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Mestrado em Biologia Marinha

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Acknowledgements

I would like to thank my supervisor for giving me the opportunity to discover this project and to collaborate on it, and for supporting me in the ordeal of writing a thesis. I would like to thank Adela Belackova for sharing her knowledge on the use of SBRUV. I would also like to thank Carolina Vieira Mourato and Inês Isabel Sousa for their hospitality and kindness during the working days. Juan Capaz for providing me with the two structures he created and helping me with species identification. Miguel Mateus for explaining the use of the PRIMER software. Finally, I would like to thank Ruben Bao Gallien and Nadège Montant for giving their time.

The thesis was financed by CCMAR in the framework of the AMPIC. COM project.

Abstract

Marine protected areas (MPAs) have been widely used as a conservation and fisheries management tool to protect fish and habitats. Measuring the effects of an MPA is difficult in dynamic and changing areas such as coastal areas. Yet, assessing the effects of the implementation of an MPA is essential to evaluate its performance in meeting its goals. For that, baseline information (before data) is critical to detect any pre-existing differences between future protection zones and disentangle habitat-associated effects from those related to the protection measures when the MPA is established. Thus, we carried out an assessment of commercial demersal fish and invertebrate communities in the largest coastal reef of the Algarve to contribute to a future MPA: the future Marine Park of the Algarve Reef, also know as AMPIC (marine protected area of community interest). This future MPA results of an innovative participatory approach that took almost 3 years involving over 70 stakeholders. The aim of this study was to obtain a first comprehensive database for the different zones to be implemented in the area, with ecological data retrieved by Stereo Baited Remote Underwater Video. Such data can be used in the future to assess the ecological effects of the MPA. We compared sites within future no-take zones with those with extractive activities, and the differences in richness, abundance, length and biomass were analyzed at community and species level. The influence of the effect of habitat complexity was accounted to distinguish it from the effect of future protection measures. Our results from the mulivariate statistics suggest the presence of pre-existing statistical differences among the proposed zones of this future protected area. Indeed, total abondance, abundance of target species above the Minimum Landing Size (MLS) and non-target species were significantly higher within the future no-take zones. This study highlights some previous differences and allows avoiding an overvaluation of the future protection measures, when implemented. Our before assessment allows conducting a Before-After-Control-Impact (BACI) approach in the future, when the MPA is implemented, which is desirable for an effective monitoring. This will also allow adapting the protection measures, if necessary, and contribute to a better MPA.

Keywords - marine protected areas, pre-implantation, stereo baited cameras, demersal fish assemblages, BACI design

Sumário Executivo

As áreas marinhas protegidas (AMP) têm sido amplamente utilizadas como instrumento de conservação da natureza e gestão de pescas para proteger peixes e habitats. Estudos anteriores observaram mais benefícios em reservas marinhas (i.e., sem pesca) ou em áreas muito regulamentadas do que noutros tipos de AMP. No entanto, continua a ser difícil medir os efeitos das AMP localizadas em áreas dinâmicas e em mudança, como as zonas costeiras. Investigar os efeitos da implementação de uma AMP é essencial para avaliar o seu desempenho no cumprimento dos seus objetivos. Para isso, obter informação de base (antes da AMP implementada) é fundamental para detectar diferenças pré-existentes entre futuras zonas com diferentes níveis de proteção; e separar os efeitos associados ao habitat daqueles relacionados com as medidas de proteção.Neste estudo realizámos uma avaliação das comunidades comerciais de peixes demersais e invertebrados no maior recife costeiro do Algarve para contribuir para o futuro Parque Marinho do Recife do Algarve – Pedra do Valado, também designado como AMPIC (área marinha protegida de interesse comunitário). O planeamento desta futura AMP resulta de uma abordagem participativa inovadora que durou quase 3 anos, envolvendo mais de 70 intervenientes, como as câmaras municipais das cidades circundantes, ONG, associações de pescadores profissionais e recreativos, laboratórios científicos como o CCMAR (da Universidade do Algarve), empresas de turismo (mergulho, observação de golfinhos, etc.), entre outros. O objectivo deste estudo foi obter a primeira base de dados ecológica das futuras zonas, com dados recolhidos através de câmaras iscadas em stereo, *Stereo Baited Remote Underwater Video* (SBRUV), cujo método pode ser repetido no futuro para avaliar os efeitos ecológicos da AMP. A campanha de amostragem foi levada a cabo no Outono de 2021 e resultou num total de 49 amostragens válidas. Comparámos os locais com futuros níveis de proteção total (i.e., sem atividades extrativas) com os de proteção parcial (i.e., com atividades extractivas), e as diferenças em riqueza, abundância, comprimento e biomassa foram analisadas ao nível da comunidade e por grupos de espécies. A influência do efeito da complexidade do habitat foi investigada a fim de o diferenciar do efeito da protecção. Além disso, analisámos em pormenor 5 espécies-chave predominantes na área (*Diplodus vulgaris, Diplodus sargus, Spondyliosoma cantharus, Coris julis, Serranus Cabrilla*). Os resultados das nossas estatísticas multivariadas sugerem a presença de diferenças pré-existentes. A abundância total, abundância de espécies comerciais acima do Tamanho Mínimo de Pesca e abundância de espécies não comerciais são significativamente maiores na futura zona de não pesca. Foram também observadas biomassas significativamente mais elevadas para espécies não alvo dentro

da zona. As espécies *Diplodus vulgaris*, *Diplodus sargus* e *Coris julis* diferiram estatisticamente entre as duas zonas com uma biomassa mais elevada na zona de protecção total. A análise do efeito habitat mostrou uma riqueza significativamente maior de espécies no habitat mais complexo. Não foram observadas outras diferenças para o efeito habitat. No decurso deste estudo observámos também que 5 das espécies observadas têm estatuto de ameaçadas de extinção na lista vermelha da IUCN. A observação de *Aetomylaeus bovinus* (Bull ray, Critically Endangered) evidência este resultado. Este estudo destaca as diferenças anteriores à implementação da AMP e evita uma sobrevalorização das futuras medidas de proteção. Esta avaliação prévia permite realizar uma abordagem Antes-Depois-Controle-Impacto (BACI) no futuro, quando a AMP for implementada, o que é desejável para uma monitorização eficaz. Isso permitirá a adaptação das medidas de proteção, se e quando necessário, o que poderá contribuir para uma melhor AMP.

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List of Abbreviations

AMPIC Marine Protected Area of Community Interest (from the Portuguese: Área Marinha Protegida de Interesse Comunitário)

CP Complementary Protection

FO Frequency of Occurrence

FP Full Protection

MLS Minimum Landing Size

MPA Marine Protected Area

PNRF Parque Natural da Ria Formosa

PNSVACV Parque Natural do Sudoeste Alentejano e Costa Vicentina

PP Partial Protection

SBRUV Stereo Baited Remote Underwater Video

UVC Underwater Visual Census

1. Introduction

Marine Protected Areas (MPAs) are considered the main tool to conserve biodiversity and reduce overfishing (Pauly et al., 2002; Halpern, 2003; Claudet et al., 2008). In addition to protecting populations directly targeted by fisheries, MPAs aim to restore habitats, ecological interactions between species, entire assemblages, and rebuild populations depleted in adjacent areas by the export of larvae, juveniles and adults (Areas, 2001; Goñi et al., 2010; Cheng et al., 2019). In line with the UN Convention on Biological Diversity (CBD) which has set a target of protecting at least 10% of the oceans by 2020 ("Aichi Target 11"), the number of MPAs is increasing rapidly (Laffoley et al., 2018). Yet, as reported by the Marine Conservation Institute (MPA atlas, https://marine-conservation.org), in January 2022 only 8.1% of the global ocean was covered by protected areas and around 2.4% of the ocean by no-take areas (also known as marine reserves or fully protected areas), MPAs where no extractive activities are allowed. Many scientists argue that the 10% target is a first step in global ocean protection, not an end point. The 2014 World Parks Congress called for ≥30% of every marine habitat to be included in highly protected MPAs. Indeed, 30% habitat coverage is claimed to be necessary to maintain habitat persistence and protect marine biodiversity (Botsford, Hastings and Gaines, 2001; Gaines et al., 2010; O'Leary et al., 2016; Krueck et al., 2017). In fact, the EU Biodiversity strategy to 2030 already committed the EU members in protecting 30% of its waters with MPAs or OECMs (other effective conservation measures), and 10% with strict protection.

Many studies have shown that marine reserves often lead to increases in fish density, size, biomass, and richness, particularly of commercial target species (Claudet et al., 2008; Lester et al., 2009; Claudet and Guidetti, 2010; Horta e Costa et al., 2013b; Belo et al., 2016). Effective marine reserves can have twice the number of large fish species within their borders compared to the outside (Graham J. Edgar et al., 2014). These studies are mainly based on Control-Impact or Before-After design (Halpern and Warner, 2003; Lester et al., 2009; Consoli et al., 2013; Graham J. Edgar et al., 2014). However, studies in which MPAs are monitored both before and after their implementation and inside and outside the reserve boundaries are relatively rare (Lester et al., 2009; Liu et al., 2018). Such studies are valuable because they effectively control for natural ecosystem dynamics and variability at the regional scale and can help distinguish spurious effects resulting from spatial differences between sites (Lester et al., 2009). Before-after-control-impact analysis (BACI) (Green, 1979; Stewart-Oaten, Murdoch and Parker, 1986) is a monitoring design that offers a simple and robust method for assessing complex effects and, when replicated in time and space, is considered the most rigorous methodological design assessing MPA impacts (Ojeda-Martínez et al., 2011; Sciberras et al., 2013; Thiault et al., 2019).

Increasing the local abundance of harvested species and restoring the structure of entire communities can take up to several decades (Micheli, S. Halpern and W.Botsford, 2004). However, for various reasons, including lack of funding and human resources, data is often not collected prior to the establishment of protection, but only after its establishment. Although some of the positive effects of protection may be seen quickly, others may not be detected for some time. In their review of 112 marine reserve studies, Halpern and Warner (2002) found that positive biological responses (e.g., higher densities and average biomass) were observed within 1-3 years of the establishment of a marine reserve. Other studies have shown that fish density and species richness increased after 3 years of protection (Russ et al.,, 2005; Claudet et al., 2006). Others even showed that decades could be needed to reach MPAs' 'natural state' (Micheli, S. Halpern and W.Botsford, 2004; Russ and Alcala, 2004). In general, for the effectiveness of MPAs and sanctuaries to be reliably quantified, long-term monitoring programs should be established, comparing marine reserve, partially protected areas (PPAs) and unprotected sites (Boersma and Parrish, 1999; Carr, 2000; Russ and Alcala, 2004; Ojeda-Martinez et al., 2007)

According to Lester et al. (2009) and Claudet and Guidetti (2010) density and size of targeted commercial species were greater in protected than unprotected areas. The exclusion of all extractive activities in marine reserves has stronger effects on the species most impacted by fisheries (Russ, 2002). The benefit does not seem to extend only to marine reserve but also to adjacent areas. In conducting the first global meta-analysis of PPAs, Zupan et al. (2018) showed that well-regulated PPAs with limited and monitored uses but with adjacent fully protected areas provided significant ecological benefits when compared to outside areas. Theorical and empirical studies have shown the positive effects of marine reserves within their boundaries and in their vicinity (Gell and Roberts, 2003; Lester et al., 2009; Sciberras et al., 2015). However, it is more difficult to quantify and generalize the effects on partially protected areas due to the heterogeneity of the measures in place (Lester and Halpern, 2008; Curley et al., 2013; Giakoumi et al., 2017)

Fishers typically show disagreement when a no-take area is established as they may lose their traditional fishing grounds, with the consequent increase in competition with other vessels and in potential costs due to displacement (Cinner et al., 2014). Yet, the benefits could also extend to the fisheries themselves. Fisheries can benefit from two different processes after biomass recovery within the marine reserve: the export of propagules (dispersion effect) and the export of adults (spillover effect) towards outside the marine reserve (Alcala and Ru, 1996; Abesamis, Russ and Alcala, 2006; Goñi et al., 2010). The size and "visibility" of the overflow effect is essential for the acceptance of marine reserves by fishers. However, many species have very long dispersions (of the order of hundreds of kilometers) so it may be difficult to detect benefits in adjacent areas (Pelc et al., 2010; Manel et al., 2019).

Traditionally, the assessment of marine demersal fauna is carried out by sampling techniques such as using fishing nets, stranded fish or fish landing data as an indicator of the underlying fauna (Horta e Costa et al., 2013a; Belo et al., 2016). However, these techniques are extractive and have an impact on fish stocks or habitats (Cole, 1994; Watson and Harvey, 2007; Pais and Cabral, 2018) which is not desirable in protected areas. Non-extractive methods for assessing fish size and density has therefore generally been preferred such as the Underwater Visual Census (UVC) performed using Self-Contained Underwater Breathing Apparatus (SCUBA). UVC has some limitations due to changes in fish behavior in the presence of divers, reduced time and depth or minimum visibility to conduct the assessments, and it is closely linked to the level of experience of the diver (Chapman et al., 1974; Cole, 1994; Watson and Harvey, 2007; Pais and Cabral, 2018; Thanopoulou et al., 2018). More recently, innovative underwater video techniques have emerged. These techniques have developed rapidly with advantages such as low cost, long immersion time and improved image quality (Cappo et al., 1999; Mallet and Pelletier, 2014; Unsworth et al., 2014; Bouchet and Meeuwig, 2015). In addition, the use of two cameras, i.e., in stereo, makes it possible to accurately measure marine organisms but also objects and distances (Cappo et al., 1999; Harvey, Fletcher and Shortis, 2002; Boutros, Shortis and Harvey, 2015; Letessier et al., 2015). Stereo Baited Remote Underwater Video (SBRUV) is now one of the most preferred methods for assessing the effects of MPAS in some regions (Stobart et al., 2007; Watson et al., 2010). The SBRUV method, allows most sampling objectives to be met at low cost, without disturbing the population assessed (Cappo et al., 2001; Mallet and Pelletier, 2014; Unsworth et al., 2014; Bouchet and Meeuwig, 2015; Letessier et al., 2015). In addition, compared to UVC which has no bait, the presence of bait allows for higher detection and abundance of predatory fish that are targeted by fisheries and thus by conservation measures (Harvey, Fletcher and Shortis, 2002; Harvey et al., 2007; Goetze et al., 2015). Last but not the least, SBRUV allows for more replicates and samplings per session especially at deeper depths where dives become more complex (Dorman, Harvey and Newman, 2012; Terres et al., 2015; Watson and Huntington, 2016)

Regionally, in the Algarve, only two marine protected areas exist: the Parque Natural da Ria Formosa (PNRF), a large coastal lagoon, and the Parque Natural do Sudoeste Alentejano e Costa Vicentina (PNSVACV), a protected area in the southwest coast of Portugal. The Algarve has a considerable anthropogenic pressure, mostly due to the massive influx of tourists and sea-oriented culture, with strong fish-based gastronomy and a majority of jobs related with the marine environment (R.Simões, 2018). This has led to strong coastal development and degradation of coastal marine environments (Lukoseviciute and Panagopoulos, 2021). At the time of implementation of these two protected areas, locals expressed negative perceptions related to lack of community participation, excessive restrictions and the occurrence of conflicts due to recreational fishing regulations(Thaman et al., 2016). Furthermore, most of the MPAs, and the PNSACV, has few data from before the MPA (Monteiro et al., 2013). For effective management of protected habitats, a clear understanding of their location and extent, assessment of biological communities, conservation importance, monitoring options and sensitivity to natural change and human disturbance must be relatively clear. To preserve and restore biodiversity, innovative and effective MPAs are needed. A new protected area is planned to be declared in the near future in the middle of the Algarve: Parque Marinho Recife do Algarve - Pedra do Valado. This MPA is the result of an innovative participatory approach that took almost 3 years involving over 70 stakeholders such as the municipal councils of the surrounding cities, NGOs, fisher's association professional and recreational fisheries, scientific laboratories such as CCMAR (from the University of Algarve), tourism companies (diving, dolphin watching, etc.), among others. Since a BACI design is central for a comprehensive monitoring of protection effects, this study aimed at contributing to this innovative process by providing the baseline information (before data) of the different protection zones of this future MPA.

Here, we provide the first assessment using SBRUV of the rocky fish communities in the future MPA of Parque Marinho Recife do Algarve - Pedra do Valado. We aimed at testing whether possible pre-existing differences in abundance, size, biomass and diversity of commercial demersal fish and invertebrates already exist between inside the no-take areas of this future MPA and outside them , but also between different habitat types. As the MPA is not yet functional, we expected to find little or no difference between the different future levels of protection. This study will be used for future studies, hopefully conducting a BACI monitoring design, to assess the effects of the MPA on the community of demersal fish and commercial invertebrate species.

2. Material and Methods

2.1. Study area

The Algarve coastal region, where the future MPA is, has been studied for the last 15 years (Leitão et al., 2008, 2009; Rangel et al., 2008, 2015; Monteiro et al., 2012; Willenbrink, 2016; Belackova, 2019). Those studies were able to highlight the ecological importance of this specific region. Particularly, from the 1,294 species identified between Lagos (Piedade Point) and Faro (Faro-Olhão bar), 889 species are present in this area, of which 703 are invertebrates, 111 are fish and 75 are algae. In addition to the presence of a high number of species, 19 species with conservation status were identified, among which the seahorse (*Hippocampus spp.*) and the dusky grouper (*Epinephelus marginatus*) (Henriques et al., 2018). Moreover, the rocky bottoms of the Algarve, are home of shallow Gorgonians (e.g. *Eunicella labiata, Eunicella gazella, Eunicella verrucosa and Leptogorgia sarmentosa*) playing a crucial role in the local ecology (Cúrdia et al., 2013). Their three-dimensional structure can modify the physical habitat, by reducing current velocity, stabilizing soft substrates, enhancing sedimentation and local accumulation of fine particles, and increasing the availability of hiding places (Idjadi and Edmunds, 2006; Norling and Kautsky, 2007). Due to its biodiversity and associated natural values, the region of the future MPA is also a hotspot of human activities, namely of commercial and recreational fishing, but also with recreational boats and tourism activities (such as dolphin watching, diving, etc.).

The future MPA, an MPA of commercial interest (Área Marinha Protegida de Interesse Comunitário no Algarve, or AMPIC) will be officially designated as Parque Natural Marinho do Recife do Algarve – Pedra do Valado. It is located between Alfanzina lighthouse and Albufeira Marina totaling 156km2 (Fig. 2.1). The maximum depth for the entire area is 50m. However, the rocky reefs justifying the high biodiversity and the proposal of MPA constitute a platform between 12 and 25m depth. The Algarve coastal region includes marine rock formations of several types (e.g., boulders, bedrock, low relief rocky areas,) and different sedimentary dynamics. The study area is characterized by heterogeneous seabed: rocky areas, mixed areas of sand and rock and areas composed only of sand.

The zoning plan of this future MPA was co-designed with the stakeholders within a participatory process (https://www.ccmar.ualg.pt/page/area-marinha-protegida-de-interesse-comunitario-do-algarve). Different protection levels were defined (Fig. 2.1), including a 4 km² (2.6%) Fully Protected zone (FP) or marine reserve, where no activities are allowed. Approximately 16.5 km² (10.6%) will be a Partial Protection zone (PP), where no extractive activities are permitted (commercial or recreational fishing), so in practice it is a no-take area or marine reserve as well. However, non-extractive activities such as diving, boating or marine mammal watching are allowed with regulations. The implementation of this zone will happen in two phases (the first part will be the area adjacent to the FP zone). The Complementary Protection zone (CP) provides an area of 135 km² (86.8%), divided in two sub-areas with regulations on the vessel size, and both banning destructive activities such as clam dredging, bottom trawling, sand extraction and aquaculture.

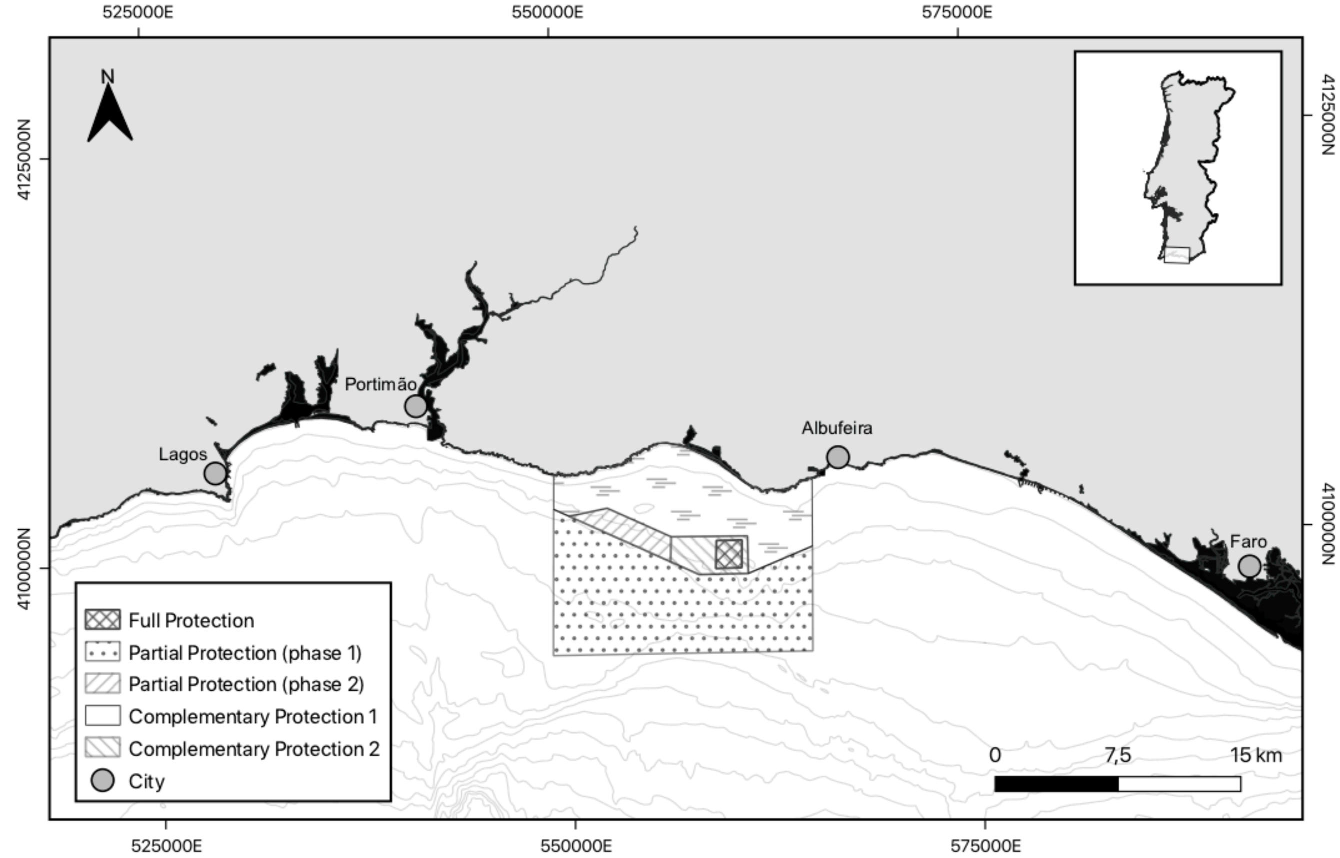


Fig. 2.1 Map of the zoning proposed for the future MPA (AMPIC), with the detail of the different zones. Fully and partially protected zones do not allow extractive activities.

2.1. Sampling design and method

Sampling methods

In order to assess the demersal fish community, the SBRUV method was used (Willenbrink, 2016). This method was derived directly from a device with a single camera built at the University of Algarve by Capaz (2013). The SBRUV installation consists of 2 small cameras (stereo) in waterproof cases spaced 40 cm apart (right and left) (Fig. 2.2). Cameras are 40 cm from the ground facing the bait basket with an inner angle of 8° (Belackova, 2019). The bait basket is at a distance of 1 meter from the cameras and at a height of 30cm from the ground. The whole system is connected by steel bars, and a PVC stick allows stability. There is a chain to connect with a rope; it contributes to a good immersion and stability during the experiments. In order to raise the structure, the structures are connected by a chain, a rope and a buoy floating on the surface. The cameras used during the sampling were SK8 4K Black Full hd quality 1080 x 30 frames. As calculated in Belackova (2019), the common FOV is estimated to be 5.15 m^2 with a 3 m distance from the cameras. Hence, to keep comparable samples, that distance represented the minimum visibility for our assessments.

The same proportion of bait (~ 200 g), by crushing bits of different sizes was put in each basket. Small pelagic fish (such as *Sardina pilchardus, Trachurus trachurus, Scomber scombrus*) and mussels (*Mytilus galloprovincialis*) was used as bait for all experiments (Capaz, 2013; Belackova, 2019). The contents of each basket were emptied and refilled identically after each deployment. The stereo-BRUVs were retrieved after recording for 30min at each station.

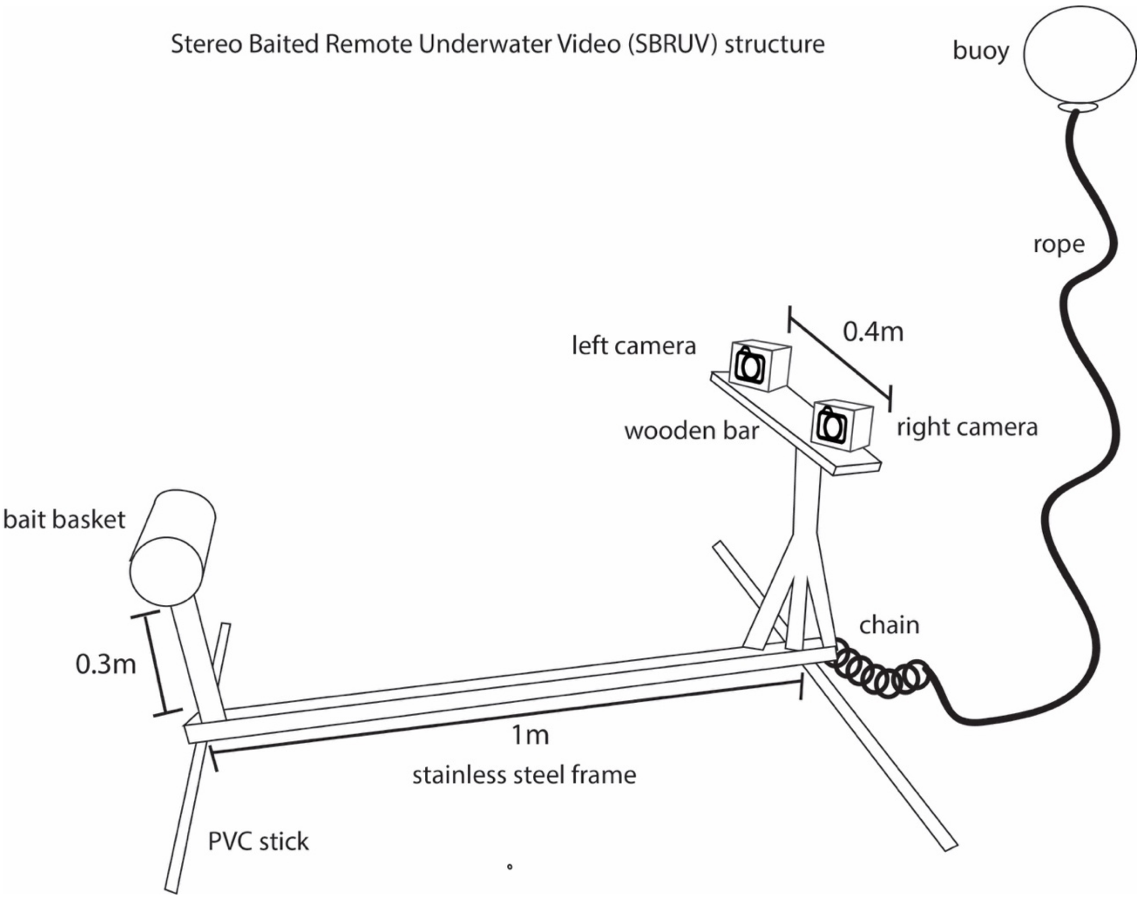


Fig. 2.2 Representation of the SBRUV structure (adapted by Belackova 2019)

Sampling design

This study was conducted in autumn with the majority of the samples done in November to December 2021. However, due to harsh ocean conditions and the loss of a camera, an additional day in February 2022 was required to acquire all the necessary data. All sampling was conducted during the daytime between 9 a.m and 4 p.m with the highest light intensity. During a fieldwork day the team was equipped with 3 SBRUVs; this allows to conduct 3 replicates per location almost simultaneously. The three replicates had a minimum distance of 250 m from each other. Based on white seabream (*Diplodus sargus*) movements, Willenbrink (2016) has shown that it is possible to space the replicates 250m apart to avoid recording the same fish. Each SBRUV was deployed underwater for a minimum of 30 minutes, and the cameras recorded during the entire immersion.

The average visibility was 3.4 m ± 0.1 SE on all usable videos with a mean depth of 16.98 m ± 0.29 SE. The sampling campaign took place from November to December with 66 samples. Of the 66 deployments carried out, 34 (51,5%) complied with the following defined criteria: bottom with rocks (proportion of rock greater than the proportion of sand), structure positioned horizontally, camera filming the bait basket, minimum visibility of 3 m. The following reasons contributed to reject 48.5% of the total original samples (n=32): sandy bottom (12% of the total), bad visibility (9.1%), cameras looking forward/backward due to a landing on the top of a rock that was not flat (4.5%), so the structure turned down, and one camera lost due to an octopus (7.6%). To avoid recording only one out of three replicates in February and relate it to the other two good samples of the same location in December (different days, season, conditions, etc.), that sample day in December was discarded (15.2% of the total original samples). For this reason, an extra day was scheduled in February, to re-perform 15 samples. With these new samples, 81 deployments in total were carried out and 49 could be accepted for analysis.

Sampling was carried out in 4 zones: inside the future fully protected area, inside the partial protection Phase I and Phase II, inside the future complementary zone I and II and also outside the MPA. As we will have few restrictions to the local fisheries in the PC, the samples from this area were merged to those outside the MPA and called outside (Out) (Table. 2.1). Similarly, in both FP and PP extractive activities will not be allowed so they were grouped together with the designation inside (In). These zones are not yet established but were agreed in a long and inclusive participatory process with the local and regional stakeholders, hence we assume these will be established when the MPA is designated (the Portuguese minister of the Environment have said this MPA is going to be declared soon).

Table. 2.1 Number of total valid samples and % at each location (PT, PP, PC, OUT). PT and PP were merged (In) and also PC and OUT (Out).

Location	Number of samples	%	Designation	Number	%
PT	7	14	In	21	43
PP	14	29			
PC	16	33	Out	28	57
OUT	12	24			

Calibration procedure

A calibration is required for each session. This procedure allows to measure the length of the fish in video analysis in the computer due to the stereo and 3D nature (Neuswanger et al., 2016). Calibration should be done in a calm environment with little current and few waves. Visibility must also be good. The calibration procedure is as follows:

- Turn on the two cameras and make sure the video is recording;
- Synchronize the two cameras with a hand clap in front of the bait basket;
- Immerse the cameras structure at a sufficient depth (~ 1 m) to use the calibration frame;
- Allow the future distortion correction (in the computer): movements back and forth of the chessboard pattern in front of each camera;
- Allow the future 3D calibration: movements of the calibration frame back and forth in front of the bait basket visible to the two cameras simultaneously.

In order to verify that the calibration was carried out correctly, bait basket measurements were taken on each sample. A margin of error of 10% was allowed; the bait basket measures 17.8 cm ± 1.8 SE (Belackova, 2019). When necessary, the calibration was redone.

2.3. Data treatment and analysis

Data treatment

VidSync software version 1.661 (Jason R. Neuswanger et al., 2016) was used to perform the video processing. Several processing steps were carried out to analyze the video samples: the synchronization of the two videos, the distortion correction and the 3D calibration. These processes are fundamental for a correct fish size measurement. For each species, the maximum number of fish observed in a given video frame (i.e., MaxN) was recorded. MaxN provides the minimum number of individuals of each species known to occur in a recording as others may appear in other frames but are not included. This ensures individuals are not counted more than once, so MaxN is considered a conservative estimator of the relative abundance of a species (Willis and Babcock, 2000; Cappo et al., 2003; Cappo, Speare and De'ath, 2004; Pomeroy et al., 2005). Therefore, MaxN may result in positively biased indices of abundance for declining fish stocks or negatively biased abundance indices when fish stocks are increasing (Schobernd, Bacheler and Conn, 2014). For each MaxN, the total length (cm) of the species in the common FOV was measured in fish and mantle length in cephalopods (Horta e Costa et al., 2013a; Unsworth et al., 2014; Wilson et al., 2018). Only 42% of the total number of fish recorded to the MaxN were measured, as some were not in a position allowing confident measurements. Some of the species, e.g., *Conger conger* or *Mureana helena*, could not been properly measured and their snake-like nature and long talus precludes having the whole individual lying in the common FOV. Cephalopods were also measured in small proportions due to the greater difficulty in measuring mantle length than total fish length.

Finally, the habitat type was assigned to each SBRUV sample (Horta e Costa et al., 2013a; Belackova, 2019). A visual assessment of the different habitats was carried out for each SBRUV sample according with two criteria: complexity and abundance of hiding places. Following the Belackova (2019) procedure, three types of habitats were identified (Table. 2.2).

Table. 2.2 Different habitat types categorized according to their physical characteristics

	hab1	**hab2**	**hab3**
Complexity	Flat	Medium	High
Hidings	No hiding	Some little hidings	Many hidings
Description	Flat habitat with little or no hiding place. Often in the presence of sedimentary rocks, sands and no source rocks.	Habitat with some small hiding places. Often in a mix of sand and rocks of varying size. Presence of some algae or gorgonians.	Habitat with numerous boulders and parts of source rocks. The hilly terrain and the presence of gorgonians and algae offer many hiding places.

The use of the SBRUV allowed the sampling of demersal fish and cephalopods. Crustacean and cryptobenthic species (Blennidae, Scorpaenidae) were not included due to an underestimation of those fish by this sampling method (Holmes et al., 2013; Cheminée et al., 2021).

The frequency of occurrence (FO) provided an indication of species abundance and richness per sample. It was expressed as follows:

$$\mathrm{FO}\ (\%) = (\mathrm{N°\ of\ samples\ containing\ the\ species/\ Total\ N°\ of\ samples}) * 100$$

The species observed were divided based on their local fishing commercial status into two categories, targeted by fisheries (commercial) and non-target (non-commercial) species. Within the commercial species group, fish were grouped according to their minimum landing size (MLS), as defined by national regulation. Commercial species with no minimum landing size were considered as above MLS. Based on the length-weight relationships, the biomass of each measured fish was calculated and multiplied by the corresponding MaxN (Gonçalves et al., 1997; Morato et al., 2001; Hernández-García, Hernández-López and Castro-Hdez, 2002; Morey et al., 2003; Krstulović-Šifner and Vrgoč, 2004; Pajuelo et al., 2006; Veiga et al., 2009; Torres, Ramos and Sobrino, 2012; Edelist, 2014; Froese et al., 2018; Jisr et al., 2018; Colombelli and Bonanomi, 2022).

Data analysis

Two potential explanatory variables: the future protection level (in/out) and the habitat (hab1/hab2/hab3) were tested using univariate statistical analysis on the response variables of richness, abundance (MaxN), length and biomass (at MaxN). This was done separately for each of the following groups of species: demersal species, target species (commercial species), target species below the MLS, target species above the MLS and non-target species (non-commercial species). Further, the same statistical tests were conducted for the most abundant demersal fish species recorded in the samples, the key-target species *Diplodus sargus*, *Diplodus vulgaris*, and *Spondyolosoma cantharus* and the non-target species, *Coris julis* and *Serranus cabrilla*.

Primer-E version 6.1.6 with PERMANOVA+package was used for the multivariate analysis. To obtain p-values, we computed 9999 permutations of the raw data units for each term in the analysis. In order to understand which species contributed the most to differences between protection levels, a SIMPER analysis was conducted (Clarke and Gorley, 2018).

Multivariate analysis is well suited to the expectations of complex systems, as it is unlikely that a single variable will be sufficient to describe the system. For this reason, multivariate analysis was preferred in this study. Univariate analyses were also carried out in a second step in order to compare possible statistical differences. The univariate analysis was done with R Studio (Version 1.4.1717) R Core Team (2020). Initially a Shapiro Wilk test was performed to test for normality, however none of the datasets showed a normal distribution. It was therefore necessary to perform non-parametric tests: Wilcoxon sum-rank for the protection factor and Kruskal-Wallis for the habitat (Bhalerao and Parab, 2010).

A Pearson's Chi-Squared Test of Independence was performed to verify that habitat was independent from biomass (Bhalerao and Parab, 2010).

3. Results

3.1. Fish assemblage description

The 49 SBRUVs deployments sampled a total of 1326 fish (mean of 23.06 ± 2.34 SE per sample) from 28 species (mean of 6.86 ± 0.39 SE per sample). A large proportion of individuals observed on SBRUVs were from 3 families, Sparidae (49.4% of the total), Labridae (22.6%) and Serranidae (14.6%) (Fig. 3.1). Of the 28 different species surveyed, 18 were species targeted by fisheries (64%) and 10 were non-target species (36%). The largest MaxN was observed with schoolings of MaxN=41 individuals for *Coris julis* and MaxN=39 individuals for *Chromis chromis.*

The following threatened species, according to the IUCN redlist (IUCN, 2021) were observed in the samples: *Aetomylaeus bovinus* (Critically Endangered), *Raja undulata* (Endangered), *Balistes capriscus* (Vulnerable), *Dentex dentex* (Vulnerable), *Trachurus trachurus* (Vulnerable).

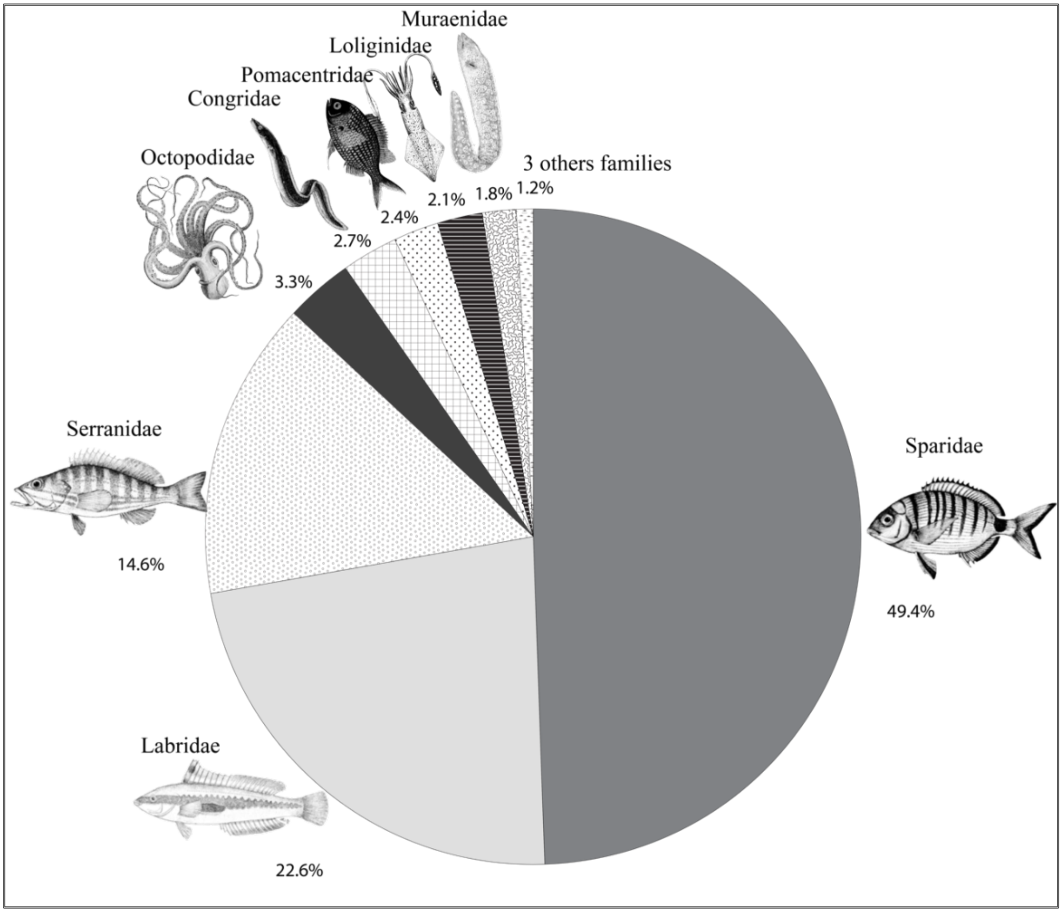


Fig. 3.1 Proportions of the total fish assemblage represented by the different fish families. Fish pictures taken from FAO (Bauchot, 1987; Schneider, 1990).

Table. 3.1 Target and non-target fish and invertebrate taxa recorded. Frequency of occurrence (FO) in %, commercial status (CS): target (T) or non-target (NT), Minimum Landing Size (MLS) in cm, % of the species in the MaxN measured, Mean length of the of individuals measured.

Family	Scientific Name	FO%	CS	MLS (cm)	% Measured	Mean length (cm)
Balistidae	*Balistes capriscus*	2%	T	0	100%	43.4
Carangidae	*Trachurus trachurus*	37%	T	15	59%	27.6
Congridae	*Conger conger*	18%	T	58	17%	67.8
Labridae	*Centrolabus exoletus*	22%	NT	0	65%	10.7
	Coris julis	84%	NT	0	36%	13.8
	Ctenolabrus rupestris	18%	NT	0	64%	12.9
	Labrus bergylta	14%	NT	0	43%	32.4
	Labrus mixtus	8%	NT	0	80%	25.6
	Symphodus bailloni	8%	NT	0	33%	19
Loliginidae	*Loligo vulgaris*	14%	T	10	29%	32.8
Muraenidae	*Muraena helena*	12%	T	0	0%	n/a
Myliobatidae	*Aetomylaeus bovinus*	4%	T	n/a	50%	115
Octopodidae	*Octopus vulgaris*	22%	T	12.4	9%	10.7
Pomacentridae	*Chromis chromis*	16%	NT	0	26%	14.4
Rajidae	*Raja undulata*	2%	T	52	100%	80.8
Serranidae	*Serranus atricauda*	6%	NT	0	67%	14.5
	Serranus cabrilla	90%	NT	0	54%	17.8
	Serranus hepatus	4%	NT	0	0%	n/a
Sparidae	*Dentex canariensis*	6%	T	0	40%	31
	Dentex dentex	2%	T	0	0%	n/a
	Diplodus annularis	6%	T	15	88%	17.6
	Diplodus cervinus	35%	T	15	55%	31.4
	Diplodus sargus	65%	T	15	52%	23
	Diplodus vulgaris	92%	T	15	41%	13.2
	Oblada melanura	2%	T	0	100%	21.8
	Pagrus auriga	29%	T	0	86%	37.2
	Sparus aurata	6%	T	19	100%	33.4
	Spondyliosoma cantharus	59%	T	23	43%	12.1

3.2. Fish assemblage comparaison

Future zones

Species richness

(Fig. 3.2) Overall, no significant statistical differences were found in species richness between inside and outside the future no-take area (Wilcoxon test; p-value = 0.18). The mean richness by protection level was 7.1 ± 0.69 SE inside and 6.71 ± 0.47 SE outside. Of the 28 species identified, 24 taxa were present inside (PP, PF; in) and 25 taxa outside (PC1, PC2; out).

Biomass

(Fig. 3.3) In the future no-take area, significant differences were found in mean biomass between the 2 zones (Wilcoxon test, p-value = 0.002). The mean biomass was higher inside the no-take area (361.63 g ± 23.40 or 70 g/m²) than outside (308.52 g ± 25.36 or 60 g/m²). Multivariate analysis showed significant differences in biomass of non-target species (Table. 3.2). The species most contributing to the differences was *Coris julis* (SIMPER 40.26%). The average biomass of *C.julis* was almost twice higher inside the future no-take area than outside. Univariate analyses showed that biomass of all community datasets, except target demersal species below MLS, differed significantly between inside and outside the no-take area (Wilcoxon test; Table. 3.2).

Abundance

(Fig 3.4) Multivariate analysis showed significant differences in the abundance of all the demersal species, the target species above the MLS and non-target species (PERMANOVA; Table. 3.2) with higher average values observed inside for most species of these groups (SIMPER; Appendix, Table. Annex 1). The difference in abundance between protection levels for all the individuals were mainly attributed to *C.julis* and *D.vulgaris*, which contributed 19.40% and 14.06% of the dissimilarity respectively. For the non-target demersal species, the mean abundance of *C.julis* was 60% higher inside, with a contribution to dissimilarity of almost half of the species (SIMPER, 47.25%).

No significant differences in terms of abundance were found in the univariate analysis

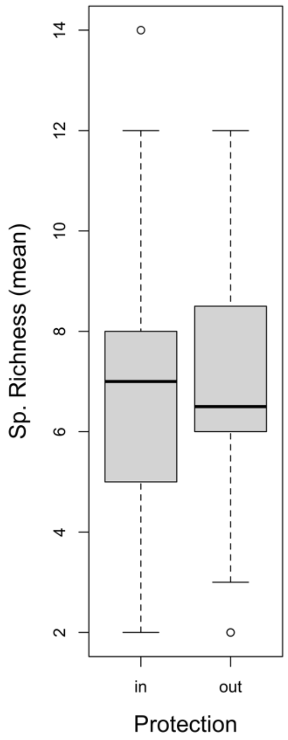


Fig. 3.2 Boxplot of mean species richness by futures protection measures

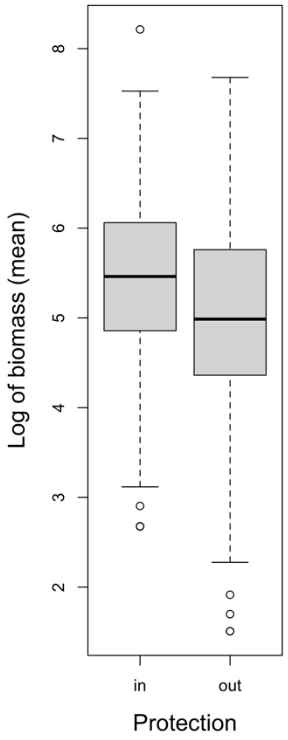


Fig. 3.3 Boxplot of the logarithm of the mean species biomass by future protection measures

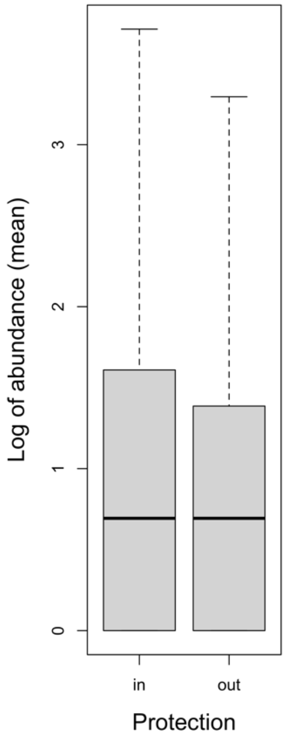


Fig. 3.4 Boxplot of the logarithm of the mean species abundance by future protection measures

Table. 3.2 Differences in community abundance and biomass between protection levels (SBRUV). Wilcoxon test and PERMANOVA results, significant p-values ($p<0.05$) highlighted in grey. (+): higher values inside the future no-take area.

	Protection in/out	
	PERMANOVA (p-values)	
	Abundance	**Biomass**
All species	0.05 (+)	0.27
Target	0.20	0.27
Above MLS	0.01 (+)	0.06
Below MLS	0.41	0.16
Non-Target	0.02 (+)	0.01 (+)

Effect of habitat

Richness

(Fig. 3.5) Of the 28 taxa identified, 21 taxa were present in hab1, 26 in hab2 and 24 in hab3. Significant differences were found between mean richness and the three habitat types (Kruskal-Wallis; p-value = 0.01). The mean richness of habitat1 = 5.4 ± 0.53 SE was much lower than that of habitat2 = 7.71 ± 0.69 SE and habitat3 = 8.34 ± 0.63 SE. The pairwise comparison shows that only hab1 and hab3 are significantly different (Wilcoxon test; p-value = 0.02).

Biomass

(Fig. 3.6) From both multivariate and univariate tests no significant differences were found. The least complex habitat had a mean biomass per sample of 307.62 g ± 19.31 SE or 60 g/m². Habitats 2 and 3 had higher biomasses with 350.22 g ± 23.73 SE per sample, respectively or 68 g/m² and 336.02 g ± 29.70 or 65 g/m².

Abundance

(Fig. 3.7) From both multivariate and univariate tests no significant differences were found.

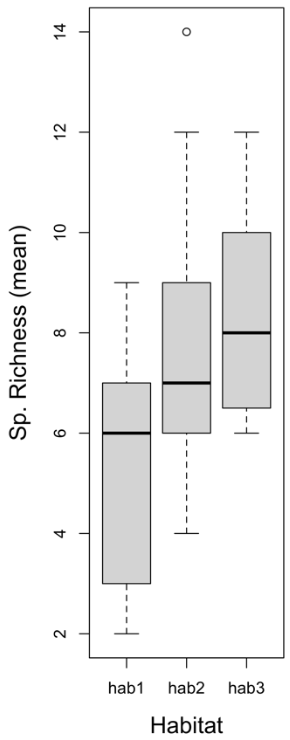


Fig. 3.5 Boxplot of mean species richness by habitat types

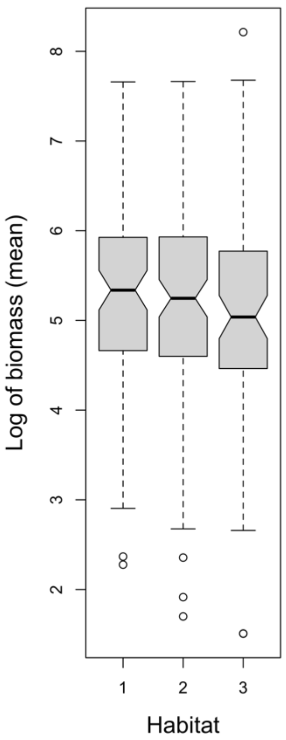


Fig. 3.6 Boxplot of mean biomass per sample by habitat types

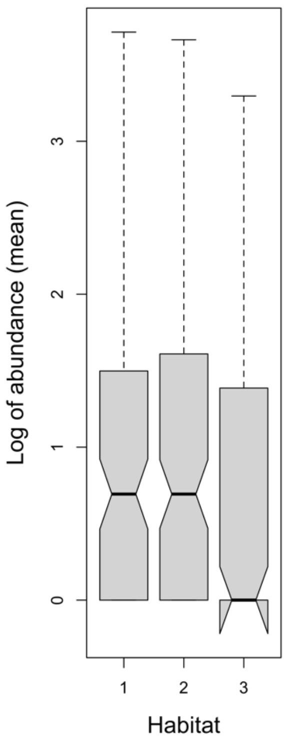


Fig. 3.7 Boxplot of mean species abundance by habitat types

Table. 3.3 Differences in community abundance and biomass between protection levels (SBRUV). Wilcoxon test and PERMANOVA results, significant p-values (p<0.05) highlighted in grey. (+): higher values inside the future no-take areas

	Habitat 1/2/3			
	PERMANOVA (p-values)		**Kruskal-Wallis (p-values)**	
	Abundance	**Biomass**	**Abundance**	**Biomass**
All species	0.48	0,41	0.44	0.52
Target	0.40	0.37	0.67	0.67
Above MLS	0.80	0.80	0.99	0.69
Below MLS	0.73	0.70	0.97	0.71
Non-Target	0.60	0.83	0.49	0.96

Interaction between protection and habitat

Habitat type 1, i.e., the least complex, was found in the greatest proportion (41%). Then the intermediate habitat 2 (35%), and the most complex, the habitat 3, was the one less abundant in the samples (24%) (Fig. 3.8). No significant variation in habitats occurrence according to protection was observed. No significant relationship was found between protection and habitat (Pearson's Chi-Squared test, p = 0.98).

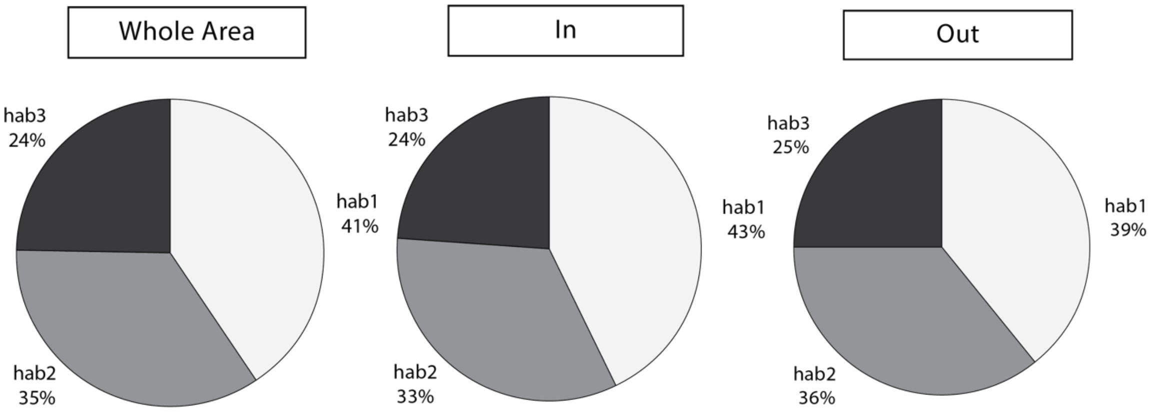


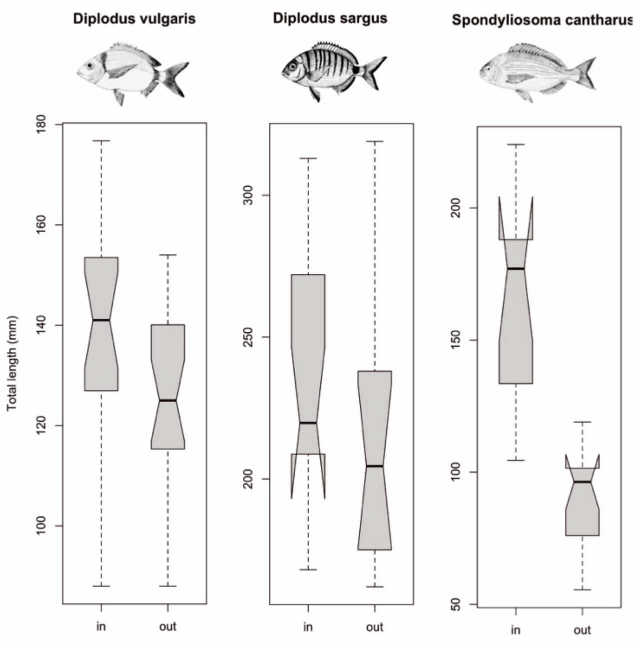
Fig. 3.8 Proportions of habitat categories in the whole area and by protection.

3.3. Key-species comparison

Target key species

Five fish species (*Didplodus vulgaris, Diplodus sargus, Spondyliosoma cantharus, Coris. julis* and *Serranus cabrilla*) were sufficiently common to facilitate statistical comparisons of length frequencies. For the key-target species, all *D. vulgaris* had significantly greater mean size (Wilcoxon test; p-value = 0.02) and all *D. vulgaris,* above and below the MLS, had significantly greater biomass (Table. 3.10) inside than outside the no-take area. Only *D. vulgaris* below the MLS had significant differences in abundance (Wilcoxon test; p-value = 0.01), with higher values inside. Differences in abundance for all *D. vulgaris* (above and below) were marginally non-significant, also with higher values inside (Wilcoxon test; p-value = 0.06). No significant differences were found for *D. sargus* and all fish measured were larger than the MLS (Table. 3.4). In contrast, no *S. cantharus* were above the MLS of 23 cm. However, significant differences were found for *S. cantharus* below 23 cm size, showing larger sizes

(Wilcoxon test; p-value < 0.001) and biomass (Wilcoxon test; p-value = 0.01) inside. No significant differences were found with the habitat types.



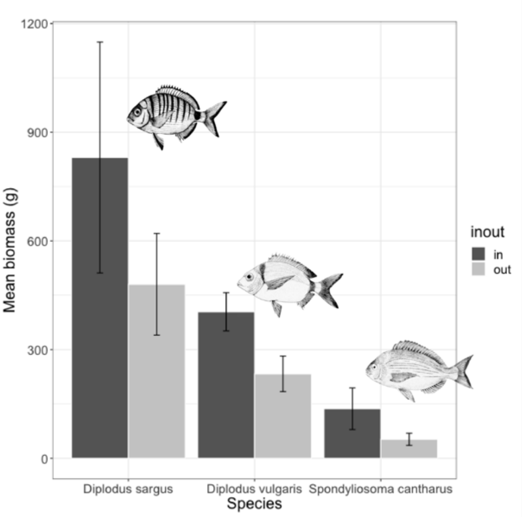


Fig. 3.9 Boxplot of the mean total length in mm for the 3 keys target species inside and outside. Fish pictures taken from FAO (Bauchot, 1987; Schneider, 1990).

Fig. 3.10 Histogram of the mean biomass in g for the 3 keys target species inside (in black) and outside (in grey). Fish pictures taken from FAO (Bauchot, 1987; Schneider, 1990).

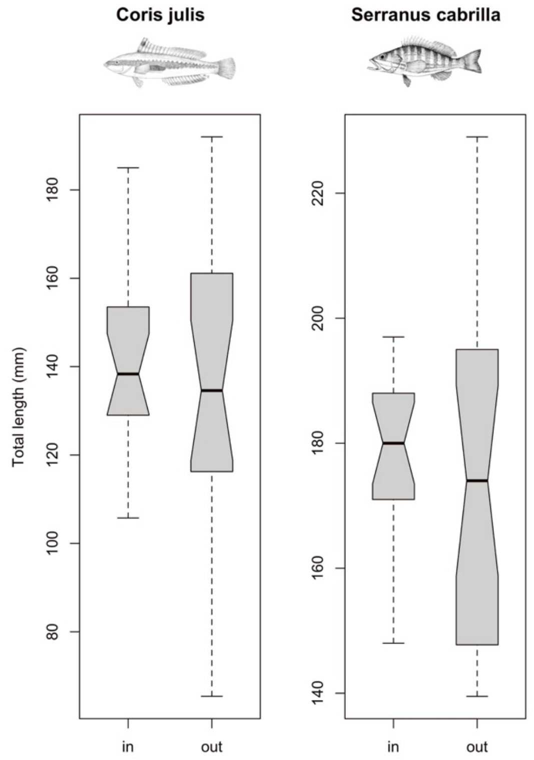
Table. 3.4 Differences in community abundance, biomass and length for the 3 key-targets species between protection levels (Wilcoxon test) and habitat (Kruskal-Wallis test). MLS: Minimum Landing Size. Significant p-values (p<0.05) highlighted in grey. (+): higher values inside the future no-take areas

		Protection			Habitat		
		Length	**Abundance**	**Biomass**	**Length**	**Abundance**	**Biomass**
D. vulgaris	**All**	0.02 (+)	0.06	0.00 (+)	0.63	0.84	0.56
	Above MLS	0.83	0.19	0.02 (+)	0.95	0.83	0.76
	Below MLS	0.40	0.01	0.01 (+)	0.19	0.60	0.23
D. sargus	**All**	0.25	0.25	0.27	0.12	0.50	0.08
	Above MLS	0.25	0.25	0.27	0.12	0.50	0.08
	Below MLS	-	-	-	-	-	-
S. cantharus	**All**	0.00 (+)	0.06	0.01 (+)	0.24	0.65	0.76
	Above MLS	-	-	-	-	-	-
	Below MLS	0.00 (+)	0.06	0.01 (+)	0.24	0.65	0.76

Non-target key species

For the 2 key non-target species, only *C. julis* had significant differences with greater length (Wilcoxon test; P=0.03) and biomass (Wilcoxon test; P=0.00) inside than outside (Table.

3.5). As for the key demersal target species, Kruskal-Wallis tests showed no significant differences with the different habitat types.



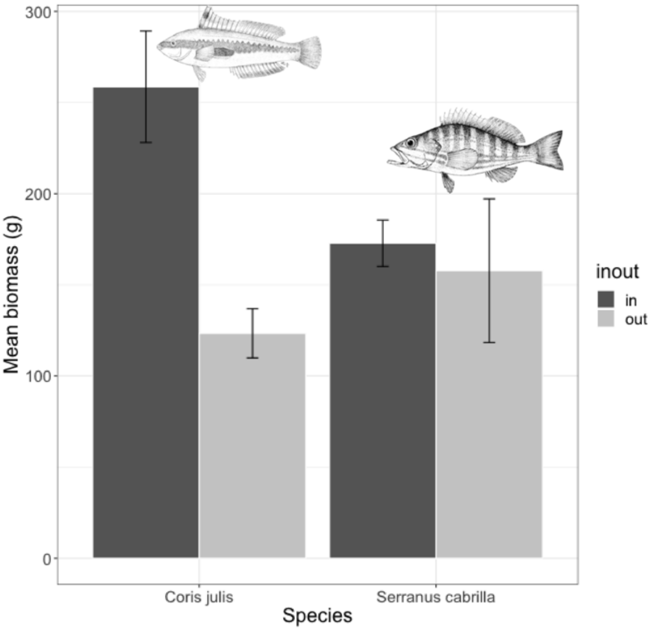


Fig. 3.7 Boxplot of the total length in mm for the 2 keys non-target species inside and outside. Fish pictures taken from FAO (Bauchot, 1987; Schneider, 1990).

Fig. 3.8 Histogram of the mean biomass in g for the 2 keys non-target species inside (in black) and outside (in grey). Fish pictures taken from FAO (Bauchot, 1987; Schneider, 1990)

Table. 3.5 Differences in community abundance, biomass and length for the 3 key-targets species between protection levels (Wilcoxon test) and habitat (Kruskal-Wallis test). Significant p-values

		Protection			**Habitat**		
		Length	**Abundance**	**Biomass**	**Length**	**Abundance**	**Biomass**
C. julis	**All**	0.77	0.03 (+)	<0.001 (+)	0.10	0.60	0.28
S. cabrilla	**All**	0.54	0.66	0.40	0.25	0.22	0.82

4. Discussion

The assessment of demersal fish and commercial invertebrate communities inside and outside the no-take areas of the future Marine Park of the Parque Natural Marinho do Recife do Algarve – Pedra do Valado (also known as AMPIC) suggests preliminary differences in the assemblages between the different future levels of protection. These differences occur even before the implementation and enforcement of any regulations. Indeed, the multivariate analyses showed a significantly higher abundance of all individuals and also a higher biomass and abundance of non-target species within the inner zone. Our results also suggest a higher biomass in the future no-take zone for some key species.

Higher biomasses or abundance in the protected zones were reported after a few years following the establishment of the reserve (Claudet *et al.*, 2008; Di Franco *et al.*, 2009; Horta e Costa et al., 2013b; Belackova, 2019). However, some of these positive effects observed within MPAs could be due to unstudied pre-existing differences (Osenberg et al., 2006, 2011). It is for this reason that a baseline data is so important, and that is why this study aims at disentangling the effects of habitat and protection in the future. Several non-mutually exclusive hypotheses can explain the pre-existing differences in the response to protection.

Firstly, marine protected zones can be strategically placed in sites where abundance and biomass are higher than in surrounding zones (Thiault et al., 2019). Preliminary scientific data mapping biodiversity hotspots have guided the design of the MPA by researchers and stakeholders. This may explain the higher total biomass found in the future no-take zones.

The second hypothesis is that the duration of the sampling period was too short and that the demersal fish and invertebrate assemblage encountered here would only be the assemblage present in November/December and February. Intra- and inter-annual movements of individuals due to foraging, breeding and environmental changes can change the assemblages among seasons (Santos, Monteiro and Lasserre, 2005). To go further, annual sampling is recommended, which is more expensive but provides a more complete analysis.

The third potential reason would be due to an unknown factor, other than the main physical habitat of the sampling site, as the biotic habitat associated was not assessed. Also, other independent factors such as environmental variability and temperature have been found to be important abiotic factors affecting the abundance distribution of the fish assemblage in coastal zones (García-Charton and Pérez-Ruzafa, 2001; García-Charton et al., 2004; Russ and Alcala, 2004; Baptista et al., 2019). Further analysis, taking into account additional parameters to those used in this study, is needed to refute or not this hypothesis.

The target species group, *Diplodus sargus*, *Diplodus vulgaris* and *Spondyliosoma cantharus*, are among the most commercial species of our samples (Erzini et al., 1998; Gonçalves et al., 2003; Veiga et al., 2010; De Biologia et al., 2013). The species *D. sargus*, unlike the other key target species, showed no difference in size, abundance or biomass between the two zones compared. Furthermore, only individuals above MLS of *D. sargus*, with a size of a mature individual (17 cm), were observed despite the large number of individuals measured. The lack of juveniles may be also an artifact of the method, attracting only adults due to the presence of the bait (Harvey et al., 2007). This species shows limited home ranges with good responses when the reserve size is adequate (D'Anna et al., 2011; Abecasis et al., 2015; Belo et al., 2016). In a small Portuguese MPA, Abecasis *et al.* (2015) found that increases in biomass and abundance were observed for this species shortly after implantation. This species should be closely monitored for future studies as it has been identified as a potentially good indicator of the effects of MPAs in the region. (Belackova, 2019). Although less prized (Veiga et al., 2010), *D. vulgaris* showed significant differences between the two future protection zones. Our results were in agreement with Belackova (2019) who observed different distributions for the two species. This may be due to divergent compartmental and spatial preferences for the two Sparids (Sala and Ballesteros, 1997; Osman and Mahmoud, 2009). The species *S. cantharus* showed even greater differences in size and biomass between protection levels than *D. vulgaris*. However, even inside, no sexually mature fish were found. This lack of data for individuals above the MLS can be explained by the fact that the size of *S. cantharus* increases with depth. Similar sizes were found by Pajuelo and Lorenzo (1999) and Erzini et al. (1998) in areas less than 30 m deep while the largest individuals were found at depths greater than 210 m. Furthermore, those authors observed that small individuals appeared from December to April following the peak of spawning activity in winter. Despite the preference of large individuals for deeper waters, it is recommended that the current legislation be strengthened to better conserve this vulnerable species (Pajuelo and Lorenzo, 1999; Gonçalves and Erzini, 2000; Pinder et al., 2017). For the key non-target species, *Coris julis* have shown a higher abundance and biomass inside the future no-take zone. Furthermore, the very high contribution to dissimilarity explains the significance in biomass of the non-target group. The high abundance and biomass of *C. julis* within the future protected area can be explained by the ecological preference for continuous and complex habitats (Vega Fernández *et al.*, 2007). Indeed, the complexity of the habitats allows protection from predators. However, the lack of significant differences between the two areas in terms of habitat complexity does not confirm this assumption. Furthermore, the high abundance and small size of this species may have underestimated the results (Campbell et al., 2015; Stobart et al., 2015). The ubiquity and high abundance of *C. julis* in a previous study in a Portuguese MPA (Horta e Costa et al., 2013b) led the authors to remove this species from their analysis. Nevertheless, *C. julis* and *Serranus cabrilla* are studied for the effects of MPAs as important species without commercial value (Harmelin, Bachet and Garcia, 1995; Claudet et al., 2006; Basterretxea et al., 2013; Belackova, 2019). The species *S. cabrilla* had a very homogeneous distribution across the whole studied zones; it is a solitary species with low MaxN. Based on responses already observed, negative effects of protection for this non-target species could be expected, after a period of regulation (Horta e Costa *et al.*, 2013b). Due to its high site fidelity, *S. cabrilla* is, as well, a good candidate to be a non-commercial indicator of MPA effects (Alós et al., 2011).

Analysis of the habitat effect is essential to separate from other sources of variation, particularly from the protection effect. Greater richness was found for the most complex habitat, which is in line with many studies (García-Charton and Pérez-Ruzafa, 2001; Gratwicke and Speight, 2005). Surprisingly, no significant differences in abundance or biomass have been shown despite the fact that a large number of studies have found effects correlated with habitat complexity (Verdiell-Cubedo *et al.*, 2013; Trebilco *et al.*, 2015; Cheminée *et al.*, 2021); and despite the fact that we used a variety of univariate and multivariate analytical approaches to test the effects of habitat on the demersal fish community. Juveniles of *D. sargus*, *D. vulgaris* and small Labridae are reported to have preference for the most complex habitats providing protection from predators (Connell and Jones, 1991; Abecasis, Bentes and Erzini, 2009; Cheminée *et al.*, 2016, 2021). Small individuals of *D. vulgaris* and small Labridae did not have a differentiated abundance between the 3 habitat types and this may be due to MaxN sampling saturation (Stobart *et al.*, 2015). MaxN measurements, such as those conducted to evaluate the effectiveness of MPAs, may not be the most appropriate method for abundance because they underestimate population size, especially at high densities (Campbell et al., 2015; Stobart et al., 2015). However, further research is needed to understand more about the distribution of species according to the type of habitat complexity. This method provides an overview of the habitat faced at the location of the structure launches but does not map the surrounding habitats. It would also be useful to consider benthic species, non-commercial invertebrates, algae, and the overlap of results with habitat mapping for a complete analysis.

Comparing the assemblage of the demersal fish and commercial invertebrate community with various similar studies, our results suggest differences in fish assemblage and frequencies of occurrence of some species. The first data were collected but not published, in this zone, between 2003-2009 through underwater visual census (UVC), in the RENSUB project led by J. Gonçalves (CCMAR) (Gonçalves et al., 2007, 2008, 2010). Taking into account only the same sampling sites, 66 species were surveyed, however, by removing the benthic or cryptobenthic species, as done in our study, 40 different species were identified whereas we only saw 28. Thus, a wider range of species have been observed using the UVC method over those 7 years. However, the duration of the study may have contributed to that difference. Our sampling was conducted over a short period of time with little or no opportunity to observe seasonal variation in species composition, size and abundance. Furthermore, the diver in the UVC may positively or negatively attract some species which will tend to flee or move closer (Chapman *et al.*, 1974; Watson and Harvey, 2007; Thanopoulou *et al.*, 2018). On the other hand, in terms of cost effectiveness, the SBRUV has shown its advantages with a constant number of species sampled at a lower cost (Langlois *et al.*, 2010). Using exactly the same method and structures, Belackova (2019) carried out an assessment of the demersal fish and rocky commercial invertebrate community in an MPA at Sagres (PNSACV), 60 km from the future MPA of this study. Some species differed but richness was similar among studies. Differences in fish and demersal invertebrate species assemblages may have been caused by the geographical difference between the two zones. The PNSACV sampling was carried out close to the cliffs in contrast to the AMPIC which is 5 to 10 km away from the shore. Taxa such as the Mugilidae (widely recorded in the PNSACV), were not observed in this study. Due to their high tolerance to saline variations, the bentho-pelagic taxa may prefer shallower environments near estuaries and coasts (Cardona, 2000; Górski, De Gruijter and Tana, 2015). More surprisingly, other demersal species such as *Dicentrarchus labrax* and *Sarpa Salpa* were not observed in our study, although they were observed in the nearshore regions of previous studies nearby (Belackova, 2019; RENSUB project).

Our study showed very encouraging results for endangered species. Indeed, 18% of the species encountered are considered by the IUCN in the red list. Most notably, two encounters with the bull ray *Aetomylaeus bovinus*, recently upgraded to CR (critically endangered) due to the continuing decline of mature individuals (Jabado *et al.*, 2021). Few studies and observations have been conducted on it, however, it seems that communities at the global level are extremely fragmented and isolated (Jabado *et al.*, 2021). Thus, each individual identification, especially by photo-identification, is vital for the monitoring of the population contributing to its conservation (Solleliet *et al.,* 2018). Unfortunately, it is not possible to assess the influence of future MPAs on rare species such as this one, whose influence on community parameters, due to its very low abundance, is extremely small (Winfree *et al.*, 2015). In order to better detecting protection effects on rare or endangered species, a huge number of samples might be needed, far in excess of the sample size of this study. By increasing the number of samples, we will also be able to better understand the future effects of fishing outside the no-take zone, by comparing the with the trends insides. New pre-establishment samples are being analyzed and may allow reinforcing the baseline data.

This study aims at gathering data before the MPA is implemented, which will allow conducting a BACI design in the future, to better monitor the effects of the protection measures. This is central to distinguish habitat influences on assemblage composition from responses to management effects. Indeed, Control-Impact studies may overestimate the effects of protection and give a false sense of confidence (Thiault et al., 2019). The BACI approach thus circumvents this problem and allows accounting for both temporal and spatial variation. However, even studies with pre-implementation analyses tend to have often only one survey before, hampering attributing with confidence observed temporal trends to the effects of regulations (Osenberg et al., 2006, 2011).

5. Conclusion

In conclusion, this study has highlighted the importance of the BACI method for a more efficient evaluation of MPAs. The presence of pre-existing differences highlights the complexity of analyzing dynamic environments containing many factors. In this study, the location of future protection levels and habitat factors were analyzed by assessing the response parameters of biomass, length, abundance and species richness. Spatial differences were noted, particularly in biomass, between the no-take zone and outside, where extractive activities will be still allowed. Habitat complexity only showed differences in species richness, but no other significant differences were detected. In addition, this study allowed the disentanglement of the protection effect from the habitat effect and thus will support further investigations to deepen the knowledge of the disparities in the zone. It was also shown that each species has a wide range of responses, even within the same species between juveniles and adults. However, more sampling will be needed to collect more data, especially on rare species which are of key importance in MPAs. This study will serve as a baseline for future work to determine the effects of protection and thus address the need for species protection. In order to obtain an effective MPA, further studies need to be carried out before the zone is implemented and this should continue over time after implementation

6. Reference

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7. Annexes

Table. Annexe 1 SIMPER results for community abundance and abondance based on protection. The table shows average abundance and average biomass in protection level (inside, outside), Av.Diss : average dissimilarity value, Diss/SD : dissimilarity to standard deviation ratio, Contrib% : percentage of contribution of each species to dissimilarity and Cum.% : cumulative percentage contribution to dissimilarity

Group	in	out				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
All species			49.26			
Coris julis	2.95	1.96	9.56	1.30	19.40	19.40
Diplodus vulgaris	2.81	2.15	6.92	0.94	14.06	33.46
Spondyliosoma cantharus	0.88	1.36	6.18	1.19	12.55	46.01
Diplodus sargus	1.11	0.76	4.39	1.06	8.91	54.92
Serranus cabrilla	1.28	1.46	3.79	0.85	7.69	62.61
Chromis chromis	0.45	0.39	3.02	0.46	6.13	68.74
Trachurus trachurus	0.46	0.42	2.87	0.92	5.82	74.56
Diplodus cervinus	0.52	0.28	2.84	0.83	5.77	80.33
Pagrus pagrus	0.29	0.29	1.99	0.75	4.04	84.37
Centrolabus exoletus	0.39	0.18	1.90	0.75	3.85	88.22
Octopus vulgaris	0.14	0.29	1.70	0.63	3.45	91.67
Conger conger	0.21	0.2	1.49	0.63	3.02	94.69
Ctenolabrus rupestris	0.26	0.16	1.46	0.65	2.97	97.66
Labrus bergylta	0.24	0.07	1.15	0.58	2.34	100.00
Target			48.32			
Diplodus vulgaris	2.81	2.23	11.03	1.03	22.83	22.83
Spondyliosoma cantharus	0.88	1.41	10.61	1.3	21.96	44.79
Diplodus sargus	1.11	0.78	7.57	1.14	15.68	60.47
Diplodus cervinus	0.52	0.29	4.91	0.92	10.17	70.64
Trachurus trachurus	0.46	0.43	4.89	0.96	10.12	80.76
Pagrus pagrus	0.29	0.3	3.57	0.77	7.38	88.14
Octopus vulgaris	0.14	0.3	3.02	0.66	6.25	94.39
Conger conger	0.21	0.21	2.71	0.63	5.61	100.00
Target above MLS			71.4			
Diplodus vulgaris	1.55	0,00	22.29	0.97	31.22	31.22
Diplodus sargus	1.25	0.82	18.21	1.13	25.5	56.72
Trachurus trachurus	0.4	0.39	10.48	0.89	14.67	71.39
Diplodus cervinus	0.47	0.21	9.12	0.84	12.77	84.16
Pagrus pagrus	0.27	0.33	8.23	0.74	11.53	95.69
Conger conger	0.12	0.04	2.09	0.32	2.92	98.61
Octopus vulgaris	0,00	0.04	0.99	0.19	1.39	100.00
Target below MLS			53.78			
Diplodus vulgaris	2.11	2.23	19.13	1.22	35.57	35.57
Spondyliosoma cantharus	0.97	1.52	16.14	1.25	30.02	65.59

Octopus vulgaris	0.16	0.28	4.40	0.64	8.18	73.76
Conger conger	0.23	0.12	3.64	0.60	6.76	80.53
Diplodus sargus	0.16	0.15	3.46	0.53	6.44	86.97
Diplodus cervinus	0.05	0.20	2.94	0.52	5.46	92.43
Trachurus trachurus	0.11	0.16	2.90	0.47	5.40	97.83
Pagrus pagrus	0.05	0.04	1.17	0.29	2.17	100.00
Non Target			44.67			
Coris julis	3.26	1.96	21.11	1.29	47.25	47.25
Serranus cabrilla	1.41	1.46	7.60	0.75	17.02	64.27
Chromis chromis	0.50	0.39	6.03	0.51	13.49	77.77
Centrolabus exoletus	0.44	0.18	4.18	0.76	9.37	87.13
Ctenolabrus rupestris	0.28	0.16	3.14	0.68	7.03	94.16
Labrus bergylta	0.26	0.07	2.61	0.59	5.84	100.00
Species	**Av.biomass**	**Av.biomass**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
All species			59.9			
Diplodus sargus	17.21	8.54	11.15	1.09	18.61	18.61
Diplodus vulgaris	17.33	11.46	8.30	1.01	13.86	32.47
Coris julis	13.14	7.51	6.54	1.11	10.92	43.39
Pagrus pagrus	5.38	5.46	5.69	0.71	9.50	52.89
Diplodus cervinus	7.70	3.79	5.61	0.76	9.37	62.26
Serranus cabrilla	10.34	10.31	5.14	0.90	8.59	70.85
Trachurus trachurus	4.49	3.66	4.32	0.72	7.20	78.05
Spondyliosoma cantharus	5.13	3.32	3.90	1.04	6.51	84.56
Chromis chromis	2.95	2.48	2.88	0.40	4.81	89.36
Labrus bergylta	1.61	1.46	1.93	0.33	3.22	92.59
Octopus vulgaris	0.00	2.37	1.67	0.26	2.79	95.38
Conger conger	0.00	2.09	1.22	0.26	2.04	97.41
Ctenolabrus rupestris	0.92	0.57	0.82	0.48	1.37	98.79
Centrolabus exoletus	1.13	0.39	0.73	0.61	1.21	100,00
Target			64.23			
Diplodus sargus	17.21	8.86	16.58	1.13	25.81	25.81
Diplodus vulgaris	17.33	11.89	12.93	0.91	20.13	45.94
Pagrus pagrus	5.38	5.66	9.06	0.70	14.11	60.05
Diplodus cervinus	7.7	3.93	8.78	0.74	13.67	73.72
Trachurus trachurus	4.49	3.79	6.52	0.74	10.15	83.87
Spondyliosoma cantharus	5.13	3.44	6.03	1.03	9.39	93.26
Octopus vulgaris	0.00	2.46	2.43	0.26	3.78	97.04
Conger conger	0.00	2.17	1.90	0.26	2.96	100.00
Target above MLS			72.49			
Diplodus sargus	19.99	12.54	20.11	1.27	27.73	27.73
Diplodus vulgaris	12.19	0,00	13.29	0.88	18.33	46.06
Diplodus cervinus	9.26	5.37	12.75	0.86	17.59	63.66
Pagrus pagrus	5.93	7.37	12.17	0.79	16.79	80.45
Trachurus trachurus	5.21	4.93	9.18	0.88	12.67	93.11
Conger conger	2.57	0.84	3.21	0.33	4.43	97.55

Octopus vulgaris	0.00	1.42	1.78	0.20	2.45	100.00
Target below MLS			57.64			
Diplodus vulgaris	11.25	11.53	35.87	1.23	62.24	62.24
Spondyliosoma cantharus	5.66	3.71	19.57	0.98	33.94	96.18
Octopus vulgaris	0.00	1.29	2.20	0.28	3.82	100.00
Conger conger	0.00	0.00	0.00	-	0,00	100.00
Diplodus sargus	0.00	0.00	0.00	-	0,00	100.00
Diplodus cervinus	0.00	0.00	0.00	-	0,00	100.00
Pagrus pagrus	0.00	0.00	0.00	-	0,00	100.00
Trachurus trachurus	0.00	0.00	0.00	-	0,00	100.00
Non Target			46.94			
Coris julis	14.52	7.51	18.9	1.09	40.26	40.26
Serranus cabrilla	11.43	10.31	12.7	0.88	27.06	67.32
Chromis chromis	3.26	2.48	6.48	0.49	13.81	81.12
Labrus bergylta	1.78	1.46	4.25	0.34	9.05	90.18
Ctenolabrus rupestris	1.01	0.57	2.31	0.52	4.92	95.10
Centrolabus exoletus	1.25	0.39	2.30	0.63	4.90	100.00

Table. Annexe 2 SIMPER results for community abundance and abondance based on habitat. The table shows average abundance and average biomass in protection level (inside, outside), Av.Diss : average dissimilarity value, Diss/SD : dissimilarity to standard deviation ratio, Contrib% : percentage of contribution of each species to dissimilarity and Cum.% : cumulative percentage contribution to dissimilarity

Group	**hab1**	**hab2**				
Species	**Av.abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
All species			50.69			
Coris julis	2.15	2.43	9.38	1.32	18.51	18.51
Diplodus vulgaris	2.15	2.63	7.61	1.07	15.01	33.51
Spondyliosoma cantharus	1.02	1.48	6.63	1.21	13.08	46.59
Serranus cabrilla	1.10	1.61	4.66	0.91	9.20	55.79
Diplodus sargus	0.78	0.92	4.56	1.04	8.99	64.78
Chromis chromis	0.10	0.80	3.64	0.44	7.18	71.97
Trachurus trachurus	0.48	0.32	2.71	0.88	5.34	77.30
Pagrus pagrus	0.20	0.47	2.44	0.87	4.82	82.12
Diplodus cervinus	0.25	0.32	2.30	0.69	4.53	86.65
Centrolabus exoletus	0.10	0.39	1.75	0.70	3.45	90.11
Octopus vulgaris	0.20	0.24	1.72	0.65	3.39	93.50
Conger conger	0.10	0.26	1.35	0.61	2.66	96.16
Ctenolabrus rupestris	0.12	0.18	1.12	0.55	2.21	98.36
Labrus bergylta	0.05	0.18	0.83	0.50	1.64	100.00
	hab3	**hab1**				
All species			50.12			
Coris julis	2.71	2.15	9.72	1.37	19.39	19.39
Diplodus vulgaris	2.63	2.15	7.60	1.13	15.17	34.57

Spondyliosoma cantharus	0.91	1.02	6.22	1.02	12.42	46.98
Diplodus sargis	1.10	0.78	3.99	1.11	7.97	54.95
Serranus cabrilla	1.53	1.10	3.71	0.87	7.41	62.36
Trachurus trachurus	0.53	0.48	3.34	0.92	6.67	69.02
Diplodus cervinus	0.70	0.25	3.14	1.04	6.27	75.30
Chromis chromis	0.40	0.10	2.06	0.61	4.12	79.41
Centrolabus exoletus	0.39	0.10	1.91	0.72	3.81	83.22
Ctenolabrus rupestris	0.37	0.12	1.89	0.74	3.77	86.99
Conger conger	0.31	0.10	1.80	0.62	3.59	90.58
Octopus vulgaris	0.25	0.20	1.79	0.68	3.58	94.16
Pagrus pagrus	0.17	0.20	1.59	0.61	3.18	97.34
Labrus bergylta	0.25	0.05	1.34	0.57	2.66	100.00
	hab3	**hab2**				
All species			42.87			
Coris julis	2.71	2.43	7.00	1.27	16.32	16.32
Spondyliosoma cantharus	0.91	1.48	6.36	1.41	14.84	31.17
Chromis chromis	0.40	0.80	4.02	0.60	9.38	40.55
Diplodus sargus	1.10	0.92	3.79	1.25	8.83	49.38
Diplodus vulgaris	2.63	2.63	3.55	1.22	8.28	57.66
Diplodus cervinus	0.70	0.32	2.90	1.20	6.76	64.41
Trachurus trachurus	0.53	0.32	2.62	0.88	6.12	70.53
Centrolabus exoletus	0.39	0.39	2.15	0.89	5.02	75.55
Serranus cabrilla	1.53	1.61	2.15	0.98	5.01	80.56
Pagrus pagrus	0.17	0.47	2.01	0.91	4.69	85.25
Conger conger	0.31	0.26	1.79	0.75	4.18	89.43
Ctenolabrus rupestris	0.37	0.18	1.68	0.79	3.91	93.34
Octopus vulgaris	0.25	0.24	1.51	0.74	3.51	96.86
Labrus bergylta	0.25	0.18	1.35	0.68	3.14	100.00
	hab 1	**hab2**				
Species	**Av.biomass**	**Av.biomass**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
All species			60.75			
Diplodus sargus	8.8	14.97	11.19	1.03	18.42	18.42
Diplodus vulgaris	12.63	14.80	8.16	1.04	13.43	31.85
Pagrus pagrus	5.10	9.07	7.63	0.82	12.56	44.41
Coris julis	8.93	11.04	6.65	1.20	10.94	55.35
Serranus cabrilla	8.97	11.03	5.70	0.78	9.38	64.73
Trachurus trachurus	4.11	4.55	4.91	0.64	8.08	72.81
Spondyliosoma cantharus	3.86	5.40	4.13	1.05	6.79	79.60
Chromis chromis	0.78	5.69	3.96	0.42	6.51	86.12
Diplodus cervinus	3.53	2.99	3.03	0.54	4.98	91.10
Octopus vulgaris	2.78	0.64	2.35	0.33	3.87	94.97
Conger conger	1.01	0.00	0.85	0.22	1.40	96.36
Ctenolabrus rupestris	0.22	1.03	0.80	0.43	1.31	97.67
Labrus bergylta	0.00	0.95	0.79	0.24	1.30	98.97
Centrolabus exoletus	0.33	0.92	0.62	0.57	1.03	100.00

	hab3	**hab1**				
All species			60.78			
Diplodus sargus	14.19	8.80	10.21	1.08	16.80	16.80
Diplodus vulgaris	15.06	12.63	8.53	1.08	14.03	30.83
Diplodus cervinus	12.2	3.53	8.47	0.94	13.94	44.77
Coris julis	10.00	8.93	6.60	1.26	10.85	44.77
Serranus cabrilla	11.58	8.97	5.29	0.81	8.710	64.33
Trachurus trachurus	3.09	4.11	4.04	0.75	6.65	70.98
Pagrus pagrus	0.81	5.10	3.70	0.54	6.09	77.07
Spondyliosoma cantharus	2.62	3.86	3.53	0.88	5.81	82.88
Labrus bergylta	4.89	0.00	3.34	0.42	5.50	88.37
Conger conger	3.21	1.01	2.35	0.37	3.87	92.24
Octopus vulgaris	0.00	2.78	2.05	0.29	3.37	95.61
Chromis chromis	1.60	0.78	1.17	0.54	1.93	97.54
Ctenolabrus rupestris	1.10	0.22	0.78	0.47	1.29	98.82
Centrolabus exoletus	1.04	0.33	0.71	0.56	1.18	100.00

	hab3	**hab 2**				
All species			55.7			
Diplodus sargus	14.19	14.97	10.82	1.20	19.43	19.43
Diplodus cervinus	12.20	2.99	7.25	0.94	13.01	32.44
Diplodus vulgaris	15.06	14.80	5.73	1.08	10.28	42.73
Pagrus pagrus	0.81	9.07	5.64	0.78	10.13	52.85
Coris julis	10.00	11.04	4.16	1.08	7.47	60.32
Trachurus trachurus	3.09	4.55	4.04	0.71	7.26	67.58
Chromis chromis	1.60	5.69	3.79	0.47	6.81	74.39
Serranus cabrilla	11.58	11.03	3.72	1.02	6.67	81.06
Spondyliosoma cantharus	2.62	5.40	3.50	1.22	6.28	87.34
Labrus bergylta	4.89	0.95	3.37	0.49	6.05	93.39
Conger conger	3.21	0.00	1.42	0.30	2.55	95.94
Ctenolabrus rupestris	1.10	1.03	1.08	0.58	1.95	97.89
Centrolabus exoletus	1.04	0.92	0.82	0.73	1.48	99.37
Octopus vulgaris	0.00	0.64	0.35	0.24	0.63	100.00

Table. Annexe 3 Comparative table of key-target species throughout the study area, inside and outside. Abundance, length (cm), biomass (g), Number of individuals counted and number of individuals measured of the same species, % of measured individuals of the most common species. Mean ± SE95% shown for abundance, length and biomass. Abundance and biomass are per sample, length is mean individual length for the specie.

TARGET SPECIES

	All area	**In**	**Out**
	Diplodus vulgaris		
abundance	7.73 ± 0.69	8.71 ± 1.06	6.88 ± 0.62
length (cm)	13.19 ± 0.31	14.03 ± 0.46	12.50 ± 0.36
biomass (g)	310.46 ± 37.67	404.22 ± 52.72	233.01 ± 48.91
N° counted	348	183	165
N°measured	144	76	68
% measured	41%	42%	41%
	Diplodus sargus		
abundance	2.16 ± 0.54	2.67 ± 0.83	1.71 ± 0.63
length (cm)	22.96 ± 19.14	23.40 ± 1.87	22.45 ± 3.74
biomass (g)	668.66 ± 255.21	830.14 ± 318.79	480.27 ± 140.41
N° counted	69	40	29
N°measured	36	21	15
% measured	52%	53%	52%
	Spondylisoma cantharus		
abundance	4.86 ± 1.28	2.83 ± 1.37	6.29 ± 1.72
length (cm)	12.06 ± 1.14	16.65 ± 2.00	8.99 ± 0.49
biomass (g)	86.06 ± 22.90	136.63 ± 57.48	52.35 ± 16.89
N° counted	141	34	107
N°measured	60	22	38
% measured	43%	65%	36%

Table. Annexe 4 Comparative table of key non-target species throughout the study area, inside and outside. Abundance, length (cm), biomass (g), Number of individuals counted and number of individuals measured of the same species, % of measured individuals of the most common species. Mean ± SE95% shown for abundance, length and biomass. Abundance and biomass are per sample, length is mean individual length for the specie.

NON-TARGET SPECIES

	All area	**In**	**Out**
		Coris Julis	
abundance	9.76 ± 1.18	13.67 ± 1.81	6.70 ± 1.17
length (cm)	13.78 ± 0.44	14.09 ± 0.35	13.50 ± 0.88
biomass (g)	187.46 ± 20.34	258.65 ± 30.57	123.39 ± 13.49
N° counted	400	246	154
N°measured	144	84	60
% measured	36%	34%	39%
		Serranus cabrilla	
abundance	2.57 ± 0.45	2.59 ± 0.16	2.56 ± 0.54
length (cm)	17.8 ± 0.94	18.1 ± 0.34	175.83 ± 9.30
biomass (g)	163.99 ± 35.15	172.82 ± 12.72	157.74 ± 39.40
N° counted	113	44	69
N°measured	61	27	34
% measured	54%	61%	49%