Intertidal species richness and abundances in a recent small Marine Protected Area in Portugal: lack of effects from protection suggests changing strategies in the future.

Justine Pagnier

University of Lund

Master's degree project

Biology, 45hp

February – August 2020

Supervisors:

Frederico Almada (MARE)

Per Carlsson (LU)

Acknowledgement

This Master's degree thesis has been performed in the Marine and Environmental Sciences Center and Instituto Universitário de Ciências Psicológicas, Sociais e da Vida of Lisbon. It is related to two projects that provided the physical and technical support: a long-term study called Rebreath and funded by the Lisbon Oceanarium, and a new project called AQUASIG3, financed by the Municipality of Cascais.

I would like to express my sincere gratitude to my two supervisors, Dr. Per Carlsson from the Biology Department of Lund University and Dr. Frederico Almada from MARE Lisbon, for their precious support, guiding and encouragement along this project. I am deeply glad that Frederico gave me this amazing opportunity as I was thrilled to be part of such a project.

I wish to thank Inês Carneiro and Pedro Coelho, with whom I learned a lot during field work as they were always present to help me, to answer my questions and to teach me their knowledge about the local biodiversity, and who also brought an important support during the data analysis.

I wish to have a special attention for the taxonomists who helped us with the identification of specimens throughout this study: Mónica Albuquerque and José Pedro Borges for mollusks, Paula Cainho for non-indigenous species in marinas.

I greatly thank Isabel Rubio Pérez, creator of the website on biodiversity in the Mediterranean and in the coastal lagoon Mar Menor (marmenormarmayor.es), for her collaboration on the sequencing of *Fulvia fragilis* barcodes and for the specimens she sent from Spain.

I would like to thank my dear friend and colleague Andrea Romero for her great work on non-indigenous species and all the interesting information that she shared with me, but also for making me feel warmly welcome as soon as I arrived in the lab and started my life in Lisbon.

I also wish to acknowledge the support of my colleague, but above all friend, Donia Kalthoumi. She always had great critics and advices that helped me along my work, and she has always been present to listen to my doubts and thoughts.

Finally, I wish to deeply thank my family and friends for being my pillars through all the challenges in my life, including this thesis, and especially my parents, for their unwavering support and without whom I could not have started this amazing journey of becoming a marine biologist.

Table of Content

Abstract

Marine protected areas (MPAs) are being created around the world as the concern on species and habitats preservation is growing. A widespread concern related to the important lack of regulation and effective conservation measures has been raised, calling the attention to the importance of no-take areas within MPA's. To justify more restrictive management decisions, it is essential to collect data and obtain baseline information that will allow to disentangle natural oscillations in ecosystems from human driven unbalances. This thesis focuses on the Avencas Marine Protected Area (AMPA), created in 2016. The intertidal biodiversity within the MPA has been compared with the one from surrounding areas. More than a hundred species of fish, invertebrates and algae have been morphologically and/or genetically identified from November 2019 to July 2020. DNA barcoding has been a key tool in this project, highlighting its high help potential to monitor MPAs and non-indigenous species (NIS) presence. The results showed no differences between species richness and abundances inside and outside the AMPA, suggesting the need of its geographical expansion, more restrictive regulations and/or stronger enforcement measures to reduce the impact of fishing or other recreative activities, and the pressure created by high human presence on this coast. Slightly higher intertidal abundances in the east side of AMPA suggest that there is an interesting community to protect there and that a potential eastward expansion of the protection would be more effective, especially knowing that larger protected areas usually have a higher effectiveness. Complementary protection measures could also include a community observed along the West coast near Cabo Raso. This region, 10km westward from the AMPA, encompasses an exposed rock coastal area with a complex topography. A new larger MPA would increase the effectiveness protecting coastal marine biodiversity in this region.

Introduction

1. Marine ecosystems

Our planet Earth is often referred to as the "Blue Planet". In fact, two third of its surface is covered by vast water bodies, and oceans can even be seen from space. Therefore, marine ecosystems form the largest aquatic system in the world, covering more than 70% of the Earth surface (NOAA, 2019).

Oceans make up for more than 90% of the biosphere so they are home to a wide variety of wildlife. It is estimated that life started to evolve within the ocean around 3 billion years before life on land (Loron *et al.*, 2019). This is one of the explanations for the high degree of species diversity found in marine systems, with more than 200,000 recognized marine species (WoRMS Editorial Board, 2020) and the estimated 91% that remain to be discovered (Mora *et al.*, 2011). Therefore, oceans are among the essential sources of life and diversity that should also be carefully monitored and preserved.

Marine systems are also key elements of the hydrosphere and biosphere as they are involved in carbon, oxygen and nutrient cycling, climate and weather regulation. As an example, the phytoplankton from the seas produces 50 to 70% of the oxygen on Earth through photosynthesis (Witman, 2017), i.e. a lot more than the rainforest which has hitherto been regarded as the most significant "lungs of the Earth".

Moreover, these ecosystems provide diverse services for human populations (Remoundou *et al.*, 2009; Martinetto *et al.*, 2020): provisioning (food security for more than 3 billion people, feed for livestock, raw materials for medicine), regulating (natural defenses against hazards such as coastal erosion and floods), cultural (aesthetic and recreation) and supporting (primary production, oxygen production, etc). Human wellbeing therefore relies on the good health of oceans.

Oceans always have experienced changes and extreme events, but the threats to marine biodiversity drastically increased during these past decades with the increasing uncertainty due to climate change.

2. Current issues and threats

Unfortunately, marine ecosystems face worldwide pressures that are increasing over time (Alder *et al.*, 2006). Some of the most important threats include habitat destruction, overfishing, invasive species, global warming, acidification, toxins and pollution, and massive nutrients runoffs (Jackson, 2009).

One of the most pervasive and transversal threats that marine ecosystems are facing is climate change. On the 25th of September 2019, the Intergovernmental Panel on Climate Change (IPCC) published the "Special Report on the Ocean and Cryosphere in a changing

climate" which highlights the urgency of the situation concerning the current state of the ocean facing global change (IPCC, 2019). In fact, climate change already has heavy consequences on biodiversity, and therefore will affect human communities through different processes. Temperature increase, for example, can impact sea level, coastlines, currents, tides, sea floor condition, weather, and climatic events (Dangendorf *et al.*, 2017; Nerem *et al.*, 2018). Also, as aquatic systems are the most sensitive to warming and acidification, this is likely to have an influence on a broad array of ecological and biological processes: the distribution of some species and the location of high-primary productivity areas, the feeding behaviors and reproductive cycles of some species up to the top of the trophic chain, etc. (Tait and Schiel, 2013).

Human activities also have a high and direct impact on marine systems and are likely to disturb key areas with important biodiversity and ecosystem services potential. A famous study on this topic analyzed the current extent of human impacts on marine ecosystems and showed that no area of the oceans is unaffected by human influence (Selig *et al.*, 2014). The highest level of disturbance is located near coasts and shores where the ocean suffers from the "tragedy of the commons" (Roopnarine, 2013). This concept can be define by " a situation in a shared-resource system where individual users, acting independently according to their own self-interest, behave contrary to the common good of all users by depleting or spoiling the shared resource through their collective action"(Loyld, 1833) and is, in this case, mainly related to overfishing.

Various fishing activities have a negative impact on marine biodiversity, especially since overfishing is becoming more and more common, putting a higher pressure on fish populations. In fact, some fish are caught in high quantities and the populations have no time to replenish, threatening the survival of the species. In addition, the overall decrease of genetic diversity with decreasing population numbers raise concerns on future recovery capability of wild marine resources (Kenchington, 2010). Fishing can also result in disrupted food webs by targeting specific taxa, increasing fish stress, damaging the sea bed with deep-sea trawling, sacrificing indiscriminate species through ghost fishing (when some nets are abandoned in the sea and drift in the ocean) and bycatch (when a non-targeted species is accidentally caught by the fishermen). This pressure on fish populations is likely to decrease their resilience against climate change because diversity is decreasing at individual, population and ecosystem levels (Planque *et al.*, 2010).

Humans are at the basis of diverse sources of pollution, including plastics, that are uniquely anthropogenic. Plastic found in the ocean has its origin in various human activities: ships and vessels (both commercial and recreational), fishing (rope, waste, gears, nets), street litter, dumping, packaging, production waste (Haward, 2018). Fishing debris represent more than two-thirds of large plastic debris found in the ocean (Eriksen et al., 2014). Another important source of pollution is runoff contamination. Oil is one of its major components, followed by fertilizers and pesticides, metals and other materials from vehicles or construction sites, soaps, accidental spills, excess nutrients like nitrates or phosphates creating eutrophication among others (Fredston-Hermann *et al.*, 2016). It is evident that these chemicals and substances can change the chemistry of the water and impact the physiology of the

organisms living in the sea (Zeng, Chen and Zhuang, 2015; Kirchner and Kleemola-Juntunen, 2018).

Lastly, marine ecosystems all around the world are suffering from the introduction of non-indigenous species (NIS) that can take over or displace the native ones and disturb their natural habitat. Human activities can play an important role in the spread of harmful species, especially through commercial and recreational shipping (Ferrario *et al.*, 2017). This transport between countries and continents usually occurs in the ballast water or as foulers on vessel hulls (Olenin *et al.*, 2016). Marinas are hot spots for the arrival and settling of exotic species. With the development of exchanges between countries and continents these past decades, the number of invasive species increased in a steady rate, as shown on figure 1 (De Poorter, Darby and MacKay, 2008).

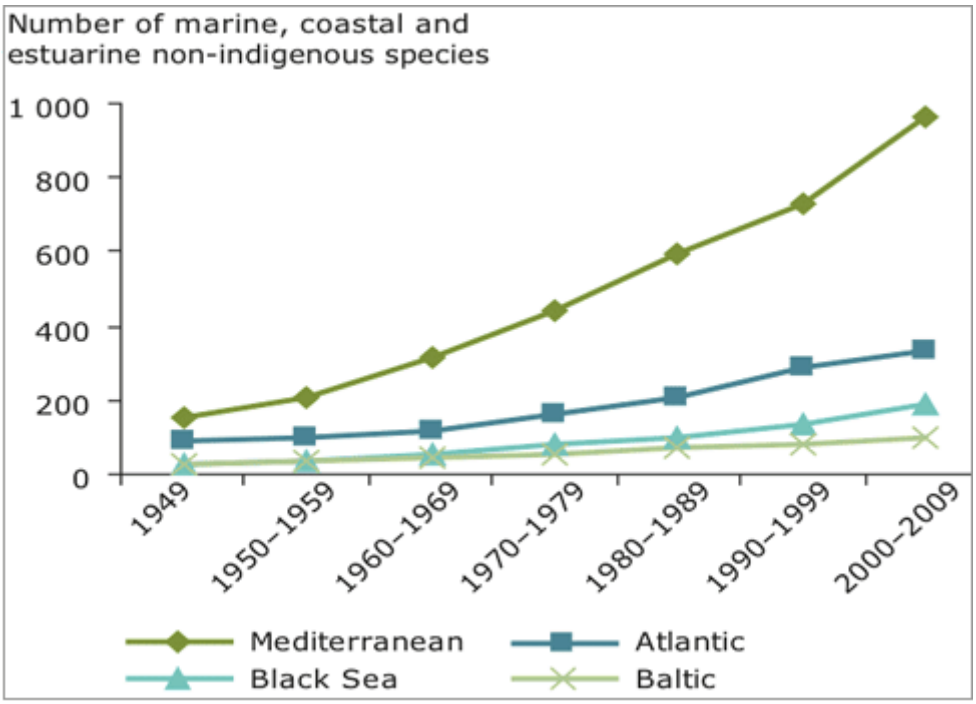


Figure 1. IUCN graph showing the evolution of the number of invasive species over time in different seas and oceans.

Until 2018, 166 NIS have been recorded on Portuguese coasts, including mainland but also Azores and Madeira archipelagos (WGITMO, 2018), and the International Convention for the Control and Management of Ships' Ballast Water and Sediments has been implemented only on the 19th of January 2018 in this country. Non-indigenous species are therefore still representing a risk for Portuguese marine ecosystems.

Since around 40% of the world's human population lives within a radius of 100km from the coasts (Small and Nicholls, 2003), and that the global population will continue to increase by more than 9 billion people by 2050 (UN, 2017), the pressure on coastal and marine ecosystems is steadily rising. It is widely recognized that maintaining the good health of natural ecosystems, especially water bodies, is a key issue of our era. The scientific community is

looking for solutions, and one of them is the establishment of Marine Protected Areas (MPAs) to shelter some species and protect important habitats.

3. Marine Protected Areas (MPAs)

As the ocean and its ecosystems are facing considerable and irreversible changes, it seems necessary to preserve its resilience, its biodiversity, and its capacity to provide services. One strategy is the implementation of Marine Protected Areas (MPAs) that could attenuate the effect of climate change, reduce or even reverse the negative anthropogenic impacts, and potentially give the ecosystems the opportunity to adapt to these changes (Lubchenco *et al.*, 2003). MPAs can be defined as "areas of sea especially dedicated to the protection and maintenance of biological diversity and of natural and associated cultural resources, managed by authorities"(IMPANA, 2019). MPAs of the world differ in many ways, including the objectives of their implementation, the ecological and human contexts in which they are located, the engagement of stakeholders in their protection, their enforcement, the strictness of their protection measures and their management (Pendleton *et al.*, 2018).

These natural reserves have the ability to help against many current issues as habitat destruction (which affects entire ecosystems by altering species richness, abundance, distribution, genetic variation and inter-population dynamics), invasive species and overfishing, by monitoring these areas and regulating all activities inside (Ardura *et al.*, 2016).

MPAs can also be efficient through climate change mitigation (Mccauley *et al.*, 2017). They might not provide resistance to global warming, but they can help to increase the resilience (Figure 2). For example, against acidification, which is mostly due to absorption of CO_2 from anthropic sources, protected areas can promote the growth of algae that capture a percentage of the atmospheric carbon, slowing down this process.

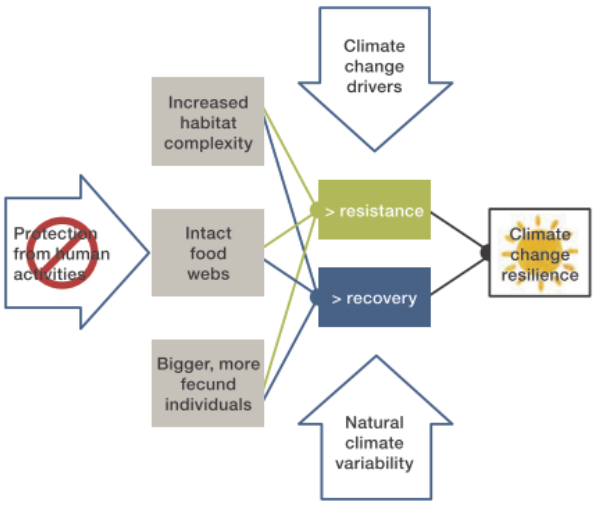


Figure 2. Mechanisms through which MPAs can increase ecosystem resilience face to climate change, source: (Baxter, 2016)

Furthermore, MPAs can provide useful protection against sea level rise and extreme weather events, especially with intact reefs or mudflats and their dense vegetation (Baxter, 2016). Protecting specific areas can also help to increase reproduction rates and genetic diversity by offering refuge to different species. This could attenuate the negative influence of global warming on marine biodiversity.

Some areas are particularly interesting to choose when creating marine protected areas because they contain unique habitats, key species to protect, ecosystems and communities with high scientific interest: as an example, coral reefs, mangroves, or rocky shores.

4. On the effectiveness of Marine Protected Areas

Since a few years, Marine Protected Areas are a hot topic in marine ecology. In the 70s, the concept of MPA developed rapidly and most of them have been created during the last two decades of the 20th Century or after (Humphreys and Clark, 2020). This gave rise to many debates and questions.

One of the main questions remains about the effectiveness of these MPAs. In fact, we have many evidence of the limitation of MPAs against all threats, one famous example being massive coral bleaching events in some iconic MPAs as the Great Barrier Reef Marine Park in Australia (Pendleton *et al.*, 2018). External factors can still have an impact on the inside of MPAs, especially concerning climate change, but to maximize their effective protection, attention should be placed on their design and management.

Edgar *et al.*, (2014) explained five key factors that influence the effectiveness of MPAs, especially on rocky reefs. The five factors have been put into the acronym NEOLI: No-take, Enforced, Old (10 years or more), Large (100 km^2 or more) and Isolated by sand or deep water.

The size of the MPA is of great importance. Large and enforced MPAs have proved their efficiency over time, but smaller ones did not always show the same results (Turnbull *et al.*, 2018). Turnbull *et al.* (2018) found that the power of small MPAs depends on many factors: they can have a real impact on biomass and diversity if they have a full no-take protection, if they consist of sheltered areas with complex habitats and if the community around is involved in its protection and surveillance. However, even if an MPA is more likely to reach its goals if it is large enough, a smaller one can be well connected to other MPAs to form a large network where individuals can move easily, therefore increasing its efficiency. Novaczek *et al.* (2017) counted over 5,000 marine protected areas around the world, but most of them of median size of ~2 km^2 and, in general, isolated.

Adequate management is also one of the foundations of efficient MPAs. However, this is where many problems arise when talking about the effectiveness of MPAs. In September 2019, the World Wildlife Fund (WWF) and Sky Ocean Rescue released a report called "Protecting our Ocean: Europe's challenges to meet 2020 deadlines". In this report, they point at the lack of adequate marine protection and therefore the poor state of European seas. According to them, 19 of 23 marine EU Member States are not developing effective

management plans for their MPAs (WWF, 2019). Portugal is among those 19 countries not coping with effective marine protection. However, the international agreements state that 10% of the ocean should be effectively protected before 2020 (Aichi, 2012). This report also shows that only 1.8% of the EU marine area is covered by MPAs having actual management plans, while 12.4% are yet designated for protection. Those areas that have not yet received the intended protection are called "Paper Parks".

Strongly protected areas can increase fish biomass and diversity (Lester and Halpern, 2008, Edgar et al., 2014; Gill et al., 2017), promote the dispersal of larvae (Harrison et al., 2012) and adults of target and non-target species to areas outside their borders, potentially benefiting both fisheries and biodiversity outside the MPA (Di Lorenzo *et al*., 2016).

The subsequent focus on an effective monitoring and management plan for all MPAs is crucial so that they can fulfill their objectives.

5. DNA barcoding as an efficient tool for MPA management and tracking non indigenous species (NIS)

In addition, as an innovative tool for MPA management, this thesis is treating uses DNA barcoding as an important tool for early detection of invasive species. The term of barcoding can be defined as the use of "a standardized DNA region as a tag for rapid and accurate species identification" (Valentini, Pompanon and Taberlet, 2009). This standardized DNA region is a part of the genome evolving quickly enough for assessing recent speciation events, subsequently allowing the distinction between closely related species.

DNA barcoding became more and more popular since 2003 and is now being developed through an international initiative (Valentini, Pompanon and Taberlet, 2009). Automatic next-generation DNA sequencers made this technique more available, not only to geneticists but to scientists from different fields of study, including ecology and conservation (Borges *et al.*, 2016). Much emphasis is currently placed on the necessity to build complete databases with a maximum of sequences, in order to make this tool even more accurate and usable. In fact, DNA barcoding will be more efficient when more scientists will use it because more species will have their barcodes sequenced and referenced.

DNA barcoding was initially used in this project to identify cryptic species to complete the species list and to distinguish potential invasive species. This lab work was tightly related to a side project of the research team aiming to develop DNA barcoding as an efficient tool for MPA management tracking NIS by sequencing DNA fragments from species that are not yet represented in public databases.

6. Portugal and the Avencas case: a micro Marine Protected Area

In 2016, a new MPA has been implemented in the Atlantic western coast of Portugal, the municipal Avencas Marine Protected Area (AMPA). It is therefore the youngest of the Portuguese marine protected areas. It is located 30 kilometers west of Lisbon (Figure 3),

stretches from the beach of São Pedro do Estoril to Parede and covers an area of 0.59 km² (Pereira, 2017). It is the first MPA in Portugal to be managed locally (by the municipality of Cascais) and not by the central Portuguese government.

Figure 3. Map of Portugal and location of the study area (source: World Atlas & Google Maps), picture of the intertidal rocky platform in the Avencas beach (source: www.mpas-portugal.org)

The AMPA is characterized by large intertidal rocky platforms which are unusually large for the Portuguese coast. This kind of habitat has been shown to play a role in the breeding cycles of different coastal fish species (Dias *et al.*, 2016), and also hosts diverse algal and invertebrates communities due to the higher abiotic stress created by the tides and waves (Scrosati *et al.*, 2011). However, it is also under the effect of several environmental pressures as climate variability and human-driven alteration of the habitat (Barceló *et al.*, 2016), and therefore needs a particular attention aiming its preservation. This coastal area between Lisbon and Cascais is more sheltered from the currents and strong waves coming from the northwest Atlantic, allowing the fixation of macroalgae and sessile invertebrates that create a typical ecosystem with dense and diversified communities.

Overall, the Portuguese coast has an interesting localization on the confluence between temperate and subtropical regions, with a clear Mediterranean influence, an ideal set-up to study the primary effects of global warming on the distribution and abundance of marine fauna and flora, using long-term monitoring.

As stated above, coastal ecosystems are among the most threatened habitats globally due to high density of human settlements, coastal development, pollution, fisheries, and tourism. AMPA is located within an urban environment with a big road axis passing nearby, a few intermittent uncontrolled sewers, many human facilities located close to the beach and numerous activities taking place on these coastal areas. These anthropogenic remedies and activities are likely to have impacts on the marine ecosystem, even inside the Protected Area. However, at the same time, the proximity of AMPA to urban area makes it an excellent platform for outreach activities such as raising environmental awareness and teaching coastal ecology.

A brief translation of the official law text concerning regulation of activities inside the AMPA can be found in Appendix A. Below, Figure 4 is the sign present of the webpage about AMPA on the Portuguese MPAs' website, summing the allowed, regulated and prohibited activities inside the protected area.

Figure 4. Indications concerning activities in AMPA from the website www.mpas-portugal.org. In the 1st column, scuba diving, cane fishing and artisanal nets fishing are regulated activities. In the 2nd column, anchoring vessels and fishing with trawl gear or gillnets are prohibited, spearfishing is regulated.

Although the Avencas represents a small-sized MPA with limited potential regarding the protection of mobile species, this could be expanded in the future under a new legal framework, which allows municipalities to manage local MPAs. However, local entities must justify the creation of new MPAs and need to monitor and manage them once they are established.

Furthermore, upon the creation of an MPA, it is essential to assess whether the objectives have been reached. This can be analyzed based on biological data obtained before and after the MPA implementation (Halpern, 2003; Horta E Costa *et al.*, 2014). However, when data from before the MPA implementation are not available, the comparison between the inside of the protected area and the outside is the only available option (Westera, Lavery and Hyndes, 2003). Natural oscillations should also be considered as they could be the reason for significant differences between before/after implementation or site/control and therefore give false positives.

7. Objectives

This work aims to:

(i) compare biodiversity inside the AMPA with the surrounding areas,

(ii) in these adjacent areas, evaluate the potential to expand the AMPA beyond its current extension

(iii) assess the differences between the South-facing coast and the West-facing coast in terms of biodiversity and communities, to evaluate if a future MPA would significantly increase the marine organisms under regulated protection.

This thesis was part of a larger project of the team from MARE, but it was also its beginning. Therefore, I have been fully involved in all the steps: the field work (sampling and identification), the lab work and genetic identifications, the data treatment and statistical analysis. The only task in which I did not really take part was the morphological identification under the stereoscope.

1) Study area and period

1.1.Location

This study has been performed on the Portuguese coast, west of Lisbon, along Cascais intertidal areas. It comprises 5 sampling areas (see Figure 5): two inside the Avencas Marine Protected Area (AMPA), two flanking the AMPA and another one further on the West coast.

Only the intertidal zone has been sampled within the framework of this thesis. In each sampling area, there are 3 sampling points: two in the midlittoral zone (M1/M2) and one in the supralittoral zone (S1). The midlittoral zone extends from the spring high tide line to the spring low tide line. The supralittoral zone is the area above the spring high tide line, it is often splashed but not submerged during high tide.

The GPS coordinates and the directions for the transects have been registered so that the survey can be replicated over time. This information can be found in Appendix B.

Figure 5. Map of the sampling points in the Cascais Area

1.2.Time period and sampling days

Field data was collected from November 2019 to July 2020, with an interruption from the middle of March to May due to the Covid19 pandemic episode. My personal collaboration in field sampling started in February 2020. The sampling was made following fortnight cycles of full and new moon defined at Instituto Hidrográfico site (https://www.hidrografico.pt/). In total, eight cycles were completed and are represented in this study. Sampling sites are located in the intertidal zone, so tides' time was a key factor to choose field days and time. Dates and hours of the lowest day tides were found on the website of Hydrografico Marinha Portugal and a schedule was made with the appropriate dates. The maximum tidal height accepted for field sampling was 0.6 meters. Based on these criteria, there were from 6 to 8 suitable days per month to sample, with a starting time between 7am and 10am (Appendix C).

2) Sampling methods

2.1.Sampling point information

At each sampling station, two fixed transects of 25 meters were carried out on the midlittoral level (M1 and M2) and one on the supralittoral level (S1). When possible, red marks have been placed on the rocks to show the start and end points of the transect. The transects followed an orientation parallel to the coastline (Figure 6), with the directions mentioned in the Appendix B.

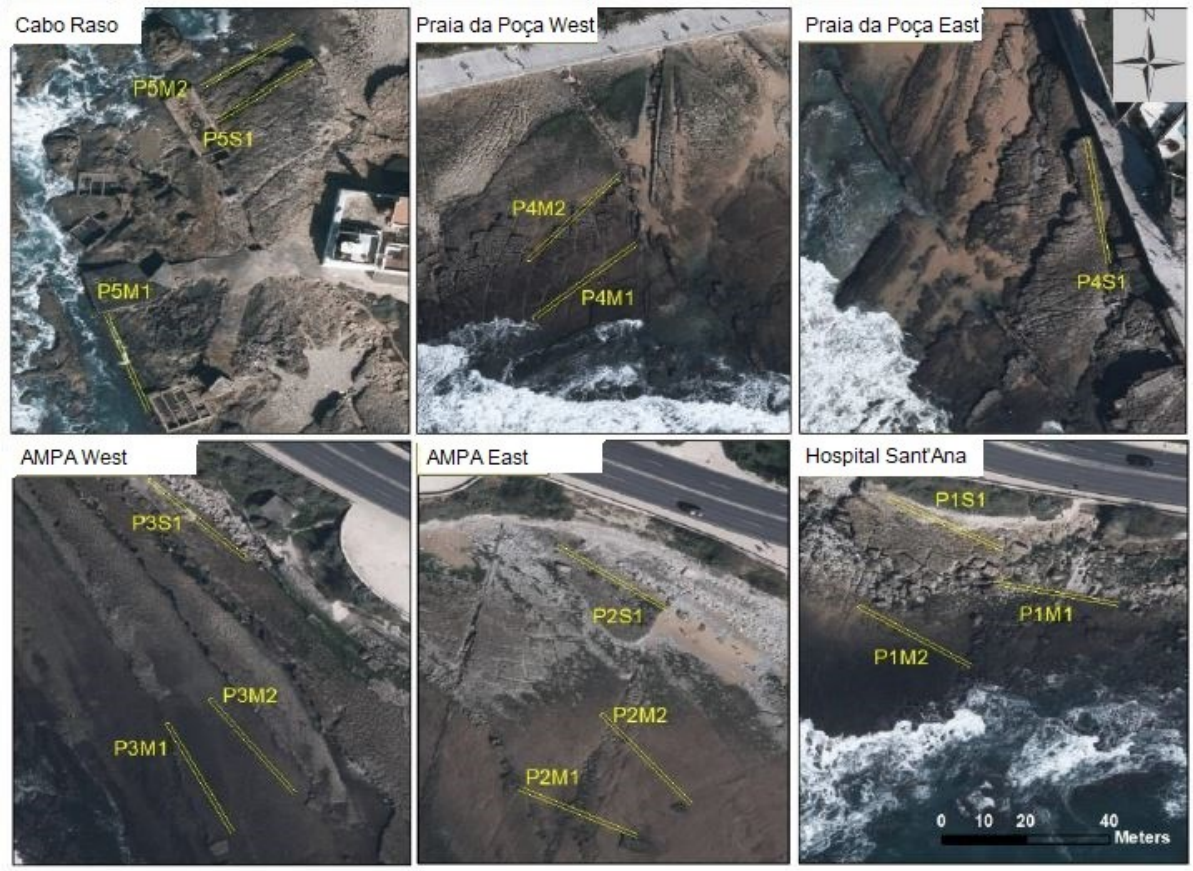


Figure 6. Aerial photos of the monitoring sites in the intertidal zone of the Cascais coast. The dimensions and location of the transects carried out associated with each sampling point shown in Appendix B are represented with yellow lines (Pais *et al.*, 2020)

For each point, some information were systematically noted: date, start and end time, total time, weather, sea conditions (with a simplified Beaufort scale), air temperature (in °C), part of the transect submerged (in meters), number of tide pools inside the strip transect which was defined as a 2x25 m corridor.

Fishermen around the sampling area were also counted and classified according to the following categories:

Collection of polychaetes - the number of people who were collecting "sea worm" with bait in pedestrian fishing;
Octopus capture - the number of people who were catching octopuses, but also those who were catching *Necora puber;*
Collection of Gastropods / Bivalves and Cirripeds - the number of people who were collecting *Steromphala* sp. and *Phorcus* sp, (located mostly on the South Coast, including within the AMPA), *Mytilus* sp. (more focus on the South and West Coast) and barnacles as *Pollicipes pollicipes* (mostly in the area of Cabo Raso);
Fishing vessels - the number of fishing vessels operating close to the place where we were sampling and within or near the area defined as MPA;

Clean fishing - the number of fishermen fishing with rod that were in the vicinity of the sampling site;
Spearfishing - the number of individuals who were seen entering the water with spearfishing equipment and or their signal buoys in the water, in the area surrounding the sampling.

These data concerning fishing activities were not used in the present analysis but stored for further studies.

2.2.Transects sampling procedure

Sampling methodologies were adapted to the different categories of species. Non-destructive sampling methods were used, causing minimal impact to the study sites, and only involving removal of organisms in case of absolute necessity (e.g. morphological identification or genetic analysis in the laboratory).

Transects were used to identify cnidarians, echinoderms, mobile macro-organisms (e.g. decapod crustaceans, fish) as well as other organisms with an aggregate distribution (e.g. Sabellaria sp. formations) which could hardly be identified using other sampling methods.

The abundances were calculated as the mean number of individuals per square meter, with the formula $\frac{N}{m^2} = \frac{n}{25\times2}$, with n being the number of counted individuals along a 25 meters transect. However, this methodology has some limitations. Some cnidarians (e.g. Actinia sp.) and echinoderms (e.g. *Paracentrotus lividus*) easily reach a count on the scale of hundreds of individuals per transect. In these cases, a maximum limit of 100 specimens per transect is fixed and the point at which this number is reached is noted in the strip transect (e.g. while sampling *Actinia equina,* if 100 individuals were counted at 5m, this would be translated into an abundance of 10 individuals per square meter.

During the transects surveys, information is also collected regarding the litter found throughout the sampling. Each item is photographed on a millimeter paper and categorized in one of the following categories: metal, plastic, glass and others. These data were not used in the present analysis but stored for further studies.

2.3.Quadrats sampling procedure

To quantify the abundance of smaller invertebrates' species and the percentage of macroalgae coverage, 0.5x0.5m (0.25m^2) quadrats have been used. These quadrats were made along each sampling transect, positioned at 5 and 20 meters (respectively to the left and right of the transect). Each of these 0.5x0.5m quadrats was subdivided into 25 identical squares. The workflow included a picture of the whole quadrat. Then, the number of submerged subquadrats was counted and the maximum height of the algae cover (canopy) was also measured in three fixed points along a diagonal, as shown in Figure 7.

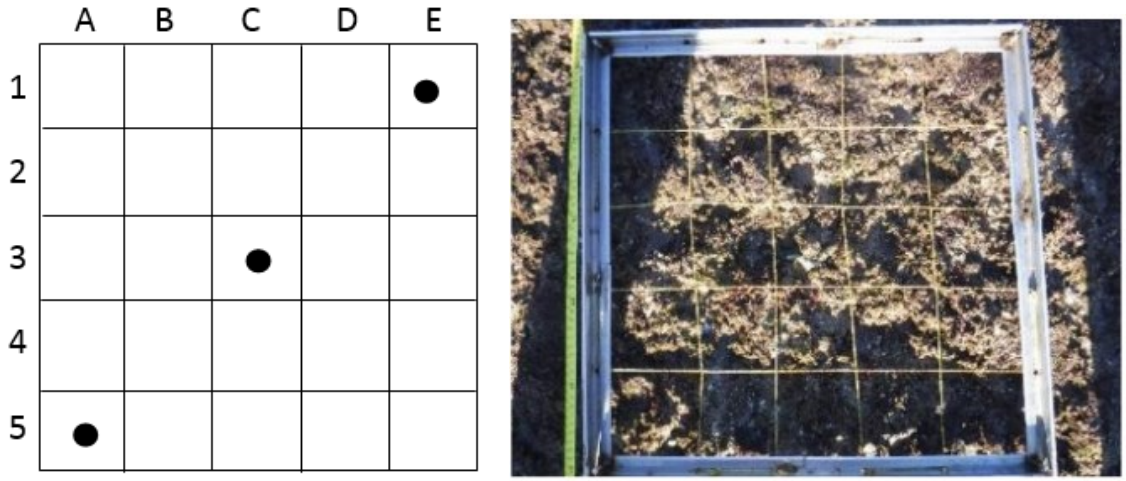


Figure 7. Scheme and picture of the grid used for quadrat sampling, with dots showing the grids where canopy was measured.

Invertebrates species were counted and the number of grids in which they were found was recorded. Then, macroalgae were identified and, as individuals were uncountable, the number of grids in which each species could be found was recorded in order to know the percentage of cover for each algae species. When the number of invertebrates was also uncountable (e.g. *Cirripedia*) the procedure adopted to estimate their abundance was the same used for macroalgae.

The abundance measure for the quadrats was the percentage cover, obtained by calculating $\frac{n}{25} \times 100$ with n being the number of grids where the species is present.

3) Species morphological identifications

3.1.Identification on the field

Prior to the field sampling, we prepared some species guides for the ones we were more likely to encounter. These guides recorded some specific characteristics to easily differentiate some species and were improved with experience along the fieldwork. A few examples can be found in Appendix D.

All the species that could be found at the sampling points were recorded. The field work team was usually composed of three elements and was led by a research assistant who was experienced with the species identification. Sometimes, some specialists of different taxa came to help the team or were available to answer some questions concerning identification (e.g. mollusks' taxonomists).

Most of the species could be identified directly on the field. However, when identification was not possible on the field (new species, cryptic species, too small individuals, etc.), pictures were taken and a sample was collected for identification in the laboratory, using

morphological keys and / or DNA sequencing with barcoding techniques. Either the whole individual or, concerning algae, partial individual samples, were conserved either in ethanol or in water and immediately labelled.

3.2.Identification in the lab

Several techniques have been used to identify the collected specimens. The first one was an identification based on pictures taken during field work, compared with specific or general literature (e.g. Hayward and Ryland, 1990). Small specimens (invertebrates or pieces of algae) were observed under a stereoscope and identified with the help of identification keys (e.g. Hayward and Ryland, 1990; Rodriguez-Prieto *et al.*, 2013). When our team was not able to identify the samples expert taxonomists that were available for some groups (e.g. mollusks) were consulted.

Whenever a taxonomic identification to the species level was not possible, the family or genus level were annotated in the species list.

When the approaches described above were not effective DNA barcoding was used as an alternative.

4) DNA barcoding: Genetic identification and NIS confirmation.

As mentioned above, some species required more precise identification. In fact, phenotypes were sometimes too similar to be distinguished either directly during field work or with a stereoscope, especially in the case of smaller individuals. Specific DNA fragments from one or several genes were sequenced and compared with available online databases (https://www.ncbi.nlm.nih.gov/genbank/). The genetic analysis work was performed in July and August 2020.

A parallel goal of these analyses was to contribute with new DNA sequences of species that are still absent from public databases, namely native species (e.g. *Irus irus*) and non-indigenous species (e.g. *Fulvia fragilis*, which is not present in Portugal yet, but was already reported in the south of Spain from where we received two specimens).

4.1.Samples preparation

In total, we had 28 samples, covering 9 genera: *Fulvia, Gibbula, Watersipora, Ocenebra, Mytilaster, Tritia, Irus, Musculus* and *Cardyta* (Table 1).

#	Samples	Reference	#	Samples	Reference
1	*Fulvia fragilis*	Spain	15	*Mytillaster minimus*	P3M120191127B001
2	*Fulvia fragilis*	Spain	16	*Mytillaster minimus*	P2M120191227B001
3	*Irus irus*	P4M120200210	17	*Ocenebra edwardsi*	P3M220191127B002
4	*Watersipora (white)*	P4M120200522T001	18	*Ocenebra edwardsi*	P2M120200112C002A
5	*Watersipora subtorquata*	P4M120200522T002	19	*Ocenebra edwardsi*	P2M120200112C002B
6	*Musculus costulatus*	P2M220191227C001	20	*Ocenebra edwardsi*	P1M220200225C002
7	*Cardyta calyculata*	P2M220200622T001	21	*Tritia incrassata*	20191126P2M1B001B
8	*Gibbula umbilicalis*	20191126P2M1B001A	22	*Tritia incrassata*	20191126P2M1B001C
9	*Gibbula pennanti*	P4S120200114B001	23	*Tritia incrassata*	20191126P2M1B001D
10	*Gibbula cineraria*	P3M120200125B001	24	*Tritia sp. (juvenile)*	20191126P2M1B001G
11	*Gibbula pennanti*	P2M120200309C001	25	*Tritia reticulata*	P2M120200112C003
12	*Gibbula cineraria*	P3M220200126T002	26	*Tritia incrassata*	P4M120200114B001
13	*Gibbula varia*	P3M220200126T004	27	*Tritia incrassata*	P2M120200309B002
14	*Gibbula varia*	P2M120200309T001	28	*Tritia incrassata*	P4S1202003128001

Table 1. Samples used for DNA purification and amplification, with their respective field work reference and label.

Alternative DNA fragments were searched in the literature. The 658bp fragment from the 5' end of mitochondrial cytochrome oxidase subunit 1 (COI) gene, also called *cox1*, is the most used barcode across taxa (Bucklin, Steinke and Blanco-Bercial, 2011), so we used this one for all our individuals. Its mutation rate is often fast enough to distinguish closely related species and its sequence is conserved among conspecifics. Divergence between COI sequences between closely related animal species is usually more than 2%, which makes this DNA fragment useful for genetic identification. In addition, COI results were confirmed with the mitochondrial 16S rRNA fragments to check for congruences.

4.2.DNA extraction and amplification

Total genomic DNA was extracted from each unidentified sample with the REDExtract-N-Amp Kit (Sigma-Aldrich) following the manufacturer's instructions. DNA fragments of interest were then amplified by PCR using the appropriate primers (Folmer *et al.*, 1994; Simon *et al.*, 1994) for each group of species/samples and appropriate temperature profiles (Table 2).

Primers	Forward	Reverse	Temperature Profile	Reference
COI	**LCOI490** (GGTCAACAAATCATAAAGATATTGG)	**HCO2198** (TAAACTTCAGGGTGACCAAAAAATCA)	2min 94°C + (60sec 94°C, 60sec 52°C, 60sec 72°C) x 35+ 3min 72°C	Folmer *et al.* ,1994
16S	**16Sar** (CGCCTGTTTATCAAAAACAT)	**16Sbr** (CCGGTCTGAACTCAGATCACGT)	2min 94°C + (60sec 94°C, 60sec 52°C, 60sec 72°C) x 35, 3min 72°C	Simon *et al.* 1994

Table 2. Table showing the detailed information of the primers used, source: (Folmer *et al.*, 1994; Simon *et al.*, 1994)

The quality of the products from the different PCRs was checked using a 1,5% agarose gel electrophoresis. A second amplification trial was necessary for some samples. In the end, only the samples containing well-amplified DNA fragments were chosen for sequencing.

5) Data processing and analysis

5.1.Database

All field data and samples' characteristics were recorded on a notebook before being entered in an Excel database. The database and species list were updated along the project, especially during the genetic analyses, and was fully completed at the end of July.

5.2.DNA barcoding: bioinformatic analysis

DNA sequences were edited with Codon Code Aligner software (Codon Code Corporation): unclear ends and chromatograms with low quality were discarded. A BLAST (Basic Local Alignment Search Tool) was performed for each DNA sequence. This software finds regions of similarity between the uploaded sequences and all the sequences available in online databases and calculates the statistical similarity. A special attention has been put on the values of "Query cover" and "Percentage Identity". Percentage identity is the percentage of residues that match up in the alignment, while the query cover is the percentage of the query sequence length that is included in the alignment (Newell *et al.*, 2013). In the end, the first match was recorded for each BLAST search.

A complementary analysis was also performed using the MEGA-X software in order to build bootstrap trees and check the position of our individuals among other individuals of the same genus already sequenced and present in the database.

5.3.Preliminary analysis of the field data

Data from transects and quadrats were separated for the statistical analyses, as their abundance units were different ("Number of individuals per m²" for the transects and "Percentage of cover" for the quadrats).

First, a species accumulation curve has been drawn for each of the two datasets in order to verify if the sampling effort was sufficient to have a good overview of the total biodiversity (Ugland, Gray and Ellingsen, 2003). In fact, it is a good way to assess the sampling effort because it shows the point at which additional sampling would lead to a very low rate of discovery of any new species (the asymptote of the curve).

Then, diversity indices (Species richness, Shannon index and Simpson index) have been calculated using PRIMER6 software. The species richness is the number of species present in the sample. Shannon index was calculated using the formula $H' = -\sum_{i=1}^{R} p_i \, ln \, pi$, with pi being the proportion of individuals belonging to the ith species. The formula used for Simpson

index was $\lambda = 1 - \sum_{i=1}^{R} p_i^2$, which assumes sampling without replacement because every individual counted was removed out of the quadrat, or ignored after being counted in the case of sessile animals or algae.

Shannon index gives more weight to rare species and is therefore more sensitive to slight changes in local biodiversity, while Simpson index gives more weight to common species, showing trends in local biodiversity. Both have been chosen in order to evaluate if differences could be observed in the data collected during field work (Pomeroy, Parks and Watson, 2004; Gotelli and Chao, 2013).

5.4.Statistical analysis

Both univariate and multivariate analyses have been performed on R software (version 4.0.2) using the *vegan* package. The Excel database has been appropriately rearranged to be used with the software (a matrix with Samples as rows and Species as columns).

For community datasets, effects of protection ("Yes" = protected, "No" = not protected), sampling sites ("Hospital", "AMPA WEST", "AMPA EAST", "Praia da Poça", "Cabo Raso") and orientation ("South" and "West") on species richness, Shannon and Simpson diversity indices were tested.

First of all, normal distribution and homogeneity of variances were tested for the 2 datasets, with Shapiro-Wilk test and Bartlett's test respectively (Dytham, 2011; Gardener, 2017). The data had a skewed distribution with a long tail as there are only a few abundant species, and many species with lower abundance, so all the following tests are not parametric and do not require a normal distribution. Differences in response variables based on the cited factors were tested using Wilcoxon rank-sum test and Kruskal-Wallis test. The significance threshold has been set to 0.05, meaning a confidence threshold of 95%. A post-hoc Dunn test with Bonferroni correction has been performed for all significant Kruskal-Wallis tests.

Secondly, the same statistical procedure has been applied to both transects and quadrats datasets. A square-root transformation has first been applied to species abundances, to uniformize the contributions of both rare and common species (Gardener, 2017). Prior to the testing, a Bray-Curtis similarity matrix was built for each dataset, with the addition of a dummy variable to counteract the problems created by empty sites that were relatively numerous for the supralittoral zone (Clarke, Somerfield and Chapman, 2006). This dummy variable had the value 1 for all the samples. Bray-Curtis quantify the similarity between all pairs of samples and is one of the most robust measure of beta diversity (Schroeder and Jenkins, 2018).

A Non-metric Multi-dimensional Scaling (NMDS) has first been performed to get a two-dimensions visualization of the distribution of the data and the potential resemblance patterns. NMDS analysis condenses multidimensional data (multiple species and factors) into a 2D graph where it is easy to interpret the distance between points as the dissimilarities between samples (Clarke, 1993).

To test the strength of the influence of each factor (Protection status, Area of sampling, Orientation) and to complement the NMDS graphs, an analysis of similarities (ANOSIM) has been performed. This type of analysis tests whether we can reject the null hypothesis that the similarity between groups is greater than or equal to the similarity within the groups. The ANOSIM test statistic (R value) is a comparative measure of the degree of separation between groups: An R value close to "1.0" suggests dissimilarity between groups while an R value close to "0" suggests an even distribution of high and low ranks within and between groups (Clarke and Warwick, 2001).

In addition, a permutational multivariate analysis of variance (PERMANOVA) has been done, along with a PERMDISP analysis and PcoA plots. PERMANOVA aims to test the null hypothesis that the centroids and dispersion of the groups are equivalent for all groups.

When significant differences have been observed with Mann-Whitney U-test or Kruskal-Wallis, a SIMPER analysis was performed. This test identifies the species that are most responsible for the observed patterns by disaggregating the Bray-Curtis similarities between samples. The more abundant a species is within a group, the more it contributes to the intra-group similarity, while a species with a consistently high contribution to the dissimilarity between groups is a good discriminating species (Clarke and Warwick, 2001).

Results

1. Metadata

This study is unique due to its large array of sampled species and taxa. During the project, 98 transects and 192 quadrats have been sampled, 138 species with different life history traits and inhabiting different reef zones have been recorded. While most of the studies on MPAs effectiveness focus on fish assemblages (Sciberras et al., 2013), this project highlights the importance of invertebrates and algae in the ecosystem's response to MPA implementation. A complete species list is available in Appendix E.

Some species, known to be present in the area (data from 2016 sampling), did not appear during this period but are listed in Appendix F.

The sampling effort was sufficient to have an accurate overview of the biodiversity present in the area, as shown on the species accumulation curves below, where the asymptotes are reached.

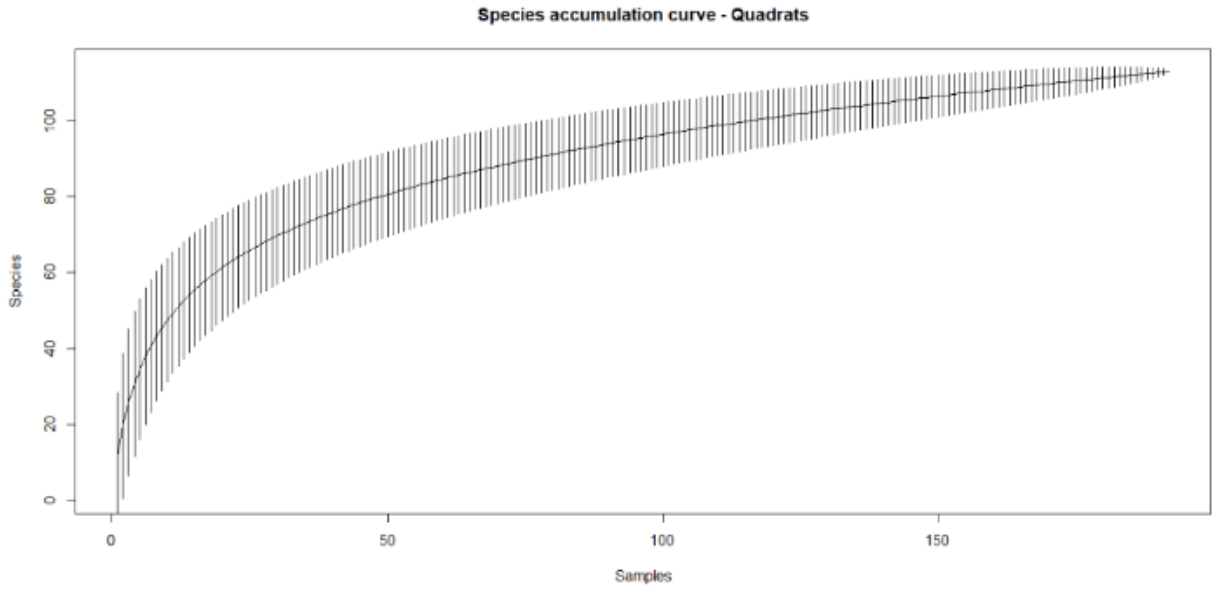


Figure 8. Number of species sampled as a function of the number of quadrats performed.

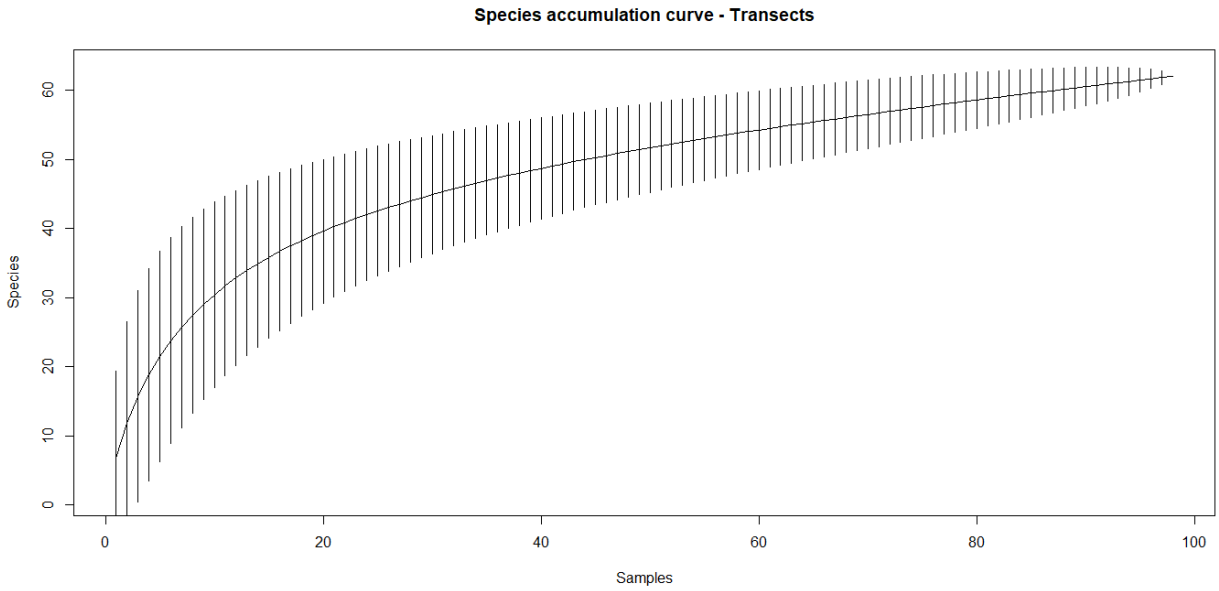


Figure 9. Number of species sampled as a function of the number of transects performed.

Despite some incomplete sampling in some cases, the number of transects and the number of quadrats for most of the points were in a close range: between 18 and 24 for transects and between 39 and 50 for quadrats, allowing comparison between them. However, due to its high expose to wave action, Cabo Raso comprises only 15 transects and 30 squares.

2.Protection status effect: overall comparison of the Avencas Marine Protected Area and unprotected adjacent areas.

2.1.Comparison of diversity indices.

The comparison of species richness, Shannon and Simpson's diversity indices between protected and unprotected areas adjacent to the AMPA did not show any significant difference, both for transects and quadrats (Table 3, all Mann-Whitney U-tests' p values > 0,05). Shannon index and Simpson index showed the same outcome, even considering their different sensibilities.

Mann-Whitney U-test p values	**Protected vs. Unprotected**	
	Transects	Quadrats
Species richness	0.928	0.774
Shannon index	0.465	0.465
Simpson index	0.286	0.346

Table 3. Results of the Mann-Whitney U-tests comparing diversity indices between protected and unprotected areas in the same region.

2.2.Distance-based analyses

The NMDS analyses presented in Figure 10 show a valid two-dimensional representation (stress level below the threshold of 0.2) of the sampling sites with different protection status (Clarke and Warwick, 2001).

Red (*unprotected*) and green (*protected*) sites are mixed in a cloud without any distinct separation or pattern.

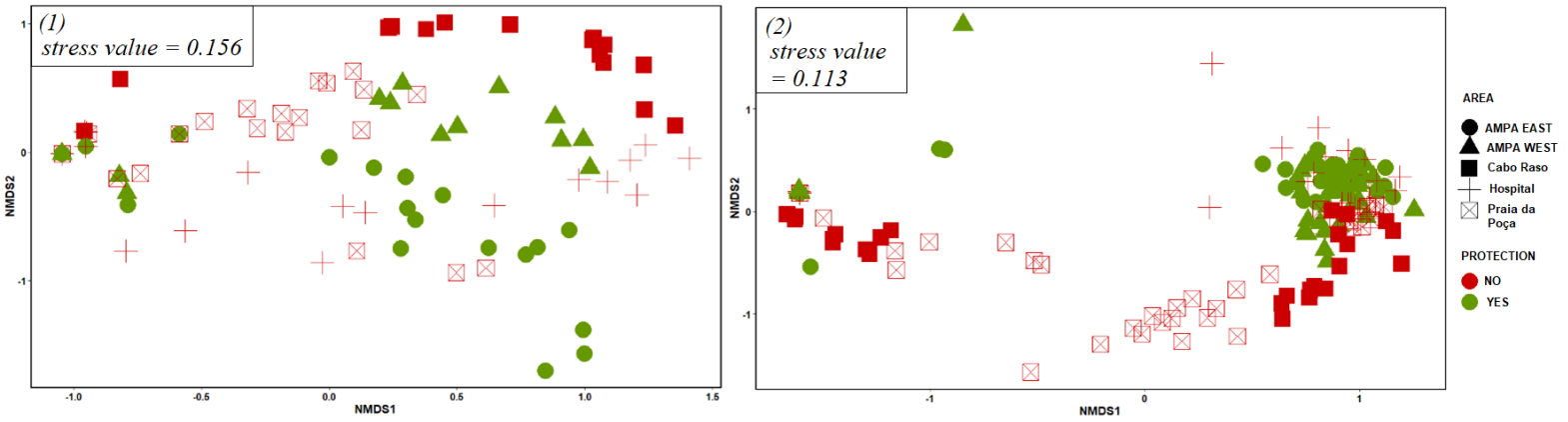


Figure 10. NMDS plots showing the distribution of the (1) transects and (2) quadrats (shape shows the different sampling sites, and color shows the protection status)

In fact, the ANOSIM R value for the factor "Protection" is equal to 0.044 (*P*=0.024) for the transects and 0.054 (*P*=0.002) for the quadrats, which suggests a very low dissimilarity between the "Protected" and "Unprotected" sampling sites. The PERMANOVA test is showing the same tendency with: the R2 value equal to 0.033 (*P*=0.018) for the transects and 0.039 (*P*=0.001) for the quadrats, meaning that around 3.3% of the variation between groups is explained by the "Protection" factor. The interactions between the factors "Protection" and "Orientation", "Protection" and "Area", "Protection" and "Orientation" and "Area" do not have more influence. All these results can be found in Table 4.

1				2			
Factors	R2 value	F value	Significance	Factors	R2 value	F value	Significance
Protection	0.033	3.232	0.018	Protection	0.039	7.821	0.001***
ProXOrient	0.088	9.453	0.001**	ProXOrient	0.024	4.861	0.004**
ProXArea	0.139	5.224	0.001***	ProXArea	0.073	5.168	0.001***
ProXOrientXArea	0.052	2.919	0.007**	ProXOrientXArea	0.049	5.214	0.001***

Table 4. Results of the PERMANOVA tests for (1) the transects and (2) the quadrats, "protection" factor.

Concerning the PERMDISP tests and PCOA plots, they show an important overlap between the protected ("Yes", in red) and unprotected ("No", in black) samples, especially in the case of transects (Figure 11). There, PCoA1 (x-axis) explains around 10.3% of the dispersion while PCoA2 (y-axis) explains only around 3.6% of the overall variation.

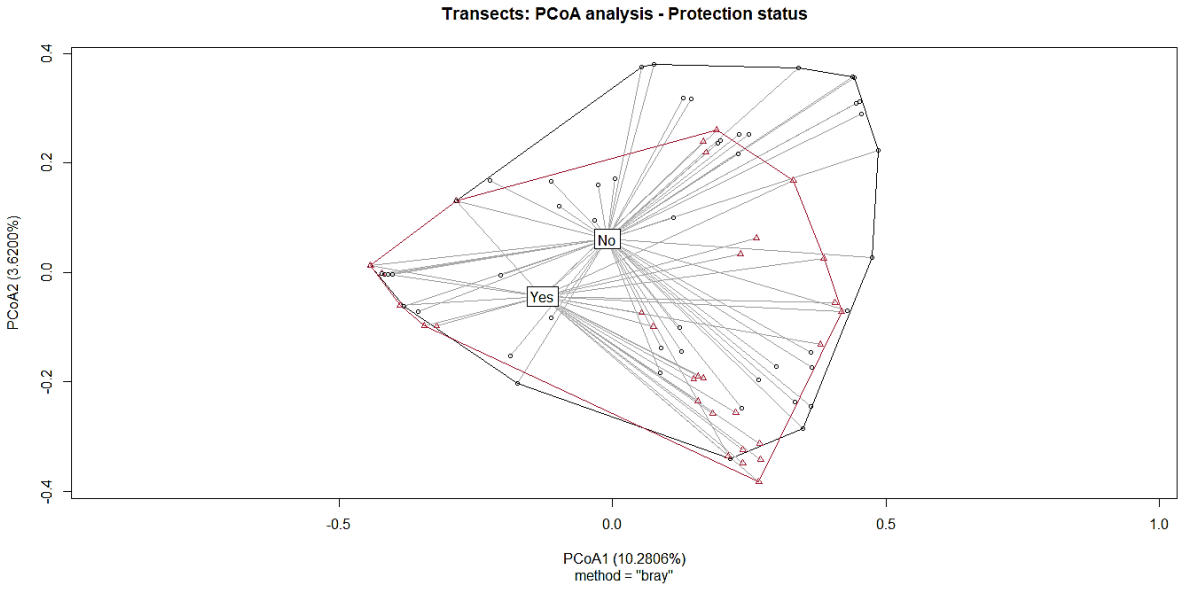


Figure 11. PCoA plot of the protection status with abundance data from transects. PCoA1 explains 10.3% of the variation and PCoA2 explains 3.6%. The species abundance in protected sites is shown in red and the species abundance of the unprotected sites is shown in black.

The quadrats data (Figure 12) shows that the unprotected sites have a larger dispersion (species abundances), and an important part is not included within the sites currently under protection. However, this variation occurs mostly on the y axis (PCoA2) which only explains around 7.0% of the dispersion, while the PCoA1 (x-axis) explains approximately 30.2%.

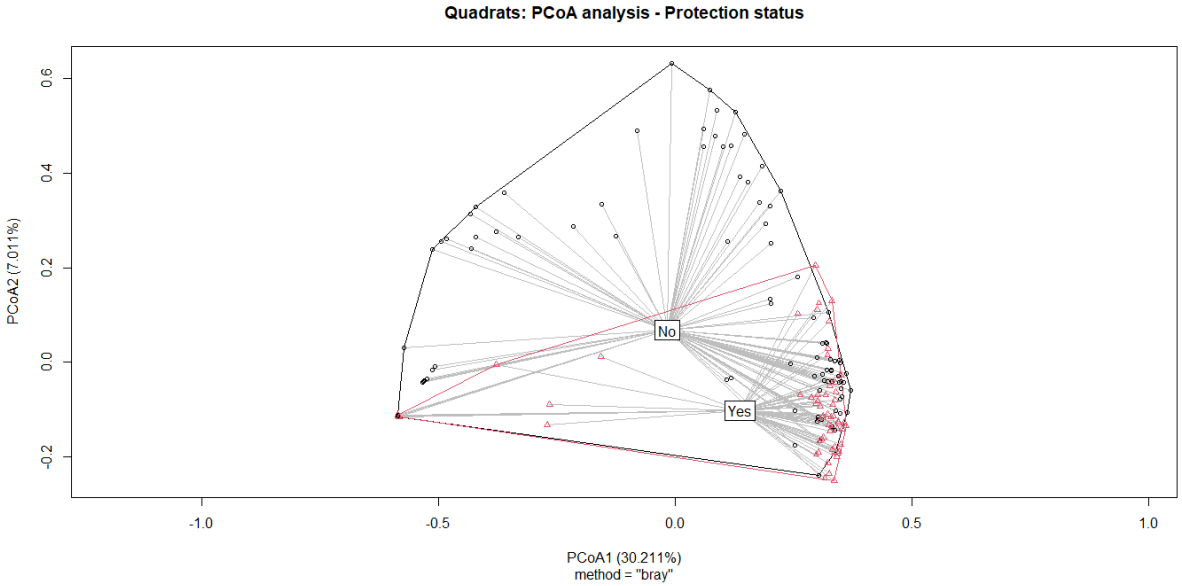


Figure 12. PCoA plot of the protection status with abundance data from quadrats. PCoA1 explains 30.2% of the variation and PCoA2 explains 7.0%. The species abundance in protected sites is shown in red and the species abundance in unprotected sites is shown in black.

3. Orientation effect: Comparison between the exposed west coast and the sheltered south coast (which includes the AMPA)

3.1. Comparison of diversity indices.

The comparison of diversity indices between the West and South coasts resulted in one significant difference. The species richness, in the case of transect sampling, is dependent on the orientation of the coastline and consequently the exposure to wave action (*P*=0.01, Table 5). Shannon index and Simpson index showed no significant differences.

Mann-Whitney U-test p values	*South vs. West*	
	Transects	*Quadrats*
Species richness	**0.011***	0.492
Shannon index	0.71	0.3
Simpson index	0.497	0.282

*Table 5. Results of the Mann-Whitney U-tests comparing diversity indices between the West and the South coast, the * shows significant results.*

The difference in species richness between South and West data is shown in Figure 13 where we can see that the West Coast has a higher mean species richness.

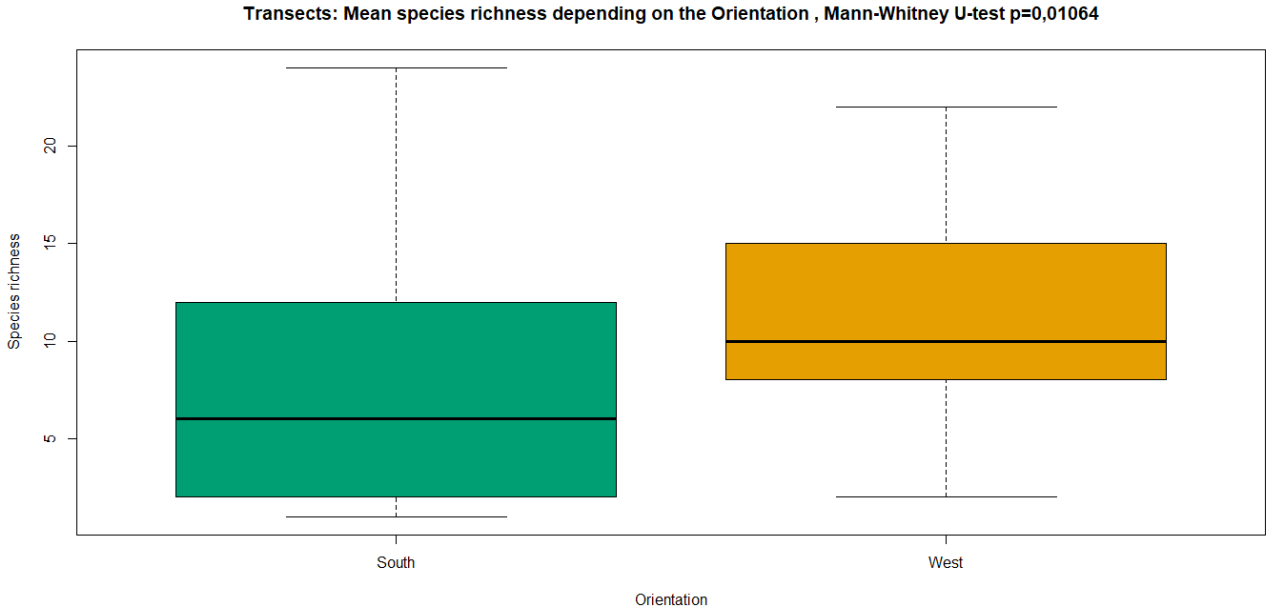


Figure 13. Boxplot showing the mean species richness of the transects from the sheltered south coast (green) and the exposed west coast (orange). The box represents the interquartile range (between the first and the third quartile, with the median shown by the bold line) and the whiskers show the lowest and highest values. Species richness is significantly higher in the west coast (Mann-Whitney U-test, P=0.01).

3.2. Distance-based analyses

On the NMDS plot (Figure 14), we can see that the samples located in the West coast (orange) are restricted to the upper part of the graph in the case of transects. However, in the case of quadrats, there is no clear distinction between the two groups.

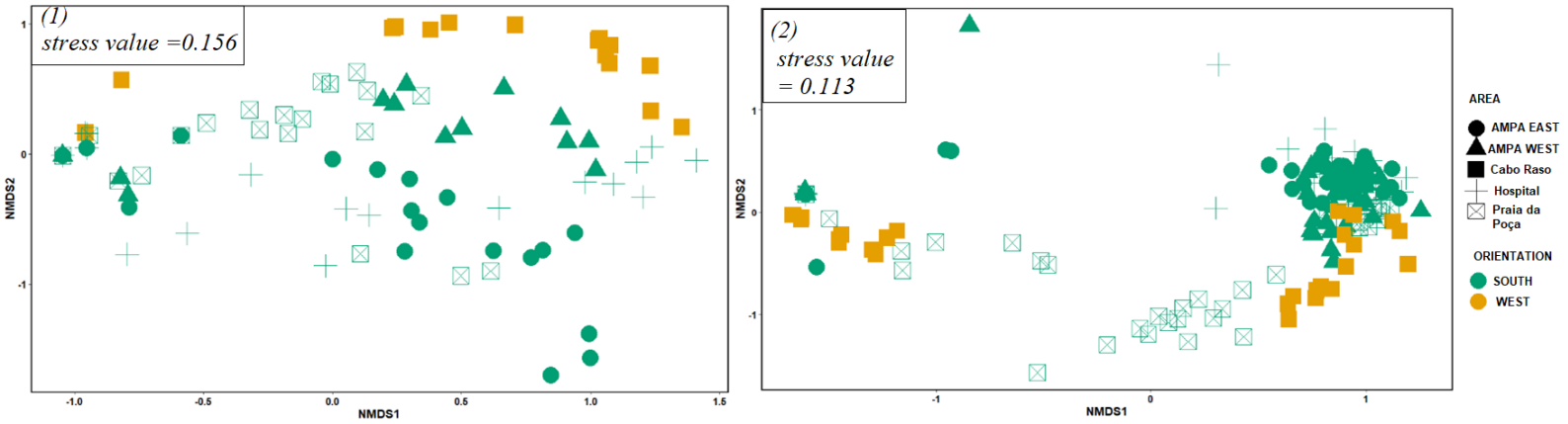


Figure 14. NMDS plots showing the distribution of the (1) transects and (2) quadrats (shape shows the different sampling points, and color shows the orientation)

In fact, the ANOSIM R value for the factor "Orientation" is equal to 0.3 (*P*=0.001) for the transects and 0.12 (*P*=0.001) for the quadrats. When R is above 0.1, dissimilarities start to appear between the groups and when R is above 0.3, we can consider that the groups are different but still have overlaps (Anderson and Walsh, 2013). The PERMANOVA test revealed an R2 value of 0.10 (*P*=0.001) for the transects meaning that around 10% of the variation is explained by the factor Orientation. However, in the case of quadrats, the R2 value is only 0.04 (*P*=0.001), suggesting a lower influence. The interactions between the factors "Orientation" and "Protection", "Orientation" and "Area", "Orientation" and "Protection" and "Area" do not have more influence (the R2 values are not higher for an interaction than for the factor itself, the result is usually the mean of the values from each tested factor). All these results can be confirmed in Table 6.

1				2			
Factors	**R2 value**	**F value**	**Significance**	**Factors**	**R2 value**	**F value**	**Significance**
Orientation	**0.104**	11.132	**0.001*** **	**Orientation**	0.035	6.834	**0.001*** **
ProXOrient	0.088	9.453	**0.001*** **	**ProXOrient**	0.024	4.861	**0.004** **
OrientXArea	0.068	2.553	**0.005** **	**OrientXArea**	0.078	5.507	**0.001*** **
ProXOrientXArea	0.052	2.919	**0.007** **	**ProXOrientXArea**	0.049	5.214	**0.001*** **

Table 6. Results of the PERMANOVA tests for (1) the transects and (2) the quadrats, using "coastline orientation" as a factor.

The SIMPER analysis revealed the 10 species that are influencing on these community differences based on transect data. *Actinia equina* and *Paracentrotus lividus* seem to be

influencing the dissimilarities between south and west coasts. Together, these two species contribute to more than 50% of the differences reported above (Figure 15).

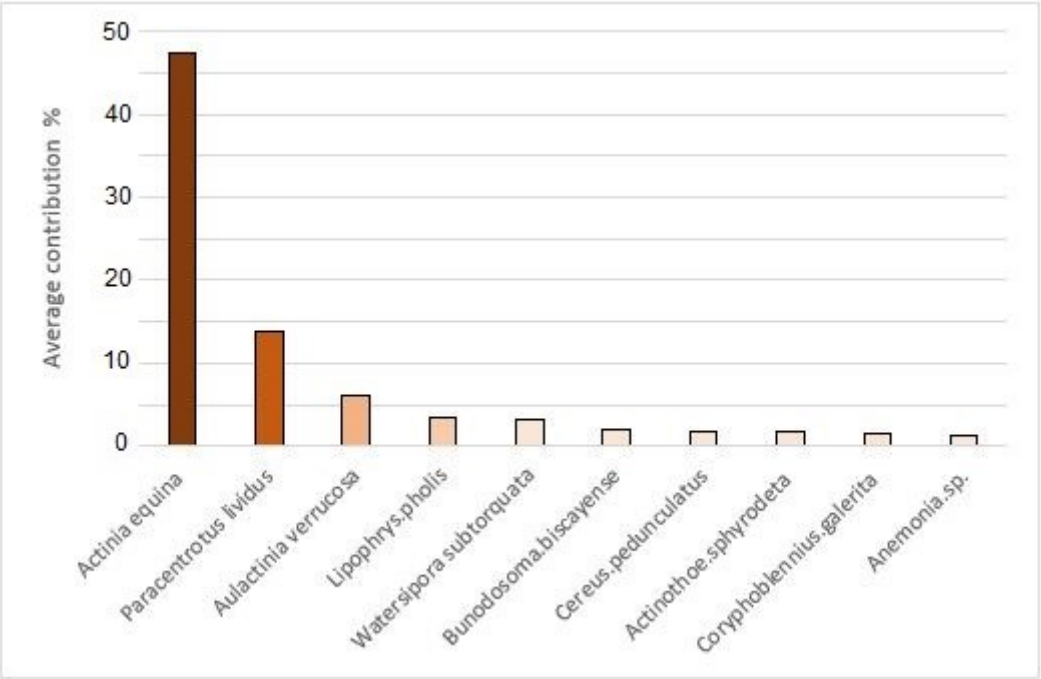


Figure 15. Average contribution of the 10 most influencing species on the differences between the sites sampled in the sheltered south and the exposed west coasts, considering data obtained from transects.

Concerning the PCOA plots, they show that the dispersion of samples from the West coast is similar to the one of samples from the South coast. In the case of the transects (Figure 16), a tendency can be observed: except a few points, the West samples are distinct from the South samples.

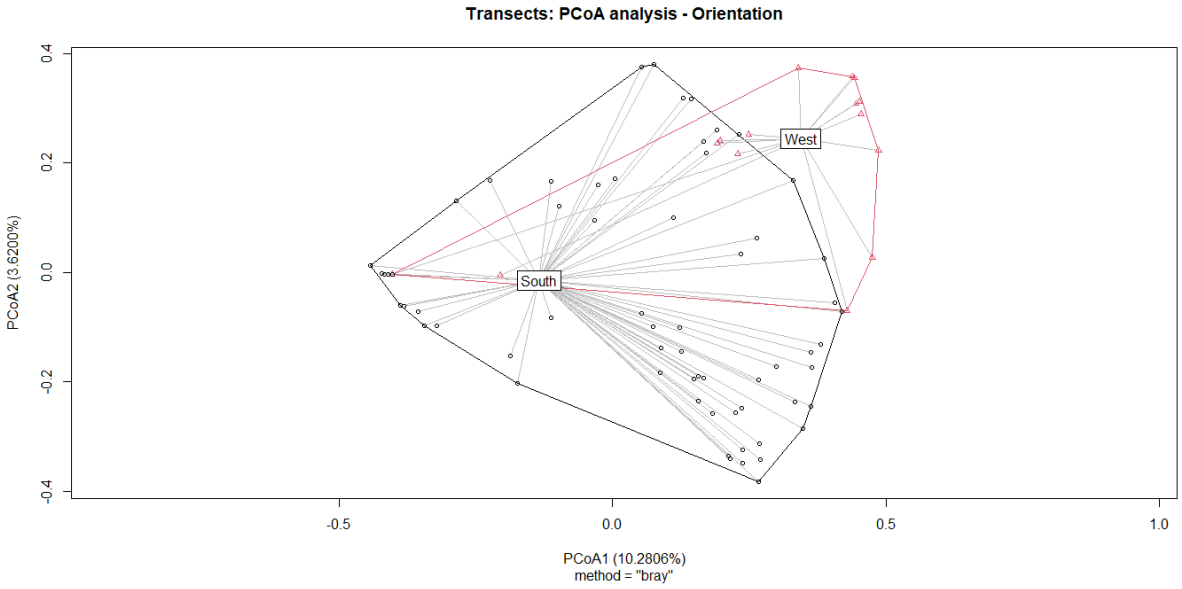


Figure 16. PCoA plot of the orientation with abundance data from transects. PCoA1 explains 10.3% of the variation and PCoA2 explains 3.6%. The species abundance in west sites is shown in red and the species abundance of the south sites is shown in black.

In the case of the quadrats (Figure 17), most of the West samples are overlapping with the distribution of the South samples, suggesting a low dissimilarity and supporting the above PERMANOVA results.

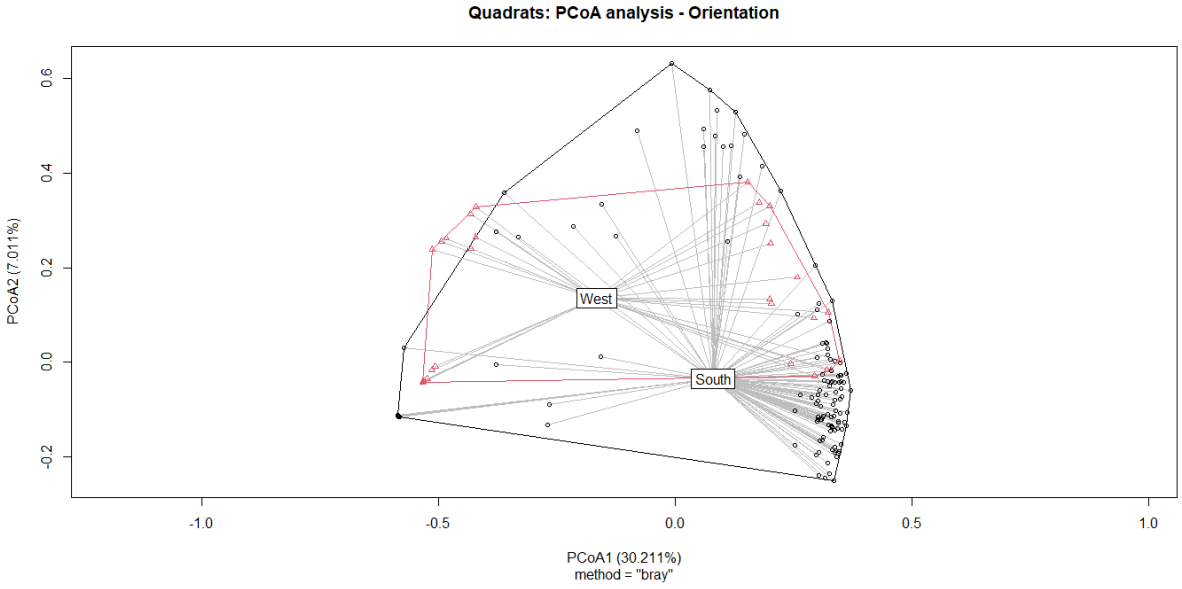


Figure 17. PCoA plot of the orientation with abundance data from quadrats. PCoA1 explains 30.2% of the variation and PCoA2 explains 7.0%. The species abundance in west sites is shown in red and the species abundance in south sites is shown in black.

4.Differences between the sampling points.

4.1.Comparison between biodiversity indices

The comparison of species richness, Shannon and Simpson's diversity indices between all the sampling points resulted in significant difference of species richness in the case of data obtained from transects (Table 7).

Kruskal-Wallis test	SAMPLE POINTS	
p values	*Transects*	*Quadrats*
Species richness	**0.034***	0.689
Shannon index	0.84	0.422
Simpson index	0.649	0.406

Table 7. Results of the Kruskal-Wallis tests comparing diversity indices between the different sampling points

The Post-hoc Dunn test with Bonferroni method revealed that the only significant difference between each point was located between Cabo Raso and Praia da Poça (P= 0.04, red star on Figure 18) with the highest species richness in Cabo Raso and the lowest species richness in Praia da Poça, both located west of the AMPA.

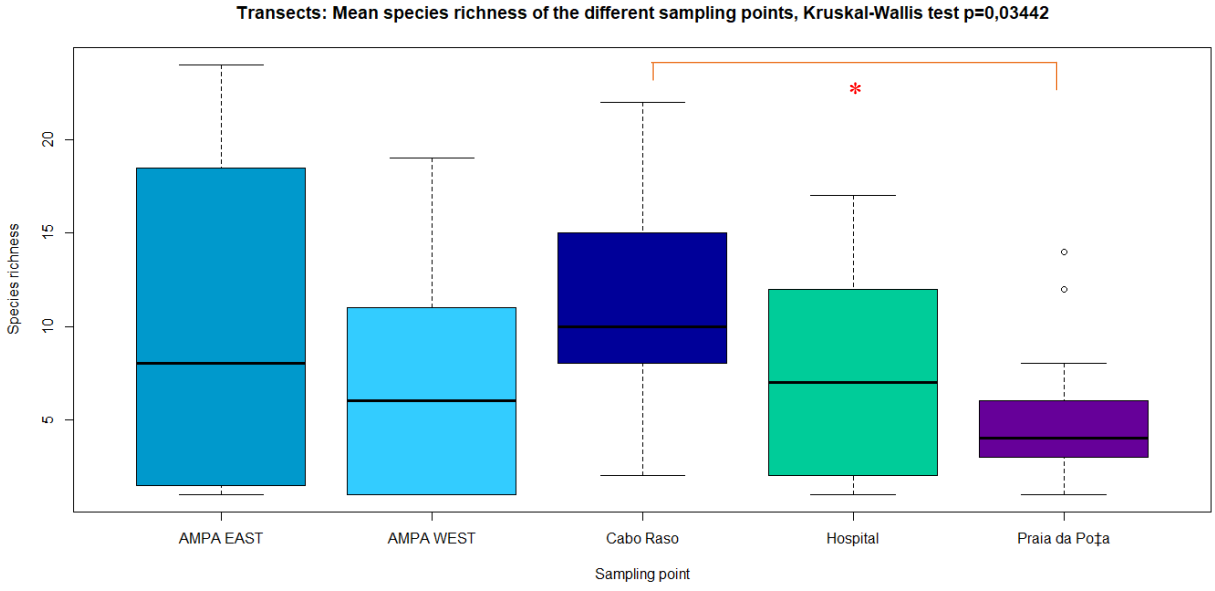


Figure 18. Boxplot showing the mean species richness of the transects from the different sampling points. The difference is significant (Kruskal-Wallis test, P=0.03). The red star indicates where the difference is located: between Cabo Raso and Praia da Poça (Post-hoc Dunn test, P=0.04)

4.2.Distance-based analyses

On the NMDS plot (Figure 19), no strong separation can be observed between the different areas, only some differences, mostly from transect data where Cabo Raso is generally apart.

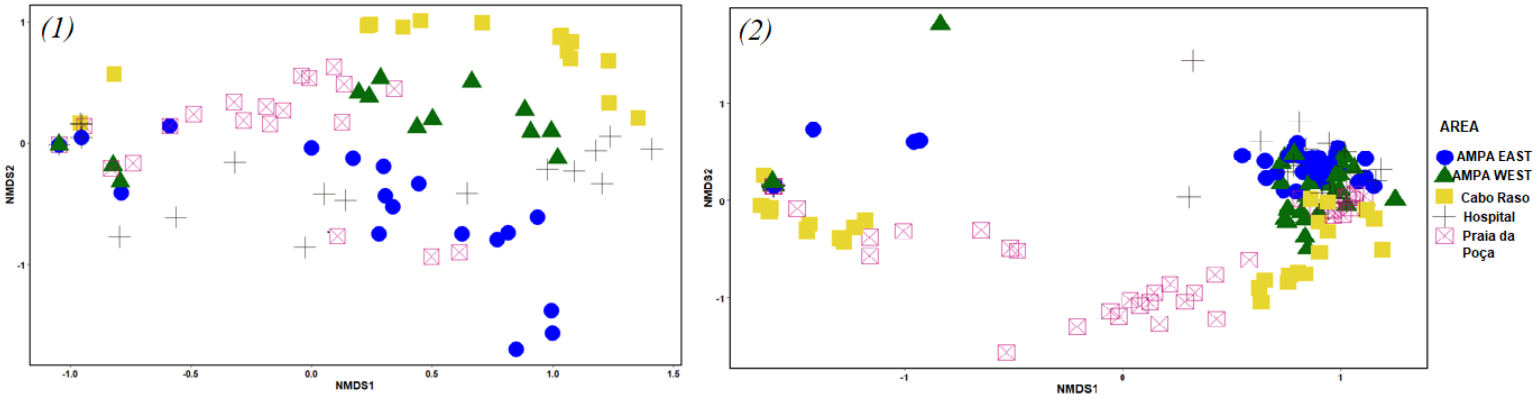


Figure 19. NMDS plots showing the distribution of the data of each sampling sites for (1) transects and (2) quadrats

The ANOSIM test revealed a moderate influence of the factor "sampling area" (Transects R value = 0.16, Quadrats R value = 0.13, *P*=0.001). The PERMANOVA confirmed that this factor was responsible for approximately 17.2% of the variation between groups in the case of transects (R2= 0.17, *P*= 0.001***) and 11.3% in the case of quadrats (R2= 0.11, *P*= 0.001***). Sampling Area was the factor that contributed the most to explain the differences observed in transect data. No interactions with other factors were detected, as no R2 value is increased in the case of an interaction. Results of the PERMANOVA test can be found in Table 8.

1				2			
Factors	R2 value	F value	Significance	Factors	R2 value	F value	Significance
Area	**0.172**	4.833	**0.001*** **	Area	**0.113**	5.960	**0.001*** **
OrientxArea	0.068	2.553	**0.005** **	OrientxArea	0.078	5.507	**0.001*** **
ProXArea	0.139	5.224	**0.001*** **	ProXArea	0.073	5.168	**0.001*** **
ProXOrientXArea	0.052	2.919	**0.007** **	ProXOrientXArea	0.049	5.214	**0.001*** **

Table 8. Results of the PERMANOVA tests for (1) the transects and (2) the quadrats, using "sampling area" as a factor.

A SIMPER analysis has been performed to know the 10 most influencing species. *Actinia equina* and *Paracentrotus lividus* were still the most important species to explain dissimilarities contributing with more than 50% of the differences (Figure 20).

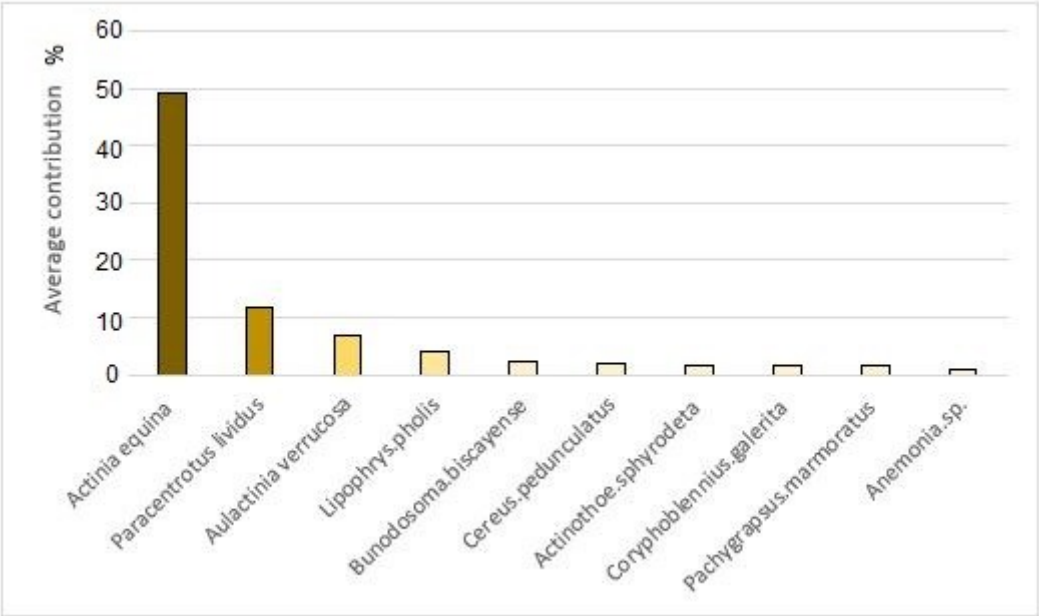


Figure 20. Average contribution of the 10 most influencing species on the differences between Cabo Raso and Praia da Poça, in the case of transects.

The PCoA graph below (Figure 21) shows the dispersion and overlapping of the transects sampling from the different sampling sites. Hospital and AMPA East seem to be similar, Praia da Poça and AMPA West too. This graph suggests that Cabo Raso is slightly different from the remaining sampling points.

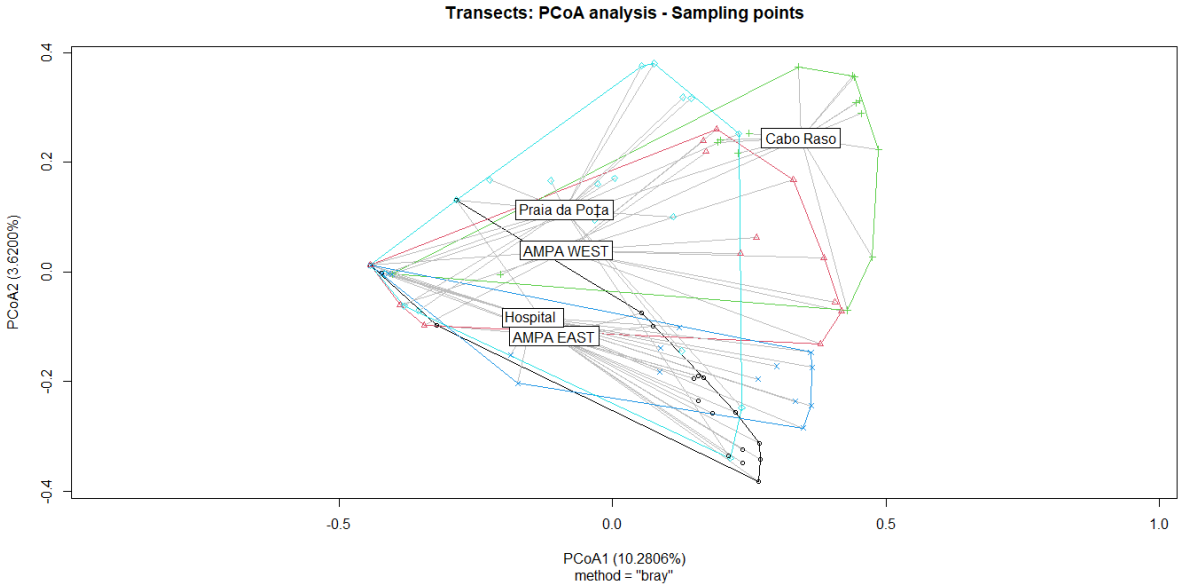


Figure 21. PCoA plot of the different sampling sites with abundance data from transects. PCoA1 explains 10.3% of the variation and PCoA2 explains 3.6%.

A large overlap was also observed on the PCoA for quadrats sampling (Figure 22), with Hospital and AMPA East grouped, together with a high similarity to AMPA West. Praia da Poça and Cabo Raso are more dissimilar with lower overlap ing species richness compared with the previous three sites.

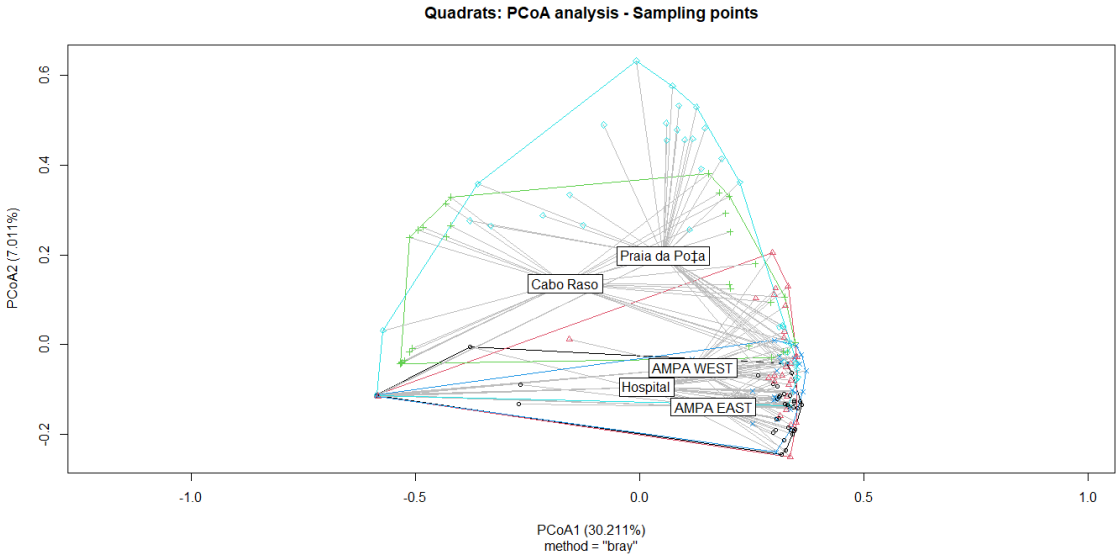


Figure 22. PCoA plot of the different sampling sites with abundance data from quadrats. PCoA1 explains 30.2% of the variation and PCoA2 explains 7.0%.

5.DNA barcoding

5.1.Confirmation of morphological identifications

The amplification success was tested on electrophoresis gels. Figure 23 is an example of the DNA amplifications: we selected only the samples with a clear band (species in bold).

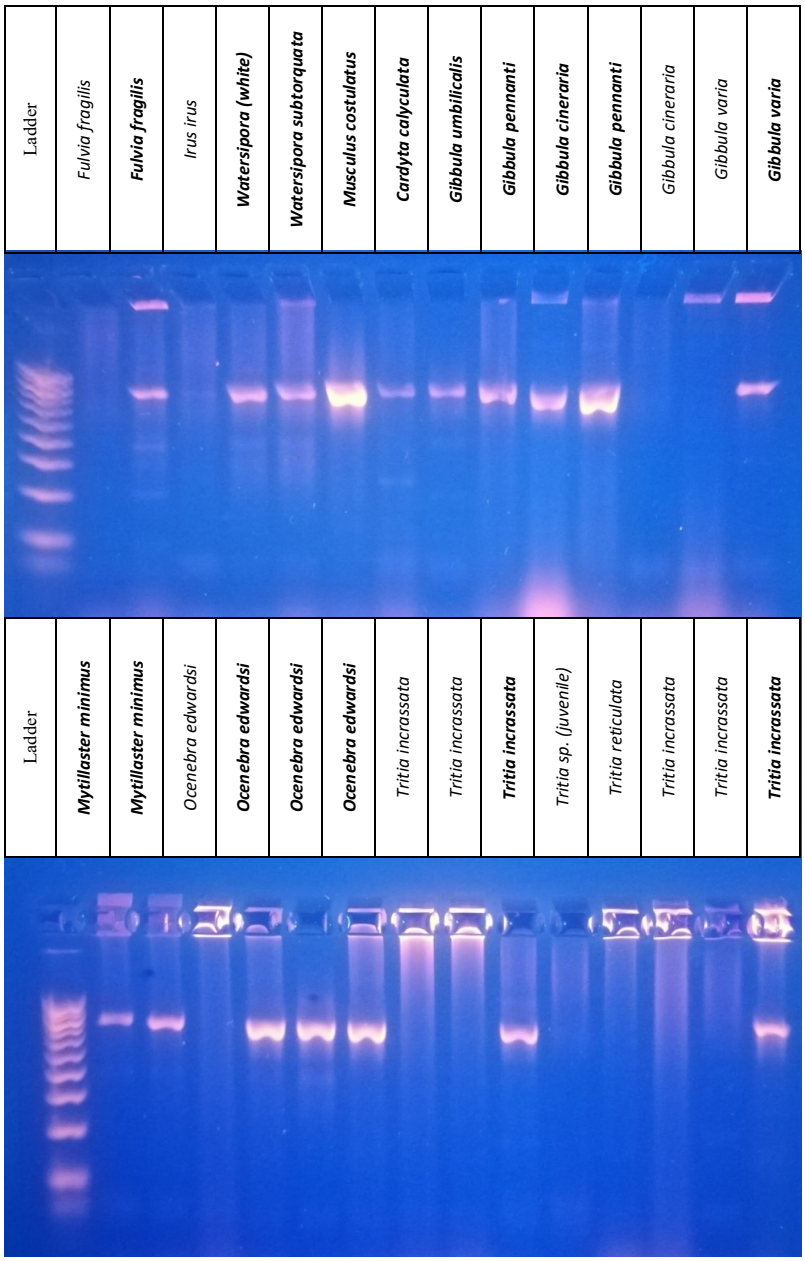


Figure 23. Example of an electrophoresis gel for the COI fragment used in DNA barcoding. Samples in bold were selected for sequencing. The primers used are LCO1490/HCO2198.

The sequencing success (i.e. when the obtained sequence was of good quality and belonging to the appropriate genus) was around 50%. In fact, for some genera the primers were not specific enough and the amplification failed. In other cases, we obtained false positives which probably resulted from contaminations. Morphological identifications matched the BLAST results for 16 cases (Table 9), i.e. around 70% of the samples for which the sequencing was successful.

Species identified	Sample	Primers	Query cover	E value	Per. Id.	Accession
Fulvia fragilis	2	16Sar/16Sbr	first contribution to Genbank			
Irus irus	3	16Sar/16Sbr	first contribution to Genbank			
Musculus costulatus	6	LCO1490/HCO2198	99%	0.0	99.85%	MT012814.1
Gibbula pennanti	11	LCO1490/HCO2198	95%	0.0	98.90%	GQ232365.1
Gibbula cineraria	12	LCO1490/HCO2198	95%	0.0	99.37%	KR084537.1
Gibbula varia	13	LCO1490/HCO2198	98%	0.0	98.90%	JQ839395.1
Gibbula varia	14	LCO1490/HCO2198	97%	0.0	99.06%	JQ839395.1
Mytilaster minimus	16	LCO1490/HCO2198	90%	0.0	97.13%	DQ836022.1
Mytilaster minimus	16	16Sar/16Sbr	95%	0.0	99.13%	DQ836017.1
Ocenebra edwardsii	18	LCO1490/HCO2198	96%	0.0	100.00%	KU566774.1
Ocenebra edwardsii	18	16Sar/16Sbr	100%	0.0	100.00%	KF153619.1
Ocenebra edwardsii	19	LCO1490/HCO2198	97%	0.0	90.02%	KU566774.1
Ocenebra edwardsii	20	LCO1490/HCO2198	98%	0.0	100.00%	KU566774.1
Ocenebra edwardsii	20	16Sar/16Sbr	99%	0.0	99.61%	KF153619.1
Tritia incrassata	27	LCO1490/HCO2198	97%	0.0	99.69%	KY489393.1
Tritia incrassata	28	LCO1490/HCO2198	97%	0.0	97.78%	KY582565.1

Table 9. Results of the DNA barcoding BLAST search for each sample

We tested several individuals from the Gibbula genus, and it appears that they were all well identified with the morphology, even considering their high variability (Figure 24).

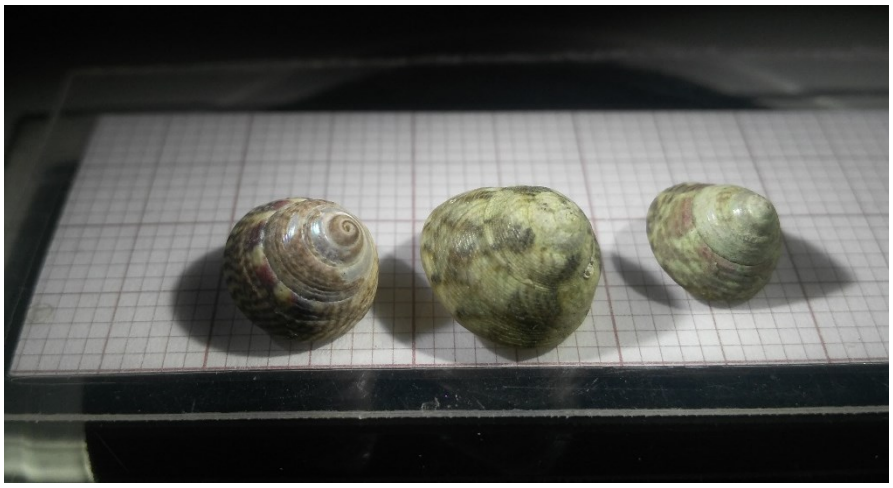


Figure 24. Within species variability, with an example for Gibbula cineraria (Photo: Pedro Duarte Coelho)

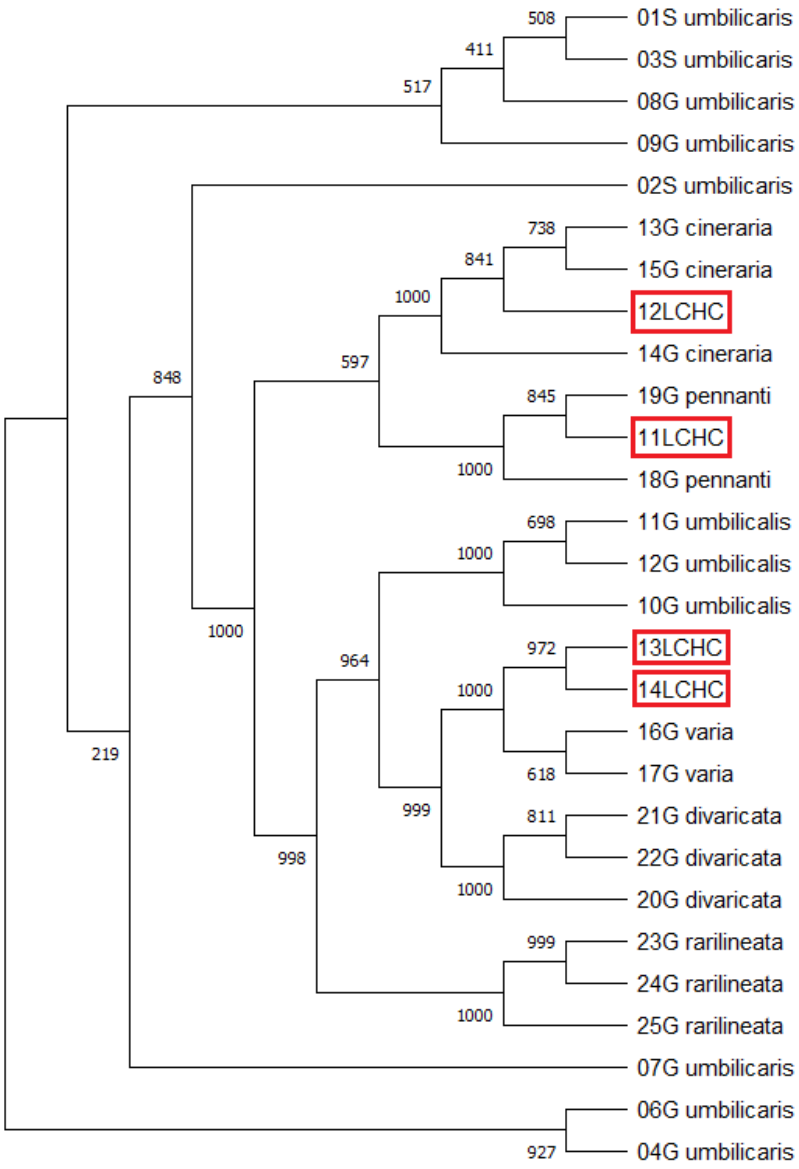


Figure 25. Phylogenic tree based on data from the COI mitochondrial DNA fragment analysed in this work, with the bootstrap values supporting each node showing the overall topology of the genus Gibbula and the position of each specimen collected during field work: 11LCHC was identified as G. pennanti, 12LCHC was identified as G. cineraria, 13LCHC and 14LCHC were identified as G.varia.

The phylogenetic tree above (Figure 25) shows where our individuals are located among individuals of the same genus (*Gibbula*) based on their COI sequences. The sample 11LCHC has been identified as *Gibbula pennanti* both morphologically and genetically, the sample 12LCHC has been identified as *Gibbula cineraria* both morphologically and genetically, the samples 13LCHC and 14LCHC have been identified as *Gibbula varia* both morphologically and genetically. In the tree, all these samples are located among individuals of the same species.

5.2. Native species monitoring: the case study of Irus irus.

The species *Irus irus* is a bivalve known to be present along the Portuguese coasts (WORMS, 2020), and it was collected during our field surveys at AMPA (Figure 26). It has been first identified by our team using a stereoscope and later confirmed by taxonomists. No COI or 16S sequences were available in GenBank, therefore a special interest was placed on this species. Our findings represent the first contribution to a public database for the 16S rDNA barcode sequences of *Irus irus* whose sequence can be found in Appendix G.

Figure 26. Picture of an individual of Irus irus *found in the AMPA (photo: Pedro Duarte Coelho)*

5.3.Tracking non-indigenous species: the case study of Fulvia fragilis.

The bivalve *Fulvia fragilis* is present along the southern Spanish coast and therefore expected to either be present in Portugal but still undetected or arrive to Portugal in the near future. Similarly to the case of *Irus irus* described above, our study contributed with the first available 16S rDNA sequence, allowing researchers to detect this species in the future. In fact, other species of *Fulvia* genus had their COI and 16S already sequenced and available in GenBank but not *Fulvia fragilis*. The whole sequence can be found in Appendix H.

Discussion

1. Avencas and MPAs effectiveness

The comparative analysis of intertidal communities between MPAs and adjacent non protected areas is an effective way to evaluate the effects of protection measures (Ferreira *et al.*, 2017). Data collected inside and the outside the AMPA suggest that there is no significant effect of the protection implemented in the area on the parameters tested in this study. In fact, the protection status is not a factor having an important influence on the biodiversity indices nor on the composition/abundance of the ecological communities. In many cases, this is a sign for a weakly protected area (Zupan *et al.*, 2018).

The NMDS plots, as well as the ANOSIM tests' R values, show that the dissimilarity between protected samples and non-protected samples is approximately the same than the dissimilarity between samples from the same group. This suggests that the protected area is not significantly different from the nearby unprotected area. The PERMANOVA results support this result as only 3% of the differences between the groups could be explained by the difference of protection status.

Given the original objectives of the creation of the AMPA, we define an "effective" protected area as having relatively high levels of biodiversity and abundance compared to reference sites (different sites outside the protected zone, or same sites before the implementation of the protected area).

This highlights the fact that the AMPA does not have an effective protection status. In fact, following the famous and widely cited "NEOLI" acronym from Edgar *et al.* (2014), the AMPA does not meet the main requirements for a protected area to be fully efficient.

First, this MPA is not a No-Take area ("N" in the acronym). In fact, many activities including fishing are still allowed, even if they must follow certain regulations. Several people collecting invertebrates were seen on different sampling days, as well as fishing boats and nets. This could contribute to the fact that the pressures were not reduced enough to produces a significant increase in species richness and abundances. Sala and Giakoumi (2018) showed the great impact of a full no-take protection on fish abundance and biomass: according to their meta-analysis of different studies "the biomass of whole fish assemblages in marine reserves is, on average, 670% greater than in adjacent unprotected areas, and 343% greater than in partially-protected MPAs". Numerous other studies showed the same trend (Pomeroy, Parks and Watson, 2004; Gaston *et al.*, 2006; Rasmussen, 2010; Vandeperre *et al.*, 2011; Sciberras *et al.*, 2013; Sadio *et al.*, 2015; Gil Fernández *et al.*, 2016). This does not only affect fish populations but also invertebrates and algae, as trophic cascades, through predation, herbivory and competition, also play a key role in this process (Gil Fernández *et al.*, 2016).

Secondly, in the acronym the "E" stands for Enforced. Enforcement and management are also a complex problem present in MPA's in general and in the AMPA in particular.

Numerous studies show that, in general, weakly protected areas differed little from unprotected areas (Zupan *et al.*, 2018).

This is what happened earlier in the same place: in 1998, the Avencas Biophysical Interest Zone (Zona de Interesse Biofísico das Avencas – ZIBA, see Figure 27) was implemented due to the exceptional intertidal biodiversity found there. It was supposed to be a "no-fishing area", but due to lack of information for visitors and lack of compliance from the recreational fishing' community, its protection status became controversial, some tensions occurred and its goals could not be reached (Ferreira, Seixas and Marques, 2015). Now, a common ground has been found but it is still evident that the current enforcement is not sufficient:

- The protected area is not clearly indicated, and people (fishermen, sailors, tourists, etc.) might not know that they are in a protected area.
- There is still an important lack of control of the fishing activities.
- There is no control of the recreational activities.
- There is an uncontrolled pollution of the area.

How old is the MPA is also an important factor: the "O" in the term "NEOLI" stands for old and implies that an MPA is old enough after 10 years of implementation. The AMPA is only 4 years old, so it is highly likely that it is not old enough to be effective. Also, it is possible that the timeframe is too short to be able to observe the results of this implementation. In fact, the ecological communities might need more time in order to benefit from protection measures. However, in some cases, effects on the biodiversity have been observed directly in the first year of implementation in some other protected areas in the world (Vandeperre *et al.*, 2011), but it does not seem to be the case for AMPA. Also, information about the evolution of potentially disturbing activities within AMPA are missing, so it is not possible to study the potential reduction of threats due to the protection over the four years.

Additionally, this similarity between the inside and the outside of the protected area can be due to its small size, which is only 0.59km². In fact, another important feature of marine protected areas is the size: "L" for large in the NEOLI acronym. Edgar *et al.*, (2014) consider that a large protected area is bigger than 100km². Some previous studies in the Avencas area concluded that size is an important factor as it influences the MPA openness, which is the ratio of periphery to area, and therefore its susceptibility to external driving forces (Ferreira *et al.*, 2017). However, there are some examples of smaller MPAs having positive consequences on the local biodiversity (Bayley *et al.*, 2019), so it is possible that the size of AMPA would not be an issue if more NEOLI criteria would be met.

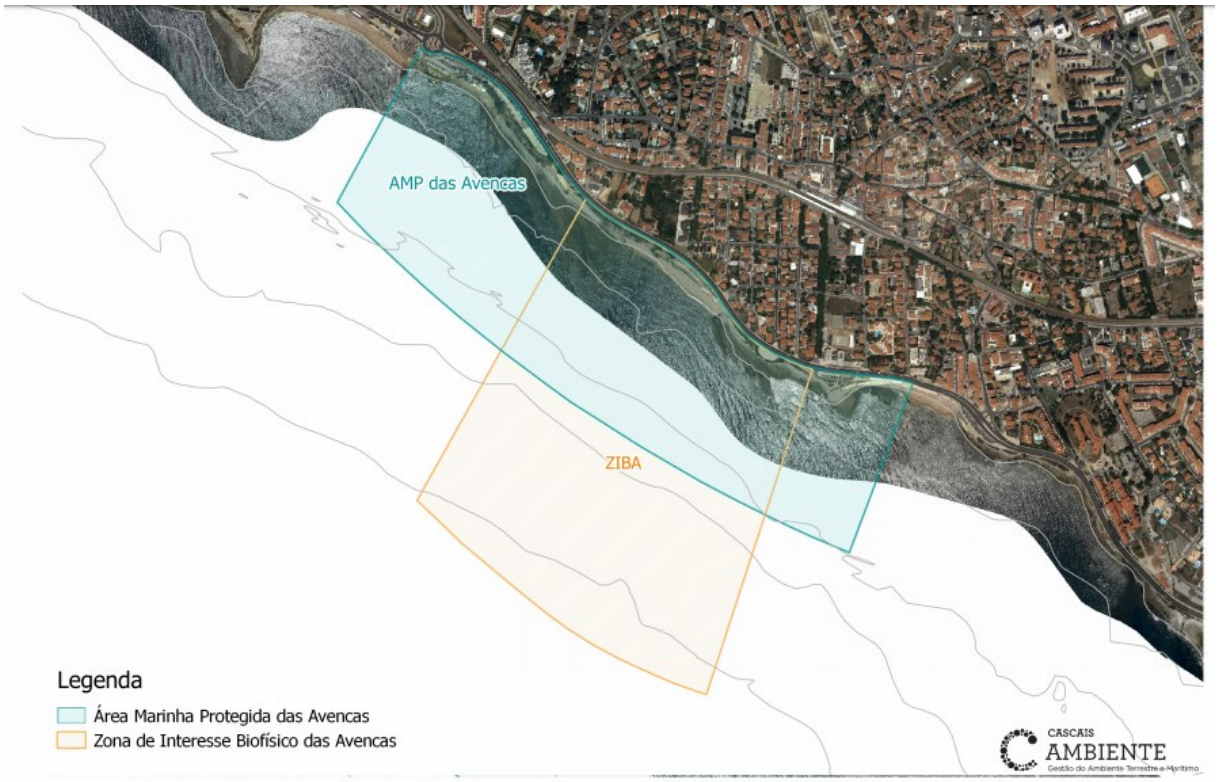


Figure 27. Satellite picture showing the AMPA and its predecessor ZIBA before 2016 (source: Ambiante Cascais)

Finally, the last letter of the acronym, "I", stands for Isolated, and describe a protected area isolated from human settlements by deep sea or sand to reinforce the protection. This is not the case for AMPA either. As shown on the figure 27, a large road and a dense habitational area runs alongside the whole upper part of the MPA. The whole coast from Lisbon to Cascais shows a high urban pressure and numerous studies show the impact of urbanization on the coastal ecosystems through different processes as resource exploitation, pollution pathways and ocean sprawl (Todd *et al.*, 2019).

In their study, Edgar et al., (2014) showed that MPAs with 3 NEOLI features had 30% more overall fish biomass than fished areas. But MPAs with 5 features had 244% higher fish biomass. In the end, they concluded that as long as an MPA can secure 4 of the NEOLI boxes, they should result in improved marine ecosystem health. As discussed above, in the case of the Avencas, none of these NEOLI features are fully guaranteed.

Implementation, regulation, and surveillance are a big concern in MPAs around the world. One example is the case of the Mediterranean sea, which is an important biodiversity hotspot. Claudet *et al.* (2020) states that "6.01 % of the Mediterranean is covered by protection" but also that "in 95% of this area, regulations are not stronger inside than outside MPAs". In the end, only 0.23% of the Mediterranean is fully or highly protected. These numbers are showing the intensity of the current issue concerning the management of MPAs and the need for more rigorous regulations.

Another problem mentioned in the same study is that protection is unevenly distributed across political boundaries (e.g. outside and inside the European Union, in the case of the Mediterranean Sea). This is mainly due to differences in governance frameworks, institutional organizations, wealth distribution, social capital, or knowledge on the environment. The same tendency can be observed worldwide, because countries with advanced economies host two-thirds of the global system of MPAs (Marinesque, Kaplan and Rodwell, 2012).

Connectivity between different MPAs is also a factor that can increase the effectiveness of protected areas. In fact, it can help biological populations to grow and develop over a larger area, especially in the case of sessile invertebrates (Marti-Puig *et al.*, 2013). Numerous benthic invertebrates are sessile and/or sedentary in the adult phase, so their gametes and larva must have the possibility to disperse easily. A network of MPAs can provide this opportunity, allowing them to spread. However, AMPA is relatively isolated from other marine reserves.

2. Comparison between West and South coasts

The south facing coast between Caxias and Cascais, just before the entry of the Tagus estuary, differs from the West coast: lower currents, less physical and hydrodynamic disturbance. Cabo Raso is the point where the coast starts facing the Atlantic Ocean (Figure 28) and is then submitted to stronger wind and waves currents. This is shaping a different coastal ecosystem. In fact, physical factors such as wave exposure, slope, and substrate complexity strongly influence heterogenous spatial distributions of species in intertidal communities (Benedetti-Cecchi *et al.*, 2003). This is why we decided to compare the diversity and species abundances between the West and the South coasts. In other words, the West coast could host another important, and different, community to preserve.

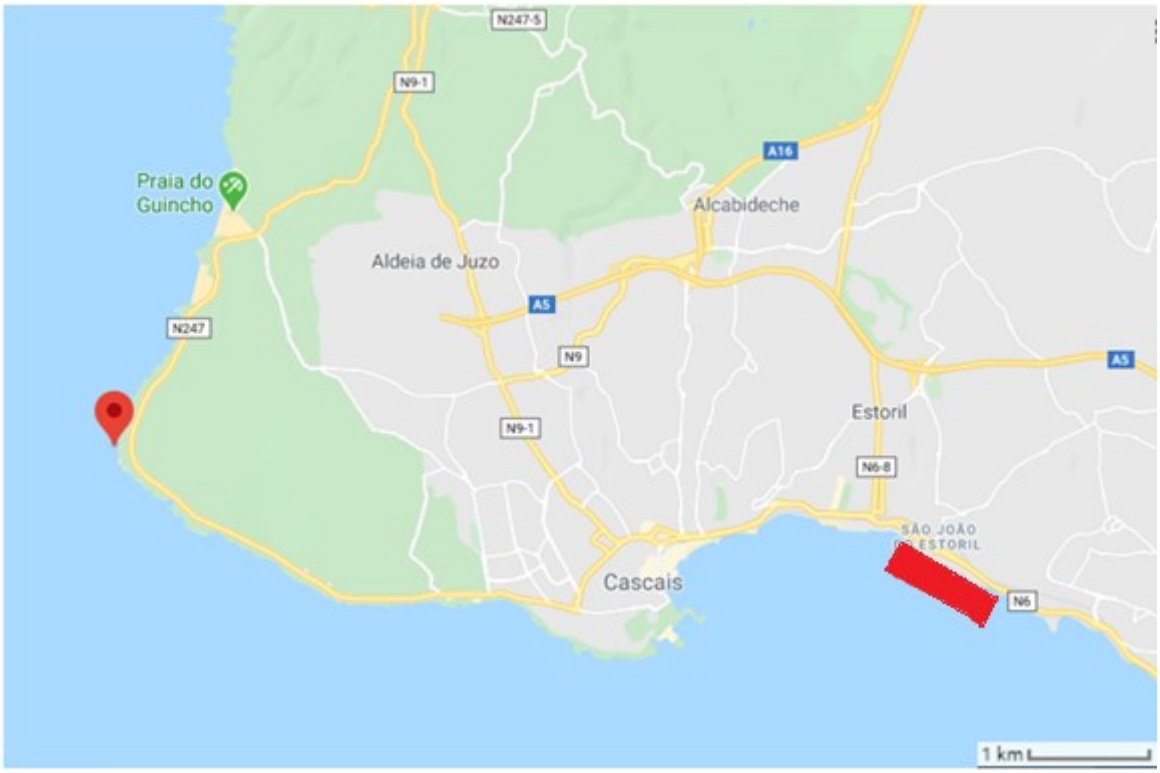


Figure 28. Map showing the location of Cabo Raso compared to the South coast near Cascais, AMPA is shown by the red rectangle.

The results did not show a clear and strong dissimilarity between those two groups. However, it seemed like the West coast had a higher species richness. The PERMANOVA test also showed that Orientation was a factor explaining around 10% of the dissimilarity between the two groups in the case of transects, while only 3% in the case of quadrats.

Our findings show a tendency that has been observed in other studies as well. Across Europe, different situations have been distinguished. Sometimes, as in our case, the exposure to waves and tidal disturbance is positively correlated with intertidal species richness (Lastra *et al.*, 2006; Kotta *et al.*, 2017) and also biomass (Ricciardi and Bourget, 1999; Rodil and Lastra, 2004). Some research teams in France found that intertidal faunal diversity was positively correlated wave-exposed conditions (Hily and Jean, 1997). However, a study in Portugal showed that a "steady community structure does not necessarily persist in similarly exposed conditions" (Gonçalves *et al.*, 2009), suggesting that other factors are also involved in the observed macrofaunal patterns. In fact, other studies along the European Atlantic coast showed that higher exposure was reducing algal and macroinvertebrates density (Junoy and Vieitez, 1992). This is highlighting the fact that in similar habitats along the Atlantic coast of Europe, the interaction between physical disturbance and biodiversity can vary.

Despite a large variety of methodologies used to examine intertidal assemblages, some studies performed in Portugal and Spain with similar protocols found similar values of diversity as in AMPA and its surroundings: H' between 0.2 and 0.8 for the midlittoral zone, and between 0 and 0.6 for the supralittoral zone (Ferreira and Andrade, 2003). Some other studies observed

similar tendencies in terms of species richness, with around 50 species sampled with quadrats (Oliveira *et al.*, 2014) and around 60 species sampled with transects (Guerra-García *et al.*, 2006). However, higher species richness and diversity are usually found in the subtidal areas, in general >200 species and H'>2 (De Montaudouin and Sauriau, 2000), which is not included in this thesis.

Our distance-based analyses, especially the SIMPER results, showed that the cnidarian *Actinia equina* and the echinoderm *Paracentrotus lividus* were, by far, the most influencing species concerning the differences between South and West. According to the AMBI ecological groups, which classifies marine invertebrates according to their tolerance to disturbance (mainly related to pollution and habitat condition), *A. equina* and *P. lividus* are sensitive to chemical disturbance (Borja, Franco and Pérez, 2000). One possible explanation of their higher presence in Cabo Raso might be that the water quality is better due to a lower urbanization of the surroundings and less human presence.

In a very physically-disturbed environment, *A. equina* and *P. lividus* are able to exhibit specific adaptative strategies in order to fight dessication and handle strong currents: individuals of smaller size (dwarves) usually living in aggregates (Boudouresque and Verlaque, 2001). This could be studied in Cabo Raso in the future as it could be another explaination for their important presence in this area compared to other species. Sea urchins are considered as important ecosystem engineers because they intensively feed on algae and coral, so the impact of their high density on the local ecosystem could be interesting to study.

Finally, a study performed in Ireland discovered that the feeding behavior of *A. equina* is influenced by the shore exposition (Davenport, Moloney and Kelly, 2011). This species can shift its preferences according to the most available food source. In highly disturbed conditions, the water becomes a rich mix of different ressources from macromolecules to whole plants or animals and *A. equina* can scavenge on those.

3. Comparison between the different sites

The aim of comparing ecological communities between sampling points is to know which area is richer, more diverse or with a different pattern. This information can be then used to spot interesting places to extend the actual protected area or create a new one.

3.1.Suggestion to expand AMPA to the East.

Concerning the potential expansion of the Avencas MPA, the present results give some leads. In fact, Praia da Poça, located on the West side of AMPA, has the lowest species richness value while Hospital, located on the East side of AMPA has similar species richness than inside the protected area. If the communities on the East are as rich as the inside of the MPA, it might be interesting to extend the protection to include them and preserve a maximum of the local biodiversity.

3.2.Suggestion to create a new MPA to the West

The analyses revealed a significant difference in species richness between sampling points, but this difference lays more precisely between Cabo Raso and Praia da Poça. This result observed with the post-hoc Dunn's test is probably an explanation for the significant difference in species richness between the West and the South coast found in the previous section, Cabo Raso being the only point in the West category.

However, even compared to the rest of the sampling sites, Cabo Raso has a higher species richness and seem to be always apart on the NMDS and PCoA plots, especially for the transects. This is can be due to a different ecological community.

Moreover, the SIMPER analysis revealed that, again, *Actinia equina* and *Paracentrotus lividus* were the species with the highest influence on the observed dissimilarity between points. The fact that more than 50% of this dissimilarity is explained by the difference of these two species' populations suggest that the rest of the community might be similar in terms of composition and abundances. This is likely that this significant dissimilarity observed between the different areas is due to the fact that Cabo Raso differ from all the others, especially in terms of *A. equina* and *P. lividus* abundances. There were, in general, 10 to 100 times more *P. lividus* in Cabo Raso than in other points, and 100 to 1000 times more *A. equina*.

These results are showing that Cabo Raso has a high potential for the creation of a new MPA as it would allow the preservation of different communities.

4. DNA Barcoding

4.1. Identification of cryptic species and MPA monitoring

In the Ocean, many genera include morphologically similar species, raising difficulties to field ecologists trying to identify a large number of different taxonomic groups. In the intertidal zone of the Avencas Marine Protected Area, many species with these characteristics have been found. A large part of them were gastropods, for which it is sometimes difficult to observe key elements of the shell or the mantle for a good and precise identification. One of the main objectives of this project is important to get an accurate estimate of the biodiversity present inside and outside this marine protected area.

As an example, the gastropod genus *Gibbula* comprises many species that can be hard to identify and to delineate (Barco *et al.*, 2013; Affenzeller, Haar and Steiner, 2017; Uribe *et al.*, 2017). But most importantly this genus is very abundant in the study area. Being able to identify the different species was then essential for the project. With practice, field identifications were possible for a large number of individuals, but DNA barcoding of some dissimilar specimens was a real asset to this study, allowing an accurate completion of the biodiversity database.

This project illustrates how DNA barcoding can be a useful tool to precisely identify some marine individuals, with little effort and in a short time. Numerous studies support the development of this technique and of the associated DNA sequences libraries to reduce current limitations (Valentini, Pompanon and Taberlet, 2009; Keele *et al.*, 2014; Trivedi *et al.*, 2016;

Weigand *et al.*, 2019). The need to enlarge the available DNA barcodes already described in public databases applies to all research teams studying ecological communities. This method can complement the work of experienced taxonomists, that are not always available for all taxonomic groups, while making the census of an area. DNA barcoding is accessible to non-geneticists as the facilities and equipment are easy to implement even in small ecology research groups.

Developing DNA barcoding for species identification could also help the discovery of new species by allowing quick sorting of specimens and highlighting divergent taxa. In fact, it is estimated that 91% of the marine species remain to be discovered (Mora *et al.*, 2011) and relying solely on morphological identification would certainly not permit the identification of all these species. However, this implies the need of sequencing more than one DNA fragment to check for congruence. Also, these results need to complemented by each species description (Hebert and Gregory, 2005). Therefore, DNA barcoding has to be performed along with traditional morphological taxonomy, in order to guarantee a correspondence between the sample and the DNA barcode. Even next generation sequencing (NGS) techniques such as eDNA (Scriver *et al.*, 2015; Ardura and Planes, 2017; Stat *et al.*, 2017) must rely on these species specific DNA barcodes to be effective. Moreover, with the development of these methods sequencing DNA is becoming cheaper, and some tools already available to perform real-time DNA barcoding directly on the field (Pomerantz *et al.*, 2018). There is a promising future for biodiversity assessment using these tools.

Monitoring the biodiversity of a Marine Protected Area and its surroundings is a key work in terms of conservation and ecosystems management. It is important to have a baseline reference in order to disentangle normal ecosystem fluctuations from trends that result from specific impacts such as habitat destruction, over-exploitation or climate change, inside and outside the MPA. Therefore, with the increasing number of protected areas, the scientific community and the other stakeholders need time- and costs-efficient tools to be able to implement a solid monitoring plan of the fauna and flora inside each MPA.

The more widely this method is used, the more complete the databases will become and the more efficient this tool will be. In fact, as shown by the case of the bivalve *Irus irus*, there are still some species present in MPAs for which we have no information on genetics or DNA barcode genes. Tracking these species with molecular tools is not only interesting to confirm their presence in a given region, but also to discover new species in an area and to make their census easier.

However, there are several limitations. For example, finding appropriate primers to perform DNA PCR amplification is not always easy because universal primers may prove to be ineffective.

4.2. Non-Indigenous Species early detection

Invasive species are one of the main current issues in ecology. Detecting their arrival as early as possible can facilitate the latter decisions to deal with them and therefore prevent them to impact native species within MPA's.

Fulvia fragilis is an exotic bivalve originated from the Indian Sea but now present in the Mediterranean Sea, documented in many countries including Turkey, Tunisia, Greece, Spain, Italy, Malta, Lebanon, and Albania (Rizgalla, Shinn and Crocetta, 2019). It is therefore expected to arrive soon to the coast of Portugal or to be already present although undetected. Although the samples were not captured within the MPA we took the opportunity to obtain the first DNA barcode sequences of this species to allow an effective identification in the future by our own team or other teams working in different geographical areas.

It can be even more important to get a precise taxonomic identification when NIS species are morphologically similar to native species. The case study that we had were the native bivalve *Musculus costulatus* and *Arcuatula senhousia* (Figure 29), which is an invasive species coming from Japan and currently present on the Portuguese coast (Lourenço *et al.*, 2018). It is challenging to distinguish them during field work so DNA barcoding may prove to be useful. For that reason, the first eight COI sequences of *M. costulatus* have been added to GenBank because *A. senhousia* already had some available sequences in Genbank database.

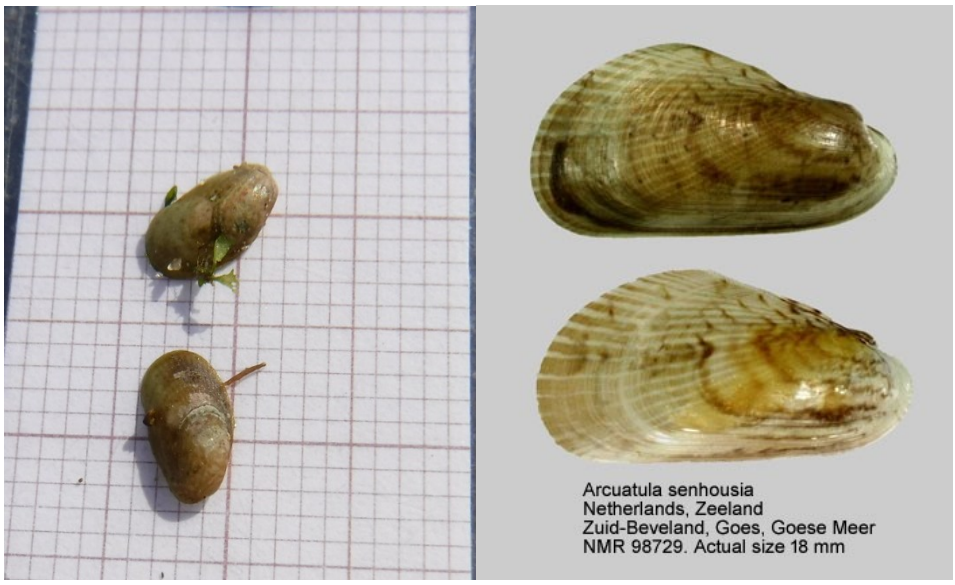


Figure 29. Left: Musculus costulatus, individuals from AMPA (photo: Pedro Duarte Coelho). Right: Arcuatula senhousia, picture from the World Register of Marine Species

In the present study, we mainly focused on bivalves as they, and mollusks in general, have a great invasion potential due to their capacity to attach to the boats' hulls, ropes, etc (Carlton, 1999). They are often called fouling organisms. However, other taxa are also fouling organisms. In AMPA, we found *Watersipora subtorquata* which is an invasive bryozoan from Asia that is well-settled along the west coast of Portugal, especially in yacht marinas (Figure 30).

Figure 30. An invasive species present in AMPA, Watersipora subtorquata

Other genera are also of great interest because they were recently reported in Portugal, as Tunicata and Crustacea for example (Chainho *et al.*, 2015). There is a wide gap to fill in terms of available sequences in GenBank and other databases, giving room for further studies on different genera in different types of habitats.

Conclusion and suggestions for the future

The focus of this study lies on the intertidal zone of AMPA, which is particularly exposed to the effect of tides and waves, which creates peculiar extreme physical conditions and a high abiotic stress. This abiotic stress is known to influence species richness and diversity in communities (Scrosati *et al.*, 2011). Thus, these conditions create a habitat hosting a large number and variety of species that forms complex communities of important ecological value and great scientific interest.

As a conclusion, this thesis showed that no differences were present between the ecological communities inside and outside the Avencas marine protected area, suggesting a low effectiveness of the protection measures and highlighting the lack of appropriate regulation and management of this area. In fact, this area is submitted to intense external pressures due to its proximity to dense human settlements and activities that have a clear impact on the coastal ecosystems (pollution, habitat destruction, fishing, etc.). If stronger protection measures would be implemented, those anthropogenic pressures could be removed or reduced, increasing the chances to observe more diverse and abundant communities inside AMPA compared to the rest of the coast.

The first suggestion to be able to observe the full potential of the current Avencas marine protected area is to improve its enforcement, alongside with other measures to maximize the effectiveness of this enforcement. A better surveillance and communication should be put in place so that the human impact could truly be reduced and give a chance to the ecosystem to take the best out of this protection. The implementation of controls or actions to make sure the

rules are respected is a real concern. First, putting more distinctive signs in the area could help people understand that there are protection measures in place and what they are allowed to do or not. This is the first step to make sure that everyone is aware of the local situation and to increase the chances that people (inhabitants, tourists, fishermen, etc.) respects the rules. Increasing the focus on the communication could also help to rise the interest on this area and the biodiversity that it hosts (through flagship species for example): on the municipality website, on other touristic or local websites, in the tourist offices of the surrounding cities, in schools, etc. Then, actively controlling what is happening in the area could enforce the implemented measures, making sure that everyone respects them.

Communication, sensibilization and control of the area require financial resources, but an effective marine protected area can also have many economic benefits that can quickly compensate this investment (Davis *et al.*, 2019). In fact, it is possible to translate ecological benefits to economic benefits, including market and non-market benefits (Figure 31). These benefits can be increased fisheries profitability, mainly due to the spillover of fish biomass from effective MPAs to the near fished areas, but also increased tourism and the provision of ecosystem services.

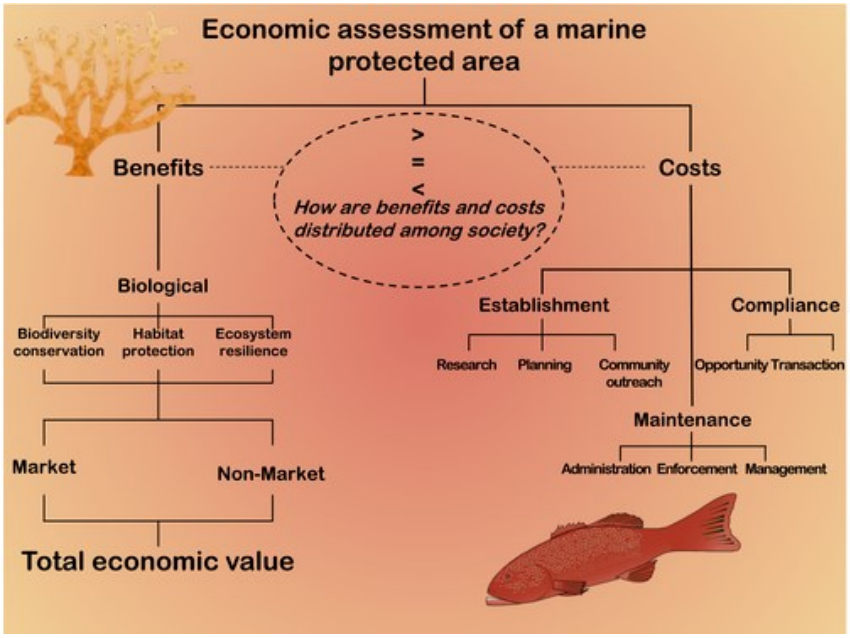


Figure 31. Components of a comprehensive economic assessment of a marine protected area, including major benefits and costs that need to be considered, source : (Davis *et al.*, 2019)

Having a good understanding of AMPA and its effectiveness through time is also a key issue, so the second suggestion is to continue the ecological monitoring work for a longer period of time. The objective would be to evaluate if the effects of protection are more visible as the AMPA approaches a minimum of 10 years since it was created. However, juvenile fish are being monitored for more than 10 years now (data not shown) and no clear tendency of recovery was observed in the last 4 years since the regulation of this MPA.

Numerous studies on MPAs effectiveness focus on fish abundances and biomass but having a closer look at invertebrates and algae can be useful to understand all the processes and dynamics occurring in the ecosystem. It is fundamental to have a better comprehension of the ecosystem's response to protection measures. Moreover, this thesis focused on effectiveness of AMPA but is only on intertidal data. A follow-up could be to perform the same work with data from the subtidal zone or, even better, to include the two datasets to obtain an overall image of the biodiversity within the AMPA. In fact, as mentioned above, the subtidal zone usually has a much higher species richness and diversity.

Despite some technical limitations, DNA barcoding has proved to be extremely useful in ecosystem monitoring and represents an essential tool that should continue to be used in the future.

For further studies and other projects, analyzing community composition more into details would also be a real asset to understand the functioning of this ecosystem. This could be performed by taking the taxonomic groups into account during the statistical analysis and also their respective biology and ecology. This would allow to have a better view over the different functional groups present, the relationships between species (e.g. predation or competition), how the different species coexist and if their distributions are related, etc. The more is known on an ecosystem, the easier it is to implement the appropriate measures to preserve it.

Another approach that could be developed are the seasonal differences. In this thesis, due to number of months dedicated to the field work and the sampling interruption during the spring, this analysis was not possible. Most of the species present in the area likely change in terms of presence and abundances along the year in different seasons. Describing the relationship between seasons and response to protection measures could also bring new and useful information to local management.

Finally, expanding the marine protected area and/or bring new areas under protection together with effective communication/education actions and enforcement measures could have a great influence on the effectiveness of MPAs in this region. This study suggests a potential expansion of the AMPA the east and a future larger MPA to the west creating a network where mobile individuals can move and where sessile individuals can spread their gametes. Having one or more effective MPAs nearby could be beneficial to preserve or even to recover the marine biodiversity in this region (Zupan *et al.*, 2018).

Overall, this thesis shows the importance of further development of the protection measures of marine ecosystems to avoid ineffective and unprotected MPAs, pointing promising leads for future improvements and increased preservation of marine biodiversity.

References

Affenzeller, S., Haar, N. and Steiner, G. (2017) 'Revision of the genus complex Gibbula: an integrative approach to delineating the Eastern Mediterranean genera Gibbula Risso, 1826, Steromphala Gray, 1847, and Phorcus Risso, 1826 using DNA-barcoding and geometric morphometrics (Vetigastropoda, Trochoidea', *Organisms Diversity and Evolution*. Organisms Diversity & Evolution, 17(4), pp. 789–812. doi: 10.1007/s13127-017-0343-5.

Aichi (2012) 'Aichi Biodiversity Targets', pp. 10–11.

Alder, J. *et al.* (2006) 'Marine and Coastal Ecosystems and Human Well-Being'.

Anderson, M. J. and Walsh, D. C. I. (2013) 'PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing?', *Ecological Monographs*, 83(4), pp. 557–574. doi: 10.1890/12-2010.1.

Ardura, A. *et al.* (2016) 'Rate of biological invasions is lower in coastal marine protected areas', *Scientific Reports*. Nature Publishing Group, 6(December 2015), pp. 1–11. doi: 10.1038/srep33013.

Ardura, A. and Planes, S. (2017) 'Rapid assessment of non-indigenous species in the era of the eDNA barcoding: A Mediterranean case study', *Estuarine, Coastal and Shelf Science*. Elsevier Ltd, 188, pp. 81–87. doi: 10.1016/j.ecss.2017.02.004.

Barceló, C. *et al.* (2016) 'Eight decades of sampling reveal a contemporary novel fish assemblage in coastal nursery habitats', *Global Change Biology*, 22(3), pp. 1155–1167. doi: 10.1111/gcb.13047.

Barco, A. *et al.* (2013) 'Testing the applicability of DNA barcoding for Mediterranean species of top-shells (Gastropoda, Trochidae, Gibbula s.l.)', *Marine Biology Research*, 9(8), pp. 785–793. doi: 10.1080/17451000.2013.765585.

Baxter, M. (2016) *Marine Protected Areas and climate change :*

Bayley, D. T. I. *et al.* (2019) 'Evaluating the efficacy of small-scale marine protected areas for preserving reef health: A case study applying emerging monitoring technology', *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29(12), pp. 2026–2044. doi: 10.1002/aqc.3215.

Benedetti-Cecchi, L. *et al.* (2003) 'Implications of spatial heterogeneity for management of marine protected areas (MPAs): Examples from assemblages of rocky coasts in the northwest Mediterranean', *Marine Environmental Research*, 55(5), pp. 429–458. doi: 10.1016/S0141-1136(02)00310-0.

Board, W. E. (2020) *World Register of Marine Species*. doi: 10.14284/170.

Borges, L. M. S. *et al.* (2016) 'With a little help from DNA barcoding: Investigating the diversity of Gastropoda from the Portuguese coast', *Scientific Reports*. Nature Publishing Group, 6(February). doi: 10.1038/srep20226.

Borja, A., Franco, J. and Pérez, V. (2000) 'A marine Biotic Index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments', *Marine Pollution Bulletin*, 40(12), pp. 1100–1114. doi: 10.1016/S0025-326X(00)00061-8.

Boudouresque, C. and Verlaque, M. (2001) 'Ecology of Paracentrotus lividus', in Lawrence, J. M. (ed.) *Edible Sea Urchins: Biology and Ecology*.

Bucklin, A., Steinke, D. and Blanco-Bercial, L. (2011) 'DNA Barcoding of Marine Metazoa', *Annual Review of Marine Science*, 3(1), pp. 471–508. doi: 10.1146/annurev-marine-120308-080950.

Carlton, J. T. (1999) ‘Molluscan invasions in marine and estuarine communities’, *Malacologia*, 41(2), pp. 439–454.

Chainho, P. *et al.* (2015) ‘Non-indigenous species in Portuguese coastal areas, coastal lagoons, estuaries and islands’, *Estuarine, Coastal and Shelf Science*, 167, pp. 199–211. doi: 10.1016/j.ecss.2015.06.019.

Clarke, K. R. (1993) ‘Non-parametric multivariate analyses of changes in community structure’, *Australian Journal of Ecology*, 18(1), pp. 117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x.

Clarke, K. R., Somerfield, P. J. and Chapman, M. G. (2006) ‘On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages’, *Journal of Experimental Marine Biology and Ecology*, 330(1), pp. 55–80. doi: 10.1016/j.jembe.2005.12.017.

Clarke, K. and Warwick, R. (2001) ‘Change in marine communities: an approach to statistical analysis and interpretation. 2nd edition. Primer-E, Plymouth’, *Plymouth, United Kingsom: PRIMER-E*, pp. 1–172. doi: 1.

Claudet, J. *et al.* (2020) ‘Underprotected Marine Protected Areas in a Global Biodiversity Hotspot’, *One Earth*. Elsevier Inc., 2(4), pp. 380–384. doi: 10.1016/j.oneear.2020.03.008.

Dangendorf, S. *et al.* (2017) ‘Reassessment of 20th century global mean sea level rise’, *Proceedings of the National Academy of Sciences of the United States of America*, 114(23), pp. 5946–5951. doi: 10.1073/pnas.1616007114.

Davenport, J., Moloney, T. V. and Kelly, J. (2011) ‘Common sea anemones Actinia equina are predominantly sessile intertidal scavengers’, *Marine Ecology Progress Series*, 430, pp. 147–155. doi: 10.3354/meps08861.

Davis, K. J. *et al.* (2019) ‘Estimating the economic benefits and costs of highly-protected marine protected areas’, *Ecosphere*, 10(10). doi: 10.1002/ecs2.2879.

Dias, M. *et al.* (2016) ‘Intertidal pools as alternative nursery habitats for coastal fishes’, *Marine Biology Research*. Taylor & Francis, 12(4), pp. 331–344. doi: 10.1080/17451000.2016.1143106.

Dytham, C. (2011) *Choosing and using statistics: A Biologist’s guide (3rd edition)*. John Wiley. Chicester, United Kingdom.

Edgar, G. J. *et al.* (2014) ‘Global conservation outcomes depend on marine protected areas with five key features’, *Nature*, 506(7487), pp. 216–220. doi: 10.1038/nature13022.

Eriksen, M. *et al.* (2014) ‘Plastic Pollution in the World’s Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea’, *PLoS ONE*, 9(12), pp. 1–15. doi: 10.1371/journal.pone.0111913.

Ferrario, J. *et al.* (2017) ‘Role of commercial harbours and recreational marinas in the spread of non-indigenous fouling species’, *Biofouling*. Taylor & Francis, 33(8), pp. 651–660. doi: 10.1080/08927014.2017.1351958.

Ferreira, A. *et al.* (2017) ‘Ecosystem response to different management options in Marine Protected Areas (MPA): A case study of intertidal rocky shore communities’, *Ecological Indicators*, 81(1830), pp. 471–480. doi: 10.1016/j.ecolind.2017.06.028.

Ferreira, A., Seixas, S. and Marques, J. C. (2015) ‘“Bottom-up management approach to coastal marine protected areas in Portugal”’, *Ocean and Coastal Management*, 118(May), pp. 275–281. doi:

10.1016/j.ocecoaman.2015.05.008.

Ferreira, M. A. and Andrade, F. (2003) 'Intertidal communities as indicators of environmental change and their potential use in biomonitoring: The Troia Resort (Portugal), a large-scale tourist development, as a case study', *Boletin - Instituto Espanol de Oceanografia*, 19(1–4), pp. 253–264.

Folmer, O. *et al.* (1994) 'DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.', *Molecular marine biology and biotechnology*, 3(5), pp. 294–299. doi: 10.1071/ZO9660275.

Fredston-Hermann, A. *et al.* (2016) 'Where does river runoff matter for coastal marine conservation?', *Frontiers in Marine Science*, 3(DEC), pp. 1–10. doi: 10.3389/fmars.2016.00273.

Gardener, M. (2017) *Statistics for Ecologists using R and Excel: Data Collection, Exploration, Analysis and Presentation (2nd edition).* Exeter, United Kingdom: Pelagic Publishing.

Gaston, K. J. *et al.* (2006) 'The ecological effectiveness of protected areas: The United Kingdom', *Biological Conservation*, 132(1), pp. 76–87. doi: 10.1016/j.biocon.2006.03.013.

Gil Fernández, C. *et al.* (2016) 'Limited differences in fish and benthic communities and possible cascading effects inside and outside a protected marine area in Sagres (SW Portugal)', *Marine Environmental Research*, 114, pp. 12–23. doi: 10.1016/j.marenvres.2015.12.003.

Gonçalves, S. C. *et al.* (2009) 'Sandy beach macrofaunal communities on the western coast of Portugal – Is there a steady structure under similar exposed conditions?', *Estuarine, Coastal and Shelf Science*, 81(4), pp. 555–568. doi: https://doi.org/10.1016/j.ecss.2008.12.004.

Gotelli, N. J. and Chao, A. (2013) *Measuring and Estimating Species Richness, Species Diversity, and Biotic Similarity from Sampling Data*, *Encyclopedia of Biodiversity: Second Edition*. Elsevier Ltd. doi: 10.1016/B978-0-12-384719-5.00424-X.

Guerra-García, J. M. *et al.* (2006) 'Assessing a quick monitoring method using rocky intertidal communities as a bioindicator: A multivariate approach in Algeciras Bay', *Environmental Monitoring and Assessment*, 116(1–3), pp. 345–361. doi: 10.1007/s10661-006-7572-y.

Halpern, B. S. (2003) 'The impact of marine reserves: Do reserves work and does reserve size matter?', *Ecological Applications*, 13(1 SUPPL.). doi: 10.1890/1051-0761(2003)013[0117:tiomrd]2.0.co;2.

Haward, M. (2018) 'Plastic pollution of the world's seas and oceans as a contemporary challenge in ocean governance', *Nature Communications*. Springer US, 9(1), pp. 9–11. doi: 10.1038/s41467-018-03104-3.

Hayward, P. and Ryland, J. . (1990) *Handbook of the Marine Fauna of North-West Europe*. Edited by O. Oxford.

Hebert, P. D. N. and Gregory, T. R. (2005) 'The promise of DNA barcoding for taxonomy', *Systematic Biology*, 54(5), pp. 852–859. doi: 10.1080/10635150500354886.

Hily, C. and Jean, F. (1997) 'Macrobenthic biodiversity in intertidal habitats of the Iroise Biosphere Reserve', *Journal of the Marine Biological Association of the United Kingdom*, 77(2), pp. 311–323. doi: https://doi.org/10.1017/S0025315400071691.

Horta E Costa, B. *et al.* (2014) 'Tropicalization of fish assemblages in temperate biogeographic transition zones', *Marine Ecology Progress Series*, 504, pp. 241–252. doi: 10.3354/meps10749.

Humphreys, J. and Clark, R. W. E. (2020) *A critical history of marine protected areas*, *Marine Protected Areas*. Elsevier Ltd. doi: 10.1016/b978-0-08-102698-4.00001-0.

IMPANA (2019) 'Preparing for the 2020 World Conservation Congress KEY DATES – MOTIONS – EVENTS', pp. 1–4.

IPCC (2019) 'IPCC Special Report on the Ocean and Cryosphere in a Changing Climate [H.-O. Pörtner Roberts, D.C. Masson-Delmotte, V. Zhai, P. Tignor, M. Poloczanska, E. Mintenbeck, K. Nicolai, M. Okem, A. Petzold, J. B. Rama, N. Weyer (eds.)]', *In press*, (September). doi: https://www.ipcc.ch/report/srocc/.

Jackson, J. B. C. (2009) 'Ecological extinction and evolution in the brave new ocean', *In the Light of Evolution*, 2, pp. 5–26. doi: 10.17226/12501.

Junoy, J. and Vieitez, J. M. (1992) 'Macrofaunal abundance analyses in the Ria de Foz (Lugo, northwest Spain)', *Cahiers de Biologie Marine*, 33(3), pp. 331–345.

Keele, J. *et al.* (2014) 'Identification of Unknown Organisms by DNA Barcoding : A Molecular Method for Species Classification', *Trends in Genetics*, 23(4), pp. 167–172. doi: Research and Development Office, Final report 2014-01 (0045).

Kenchington, E. L. (2010) 'The effects of fishing on species and genetic diversity.', *Responsible fisheries in the marine ecosystem*, (May), pp. 235–253. doi: 10.1079/9780851996332.0235.

Kirchner, S. and Kleemola-Juntunen, P. (2018) 'Dumping and oil pollution: Regulatory approaches for vessel operations in an ice-free central Arctic Ocean', *Review of European, Comparative and International Environmental Law*, 27(1), pp. 28–34. doi: 10.1111/reel.12246.

Kotta, J. *et al.* (2017) 'Essence of the patterns of cover and richness of intertidal hard bottom communities: A pan-European study', *Journal of the Marine Biological Association of the United Kingdom*, 97(3), pp. 525–538. doi: 10.1017/S0025315416001351.

Lastra, M. *et al.* (2006) 'Ecology of exposed sandy beaches in northern Spain: Environmental factors controlling macrofauna communities', *Journal of Sea Research*, 55(2), pp. 128–140. doi: https://doi.org/10.1016/j.seares.2005.09.001.

Loron, C. C. *et al.* (2019) 'Early fungi from the Proterozoic era in Arctic Canada', *Nature*, 570(7760), pp. 232–235. doi: 10.1038/s41586-019-1217-0.

Lourenço, P. M. *et al.* (2018) 'First record of the invasive asian date mussel arcuatula senhousia (Benson, 1842) (mollusca: Bivalvia: Mytilidae) in west africa', *Journal of Natural History*. Taylor & Francis, 52(39–40), pp. 39–40. doi: 10.1080/00222933.2018.1545058.

Loyld, W. F. (1833) *Two Lectures on the Checks to Population*. Oxford.

Lubchenco, J. *et al.* (2003) 'Plugging a hole in the ocean: The emerging science of marine reserves', *Ecological Applications*, 13(1 SUPPL.), pp. 3–7. doi: 10.1890/1051-0761(2003)013[0003:PAHITO]2.0.CO;2.

Marinesque, S., Kaplan, D. M. and Rodwell, L. D. (2012) 'Global implementation of marine protected areas: Is the developing world being left behind?', *Marine Policy*. Elsevier, 36(3), pp. 727–737. doi: 10.1016/j.marpol.2011.10.010.

Marti-Puig, P. *et al.* (2013) 'Patterns of genetic connectivity in invertebrates of temperate MPA networks', *Advances in Oceanography and Limnology*. Taylor & Francis, 4(2), pp. 138–149. doi: 10.1080/19475721.2013.850445.

Martinetto, P. *et al.* (2020) 'Linking the scientific knowledge on marine frontal systems with ecosystem services', *Ambio*, 49(2), pp. 541–556. doi: 10.1007/s13280-019-01222-w.

Mccauley, D. *et al.* (2017) 'Marine reserves can mitigate and promote adaptation to climate change', (June). doi: 10.1073/pnas.1701262114.

De Montaudouin, X. and Sauriau, P. G. (2000) 'Contribution to a synopsis of marine species richness in the Pertuis Charentais Sea with new insights in soft-bottom macrofauna of the Marennes-Oleron Bay', *Cahiers de Biologie Marine*, 41(2), pp. 181–222. doi: 10.21411/CBM.A.2E300127.

Mora, C. *et al.* (2011) 'How many species are there on earth and in the ocean?', *PLoS Biology*, 9(8), pp. 1–8. doi: 10.1371/journal.pbio.1001127.

Nerem, R. S. *et al.* (2018) 'Climate-change–driven accelerated sea-level rise detected in the altimeter era', *Proceedings of the National Academy of Sciences of the United States of America*, 115(9), pp. 2022–2025. doi: 10.1073/pnas.1717312115.

Newell, P. D. *et al.* (2013) 'A Small-Group Activity Introducing the Use and Interpretation of BLAST †', *Journal of Microbiology & Biology Education*, 14(2), pp. 238–243. doi: 10.1128/jmbe.v14i2.637.

NOAA (2019) *How much water is in the ocean?* Available at: https://oceanservice.noaa.gov/facts/oceanwater.html (Accessed: 18 March 2020).

Olenin, S. *et al.* (2016) 'New arrivals: An indicator for non-indigenous species introductions at different geographical scales', *Frontiers in Marine Science*, 3(OCT). doi: 10.3389/fmars.2016.00208.

Oliveira, J. P. *et al.* (2014) 'Urban vs. extra-urban environments: Scales of variation of intertidal benthic assemblages in north Portugal', *Marine Environmental Research*. Elsevier Ltd, 97, pp. 48–57. doi: 10.1016/j.marenvres.2014.02.005.

Pais, M. P. *et al.* (2020) *AquaSig 3 Relatório Fevereiro 2020*.

Pendleton, L. H. *et al.* (2018) 'Debating the effectiveness of marine protected areas', *ICES Journal of Marine Science*, 75(3), pp. 1156–1159. doi: 10.1093/icesjms/fsx154.

Pereira, A. (2017) 'Parte I - Relatório Geral'.

Planque, B. *et al.* (2010) 'How does fishing alter marine populations and ecosystems sensitivity to climate?', *Journal of Marine Systems*. Elsevier B.V., 79(3–4), pp. 403–417. doi: 10.1016/j.jmarsys.2008.12.018.

Pomerantz, A. *et al.* (2018) 'Real-time DNA barcoding in a rainforest using nanopore sequencing: Opportunities for rapid biodiversity assessments and local capacity building', *GigaScience*, 7(4), pp. 1–14. doi: 10.1093/gigascience/giy033.

Pomeroy, R. S., Parks, J. E. and Watson, L. M. (2004) *How is your MPA doing ? A guidebook of natural and social indicators for evaluating marine protected areas management effectiveness*, *How is your MPA doing ? A guidebook of natural and social indicators for evaluating marine protected areas management effectiveness*. doi: 10.2305/iucn.ch.2004.paps.1.en.

De Poorter, M., Darby, C. and MacKay, J. (2008) 'Marine Menace', *Marine Menace: Alien invasive species in the marine environment*, pp. 1–32. Available at: http://www.issg.org/pdf/publications/marine_menace_english.pdf.

Rasmussen, D. (2010) 'Comparisons of Fish Species Inside and Outside of Marine Protected Areas Off the South Central Coast of California', (September), p. 104.

Remoundou, K. *et al.* (2009) 'Valuation of natural marine ecosystems: an economic perspective', *Environmental Science and Policy*, 12(7), pp. 1040–1051. doi: 10.1016/j.envsci.2009.06.006.

Ricciardi, A. and Bourget, E. (1999) 'Global patterns of macroinvertebrate biomass in marine intertidal communities', *Marine Ecology Progress Series*, 185, pp. 21–35. doi: 10.3354/meps185021.

Rizgalla, J., Shinn, A. P. and Crocetta, F. (2019) 'First documented record of the invasive cockle fulvia fragilis (Forsskål in niebuhr, 1775) (mollusca: Bivalvia: Cardiidae) in libya', *BioInvasions Records*, 8(2), pp. 314–319. doi: 10.3391/bir.2019.8.2.13.

Rodil, I. F. and Lastra, M. (2004) 'Environmental factors affecting benthic macrofauna along a gradient of intermediate sandy beaches in northern Spain', *Estuarine, Coastal and Shelf Science*, 61(1), pp. 37–44. doi: https://doi.org/10.1016/j.ecss.2004.03.016.

Rodriguez-Prieto, C. *et al.* (2013) *Guia de las Macroalgas y fanerogamas marinas del mediterraneo occidental*. Omega.

Roopnarine, P. (2013) 'Ecology and the tragedy of the commons', *Sustainability (Switzerland)*, 5(2), pp. 749–773. doi: 10.3390/su5020749.

Sadio, O. *et al.* (2015) 'Effect of a marine protected area on tropical estuarine fish assemblages: Comparison between protected and unprotected sites in Senegal', *Ocean and Coastal Management*, 116, pp. 257–269. doi: 10.1016/j.ocecoaman.2015.08.004.

Sala, E. and Giakoumi, S. (2018) 'No-take marine reserves are the most effective protected areas in the ocean', *ICES Journal of Marine Science*, 75(3), pp. 1166–1168. doi: 10.1093/icesjms/fsx059.

Schroeder, P. J. and Jenkins, D. G. (2018) 'How robust are popular beta diversity indices to sampling error', *Ecosphere*, 9(2). doi: 10.1002/ecs2.2100.

Sciberras, M. *et al.* (2013) 'Evaluating the biological effectiveness of fully and partially protected marine areas', *Environmental Evidence*, 2(1), pp. 1–31. doi: 10.1186/2047-2382-2-4.

Scriver, M. *et al.* (2015) 'Development of species-specific environmental DNA (eDNA) markers for invasive aquatic plants', *Aquatic Botany*. Elsevier B.V., 122, pp. 27–31. doi: 10.1016/j.aquabot.2015.01.003.

Scrosati, R. A. *et al.* (2011) 'Species richness and diversity in different functional groups across environmental stress gradients: A model for marine rocky shores', *Ecography*, 34(1), pp. 151–161. doi: 10.1111/j.1600-0587.2010.06119.x.

Selig, E. R. *et al.* (2014) 'Global priorities for marine biodiversity conservation', *PLoS ONE*, 9(1), pp. 1–11. doi: 10.1371/journal.pone.0082898.

Simon, S. *et al.* (1994) 'Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers', *Ann Ent Soc Am*.

Small, C. and Nicholls, R. J. (2003) 'A global analysis of human settlement in coastal zones', *Journal of Coastal Research*, 19(3), pp. 584–599.

Stat, M. *et al.* (2017) 'Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment', *Scientific Reports*. Springer US, 7(1), pp. 1–11. doi: 10.1038/s41598-017-12501-5.

Tait, L. W. and Schiel, D. R. (2013) 'Impacts of Temperature on Primary Productivity and Respiration in Naturally Structured Macroalgal Assemblages', *PLoS ONE*, 8(9), pp. 1–10. doi:

10.1371/journal.pone.0074413.

Todd, P. A. *et al.* (2019) 'Towards an urban marine ecology: characterizing the drivers, patterns and processes of marine ecosystems in coastal cities', *Oikos*, 128(9), pp. 1215–1242. doi: 10.1111/oik.05946.

Trivedi, S. *et al.* (2016) 'Role of DNA barcoding in marine biodiversity assessment and conservation: An update', *Saudi Journal of Biological Sciences*. King Saud University, 23(2), pp. 161–171. doi: 10.1016/j.sjbs.2015.01.001.

Turnbull, J. W. *et al.* (2018) 'Key drivers of effectiveness in small marine protected areas', *Biodiversity and Conservation*. Springer Netherlands, 27(9), pp. 2217–2242. doi: 10.1007/s10531-018-1532-z.

Ugland, K. I., Gray, J. S. and Ellingsen, K. E. (2003) 'The species-accumulation curve and estimation of species richness', *Journal of Animal Ecology*, 72(5), pp. 888–897. doi: 10.1046/j.1365-2656.2003.00748.x.

UN (2017) *World population projected to reach 9.8 billion in 2050, and 11.2 in 2100*.

Uribe, J. E. *et al.* (2017) 'Phylogenetic relationships of Mediterranean and North-East Atlantic Cantharidinae and notes on Stomatellinae (Vetigastropoda: Trochidae)', *Molecular Phylogenetics and Evolution*, 107, pp. 64–79. doi: 10.1016/j.ympev.2016.10.009.

Valentini, A., Pompanon, F. and Taberlet, P. (2009) 'DNA barcoding for ecologists', *Trends in Ecology and Evolution*, 24(2), pp. 110–117. doi: 10.1016/j.tree.2008.09.011.

Vandeperre, F. *et al.* (2011) 'Effects of no-take area size and age of marine protected areas on fisheries yields: A meta-analytical approach', *Fish and Fisheries*, 12(4), pp. 412–426. doi: 10.1111/j.1467-2979.2010.00401.x.

Weigand, H. *et al.* (2019) 'DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work', *Science of the Total Environment*, 678, pp. 499–524. doi: 10.1016/j.scitotenv.2019.04.247.

Westera, M., Lavery, P. and Hyndes, G. (2003) 'Differences in recreationally targeted fishes between protected and fished areas of a coral reef marine park', *Journal of Experimental Marine Biology and Ecology*, 294(2), pp. 145–168. doi: 10.1016/S0022-0981(03)00268-5.

WGITMO (2018) 'ICES WGITMO Report 2018 Working Group on Introductions and Transfers of Marine Organisms (WGITMO)', (March), pp. 16–17.

Witman, S. (2017) 'World's Biggest Oxygen Producers Living in Swirling Ocean Waters', *Eos*. doi: 10.1029/2017EO081067.

WWF (2019) 'Protecting Our Ocean: Europe's challenges to meet the 2020 deadlines'. Available at: https://wwf.panda.org/knowledge_hub/all_publications/?352798/Protecting-Our-Ocean-Europes-challenges-to-meet-the-2020-deadlines.

Zeng, X., Chen, X. and Zhuang, J. (2015) 'The positive relationship between ocean acidification and pollution', *Marine Pollution Bulletin*. Elsevier Ltd, 91(1), pp. 14–21. doi: 10.1016/j.marpolbul.2014.12.001.

Zupan, M. *et al.* (2018) 'Marine partially protected areas: drivers of ecological effectiveness', *Frontiers in Ecology and the Environment*, 16(7), pp. 381–387. doi: 10.1002/fee.1934.

Appendices

Appendix A.

1.Within the limits of the Avencas MPA, the following acts and activities are prohibited:
a) Introduction of non-indigenous species, flora or fauna, according to the legislation in force
b) Collection of biological and geological samples or any acts that contribute to the degradation or destruction of the natural heritage, except for studies carried out for exclusively scientific purposes and duly authorized by the Portuguese Environmental Agency
c) Changes in soil morphology and modification of the vegetation cover, except for environmental recovery interventions authorized by the Portuguese Environmental Agency
d) Actions that may introduce changes in the coastal dynamics and coastal modification, except for maintaining coastal defense structures existing
e) Carrying out artificial feeding operations on the beaches within the limits of the AMPA
f) Anchoring of any type of vessel, except for cases of vessels inserted in scientific research or conservation projects nature, under the conditions set out in the respective licenses or authorizations
g) Installation of aquaculture units
h) Practice of motorized water sports
i) Sport fishing competitions
j) Picking, playful or professional, of any specimens of local fauna and flora
k) Fishing with any trawl gear, including the hook
l) The use of gillnets

2.Within the limits of the Avencas MPA, recreational fishing is only allowed in the cane and underwater fishing modes, in the following terms:
a) Be a holder of the 'Sustainable Fisherman' card obtained in the mandatory training for the purpose and issued by the Directorate-General for Natural Resources, Security and Maritime Services
b) Practitioners must respect minimum distance of 10 m from each other and only use one line with a hook per practitioner
c) Spearfishing practitioners are conditioned to a maximum total daily catch weight of 7.5 kg

3.Within the limits of the Avencas AMP, activities authorized by the Captaincy of the Port of Cascais must follow some guidelines:
a) They should enter in these categories: Carrying out research / monitoring work, carrying out of nature tourism activities or environmental education and awareness actions
b) Environmental education and awareness actions should contemplate the existence of two responsible for every 15 participants.
c) The movement of users on rocky platforms at low tide must follow the marked paths and / or other orientations for that purpose.

Appendix B.

Point	Zone	N Coordinates	W Coordinates	Error	Direction	Area
P1M1	Midlittoral 1	38°41.06590'N	9°21.0740'W	3,2	280°	Hospital de Sant'Ana
P1M2	Midlittoral 2	38°41.0580'N	9°21.0930'W	3,2	298°	Hospital de Sant'Ana
P1S1	Supralittoral	38°41.0730'N	9°21.0890'W	4,3	294°	Hospital de Sant'Ana
P2M1	Midlittoral 1	38°41,1480´N	9°21,4500'W	3,2	292°	**AMPA EAST**
P2M2	Midlittoral 2	38°41,1520´N	9°21,4430'W	3,2	315°	**AMPA EAST**
P2S1	Supralittoral	38°41,1770´N	9°21,4460'W	3,2	300°	**AMPA EAST**
P3M1	Midlittoral 1	38°41,1670´N	9°21,5150'W	3,2	329°	**AMPA WEST**
P3M2	Midlittoral 2	38°41,1720´N	9°21,5070'W	3,2	318°	**AMPA WEST**
P3S1	Supralittoral	38°41.2020'N	9°21.5130'W	3,2	309°	**AMPA WEST**
P4M1	Midlittoral 1	38°42.0990'N	9°23.6960'W	3,2	234°	Praia da Poça
P4M2	Midlittoral 2	38°42.1080'N	9°23.6980'W	3,2	227°	Praia da Poça
P4S1	Supralittoral	38°42.0540'N	9°23.4290'W	4,3	170°	Praia da Poça
P5M1	Midlittoral 1	38°42.5550'N	9°29.1860'W	3,2	161°	Cabo Raso
P5M2	Midlittoral 2	38°42.5880'N	9°29.1760'W	3,2	62°	Cabo Raso
P5S1	Supralittoral	38°42.5840'N	9°29.1720'W	3,2	44°	Cabo Raso

Appendix C.

Point	Location	Zone	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8
P1S1	Hospital	Supralittoral	15/11/2019	13/12/2019	11/01/2020	25/02/2020	13/03/2020	24/05/2020	05/06/2020	08/07/2020
P1M1	Hospital	Midlittoral 1	tide too high	tide too high	11/01/2020	25/02/2020	13/03/2020	24/05/2020	05/06/2020	23/07/2020
P1M2	Hospital	Midlittoral 2	tide too high	13/12/2019	11/01/2020	25/02/2020	13/03/2020	24/05/2020	05/06/2020	08/07/2020
P2S1	AMPA East	Supralittoral	26/11/2019	27/12/2019	12/01/2020	12/02/2020	09/03/2020	23/05/2020	22/06/2020	06/07/2020
P2M1	AMPA East	Midlittoral 1	26/11/2019	27/12/2019	12/01/2020	12/02/2020	09/03/2020	23/05/2020	22/06/2020	06/07/2020
P2M2	AMPA East	Midlittoral 2	26/11/2019	27/12/2019	12/01/2020	12/02/2020	09/03/2020	23/05/2020	22/06/2020	06/07/2020
P3S1	AMPA West	Supralittoral	27/11/2019	not sampled	25/01/2020	24/02/2020	10/03/2020	not sample	04/06/2020	21/07/2020
P3M1	AMPA West	Midlittoral 1	27/11/2019	not sampled	25/01/2020	24/02/2020	10/03/2020	not sample	04/06/2020	21/07/2020
P3M2	AMPA West	Midlittoral 2	27/11/2019	not sampled	26/01/2020	24/02/2020	10/03/2020	not sample	04/06/2020	21/07/2020
P4S1	Praia da Poça	Supralittoral	not sampled	11/12/2019	14/01/2020	10/02/2020	12/03/2020	22/05/2020	21/06/2020	07/07/2020
P4M1	Praia da Poça	Midlittoral 1	not sampled	11/12/2019	14/01/2020	10/02/2020	12/03/2020	22/05/2020	21/06/2020	07/07/2020
P4M2	Praia da Poça	Midlittoral 2	not sampled	11/12/2019	14/01/2020	10/02/2020	12/03/2020	22/05/2020	21/06/2020	07/07/2020
P5S1	Cabo Raso	Supralittoral	13/11/2019	28/12/2019	13/01/2020	11/02/2020	11/03/2020	not sample	06/06/2020	22/07/2020
P5M1	Cabo Raso	Midlittoral 1	tide too high	tide too high	tide too hig	tide too high	11/03/2020	not sample	06/06/2020	tide too hig
P5M2	Cabo Raso	Midlittoral 2	tide too high	28/12/2019	13/01/2020	11/02/2020	11/03/2020	not sample	06/06/2020	22/07/2020

Appendix D.

Phylum (Division) **Cnidaria**;
Class **Anthozoa**;
Order **Actinaria**

Actinia equina

From Naturdata; Miguel Pais, Cabo Raso

Physical characteristics:
- Up to 50mm in height and diameter
- Around 200 tentacles of moderate length and retractable, arranged in 6 circles.
-Base with blue border
-Variable color: red, orange, green or brown.

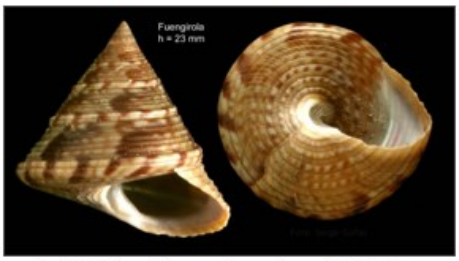
From Naturdata; peterwirtz2004@yahoo.com

From Naturdata: Miguel Berkemeier

Location:
-Both in exposed and sheltered situations, until 20m deep.
-Attached to hard substrata.
-Highly adapted to the intertidal zone as it can tolerate both high temperatures and desiccation
-May also be found in regions of variable salinity (e.g. estuaries)

Phylum (Division) **Mollusca**;
Class **Gastropoda**;
Order **Trochida**

Calliostoma zizyphinum



Retirada de http://www.marinespecies.org/; Gofas, Serge

Physical characteristics:
-Very conical, pointed and shiny shell
-30 mm high
-8 to 10 turns of striated whorl separated by a thick cord
-Variable color bands, usually pink, red or mauve
-Wide and oblique opening blocked by a horny cover
-Very flat and striated base

Location:
-Infralittoral
-Drop-offs and rock overhangs
-Fronds of aminaria
-Bottom of foreshore, protected from light
-Up to 100m deep

Retirada de http://www.marinespecies.org/; Natural History Museum Rotterdam

Phylum (Division) **Cnidaria**;
Class **Anthozoa**;
Order **Actinaria**

Cereus pedunculatus

Pedro Coelho, Portugal

Physical characteristics:
-Oral disc wide (40-50mm)
-Body up to a diameter of 100mm
-Numerous short tentacles (usually >700) arranged hexamerously
-Flattened body
-Dark column
-Trunk covered with small dots and can be cream, pink, brown or violet
-Whittish verrucae

Location:
-Often with the base and column concealed in holes and crevices
-Typically in rock pools
-Or in muddy gravel where it is anchored to a stone

Pedro Coelho, Portugal

Phylum (Division) **Arthropoda**;
Class **Malacostraca**;
Order **Decapoda**

Clibanarius erythropus

Retirada de http://biodiversidade.eu/ Antúnez Glez - CC BY-NC

Physical characteristics:
-Carapace length about 15mm
-Abdomen is soft-shelled and sheltered in a gastropod shell
-Fixed, short and triangular rostrum
-Cylindrical, long and narrow peduncles
-Eyes with black cornea and white dots
-Fingers of the pincers of the second and third legs have red and blue dots
-Cephalothorax often tinted white

Location:
-Rock pools and sublittoral waters
-Sand/gravel/algae
-Low depths

Differences with Paguridae:
-*Clibanarius erythropus* has 2 claws with same size
- *Clibanarius erythropus* rarely goes below 4m deep

Phylum (Division) **Arthropoda**;
Class **Malacostrata**;
Order **Decapoda**

Palaemon serratus

From http://www.marinespecies.org/, Decleer Misiel

From http://www.marinespecies.org/, Roberto Pillon

Physical characteristics:
-Length up to 110mm
-Variable color (pinkish-brown)
-Tranxparant body
-Pereon and pleon often banded with brownish red
-Yellow points on legs and carapace
-Rostrum curves upwards, bifurcated at the tip and has 6-7 teeth on the lower edge

Location:
-Rocky/sandy crevices (prefer shadow)
-Depths of up to 40 meters

Difference with *Palaemon elegans*:
P. elegans is smaller, with a smaller rostrum, and the space between the first and the second spine of the rostrum is the same than between the 2nd and 3rd spine (larger in *P.serratus*)

Appendix E

TOTAL OBSERVED SPECIES IN ALL THE AREA SINCE NOVEMBER:		
Species	**Taxon**	**Category**
Acanthochitona crinita	Polyplacophora	Invertebrate
Acanthochitona sp.	Polyplacophora	Invertebrate
Acrosorium ciliolatum	Algae	Algae
Actinia equina	Cnidaria	Invertebrate
Actinia fragacea	Cnidaria	Invertebrate
Actinia striata	Cnidaria	Invertebrate
Actinothoe sphyrodeta	Cnidaria	Invertebrate
Anemonia sp.	Cnidaria	Invertebrate
Anotrichium tenue	Algae	Algae
Aplysia sp.	Gastropoda	Invertebrate
Apoglossum ruscifolium	Algae	Algae
Asparagopsis armata	Algae	Algae
Asparagopsis armata (fase Falkenber	Algae	Algae
Asterias rubens	Echinodermata	Invertebrate
Asterina gibbosa	Echinodermata	Invertebrate
Aulactinia verrucosa	Cnidaria	Invertebrate
Balanus sp.	Cirripedia	Invertebrate
Bifurcaria bifurcata	Algae	Algae
Bittium sp.	Gastropoda	Invertebrate
Bornetia secundiflora	Algae	Algae
Bryopsis hypnoides	Algae	Algae
Bryopsis pennata	Algae	Algae
Bryopsis plumosa	Algae	Algae
Bryopsis sp.	Algae	Algae
Bunodosoma biscayense	Cnidaria	Invertebrate
Callianassa sp.	Decapoda	Invertebrate
Callionymus lyra	Pisces	Vertebrate
Calliostoma zizyphinum	Gastropoda	Invertebrate
Cancer pagurus	Decapoda	Invertebrate
Carcinus maenas	Decapoda	Invertebrate
Cardita calyculata	Bivalvia	Invertebrate
Caulacanthus ustulatus	Algae	Algae
Ceramium sp.	Algae	Algae
Cereus pedunculatus	Cnidaria	Invertebrate
Chaetomorpha sp.	Algae	Algae
Champia parvula	Algae	Algae
Chondracanthus acicularis	Algae	Algae
Chondria coerulescens	Algae	Algae
Chondrus crispus	Algae	Algae
Chthamalus montagui	Cirripedia	Invertebrate

Ciliata mustela	Pisces	Vertebrate
Cladostephus spongiosum	Algae	Algae
Clibanarius erythropus	Decapoda	Invertebrate
Codium sp.	Algae	Algae
Colpomenia sp.	Algae	Algae
Coryphoblennius galerita	Pisces	Vertebrate
Cryptopleura ramosa	Algae	Algae
Cystoseira sp.	Algae	Algae

Dictyota cyanoloma	Algae	Algae
Dictyota dichotoma	Algae	Algae
Dictyota sp.	Algae	Algae
Diplodus sargus	Pisces	Vertebrate
Ellisolandia elongata	Algae	Algae
Eriphia verrucosa	Decapoda	Invertebrate
Eulalia viridis	Polychaeta	Invertebrate
Felimida krohni	Nudibranchia	Invertebrate
Fucus sp.	Algae	Algae
Gastroclonium reflexum	Algae	Algae
Gelidium corneum	Algae	Algae
Gelidium sp.	Algae	Algae
Gobius cobitis	Pisces	Vertebrate
Gobius paganellus	Pisces	Vertebrate
Gymnogongrus crenulatus	Algae	Algae
Halopteris filicina	Algae	Algae
Halopteris sp.	Algae	Algae
Hildenbrandia sp.	Algae	Algae
Holothuria (Panningothuria) forskali	Echinodermata	Invertebrate
Hymeniacidon perlevis	Porifera	Invertebrate
Hypoglossum sp.	Algae	Algae
Irus irus	Bivalvia	Invertebrate
Laurencia sp.	Algae	Algae
Lepadogaster sp.	Pisces	Vertebrate
Lepidochitona cinerea	Polyplacophora	Invertebrate
Leptochiton algesirensis	Polyplacophora	Invertebrate
Lipophrys pholis	Pisces	Vertebrate
Lipophrys trigloides	Pisces	Vertebrate
Lithophyllum byssoides	Algae	Algae
Lithophyllum incrustans	Algae	Algae
Lomentaria articulata	Algae	Algae
Marthasterias glacialis	Echinodermata	Invertebrate
Mastocarpus sp. (Petrocelis phase)	Algae	Algae
Mastocarpus stellatus	Algae	Algae

Melarhaphe neritoides	Gastropoda	Invertebrate
Mesophyllum lichenoides	Algae	Algae
Musculus costulatus	Bivalvia	Invertebrate
Mytillaster minimus	Bivalvia	Invertebrate
Mytilus sp.	Bivalvia	Invertebrate
Necora puber	Decapoda	Invertebrate
Nemalion elminthoides	Algae	Algae
Nereis sp.	Polychaeta	Invertebrate
Nerophis lumbriciformis	Pisces	Vertebrate
Nitophyllum punctatum	Algae	Algae
Nucella lapillus	Gastropoda	Invertebrate
Ocenebra edwardsii	Gastropoda	Invertebrate
Ocenebra sp.	Gastropoda	Invertebrate
Onchidella celtica	Gastropoda	Invertebrate
Onchidella sp.	Gastropoda	Invertebrate
Ophiuridae	Echinodermata	Invertebrate

Osmundea sp.	Algae	Algae
Pachygrapsus marmoratus	Decapoda	Invertebrate
Paguridae	Decapoda	Invertebrate
Palaemon serratus	Decapoda	Invertebrate
Palaemon sp.	Decapoda	Invertebrate
Parablennius gattorugine	Pisces	Vertebrate
Parablennius pilicornis	Pisces	Vertebrate
Paracentrotus lividus	Echinodermata	Invertebrate
Patella depressa	Gastropoda	Invertebrate
Patella sp.	Gastropoda	Invertebrate
Patella ulyssiponensis	Gastropoda	Invertebrate
Patella vulgata	Gastropoda	Invertebrate
Perforatus perforatus	Cirripedia	Invertebrate
Phorcus lineatus	Gastropoda	Invertebrate
Phorcus sauciatus	Gastropoda	Invertebrate
Phyllariopsis brevipes	Algae	Algae
Pirimela sp.	Decapoda	Invertebrate
Plocamium sp.	Algae	Algae
Pollicipes pollicipes	Cirripedia	Invertebrate
Porcellana platycheles	Decapoda	Invertebrate
Porphyra sp.	Algae	Algae
Sabellaria alveolata	Polychaeta	Invertebrate
Siphonaria pectinata	Gastropoda	Invertebrate
Sphacelaria sp.	Algae	Algae
Steromphala cineraria	Gastropoda	Invertebrate
Steromphala pennanti	Gastropoda	Invertebrate

Steromphala umbilicalis	Gastropoda	Invertebrate
Steromphala varia	Gastropoda	Invertebrate
Stramonita haemastoma	Gastropoda	Invertebrate
Tritia incrassata	Gastropoda	Invertebrate
Tritia pygmaea	Gastropoda	Invertebrate
Tritia reticulata	Gastropoda	Invertebrate
Turbonilla sp.	Gastropoda	Invertebrate
Ulva clathrata	Algae	Algae
Ulva intestinalis	Algae	Algae
Ulva sp.	Algae	Algae
Verrucaria maura	Fungi	Invertebrate
Vertebrata fruticulosa	Algae	Algae
Watersipora subtorquata	Bryozoa	Invertebrate
Xantho sp.	Decapoda	Invertebrate

Appendix F. Species known to be present in the area (data from 2016) but not appearing in the database (not observed in 2019/2020

Species known to be present in the area but not appearing in the database:		
Species	**Taxon**	**Category**
Acar clathrata	Bivalvia	Invertebrate
Anapagurus laevis	Decapoda	Invertebrate
Atherina presbyter	Pisces	Vertebrate
Bittium latreillii	Gastropoda	Invertebrate
Calliblepharis jubata	Algae	Algae
Carcinus sp.	Decapoda	Invertebrate
Chylocladia verticillata	Algae	Algae
Cladophora prolifera	Algae	Algae
Cutleria adspersa	Algae	Algae
Doriopsilla areolata	Nudibranchia	Invertebrate
Doriopsilla sp.	Nudibranchia	Invertebrate
Ervilia castanea	Bivalvia	Invertebrate
Felimare sp.	Nudibranchia	Invertebrate
Ischnochitonidae	Polyplacophora	Invertebrate
Lysmata sp.	Decapoda	Invertebrate
Octopus vulgaris	Cephalopoda	Invertebrate
Polysiphonia sp.	Algae	Algae
Pomatoschistus sp.	Pisces	Vertebrate
Pterosiphonia sp.	Algae	Algae
Scinaia furcellata	Algae	Algae
Velella velella	Cnidaria	Invertebrate

Appendix G. New contributions to GenBank (*Irus irus*)

Irus irus, 16S sequence.

ATGAGTCCGGCCTACCCGGTGAGATTAAACGGTTGCAACTGTGTTGTACTAAGGTAGCAA
AATCAGTCGTTTCTTAATTGGAAAATAGAATGAAGGGTTAGACGTAAAGCAGCTGTTTCT
TTAAAATAGTATGAAGTTATCTTTTAGGTGAAAAGACCTAAGTTTTTGTAAAAGACGAGA
AGACCCCGTCGAGTTTAATTTAAAAGTAGGAGGTTCTGCTTTTCTAAGTTTTGTTGGGGCA
ATACAAGGTAAAATTTATCACCTTTTGAATTACGAACCTTTTATGAAAAAGAGAGAAAAA
ACTACCGCGGGGATAACAGCGTTATCTTTCTTAAGAGTTCTTATTGATGGAAAGGTTTGC
GACCTCGATGTTGGATTAAAGAAACTTTATGGCGCAGCAGCTATAGGAGTGAGACTGTTC
GTCTTTTAATACTTTACGTGATCTGAGTTCAGACCGGA

Appendix H. New contributions to GenBank (*Fulvia fragilis*)

Fulvia fragilis, 16S sequence.

GGGGGTAGGCCCTGCCCAGTGGAGTATTTCTAAACGGAAAGGATAACTTTTAAAGTAGCG
TAATAATTTGTCCCTTAATTAGGGTCTGTATGAACGGGTTGACGTGGGATAACTGTCTTGA
AAAAATAATTCGAAATTTTCTTCTTAGTGAAAAGCCTAAGATAAATTTAAAAGACGAGAA
GACCCCGTCGAGCTTATGAGAAAATGAGATTAATGCTTCTTCCTATTTTCCAGGTTTGTTG
GGGTAACAAAGGAGAAATTAAACCTCCTATTTATAATATAGATCCACTATTTAGTGATAA
AAAGAAAAAGCTACCGCGGGGATAACAGCGCAAGACAGCCAGAGAGTTCTTATCTAAGG
TTGTAAGTGCGACCTCGATGTTGGATTAAGGTGGGCTCAAGGGTGCAGCAGCTCTTGAAG
CGGGACTGTTCGTCCTTTAAATCCTTACGTGATCTGAGTTCAGACCGGA