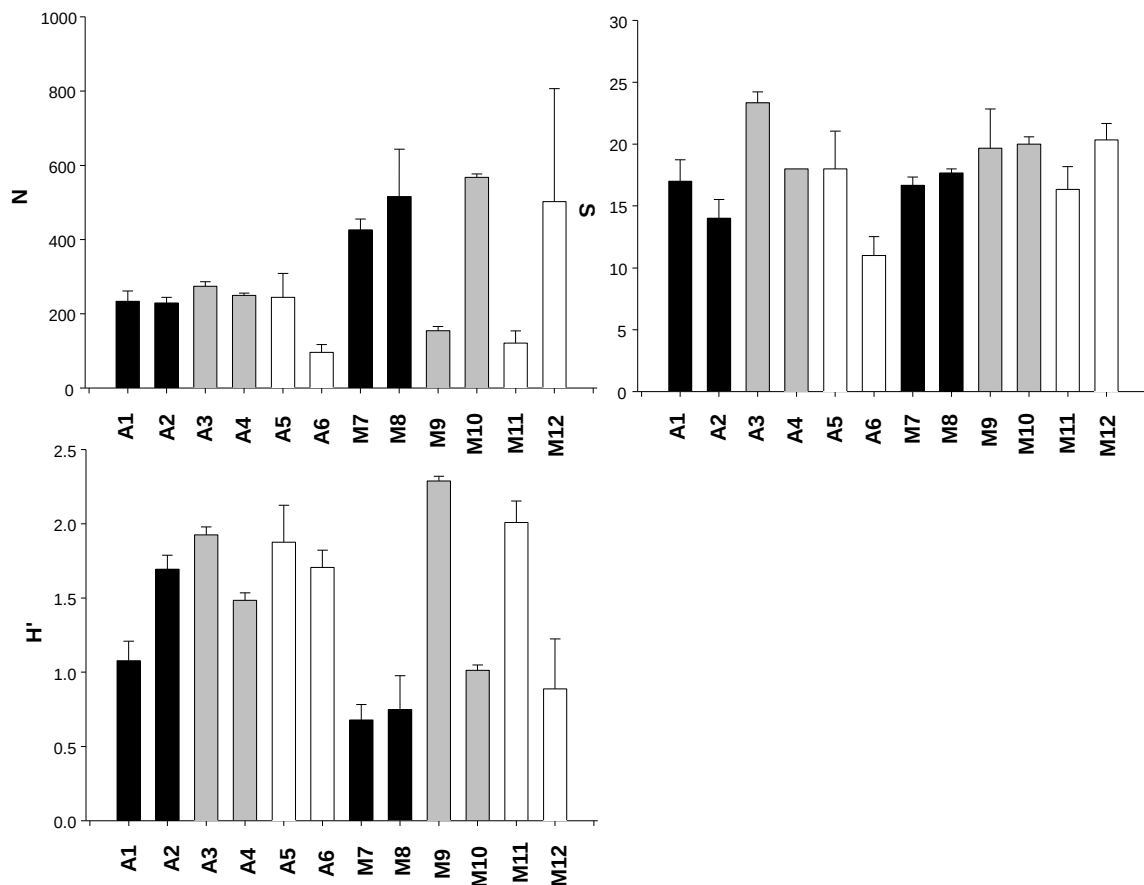


Figure 2. Mean values ($\pm$ SE) of the number individual (N), number of taxa (S) and Shannon index (H') of faunal assemblage of mussels. Black: High trampling (A1/A2: Vila Praia de Âncora plot 1 and 2, M7/M8: Moledo plot 7 and 8); Grey: Low trampling (A3/A4: Vila Praia de Âncora plot 3 and 4, M9/M10: Moledo plot 9 and 10); White: control (A5/A6: Vila Praia de Âncora plot 5 and 6, M11/M12: Moledo plot 11 and 12).

Results of PERMANOVA analysis of the structure of the total assemblage indicated no significant differences between treatments, only significant variability at the scale of plot

(Table 5). The nMDS ordination showed a no clear separation between treatments (Figure 3).

Table 5. Summary of PERMANOVAs for total assemblage. **: $p < 0.01$

Source	Df	Total assemblage		
		MS	Pseudo-F	Unique Perms
Treatment	2	5381.3	1.1058	15
Shore	3	4866.2	0.85558	996
Plot	6	5687.6	8.2869**	997
Residual	24	686.34		
Total	35			

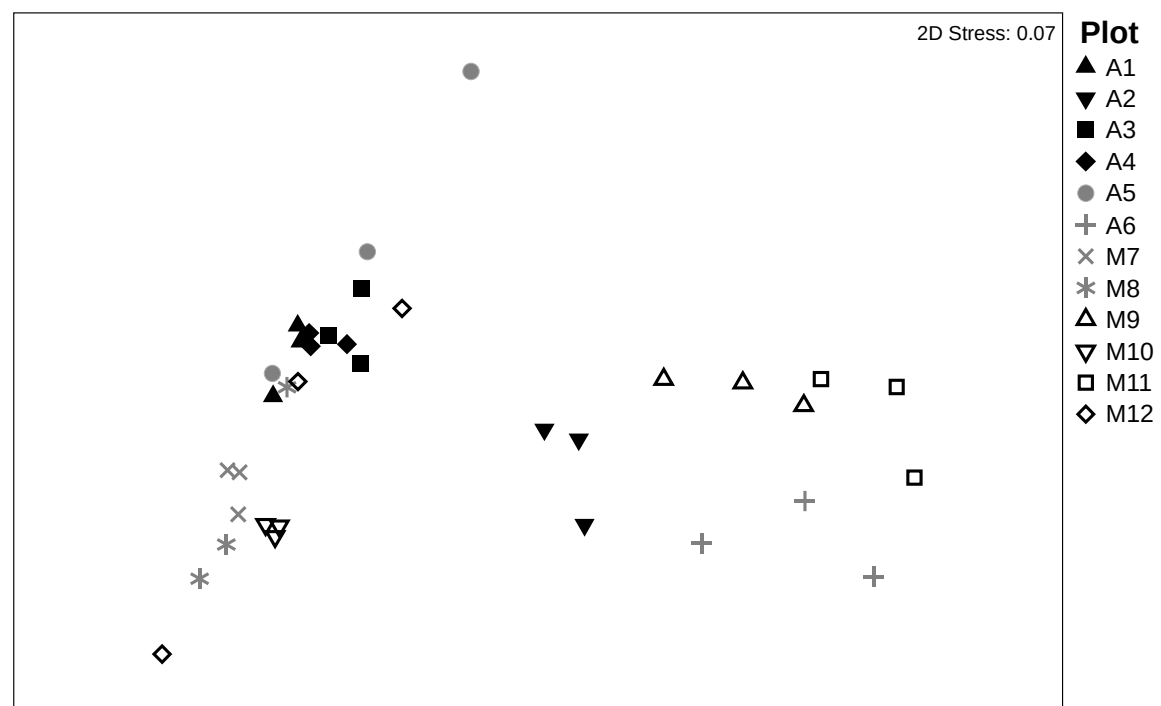


Figure 3. nMDS plots for plots of each shore for high trampling (black), low trampling (grey) and control (white).

4. DISCUSSION

Increased trampling on rocky shores has become a major anthropogenic disturbance (Araújo et al., 2009b; Plicanti et al., 2016), but its effects on the communities therein present will depend on different factors inherent to the species and to the characteristics of the disturbance and the habitat itself (Araújo et al., 2009b; 2012; Bertocci et al., 2011; Mendez et al., 2018).

M. galloprovincialis and its associated communities, by being intertidal organisms, are often subjected to natural disturbances, making them more adapted and less vulnerable to anthropogenic impacts (Micheli et al., 2016; Nicastro et al., 2018). Yet, it is important to understand how different trampling intensities influence mussel attributes and their associated fauna.

In this study, the density, condition index and size of mussels did not differ significantly between the different trampling intensities studied. Contrary to the results of our study, Micheli et al. (2016), who explored the effects of different trampling intensities on habitat forming organisms, found that trampling caused a significant reduction in mussel abundance, especially when mussels were more exposed to tidal waves. In that study, authors hypothesised that mussels more exposed to waves would be more resistant to trampling, which they ended up finding otherwise. Based on our data, that hypothesis makes sense, as no significant differences between trampling intensities were recorded among mussel attributes. In contrast, Mendez et al. (2018), who studied the effect of different trampling intensities on the mussel *Brachidontes* spp. found that the percentage of mussel cover decreased as the treatment progressed, especially at the higher trampling intensities. The differences between their outcomes and ours may be related to the mussel species studied, the study area, and the treatment intensity, as in that study additional trampling events were carried out in a shorter period. Yet, in agreement with our data, Ferreira and Rosso (2009) found that the percentage cover of the bivalve *Isognomon bicolor* was not affected by increasing trampling. According to the authors, the absence of differences was mainly related to the resistance of the bivalve to trampling with the high-density rates and also with the capacity of this organism to move laterally, thus being able to colonize rapidly other surrounding areas. In our study, despite being a different species of mussel, a similar response to trampling was observed. However, in this case, we do not know if it was due to the high-density rates, since this varied significantly between plots. This result which suggests there must be other factors interfering in the absence of differences between trampling intensities.

Another study by Plicanti et al. (2016), which aimed to explore the experimental effects of trampling on *Sabellaria alveolata* reefs and their associated fauna, found that trampling caused physical damage to the reefs, but mussels of the family Mytilidae, one of the taxa associated with these reefs, had an increased abundance in most of the trampling areas relative to the control. Interestingly, according to the authors, that result can be linked to a spawning event for *Mytilus* spp. which may have led to increased abundance. Similarly, in the present study, the experiment was also undertaken in the same period of the year, so

a possible explanation for the absence of differences in mussel density between the different trampling intensities may be related to the spawning season and possible homogenisation between treatments.

Although another result found in our study was the significant differences in density between selected plots and between shores and squares for the condition index. Even among the control plots, differences were found, which means that there are other factors that may have influenced the mussel beds. One of these factors could be the natural variability in the spatial distribution of *M. galloprovincialis*. Ramos-Oliveira et al. (2021), by studying the spatio-temporal variability of *M. galloprovincialis*, found differences for density between sites within each shore and for the condition index between shores and plots, which is equivalent to our plots.

Regarding mussels' associated fauna, no significant differences between the different trampling intensities were found, neither for abundance and diversity nor for the structure of the total assemblage. Actually, a variability was only recorded between plots, for S, H' and the structure of total assemblage. Contrary to our study, Ferreira and Rosso (2009) found an increase in taxa associated with *I. bicolor* after 4 months of trampling. Their explanation has to do with the fact that trampling may indirectly increase the abundance of certain herbivores, for example. In our case, we do not have the evolution of values throughout the treatment, we only have the final values. So, we cannot verify if there was an increase or decrease in abundance and diversity throughout the treatment. Similarly to our study, Plicanti et al. (2016) found no significant differences in associated fauna between the different trampling treatments. According to them and supporting our results the associated fauna seemed to be less vulnerable and resistant, as they have the ability to move away from the disturbed site and return after the disturbance. This event may actually have happened in our study, since the samples were collected in the following month after the last experimental trampling event, which could have given time for the mobile fauna to recolonize the disturbed area.

Smith et al. (2008), who studied the impacts of human visits on mussel beds, found no significant differences for either species richness or diversity (Shannon-Wiener index) between the most and least frequented sites. Also, Micheli et al. (2016) found that some taxa had no influence from trampling, although they found others that increased their abundance. In our study, no significant differences were found between treatments, but there was an increase in the numerical values of S and H' at the intermediate trampling level. These results may indicate that trampling can increase diversity, especially at

intermediate levels of disturbance (Ferreira and Rosso, 2009). The intermediate level of disturbance may interfere with competitive exclusion processes, favouring recolonization by new species and thus changing the community structure (Ferreira and Rosso, 2009). In the case of our study, there was no significant increase in fauna and the numerical increase verified was not sufficient to cause changes at the level of community structure.

Following our results and comparing them with other available studies, it is suggested that the effects of trampling on communities is variable and indeed depend on several other factors that are acting at simultaneously.

Moreover, as we have already mentioned, a spatial variability between plots for S, H' and for the structure of the total assemblage was recorded. Being a field experiment, it is difficult to control the normal beach trampling, even if it is an area with a low population density. Furthermore, as the study was carried out between spring and summer, the increase of tourism activities in these months may also have conditioned the obtained results. Furthermore, during the treatment, a clear difference in the quantity of sediment trapped between the mussel beds was found across the selected plots. Thus, mussels with a lower quantity of sediment were much more fragile to trampling than those with more sediment. Moreover, the amount of sediment influenced the associated fauna (unpublished data). Additionally, to this shortcoming, we also observed that, in certain plots, the growth of algae on mussel shells. These algae, besides being able to attenuate the effects of trampling, can also act as co-engineer (unpublished data), significantly increasing the diversity of organisms, which may have caused the differences between plots, regardless of trampling intensity.

The effects of experimental trampling disturbance seem to have been attenuated by other factors inherent to the habitat and species present, indicating that the effects and their interpretation are complex and require more detailed and experimentally controlled studies. Despite this, these experimental studies are important for environmental management in order to predict and minimize the risks for species.

CHAPTER VII. FINAL REMARKS

General conclusions

Due to the substantial increase in population density along the coasts, coastal ecosystems are particularly exposed to human pressures, consequently threatening the organisms living in such areas (Martinez-Crego et al., 2010; de la Ossa-Carretero et al., 2012). To minimize or mitigate these effects, different strategies have been created, such as the implementation of environmental protected areas (Cole et al., 2011). However, for such strategies to be effectively implemented, monitoring studies of environmental quality are necessary (Borja et al., 2000; Simboursa and Zenetos, 2002). In environmental monitoring studies, benthic invertebrates are often used as biological indicators of the marine environment because they respond relatively quickly to anthropic and natural stress (Borja et al., 2000; de la Ossa-Carretero et al., 2012). From all benthic invertebrates, there are some species considered particularly relevant for environmental assessment, due to their roles in the functioning of ecosystems, such as ecosystem engineers (Jones et al., 1994;1997). In the case of this study, the mussel *Mytilus galloprovincialis* was selected as a model, since in addition of being considered as a good bioindicator and to provide various ecosystem goods and services, it is also considered an ecosystem engineer (Bertocci et al., 2012b; Gundersen et al., 2017; Romero-Freire et al., 2020).

However, mussel beds, particularly in the intertidal, are subjected to various anthropic disturbances, which may have a great impact on the functioning and stability of their aggregates (Thompson et al., 2002; Carranza et al., 2009). One of these disturbances is increasing urbanisation, which is one of the most widespread and growing threats to coastal ecosystems (Barragan and de Andrés, 2015; Momota and Hosokawa, 2021). Therefore, it is imperative to study the effects of urbanisation, because it is a very accessible area to human activities (Thompson et al., 2002). The effect of urbanisation on mussel beds has also received some attention (e.g. Brosnan and Crumrine, 1994; Smith and Murray, 2005; Robinson et al., 2007; Veiga et al., 2020) but these studies were focused on mussel populations. However, studies focused on the urbanisation effect on assemblages associated with mussels are still scarce.

So, this thesis focused on the study of four different anthropogenic impacts and their effects on mussel beds of *Mytilus galloprovincialis* and its associated macrofauna.

In the first chapter, we studied the differences in mussel attributes and associated macrofauna between urban and non-urban shores. The results showed that larger mussels were more frequent on urban shores, while the smallest mussels were only found on non-urban shores. With regards to the associated macrofauna, only the number of species was significantly higher on non-urban shores, although both the number of individuals and the Shannon index showed tendentially higher values on non-urban

shores. In addition, the whole structure of the associated macrobenthic community was significantly different between urban and non-urban shores.

In the second chapter, it was explored how a greater quantity of sediment trapped among mussel beds influenced their associated fauna. Overall, data revealed significant correlations for the Pielou and Shannon indexes and for the abundance and diversity of the associated fauna. In particular, the abundance and the diversity showed positive correlations, while the Shannon index and the Pielou index showed negative correlations. For the entire associated community, a significant high correlation with the quantity of sediment, was found and the size being 0.500 mm of the sediment that most influenced the fauna. Particularly, the results of samples with high and low quantity of sediment, showed significant differences in the interaction between shore and quantity of sediment for the number of individuals, with higher values in samples with a high quantity of sediment, but with significant differences only on the shore of Moledo. For the indexes of Shannon and Pielou, a significantly higher values in samples with low quantity of sediment and also for the whole structure of the community.

In the third chapter, focus was placed on the effects the combination of two ecosystem engineers (*M. galloprovincialis* and alga turf of the genus *Caulacanthus* sp.), living together in certain places of the northern of Portugal on their associated fauna, throughout the year. In parallel, the effect of algae on the attributes of mussels was also evaluated. For the results of the mussel attributes, the presence of the algae affected only the condition index, though this observation depended on the sampling date. With regard to macrofauna associated with mussels, differences were found between samples with and without algae for number of taxa and for Shannon and Pielou indexes, where the values were always significantly higher in algal samples. For the entire structure of the community, differences were also found between conditions, but these were also dependent on the sampling date.

In the fourth chapter, we simulate a trampling experiment with different intensities to test the effects of trampling on the attributes of the mussel *M. galloprovincialis* and its associated fauna. The results showed that trampling did not significantly affect the attributes of mussels (density, condition index and size), the abundance, diversity and structure of the total assemblage of associated fauna. Actually, significant variability was only found among plots for mussel density and for S, H' and structure of the total assemblage.

Taking together all of these data, the results obtained in the four tasks show that the different impacts due to urbanisation of rocky intertidal areas causes significant changes on the attributes of the mussel *M. galloprovincialis* and its associated macrofauna. In the

case of mussels, they were shown to have a lower sensitivity to anthropic variations, i.e. significant differences only on size frequencies (urbanisation level) and on condition index (presence of *Caulacanthus*), while the associated macrofauna seemed to have a greater sensitivity, i.e. significant differences on N and whole assemblage (urbanisation), on N, H', J' and whole assemblage (quantity of sediment) and on S, H', J' and whole assemblage (presence of *Caulacanthus*). In all tasks, Nematoda, *Idotea* sp., *Nucella lapilus* and *Hyalle* spp. are the taxa that most contributed to the significant differences found, but their abundances depended on the anthropogenic disturbance considered. In particular, Nematoda were more abundant on non-urban shores, but when we consider the presence of algae or the high amount of sediment (activities resulting from increased urbanisation), they were already more abundant in these samples. Therefore, Nematoda seems to be a suitable bioindicator of the ecological status of mussel beds.

In general, anthropic activities caused effects on the communities present, but these impacts were dependent on several factors, such as the anthropic disturbance (type, duration, magnitude and frequency) and the present species (Burnett and Sarà, 2019).

Although mussel beds appear to be more resistant to anthropogenic variations, due to their extreme importance as a biodiversity hotspot and also due to the important role they play in coastal areas, small changes in mussel communities could put other dependent species at risk, can bring irreversible changes to the ecosystem.

Therefore, the results obtained in this study may contribute to support measures for the conservation of mussel beds *M. galloprovincialis* on the north coast of Portugal, as well as encourage similar studies to be carried out in other places, where there are similar species in order to preserve them and avoid further losses.

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