Effects of the implementation of the MPAs from the Alentejo Coast on local fish communities and on some species with commercial/conservation interest

Tadeu José Faria de Sousa Pereira

Tese apresentada à Universidade de Évora
para obtenção do Grau de Doutor em Biologia

ORIENTADORES: *Professor Doutor Pedro Miguel Raposo de Almeida*
Professor Doutor José Lino Vieira de Oliveira Costa

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aos meus Pais

"Fish," he said softly, aloud, "I'll stay with you until I am dead."

Ernest Hemingway | The Old Man and the Sea

- Who are you, who are so wise in the ways of science?

- I am Arthur, King of the Britons.

Monty Python and the Holy Grail

I won't lie to you, boys, I was terrified! But I pressed on - and as I made my way past the breakers, a strange calm came over me. I don't know if it was divine intervention or the kinship of all living things, but I tell you, Jerry, at that moment - I was a Marine Biologist!

George Costanza in *Seinfeld 'The Marine Biologist'*

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Mira, Andrade, Robalo, Marco, Berm, Frade. Não é preciso dizer nada, certo?

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Resumo

Efeitos da implementação das AMPs da Costa Alentejana nas comunidades piscícolas locais e em populações de algumas espécies com interesse comercial e/ou conservacionista.

Tendo em conta o seu sucesso, as Áreas Marinhas Protegidas (AMP) têm sido implementadas como ferramenta de gestão de recursos pesqueiros. Contudo, o uso desta ferramenta em portugal é ainda relativamente recente. Tendo em conta esta necessidade, em 2011 foi criado o Parque Marinho do Sudoeste Alentejano e Costa Vicentina, que inclui várias AMPs. Duas destas estão localizadas na costa alentejana do referido parque. A eficiência destas ferramentas depende dum planeamento e gestão adequados. De facto, a dimensão duma AMP tem um papel importante na sua eficácia e no tempo necessário para que alterações nas suas comunidades piscícolas seja evidentes. AMPs de maior dimensão e mais antigas tendem a apresentar maiores densidades e exemplares de maior tamanho. Nesse sentido, o principal objectivo desta tese foi avaliar se a designação de pequenas AMPS podem causar impactos positivos a curto prazo em espécies de interesse comercial, nas suas comunidades piscícolas e nas actividades de pesca local. No caso da AMP da Ilha do Pessegueiro, esta provou ser importante e adequada para proteger algumas espécies de interesse comercial, como moreias, safios e sargos, que encontram na zona refúgio e alimento. As comunidades de peixes locais também foram positivamente impactadas, vendo um aumento significativo na sua abundância e diferenças na sua composição quando comparadas com áreas não protegidas. O efeito mais imediato destas medidas é a perda de área de pesca para a frota pesqueira. No entanto, a deslocalização da frota para áreas próximas resultou num aumento das descargas com o tempo. De um modo geral, este trabalho valida a implementação destas medidas como adequadas e eficazes para a proteção marinha e para a exploração sustentável dos recursos. Confirma também que pequenas AMPs podem ser eficazes a curto prazo. No entanto, uma monitorização contínua dos impactos é indispensável. Assim, é aconselhada a manutenção destas medidas de protecção na costa Alentejana.

Palavras – chave

Área Marinha Protegida, telemetria acústica, comunidades piscícolas, ecologia trófica, descargas de pescado, Parque Marinho do Sudoeste Alentejano e Costa Vicentina

Summary

Effects of the implementation of the MPAs from the Alentejo Coast on local fish communities and on some species with commercial/conservation interest.

Given their success, MPAs have been widely implemented as fisheries management tools. However, the use of MPAs as conservation and management tools in Portugal is recent. In 2011, the Costa Vicentina and Sudoeste Alentejano (PNSACV) Marine Park was implemented which included several no take MPAs. Two of these were in the Alentejo coast of the Park. MPA efficiency depends on adequate planning and an appropriate management. In fact, the size of the MPA plays an important role on how effective it can be, and how fast changes on fish assemblages may occur. Larger and older MPAs tend to present higher densities and larger specimens than younger or smaller MPAs. The main objective of this dissertation was to evaluate if the designation of small no take MPAs can cause positive impacts at a short term on commercially important fish species, local fish communities and local fisheries. The implementation of the Pessegueiro Island no take MPA proved to be important and adequate for protecting commercially important species, such as morays, congers and seabreams, who find in this area optimal feeding and sheltering areas. Local fish assemblages were also positively impacted by the designation of both no take MPAs, with a significant increase in fish abundance and significant differences in their structure between protected and neighbouring areas. The most immediate consequence of these protective measures was the loss of available fishing grounds, but fleet relocation resulted in the increase in fish landings over time. Overall, this work validates the implementation of these measures as adequate and effective protection tools for marine conservation and sustainable resource exploitation. It also confirms that small no take MPAs can be short term effective. However, continuous monitoring is of the uttermost importance to adapt protective measures. Given this, the continuity of the protective measures in place is advised.

Keywords

Marine Protected Areas, acoustic telemetry, fish assemblages, trophic ecology, fish landings, PNSACV Marine Park

Index

Chapter 3

Chapter 4

CHAPTER 1

GENERAL INTRODUCTION

1.1. General introduction

The growth of the human population together with the technological development observed in the recent decades have contributed to the expansion of fishing industries and consequent increase in fish consumption (FAO 2014). Inversely, however, the capture of commercially important species has been decreasing as a consequence of the pressure of the fishing industry on marine resources (FAO 2014). In fact, in 2011, 615 of fish stocks were considered near the limit of their Maximum Sustainable Yield (MSY) (FAO 2014), raising serious questions on their sustainability (Pauly et al. 2002). In 2009, the North Atlantic region was the third most exploited area in the world, having 93% of fish stocks near MSY or over exploited (Ye and Cochrane 2011).

Due to biological and ecological aspects as well as to technological issues, demersal fish stocks have been the most affected by over exploitation with 38% of stocks classified as over exploited (Ye and Cochrane 2011).

Portugal is the second largest fish consumer per capita worldwide (FAO-FIGIS 2015). In 2012, Portuguese fishing industry employed 13 156 people and had 16 559 registered fishermen (INE 2007-2015). Nevertheless, fish captures (gross tonnage) has decreased 65% over the past 50 years (FAO-FIGIS 2015). This can be explained by either the displacement of workforce to other activities or by the near over exploitation of fish stocks. Therefore, the adoption of scientific based management plans for marine resources is key.

Considering the recurrent failure of traditional measures of fisheries management, such as fishing effort control or capture control, the designation of Marine Protected Areas (MPA) has been proposed as a management tool that can effectively contribute to the sustainable exploitation of marine resources (e.g. Plan Development Team 1990, Allison et al. 1998, Pauly et al. 2002). An MPA can be defined as any intertidal or subtidal areas, together with their fauna, flora, and cultural and historical heritage, protected by law in order to maintain biodiversity and guarantee a sustainable use of their resources (Kelleher and Kenchington 1992). The objectives of establishing an MPA go from maintaining ecological and life support processes to protecting biodiversity, and assuring a sustainable use of marine resources, both species and ecosystems (Kelleher and Kenchington 1992).

From a conservationist point of view, MPAs have been considered an effective tool, namely by promoting the increase in biomass, density, abundance, species richness and specimens' size (e.g. García-Rubies and Zabala 1990, Roberts 1995, Rius 2007). The increase in density may also increase competition for space and food, with prey-predator interactions, leading to the dispersion of specimens to outside MPA boundaries, benefiting fisheries in adjacent areas (e.g. Sánchez-Lizaso et al. 2000, Abesamis and Russ 2005, Grüss et al. 2011). Biomass transport to outside MPA boundaries may also be independent from density increase, namely through pelagic eggs and larvae (Russ 2002, Grüss et al. 2011), and juvenile and adult migration, either onthogenic (Roberts and Polunin 1993, Vandeperre et al. 2008, Grüss et al. 2011) or caused by home range reallocation (Roberts and Polunin 1993, Kramer and Chapman 1999). Through this spill over phenomena, MPAs contribute not only for local fish stocks (Harmelin-Vivien et al. 2008, Forcada et al. 2009), but also at regional level, by exporting pelagic eggs and larvae (Forcada et al. 2009). This way, MPAs act as buffering zones in protecting marine resources against the inefficiency of the traditional measures for fisheries management and against the unpredictable and uncontrollable breaks in fish recruitment (Allison et al. 1998, Pauly et al. 2002, Pitchford et al. 2007).

Given their success, MPAs have been widely implemented as fisheries management tools (García-Charton and Pérez-Ruzafa 1999, Lubchenco et al. 2003). However, the use of MPAs as conservation and management tools in Portugal is only recent. In mainland Portugal, only four MPAs are in place and each with different protection levels: Litoral Norte Park (since 1987, north coast), Berlengas Natural Reserve (since 1981, central coast), Arrábida Marine Park (since 1998, central coast), and the Marine Park of Costa Vicentina and Sudoeste Alentejano Natural Park (PNSACV Marine Park, since 1995, south western coast). The PNSACV started as a 2 km wide maritime extension of the Sudoeste Alentejano and Costa Vicentina Natural Park (PNSACV) (Castro and Cruz 2009), following the entire coast of this park (FIGURE 1.1) (Gonçalves 2000).

Biophysical and ecological features of PNSACV result in highly productive marine systems, so fishing activities have an important role in local socio-economy (Gonçalves 2000, Reis 2011). Despite being mostly artisanal and traditional, local fisheries are performed intensively (Castro and Cruz 2009). This resulted in the need to regulate local fishing activities in the park and adjacent coastal area through a revised General Development Plan. As a result, the Sudoeste Alentejano and Costa Vicentina Marine Park (PNSACV Marine Park) was designated, comprising different areas with distinct protection levels. These included Type I Protection Areas, commonly referred to as no take MPAs, in which all fishing activities are forbidden with the exception of commercial harvesting of stalked barnacle, *Pollicipes pollicipes*, Gmelin 1789 (Ordinance 11B/2011 February 4).

MPA efficiency depends on adequate planning, namely location, size and design (Claudet et al. 2008, Pérez-Ruzafa and García-Charton 2008, Forcada et al. 2009), as well as an appropriate management with an effective monitoring and law enforcement system (Allison et al. 1998, Claudet and Pelletier 2004, García-Charton et al. 2008). In fact, the size of an MPA plays an important role on how effective it can be and how fast changes on fish assemblages may occur, with a larger and older MPAs presenting higher densities and larger specimens than younger or smaller MPAs (Claudet et al, 2008).

Solid knowledge on the area to be protected, such as oceanographic conditions, ecosystem structure, species biology and ecology, and socio-economy are determinant factors for an adequate planning (e.g. Allison et al. 1998, Claudet and Pelletier 2004, García-Charton et al. 2008).

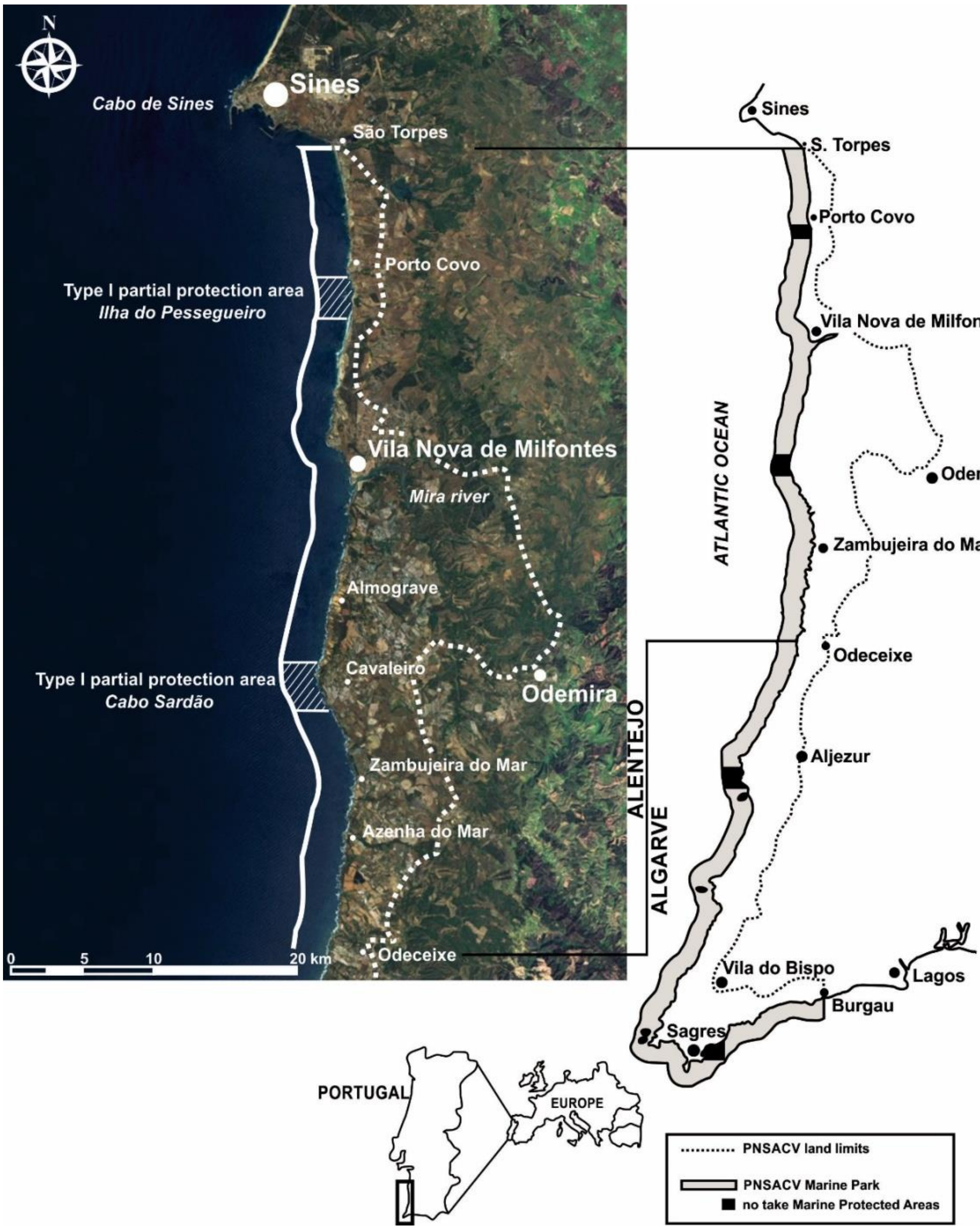

FIGURE 1.1 – Location of the Sudoeste Alentejano and Costa Vicentina Marine Park (PNSACV Marine Park), in South Western coast of Portugal, and of the Pessegueiro Island and Cabo Sardão Type I protection areas (no take MPAs).

Knowledge on the ecology and biology of targeted species are also key in planning and implementing MPAs that aim to restore fish stocks (Allison et al. 1998, Gonçalves 2000, Claudet and Pelletier 2004). In this context, movements and activity patterns of fish and their use of the territory are relevant (e.g. Kramer and Chapman 1999, Claudet and Pelletier 2004, Topping et al. 2005). A species' home range is a relatively circumscribed area over which an organism moves to acquire vital resources for survival and reproduction (Dingle 1996). Therefore, an efficient no take MPA should include the entire or most of the species home range in order to reduce the probability of capture (Krammer and Chapman 1999, Pauly et al. 2002). This way, it is important to assess if the size of no take MPAs encompasses the home range of some commercially important fish species and is, therefore, adequate to protect them.

However, the application of single-species management tools to restore fisheries has proven quite ineffective when applied to multispecies fisheries. This scenario is particularly evident concerning artisanal fisheries (Vinther et al. 2004, Tzanatos et al. 2005). While in some cases the increment of fish species and density inside MPAs can be visible after only 2-3 years upon implementation (Halpern 2003), improvements in fisheries harvests and profits in adjacent areas are usually only visible in the mid to long-term (e.g. Russ et al. 2003, Goñi et al. 2010). Immediately after implementation, marine reserves usually reduce catches because fishers lose fishing grounds (Smith et al. 2010), which will lead to a redistribution of fishing effort through activity displacement (Halpern et al. 2004).

After the initial decrease in harvests, however, catch rates outside no take areas seem to develop (Vandeperre et al. 2011). Several studies focusing on global fish landings in the Portuguese coast indicate a decreasing trend in terms of gross tonnage (Baeta et al. 2009, Gamito et al. 2013, Ferreira et al. 2013, Teixeira et al. 2014). However, despite several studies focusing on the impact of MPA implementation on its animal communities becoming increasingly available (e.g. Gonçalves et al. 2003, Jacinto et al. 2011, Bertocci et al, 2012, Henriques et al. 2013, Haug et al. 2015), the effect of no take MPA implementation on the quantity of fish captured is yet to be evaluated, with the only available approach the work by Viegas (2013), precisely in the PNSACV Marine Park. In fact, the recent implementation, in 2011, of four partial protection areas within the PNSACV Marine Park, where fishing activities are prohibited, provided the opportunity to monitor the impact of these protective measures on local fish landings as well as the evolution of fish assemblages from an early stage and to determine how fast protection effects can become evident. Also, the insufficiency of updated data regarding fish assemblage characterization in this region (Castro 2004) urged to act rapidly in order to gather baseline information that can be used in future studies assessing reserve effects. More recently, following the implementation of the no take areas in the

PNSACV Marine Park, evaluating the effectiveness of these management tools to protect commercially important species has become a key necessity (Silva 2015).

1.2. Main objectives

The main objective of this dissertation was to evaluate if the designation of small no take MPAs can cause positive impacts at a short term on local fisheries, local fish communities and local commercially important fish species by studying two small no take MPAs (Pessegueiro Island and Cabo Sardão) on the southwestern coast of Portugal since the early stages of their implementation. More specifically the following questions were investigated:

a) *Is the small size of the Pessegueiro Island no take MPA adequate for protecting commercially important fish species?* It is expected that despite its small size, this no take MPA can be effective in protecting species that are highly targeted by local fisheries, especially species with reduced home range and high site fidelity. The Mediterranean moray, the European conger and the white seabream are such species and it is anticipated that the features of Pessegueiro Island are adequate for protecting the local populations of these species.

b) *Did the implementation of the two northernmost protected areas of the PNSACV Marine Park lead to positive changes in fish assemblage trophic ecology, fish species diversity and fish assemblage structure?* It is expected to observe positive impacts in these parameters with time, but is also important to assess how long do the fish communities take to respond to protective measures. However, if differences between protected and unprotected areas exist at an initial phase, there is a high probability that those are inherent to each locality's characteristics and that protected sites naturally present a higher potential to be marine reserves. If those differences emerge with time, then no take MPA designation could be responsible for those changes to happen. In both cases, no take MPA designation is justified, as its purpose is to maintain existing biodiversity or create conditions to enhance biodiversity (Halpern 2003);

c) *Did MPA designation impact fish landings between before and after MPA designation inside and outside this marine park?* By answering this question, it will be possible to evaluate how these protective measures impacted local (mainly artisanal) fishing communities at short term, and create a basis from which the evolution of fish landings can be monitored and compared in order to assess eventual mid and long term benefits of no take MPA designation to local fisheries.

1.3. Thesis outline

The present work is divided into seven chapters.

In Chapter 1 a global framing of the study is provided and main objectives are described.

Chapter 2 encompasses the analysis of the movements of morays and congers inside Pessegueiro Island no take MPA to assess its adequacy for protecting these two commercially important fish species. Chapter 3 addresses if the same no take area has an adequate size for protecting local white seabream populations by analysing fish movements inside this area. These two chapters answer the question about the adequacy of this small no take MPA in particular, and small no take MPAs in general, to effectively protect commercially important fish species from different habitats, namely demersal (seabreams) and benthic (congers and morays) species.

Chapter 4 comprises the analysis made to local fish communities to assess how no take MPA designation may have impacted local fish assemblages in terms of diversity and abundance by comparing between protected and unprotected areas as well as over time. Chapter 5 consists in assessing how protective measures impacted the trophic ecology of local fish assemblages by comparing the respective diets between protected and unprotected areas as well as over time. These two chapters altogether aim to answer if fish species diversity, fish assemblage structure and fish assemblage trophic ecology were impacted and in what extent.

In Chapter 6 fish landings data from inside and outside PNSACV Marine Park are compared to assess how the implemented protective measures impacted local fisheries.

Chapter 7 includes the general conclusions and the final remarks of the present work.

1.4. General methodology

1.4.1. Study area

The present work was conducted in the Sudoeste Alentejano and Costa Vicentina Marine Park (PNSACV Marine Park), a marine complement to the inland Sudoeste Alentejano and Costa Vicentina Natural Park (PNSACV).

The PNSACV extends over 120 km of coast, between Sines (Alentejo - southwestern coast) and Lagos (Algarve - South coast) (FIGURE 1.1). The inland park itself was first established as Protected Landscape in 1988, becoming a Natural Park in 1995. With limited human pressure, this natural park contains some of the most well preserved shores of southern Europe (ICNB 2008, Bastos et al. 2012). Its coastal line is characterized by cliffs interspaced by small near pristine waterlines (Bastos et al. 2012).

Since its inception, this park included a maritime extension, comprising a 2 km wide coastal strip along the Natural Park shore, comprising a marine area of about 29 000 ha. However, only in 2011 was it officially designated as Marine Park through the Ordinance 11B/2011. Since then, this large protected area is divided into three different protection regimes: total protected areas (42 ha), type I partial protected areas (2 478 ha), and type II partial protected areas (26 521 ha) (Ordinance 11B/2011). While on total protected areas all fishing activities are forbidden, inside type I partial protected areas only commercial harvest of stalked barnacle *Pollicipes pollicipes* (Gmelin 1790) is allowed. For this reason, these areas are commonly referred to as no take MPAs. Inside type II partial protected areas fishing restrictions mostly consist in species specific legislation and fishing gear regulation (Ordinance 11B/2011).

The western Portuguese coast is predominantly under north/northeastern winds, especially during summer (Relvas and Barton 2002). Also, upwelling occurs during this period with colder but nutrient enriched water emerging near the coast (Fiúza et al. 1982, Relvas and Barton 2002). Sea water temperature in the region ranges between 15 ºC during winter and 21 ºC during summer, although during upwelling water temperature may decrease down to 17 ºC (Portuguese Geographical Institute 2005, ICNB 2008). As in the rest of the western coast of Portugal, sea swell in the region of the PNSACV derives predominantly from Northwest with wave height ranging in average between 1 and 2 m (Institute of Meteorology 2004, ICNB 2008). The complexity of water current patterns and confluence of distinct water bodies in this region originate high productivity and consequent high biodiversity, including important fishing resources (e.g. Fiúza et al. 1982, Abrantes and Moita 1999, Peliz and Fiúza 1999).

Fish assemblages from the southwestern coast of Portugal encompass 149 species (ICNB 2008). Small pelagic, for example sardine [*Sardina pilchardus* (Walbaum, 1792)] and chub mackerel (*Scomber colias* Gmelin*, 1789*), and small demersal fish, such as horse mackerel [*Trachurus trachurus* (Linnaeus, 1758)] and boarfish [*Capros aper* (Linnaeus, 1758)], are dominant in terms of biomass (Gomes et al. 2001, Sousa et al. 2005, ICNB 2008).

Despite usually considered without significant impact on the Portuguese panorama, local fishing activities are economically important to the region. Typically, artisanal fishing activities mostly use multi gear or purse seine (Castro 2004, INE 2007-2015)

A total of 12 fishing harbours are at use in the PNSACV Marine Park, being Sines, Sagres and Vila Nova de Milfontes the most important. Despite the increase in tourism for the past years, agriculture and fisheries are the activities that provide the most employment in the region (INE 2007-2015). Either by recreation or profession, fishing is an intense and traditional activity inside the PNSACV Marine Park (Castro and Cruz 2009).

The present work focused exclusively in the Alentejo coast of the PNSACV Marine Park. In this region two Type I Partial Protected Areas (no take MPAs) were designated in 2011: Ilha do Pessegueiro to the north and Cape Sardão to the South. Both no take MPAs are relatively small. Pessegueiro Island has an area of ca. 6 km^2 and Cape Sardão ca. 7 km^2. Maximum depth on both areas is ca. 25 m and the bottom is composed by rock and sand in Pessegueiro Island no take MPA, while in Cape Sardão it is mainly rocky reef. The overall fish assemblage of these areas is very diverse, some of which of high commercial value for regional fisheries, such as breams *Diplodus sargus* (Linnaeus, 1758), *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), conger eel *Conger conger* (Linnaeus, 1758), sole *Solea solea* (Linnaeus, 1758) and moray eel *Muraena helena* (Linnaeus, 1758) (ICNB 2008).

1.4.2. Data collection

Acoustic telemetry twas used o investigate area use and movement patterns of some selected fish species inside no take MPAs. Specifically, this method was applied to evaluate the movements and area use by *Muraena Helena* Linnaeus 1758, *Conger conger* (Linnaeus, 1758) and *Diplodus sargus* (Linnaeus, 1758) inside the Pessegueiro Island Marine no take MPA.

An array of 20 automatic underwater acoustic receivers VR2W-69kHz (Vemco) was deployed around the Pessegueiro Island allowing them to cover most of the no take MPA. Since these species were monitored in different years, acoustic receivers display was adapted between years to better address the specific traits of each species. 16 morays and five congers were captured inside Pessegueiro Island no take MPA using baited fish traps deployed around the island and left fishing overnight. Captured specimens were transported to land, anesthetized and implanted with V9 2H-69 kHz (Vemco) acoustic transmitters. After surgery, specimens were taken inside the MPA to be released and monitored by the acoustic receiver array. Regarding seabreams, 19 adult *D. sargus* were captured by angling from Pessegueiro Island. Contrary to morays and congers, these specimens were immediately anaesthetized, surgically implanted with the same model coded acoustic transmitters and released at capture site (SEE CHAPTERS 2 AND 3).

In both years, tagged specimens were continuously monitored for 60 days. Adverse sea conditions, typical of the region during most of the year, limited telemetry studies in the area to the summer periods. Additionally, mark-recapture was used on *D. Sargus* to evaluate its population dynamics and movements inside the entire Marine Park.

Data used for the analysis of fish assemblages from the Alentejo Coast of the PNSACV Marine Park was obtained by means of experimental fishing. Sampling consisted of 4 surveys: August 2011 (summer), February 2012 (winter), August 2013 (summer) and December 2013 (winter). This temporal design allowed for obtaining data from the first year the no take MPAs were implemented (2011/12) and from almost three years after protection (end of 2013), before and after eventual early effects of that protection would become evident. The first two sampling seasons comprised year 1 of protection while the remaining corresponded to year 3. Surveys were performed aboard a professional fishing vessel using trammel nets with inner mesh of 100 mm and two outer layers of 500 mm mesh. Trammel nets were the only fishing gear that could be used simultaneously in both areas in a cost efficient manner. Also, this gear provides a global sampling of the entire fish assemblage, rather than more selective gears such as fish traps and longlines. However, in the Pessegueiro Island no take MPA a bottom otter trawl was also used to sample juveniles and small size fish species. Further details on these methods are provided in the following chapters (SEE CHAPTERS 4 AND 5).

Fish landings data for the 6th chapter of this work was obtained either from yearly surveillances performed and published by the Portuguese National Institute for Statistics (Instituto Nacional de Estatística – INE 2007-2015) or provided by the National Authority for Natural Resources and Maritime Services and Security (Direção Geral dos Recursos Naturais, Segurança e Serviços Marítimos – DGRM). Further information is provided in the chapter (SEE CHAPTER 6).

All procedures were carried out in accordance with the Portuguese legislation regarding animal capture, manipulation and experimentation for scientific purposes. This includes certification on laboratory animal science that meets the requirements of FELASA level C courses to license people responsible for directing animal experiments and Veterinary National Authority proper accreditation.

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Chapter 2

ASSESSING THE SIZE ADEQUACY OF A SMALL NO TAKE MARINE PROTECTED AREA FOR MEDITERRANEAN MORAY AND EUROPEAN CONGER

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Abstract

In 2011, several type I Marine Protected Areas (no take MPAs) were implemented inside the Sudoeste Alentejano and Costa Vicentina Marine Park, along the southwestern coast of Portugal. This study quantified the home range, movement patterns and activity levels of 19 moray (*Muraena Helena*) and 6 conger (*Conger conger*) eels to assess if the size of one of these MPAs (Pessegueiro Island) is adequate to protect these heavily fished species. Individuals were captured inside and outside the no take MPA, tagged with acoustic transmitters and monitored for 2 months during the summer of 2013. Morays and congers showed mean residency index of 0.48, and home ranges of 19.4 and 34.4 ha, respectively, corresponding to 4% and 8% of the MPA. Morays and congers were active for 3.3 % and 12.0 % of the time, respectively, mainly at night and during quarter lunar phases. Morays moved less than 1 km per day around reefs near the island, while congers roamed more widely. Results highlight the importance of this no take MPA as a refuge and feeding area for both species at least during the summer period, and suggest that its size is adequate for protecting these species against fishing activity. Ultimately, the present study provides evidence that small no take MPAs can be adequate for protecting species with reduced home ranges.

Keywords:

Activity patterns, acoustic telemetry, *Conger conger*, home range, *Muraena helena*, PNSACV Marine Park

2.1. Introduction

Human population growth is leading to an increase in fish consumption and a decrease in the abundance of commercially fished species (FAO 2014), with associated problems regarding fisheries sustainability (Pauly et al. 2002). In face of this, management plans for marine resources based on scientific evidence are essential. Given the limited success of traditional management tools such as fishing effort control, implementation of Marine Protected Areas (MPAs) has been highly advocated in promoting fisheries sustainability and biodiversity preservation (Allison et al. 1998, Pauly et al. 2002). These protected areas are generally accepted as being effective and contributing to increases in the number, biomass, abundance and size of individuals of multiple key species (e.g. García-Rubies and Zabala 1990, Roberts 1995, Russ 2002). An MPA can be defined as any intertidal or subtidal area that is protected by law to preserve biodiversity and assure a sustainable use of its resources (Kelleher and Kenchington 1992). In 1995 a 2-km wide coastal stretch was designated as an extension of the Sudoeste Alentejano and Costa Vicentina Natural Park (PNSACV), southwest Portugal. At the time no fishing restrictions were implemented. However, in 2011 this area was designated as a Marine Park and no take and type I partial protection areas were implemented. In these areas, commonly referred to as no take MPAs, almost all fishing activities are now forbidden.

The efficiency of an MPA depends on its location and size (e.g. Claudet et al. 2008) together with an appropriate management plan and adequate monitoring and surveillance (Allison et al. 1998, Claudet and Pelletier 2004, García-Charton et al. 2008). Furthermore, knowledge of fish species biology and ecology is a key factor when designating an MPA or a network of MPAs (Allison et al. 1998, Claudet and Pelletier 2004). Thus, knowledge on fish movements, activity patterns and habitat use throughout MPA and adjacent areas is paramount (e.g. Kramer and Chapman 1999, Claudet and Pelletier 2004, Topping et al. 2005). Furthermore, a no take MPA to be efficient should encompass at least most of the home ranges of the species of interest, to reduce the probability of their capture by commercial or recreational fisheries (Kramer and Chapman 1999, Pauly et al. 2002); with home range defined as the relatively circumscribed area over which an organism moves to acquire vital resources for survival and reproduction (Dingle 1996).

Home range estimation can be based on presence indicators (Powell 2000) and acoustic telemetry is a widely used method to study the movement of marine organisms (Zeller 1999, Heupel et al. 2006) which has been used to help plan and manage several MPAs (e.g. Abecasis and Erzini 2008, Abecasis et al. 2009, Alós et al. 2012). Although acoustic telemetry is often used to study the spatial ecology of many marine fish (e.g. Finn et al. 2014, Farris et al. 2016, Lédée et al. 2016), this technique has not been previously used on several commercially important species, such as the Mediterranean moray (*Muraena helena* Linnaeus, 1758) and the European conger (*Conger conger* Linnaeus, 1758) (Pita and Freire 2011, Matić-Skoko et al. 2014).

The Mediterranean moray (Muraenidae) is a territorial fish, which lives in crevices on rocky reefs (La Mesa et al. 2008) and forages mainly during the nighttime on crustaceans, cephalopods, and other fish (e.g. Bauchot and Saldanha 1986a, Matić-Skoko et al. 2014). Moray eels inhabit shallow waters near the coast to up 800 m in depth (Jiménez et al. 2007). Near shore, where fisheries targeting *M. helena* generally occur, individuals are mostly females and juveniles (Matić-Skoko et al. 2014). The European conger (Congridae) inhabits coastal areas from a few meters to over 1000 m in depth (Mytilineou et al. 2005). Juveniles are demersal and display cryptic behaviour and high site fidelity (Correia et al. 2012) typically within rocky reefs associated with sandy bottoms (Day 1880, Bauchot and Saldanha 1986b, La Mesa et al. 2008). Individuals seem to be more active during sunset and nighttime (Day 1880, Morato et al. 1999, Pita and Freire 2011), feeding mostly on crustaceans, cephalopods (octopus) and other fish (Morato et al. 1999, O'Sullivan et al. 2004, Matić-Skoko et al. 2012). Due to their semelparitiy, congers are particularly vulnerable to intense targeted fishing (Morato et al. 1999).

Both species are common inside the Pessegueiro Island no take MPA, where, unlike in adjacent areas, individuals are supposedly protected from fishing activities. No previous study has examined whether the size of the no take MPA around Pessegueiro Island encompasses most of the home range of conger and moray eels. Previous studies indicated that this small, no take MPA was adequate to protect white seabream (*Diplodus sargus* Linnaeus, 1758), a species which is also highly targeted by fishers and displays a reduced home range and high site fidelity (e.g. Belo et al. 2016). Nevertheless, it is important to test an MPA's suitability for a broad array of commercially exploited fish species in order to further confirm the effectiveness of this management tool.

Regardless of their wide distribution and commercial importance, most studies on conger and moray focus on diet (e.g. Morato et al. 1999, O'Sullivan et al. 2004, Matić-Skoko et al. 2014), age and growth (e.g. Matić-Skoko et al. 2011, 2012), reproduction (e.g. Cau and Manconi 1984, Sbaihi et al. 2001, Matić-Skoko et al. 2011) and population structure (e.g. Correia et al. 2012). Very few studies have examined the home range, movement patterns, space use and site fidelity of moray and conger eels. To the best of our knowledge, only one study (Pita and Freire 2011) has provided preliminary information on these aspects of the ecology of conger eel. This lack of knowledge makes it challenging to promote management measures for the sustainable exploitation of both species.

The main objective of this study was to assess whether the size of the Pessegueiro Island no take MPA is adequate for the protection Mediterranean moray and the European conger. Activity patterns and habitat use of both species were studied by means of acoustic telemetry and the influence of environmental factors on the behaviour of both species was also evaluated. The results from this study provide important and useful information on the adequacy of small no take MPAs to protect fish species, whilst delivering valuable information on moray and conger ecology and behaviour.

2.2. Methods

2.2.1 Study area

The study was carried out in the Pessegueiro Island type I MPA (fishing activities forbidden with the exception of commercial harvest of stalked barnacle *Pollicipes pollicipes* (Gmelin, 1790)), the northernmost protected area in the PNSACV Marine Park (FIGURE 2.1). This protected area belongs to a network of several small no take (all fishing activities forbidden) and type I protected areas, implemented within the PNSACV Marine Park in 2011. All these small areas are commonly referred to as no take MPAs. This no take MPA has an area of approximately 450 ha surrounding a small rocky island. Maximum depth is ca. 18 m and the seabed is mainly composed of rocky reefs delimited by sandy bottom. Several islets surround the island. On the northern and western sides, a rocky platform extends over 400 m after which sandy bottoms abound. These areas are exposed to the dominant north-western wind and swell. The fish community of this region is diverse, with 149 fish species described, some with high commercial value, such as morays and congers (ICNB 2008).

2.2.2 Field work

In July 2013 an array of 20 underwater acoustic receivers (VEMCO VR2W 69 Hz) was deployed inside the no take MPA and covering most of its area (FIGURE 2.1). Receivers were attached to a removable cable secured with stainless steel snap hooks on each end and connected to the main cable fixed to a 100kg cement block. A rigid plastic buoy maintained the receiver in a vertical position. The entire mooring apparatus measured ca. 2 m in height and was permanently submersed. Mooring geographical coordinates were recorded by GPS.

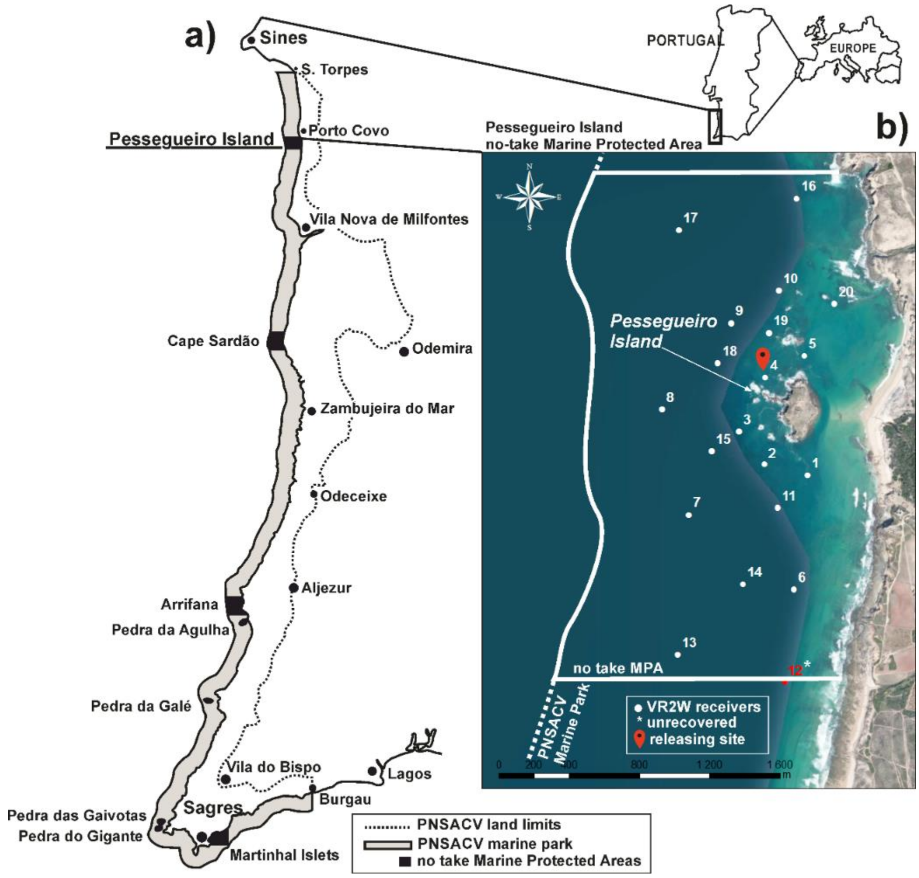

FIGURE 2.1 – a) Sudoeste Alentejano and Costa Vicentina Marine Park (grey) with its network of no take Marine Protected Areas (black). b) Detail of the Pessegueiro Type I Protection Area (i.e., no take MPA) and location of acoustic receivers.

Detection range depends on environmental conditions and habitat type, from 600 m in deep water and low turbulence conditions to 30 m in the shallow waters of tidal creeks (e.g. Finstad et al. 2005, O'Toole et al. 2011, Welsh et al. 2012). Range testing was not conducted, since detection range would greatly vary among receivers. However, it was assumed that individuals would be detected within at least 100 m a receiver, as previously observed in this area with an almost identical receiver array (e.g. Belo et al. 2016). Sentinel tags would be the most adequate method of continuously measuring home range (e.g. Payne et al. 2010, Currey et al. 2014) but this method was discarded since both studied species potentially display high site fidelity (e.g. Pita and Freire 2011, Correia et al. 2012). This fact would increase the chance of code transmission collision. To optimize signal coverage near the island, 10 receivers were deployed less than 200 m from each other.

Remaining receivers located farther from the island were positioned farther apart (*ca*. 400 m), since bottom characteristics in those areas enable higher detection range.

Individuals were caught in early August 2013 inside Pessegueiro Island no take MPA using overnight fishing with baited traps. A total of 16 morays and 5 congers was captured. Three additional morays and one conger captured southwest outside the protected area were also tagged. Individuals were placed inside a 600L oxygenated renewed sea water tank and individually anesthetized with a 2-phenoxyethanol (0.4 $ml.dm^{-3}$) solution. After measuring for length (mm) and weight (g), a 9 mm diameter coded acoustic transmitter (VEMCO V9 2H-69kHz) was implanted in the intraperitoneal cavity. Each transmitter measured 29 mm in length and weighed 2.9 g (in water). Power output was 151 dB, with a 99-day expected battery life and transmission rate of 60 s (30-90 s) nominal delay. After sugery, individuals were placed in a recovering tank for a minimum of 2 hours before being released inside the MPA (FIGURE 2.1). Tagged fishes were monitored until receivers were collected in early October 2013 to avoid winter storms season. Data from the first two days after release was discarded as tagged individuals were presumably recovering from surgery and eventually relocating to the capture sites, resulting in a 60-day study period. Adverse sea conditions, typical of the region during most of the year, restricted this study to summer.

2.2.3. Data analysis

After removing false detections and assessing noise quotient, each individual detection was assigned location, date, time, lunar cycle and swell height at the time of detection (Appendix I).

2.2.3.1 Residency, Activity and Covered Distance Index

Residency Index (I_r) was calculated for each individual as the ratio between number of days the fish was detected and total number of days of monitoring (Afonso et al. 2008). Individuals were considered active when intervals between consecutive detections were less than 10 minutes. Activity index (I_a) was calculated as the ratio between individual activity time and total time of monitoring. Individual Covered Distance Index (I_{cd}) was calculated as the sum of the minimum distances covered between consecutive detections (Lino 2012).

Average daily distance covered (by day with recorded activity) and final displacement vectors ($\vec{d}$) were also calculated to assess tagged individuals displacement tendency (see Appendix I).

2.2.3.2. Kernel Density Estimation (KDE) analysis

Kernel Density Estimation (KDE) was computed to determine home and core ranges of morays and congers. This tool estimates a bivariate density probability function, “utilization distribution”, corresponding to the spatial distribution of a certain individual, thereby providing information on habitat use (Jacoby et al. 2012). Home and core range areas and contours were obtained using the ESRI software package ArcGIS 9.3® with Hawth’s Analysis© extension (Beyer 2004), applying an h_{ref} value (Worton 1989) of 90 for morays and 135 for congers. The percentage of the MPA used by each individual was calculated (see Appendix I).

2.2.3.3. Network analysis (NA)

Network analysis evaluates the type and degree of interactions between activity centers (Jacoby et al. 2012) and it has been used as a complement to KDE (e.g. Espinoza et al. 2015, Lédée et al. 2015, Belo et al. 2016). Spatial networks are complex systems of nodes, representing acoustic receivers, connected by edges, i.e., fish movements. Centrality, a node-type metric, was calculated as both degree centrality (C_d) and betweenness centrality (C_b), measuring the affluence to the focal node (C_d) and its importance as a middle location between several paths (C_b) (Jacoby et al. 2012, Makagon et al. 2012). Network analysis was performed using UCINET software package (Borgatti et al. 2002) after testing each network for non random patterns using the *igraph* R package (Csardi and Nepusz 2006), through an edge rearrangement and bootstrap approach (Croft et al. 2011). By crossing this information with KDE analysis, it is possible to determine the type of area use of each individual (Lédée et al. 2015). Areas with high C_d indicate evident space fidelity (Jacoby et al. 2012). Areas with high C_d overlapping core range correspond to refuge areas. Conversely, areas with high C_b may represent ecological paths or access areas to valuable resources (Jacoby et al.

2012), such as feeding areas. Regarding individual fish, high individual C_d values indicate movements centred in a particular area, concordant with refuging behaviour. High individual C_b indicates broader movements, consistent with roaming behaviour (Jacoby et al. 2012) (see Appendix I).

2.2.3.4. Environmental parameters

Influence of circadian cycle, lunar cycle, tidal cycle and swell on tagged fish behaviour was also analysed through multivariate PERMANOVA (Anderson et al. 2008) performed in PRIMER 6 and PERMANOVA+© (PRIMER-E Lda 2009) and Mantel tests using ade4 R package (Chessel et al. 2004, Dray and Dufour 2007, Dray et al. 2007). Given the low sample size for congers, these analyses were only performed for morays (see Appendix I).

2.3. Results

The mean noise quotient (nq) was -222.22, a value concurrent with reduced environmental noise and occurrence of code collision. This suggests that individuals might be within receiver range, despite not being detected.

2.3.1. Residency, Activity and Covered Distance Index

In total, 69936 single and individual codes were detected and recorded by the VR2W array, with detections varying considerably amongst individuals from both species, i.e. standard deviation of 3 467 detections for morays and 13 902 detections for congers.

Both morays and congers had high mean I_r (morays 0.48; congers 0.48) (TABLE 2.1) and most of the tagged individuals were detected throughout the study period (FIGURE 2.2). Congers were generally more active than morays (morays 3.25%; congers 12.02%) (TABLE 2.1).

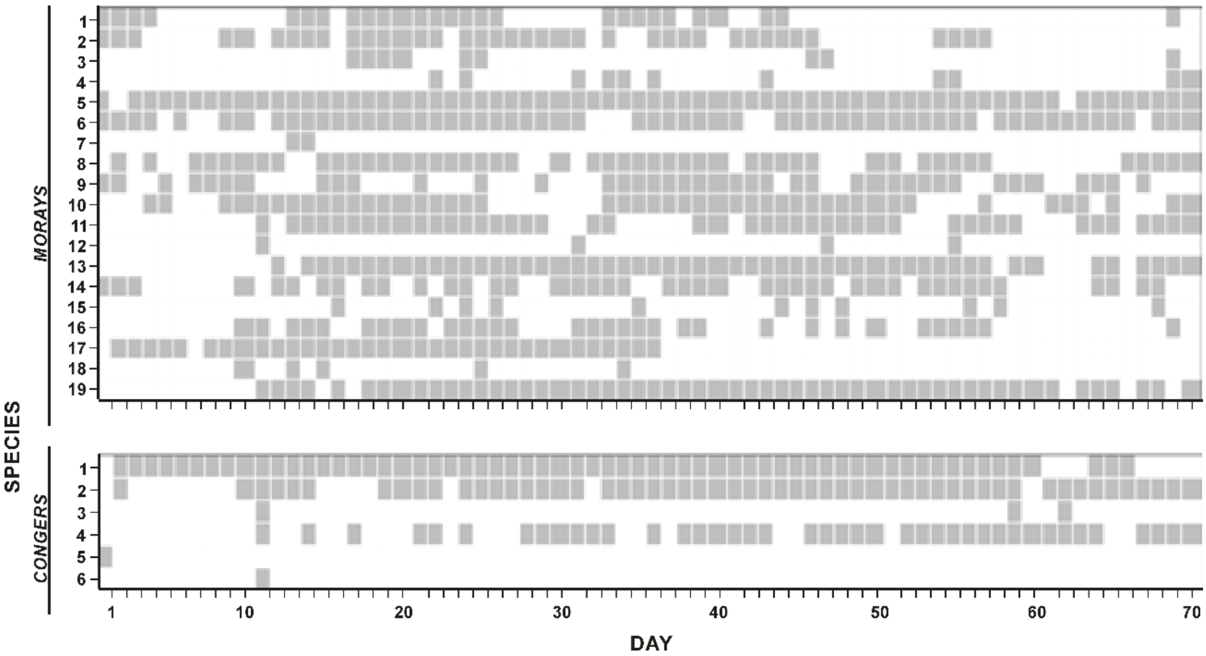

FIGURE 2.2 – Daily presence/absence map of tagged morays and congers throughout the monitoring period.

Morays covered (I_{cd}) around 11.0 km during the 60-day study period with a median daily covered distance (DCD) of 0.8 km, while congers covered about 334.9 km (DCD = 8.7 km) (TABLE 2.1). Displacement vectors confirm that morays moved mainly around their release location (near receiver R#4), presenting final displacement vectors directed towards nearby receivers (FIGURE 2.3). Congers had less uniform behaviour, with conger C#1, C#2 and C#4 staying and/or moving mainly around their release location, whereas conger #5 and #6 moved to receiver R#7 and R#13, the last one near the south limit of the area (FIGURE 2.3). Overall, these two individuals were detected only during the first 150 min after their release (FIGURE 2.2).

2.3.2. Kernel density estimation (KDE) analysis

Morays presented an average home range of 19.4 ha (4 % of the no take MPA) and core range of 3.4 ha. Congers presented mean home ranges of 34.4 ha (8% of the no take MPA) and core ranges of 8.6 ha. KDE analysis showed that morays were generally confined to a specific and restricted area near the island (FIGURE 2.4a and 2.4b) while congers showed wider areas coverage, although having home and core ranges around few locations too (FIGURE 2.5a and 2.5b).

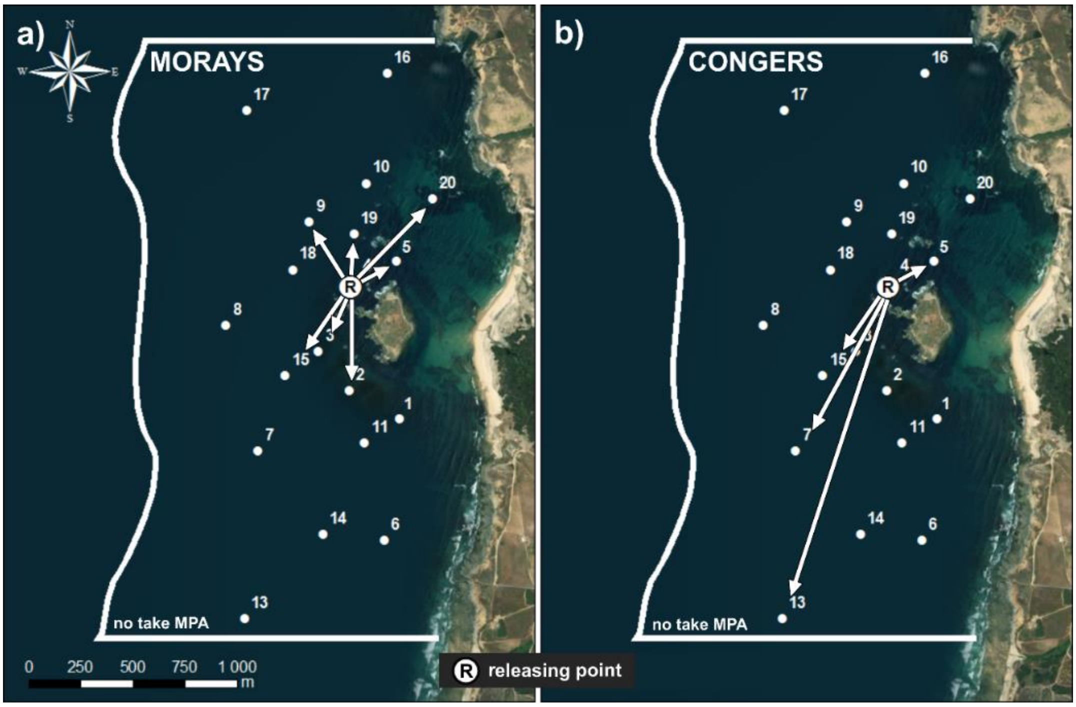

FIGURE 2.3 – Final displacement vectors of tagged a) morays and b) congers captured inside the AMP in relation to releasing point.

TABLE 2.1 – Summary table of moray and conger individual total length (TL), residency index (I_r), total activity time (AT – hh:mm), Activity Index (I_a), core range (CR), home range (HR), percentage of MPA correspondent to home range (%MPA), Covered Distance Index (I_{cd}) and Daily Distance Covered (DCD, in days with registered activity); M – morays, C – congers

Fish ID	TL (cm)	I_r (%)	AT	I_a (%)	CR (ha)	HR (ha)	% MPA	I_{cd} (km)	DCD (km)
M 1	74.0	0.39	1:55	0.13	4.3	23.8	5.32	10.48	0.40
M 2	72.0	0.54	10:06	0.70	3.7	20.5	4.59	13.17	0.42
M3	96.0	0.15	0:55	0.06	4.4	31.9	7.15	4.97	0.87
M 4	105.0	0.16	0:38	0.04	4.0	24.7	5.53	2.81	0.82
M 5	76.0	0.97	338:21	23.50	1.9	12.6	2.83	267.98	4.32
M 6	79.5	0.84	104:04	7.23	3.1	21.9	4.90	228.86	4.30
M 7*[1]	88.0	0.03	2:23	0.17	9.2	44.0	9.86	11.55	5.78
M 8	81.5	0.69	46:19	3.22	2.4	14.4	3.22	76.49	1.98
M 9	85.5	0.57	9:26	0.66	2.7	13.7	3.08	4.40	0.16
M 10	84.0	0.67	19:56	1.38	2.3	17.0	3.81	16.67	0.40
M 11*[1]	76.5	0.72	48:10	3.35	3.5	32.2	7.20	93.21	2.25
M 12	83.5	0.06	0:00	0.00	3.2	14.8	3.31	0.69	-*[2]
M 13	97.0	0.87	158:49	11.03	2.7	18.6	4.17	188.27	7.82
M 14	86.0	0.60	13:44	0.95	1.8	8.20	1.84	4.57	0.14
M 15	98.0	0.18	0:14	0.02	2.9	14.4	3.21	1.37	0.34
M 16*[1]	73.0	0.52	19:30	1.35	7.5	58.4	13.07	67.83	2.40
M 17	78.0	0.49	19:36	1.36	3.0	21.5	4.82	116.53	4.65
M 18	97.0	0.08	0:04	0.01	4.4	19.9	4.46	0.42	0.25
M 19	87.5	0.89	92:51	6.45	2.6	24.4	5.46	129.62	2.47
average	85.2	0,48	46:28	3.25	3.4	19.4	4.23	11,02*[3]	0,82*[3]
C 1	82.0	0.89	673:09	47.14	3.9	15.4	3.46	0.00	-
C 2	87.5	0.80	112:45	7.83	12.3	45.0	10.07	447.08	9.11
C 3*[1]	78.5	0.05	5:13	0.36	29.7	133.8	29.94	24.33	9.93
C 4	85.0	0.67	70:42	4.91	9.7	42.7	9.57	334.88	8.28
C 5	98.0	0.01	00:00	0.00	-	-	-	-	-
C 6	96.0	0.02	1:57	0.14	-	-	-	-	-
average	87.8	0.48	171:42	12.02	8.6	34.4	7.70	334.88*[3]	88.69*[3]

*[1] individuals captured outside the no take MPA; *[2] impossible to assess; *[3] median

2.3.3. Network analysis

Network analysis confirmed that morays moved over smaller areas, occasionally moving between zones (FIGURE 2.4c and 2.4d) while congers displayed wider ranging movements (FIGURE 2.5c and 2.5d). Centrality values indicate the area around receiver R#4 as the most used by morays (FIGURE 2.6). Degree centrality values (C_d) show that the area around receiver R#19 was highly frequented too, while betweenness centrality values (C_b) for areas around R#5, R#1, #R2 and #R8 emphasize their importance as passageway (TABLE A2.1 Appendix II, FIGURE 2.6). Overall, refuge areas and crossing and/or feeding areas were mostly located in rocky reefs near the island in the northeast quadrant (FIGURE 2.6).

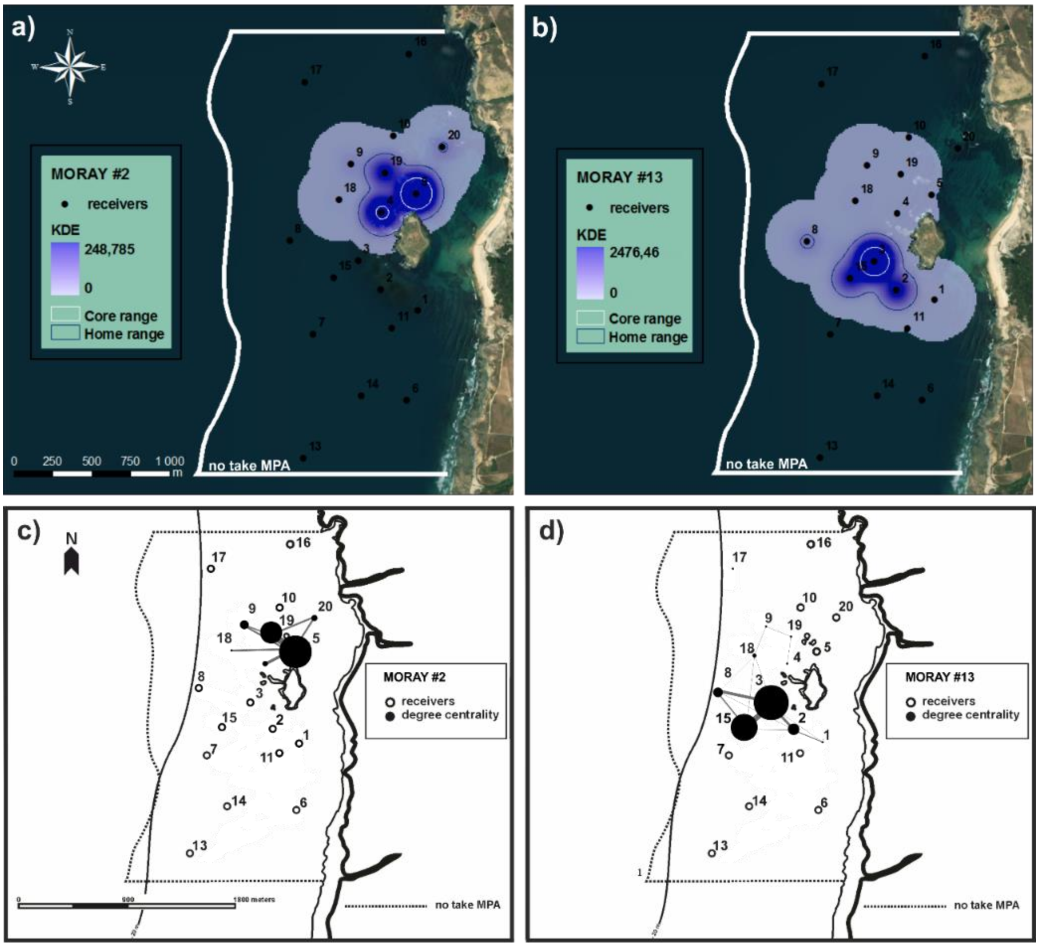

FIGURE 2.4 – Example of a), b) Kernel Density Estimation (KDE) analysis and c), d) example of movement patterns determined through network analysis for morays.

2.3.4. Environmental parameters

Moray activity (I_a) was significantly influenced by environmental parameters since tagged individuals were significantly more active during nocturnal, first quarter lunar phase and low swell periods (TABLE 2.2, FIGURE 2.7a-d). PERMANOVA analysis revealed interactions between lunar phase and circadian cycle and swell (TABLE 2.2), with significantly higher I_a in first quarter lunar phases during both day and night (FIGURE 2.7e-f TABLE A2.2, Appendix II) and in third quarter lunar phase with low swell (FIGURE 2.7g, TABLE A2.2 Appendix II). Graphical analyses show congers with higher median I_a during nighttime, third quarter lunar phase, ebbing tide and high swell (FIGURE 2.7h-k). Regarding C_b values, Mantel tests pointed out ($r<0.4$, $p>0.05$) that moray movement patterns varied with environmental variables. Betweenness values suggest morays preferred to use areas near the island as passageways or/and feeding areas during daytime (FIGURE 2.8a) with high swell (FIGURE 8d) in full moon periods (FIGURE 2.8g). During night, low swell, and other lunar periods morays tended to explore farther.

TABLE 2.2 – Results of PERMANOVA analysis regarding activity index I_a variation according to environmental parameters where df: degrees of freedom; SS: sum of squares; MS: mean squares; Pseudo-*F*: test statistic; *p*-value: calculated probability to significance level α=0.05; Perm: number of permutations

Factors	df	SS	MS	Pseudo-*F*	*p*-value	Perm
Circadian cycle	1	18550.0	18550.0	21.96	0.00	999
Lunar cycle	3	13138.0	4379.4	5.19	0.00	998
Tidal cycle	1	797.3	797.3	0.94	0.37	999
Swell	1	5959.8	5959.8	7.06	0.01	998
Circadian cycle x Lunar cycle	3	7358.6	2452.9	2.90	0.02	999
Circadian cycle x Tidal cycle	1	1112.0	1112.0	1.32	0.24	997
Circadian cycle x Swell	1	520.5	520.5	0.62	0.52	999
Lunar cycle x Tidal cycle	3	834.4	278.1	0.33	0.91	999
Lunar cycle x Swell	3	6833.0	2277.7	2.70	0.03	997
Tidal cycle x Swell	1	762.2	762.2	0.90	0.38	999
Circadian cycle x Lunar cycle x Tidal cycle	3	1168.8	389.6	0.46	0.83	998
Circadian cycle x Lunar cycle x Swell	3	5030.1	1676.7	2.00	0.09	999
Circadian cycle x Tidal cycle x Swell	1	469.96	470.0	0.56	0.53	998
Lunar cycle x Tidal cycle x Swell	3	2332.6	777.5	0.92	0.46	999
Circadian cycle x Lunar cycle x Tidal cycle x Swell	3	1015.3	338.4	0.40	0.85	997

2.4. Discussion

Pessegueiro Island no take MPA proved to be an important refuge and foraging area for both morays and congers. The size and location of this small protected area seem adequate to protect these species during the summer, when fishing pressure is highest. These results, together with previous findings (e.g. Belo et al. 2016), suggest that Pessegueiro Island no take MPA, and other small no take MPAs in general, can provide effective protection against fishing activities for highly targeted species with reduced home range and high site fidelity. Our results highlight the appropriateness of MPAs as management tools for these species. Our measurements also corroborated and complemented previous findings (e.g. Cau and Manconi 1984, Morato et al. 1999, O'Sullivan et al. 2004, Correia et al. 2012, Matić-Skoko et al. 2012, 2014).

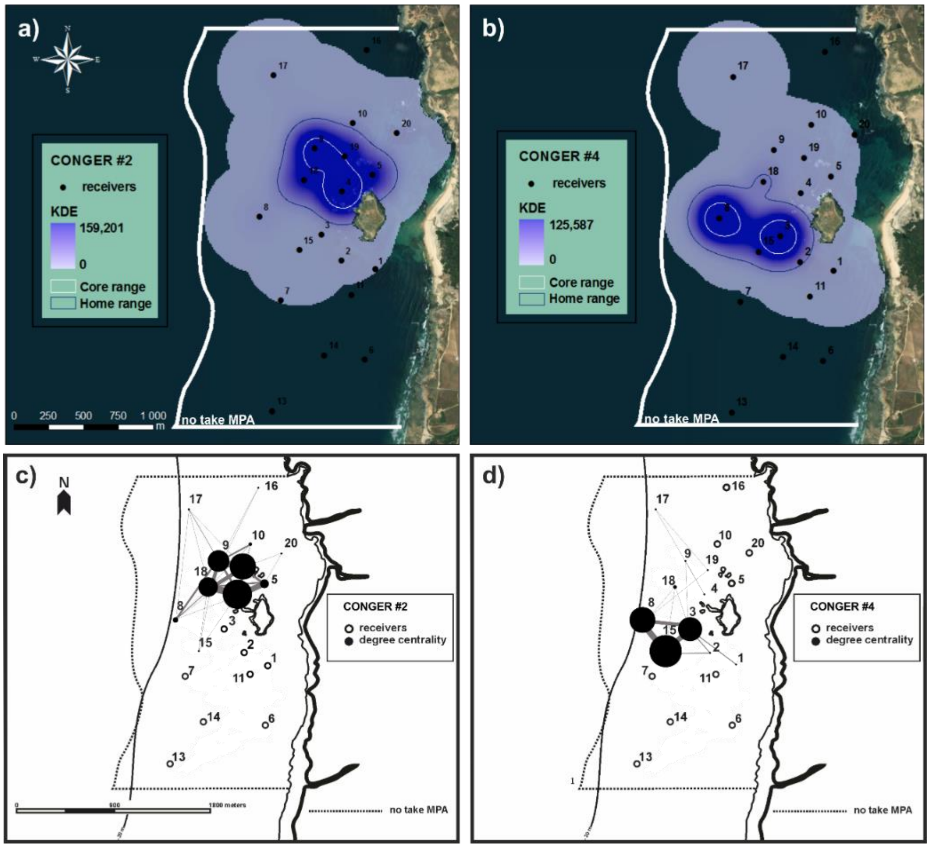

FIGURE 2.5 – Example of a), b) Kernel Density Estimation (KDE) analysis and c), d) example of movement patterns determined through network analysis for congers.

The cryptic behaviour of morays and congers, which use rocky reefs as shelters, can disturb sign transmission, reducing detection range of receivers (Lino 2012, Welsh et al. 2012) and likely contributed to some of the interference in code transmission observed in this study. Noise quotient values suggested that using sentinel tags would not have solved this issue but, rather, likely would have increased code collision. Although a range test was not performed to assess how habitat complexity and variable ocean conditions influenced the efficiency of signal detection, the deployment design of the array of receivers used here was based on a reference detection range of 100 m to provide an optimal coverage of the MPA, following previous studies in this area (Belo et al. 2016). Despite a low level of detections, the residency index of both congers and morays was high (*c.a.* 50%), while the latter were about four times more active than the former (12 vs 3%). Both species have well defined home ranges which were restricted to < 8% of MPA's area.

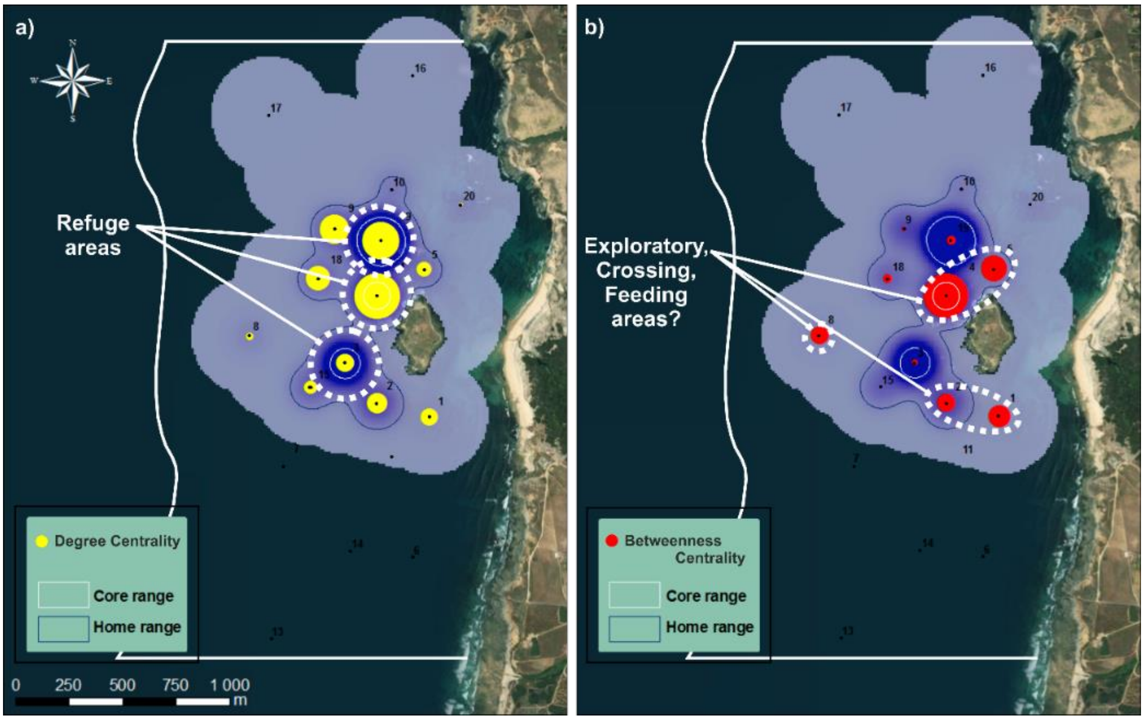

FIGURE 2.6 – a) Refuge areas and b) passageways/feeding areas of morays according to KDE and network analyses.

In this study, the mean home range of morays (19 ha) was about 50% that (34 ha) of congers. Pita and Freire (2011) previously reported a very small (0.6 ha) home range for a single conger tracked manually for 48 h. Despite a larger number of individuals tagged in this study, conclusions regarding the activity and home range of congers should be addressed with some caution. Furthermore, conger C#1 was registered on only one receiver, though detection cycles were concordant with a living individual. On the other hand, centrality measures for morays were high, revealing a more pronounced territorial and a less pronounced roaming behaviour compared to congers. Both species centered their movements closer to the island, frequently on the northwestern side, where shelter is afforded by complex rocky habitats (J. Parrinha unpublished data). Morays refuge areas overlapped with three well-known complex rocky reefs, whereas crossing and other areas which may be foraging habitats were somewhat to the east. The southernmost of these areas is dominated by sandy habitats with a few flat rock platforms.

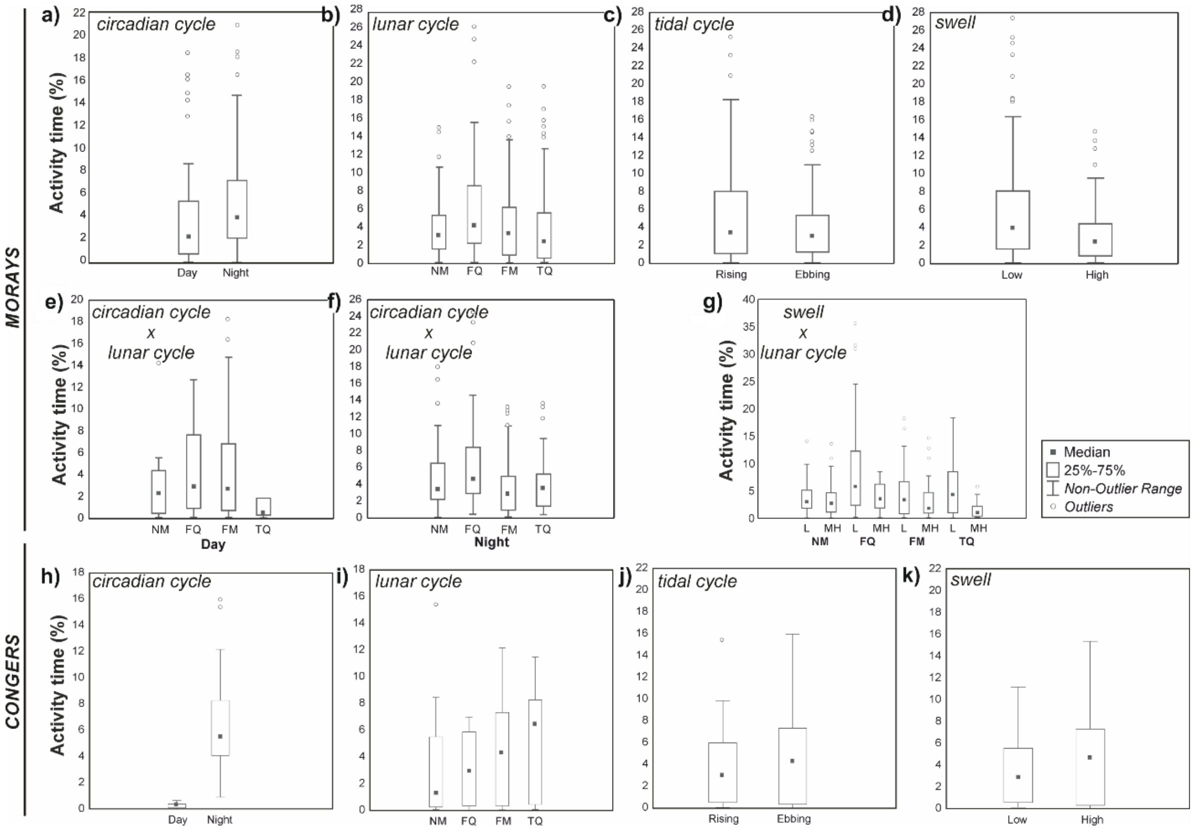

FIGURE 2.7 – Box-and-whiskers plot of moray and conger activity index I_a for each environmental variable tested, for morays: a) circadian cycle, b) lunar cycle, c) tidal cycle, d) swell, e) and f) interaction between lunar and circadian cycle, g) interaction between swell and lunar cycle; for congers: h) circadian cycle, i) lunar cycle, j) tidal cycle and k) swell.

Inside this no take MPA, morays and congers displayed similar habits. Both presented low activity levels and nocturnal behaviour, corroborating previous research (Pita and Freire 2011). However, differences in their patterns of activity and size of their home range suggest that these species have adopted distinct behaviours, probably related to differences in foraging. Morays were significantly more active during the night and first quarter lunar phase with low swell conditions. Indeed, centrality values indicated that morays were more active during the night in offshore areas, with broader, more long-distance movements. Despite being more active in offshore areas during low swell and quarter lunar phase periods, in these periods morays movements were spatially restricted. During the day, high swell and full moon periods, morays centered their activities around their refuge reefs. During nighttime, morays may successfully forage using olfaction (Santos and Castro 2003) which may allow these fish to forage in darkness and remain inactive to avoid predators during nights with a full moon. Digestive tract contents from morays captured around the no take MPA indicated that these species mostly feed on octopus and bony fish, including seabreams (Silva 2015). The nocturnal activity of morays may be related to the similar activity pattern of cephalopods (Altman 1966, Kayes 1973) and predator-prey interactions between these species (Meisel et al. 2013). Additionally, during daytime, in high swell and full moon periods, morays centered their activities near the island around known feeding areas of seabreams (Belo et al. 2016). This suggests that morays may maximize their predatory success via prey switching (e.g. Matić-Skoko et al. 2014). Although far fewer individuals were monitored, congers tended to be active for longer periods during the night, as also described by Pita and Freire (2011), and during the third quarter and full moon. Conger possess relatively large eyes, they are known to avoid capture during full moon periods (Day 1880) and low activity levels in new moon periods indicate that they may be essentially visual predators. Since octopus are also the preferred prey of conger (Xavier et al. 2010, Matić-Skoko et al. 2012, Silva 2015), higher activity by conger during nocturnal full moon may also reflect trophic interactions between these two species.

In contrast to individuals captured within the MPA, morays and congers captured outside the MPA appeared to have larger home ranges which, in some cases, extended towards the southern limit of the no take zone, an area intensively used as passageway. All morays captured outside the MPA were ultimately recaptured by fishermen and, unlike morays captured inside the MPA, detected most frequently within 800 m of their release location, two of these three individuals moved south by the end of the monitoring period. These non-resident individuals may have been moving towards their original home range (FIGURE IIIa, Appendix III) and were subsequently captured by fishermen 2 nm west-southwest from the no take MPA near their original capture location (FIGURE IIIb, Appendix III). Similar patterns cannot be confirmed for congers as no individuals were recaptured, although congers displayed a similar displacement during the monitoring period. Ultimately, these observations support site fidelity behaviour, typical from life strategies of cryptic and sedentary species.

A network of small MPAs is commonly accepted as an effective tool for protecting species with movement patterns similar to those found for the Mediterranean moray and European conger (Moffit et al. 2009). Monitoring a larger number of individuals over longer periods would help define the environmental factors influencing patterns of movement. Especially important will be documenting any intrinsic, seasonal changes in activity patterns which may influence the effectiveness of the no take MPA. Comprehensive bathymetric and geomorphological mapping of the area will underpin future investigations on the habitat use of these and other rocky reef species (e.g. Topping et al. 2005, Friedlander and Monaco 2007). In addition, combining passive and active telemetry techniques (e.g. Afonso et al.

2009, Pita and Freire 2011, Lino 2012) and sensor tags such as the AccelTag (Almeida et al. 2013) will further advance knowledge on the movement and activity patterns of these fish and the effective design of small no take MPAs.

FIGURE 2.8 – Moray movement patterns determined by network analysis (C_b) according to a,b) circadian cycle, c, d) swell and e-h) lunar cycle.

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CHAPTER 3

MOVEMENTS OF DIPLODUS SARGUS (SPARIDAE) WITHIN A PORTUGUESE COASTAL MARINE PROTECTED AREA: ARE THEY REALLY PROTECTED?

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Abstract

Mark-recapture tagging and acoustic telemetry were used to study the movements of *Diplodus sargus* within the Pessegueiro Island no take Marine Protected Area (MPA), (Portugal) and assess its size adequacy for this species' protection against fishing activities. Therefore, 894 *D. sargus* were captured and marked with conventional plastic t-bar tags. At the same time, 19 *D. sargus* were tagged with acoustic transmitters and monitored by 20 automatic acoustic receivers inside the no take MPA for 60 days. Recapture rate of conventionally tagged specimens was 3.47%, most occurring during subsequent marking campaigns. One individual however, was recaptured by recreational fishermen near Faro (ca. 250 km from the tagging location) 6 months after release. Furthermore, three specimens were recaptured in October 2013 near releasing site, one year after being tagged. Regarding acoustic telemetry, 18 specimens were detected by the receivers during most of the study period. To analyse no take MPA use, the study site was divided into five areas reflecting habitat characteristics, three of which were frequently used by the tagged fish: Exterior, Interior Protected and Interior Exposed areas. Information on no take protected area use was also analysed according to diel and tidal patterns. Preferred passageways and permanence areas were identified and high site fidelity was confirmed. The interaction between tide and time of day influenced space use patterns, with higher and more variable movements during daytime and neap tides. This no take MPA proved to be an important refuge and feeding area for this species, encompassing most of the home ranges of tagged specimens. Therefore, it is likely that this no take MPA is of adequate size to protect *D. sargus* against fishing activities, thus contributing to its sustainable management in the region.

Keywords:

Movements; Residency patterns; Mark-recapture; Acoustic telemetry; Diplodus sargus; Portugal; PNSACV; MPA size adequacy

3.1. Introduction

The human population significantly depends on the ocean for vital resources (Holmlund and Hammer 1999). In fact, fishing activities provide over 54.8 million jobs and are deeply rooted in the culture of many populations (FAO 2012a), yet fisheries over exploitation and consequent biodiversity loss has had a proven impact at a global level (Worm et al. 2006). To reduce further negative effects on the ecosystem and promote ocean recovery, Marine Protected Areas (MPA) have been widely implemented to stimulate the recovery of fishing stocks and preservation of biodiversity, thus combining marine conservation and human needs (Allison et al. 1998, Russ 2002, Botsford et al. 2003, Chateaux and Wantiez 2009). An MPA can be defined as any intertidal or subtidal area, together with its fauna, flora and cultural and historical heritage, that is protected by law in order to maintain its biodiversity and assure a sustainable use of its resources (Kelleher and Kenchington 1992). Restrictions often include no take zones, where all fishing activities are prohibited. The efficiency of this tool has been debated in several studies over the past years (e.g. García- Rubies and Zabala 1990, Guidetti and Sala 2007, La Mesa et al. 2011, Horta e Costa et al. 2013). However, for an MPA to be effective, an established fish population should subsist within its limits (Kaplan et al. 2006), being its efficiency directly dependent of the home range of the fish targeted for protection (Kramer and Chapman 1999, Sale et al. 2005, Chateaux and Wantiez 2009, Alós et al. 2012). A species' home range is a relatively circumscribed area over which an organism moves to acquire vital resources for survival and reproduction (Dingle, 1996). Therefore, an efficient no take MPA should include the entire or most of the specimens' home range in order to reduce the probability of the protected species being captured (Afonso et al. 2011). It is important to assess and understand the movements of fish species to estimate effectiveness of the MPA protection, especially for species with high commercial or conservation value (Abecasis et al. 2009, Afonso et al. 2009).

The knowledge of where a particular animal is located at a given time provides important information about its behaviour, ecology and social interactions. However, studying animals in their natural habitat can be very complex, especially in marine environments (Rutz and Hays 2009). The mark-recapture method is the most traditional to assess a population's spatial distribution and large scale movements (Kerwarth et al. 2007a, b). Despite being relatively low cost, recapture rates are usually low and the obtained information is limited, since it only provides the minimum distance between the tagging and the recapture locations with no details on the route fish underwent between sites (Kohler and Turner, 2001, Andrews et al. 2007). However, in the last decade, an increasing number of studies use acoustic telemetry, producing large amounts of data on tagged marine fishes (Rutz and Hays 2009, Pita and Freire 2011, Campbell et al. 2012) and enabling continuous monitoring of specimens at distance without the need to recapture them. By using this technology, it is possible to determine a particular species vital area and study its movement patterns within it, thus obtaining important information for its conservation and management (Jacoby et al. 2012). Despite its costs, which limit the number of possible tagged specimens as well as the area covered by the acoustic receivers (Rutz and Hays 2009), acoustic telemetry has been successfully used in several studies, including for the white seabream *Diplodus sargus* (Linnaeus, 1758) (Abecasis et al. 2009, 2013, 2015, D'Anna et al. 2011, Alós et al. 2012, Koeck et al. 2013). Overall, it is possible to study with high resolution the space use of a relatively small area via telemetry, whilst, large-scale movements can be assessed by conventional mark-recapture techniques, therefore complementing the more detailed information obtained with acoustic telemetry (Kerwarth et al. 2007a).

Being a coastal country, Portugal has an ancient and long lasting tradition regarding the use of marine resources, both by professional and recreational fishers. This has resulted in the necessity to implement protective measures, such as no take protected areas, to encourage the sustainable use of marine resources in the Portuguese southwestern coast. In this region, one of the most targeted fish families is the Sparidae, especially *D. sargus*. This species is abundant in this area and has relatively high commercial value. Adults prefer rocky bottoms from near the shore to over 50 m in depth (Bauchot and Hureau 1986, Morato et al. 2003). Adults are considered to be omnivore, and their prey includes decapods, gastropods, echinoderms, polychaets and cnidarians, with algae also an important food item (Rosecchi 1985, Sala and Ballesteros 1997, Cejas et al. 2003, Figueiredo et al. 2005, Pallaoro et al. 2006, Leitão et al. 2007). In north Atlantic waters, *D. sargus* size at first maturity is 16.7 cm and reproduces during spring, while the recovery period occurs in summer and autumn (Martínez and Villegas 1996, Morato et al. 2003).

Considering the above, the main objective of this study was to evaluate the importance of the Pessegueiro Island no take MPA for *D. sargus* by studying their movements within and around this area during summer. First, mid and large scale movements of this species were studied by means of conventional mark-recapture techniques, having the Pessegueiro Island as capture and release location. Second, residency and home range was assessed through acoustic telemetry. Finally, the influence of environmental parameters on this species movement patterns was also evaluated, providing relevant information on how and when this species uses this no take MPA. These objectives would also allow identifying potential summer refuge and feeding areas. Obtained information will allow characterizing the use of

Pessegueiro Island no take MPA by *D. sargus* and evaluating if this area can provide an efficient protection for this highly targeted species during summer period.

3.2. Methods

3.2.1. Study area

The study was conducted in the marine section of the Sudoeste Alentejano and Costa Vicentina Natural Park (PNSACV), in the southwestern coast of Portugal (FIGURE 3.1). This area was established as an extension of the land area of the PNSACV in 1995 and designated Marine Park in 2011. It comprises a coastal strip 2 km wide along the entire coast of the land park and despite being itself a large protected area, it comprises several smaller Type I Protection Areas with more restrictive measures, commonly referred to as no take MPAs (FIGURE 3.1). In these areas, all fishing activities are prohibited, with the exception of professional harvest of stalked barnacle *Pollicipes pollicipes* (Gmelin, 1790).

Pessegueiro Island no take MPA is the northernmost protected area inside the Marine Park. This area surrounds a small rocky island (0.4 x 0.3 km) and has an approximate area of 7 km2 (FIGURE 3.1). Maximum depth is ca. 18 m and the seabed around the island is mainly rocky, with the exception of the mostly arenous eastern side. Several smaller rocky islands are located nearby the main island. The rocky seabed extends to the southern limit of the no take MPA while on the northern and western sides extends over 400 m and ending as a 2 m vertical wall, after which sandy bottoms ensue. The north and west areas of the no take MPA are exposed to the dominant northwestern swell.

The fish community of the southwestern coast is diverse (149 fish species), comprising commercially valued species such as the Sparidae family, which are targeted both by professional and recreational fishermen (Gomes et al. 2001, INE 2012).

3.2.2. Field work

Mark-recapture was used to evaluate population dynamics and movements of *D. sargus* within the entire Marine Park. Four tagging campaigns of three days each were conducted between June and October 2012 and a fifth one in October 2013. *D. sargus* were captured by angling from the same location onshore at Pessegueiro Island. Immediately after, total length (TL, mm) was measured, specimens tagged with T-bar plastic tags under the dorsal fin, and released straightaway. Prior and during these campaigns, fishermen from the south western coast were given information about this study and a reward was offered for info regarding the recapture of tagged specimens outside of the no take MPA.

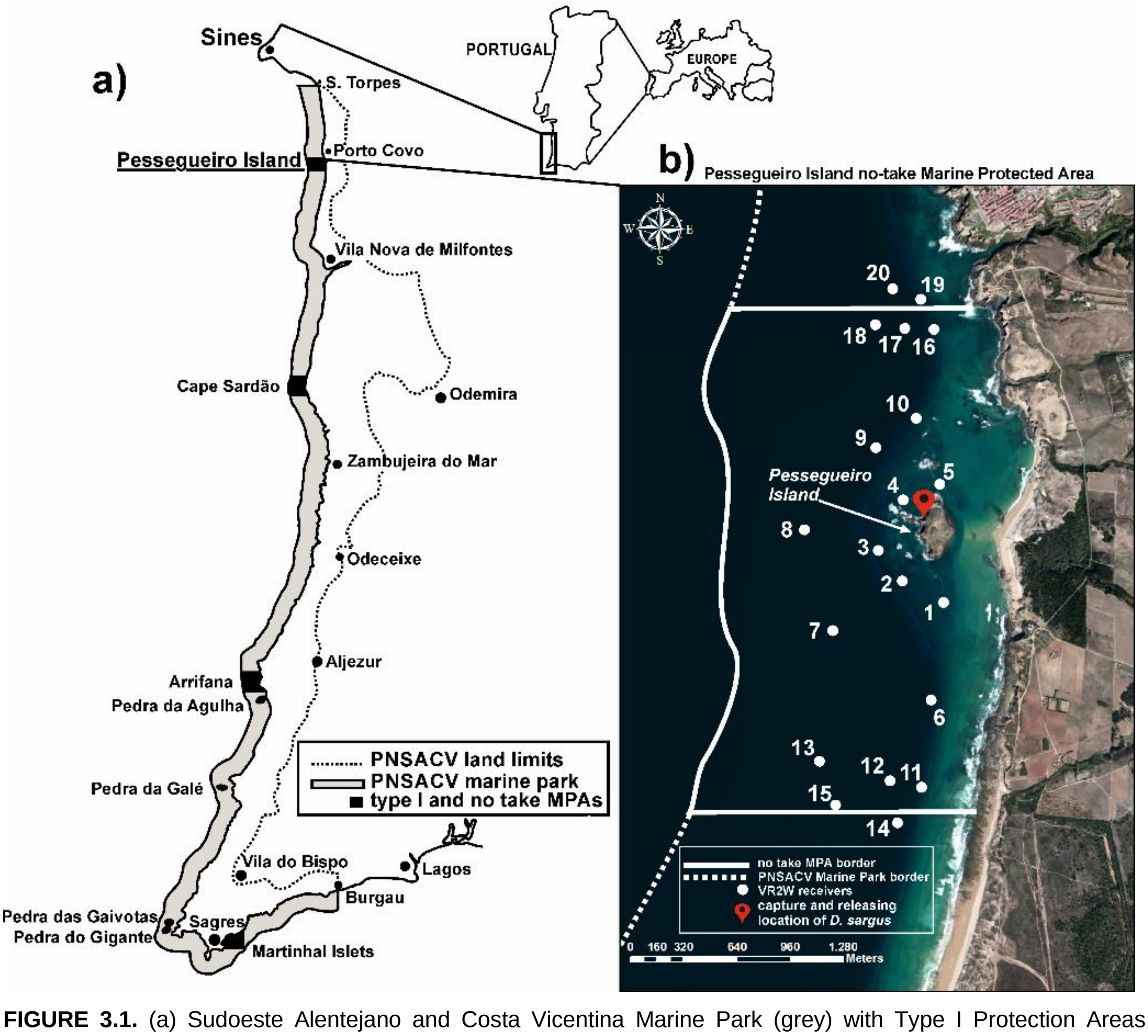

FIGURE 3.1. (a) Sudoeste Alentejano and Costa Vicentina Marine Park (grey) with Type I Protection Areas (black) defined by specific legislation (Ordinance 143/2009). (b) Map of the Pessegueiro Type I Protection Area (no take MPA) with *D. sargus* capture and release site (mark-recapture and acoustic telemetry) and numbers assigned to the location of each acoustic receivers.

To study fine scale movements of *D. sargus* and assess the importance of the Pessegueiro Island no take MPA as refuge and feeding area for this species, passive acoustic telemetry was used. For that purpose, an array of 20 automatic underwater acoustic receivers VR2W-

69 kHz (Vemco) was deployed in mid July 2012 around the Pessegueiro Island, positioned in a design that covered the entire no take MPA (FIGURE 3.1). Receivers were attached to a small rope with stainless steel snap hooks on each end that connect with two ring ropes to a 2 m main cable, attached in turn through a stainless steel snap hook to a 100 kg cement block. At the top of the main cable a rigid plastic buoy was placed, in order to keep the receiver in a vertical position. The entire apparatus was placed from a boat, being completely submersed at all times to avoid boat collision and/or theft, and location recorded by GPS. Receiver deployment and retrieving for data download was made through SCUBA diving. Two sets of five receivers each were deployed in a half circle on the western area and another five receivers were placed near each MPA border, north and south (FIGURE 3.1).

Receiver detection range varies with environmental conditions and habitat type, reaching 500 m in ideal conditions, 200 m in shallow turbulent waters, and as little as 30 m in some environments (O'Toole et al. 2011, Welsh et al. 2012). Sampling sites in this study were <18 m in depth, exposed to turbulent conditions and comprised of underwater rock formations. Detection range of receivers was therefore estimated as approximately 200 m, since tagged individuals were simultaneously detected on multiple receivers positioned 200 m apart (26% of detections). Although range testing was not conducted, since its values would greatly vary among receivers and from one day to another according to sea conditions and receiver location, it is likely that the receivers would detect individuals within at least 100 m due to the array coverage. The use of sentinel tags would be the most adequate method of continuously range testing (e.g. Payne et al. 2010, Currey et al. 2014) but was discarded since the studied species potentially displays high site fidelity (e.g. Abecasis et al. 2009, D'Anna et al. 2011), increasing the chance of transmission collision and consequent data loss.

While near the island rocky formations abound, in the western region of the no take MPA sandy bottom predominates and detection range was expected to be higher. So, receivers in the set closer to the island, as well as those deployed in the northern and southern limits, were placed no further than 200 m from each other while the remaining were deployed around 500 m apart. The deployment of the receivers in three major sets allowed an optimum coverage of the tagged fish and monitoring their movements between areas and from or to the no take MPA.

After receiver deployment, 19 *D. sargus* were captured by angling onshore from Pessegueiro Island (FIGURE 3.1) and immediately anaesthetized in a solution of 0.4 ml 2-phenoxyethanol per litre of seawater. Specimens were surgically implanted with coded acoustic transmitters V9 2H-69 kHz (Vemco) with 9 mm diameter, 29 mm in length, 2.9 g weight (in water), 151 dB power output, 99 days expected battery life and transmission rate with 60 s (30 - 90 s) nominal delay. After recovering for 1 hour, fish were released at capture site and continuously monitored by the receiver array. Tagged specimens were all adults over 270 mm TL and over 0.2 kg in weight. Transmitter weight did not exceed 1% of the fish total weight (Winter 1983). Despite transmitter battery life being almost 100 days, receivers were retrieved before autumnal equinox spring tides (late September), resulting in a total monitoring period of 60 days. Adverse sea conditions typical of the region during most of the year and consequent risk of losing equipment restrict telemetry studies in the area, such as the present work, to the summer periods.

All procedures were carried out in accordance with all the Portuguese legislation regarding animal capture, manipulation and experimentation for scientific purposes. This includes certification on laboratory animal science that meets the requirements of FELASA level C courses to license people responsible for directing animal experiments and Veterinary National Authority proper accreditation.

3.2.3. Data analysis

After receiver retrieval, data was downloaded, filtered and false detections removed. Files downloaded from VR2W receivers contain parameters that were used to calculate the noise quotient $nq = P - (S.cl)$, where P is the number of pulses detected, S is the number of synchs, and cl is the number of pulses used to make a valid code (Simpfendorfer et al. 2008, Garcia et al. 2015). Positive values of nq indicate environmental noise while negative values indicate code collision. Noise quotient was then correlated against swell height (Pearson correlation) to investigate how this parameter influenced receiver performance and, therefore, detection range.

Due to the large amount of data obtained, simple Visual Basic routines in Microsoft Office Excel© were used for preliminary analysis. Intervals between detections were also analysed and multiple detections identified. Codes from the same transmitter detected by different receivers with intervals less than 20 s meant that a single transmission was detected by more than one receiver and therefore considered to be multiple (or repeated). Additionally, number of detections per hour and per day were also determined.

Data from each tagged specimen was then sorted and categorized according to environmental parameters and local of detection. This way, it was possible to determine when and where each specimen was detected and assign each detection to a specific location and environmental parameter (time of day and tidal cycles) for posterior statistical analysis. For subsequent analyses, with the exception of Kernel Density Estimation, only the first detected code of a set of multiple detections was considered, thus removing repeated detections.

Residency Index (Ir) was calculated for each specimen as the ratio between number of days that fish were detected (minimum of two detections per day with less than 5 min apart) and total number of days of monitoring (60 days) (Afonso et al. 2008). If the interval between individual detections by the same receiver was inferior to 5 min, the tagged fish was considered to be within range and, therefore, inside the no take MPA for the duration of that interval. The sum of these intervals resulted in residency time of each specimen. The ratio between this parameter and total study period resulted in the percentage of time each fish spent inside the no take MPA. Residency index is a measure of the number of days each fish was present inside the no take MPA, while residency time is a measure of the amount of time each fish spends inside the protected area. *D. sargus* home range was determined by means of Kernel Density Estimation (KDE), which provides the most accurate results (Seaman and Powell 1996) and allows the analysis of more complex patterns (Worton, 1989), making it the best option (Powell 2000) from the various available methods. This method estimates the density probability that corresponds to the distribution of the use of a certain area by an animal, providing important information on tagged animal movements and habitat use (Jacoby et al. 2012). KDE was calculated using raw data and multiple detections were used to calculate the intermediate position (average coordinates) among receivers that detected the same transmitted code in order to expand spatial definition. Other approaches to simplify raw data obtained using omnidirectional receivers have been described and widely used, such as determining center of activity locations by using the “mean position algorithm” (Simpfendorfer et al. 2002, 2008, Heupel et al. 2006). The smoothing factor (h) plays an important role in obtaining the KDE. The higher its value, the less detailed is the home range estimation (Silverman 1986, Worton 1989, Hemson et al. 2005).

The smoothing factor was calculated using the ad hoc method (Worton, 1989, Topping et al. 2005, Topping and Szedlmayer, 2011) and the obtained value h = 100 was in line with the expected detection range of 100 m. Cell size used was of 20 m and KDE was then obtained using the ESRI software package ArcGIS 9.3® with Hawth's Analysis© extension (Beyer 2004). Percent volume contours of 50% (i.e. core range) and 95% (i.e. home range), were also determined. This resulted in the identification of preferential zones used by the tagged specimens within the no take MPA.

Results obtained by KDE were complemented by network analysis. KDE provides important information on movements and habitat use, but network analysis allows the identification of more frequently used zones as well as movement patterns within and between those zones. Results from this analysis, together with KDE, allowed a comprehensive investigation of how this species uses and moves within the study area. In fact, this method has been used as a complement to KDE (e.g. Finn et al. 2014, Fox and Bellwood 2014, Espinoza et al. 2015, Lédée et al. 2015) by evaluating the type and degree of interactions between determined activity centres (Jacoby et al. 2012, Makagon et al. 2012). This method is based on a complex system of interactions composed of nodes connected by edges in which the metrics based on the nodes describe the influence of each one on the entire network (Jacoby et al. 2012). These metrics are based on five basic principles: prominence/centrality, amplitude, cohesion, structural equivalence and intermediation (Makagon et al. 2012). Centrality measures the importance of a node in a network and its importance to the same network (Gómez et al. 2013). This kind of analysis has been used in the study of animal behaviour and relations between individuals of a population. However, it can also be applied to acoustic detections to understand which zones are more relevant and the level of interaction between them (Jacoby et al. 2012). Network analysis was performed using the software package UCINET (Borgatti et al. 2002), where receivers corresponded to nodes and interactions between receivers corresponded to edges. An interaction between receivers was considered when a tagged fish was detected by a receiver other than the one that detected it previously. Centrality was determined as degree centrality, which takes into account the number of edges that connect to a node. This metric is adequate for large and complex networks (Coleing 2009, Jacoby et al. 2012, Makagon et al. 2012, Gómez et al. 2013). Once the data showed the direction of the movements, indegree centrality (number of edges directed to a node) and outdegree centrality (number of edges that depart from a node) were determined as well. Values of betweenness were also calculated. This metric is based on the number of shortest paths between any two nodes which cross the node in question. High values of betweenness indicate an intermediate position between many paths (Makagon et al. 2012).

The influence of environmental variables on the use of the no take MPA by *D. sargus* and on the use of their preferential zones assigned through KDE was analysed by means of Factorial ANOVA using SPSS 20.0 statistical package (IBM 2011). The independent variables considered were time of day (day vs. night), tidal phase (ebbing vs. rising tide) and tidal cycle (spring vs. neap tide), being residency time the dependent variable. Data on time of day was obtained from Lisbon Astronomical Observatory (for 2012) and tide data from the Portuguese Hydrographic Institute (for 2012).

3.3. Results

3.3.1. Mark-recapture

In total, 894 specimens of *D. sargus* were captured and tagged with plastic t-bars. The largest *D. sargus* tagged measured 42.0 cm while the smallest measured 17.0 cm. However, most of the tagged fish were within the length interval of 200 - 300 mm. 31 specimens (3.47%) were re-captured, 27 of which during 2012 tagging campaigns of (1 - 4 months after tagging) and three during the 2013 campaign (one year after tagging). Since these recaptures took place at the original capture and releasing site, no information on the distance covered by recaptured specimens could be obtained. Also, this method did not provide information on if specimens recaptured one year after being tagged and released abandoned the no take MPA and, if so, when they did it and when returned. Only one individual was captured and reported by a fisherman. However, this specimen was captured in Faro (south coast of Portugal), in February 2013 (6 months after tagging), after covering a minimum distance of 250 km at 1.4 km/day. All recaptured fish belonged to the 200 - 300 mm length interval.

3.3.2. Acoustic telemetry

Receiver array detected 18 out of 19 white *D. sargus* tagged with acoustic transmitters throughout the study period. This resulted in a total of 236049 recorded codes, corresponding to 14.4% of the total codes transmitted by the 19 tagged fish (TABLE 3.1). Fish #17 was never detected during the course of the study, while fish #5 had the lowest number of detections and fish #7 the highest. Overall, 21% of the fish (n = 4) were detected every day of the study period.

Average noise quotient (nq) was considerably low and negative (1662.87), indicating that environmental noise was reduced but code collision occurred. Therefore, despite not detected, tagged specimens were within receiver range. Noise quotient was not correlated with swell height ($r_{Pearson}$ = 0.05), suggesting that this parameter was not the main cause of environmental noise and did not significantly affect receiver performance and detection range in the study area.

Time spent by each fish on the no take MPA greatly varied, from a maximum of 42% (fish #7) to a minimum of 0.31% (fish #5) within receivers range. On average, tagged fish spent 14% of the time within the no take MPA boundaries. Considering residency index (Ir), 3 specimens (#1, #2, and #15) were detected at least twice every day during the 60 day study period and thus presented Ir = 1 (TABLE 3.1). Most specimens were detected in the no take MPA throughout the study period, although not on a daily basis (FIGURE 3.2).

KDE analysis led to the identification of five areas used differently by the tagged fish: N - North (receivers 16 to 20), S - South (receivers 11 to 15), E - Exterior (receivers 6 to 10), IE - Interior Exposed (receivers 3 to 5), and IP - Interior Protected (receivers 1 and 2) (FIGURE 3.3a). Despite the south area of the no take MPA having more detections than the north, both were scarcely visited by the tagged specimens when compared to the areas surrounding the island (FIGURE 3.3b, c, d). It was also possible to identify three groups of *D. sargus* that used the area surrounding the island with distinct patterns.

TABLE 1. Summary table of the passive acoustic telemetry data regarding detections obtained from tagged *D. sargus* in the Pessegueiro Island no take MPA

Fish	ID code	Total length (cm)	Tagging/release date	Nº of days detected	N detections	Residency time (h)	% Time MPA	Residency Index (Ir)
# 1	ID5732	27.7	21/07/2012	60	27670	566.70	39.35	1.00
# 2	ID5733	34.0	21/07/2012	60	43450	550.84	38.25	1.00
# 3	ID5734	28.5	21/07/2012	47	7262	112.46	7.81	0.78
# 4	ID5735	34.5	21/07/2012	58	10216	196.00	13.61	0.97
# 5	ID5736	28.5	21/07/2012	18	354	4.53	0.31	0.30
# 6	ID5737	28.5	21/07/2012	57	19494	323.27	22.45	0.95
# 7	ID5738	28.0	21/07/2012	60	46589	609.87	42.35	1.00
# 8	ID5739	33.2	22/07/2012	50	11939	205.39	14.26	0.83
# 9	ID5740	29.1	22/07/2012	51	6549	91.36	6.34	0.85
# 10	ID5741	36.5	22/07/2012	57	6953	113.97	7.91	0.95
# 11	ID5742	28.0	22/07/2012	52	6287	114.21	7.93	0.87
# 12	ID5743	27.5	23/07/2012	23	696	11.30	0.78	0.38
# 13	ID5744	28.2	23/07/2012	25	771	14.63	1.02	0.42
# 14	ID5745	28.5	23/07/2012	58	18433	331.96	23.05	0.97
# 15	ID5746	28.1	23/07/2012	60	12311	243.91	16.94	1.00
# 16	ID5747	32.7	23/07/2012	38	6054	120.91	8.40	0.63
# 17	ID5748	28.4	23/07/2012	0	0	0.00	0.00	0.00
# 18	ID5749	28.0	23/07/2012	39	5128	111.40	7.74	0.65
# 19	ID5750	28.6	23/07/2012	32	5893	123.82	8.60	0.53

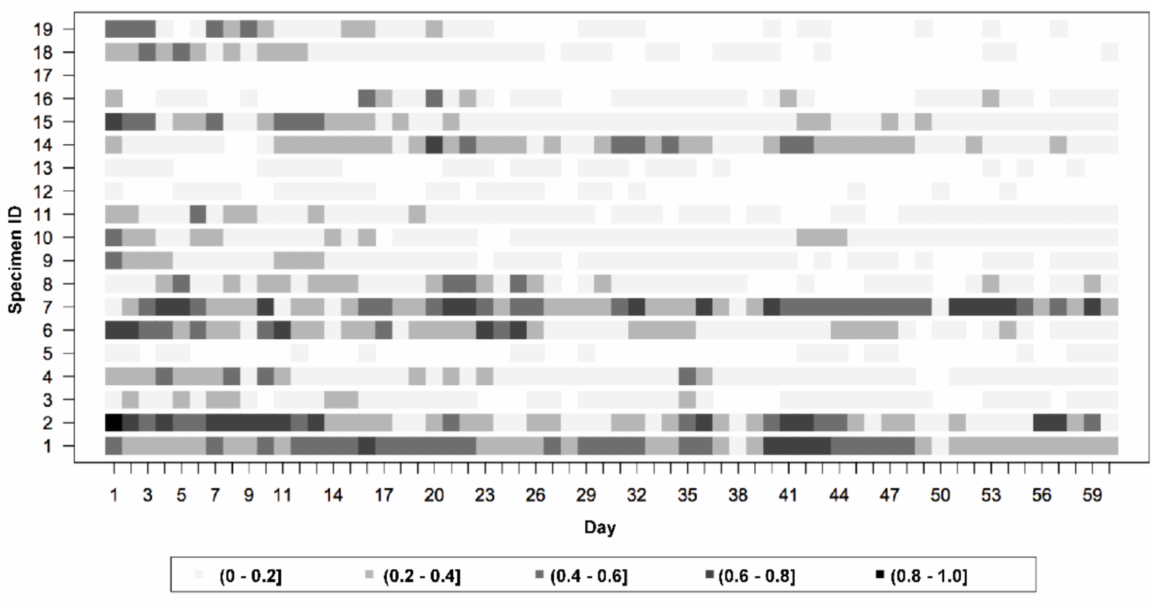

FIGURE 3.2. Residency of *D. sargus* tagged and released at the Pessegueiro Island no take MPA. Tone of each bar indicates the percentage of time spent per day at the study site.

One group of specimens mostly used Exterior (around receivers 8 and 9) and Interior Exposed (around receivers 3, 4 and 5) areas (FIGURE 3.3b) and a second group that was mostly detected in the Interior Protected area (around receivers 1 and 2) and also around receiver 8 in the Exterior area (FIGURE 3.3c). The third group showed a preference for Exterior and Interior areas but with a wider range of dispersion throughout the study area, including receivers near the southern border (FIGURE 3.3d).

Network analysis was used to study the movements of the fish between different areas (receivers) and evaluate its importance, supporting and complementing KDE analysis and providing detailed information on the type of movements and use of the no take MPA by this species. As the KDE, the network analysis revealed three groups of fish with distinct movement patterns and area use (FIGURE 3.4). One group prefers northern areas near the island and performs frequent movements between Exterior and Interior Exposed areas (FIGURE 3.4a). The second group was more active in the southern areas around the island, also with a marked interaction between the Exterior and the Interior Protected area (FIGURE 3.4b). A third group performed broader movements throughout the no take MPA with important connections between areas surrounding the island and South (FIGURE 3.4c). For most tagged specimens, receivers closer to the island revealed high centrality values (Appendix IV). Regarding betweenness, areas encompassing part of the Interior Exposed area and a mixture of the areas further from the coast line presented higher values of this parameter.

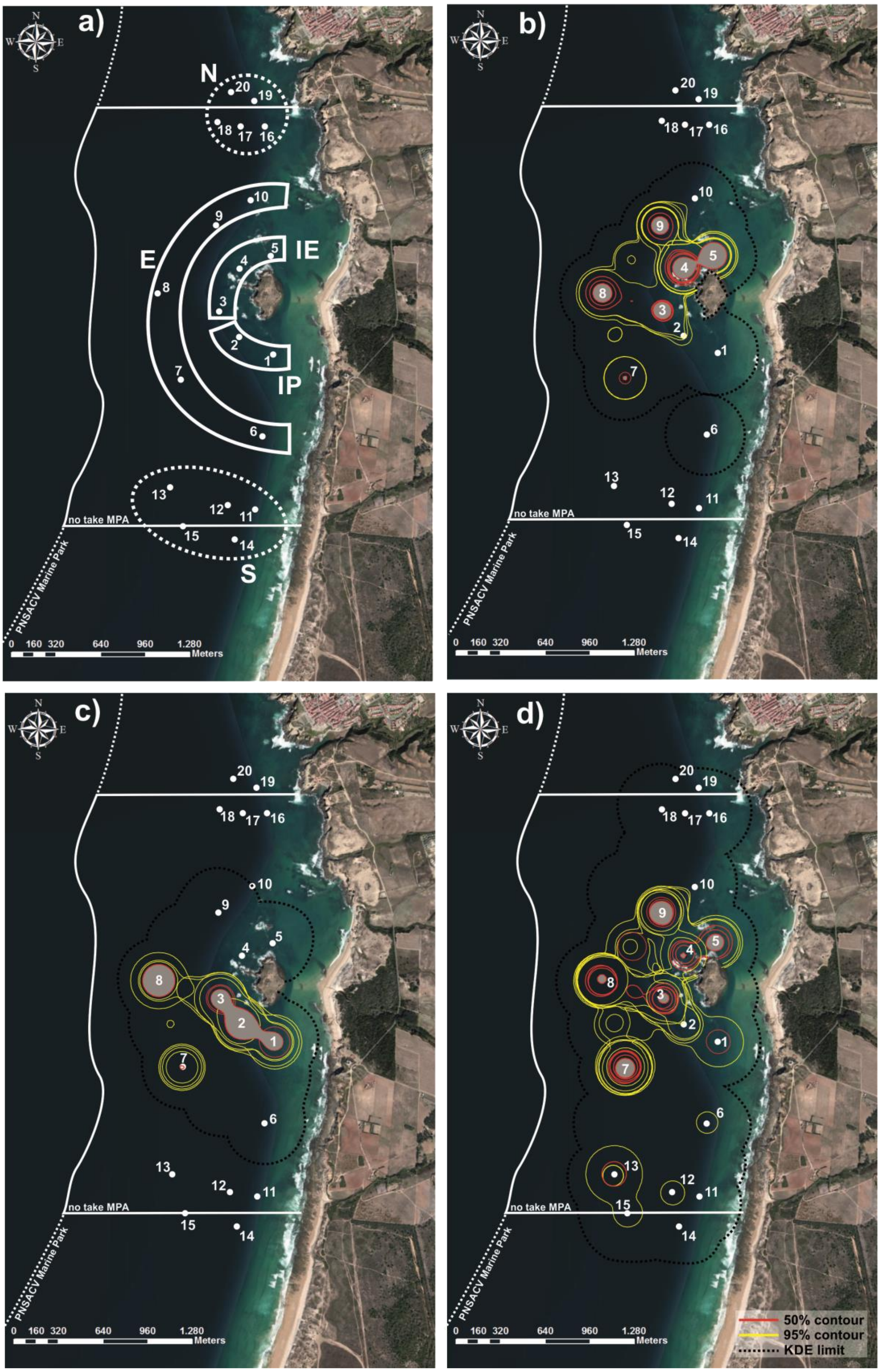

FIGURE 3.3. Maps detailing the (a) use based areas assigned according to KDE analysis (N – North; S – South; E – Exterior; IE – Interior Exposed; IP – Interior Protected) and (b-d) individual KDE of the 3 groups of *D. sargus* sorted according to its use of the no take MPA with percent volume contours of 50% (core range) and 95% (home range), for the 18 specimens for which detections were obtained. Grey areas indicate overlapping core ranges within each group and dashed line indicates the KDE limit for each group. Note: b) fish #1, #3, #5, #8, #9, #10 c) fish #12, #13, #14, #15, #18 d) fish #2, #4, #6, #7, #11, #16, #19.

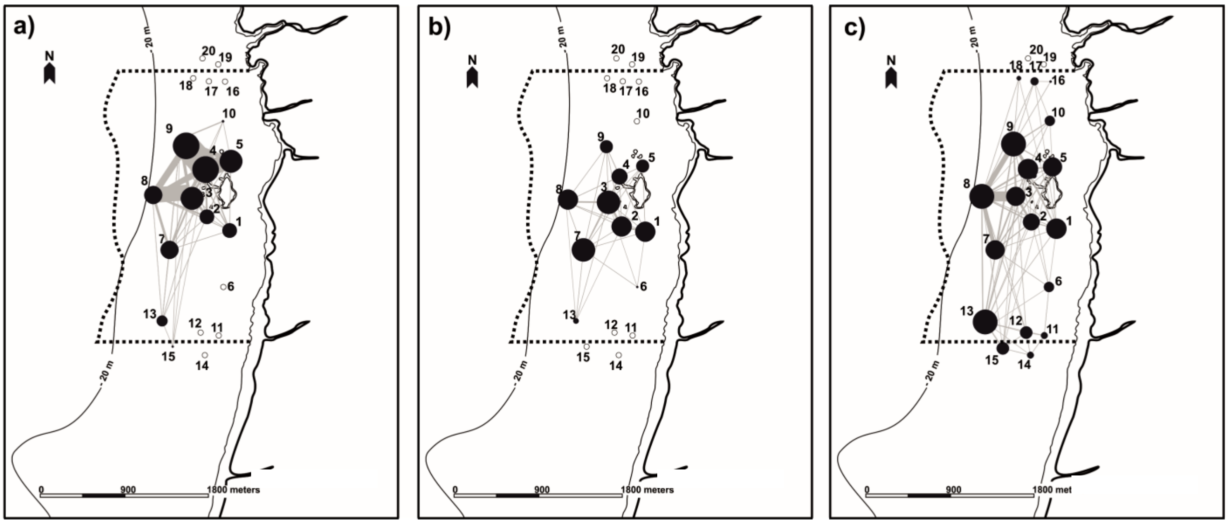

FIGURE 3.4. Graphic representation of network analysis (UCINET) illustrating the movements conducted by each group of tagged *D. sargus* within the no take MPA. Circles correspond to nodes (receiver stations) and their size reflects centrality. Vector width reflects frequency of movements between zones. Note: a) specimens #1, #3, #5, #8, #9, #10 b) specimens #12, #13, #14, #15, #18 c) specimens #2, #4, #6, #7, #11, #16, #19. Dashed line – MPA border.

Degree centrality values varied between 0 and 12. Higher values of outdegree centrality were observed on the receivers 7, 8, 9 and 13, while the higher values of indegree centrality were obtained for receivers 4, 8 and 9 (Appendix IV). The highest mean values of both these metrics were obtained for receivers 2, 3, 4, 7, 8 and 9, confirming the results from the previous analysis that the areas surrounding the island were important for these fish. Compilation of results obtained with network analysis led to the identification of three different areas according to their use by the tagged fish: passage, permanence and mixed areas (FIGURE 3.5).

Factorial ANOVA allowed identifying environmental factors that significantly influenced *D. sargus* use of the no take MPA (TABLE 3.2). Residency time was significantly influenced by time of day [$F (1, 104) = 15.104$, $p < 0.001$], and by its interaction with tidal cycle [$F (1, 104) = 4.337$, $p < 0.05$] (TABLE 3.2). During day time, residency time was higher and more variable, with 63.3% of the time spent within the no take MPA occurring during this period, being this pattern more evident during neap tides (FIGURE 3.6, TABLE 3.2). North and South zones were excluded from the analysis regarding the influence of environmental factors in the use of each zone assigned by KDE analysis due to the reduced amount of detections. Overall, time of day was the most important influence on the residency time [$F (1, 302) = 12.428$, $p < 0.001$].

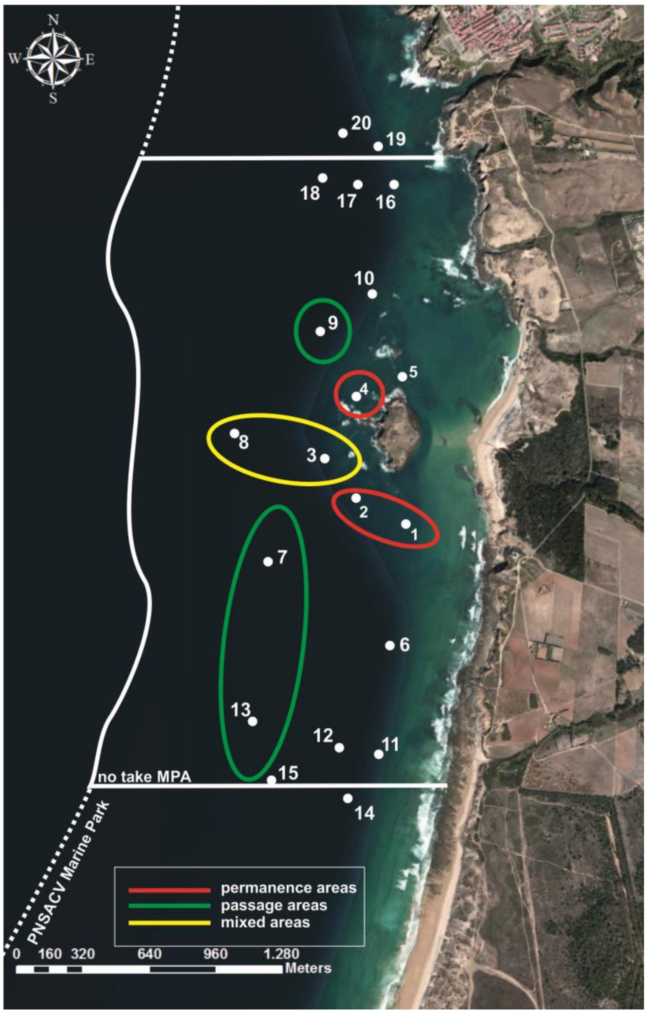

FIGURE 3.5. Map of Pessegueiro Island no take MPA illustrating the three different areas assigned through network analysis according to its use by *D. sargus* (passage, permanence and mixed areas).

TABLE 3.2. Output of the Factorial ANOVA applied to analyse the influence of three environmental parameters (time of day, tidal phase and tidal cycle) on the time spent by *D. sargus* within the no take MPA boundaries, regarding the whole study period

Dependent variable: Time of residency					
Variability source	**SS**	**df**	**MS**	**F-value**	**P-value**
Time of day	0.178	1	0.178	15.104	0.00
Tidal phase	0.009	1	0.009	0.792	0.376
Tidal cycle	0.040	1	0.040	3.427	0.067
Time of day and Tidal phase	0.004	1	0.004	0.338	0.562
Time of day and Tidal cycle	0.051	1	0.051	4.337	0.040
Tidal phase and Tidal cycle	0.016	1	0.016	1.366	0.245
Time of day and Tidal phase and Tidal cycle	0.000	1	0.000	0.040	0.842
Error	1.228	104	0.012		

Note: SS (sum of squares); df (degrees of freedom); MS (mean square); F-value (value of statistic F); p-value (estimated probability).

Furthermore, the permanency in each zone was also related with the tidal phase [$F(2, 302) = 3.821$, ($p < 0.05$)]. *A posteriori* tests showed that tagged specimens spent significantly less time in the Interior Protected area ($p < 0.001$) when compared with the other two areas. This result is also evident when analysing the residency time by means of box and whiskers plot (FIGURE 3.7). Also noteworthy is the fact that tagged fish spent more time in the Exterior area during ebbing tide but more time in the remaining areas during rising tide (TABLE 3.3, FIGURE 3.7). Similarly to the analysis regarding residency time inside versus outside the no take MPA, the amount of time spent by the specimens in each of the three areas was higher during day time (TABLE 3.4, FIGURE 3.7).

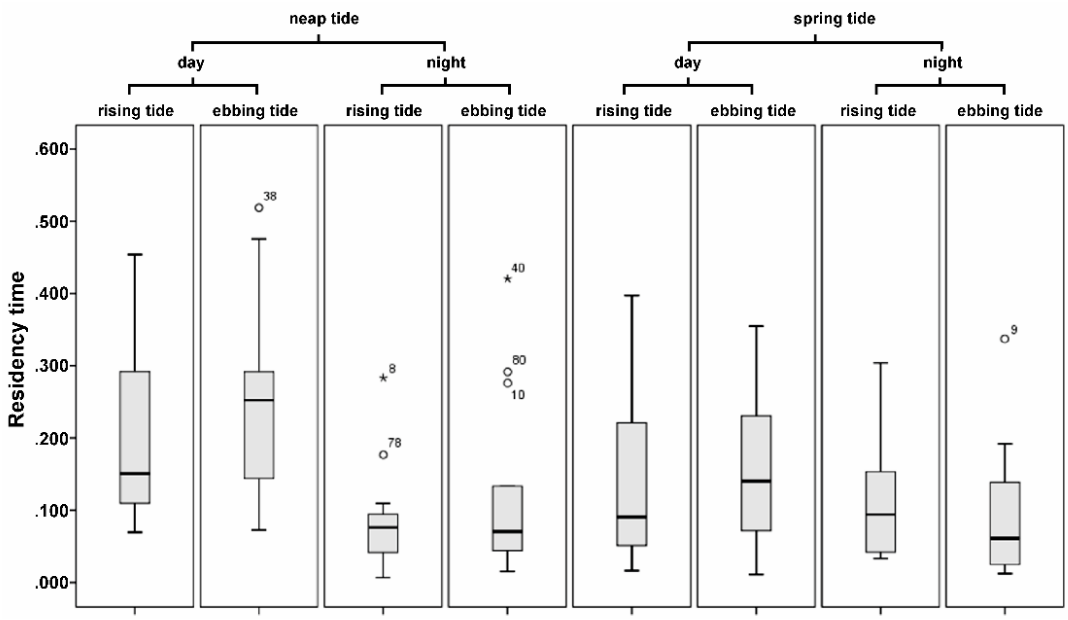

FIGURE 3.6. Box and whiskers plots relative to the variation of residency time of *D. sargus* according to time of day, tidal phase and tidal cycle.

3.4. Discussion

Conventional tagging and acoustic telemetry provided valid information to characterize *D. sargus* movements inside Pessegueiro Island no take MPA and assess its importance to potentially provide protection for this highly targeted species. Data analysis techniques were successfully applied with highlight for the use of network analysis with passive acoustic telemetry data, for which its application with marine organisms is still recent (e.g. Jacoby et al. 2012, Makagon et al. 2012, Gómez et al. 2013, Espinoza et al. 2015, Garcia et al. 2015, Lédée et al. 2015). Despite previous studies on movement patterns, home range, and site fidelity of this species (e.g. Abecasis et al. 2009, 2013, 2015, D'Anna et al. 2011), the differences observed between them support the need for result validation at local sites, especially when MPAs are concerned (Abecasis et al. 2015). In that sense, not only the present study confirms some of the results previously obtained but also provides new insights to assess and evaluate *D. sargus* movements and MPA size adequacy.

Recapture rates for *D. sargus* (3.5%) were in agreement with the ones obtained in previous studies (Santos et al. 2006, Abecasis et al. 2008). Most recaptures were obtained during the subsequent tagging campaigns shortly after release (1-4 months) with the exception of one individual recaptured by a recreational fisherman in February 2013, 6 months after tagging, and another three recaptured one year later during October 2013 tagging campaign. Since most recaptures occurred at releasing site and during summer, no information could be inferred on the distance covered between releasing and recapturing. However, it suggests that this species displays high site fidelity at least during summer, concurrent with the results obtained by acoustic telemetry. Nevertheless, one specimen was captured in Faro (ca. 250 km from tagging and releasing site), indicating that at least some fish may perform long distance movements. There are strong similarities between these results and those obtained by Abecasis et al. (2009) in Ria Formosa, a shallow mesotidal lagoon on the south coast of Portugal, where a long distance displacement by a tagged specimen (90 km away from releasing site) is also reported. Kerwarth et al. (2007b) also observed erratic movements out of home range for other Sparidae species. In fact, several studies refer to species where a proportion of the population remains sedentary while the remainder move more broadly (Kaunda-Arara and Rose 2004, Marshell et al. 2011, O'Toole et al. 2011, Chapman et al. 2012), which may be the case of *D. sargus* population from Pessegueiro Island no take MPA. Recaptures on the study site in October 2013 reinforce the importance of the island for this population, as they proved that some of the individuals either remained in the study site or returned one year later after they eventually left the area. However, this method did not provide information on if or for how long tagged specimens were absent from the no take MPA or on the distance these specimens covered.

The fact that all the animals recaptured in the study site belong to the 200 - 300 mm length interval may indicate that larger individuals (over 300 mm) probably leave the area more frequently during summer and abandon it definitively after this season, reinforcing a partial population migration scenario (Chapman et al. 2012). In fact, specimens over 300 mm are known to be captured in the region all year round, according to information provided by local fishermen and authorities (unpublished data).

TABLE 3.3. Output of the Factorial ANOVA applied to analyse the influence of environmental patterns (time of day, tidal phase and tidal cycle) on the time spent by *D. sargus* in each zone assigned by KDE analysis, regarding the whole study period

Dependent variable: Time of residency (zone)					
Source	**SS**	**df**	**MS**	**F-value**	**P-value**
Zone	0.226	2	0.113	35.995	0.000
Tidal cycle	0.005	1	0.005	1.565	0.212
Time of day	0.039	1	0.039	12.428	0.000
Tidal phase	0.004	1	0.004	1.224	0.269
Zone and Tidal cycle	0.002	2	0.001	0.309	0.734
Zone and Time of day	0.016	2	0.008	2.621	0.074
Zone and Tidal phase	0.024	2	0.012	3.821	0.023
Tidal cycle and Time of day	0.010	1	0.01	3.065	0.081
Tidal cycle and Tidal phase	0.002	1	0.002	0.647	0.422
Time of day and Tidal phase	0.005	1	0.005	1.581	0.209
Zone and Tidal cycle and Time of day	0.006	2	0.003	0.910	0.403
Zone and Tidal cycle and Tidal phase	0.002	2	0.001	0.323	0.724
Zone and Time of day and Tidal phase	0.005	2	0.003	0.811	0.445
Tidal cycle and Time of day and Tidal phase	0.000	1	0.000	0.050	0.824
Zone and Tidal cycle and Time of day and Tidal phase	0.002	2	0.001	0.249	0.780
Error	0.978	312	0.003		

Note: SS (sum of squares); df (degrees of freedom); MS (mean square); F-value (value of statistic F); p-value (estimated probability).

Despite the advertising effort and reward for tag return and further information, only one recapture was reported by a recreational fisherman. Hence, and since *D. sargus* is highly targeted in the region, unreported recaptures might have occurred due to lack of information or will to cooperate by local fisherman. This situation is not unusual with this type of work and can be explained by the fact that some of the tagged animals may have been captured illegally inside the no take MPA (Abecasis et al. 2009, Marsh et al. 2011).

A higher level of cooperation from the recreational and commercial fishing community and information concerning recaptures would allow a broader understanding of dispersal patterns of *D. sargus* beyond the study area, and perhaps to calculate mortality rates associated with recreational and commercial fisheries. Nevertheless, recaptured specimens were found in good physical condition but tags presented high algae fouling, contributing to an increased drag and to the formation of small necrosis, also observed in other studies (Abecasis et al. 2009, Marsh et al. 2011). In fact, three specimens recaptured in October 2013 had small scars under the dorsal fin, suggesting that these animals might have lost or removed their tags. Altogether, these occurrences might have contributed for an under estimation of the recaptured animals.

TABLE 3.4. Median Time of Residency (%) by zone of the study site and time of day

	Median Time of Residency (%)		
	E	**IE**	**IP**
Day	4.08	4.21	0.35
Night	2.13	2.87	0.15

Note: Interior Exposed Zone - IE; Interior Protected Zone - IP; Exterior Zone – E

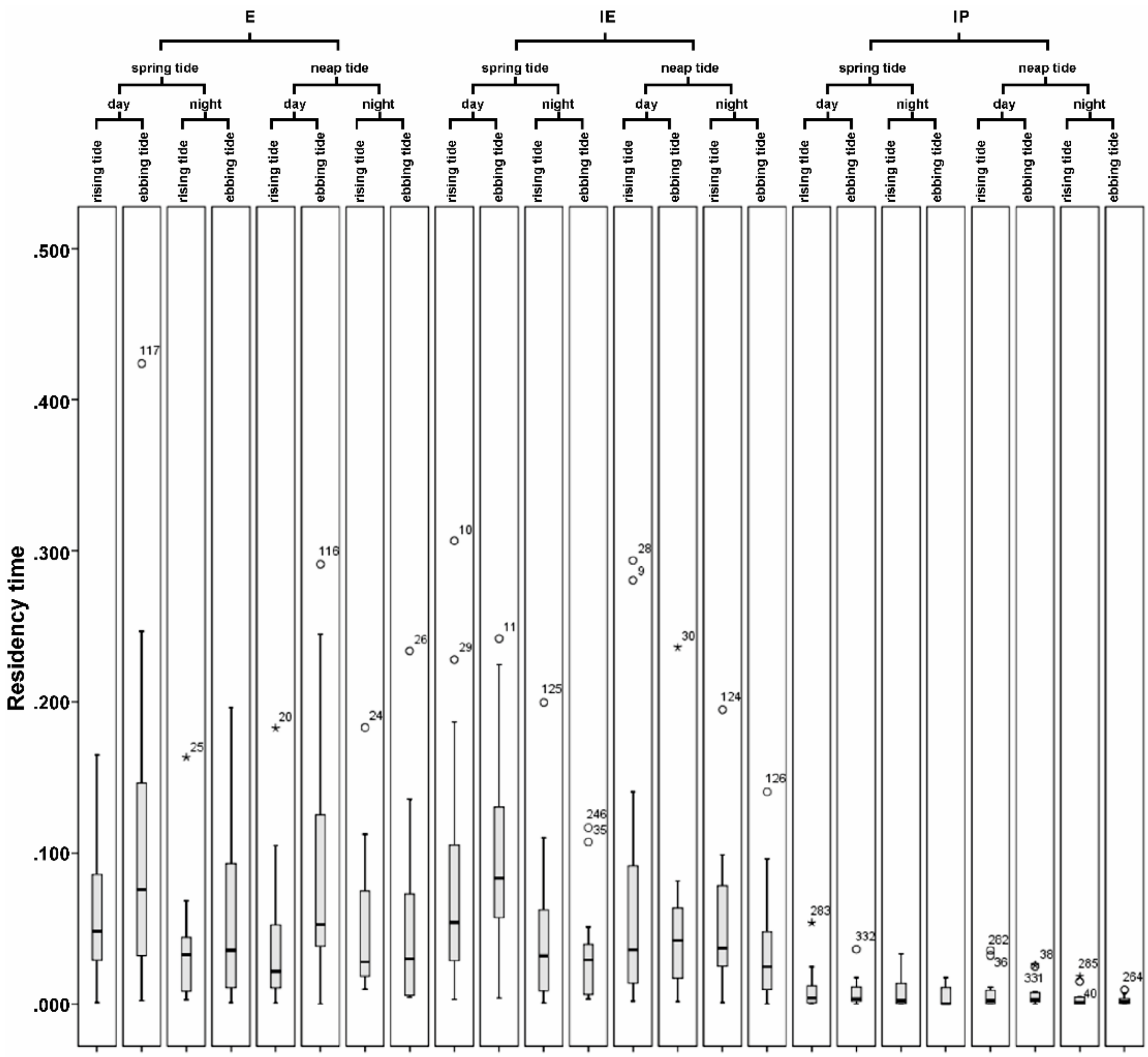

FIGURE 3.7. Box and whiskers plot detailing time of residency variation according to zone of the study area, tidal cycle and phase and time of day. E (Exterior), IE (Interior Exposed) and IP (Interior Protected).

Regarding the use of acoustic telemetry, total number of detections obtained was relatively high (236 099), which accounts for 14.4% of all possible detections for the 60 days of the study. However, this volume of data may be underestimated, since the absence of detections may not imply the absence of the specimen from the theoretical receiver range. The study area is characterized by a very complex seabed (very irregular rocky bottom), especially near and around the island, which together with the noise created by the hydrodynamics of the area, may reduce the detection capacity of the receivers, as described for other locations (Welsh et al. 2012). *D. sargus* is a species associated with rocky bottoms and uses the crevices as refuge, enhancing the obstacles' effect in the detection range (Guidetti 2000, D'Anna et al. 2011, Abecasis et al. 2013, 2015). In these conditions, the acoustic signal's range sent by the transmitter is considerably reduced and the number of detections may be considerably lower. However, noise quotient (nq=1662.87), based on VR2W receiver parameters, indicates substantially low levels of environmental noise, suggesting that missing codes where probably caused by physical obstacles, such as rocky formations, together with tagged fish behaviour and movements during code transmission. On the other hand, negative value of nq indicates code collision, suggesting that tagged specimens were within receiver range but frequently not detected. In optimal conditions, a range test should be performed to correctly assess receiver range. However, the study area displays unpredictable sea conditions with frequent high swell height, allowing range tests to be performed only during low swell. This fact together with the complexity of the sea bottom with abundant rocky formations that difficult code transmission, would provide biased receiver range values. Nevertheless, global high percentage of multiple detections (26%) and negative noise quotient suggest that, despite highly variable, receiver range would be no less than 100 m and receiver deployment design was adequate to provide an optimal study area coverage, since tagged fish were probably within receiver range despite not detected. Therefore, the reduced number of detections was probably caused by tagged fish movements and activity and results obtained were, in our opinion, a reflection of *D. sargus* behaviour. In accordance with literature, all the individuals implanted with the transmitter (from 27.7 to 36.5 cm in TL) were adults (Martínez and Villegas 1996, Morato et al. 2003, Mouine et al. 2007, Benchalel and Kara 2013). The results obtained indicate a strong use of the no take MPA by *D. sargus* during summer, since tagged specimens stayed in the area during the total length of the study showing high residency index values. A high spatial fidelity was also observed in other populations of the same species (Abecasis et al. 2009, D'Anna et al. 2011, Abecasis et al. 2013) as well as other Sparidae (Jadot et al. 2006, Kerwarth et al. 2007a).

Since North and South zones of the no take MPA were very little used, movement to the boundary areas should be scarce. A coarse description of the seabed attained through SCUBA diving and by using a probe from the support vessel revealed that receivers 7, 8 and 9 (exterior receiver semi-circle) were located on the edge of a rocky platform ending with a one metre high vertical wall, beyond of which the seabed is mostly sand. Additionally, receivers 13 and 15 (south limit) were located near a rocky chain called "the castle" extending from shore towards southwest, passing between the exterior and interior areas. This fact may explain a preference for this southern area by the tagged fish as revealed by the relatively high number of detections observed in this area, as the rocky bottom may provide shelter and food. In general, tagged specimens presented three different area use patterns according to KDE: using more frequently both the Exterior and Protected Interior areas, using the Exterior and Exposed Interior areas, and a combination of both, i.e. frequently using these three areas of the no take MPA. This evidence indicates that tagged specimens have high spatial fidelity associated with different territories. The fact that all specimens were released at the same location where captured could affect behavioural and area use patterns and, ideally, specimens should have been released throughout the no take MPA, but given the small size of this area and the marked behaviours in accordance with environmental variables, area use by these fish was probably not affected by capture and release site. This evident display of very distinct movement patterns may also be associated with gender, as spatial segregation based on gender has been observed in *D. sargus* outside the reproductive season (Mouine et al. 2007). However, it was not possible to identify the gender of the individuals in this study and compare movement patterns and area use between males and females.

Results obtained with both components of the network analysis (betweenness and degree centrality) indicate that the interior areas together with the ones covered by receivers 7, 8 e 9 were the most used. The area around receiver 13 was also important for some individuals. Fish #2, #6, #7, #11, #16 showed higher centrality values for both centrality measures, especially for betweenness. These individuals also presented more dispersed KDE, which is associated with broader movements and larger vital areas. According to Jacoby et al. (2012) higher values of betweenness and lower values of degree centrality are characteristic of roaming behaviours, i.e. when fish are actively moving throughout the study area. This pattern was observed in most tagged animals (n = 14). On the other hand, lower betweenness values and higher degree centrality are typical of refuging behaviours, i.e. when tagged fish remain within a restricted area for long periods of time. This behaviour was less frequent in the present study (n = 4). Given this, and considering the study site, it was possible to identify, with this method, three areas used by *D. sargus* with distinct purposes. Receivers 7, 9 and 13 displayed high levels of betweenness, indicating that these areas were used as passageways, possibly to enter and exit the no take MPA, whereas receivers 1, 2 and 4 presented higher values of degree centrality and were probably used as permanence areas. Receivers 3 e 8 showed high values for both centrality measures, which can be explained by their location, since receivers 8, 7 and 9 are located on the border of a rocky platform, and receiver 3 is located at the westernmost point of the island, a rocky formation that extends from the island dividing the west zone of the island into two (see FIGURE 1). This suggests that the areas associated with receivers 3 and 8 had a refuge function while areas closer to the island were mainly used as feeding areas. These results corroborate and complement the KDE analysis.

D. sargus used the Pessegueiro Island no take MPA differently according with environmental parameters. Tagged specimens were more detected during day time, and a more active behaviour during this period is usually observed in this species (Abecasis et al. 2013, Koeck et al. 2013), since it is a diurnal feeder and its feeding activity is highly influenced by light intensity (Figueiredo et al. 2005). This suggests that *D. sargus* associated to the Pessegueiro Island may use this area mainly as feeding ground. On the other hand, the number of detections decreased at night, suggesting that tagged specimens might seek shelter in areas adjacent to the no take MPA or in rocky crevices inside it (D'Anna et al. 2011). In fact, percentage of multiple detections was lower during night time (20.19% versus 29.83% during day time), suggesting detection range may be lower during this period, as also referred by Payne et al. (2010). Nevertheless, since average noise quotient was low throughout the study duration, detection range variation is likely to be caused by behavioural features, such as sheltering (D'Anna et al. 2011), rather than environmental noises. Tidal cycle also seemed to influence the amount of time spent inside no take MPA. This species seeks areas and periods of great hydrodynamic activity to feed (Sala and Ballesteros 1997) and spring tides are usually associated with greater hydrodynamic activity as well as higher tidal range. This provides access to feeding areas that are not available during neap tides, namely during high tides. However, spring tides and consequent hydrodynamism may influence the number of detections. Nevertheless, percentage of multiple detections during spring and neap tides was similar (26.51% and 26.30%, respectively), indicating detection range was not influenced by hydrodynamic conditions and *D. sargus* prefer in fact areas near the island during tides of higher amplitude. Factorial ANOVA, complemented by the box and whiskers plot analysis, revealed significant differences among the use of the three KDE assigned areas, of which the Interior Protected was the less used. The Exterior area seemed to be preferred during ebbing tide while the other two were more used during rising tide. Since the areas closer to the island present higher substrate complexity (B.R. Quintella, unpublished data) and hydrodynamic movement, favouring this species' feeding activity, the fact that these areas were more frequently used in rising/high tide, when some intertidal areas become accessible, may also be indicative that they are mainly used as feeding grounds. This observation is in accordance with other works stating that *D. sargus* seeks intertidal areas for feeding purposes during rising/ high tide (Faria and Almada 2006, Abecasis 2008). Furthermore, the exterior areas were more used in ebbing tide, suggesting it is used as a refuge area. Interior areas would therefore be separate feeding areas, whereas the exterior zone would be used for refuge. Nevertheless, it should also be taken into account that an increase in detections during high tide can be associated with an increase in detection range in shallow areas due to the rising tide (Abecasis 2008) and lower hydrodynamic background noise. However, as stated before, low average noise quotient throughout the study period and similar multiple detection percentage during ebbing and rising tides (27.50% and 25.29%, respectively), suggests that detection range was not affected by noise and consequently obtained data reflects the behaviour of the tagged fish.

The present work allowed assessing the importance of Pessegueiro Island no take MPA for *D. sargus*, identifying potential feeding and refugee areas, identifying preferred movements between those areas and identifying when, according to environmental variables, those fish engaged in those activities.

With this outcome it is possible to conclude that most fish reside in the study area during the summer period, displaying high spatial fidelity. Also, areas surrounding the island are very important for feeding purposes and possibly refuge. Additionally, the no take MPA size comprises the home range of most of the specimens at least during the summer period, reinforcing the protection provided for this group of individuals associated with the Pessegueiro Island during this time of year. This is in agreement with other studies for several reef species stating that small MPA's can provide efficient protection for reef species with small home ranges, such as *D. sargus* and other Sparidae, at least for certain periods of the year (Kerwarth et al. 2007a, Afonso et al. 2011, Alós et al. 2012, La Mesa et al. 2013, Taylor and Mills 2013). Consequently, the protection granted to the area with the creation of the no take MPA is an adequate and relevant measure for the protection and sustainable exploitation of *D. sargus* in the PNSACV, ensuring the protection of this species at least during summer.

The creation of the no take MPA (in 2011) together with the fishing closure established for *D. sargus* (ended in 2014) in the PNSACV may effectively contribute for its protection during the two most critical periods (i.e. feeding and reproduction) of their cycle, considering the probability of being caught by fishing activities. So, even small MPAs can provide an effective measure to protect commercially important fish species, as stated before for the Portuguese coast (Abecasis et al. 2015) and for other Sparidae (Kerwarth et al. 2007a). These measures are particularly effective and important for species with high site fidelity and small home ranges since they are more exposed to over fishing (Cowley et al. 2002). In addition, even if all tagged specimens in this study were adults, previous studies also highlighted small home range and high site fidelity for juvenile Sparidae (Watt- Pringle et al. 2013), indicating small

MPAs in general, and Pessegueiro Island in particular, as potentially effective in protecting smaller specimens.

Since adverse sea conditions and hydrodynamics of the region make it very difficult to conduct telemetry studies outside summertime due to navigation difficulties, high risk of losing acoustic receivers and the probable low level of efficiency of the equipment in detecting the acoustic signals, all the data was obtained during summer period, providing only a reduced time frame of this species behaviour. For this reason, the use of this data for a comparative analysis through the rest of the annual cycle is not possible. However, results from mark-recapture and acoustic telemetry suggest that the movement patterns and Pessegueiro Island no take MPA use by *D. sargus* may be consistent and repeatable from one year to another during summer time. Nevertheless, it would be interesting to conduct similar works in different years to confirm these findings. Despite the evidence of partial migrations in other species (e.g. Chapman et al. 2012) and mark-recapture results suggesting that some *D. sargus* specimens may leave this no take MPA and cover large distances, acoustic telemetry data indicates that the home ranges and preferred movements of most tagged specimens are within MPA borders. This adds more relevance to this no take MPA and to its potential to protect most *D. sargus* during summer periods. Also, summer is when sea conditions are more favourable for fishing activities, both professional and leisure, adding relevance to this no take MPA for *D. sargus* protection during this period.

Based on the conclusions presented and the interest inherent to this type of work, future tagging campaigns might result in higher recapture rates and consequently lead to a better understanding of this species' behaviour. It would also be relevant to verify if these patterns are kept throughout the year, particularly during reproductive season, to understand if this is also an important reproductive area. The information collected during the present work can also be an important contribution towards the eventual re-evaluation of the no take MPA spatial limits and to improve its management, especially regarding species with significant socio-economical interest.

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CHAPTER 4

CHANGES IN FISH ASSEMBLAGE STRUCTURE AFTER IMPLEMENTATION OF MARINE PROTECTED AREAS IN THE SOUTH WESTERN COAST OF PORTUGAL

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Abstract

Marine Protected Areas (MPAs) are increasingly being recommended as management tools for biodiversity conservation and fisheries. With the purpose of protecting the region's biodiversity and prevent the over exploitation of marine resources, in February 2011 the no take MPAs of Ilha do Pessegueiro and Cabo Sardão were implemented within the Parque Natural do Sudoeste Alentejano e Costa Vicentina (PNSACV) Marine Park, south western coast of Portugal. As such, commercial and recreational fishing became prohibited in these areas. In order to evaluate the effects of these no take MPAs, the structure of their fish assemblages and of adjacent control areas without fishing restrictions were studied between 2011 (immediately after implementation) and 2013 (two years after implementation). A total of 4 sampling campaigns were conducted (summer 2011, winter 2012, summer 2013 and winter 2013) using trammel nets and bottom trawl. Ichthyofaunal assemblages from the no take MPAs (treatment) were compared with adjacent areas (controls) and changes evaluated as a function of time since protection. Results revealed significant increase in fish abundance after the implementation of the no take MPAs. Furthermore, significant differences in the structure of fish assemblages (abundance and fish size) between protected and neighbouring areas were rapidly observed upon the implementation of the no take MPAs. In addition, specimens of larger size occurred more frequently within Ilha do Pessegueiro no take MPA in the last year of the study. Overall, despite the young age of these no take MPAs, changes on the structure of their fish assemblages were already evident after only two years of protection, indicating that management measures such as MPA designation may play an important role to promote fisheries sustainable exploitation as well as to protect species with conservation interest.

Keywords

Management measures; MPA effectiveness; Fishing prohibition; No take zone; PNSACV Marine Park

4.1. Introduction

Marine Protected Areas (MPA) are any intertidal or subtidal areas protected by law in order to maintain its biodiversity and guarantee a sustainable use of their resources (Kelleher and Kenchington 1992). Over the years, these tools have been widely implemented to manage fishing stocks and promote biodiversity conservation (Allison et al. 1998, Russ 2002, Botsford et al. 2003, Chateau and Wantiez 2009). Several studies highlight the effectiveness of MPA implementation towards these purposes (e.g. Fenberg et al. 2012, García-Charton et al. 2008, Harmelin et al. 1995). Amongst others, regulations may include partial or total prohibition of fishing inside the MPA. By limiting or eliminating fishing activities in these areas, species richness, fish size and density are expected to increase (Fenberg et al. 2012, Lester et al. 2009) and by removing top predators, larger amounts of juvenile fish and small fish species may occur inside marine reserves (Gell and Roberts 2003, Russ 2002). The size of an MPA plays an important role on how effective it can be and how fast changes on fish assemblage may occur. Larger and older areas tend to present higher densities and larger specimens than younger or smaller MPAs (Claudet et al. 2008). Nevertheless, several documented cases have shown that positive effects on fish assemblages become evident only after some years of protection (e.g. Claudet et al. 2008). In some cases, for example, the increment of fish species and density can be visible after only 2e3 years upon MPA implementation (Halpern 2003). Therefore, for an MPA to be successful, especially in the short term, level of protection, size and location should be taken into account when designing it. Only 4.6% of the MPAs have simultaneously an adequate level of protection (no take zones) and are old, large and well located enough to be effective and ecologically distinctive from adjacent areas (Edgar et al. 2014).

The use of MPAs as conservation and management tools in Portugal is only recent and still uncommon. In mainland Portugal, only 4 MPAs are in place and each with different protection levels: Litoral Norte Park (1987, north coast), Berlengas Natural Reserve (1981, central coast), Arrábida Marine Park (1998, central coast), and the Marine Park of Costa Vicentina and Sudoeste Alentejano Natural Park (PNSACV Marine Park, 1995, south western coast). Litoral Norte Park does not include any no take zone, while Arrábida Marine Park, Berlengas Natural Reserve and PNSACV Marine Park contain both total and partial protection areas as well as buffering zones, where fishing activities by licensed vessels and recreational fishermen are allowed. While studies for Litoral Norte and Berlengas MPAs are scarce or mainly focused on intertidal organisms (e.g. Bertocci et al. 2012, Haug et al. 2015, Jacinto et al.2011), Arrábida Marine Park fish assemblages have been frequently studied for the past years (e.g. Gonçalves et al. 2003, Henriques et al. 2013, Sousa 2011). This park was implemented in 1995 without specific protection areas, which were only posteriorly designated in 2005 (Horta e Costa et al. 2013). Studies have since shown that fish species richness and abundance became higher with time in both total and partial protection areas when compared with buffering and adjacent zones (Sousa 2011).

Fishing activities, both professional and recreational, are very common in the entire southwestern coast of Portugal, being intensively exploited (Reis 2011). The recent implementation, in 2011, of four partial protection areas within the PNSACV Marine Park provided the opportunity to monitor the evolution of fish assemblages from an early stage and to determine how fast protection effects can become evident. These areas are commonly referred to as no take MPAs and fishing activities are prohibited inside its boundaries. Also, the insufficiency of updated data regarding fish assemblage characterization in this region (Castro 2004) urged to act rapidly in order to gather baseline information that can be used in future studies assessing reserve effects. Sampling campaigns started 6 months after no take MPA implementation, when protection effects were likely still insignificant, in order to more accurately evaluate changes in the corresponding fish assemblages. If differences between protected and unprotected areas exist at an initial phase, there is a high probability that those differences are inherent to each locality's characteristics and that protected sites present a naturally higher potential to be marine reserves. If those differences emerge with time, then no take MPA designation could be responsible for those changes to happen. In both cases, no take MPA designation is justified, as its purpose is to maintain existing biodiversity or create conditions to enhance biodiversity (Halpern 2003).

The aim of this study was therefore to evaluate if the implementation of the two northernmost protected areas of the PNSACV Marine Park led to changes in fish species diversity and fish assemblage structure (relative species abundance and size). This was achieved by comparing fish assemblages of the study area between an early stage after no take MPA designation and almost 3 years later. Variations on species richness, abundance and fish size were assessed over the first years after protection was implemented. Given the effect of protected areas on fish assemblages demonstrated by previous studies, it is expected that the designation of areas with highly restrictive fishing measures will lead to an increase in species richness, abundance and size of both targeted and non targeted fish species (Branch and Odendaal 2003). Therefore, changes in the structure of fish assemblages over time within the protected areas, in comparison to adjacent areas, were anticipated.

4.2. Methods

4.2.1. Study area

This study was carried out in the PNSACV Marine Park, southwest Portugal (FIGURE 4.1). This park was designated in 2011 as a maritime extension of the Sudoeste Alentejano e Costa Vicentina Natural Park (PNSACV). The marine park extends 2 km offshore along the 120 km coast of the natural park, crossing through the regions of Alentejo (southwestern coast) and Algarve (south and southwestern coasts) (FIGURE 4.1). Upon its designation, four type I partial protection areas (no take MPAs) where almost all fishing activities are forbidden were implemented along the marine park. The only exception is the commercial capture of stalked barnacle *Pollicipes pollicipes* (Gmelin, 1789) on coastal cliffs. Law enforcement and surveillance are under Maritime Police jurisdiction and, globally, fishermen comply with the regulations (J. Parrinha, personal communication). Two of these no take MPAs are located in the Alentejo coast of the park: Pessegueiro Island no take MPA in the northern Alentejo coast and Cape Sardão no take MPA in the southern (FIGURE 4.1). Both no take MPAs are relatively small. Pessegueiro Island has an area of 6 Km^2 and Cape Sardão 7 Km^2, approximately. Maximum depth on both areas is about 25 m and the bottom is composed by rock and sand in Pessegueiro Island no take MPA and adjacent control areas, while in Cape Sardão and adjacent control areas mainly rocky reef occur. The overall fish assemblage of these areas is very diverse, encompassing 149 fish species, some of which of high commercial value for regional fisheries. These include breams *Diplodus sargus* (Linnaeus, 1758), *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), conger eel *Conger conger* (Linnaeus, 1758), sole *Solea solea* (Linnaeus, 1758) and moray eel *Muraena helena* (Linnaeus, 1758) (ICNB 2008).

4.2.2. Sampling

Sampling consisted of 4 surveys: August 2011 (summer), February 2012 (winter), August 2013 (summer) and December 2013 (winter). This temporal design allowed characterizing the fish assemblages during the first year (2011/12) and after almost three years of protection (end of 2013), before and after eventual early effects would become evident, respectively. August 2011 and February 2012 sampling seasons comprised year 1 of protection and August and December 2013 corresponded to year 3. Sampling in distinct seasons allowed detecting eventual intra-annual differences. Surveys were performed aboard a professional fishing vessel using trammel nets with inner mesh of 100 mm and two outer layers of 500 mm mesh. In each sampling season, two replicates of trammel nets 100 m long and 2 m high were deployed between 10 m and 20 m depth inside each no take MPA (treatment) and corresponding adjacent control areas. In order to sample different habitats, one replicate was deployed over sandy bottom and one over rocky reef.

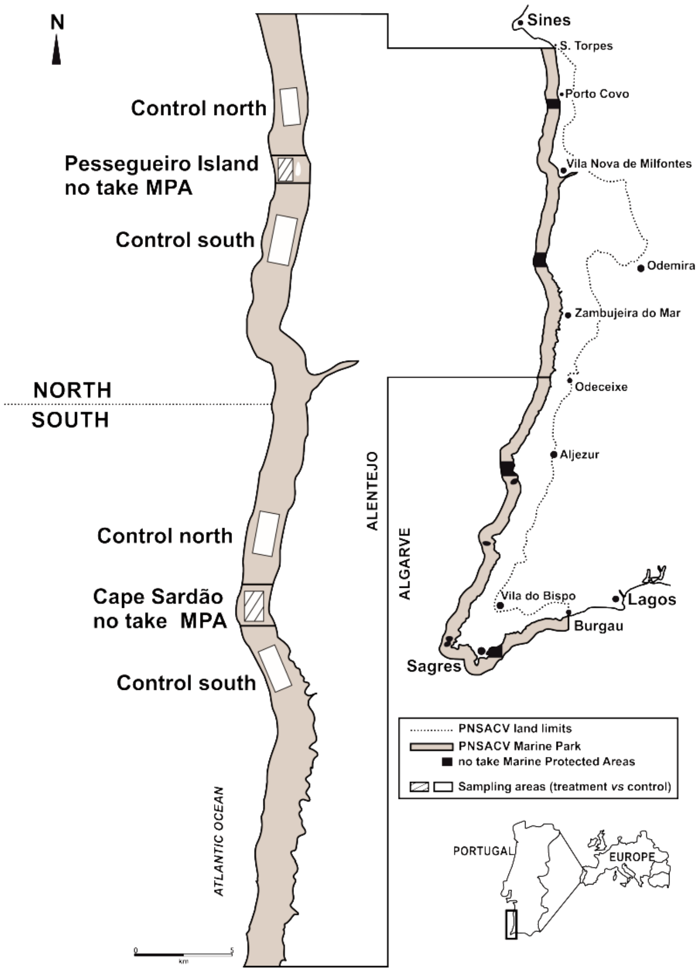

FIGURE 4.1 – Location of the study area with the monitored no take MPAs (treatment) and corresponding adjacent control areas.

Trammel nets were left fishing overnight for a minimum period of 8 and a maximum of 12 h. Each habitat was sampled for two consecutive days, resulting in 200 m of trammel nets per habitat per sampling season. This gear provides a global sampling of the fish assemblage when compared with more selective ones such as fish traps and longlines. Trammel nets mostly capture adults of benthic and demersal fish species and it's the fishing gear local fishermen use the most. However, it is not suitable for small size and cryptic species. Trammel nets were the only fishing gear that could be used simultaneously in both areas since the high abundance of rocky reef in Cape Sardão impaired the use of bottom trawling.

Alternative sampling methods (visual censuses) and gears (fish traps) were considered to sample juvenile and cryptic fish from both areas but its execution would not be practical. The study area was divided into two sub-areas, north and south, each corresponding to a set of no- take MPA and corresponding adjacent areas (FIGURE 4.1). By evaluating both no take MPAs and their corresponding adjacent control areas separately, local fish assemblages were characterized and the response of each to the protective measures was compared.

The analysis of the effect of the creation of no take MPAs on the proportion of juveniles and on fish size structure was made only for the Pessegueiro Island no take MPA fish assemblage. The reason for this was that juvenile fish collection could only be performed through bottom otter trawling. This type of gear captures a broader size range of benthic and demersal fish species when compared with trammel nets, and allowed the comparison of fish size between sampling seasons and protected and non protected areas. This gear could not be used in Cape Sardão no take MPA given the abundance of rocky bottom. Additional sampling surveys were therefore performed only in this area using a bottom otter trawl with 20 mm cod-end mesh. Trawling operations were performed at the same sampling occasions as trammel net sampling. The surveys consisted of three trawls of 15 min between 10 m and 20 m depth at an average speed of two knots inside the no take MPA and each adjacent control area. Each sampling campaign with trammel nets and bottom trawling lasted 5 days.

In each sampling survey, whenever possible, captured specimens were identified, weighed (±0.01 g) and measured (total length ± 1 mm) on board and then released. The remaining specimens were processed in the laboratory. Identification of the fish specimens was made according to Whitehead et al. (1984/1986).

4.2.3. Data analysis

Species richness, abundance and assemblage structure were compared between protected areas (Pessegueiro Island and Cape Sardão) and between years 1 and 3 of protection. Furthermore, the assemblages from each no take MPA were compared in each year with those from corresponding adjacent control areas. Additionally, the influence of habitat and season on variations of fish assemblages was also analysed. Pelagic species such as *Boops boops* (Linnaeus, 1758), *Sardina pilchardus* (Walbaum, 1792) and *Scomber japonicus* Houttuyn, 1782 were removed from comparing analysis given their mobility and schooling behaviour.

4.2.3.1. Abundance and species richness

The total abundance and number of fish species captured with trammel nets in both no take MPAs and respective adjacent control areas were compared by means of univariate PERMANOVA considering 5 factors: Locality (Pessegueiro Island and Cape Sardão), Year (1 and 3), Protection (with and without) nested in Locality, Season (summer and winter) nested in Year and Habitat (sand and rock) nested in Protection (Anderson et al. 2008).

4.2.3.2. Fish assemblage structure

Effect on local fish assemblages was analysed by comparing their composition taking into account the number of specimens of each fish species by means of multivariate PERMANOVA with 5 factors: Locality (Pessegueiro Island and Cape Sardão), Year (1 and 3), Protection (with and without) nested in Locality, Season (summer and winter) nested in Year and Habitat (sand and rock) nested in Protection (Anderson et al. 2008). When interaction between factors occurred, pair-wise PERMANOVA tests were performed to assess in which way each interactive factor influenced fish assemblage structure (Anderson et al. 2008). Additionally, SIMPER analysis was used to investigate which fish species contributed the most for the obtained differences (Anderson et al. 2008). PERMDISP analysis was applied to evaluate data dispersion and validate PERMANOVA results (Anderson et al. 2008).

4.2.3.3. Fish size structure

Variations on fish size structure were assessed by analysing the lengths of specimens from the most abundant species captured only with bottom otter trawl in Pessegueiro Island no take MPA and adjacent areas. Analysis focused on European scaldfish *Arnoglossus laterna* (Walbaum, 1792), soles *Pegusa lascaris* (Risso, 1810), *Solea senegalensis* (Kaup, 1858) and *S. solea*, longfin gurnard *Chelidonichthys obscurus* (Walbaum, 1792) and rays *Raja clavata* Linnaeus, 1758 and *Raja undulata* Lacepède, 1802. Comparisons were made by means of univariate ANOVA considering 3 factors (protection, year and season).

4.2.3.4. Percentage of juvenile fish

The influence on the nursery function was assessed by analysing the percentage of juveniles of the most abundant species captured only by trawling on the Pessegueiro Island area. Juvenile proportion of *A. laterna*, *P. lascaris*, *C. obscurus* and *R. undulata* was determined considering the length at first maturation (L50%) assigned to each species based on literature. Comparisons between no take MPA and control areas and between 1st and 3rd years of protection were made by means of RxC G-test-of-independence (Sokal and Rohlf 2012).

4.3. Results

4.3.1. Global composition

In total, 1740 specimens from 60 species were captured and identified during the study: 53 species in Pessegueiro Island no- take MPA and adjacent control areas, and 40 species in Cape Sardão. *S. japonicus*, *P. lascaris* and *D. sargus* were globally the most abundant species with 284, 221 and 143 specimens, respectively. More specimens were captured in the Pessegueiro Island area than in Cape Sardão, both in total (1187 vs. 573) and inside the no take MPA (429 vs. 215).

4.3.2. Abundance and species richness

Trammel net sampling resulted in a total of 1354 specimens belonging to 52 fish species. Number of species and specimens was higher in the northern study area than in the southern. The same occurred when comparing each no take MPAs with corresponding control areas (FIGURE 4.2). However, PERMANOVA tests showed that these parameters were not statistically different between study areas or according with protection level. Similarly, these parameters did not vary according with sampling season and habitat. Nonetheless, despite the number of species being similar over time, number of captured specimens in both studied localities significantly increased from year 1 to year 3 of protection (PERMANOVA d.f. = 1, Pseudo-F = 4.196, p = 0.028).

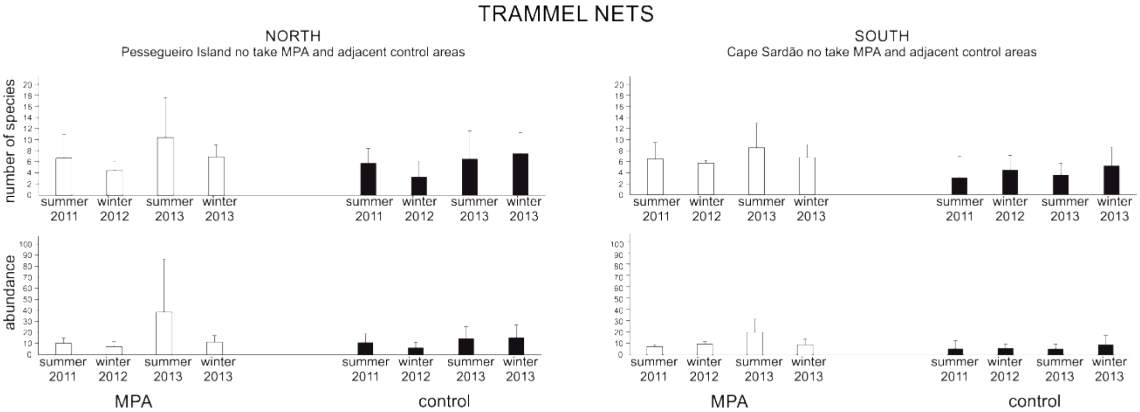

FIGURE 4.2 – Number of species and abundance of specimens captured in both no take MPAs and respective control areas by means of trammel nets in each sampling season.

4.3.3. Fish assemblage structure

Fish assemblage structure varied with time and with locality, being significantly different between years, localities, level of protection and seasons. On the other hand, assemblages from sandy habitats revealed no significantly different structure from those from rocky habitats (TABLE 4.1). PERMDISP analysis showed no significant differences for all factors, thus validating PERMANOVA results. Additionally, significant interactions between factors were found, namely between factors Year and Protection and between Habitat and Year (TABLE 4.1).

Principal Coordinates Analysis (PCO) for the fish assemblage considering factors Protection and Year separates the corresponding groups, illustrating the differences between years and between levels of protection (FIGURE 4.3).

Pair-wise tests showed that the structure of the fish assemblage from Pessegueiro Island no take MPA during year 1 was significantly different from the assemblage from the adjacent non protected areas (TABLE 4.2). During year 3, those differences reduced and the structure of the assemblages from inside and outside Pessegueiro Island no take MPA were not statistically different (TABLE 4.2). When considering the interaction between factors Habitat and Year, pair-wise tests showed that assemblages from rocky and sandy habitats from inside Pessegueiro Island no take MPA were similar in structure during year 1 of protection. However, over time those assemblages became significantly distinct (TABLE 4.2). Outside this no take MPA, fish assemblages from rocky and sandy habitats were structurally similar over time (TABLE 4.2).

TABLE 4.1 – Results from multivariate PERMANOVA to compare fish assemblage structure according to 5 factors: Locality (Pessegueiro Island and Cape Sardão), Year (1 and 3), Protection nested in Locality (with and without), Season nested in Year (summer and winter) and Habitat nested in Protection (sand and rock)

Factors	d.f.	SS	MS	Pseudo-F	P(perm)	perms
Year	1	16852.0	16852.0	5.763	0.001	998
Locality	1	5921.6	5921.6	2.025	0.021	999
Protection (Locality)	2	12092.0	6045.8	2.068	0.003	999
Season (Year)	2	16320.0	8160.1	2.791	0.001	999
YearxLocality	1	2540.1	2540.1	0.869	0.564	998
Habitat [Protection (Locality)]	4	15172.0	3793.0	1.297	0.090	998
Protection (Locality) x Year	2	9436.1	4718.0	1.614	0.042	998
Season (Year) x Locality	2	7644.7	3822.4	1.307	0.157	997
Protection (Locality) x Season (Year)	4	1209.0	3022.4	1.034	0.397	998
Habitat [Protection (Locality)] x Year	4	16782.0	4195.5	1.435	0.023	999
Habitat [Protection (Locality)] x Season (Year)	8	25455.0	3181.9	1.088	0.269	996
Residual	57	1.7E5	2924.1			
Total	88	3.1E5				

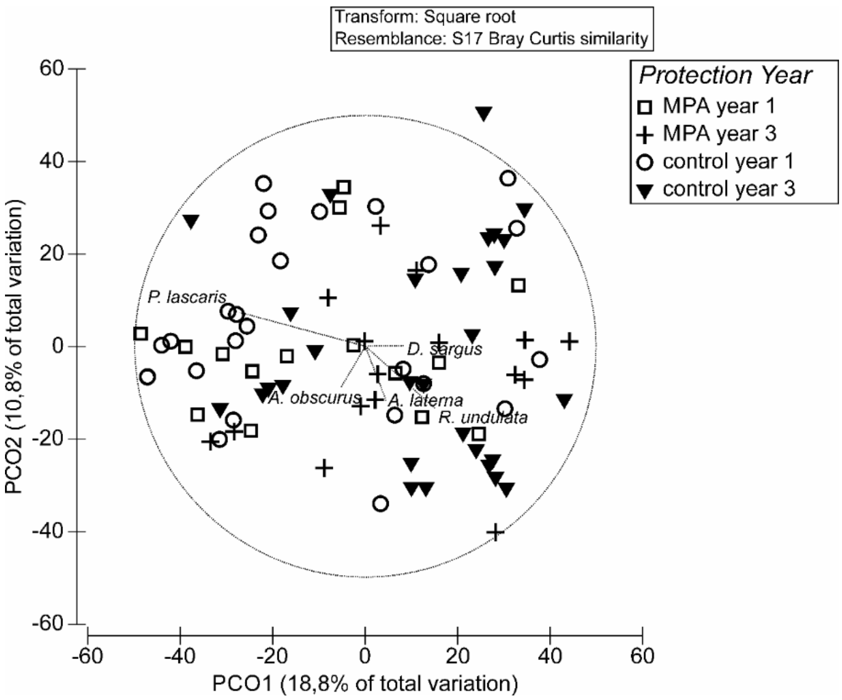

FIGURE 4.3 – Principal Coordinates Analysis (PCO) for the fish assemblage structure of both north and south areas regarding factors Protection and Year.

Regarding the southern study area, fish assemblages from within the no take MPA were not significantly different from those from adjacent areas during year 1, becoming significantly different during year 3 of protection (TABLE 4.2). In this study area the structure of fish assemblages from rocky and sandy habitats inside the no take MPA were similar during year 1 of protection but became significantly different during year 3, as found in the northern area (TABLE 4.2). Outside Cape Sardão no take MPA, assemblages from both habitats remained similar throughout the study period (TABLE 4.2).

TABLE 4.2 – Results from PERMANOVA pairwise tests to compare fish assemblage structure according to significantly interacting factors, Protection x Year and Habitat x Year

		Factor	
	Factor	**Year 1**	**Year 3**
North (Pessegueiro Island no take MPA and adjacent control areas)			
MPA	Protection (with *vs*. without)	0.043	0.095
	Habitat (sand *vs*. rock)	0.458	0.026
Control	Habitat (sand *vs*. rock)	0.556	0.535
South (Cape Sardão no take MPA and adjacent control areas)			
MPA	Protection (with *vs*. without)	0.070	0.021
	Habitat (sand *vs*. rock)	0.055	0.026
Control	Habitat (sand *vs*. rock)	0.228	0.074

SIMPER analysis revealed that *P. lascaris* and *D. sargus* were the species that contributed the most for the differences between fish assemblages, followed by *S. senegalensis*, grey triggerfish *Balistes capriscus* Gmelin, 1789 and ballan wrasse *Labrus bergylta* Ascanius, 1767 (TABLE 4.3). Both *P. lascaris* and *D. sargus* were more abundant in the Pessegueiro Island study area than in Cape Sardão (factor Locality - SIMPER: contribution of 9.80% and 6.96%, respectively) and inside both no take MPAs than in corresponding adjacent control areas (factor Protection - SIMPER: contribution of 9.79% and 7.24%, respectively) (TABLE 4.3). On the other hand, *D. sargus* was more abundant during year 1 of protection (factor Year - SIMPER: contribution of 6.05%) and on rocky habitat (factor Habitat - SIMPER: contribution of 6.64%), while *P. lascaris* was more abundant during year 3 (factor Year - SIMPER: contribution of 10.61%) and on sandy habitat (factor Habitat - SIMPER: contribution of 9.85%) (TABLE 4.3).

4.3.4. Fish size structure

The length of rays and soles sampled with otter trawl in the northern study area varied significantly over time (TABLE 4.4). Both rays and soles captured in this locality were significantly larger in year 3 when compared with the specimens captured earlier. Additionally, an interaction between factors Protection and Year was found regarding soles, with specimens captured inside Pessegueiro Island no take MPA during year 1 significantly smaller than those captured in year 3 (TABLE 4.3, FIGURE 4.4). Inversely, scaldfish specimens, a species with low commercial value, significantly decreased in length from year 1 to year 3 (TABLE 4.3, FIGURE 4.4). The gurnard, on the other hand, only revealed significant differences in size considering sampling season, with larger specimens more abundant in summer (TABLE 4.4, FIGURE 4.4).

TABLE 4.3 – Percentage of contribution (%, first column of each factor) and corresponding average abundance (2nd and 3rd columns of each factor) of the species that contributed the most for obtaining significant differences between factors according to SIMPER analysis

Species	Factor											
	Locality			Protection			Year			Habitat		
	%	north	south	%	MPA	Cont.	%	1	3	%	sand	rock
P. lascaris	9.80	0.94	0.85	9.79	1.09	0.78	10.61	1.15	0.65	9.85	0.95	0.84
D. sargus	6.96	0.84	0.31	7.24	0.94	0.37	6.05	0.41	0.75	6.64	0.37	0.77
S. senegalensis	6.27	0.61	0.28	5.81	0.46	0.44	5.89	0.57	0.32	5.93	0.55	0.35
B. capriscus	4.69	0.38	0.32	5.11	0.48	0.28	4.92	0.07	0.61	4.78	0.42	0.28
L. bergylta	4.11	0.41	0.24	4.12	0.35	0.31	4.03	0.35	0.30	4.33	0.14	0.51

4.3.5. Percentage of juvenile fish

Size-frequency plot for juvenile fish from Pessegueiro Island showed that *A. laterna* and *C. obscurus* presented reduced percentage of juveniles both inside and outside the no take MPA. Conversely, *P. lascaris* showed higher juvenile proportion both inside and outside the protected area. *R. undulata* presented relatively high percentage of juveniles inside the no take MPA when compared with outside. However, young specimens were more abundant in the adjacent control area (FIGURE 4.5). G test-of-independence between percentages of juveniles according to Protection, Year and Habitat only revealed significant differences according to Protection for *P. lascaris*. Post-hoc tests showed that *P. lascaris* juveniles were significantly more abundant inside the no take MPA during the entire study period (TABLE 4.5).

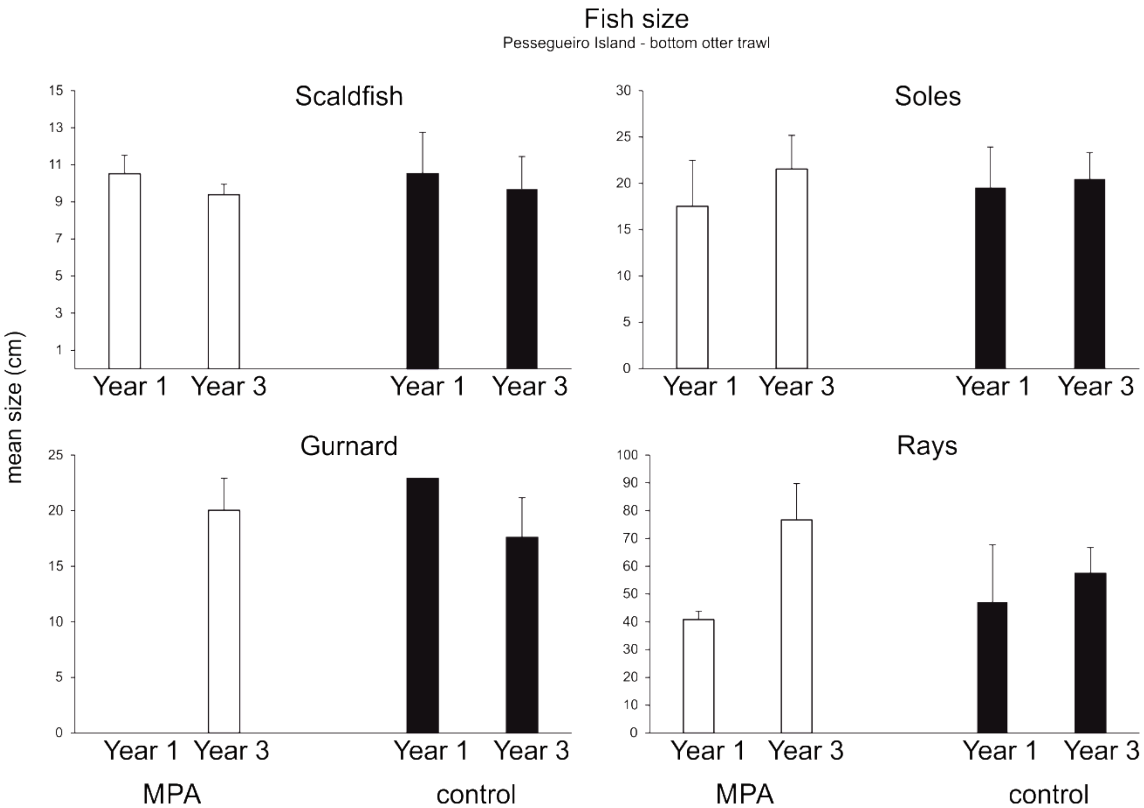

FIGURE 4.4 – Mean size and standard error of the most abundant species captured with bottom otter trawl according to sampling season and protection level.

TABLE 4.4 – Univariate ANOVA summary to compare the length of the most abundant species captured with otter bottom trawl in Pessegueiro Island area

	Fish size – ANOVA (bottom otter trawl)			
	Factors	**d.f.**	**F**	**p**
Scaldfish (*A. laterna*)	Protection	1	0.016	n.s.
	Year	1	5.087	0.027
	Season(Year)	1	0.055	n.s.
	Protection x Year	1	0.164	n.s.
	Protection x Season(Year)	1	0.039	n.s.
Soles (*Solea* spp., *P. lascaris*)	Protection	1	1.687	n.s.
	Year	1	15.496	0.000
	Season(Year)	1	2.889	n.s.
	Protection x Year	1	8.813	0.004
	Protection x Season(Year)	1	0.680	n.s.
Gurnard (*C. obscurus*)	Protection	1	0.150	n.s.
	Year	1	0.176	n.s.
	Season(Year)	1	4.633	0.040
	Protection x Year	1	-	-
	Protection x Season(Year)	1	3.966	n.s.
Rays (*Raja* spp.)	Protection	1	1.444	n.s.
	Year	1	5.396	0.031
	Season(Year)	1	0.007	n.s
	Protection x Year	1	0.255	n.s.
	Protection x Season(Year)	1	1.387	n.s.

n.s. Not significant, Significance level p<0.05.

TABLE 4.5 – Juvenile percentages of the most abundant species captured with bottom otter trawl in the Pessegueiro Island and G test-of-independence with corresponding *post-hoc* tests

Species	MPA (%)		Control (%)		$G_{Williams}$	p	*Post-hoc*
	Year 1	Year 3	Year 1	Year 3			
A. laterna	0,0	0,0	4,6	0,0	1,631	n.s.	-
P. lascaris	100,0	91,7	50,0	9,1	12,262	0,007	P1 P3 C1 C3
C. obscurus	0,0	0,0	0,0	6,3	0,273	n.s.	-
R. undulata	100,0	25,0	85,7	100,0	7,365	n.s.	-

$G_{williams}$ – independence test, p – significance level (p<0,05), P1 – protected area year 1, P3 – protected area year 3, C1 – control area year 1, C3 – control area year 3, Underline connects subsets without significant differences.

4.4. Discussion

Overall, the present work revealed that, despite their small size and recent designation, both no take MPAs produced effects on fish abundance that eventually led to changes in their fish assemblages. Furthermore, Pessegueiro Island no take MPA seemed to be adequate to protect juvenile fish and benefit commercially important species, as the number of larger specimens of these fish increased over time. Yet, these changes should be addressed carefully as they may be caused not only by the implementation of these protected areas but also by its interaction with other factors such as locality and elapsed time.

In order to better assess the effects of MPA implementation, monitoring should have started before protection designation to determine if differences already existed between the area to be protected and adjacent control areas. However, logistic and meteorological constraints did not allow starting sampling surveys before protective measures were in place, in February 2011. Still, in this particular study case, monitoring started a few months after the implementation of the no take MPAs, which enabled the identification of the fish assemblage status close to the outset of the protection measures. Furthermore, monitoring should also continue for several years in order to track the evolution of both protected and non protected fish assemblages.

Studies focusing on the effectiveness of MPAs and resulting benefits for biological resources are common worldwide for several years (e.g. Fenberg et al. 2012, García-Charton et al. 2008, Harmelin et al. 1995, Harmelin-Vivien et al. 2008). Along the mainland Portuguese coastal MPAs, this type of works are increasingly becoming available. Arrábida Marine Park early reserve effects have been assessed on single fish species (Abecasis et al. 2015), on fisheries (e.g. Batista et al. 2011) and on fish assemblages (e.g. Gonçalves et al. 2003, Henriques et al. 2013, Sousa 2011). However, studies regarding fish species are still rare for the Berlengas Natural Reserve (Rodrigues et al. 2011) and Alentejo coast (Fernandéz et al.

2016). Considering this, the designation of the Pessegueiro Island and Cape Sardão no take MPAs provided an opportunity to assess the effects of these management tools starting at an early stage, only six months after MPA designation, which is uncommon for the Portuguese coast. If differences develop with time, then protective measures might have been a determinant factor.

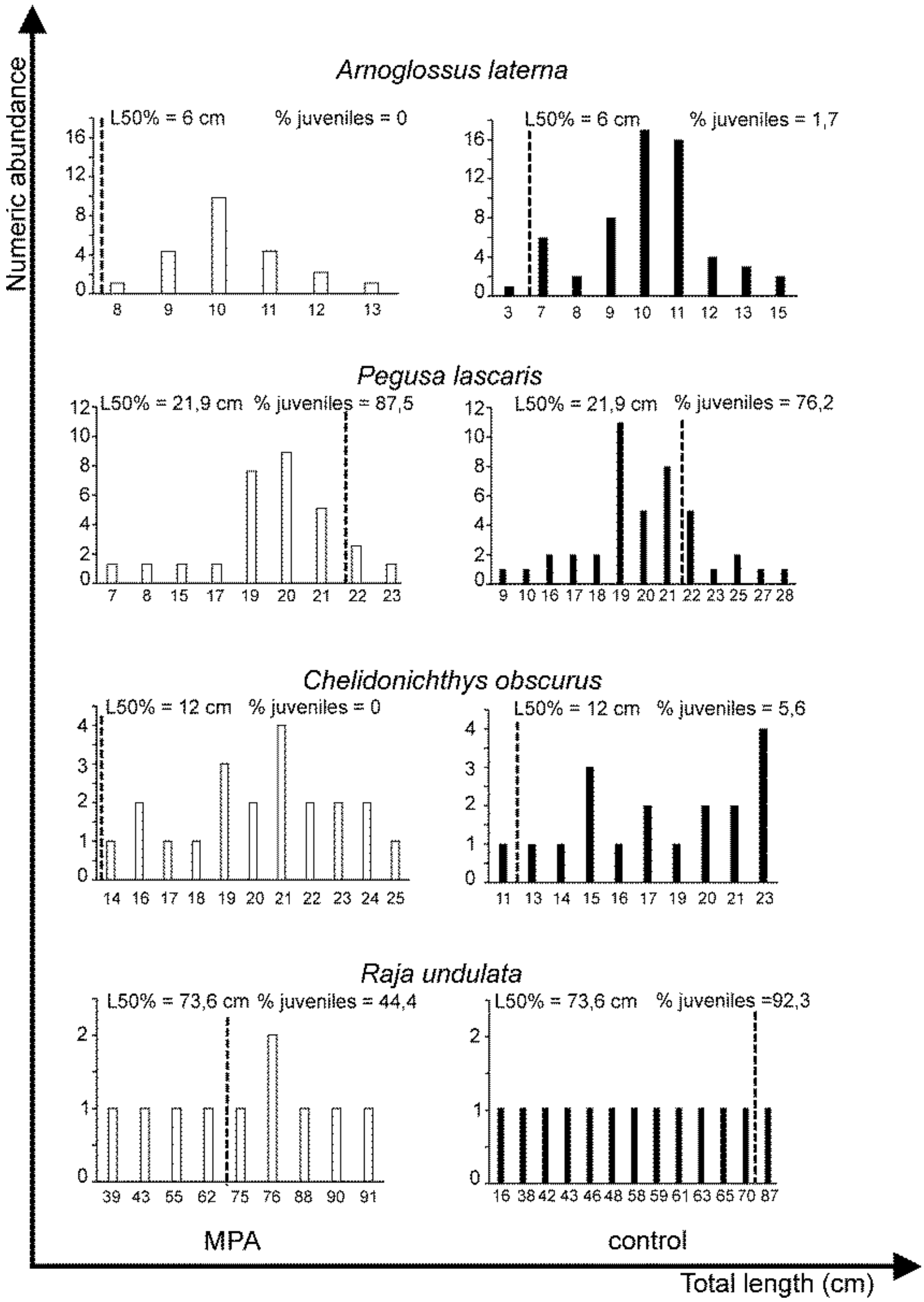

FIGURE 4.5 – Plot of numeric abundance against total length of the 4 most abundant fish species sampled in Pessegueiro Island area with bottom otter trawl. Dashed line represents length at first maturity (L50%) according to: Gibson and Ezzi (1980) for *A. laterna*, Teixeira et al. (2009) for *P. lascaris*, Muñoz et al. (2001) for *C. obscurus*, and Coelho and Erzini (2006) for *R. undulata*.

So far, no take MPA implementation in the Alentejo coast has not significantly impacted species richness in the area. Also, protected and non protected areas were similar regarding this parameter throughout the study period. Species richness increase was observed in several MPAs worldwide but the amount of time after which those changes became evident surpasses 5 years in most cases. Such is the case of the Arrábida Marine Park (Sousa 2011). Roberts and Hawkins (2000) highlight evident species richness increase after 5 years in MPAs in New Caledonia and Philippines, while Harmelin-Vivien et al. (2008) found the same tendency in 6 Mediterranean MPAs after 10 years of implementation. Therefore, the absence of a significant effect on the number of species of the Alentejo coast may result from the small amount of time elapsed but may also be an evidence of a balanced and rich fish assemblage in these areas. On the other hand, implementation of these two no take MPAs seemed to influence the number of captured specimens as a significant increase in fish number was observed with time. This increase was observed in both no take MPAs as well as in adjacent control areas. Even if not statistically significant, this increase was more evident inside no take MPAs, suggesting that applied protective measures may not yet have enhanced biodiversity but may have been already influencing fish abundance. In fact, the increase in the number of specimens at relatively early stages after protection implementation has been recorded several times (e.g. Fenberg et al. 2012, Lester et al. 2009). The higher number of specimens observed in Pessegueiro Island compared to Cape Sardão may be due to geographical variables, such as depth and sea conditions. The northern area is shallower and less exposed to wind and swell than its southern counterpart, which may provide shelter and calmer sea conditions, therefore overall benefiting fish abundance.

The implementation of these no take MPAs and likely consequent increment on fish abundance may have been an important factor that led to significant changes on the structure of its fish assemblages. In fact, the number of specimens increased with time in Pessegueiro Island and Cape Sardão since MPA designation, and this was more evident inside the protected areas in comparison with control areas. The increase in abundance inside no take areas led the northern area fish assemblages from being significantly different during year 1 of protection to become similar in year 3, after the increase of specimens inside its no take MPA. Inversely, in the southern region, the higher increase of specimens inside the no take MPA led to significant differences between assemblages of protected and non protected areas. In both cases, *D. sargus* and *P. lascaris* were the species that contributed the most for these changes. In fact, the captures of both species increased significantly from year 1 to year 3, especially inside protected areas. These two species were also the most important in defining the differences between localities, being both sand soles and white seabreams significantly more abundant in the northern region. Despite both regions offering optimal conditions for seabreams to feed, sea conditions in the north are more favourable for these fish to reach the surf zone near rocky reefs where they preferentially obtain food (Faria and Almada 2006, Sala and Ballesteros 1997). Additionally, the northern region features larger areas of sandy bottom, the preferred habitat of sand sole (Teixeira et al. 2009). This way, in addition to protection, sea conditions and habitat also seemed to play an important role in structuring fish assemblages from both areas. The fact that most fish species presented more specimens during summer, when swell and winds are lower, led to significant differences in the structure of fish assemblages between seasons. During winter, when sea conditions tend to be less favourable, most fish probably relocate to areas offshore in search of better shelter and feeding conditions.

The analysis of the fish size of the specimens from Pessegueiro Island area indicated the occurrence of larger fish specimens over time, in this no take MPA, namely soles *Solea* spp. and *P. lascaris*. In fact, specimens of soles were significantly larger inside this no take area during year 3 of protection, whereas in year 1 there were no significant differences between the size of specimens captured inside and outside the protected area. This fact suggests that this no- take MPA may provide effective protection for these fish against fishing activities, allowing for the increase in abundance of larger specimens. Also, a significant increase in mean size of rays was observed both inside and outside this protected area with time. This fact may result from the combination of the general protective measures in place (MPA designation) with the specific protective measures towards rays. These fish are in fact protected by law and its captured is prohibited in the entire PNSACV Marine Park. Inversely, the decrease in mean size of *A. laterna* over time both inside and outside the no take MPA may be related with the increase of larger predators, such as rays. In fact, specimens of *A. laterna* were commonly found in stomach contents of the rays captured in the study area (unpublished data).

The particular study focused on the juvenile assemblage from the Pessegueiro Island area showed that protective measures in place seemed to benefit juveniles of several fish species inside its no take MPA, particularly *P. lascaris*. Juveniles of this species were significantly more abundant inside the no take MPA throughout the study period, suggesting that this area may be a natural nursery for this species. This occurrence is particularly relevant since *P. lascaris* is highly targeted by local fishermen and the designation of this particular no take MPA has the potential to protect this species in different stages of its life cycle.

These results suggest that the designation of these measures was appropriate and may benefit local fish communities. *D. sargus* and *P. lascaris* seemed to be the species that benefited the most from protective measures in place, as their abundance inside both protected areas significantly increased with time. Additionally, Pessegueiro Island proved to be a naturally important area for soles, given the higher abundance of juveniles throughout the study period and the increase in number of larger specimens over time inside the protected area. These protective measures, especially the designation of Pessegueiro Island MPA together with specific legislation towards rays, also allowed the increase in abundance and the occurrence of larger specimens of these fish. On the other hand, higher abundance of rays, a large predator, may have had a negative effect on smaller fish, such seems to be the case of *A. laterna*. The increase of seabream abundance inside protected areas may also cause a negative effect on other fish species that reproduce in rocky substrata and on mussels and stalked barnacles, food items regularly found on their diets (Figueiredo et al. 2005, Leitão et al. 2007, Pallaoro et al. 2006). Therefore, MPA designation seems to benefit, at short term, fish species of commercial and conservational importance and larger in size. However, it can have the opposite effect on small non targeted fish species of particular groups of species (e.g. cryptic species) that are commonly preys of larger and economically important fish species (Claudet et al. 2008).

Overall, and despite their young age and small size, it was possible to identify positive effects that may have resulted from the designation of these no take MPAs, especially regarding commercially important fish species. These results also illustrate how fast positive effects on fish assemblages can occur. Also, these no take MPAs seem to have an appropriate size and are well located enough to be effective and ecologically distinctive from adjacent areas, at least for the analysed species. In future cases, MPA design should be validated through preliminary studies in order to determine correct size and locality, and to ensure effectiveness and benefits for fish assemblages from an early stage. In addition, effects and MPA effectiveness must continue to be monitored for larger periods of time to assess how the protective potential (positive or negative) evolves and if adjustments in MPA size and restrictive measures are needed.

In conclusion, small and young MPAs can be short term effective in protecting commercially important fish species. When designated in ecologically important areas, they also have the potential to protect species richness and promote fish abundance increase. Also, when implemented as a network of small MPAs, such as those implemented inside PNSACV Marine Park, the potential to be long term effective and benefit larger geographic areas increases, especially if connectivity between MPAs occurs (Chateau and Wantiez 2009). Nevertheless, negative effects on small fish species caused by predator increase inside MPAs are possible and only long term monitoring can provide answers regarding biodiversity evolution and determine additional measures necessary to achieve an adequate balance. Hence, MPA management should be flexible. Fish assemblages status should be periodically assessed in order to adapt protective measures accordingly either by maintaining or reinforcing current measures (adapt MPA size, ban all activities) or by temporarily adopting less restrictive ones (open recreational and/or professional fishing seasons for certain species). Only a large time scale protection programme can provide accurate information towards the successful management of an MPA or MPA network from which both nature and society can effectively benefit from.

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CHAPTER 5

CHANGES IN TROPHIC ECOLOGY OF FISH ASSEMBLAGES AFTER NO TAKE MARINE PROTECTED AREA DESIGNATION IN THE SOUTHWESTERN COAST OF PORTUGAL

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Abstract

Changes in fish assemblage structure caused by human activities, such as fishing, can alter trophic relations in fish assemblages. In this context, Marine Protected Areas (MPA) are efficient tools for habitat recovery and ideal environments for evaluating changes on the trophic structure resulting from human activities. The present work targeted fish assemblages from two no take MPAs from the northern half of South Alentejo and Costa Vicentina Marine Park, established in 2011. Previous works reported positive effects on local fish assemblages after no take MPA designation, and it is therefore important to further study its impact on local fish assemblages, especially concerning trophic interactions. Local fish assemblages were sampled (summer 2011, winter 2012, summer 2013 and winter 2013) using trammel nets. Diets were characterized and digestive tract contents of the 10 most abundant fish species were compared between the no take MPAs (treatment) and adjacent areas (controls), and changes evaluated as a function of time since protection. Results revealed significant differences between the diets of fish from protected and non protected areas, with crabs being the preferential prey in both protected and control areas but being more ingested outside the no take areas. However, these differences were evident since the beginning of the study. Fish assemblages from the northern area presented significantly larger niche breadth and significantly increasing with time. This way, the main effects of no take MPA implementation were directly visible on the niche breadth but did not directly impact the diet composition of the sampled fish assemblages, contributing however to reinforce the already naturally existent differences. This work provides important information regarding the effect of changes in the fish assemblage caused by MPA designation on the trophic ecology of fish.

Keywords

MPA impact; Fish diet; Niche breadth; No take zone; PNSACV marine park

5.1. Introduction

Marine Protected Areas (MPAs) have been widely implemented to stimulate the recovery of fishing stocks and preservation of biodiversity (Allison et al. 1998, Russ 2002, Botsford et al. 2003, Chateaux and Wantiez 2009), and can be defined as any intertidal or subtidal area that is protected by law in order to maintain its biodiversity and assure a sustainable use of its resources (Kelleher and Kenchington 1992). Ideally, a functional MPA should have four of the following: no take zones, size over 100km2, age over 10 years, enforcement measures, and be isolated from human activity (Edgar et al. 2014). Additionally, to be effective, an established fish population should subsist within MPA limits in order to gain benefits from protection (Kaplan et al. 2006), being its efficiency also directly dependent of the home range of the species targeted for protection (Kramer and Chapman 1999, Sale et al. 2005, Alós et al. 2012). MPA designation does not guarantee the return of fish assemblages to or near pristine condition but the implementation and efficiency of no take zones, where all fishing activities are prohibited, have been debated in several studies over the past years, suggesting that he majority of MPAs are effective protective tools and help the recovery of fish assemblages (e.g. García-Rubies and Zabala 1990, Guidetti and Sala 2007, Horta e Costa et al. 2013).

Changes in fish community structure caused by human activities, such as fishing, can alter trophic relations (Villamor and Becerro, 2012). In this context, Marine Protected Areas (MPAs) are ideal environments for evaluating changes on the trophic structure resulting from human activities (Villamor and Becerro, 2012). By removing top predators through fishing activities, organisms from lower trophic levels may prosper and cause algae depletion together with habitat complexity to decline (Seytre et al. 2013). By eliminating fishing activities through MPA implementation, top predators abundance may increase, causing a chain of events, or cascades, that may result in the decrease of prey abundance (Halpern 2003). Also, changes in predator density, size, or behaviour impact the entire trophic chain and influence assemblage structure, resulting in different fish assemblages from MPAs to non protected areas (Fenberg et al. 2012, Consoli et al. 2013, Sadio et al. 2015). Works focusing on trophic relations inside MPAs are becoming increasingly available (e.g. Vizzini and Mazzola 2009, Faye et al. 2011, Villamor and Becerro 2012, Soler et al. 2015) but most focus only on the characterization of the trophic chain regarding flows between levels and not on the impact of these protective measures on the trophic ecology of their fish assemblages (Vizzini and Mazzola 2009, Faye et al. 2011). Additionally, some of these works that characterize trophic relations and functional groups inside MPAs, and compare those with assemblages from unprotected areas, started several years after protection designation (e.g. Villamor and Becerro, 2012, Fernandéz et al. 2016,

Soler et al. 2015). The implementation of protected areas often leads to an increase in carnivore, planktivore and invertivore densities inside them (Halpern 2003). Villamor and Becerro (2012) report that functional diversity increase inside MPAs is more evident than the increase of fish abundance and species richness, with predators and carnivores being the groups that benefited the most by protective measures. Similarly, Colléter et al. (2012) observed predator biomass increasing and prey biomass decreasing after protection designation. Yet, Soler et al. (2015), based on 79 MPAs worldwide, affirm that MPA designation has positive effects at all trophic levels, as fishing activities harm the entire trophic chain, especially in shallow waters and reefs. Altogether, MPA designation may result in higher fish abundance and consequent increase in competition for resources (Halpern 2003). This occurrence may lead to changes in diet composition and feeding strategies, causing fish species to increase their niche breadth and thus becoming more generalists (Svanbäck and Persson 2004). Nevertheless, studies focusing on the impact of these protective measures on the diets and trophic ecology of their fish assemblages is yet almost unavailable (Murawski et al. 2005, White et al. 2010).

For the Portuguese coast, works focusing on coastal fish feeding ecology and diet mainly target commercially important species (e.g.: Morato et al. 2000, Vinagre et al. 2005, Leitão et al. 2007, Garrido et al. 2008), being works on the diets of coastal assemblages scarce (Castro et al. 2013). The same way, studies regarding the impacts caused by MPA designation on the trophic ecology of its fish assemblages are limited to one work that focus on the impact of predator increase on prey availability rather than changes on the diets of the global fish assemblage (Fernandéz et al. 2016).

The present work targeted fish assemblages from the northern half of South Alentejo and Costa Vicentina Marine Park, established in 2011 as an extension of the terrestrial natural park (PNSACV). The main purpose of its designation was to implement a long term tool to protect the southwestern Portuguese coastal strip, including its ecosystems, habitats and marine species and assemblages, by regulating human activities inside the area (Ordinance 143/2009). The designation of this large MPA comprised the implementation of a network of small type I and no take protected areas within its boundaries, commonly referred to as no take MPAs. In both MPA categories, no fishing activities are allowed, except for commercial harvesting of stalked barnacle *Pollicipes pollicipes* (Gmelin, 1790) inside type I protected areas. Previous works in this area reported positive effects on local fish assemblages after no take MPA designation, namely in fish abundance, species richness and specimens size, at an early stage (3 years) after implementation (Silva 2015a). It is therefore important to further study the impact of these protective measures on local fish assemblages, especially concerning trophic interactions. This need assumes particular relevance considering the almost absence of studies regarding MPA impact on trophic ecology of fish assemblages, particularly when assessed from an early stage after implementation (Colléter et al. 2012). Some of the most targeted species in this study are low mobility commercially important fish species, highly targeted by fishermen (e.g. breams, phycid hakes and soles) that find in this area ideal reproducing and feeding grounds, (Silva 2015a, b, Belo et al. 2016), and the designation of these no take MPAs may affect not only their abundance but also their behaviour and trophic ecology. Considering the above, the aim of this study was to assess variations in the trophic ecology of the fish assemblages from two small no take MPAs from the PNSACV Marine Park, southwestern coast of Portugal and test if MPA designation affects fish assemblage trophic ecology. Specifically, this work aims to assess how and how fast changes in diet composition, feeding habits and niche breadth of these assemblages after no take MPA designation. Are these parameters different between assemblages from protected and non protected areas? Did these parameters become different with time? To answer these questions, diets of fish from both protected and non protected areas were characterized and compared according to their composition and frequency of occurrence of each food item on their gut contents. Similarly, diets from specimens captured at different times after MPA designation were characterized and compared, thus allowing capturing probable temporal variations in feeding ecology after protective measures took place.

5.2. Methods

5.2.1. Study area

This study was conducted in the PNSACV Marine Park, southwest coast of Portugal (FIGURE 5.1). This marine park extends 2 km offshore and along the ca. 120 km coast of the natural park, crossing through two regions, Alentejo (southwestern coast) and Algarve (southwestern and south coasts) (FIGURE 5.1).

In 2011, several no take MPAs (no take and type I partial protected areas) were implemented along the marine park, two of which in the Alentejo coast of the park: Pessegueiro Island no take MPA in the northern Alentejo coast and Cape Sardão no take MPA in the southern Alentejo coast (FIGURE 5.1). Both are relatively small, having Pessegueiro Island an area of ca. 6 Km2 and Cape Sardão ca.7 Km2. Maximum depth on both areas is ca. 25 m, with the bottom composed of rock and sand in Pessegueiro Island no take MPA and adjacent areas, and mainly rocky reefs in Cape Sardão and adjacent areas. The overall fish assemblage of PNSACV is highly diverse, encompassing 149 fish species, some of which of high value for regional fisheries such as breams *Diplodus sargus* (Linnaeus, 1758), *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), conger eel *Conger conger* (Linnaeus, 1758), sole *Solea solea* (Linnaeus, 1758) and moray eel *Muraena helena* (Linnaeus, 1758) (ICNB 2008). In fact, breams and soles, two of the most targeted fish groups in the present study, comprise about 12% and 4% of total catches (ton) by local fishermen over the past 10 years (DGRM and National Authority for Marine Resources, unpublished data). Despite relatively low percentages, the market value of these fish groups usually reaches high values, making them important for local economy (Viegas 2013). Also, phycid hakes and triggerfish each represented about 6% of total catches in the region (DGR and National Authority for Marine Resources, unpublished data).

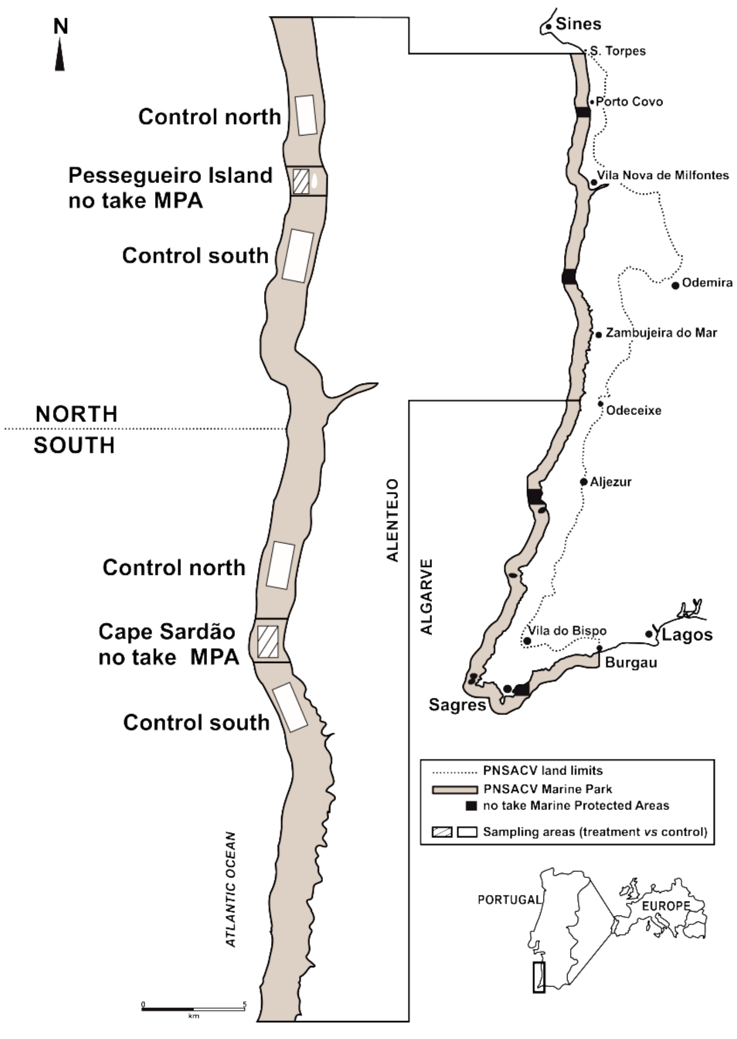

FIGURE 5.1 - Location of the study area with the monitored no take MPAs (treatment) and correspondent adjacent control areas.

5.2.2. Sampling and laboratorial procedures

Given the unfeasibility of bottom trawl use in several stretches of the study area due to the abundance of rocky bottoms, especially in the southern area, specimens from both MPAs were captured with a professional fishing vessel using trammel nets with inner mesh of 100 mm and two outer layers of 500 mm mesh, deployed near the bottom. Despite this gear mostly captures adults of benthic and demersal fish species when deployed near the bottom and not being suitable for sampling small sized and cryptic species, this is the most used fishing gear by local fishermen and the most adequate for general sampling of the local fish assemblage, in contrast with more selective gears such as fish traps and longlines. Sampling consisted of 4 surveys, August 2011 (summer), February 2012 (winter), August 2013 (summer), and December 2013 (winter) to allow characterizing the fish assemblages' diets in the year that the no take MPAs were implemented (2011/12) and after two years of protection (2013). August 2011 and February 2012 sampling seasons comprised year 1 of protection and August and December 2013 corresponded to year 3. Sampling in different seasons allowed detecting probable intra-annual differences related to natural variations. In order to compare the impact of the creation of each no take MPA on its fish assemblages' trophic ecology, sampling was conducted inside each protected area (treatment) and in adjacent control areas north and south of each. This way, the study area was divided into two sub-areas: north, encompassing Pessegueiro Island no take MPA and adjacent control areas, and south, composed of Cape Sardão no take MPA and adjacent control areas (FIGURE 5.1). This design allowed comparing trophic ecology of fish from inside and outside the no take MPA starting at an early stage after implementation, this way assessing if probable differences were already present or only appeared over time. Furthermore, by evaluating each no take MPA and their correspondent adjacent control areas separately, the response of each local fish assemblages' trophic ecology to the protective measures allowed to assess if protection effects were similar in both areas. Additionally, sampling was performed in rocky and sandy bottoms to assess if hypothetical alterations in trophic ecology varied according to habitat.

In each sampling season, two sets of trammel nets 200 m long and 2 m high were deployed between 10 m and 20 m depth inside each no take MPA and adjacent control areas, one over sandy bottom and other over rocky reef. Each set was deployed as two 100 m subsets and used as replicate. Trammel nets were left fishing overnight for a maximum period of 12 hours. This resulted in a total of 5 days of survey in each sampling season.

After capture, fish specimens were frozen for posterior analysis. In the laboratory, sampled specimens were defrosted and identified according to Whitehead et al. (1984/1986). The digestive tract from each specimen was removed and preserved in a separate labeled plastic jar with 70% alcohol solution for subsequent content analysis. Stomachs were then separated from the rest of the digestive tract and opened for content removal. For species without separated stomach, the contents of the entire digestive tract were considered for analysis. Food items were identified to the lowest taxonomic level possible, under 8x magnification, and according to Whitehead et al. (1984/86), Falciai and Minervini (1995) and Hayward and Ryland (1995).

5.2.3. Data analysis

Digestive tracts from all captured specimens were analysed, except those from high mobility planktonivore species [*Boops boops* (Linnaeus, 1758), *Sardina pilchardus* (Walbaum, 1792), *Trachurus trachurus* (Linnaeus, 1758)] since they probably fed in other areas in addition to those of capture. To simplify diet comparison amongst assemblages, food items were grouped in broader sets, resulting in 19 food categories (TABLE 5.1). Whenever items were too damaged for further identification or too low in number, they were grouped within categories with similar taxonomical and ecological features. Such were the cases of the categories of Small Crustaceans, which includes amphipods, cumaceans and isopods but excludes mysids due to their distinct bio-ecology, and Crabs, which includes anomurans and brachyurians.

In order to obtain a general overview of the feeding activity of the entire fish assemblage of the Alentejo coast, Vacuity Index was calculated as I_v = (empty digestive tracts / total number of digestive tracts)*100 as an indicator of the number of specimens feeding at the time of capture (Assis 1992). To globally characterize fish assemblage diets regarding the type and amount of ingested preys, Numeric Frequency of each item was calculated for every species considered for analysis as %F*i* = (number of prey *i* / total number of prey)*100 (Hyslop 1980). For subsequent analysis, namely diet comparison between fish assemblages, Frequency of Occurrence (Hyslop 1980, Tirasin and Jørgensen 1999) of each food item was determined for every species considered for analysis as: %O*i* = (number of stomachs with the item *i* / total number of stomachs with food)*100. According to the same authors, this parameter is the most adequate for analysis regarding fish species with distinct trophic ecology since it only accounts for the type of food item and not the ingested amount of each item. This way, differences in diets would be due to its composition instead of the quantity of each item. Despite low the %O*i* values obtained, trammel nets sampling provided similar results to those obtained with bottom trawl in some areas of the study area, as reported by Silva (2015a), suggesting that this sampling method was adequate and provided consistent results.

TABLE 5.1 – Considered categories of the food items identified in the digestive contents of the sampled species

Food item category	**Taxonomic groups included**
Algae	All types of Algae
Sponges	Phyllum Porifera
Anthozoans	Cnidarians from Class Anthozoa
Nematods	Phyllum Nematoda
Polychaetes	Annelids from Class Polychaeta
Barnacles	Crustaceans from Infraclass Cirripedia
Mysids	Crustaceans from Order Mysidacea
Small crustaceans	Crustacens from Orders Isopoda, Amphipoda and Cumeacea
Shrimps	Crustaceans from Infraorder Natantia
Crabs	Crustaceans from Infraorder Anomura and Order Brachyura
Other crustaceans	Unidentified Crustaceans
Chitons	Molluscs from Class Polyplacophora
Gastropods	Molluscs from Class Gastropoda
Bivalves	Molluscs from Class Bivalvia
Cephalopods	Molluscs from Class Cephalopoda
Other molluscs	Unidentified Molluscs
Britle stars	Echinoderms from Order Ophiurida
Sea urchins	Echinoderms from Class Echinoidea
Bony fish	Superclass Osteichthyes

Diet overlap was calculated, as Horn index (R), according to the formula (Krebs 1989):

$$R = \frac{\sum(p_{ij}+p_{ik})\ln(p_{ij}+p_{ik}) - \sum p_{ij} ln p_{ij} - \sum p_{ik} ln p_{ik}}{2ln2}$$

where p_{ij} and p_{ik} represent the proportion of each prey group [(standardized frequency of occurrence as defined by Gunn and Milward (1985)] for species *j* and *k*, respectively. This index ranges between 0 and 1 and significant dietary overlap is considered to occur for values higher than 0.6 (Wallace and Ramsey 1983). This index can be used to hypothesize the presence and degree of interspecific competition for food resources in order to assess the influence of changes in this parameter on niche breadth variations.

Niche breadth was calculated to assess the degree of diet specialization of each species. This parameter was calculated according to Cardona (1991) as:

$$B' = \frac{\sum_{i=1}^{n} \%O_i - \delta}{100 - R}$$

where, $\%O_i$ is the frequency of occurrence of item *i*, δ is the standard deviation of %O and R is the number of food items ingested by the total analysed specimens. This method is the most appropriate when using frequency of occurrence data and does not require information about prey availability in the surrounding environment (Cardona 1991).

To assess if local fish assemblages trophic ecology differed between protected and non protected areas, and with time after MPA designation as well as between localities, seasons and habitats, %O and B' were compared (TABLE 2) by means of multivariate PERMANOVA, regarding %O, and univariate PERMANOVA, regarding B', considering 5 fixed factors: Locality (Pessegueiro Island-north and Cape Sardão-south), Year (1 and 3), Protection nested in Locality (with and without), season nested in year (summer and winter) and Habitat nested in Protection (sand and rock) (Anderson et al. 2008). Given the fact that several species were captured in reduced numbers or in only a few sampling seasons and/or localities, this analysis was performed using only the 10 most abundant fish species (TABLE 5.2). This way, probable variations caused by the diets of sporadic specimens were removed.

Principal Coordinate Analysis (PCO) considering $\%O_i$ was performed to illustrate results obtained through PERMANOVA and the items that contributed the most for those differences were assessed by means of SIMPER analysis (Anderson et al. 2008).

PERMANOVA, PCO and SIMPER analysis were performed using PRIMER 6 & PERMANOVA + statistical software package (Anderson et al. 2008). PERMDISP analysis was applied to evaluate data dispersion and validate PERMANOVA results (Anderson et al. 2008).

TABLE 5.2 – Most abundant fish species captured along the study area and correspondent amount

Species	N
Phycis phycis (Linnaeus, 1766)	30
Mullus surmuletus Linnaeus, 1758	28
Diplodus sargus (Linnaeus, 1758)	143
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817	36
Labrus bergylta Ascanius, 1767	50
Scorpaena porcus Linnaeus, 1758	39
Chelidonichthys obscurus (Walbaum, 1792)	36
Pegusa lascaris (Risso, 1810)	151
Solea senegalensis Kaup, 1858	54
Balistes capriscus Gmelin, 1789	51

5.3. Results

A total of 710 digestive tracts from 42 fish species were analysed, 134 of which were empty (I_v = 18.9%), resulting in the identification of 859 food items grouped in 19 categories (TABLE 5.3). Crustaceans in general and crabs in particular, mostly *Polybius henslowii* Leach, 1820, played an important role on the diets of the fish from the Alentejo coast of the PNSACV Marine Park. Overall, those decapods were the most ingested prey (N=142), followed by bivalves (N=106) and shrimps (N=98) (TABLE 5.3). Considering the 10 most abundant species in the study area, crabs remained as the most frequently ingested item (%O = 6.5%). However, bivalves became an important item, being the third most ingested prey (%O = 4.6%) after small crustaceans (%O = 5.1%) (TABLE 5.4). In fact, these mollusks played an important role in the diets of the fish from inside Pessegueiro Island no take MPA, where they were the most frequently ingested item, especially in year 3 of protection (TABLE 5.4). Overall, in the northern study area, the importance of bivalves concerning %O was only surpassed by crabs (TABLE 5.4). In the southern counterpart, crabs remained the most important item in terms of %O. Also noteworthy, the relevance of bony fish in the diets of the fish from outside Pessegueiro Island no take MPA, namely in year 3 of protection where it was the second most frequently ingested prey (%O = 6.7%), after crabs (%O = 8.3%) (TABLE 5.4).

TABLE 5.3 – Numeric Frequency (%F) of each food item identified in the digestive contents of all the sampled specimens according to locality, protection and year

Food items	North							South							GRAND TOTAL
	MPA		Controls		total		TOTAL	MPA		Controls		total		TOTAL	
	Y1	Y3	Y1	Y3	Y1	Y3		Y1	Y3	Y1	Y3	Y1	Y3		
Algae	10.00	9.52	8.73	5.24	9.04	7.48	7.94	7.69	13.33	0.00	6.86	2.86	10.36	8.56	8.92
Sponges	0.00	0.48	0.00	0.00	0.00	0.25	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
Anthozoans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00	0.00	0.00	0.45	0.34	0.13
Nematodes	2.50	0.48	0.00	0.00	0.60	0.25	0.35	0.00	0.00	2.27	0.00	1.43	0.00	0.34	0.38
Polychaetes	7.50	9.05	6.35	5.24	6.63	7.23	7.05	11.54	5.00	18.18	7.84	15.71	6.31	8.56	8.28
Barnacles	0.00	3.33	0.00	0.00	0.00	1.75	1.23	0.00	0.83	0.00	0.00	0.00	0.45	0.34	1.02
Mysids	5.00	0.00	0.79	2.09	1.81	1.00	1.23	0.00	1.67	0.00	0.00	0.00	0.90	0.68	1.15
Small crustaceans	17.50	10.00	11.90	5.24	13.25	7.73	9.35	11.54	10.00	22.73	14.71	18.57	12.16	13.70	11.85
Shrimps	17.50	7.14	12.70	14.14	13.86	10.47	11.46	7.69	14.17	0.00	13.73	2.86	13.96	11.30	12.48
Crabs	15.00	7.14	18.25	17.80	17.47	12.22	13.76	26.92	14.17	22.73	29.41	24.29	21.17	21.92	18.09
Other crust.	17.50	6.67	7.14	7.85	9.64	7.23	7.94	19.23	10.00	11.36	13.73	14.29	11.71	12.33	10.32
Chitons	0.00	0.00	0.00	0.52	0.00	0.25	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
Gastropods	2.50	9.05	11.90	7.33	9.64	8.23	8.64	0.00	4.17	4.55	2.94	2.86	3.60	3.42	7.52
Bivalves	0.00	29.05	5.56	11.52	4.22	20.70	15.87	3.85	10.00	6.82	0.00	5.71	5.41	5.48	13.50
Cephalopods	0.00	0.48	0.00	2.09	0.00	1.25	0.88	0.00	2.50	0.00	2.94	0.00	2.70	2.05	1.40
Other moll.	0.00	0.48	1.59	0.00	1.20	0.25	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38
Britle stars	0.00	0.95	3.17	2.09	2.41	1.50	1.76	0.00	2.50	4.55	0.98	2.86	1.80	2.05	2.04
Sea urchins	0.00	2.86	2.38	4.19	1.81	3.49	3.00	0.00	0.83	0.00	0.00	0.00	0.45	0.34	2.29
Bony fish	5.00	3.33	9.52	14.66	8.43	8.73	8.64	11.54	10.00	6.82	6.86	8.57	8.56	8.56	9.43
Empty tracts	6	30	31	34	36	65	101	12	7	8	6	19	14	33	134

TABLE 5.4 – Frequency of Occurrence (%O) of the food items identified in the digestive contents of the 10 most abundant fish species according to locality, protection and year

Food items	North							South							GRAND TOTAL
	MPA		Control		total		TOTAL	MPA		Control		total		TOTAL	
	Y1	Y3	Y1	Y3	Y1	Y3		Y1	Y3	Y1	Y3	Y1	Y3		
Algae	2.5	7.2	3.8	1.6	3.2	4.4	3.8	2.5	10.2	0.0	0.3	1.3	5.3	3.3	3.5
Sponges	0.0	0.4	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Anthozoans	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.2	0.1	0.1
Nematodes	0.6	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Polychaetes	1.0	10.3	2.2	2.6	1.6	6.5	4.0	1.3	4.4	3.6	1.2	2.4	2.8	2.6	3.3
Barnacles	0.0	3.0	0.0	0.0	0.0	1.5	0.8	0.0	0.6	0.0	0.0	0.0	0.3	0.2	0.5
Mysids	1.3	0.0	0.2	0.6	0.7	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Small crust.	4.6	11.4	5.7	2.4	5.2	6.9	6.0	1.3	6.9	3.7	4.5	2.5	5.7	4.1	5.1
Shrimps	1.3	9.1	5.0	6.5	3.1	7.8	5.4	0.0	11.3	0.0	2.8	0.0	7.0	3.5	4.5
Crabs	3.1	8.1	9.2	8.3	6.1	8.2	7.2	2.5	10.6	3.9	6.0	3.2	8.3	5.8	6.5
Other crust	2.3	7.8	3.0	1.6	2.7	4.7	3.7	1.3	5.6	2.1	3.3	1.7	4.5	3.1	3.4
Chitons	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gastropods	0.6	10.3	3.9	4.6	2.3	7.4	4.9	0.0	4.4	0.8	0.6	0.4	2.5	1.5	3.2
Bivalves	0.0	20.5	2.0	4.9	1.0	12.7	6.9	1.3	6.9	1.2	0.0	1.2	3.4	2.3	4.6
Cephalopods	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.0	1.0	0.0	0.9	0.0	1.0	0.5	0.3
Other moll.	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Britle stars	0.0	2.5	1.0	0.3	0.5	1.4	0.9	0.0	1.9	1.2	0.2	0.6	1.0	0.8	0.9
Sea urchins	0.0	3.2	1.3	1.8	0.6	2.5	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8
Bony fish	1.3	3.9	3.0	6.7	2.1	5.3	3.7	1.3	5.4	0.6	0.6	0.9	3.0	2.0	2.8

Diet overlap was reduced between years and between no take MPAs and adjacent control areas (TABLES 5.5 and 5.6). In fact, in the first year of protection the diet of the different species from both study areas did not overlap among them (TABLE 5.5). Nevertheless, during year 3 of protection, diet of *L. bergylta* overlapped with *D. sargus* and *P. lascaris* diet overlapped with that of *M. surmuletus* (TABLE 5.5). Regarding protection level, diet of *D. sargus* from Pessegueiro Island no take MPA overlapped with those of *M. surmuletus* and *L. bergylta* while the diet of *P. lascaris* of the same locality overlapped with those of *D. sargus* and *M. surmuletus* (TABLE 5.6). In the south study area, diets of species from inside the no take MPA and from the correspondent adjacent control areas did not overlap between them (TABLE 5.6).

PERMANOVA analysis comparing the diets according to %O only revealed significant differences between protected and non protected areas (factor Protection – PERMANOVA: d.f.=2, pseudo-F=1.719, p=0.044), with crabs confirming their importance as preferential prey by being the most frequently consumed item in both protected and non protected areas but with higher incidence outside MPAs (factor Protection – SIMPER: contribution of 14.73%), followed by small crustaceans (factor Protection – SIMPER: contribution of 11.80%), shrimps (factor Protection – SIMPER: contribution of 10.38%) and other crustaceans (factor Protection – SIMPER: contribution of 10.35%). PCO analysis illustrates these differences and highlight the contribution of these food categories for the differences obtained (FIGURE 5.2).

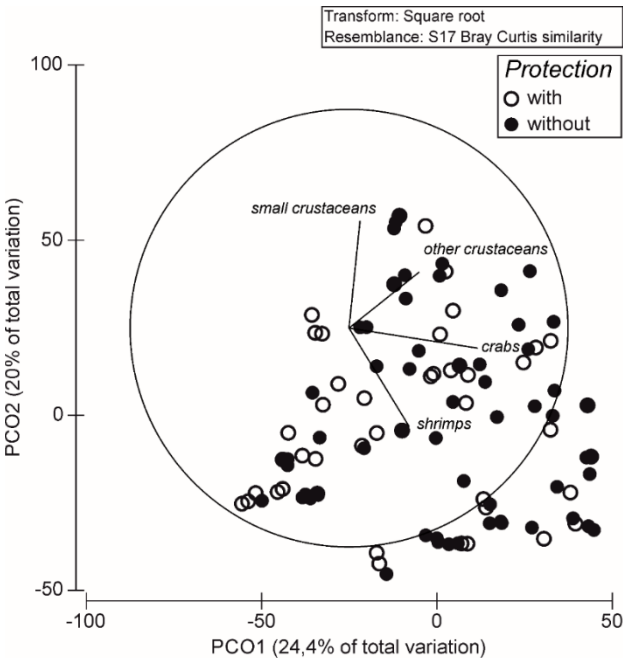

FIGURE 5.2 – Principal Coordinate Analysis (PCO) regarding Frequency of Occurrence (%O) for the 10 most abundant fish species in the study area according to protection. Vectors indicate groups that most contributed for the obtained differences.

The frequency each food category or item was consumed by the fish from the study area was not significantly influenced by the year of protection, despite a tendency for an increase in food consumption with time, both in abundance and frequency of occurrence in (TABLES 3 and 4). The same way, intra-annual variations and differences between localities and habitats on the diets of the fish assemblages of the study area were not visible (TABLES 3 and 4).

Niche amplitude was assessed by means of B' and it was possible to observe marked variations with locality and time (FIGURE 5.3). In fact, PERMANOVA analysis showed significant differences regarding niche breadth according to Locality and Year of protection. Assemblages from the northern area presented significantly larger niche breadth (d.f.=1, pseudo-F=5.167, p=0.012), indicating a more generalist strategy when compared with their southern counterpart (FIGURE 3). Additionally, niche breadth significantly increased from year 1 to year 3 of protection (d.f.=1, pseudo-F=4.377, p=0.026), being more evident inside both no take MPAs despite without statistical significance (FIGURE 3), indicating that these assemblages became more generalist feeders with time. PERMDISP analysis showed no significant differences for all factors, thus validating both PERMANOVAs results.

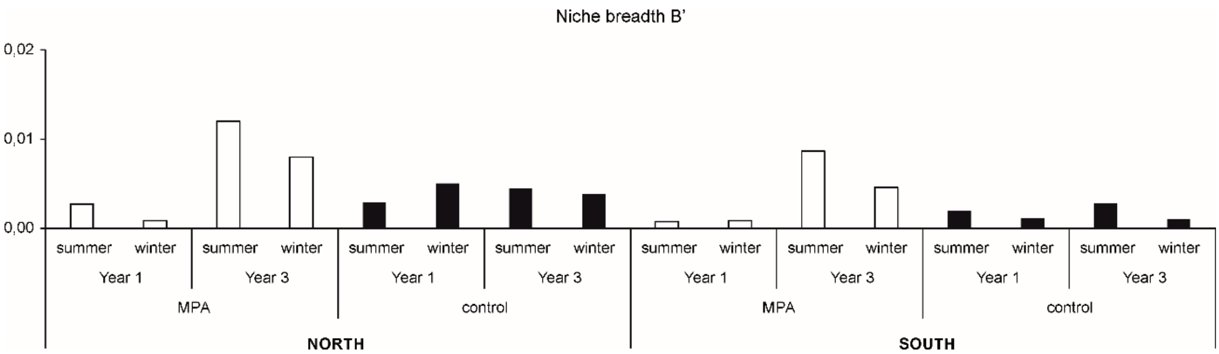

FIGURE 4.3 – Niche breadth (B') for the 10 most abundant species in the study area according to season, year, protection and locality.

5.4. Discussion

By prohibiting fishing activities, the establishment of no take MPAs can lead to changes in the structure and composition of their fish assemblages (Halpern 2003, Claudet et al. 2008). In several cases, those changes can be visible after only 4 to 6 years, as described by Colléter et al. (2012) for a Senegalese MPA, by Seytre et al. (2013) for Cap Roux, in the Mediterranean, or by Sousa (2011) for the Arrábida Marine Park, in Portugal. In the particular case of the no take MPAs from Alentejo coast of PNSACV Marine Park, recent studies showed changes in their fish assemblage when compared with adjacent unprotected areas after only 3 years of protection (Silva 2015a). The number of specimens increased inside both no take MPAs and, in the specific case of Pessegueiro Island no take MPA, an increase in average size of some commercial fish species after MPA designation was observed (Silva 2015a). Increase in fish abundance may cause competition for food resources (Svanbäk and Persson 2004) and the scarcity of studies focusing on how changes in the assemblage structure affect its trophic relations are practically inexistent in the literature (Murawski et al. 2005, White et al. 2010), making the present work on this theme valuable, even more considering that those changes might have been induced by MPA implementation.

Considering the frequency of occurrence, crabs were also the most frequently preyed group among the most abundant fish species. Bivalves appear as the second most frequent prey, also mostly inside Pessegueiro Island no take MPA and during the 3rd year of protection. This increase in bivalve intake with time may be a reflection of the rise of the number of specimens inside this protected area from year 1 to year 3 of protection (Silva 2015a). Bony fish also became an important prey with time, especially outside Pessegueiro Island no take MPA. The increase in abundance of larger predators in the northern area of the PNSACV Marine Park, especially rays, which are targeted by specific protective legislation (Silva 2015a) may have resulted in higher consumption of small bony fish.

Despite the global dominance of crabs as the most ingested item, both in quantity and frequency of ingestion, the diets of the most abundant fish were significantly different between protected and non protected areas. However, these differences were evident since the beginning of the present study, indicating that specimens from inside the protected areas naturally displayed different feeding habits from those from adjacent control areas. This occurrence is probably a reflection of the naturally distinct prey availability in each area, regardless protection measures in place. However, given the changes in fish assemblage structure described for this area by Silva (2015a), these differences between protected and no-protected areas can become more evident with time. The increase in number of specimens is usually the first visible effect after MPA designation (e.g. Fenberg et al., 2012, Lester et al., 2009) and the first species to benefit from protection are especially those with higher commercial importance (Claudet et al. 2008, Silva 2015a). Given that the most targeted species often are also predatory fish (Myers and Worm 2003), it is expected that their abundance increase will negatively affect smaller fish. This way, protective measures in place may carry negative impacts to smaller fish species that are becoming more evident with time but may result in a more balanced ecosystem at long term (e.g. Soler et al. 2015).

TABLE 5.5 – Diet overlap (Horn index – R) for the 10 most abundant fish species according to locality and year. Dietary overlap is considered to occur for values higher than 0.6 (highlighted in grey). *P.phy – Phycis phycis*; *M.su – Mullus surmuletus*; *D.sar – Diplodus sargus*; *D. vul – Diplodus vulgaris*; *L.ber – Labrus bergylta*; *S.por – Scorpaena porcus*; *C.obs – Chelodonichthys obscurus*; *P.las – Pegusa lascaris*; *S.sen – Solea senegalensis*; *B.cap – Balistes capriscus*

		Species	***P.phy***	***M.sur***	***D.sar***	***D.vul***	***L.ber***	***S.por***	***C.obs***	***P.las***	***S.sen***	***B.cap***
YEAR 1	NORTH	*P.phy*	-									
		M.sur	0.17	-								
		D.sar	0.11	0.33	-							
		D.vul	0.09	0.20	0.24	-						
		L.ber	0.17	0.20	0.19	0.17	-					
		S.por	0.18	0.30	0.17	0.11	0.17	-				
		C.obs	0.06	0.31	0.14	0.11	0.25	0.16	-			
		P.las	0.05	0.42	0.26	0.18	0.16	0.09	0.23	-		
		S.sen	0.04	0.00	0.00	0.03	0.03	0.00	0.00	0.00	-	
		B.cap	0.00	0.00	0.00	0.03	0.03	0.00	0.04	0.00	0.00	-
	SOUTH	*P.phy*	-									
		M.sur	0.00	-								
		D.sar	0.00	0.00	-							
		D.vul	0.00	0.00	0.00	-						
		L.ber	0.00	0.00	0.06	0.00	-					
		S.por	0.04	0.00	0.00	0.00	0.00	-				
		C.obs	0.08	0.00	0.05	0.00	0.07	0.08	-			
		P.las	0.13	0.00	0.07	0.00	0.06	0.06	0.28	-		
		S.sen	0.06	0.00	0.05	0.00	0.00	0.00	0.07	0.20	-	
		B.cap	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	-
YEAR 3	NORTH	*P.phy*	-									
		M.sur	0.57	-								
		D.sar	0.34	0.55	-							
		D.vul	0.18	0.22	0.30	-						
		L.ber	0.38	0.38	0.67	0.30	-					
		S.por	0.41	0.51	0.17	0.00	0.10	-				
		C.obs	0.16	0.16	0.03	0.00	0.00	0.14	-			
		P.las	0.29	0.62	0.42	0.24	0.34	0.28	0.07	-		
		S.sen	0.02	0.09	0.08	0.04	0.05	0.02	0.00	0.06	-	
		B.cap	0.27	0.18	0.31	0.06	0.21	0.13	0.00	0.09	0.02	-
	SOUTH	*P.phy*	0.23	-								
		M.sur	0.12	0.24	-							
		D.sar	0.13	0.31	0.16	-						
		D.vul	0.00	0.04	0.05	0.09	-					
		L.ber	0.42	0.41	0.14	0.27	0.04	-				
		S.por	0.27	0.39	0.08	0.24	0.06	0.44	-			
		C.obs	0.11	0.44	0.20	0.33	0.10	0.33	0.32	-		
		P.las	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	
		S.sen	0.03	0.09	0.18	0.10	0.04	0.06	0.00	0.11	0.00	
		B.cap										

At the same time, abundance increase may lead to higher fish density inside the no take MPAs, where fish are not subject to fishing activities. This way, competition for food resources may occur as a result from high fish density (Svanbäk and Persson 2004), causing variations in the niche breadth of the populations that compose its fish assemblages. In fact, niche breadth was significantly higher in the northern study area, where fish abundance was also higher and increasing with time (Silva 2015a). These occurrences could suggest that the previously observed increase in fish abundance may have led to a potential increased competition for food in top levels which in turn resulted in a significant expansion of the niche breadth.

TABLE 5.6 - Diet overlap (Horn index – R) for the 10 most abundant fish species according to locality and protection. Dietary overlap is considered to occur for values higher than 0.6 (highlighted in grey). *P.phy – Phycis phycis*; *M.su – Mullus surmuletus*; *D.sar – Diplodus sargus*; *D. vul – Diplodus vulgaris*; *L.ber – Labrus bergylta*; *S.por – Scorpaena porcus*; *C.obs – Chelodonichthys obscurus*; *P.las – Pegusa lascaris*; *S.sen – Solea senegalensis*; *B.cap – Balistes capriscus*

		Species	***P.phy***	***M.sur***	***D.sar***	***D.vul***	***L.ber***	***S.por***	***C.obs***	***P.las***	***S.sen***	***B.cap***
NO-TAKE MPA	*NORTH*	*P.phy*	-									
		M.sur	0.47	-								
		D.sar	0.30	0.87	-							
		D.vul	0.05	0.05	0.03	-						
		L.ber	0.22	0.36	0.77	0.06	-					
		S.por	0.00	0.00	0.00	0.00	0.00	-				
		C.obs	0.06	0.25	0.14	0.00	0.03	0.00	-			
		P.las	0.20	0.70	0.72	0.06	0.45	0.00	0.16	-		
		S.sen	0.00	0.13	0.06	0.00	0.00	0.00	0.00	0.06	-	
		B.cap	0.00	0.15	0.29	0.00	0.16	0.00	0.00	0.09	0.00	-
	SOUTH	*P.phy*	-									
		M.sur	0.38	-								
		D.sar	0.15	0.31	-							
		D.vul	0.25	0.33	0.29	-						
		L.ber	0.00	0.09	0.09	0.08	-					
		S.por	0.41	0.22	0.06	0.15	0.00	-				
		C.obs	0.09	0.18	0.00	0.06	0.09	0.09	-			
		P.las	0.22	0.38	0.33	0.25	0.15	0.06	0.12	-		
		S.sen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	
		B.cap	0.10	0.17	0.36	0.14	0.07	0.05	0.00	0.17	0.00	-
CONTROL	*NORTH*	*P.phy*	-									
		M.sur	0.31	-								
		D.sar	0.23	0.25	-							
		D.vul	0.20	0.19	0.33	-						
		L.ber	0.33	0.21	0.28	0.26	-					
		S.por	0.40	0.44	0.19	0.17	0.25	-				
		C.obs	0.25	0.26	0.08	0.10	0.19	0.27	-			
		P.las	0.16	0.35	0.29	0.24	0.23	0.32	0.15	-		
		S.sen	0.05	0.02	0.10	0.05	0.06	0.01	0.00	0.02	-	
		B.cap	0.18	0.05	0.07	0.05	0.13	0.11	0.07	0.04	0.03	-
	SOUTH	*P.phy*	-									
		M.sur	0.00	-								
		D.sar	0.00	0.00	-							
		D.vul	0.00	0.00	0.00	-						
		L.ber	0.00	0.00	0.00	0.00	-					
		S.por	0.06	0.06	0.00	0.06	0.00	-				
		C.obs	0.04	0.12	0.00	0.05	0.03	0.33	-			
		P.las	0.03	0.12	0.00	0.11	0.03	0.28	0.38	-		
		S.sen	0.00	0.08	0.00	0.05	0.00	0.09	0.06	0.15	-	
		B.cap	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	-

On the other hand, the increase in predation could reduce competition in lower trophic levels, increasing their diversity (Paine 1980). However, results showed reduced diet overlap between the studied fish species. During year 3 of protection, in the northern study area, the diet of *L. bergylta* overlapped with the diet of *D. sargus* and that of *P. lascaris* overlapped with the one of *M. surmuletus*, probably as a result of the increase in fish abundance.

However, despite this occurrence, diet overlap remained reduced, confined only to 2 pairs of species, and eventual competition for food was probably more intense between individuals of the same species. Nevertheless, the combination of the increase in fish abundance and the reduced diet overlap may have been responsible for the observed expansion in niche breadth from year 1 to year 3 in the northern area, since this parameter may increase due to high intraspecific competition or reduced interspecific competition (e.g. Robinson and Wilson 1994, Robinson and Schluter 2000).

Habitat did not play a relevant role in structuring the diets of the studied assemblages since both MPAs are relatively small and with both rocky and sandy bottom, especially in the northern area. Therefore, sampled fish could easily move from one habitat to another according to their feeding needs and the presence of items from sandy bottoms, such as bivalves, or from rocky reefs, such as small fish, ended up being equally present in their diets .This was particularly evident for the Pessegueiro Island no take MPA, where bivalves were frequently preyed upon and common on the diets of the fish from this area, probably due the higher presence of sandy bottom in this locality. However, the relevance of this item was not sufficient to significantly discriminate diets according to habitat. Differences in the diets of the assemblages from different habitats, namely between rocky reefs and sandy bottoms, could only be visible if the assemblage of cryptic fish was sampled, since these specimens present low mobility and are confined to very small areas around rocky substrate (Henriques et al. 2013, Silva 2015b), which was not the case.

The influence of intra-annual variations in prey availability was not visible on the diets of the fish assemblages of the study area. However, temperature can influence prey availability (Adams et al. 1982) and several studies document seasonal variations in fish diets (e.g. Vinagre et al. 2005, Castro et al. 2013). In the particular case of the sampled fish assemblages, the absence of significant seasonal variations in the diets may once again be related with the captured and analysed species. In fact, species targeted by trammel nets are mostly demersal fish that perform movements large enough to feed on different habitats but that usually do not perform large enough seasonal displacements to influence the type of preys they ingest. In this specific case, the comparison of the diets comprised the ten most abundant fish species captured in the Alentejo coast of the PNSACV and most of them are known to display small displacements and reduced home ranges (García-Chartom and Pérez-Ruzafa 2001, Simmons and Szedlmeyer 2012, Abecasis et al. 2015, Belo et al. 2016), resulting in a diet with temporal homogeneity with reduced seasonal variation, or at least not enough to be detected at community level.

Considering all of the above, it is possible to conclude that the main effects of no take MPA implementation in the Alentejo on the trophic ecology of its fish assemblages, were directly visible on the niche breadth of the most abundant species. With the previously observed increment in fish abundance in the area (Silva 2015a) and eventual, but not confirmed, consequent increase in intraspecific competition for food resources, specimens needed to broaden their diets, becoming more generalist (Svanbäk and Persson 2004). On the other hand, the natural differences observed in the diets between the assemblages from no take MPAs and correspondent adjacent control areas may be emphasized with time as consequence of protection implementation. As stated before, the increase in larger specimens' abundance inside protected areas may generate predatory pressure on several animals, as it was already visible with the increase of the presence of bivalves and small fish in the diets from year 1 to year 3 of protection. This way, MPA designation did not directly impact the diet composition of the sampled fish assemblages but may contribute to reinforce the already naturally existent differences.

The first visible effects of MPA designation on fish assemblages are usually the increase in fish abundance and diversity, with effects on trophic ecology taking longer to occur (Edgar and Barrett 1999, Colléter et al. 2012, Seytre et al. 2013). However, in both studied MPAs some effects were already visible only 3 years after implementation. Additionally, already detected changes in the structure of these fish assemblages are likely to cause further impacts on its diets that can be problematic for small fish species as they started to appear more frequently in the diets in year 3 of protection, and the tendency could be to become more predated as larger predatory fish continue to increase. However, at some point in time a more balanced ecosystem may be attained, since protective measures are known to benefit all trophic levels with time (e.g. Soler et al. 2015). This way, it is important to continue to monitor changes in fish assemblage structure and in fish diets to assess the evolution of its impacts. It is relevant to investigate if the initial predatory pressure on small fish and other items continues in time and if it causes impacts that can be negative to the entire assemblage at long term. Overall, small MPAs seem in fact to initially benefit fish assemblages, especially concerning commercially targeted fish species, but their small size may lead to rapid effects on the trophic chain by rapidly increasing the consumption of small organisms, both inside and in adjacent non protected areas.

Altogether, the present work provides important information regarding the effect of changes in the fish assemblage caused by MPA designation on the trophic ecology of its assemblages, contributing to a global understanding of the impacts of this tool for ocean conservation and biodiversity preservation.

It is therefore important, when designing and implementing a no take MPA, to take into account not only short term impacts, usually positive and rapidly observable for commercially important species, but also mid and long term effects, namely on the assemblage of potential preys, that take into account fish assemblages life cycles and habitat requirements. In this context, MPA size plays an important role and continuous monitoring is essential to adopt appropriate mitigating measures in time.

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CHAPTER 6

DID NO-TAKE MARINE PROTECTED AREA DESIGNATION AFFECT FISH LANDINGS ON THE SOUTHWESTERN COAST OF PORTUGAL?

IN PREPARATION FOR PUBLICATION

Abstract

Regulations inside MPAs may include partial or total prohibition of fishing and immediately after implementation, marine reserves can reduce catches because fishers lose fishing grounds, consequently negatively affect local fishers at short term. This way, the present work aimed to assess the impact of the designation of no take MPAs in the PNSACV Marine Park on local commercial fisheries immediately after implementation. A global decrease in fish landings was observed over the years within the Sines maritime jurisdiction as well in adjacent areas, with a drop in gross tonnage between the years before and after protective measures were in place. The impact of these measures on small scale fisheries, intensifying an already global decreasing tendency in the area, was evident not only on fishing communities but also on targeted animal groups. This work contributed to understand how local fisheries were affected by no take MPA designation in the Alentejo coast of the PNSACV Marine Park and, globally, how the implementation of protective measures including closed areas can impact fish landings at short term. At the same time, it showed how small scale fisheries can recover rapidly. In fact, the abundance of fishing grounds allowed for fleet relocation and consequent increase in landings over time. However, the direct contributions of these no take MPAs for fisheries, namely as a result of spillover is yet to be shown.

Keywords

Marine reserve, Fish landings; Small scale fisheries; MPA Impact PNSACV Marine Park

6.1. Introduction

The application of single-species management tools to restore fisheries has been proved quite ineffective when applied to multispecies fisheries. This scenario is particularly evident concerning artisanal fisheries, which usually present high variation related to the use of different gears and multiple target species (Vinther et al. 2004, Tzanatos et al. 2005). In this context, Marine Protected Areas (MPAs) have been widely implemented to manage fishing stocks and promote biodiversity (Allison et al. 1998, Russ 2002, Botsford et al. 2003, Chateau and Wantiez 2009) becoming increasingly popular within the context of an ecosystem approach to fisheries management (Gell and Roberts 2003). At the same time, MPAs have the potential to sustain the fisheries in areas adjacent to the MPA while protecting species and habitats inside (Vandeperre et al. 2011).

Regulations inside MPAs may include partial or total prohibition of fishing, potentially leading to an increase in species richness, fish size and density inside protected areas (Fenberg et al. 2012, Lester et al. 2009) as well as larger amounts of juvenile fish and smaller sized fish species (Russ 2002, Gell and Roberts 2003). In some cases, the increment of fish species and density inside MPAs can be visible after only 2-3 years upon implementation (Halpern 2003). On the other hand, improvements in fisheries harvests and profits in adjacent areas are usually only visible in the mid to long-term (e.g. Russ et al. 2003, Goñi et al. 2010), and helping to recover overfished populations (Lester et al. 2009).

However, immediately after implementation, marine reserves usually reduce catches because fishers lose fishing grounds (Smith et al. 2010). At this stage fisheries will not benefit from the spillover of adult fish and increased larval supply, for at least several years after implementation (Brown et al. 2014). Additionally, the implementation of an MPA will lead to a redistribution of fishing effort through activity displacement from the closed area to the surroundings where fishing is still permitted (Halpern et al. 2004). Altogether, these impacts may negatively affect local fishers in the short term. After the initial decrease in harvests, however, catch rates outside no take areas seem to develop over long time periods and continue at least until 30 years of protection (Vandeperre et al. 2011).

Fishing activities, both professional and recreational, are very common in the entire coast of Portugal, playing a relevant role on its local economy, and the southwestern coast is no exception (Reis 2011). Several studies focusing on global fish landings in the Portuguese coast are available and indicate a decreasing trend in terms of gross tonnage (Baeta et al. 2009, Ferreira et al. 2013, Gamito et al. 2013, Teixeira et al. 2014). When considering Landings Per Unit Effort (LPUE), global purse seine landings increased while multi-gear landings, usually associated with artisanal fisheries, decreased over the past decade (Ferreira et al. 2013, Teixeira et al. 2014). Also, annual mean trophic levels (T_{Lm}) of mainland landings have shown a decreasing trend, reflecting changes in the structure of marine food webs (Baeta et al. 2009).

In mainland Portugal, only four MPAs are in place and each with different protection levels: Litoral Norte Park (implemented in 1987, north coast), Berlengas Natural Reserve (implemented in 1981, central coast), Arrábida Marine Park (implemented in 1998, central coast), and the Marine Park of Sudoeste Alentejano and Costa Vicentina Natural Park (PNSACV, implemented in 1995, designated as Marine Park in 2011, south western coast). The implementation, in 2011, of four partial protection areas where fishing activities are prohibited, commonly referred to as no take MPAs, within the PNSACV Marine Park, provided the opportunity to evaluate how these measures affected local fisheries.

Studies from Litoral Norte and Berlengas MPAs have been mainly focused on intertidal organisms (e.g. Jacinto et al. 2011, Bertocci et al. 2012, Haug et al. 2015). Arrábida Marine Park fish assemblages, on the other hand, have been frequently studied in the past years (e.g. Gonçalves et al. 2003, Sousa 2011, Henriques et al. 2013) and studies have since shown that, as in several other MPAs, fish species richness and abundance increased with time in total and partial protection areas when compared with buffering and adjacent zones (Sousa 2011). More recently, information on the impact of the PNSACV Marine Park no take MPAs on local fish species and fish assemblages is becoming available and, also confirms the effectiveness of these management tools in protecting commercially important fish species (Silva 2015a, Belo et al. 2016) as well as contributing for the increment in fish abundance and larger specimens inside some of those no take MPAs (Pereira et al. 2017). Also, Fernandéz et al. (2016) showed higher biomass of some commercially important fish species inside one no take MPA in the PNSACV Marine Park.

Despite information regarding global landings along the Portuguese coast, (INE 2015) and several studies focusing on the impact of MPA implementation on its animal communities becoming increasingly available (e.g. Gonçalves et al. 2003, Jacinto et al. 2011, Bertocci et al. 2012, Henriques et al. 2013, Haug et al. 2015), the effect of no take MPA implementation on the quantity of fish captured by commercial fisheries is yet to be evaluated, with the only available approach the work by Viegas (2013), based on inquiring the PNSACV Marine Park fishers community.

Considering the above, the present work aimed to assess the impact of the designation of no take MPAs in the PNSACV Marine Park on local commercial fisheries immediately after its implementation by comparing fish landings data before and after MPA designation, inside and outside this marine park. Also, fish landings were compared among local harbours to evaluate if these impacts varied according to location. This way, it was possible to evaluate if and how these protective measures impacted local artisanal fishing communities at short term and create a basis from which the evolution of fish landings can be monitored and compared in order to assess eventual mid and long term benefits from no take MPA designation to local fisheries. Additionally, this work can also act as a call out to take into account short term impacts on local small-scale fisheries communities when planning future no take MPAs.

6.2. Methods

6.2.1. Study area

This study focused on fish landings data from the four most important fishing harbours near or inside the northern half of the PNSACV Marine Park, southwest coast of Portugal: Sines, Vila Nova de Milfontes, Zambujeira do Mar and Azenha do Mar (FIGURE 6.1). This marine park extends 2 km offshore along a coastline of ca. 120 km, crossing through Alentejo (southwestern coast) and Algarve (southwestern and south coasts). In February 2011, several no take MPAs (no take and type I partial protected areas) were implemented inside this marine park, two of which in the Alentejo coast of the park: Pessegueiro Island no take MPA in the northern Alentejo coast and Cape Sardão no take MPA in the southern Alentejo coast (FIGURE 1). Both are relatively small, with the Pessegueiro Island MPA covering an area of ca. 6 km^2 and Cape Sardão MPA ca.7 km^2. The overall fish assemblage of PNSACV is highly diverse, encompassing 149 fish species, some of which of high value for regional fisheries such as breams *Diplodus sargus* (Linnaeus, 1758) and *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), conger eel *Conger conger* (Linnaeus, 1758), sole *Solea solea* (Linnaeus, 1758) and moray eel *Muraena helena* (Linnaeus, 1758) (ICNB 2008). The city of Sines (18 298 inhabitants, INE 2015) is the largest of the four harbours considered in the present work and it is located outside the PNSACV, near its northern border but most of its multi gear operating vessels fish inside the marine park (Viegas 2013). This harbour is also the main receiver of catches by seine purse fishing vessels operating offshore Alentejo, but almost all these catches are made outside the PNSACV Marine Park limits (Viegas 2013). Inside the PNSACV Marine Park, between Pessegueiro Island and Cape Sardão no take MPAs, is located Vila Nova de Milfontes harbour, belonging to a small village with around 5000 people (INE 2012). The remaining two harbours are located south of the Cape Sardão no take MPA, in small villages belonging to the same municipality, São Teotónio, with around

5500 people in total (INE 2012). However, most of the population inhabits areas inland, with Zambujeira and Azenhas do Mar comprising ca. 1000 habitants each (INE 2012). Despite having a relatively large population (757 302), only 538 people are registered as professional fishermen in the Alentejo region (INE 2015). Additionally, only 150 fishing vessels registered in the Alentejo were operating in 2014, although vessels from other areas are allowed to fish in the area and land their catches in these harbours, with most of these mostly purse seine vessels (INE 2015). Nevertheless, the vast majority of fishermen registered in these harbours develop their activity inside PNSACV Marine Park (Viegas, 2013).

6.2.2. Data collection and analysis

For this work, data concerning gross tonnage of landed fish and LPUE (Landings per Unit Effort = gross tonnage/number of vessels registered in the region) obtained from yearly surveillances performed and published by the Portuguese National Institute for Statistics was used (Instituto Nacional de Estatística – INE 2008-2015). Data available is divided by maritime jurisdiction. The northern half of the PNSACV Marine Park belongs to Sines maritime jurisdiction. Unpublished data concerning landings from the four fishing harbours from inside this jurisdiction were provided by the National Authority for Natural Resources and Maritime Services and Security (Direção Geral dos Recursos Naturais, Segurança e Serviços Marítimos – DGRM).

In order to evaluate if no take MPA designation inside PNSACV Marine Park affected local fisheries and if and how eventual differences occurred or not throughout the entire central and southwestern coast of Portugal, global fish landings were compared between four years before (2007-2010) and four years after (2011-2014) MPA designation in the Sines maritime jurisdiction, and in Setúbal (north) and Portimão (south) maritime jurisdictions (FIGURE 1). Global landings data from Sines jurisdiction comprised the data from its smaller harbours, namely Vila Nova de Milfontes, Zambujeira do Mar and Azenha do Mar (INE 2015). Landings from Setúbal included Sesimbra and Carrasqueira, and data from Portimão also included Albufeira and Quarteira (INE 2015).

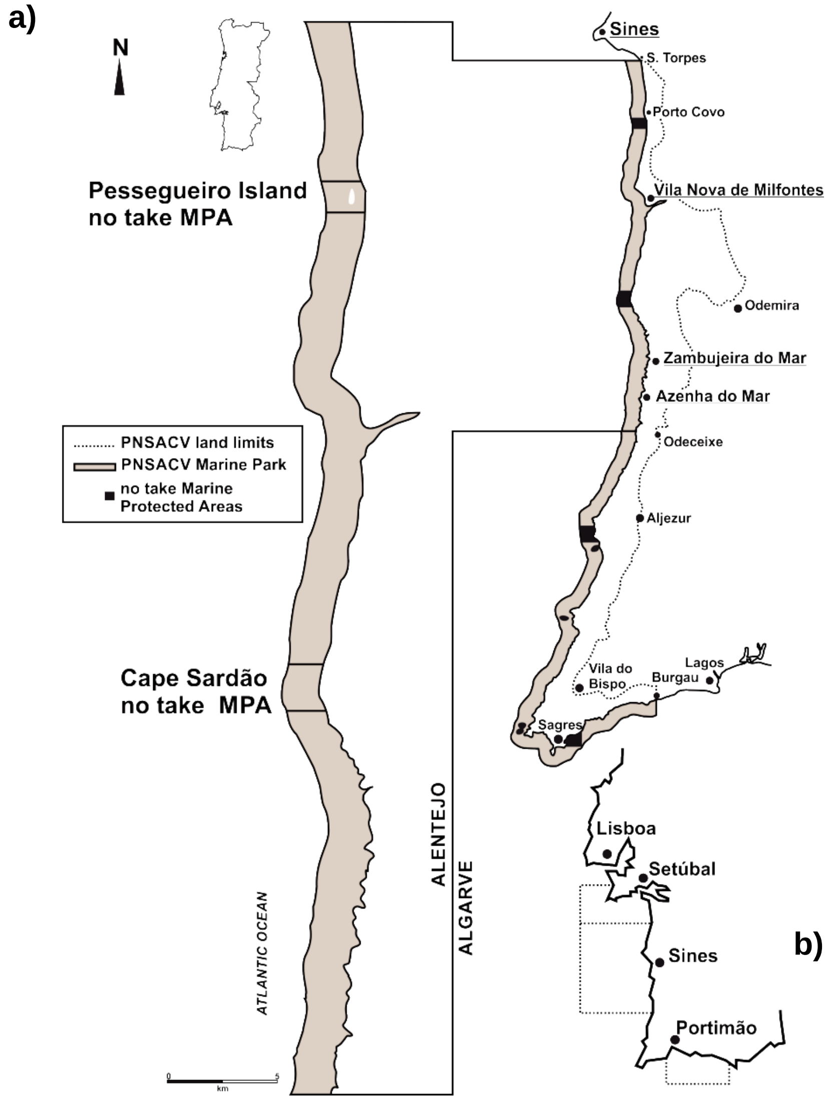

FIGURE 6.1 – Map of the Sudoeste Alentejano and Costa Vicentina (PNSACV) Marine Park with a) location of the no take MPAs of its northern half and b) Sinesmaritime jurisdiction with northern (Setúbal) and southern (Portimão) adjacent maritime jurisdictions (dashed line box).

For all the analysis, only fish species that are described for the PNSACV Marine Park and targeted by local multi gear artisanal fisheries (ICNB 2008, Viegas 2013) were considered. Therefore, most pelagic species targeted by purse seine vessels were not included in the analysis.

Since the data fell within ANOVA usage criteria, gross tonnage of landed fish and LPUE in each harbour were compared by means of univariate ANOVA between the periods before and after protective measures were implemented. Analysing gross tonnage allowed the comparison of global landings in each harbour between time periods, reflecting the impact on the entire community. Using LPUE allowed comparing fish landings per average fishing vessel in each harbour between time periods, thus allowing to understand if the protective measures impacted the landings per vessel.

Since in the maritime jurisdictions of Setúbal and Portimão no protective measures were implemented during the considered time scale, it is expected that eventual differences in fish landings within the PNSACV Marine Park, before and after the implementations of the protective measures and without correspondence in the other two regions, were probably caused by no take MPA designation.

Subsequently, fish landings before and after the no take MPA designation, and among four fishing harbours (Sines, Vila Nova de Milfontes, Zambujeira do Mar and Azenha do Mar), inside the northern half of the PNSACV Marine Park were to evaluate if the no take MPA affected fisheries the same way throughout the northern part of the marine park.

Since the available data from national authorities did not allow splitting the number of registered vessels by harbour inside the PNSACV Marine Park, comparisons were made using only gross tonnage of landed fish by means of multivariate PERMANOVA (Anderson et al. 2008) considering two fixed factors (Protection: before and after, and harbour) and using landing data of each target animal or group of animals. When interactions between factors occurred, pair-wise PERMANOVA tests were performed to assess in which way each interactive factor influenced fish landings complemented by SIMPER analysis (Anderson et al. 2008), to investigate which target group contributed the most for the obtained differences. PERMDISP analysis was then applied to evaluate data dispersion and validate PERMANOVA results. If data dispersion is constant across groups, then PERMANOVA results are valid (Anderson et al. 2008).

Regional (Setúbal, Sines and Portimão maritime jurisdictions) and local (inside PNSACV Marine Park) comparisons were made considering total landings of marine animals. Comparisons were also made according to animal group (crustaceans, molluscs and fish) in order to assess how protection designation affected fisheries of distinct target groups. Additionally, comparisons were made for captures according to fish habitat (demersal and benthic) in order to assess how protective measures affected fisheries with distinct gears used for each group. Pelagic fish were left out of the analysis because most of them may have been caught outside the PNSACV Marine Park by purse sein vessels (Viegas 2013).

Analyses were performed separately among location, animal group and habitat since the low N did not allow a global comparison.

ANOVA analysis was performed using SPSS 22 software (IBM Corp. 2013) and PERMANOVA, PERMDISP and SIMPER analysis were performed using PRIMER 6 & PERMANOVA+ statistical software package (Anderson et al. 2008).

6.3. Results

A total of 80641 ton of marine animals (fish, molluscs and crustaceans) were landed in the Sines maritime jurisdiction between 2007 and 2014, representing an average of 10080.1 ton per year.

A global decrease in fish landings was observed over the years within Sines jurisdiction, with a significant drop in gross tonnage before (45431 ton) and after (35210 ton) protective measures were in place (FIGURE 6.2, TABLE 6.1). However, no significant differences were found concerning LPUE, despite its decrease with time (FIGURE 6.2, TABLE 6.1). This decrease was also evident in the maritime jurisdictions to the north (before: 17714 ton, after: 12363 ton) and to the south (before: 29840 ton, after: 21050 ton), being more noticeable in Portimão where significant decreases were observed in both gross tonnage and LPUE before and after protective measures were implemented.

When considering only fish species, gross tonnage of fish landings was significantly lower after no take MPA implementation in both Sines and Portimão jurisdictions (TABLE 6.1). LPUE only present significant differences between time periods the period before for Portimão, even if values decreased after protective measures in all maritime jurisdictions (TABLE 6.1). On the other hand, landings of crustaceans and molluscs in all jurisdictions did not significantly vary with protective measure implementation (TABLE 6.1).

The comparison of fish landings by habitats revealed a significant decrease in Sines and Portimão jurisdictions' gross tonnage for both demersal and benthic fish (TABLE 6.2, FIGURE 6.3). When considering LPUE, however, significant decreases were only observed for the jurisdiction of Portimão, despite de steady decrease in the remaining maritime jurisdictions over time (TABLE 6.2, FIGURE 6.3).

Total and fish landings in the small harbours from inside the PNSACV Marine Park and from Sines harbours followed the same tendency observed for the global landings in the south central region of the mainland Portuguese coast, with a global decrease in terms of gross tonnage over the years, especially in Sines (FIGURE 6.4). This northern harbour is the largest and presented greater amounts of landings in comparison to the remaining smaller harbours. In fact, significant differences were obtained when comparing total and fish landings between before and after protection as well as among harbours (TABLE 6.3). Pair-wise tests showed that total landings decreased in all harbours after no take MPA designation (pair-wise PERMANOVA: $p_{sines,\ before\ vs\ after}$ = 0,031, $p_{milfontes,\ before\ vs\ after}$ = 0,033, $p_{zambujeira,\ before\ vs\ after}$ = 0,03, $p_{azenha,\ before\ vs\ after}$ = 0,021) as well as fish landings (pair wise

PERMANOVA: $p_{sines,\ before\ vs\ after}$ = 0,029, $p_{milfontes,\ before\ vs\ after}$ = 0,025, $p_{zambujeira,\ before\ vs\ after}$ = 0,034, $p_{azenha,\ before\ vs\ after}$ = 0,027), except for Zambujeira do Mar ($p_{zambujeira,\ before\ vs\ after}$ = 0,034, $p_{azenha,\ before\ vs\ after}$ = 0,027) (FIGURE 6.4).

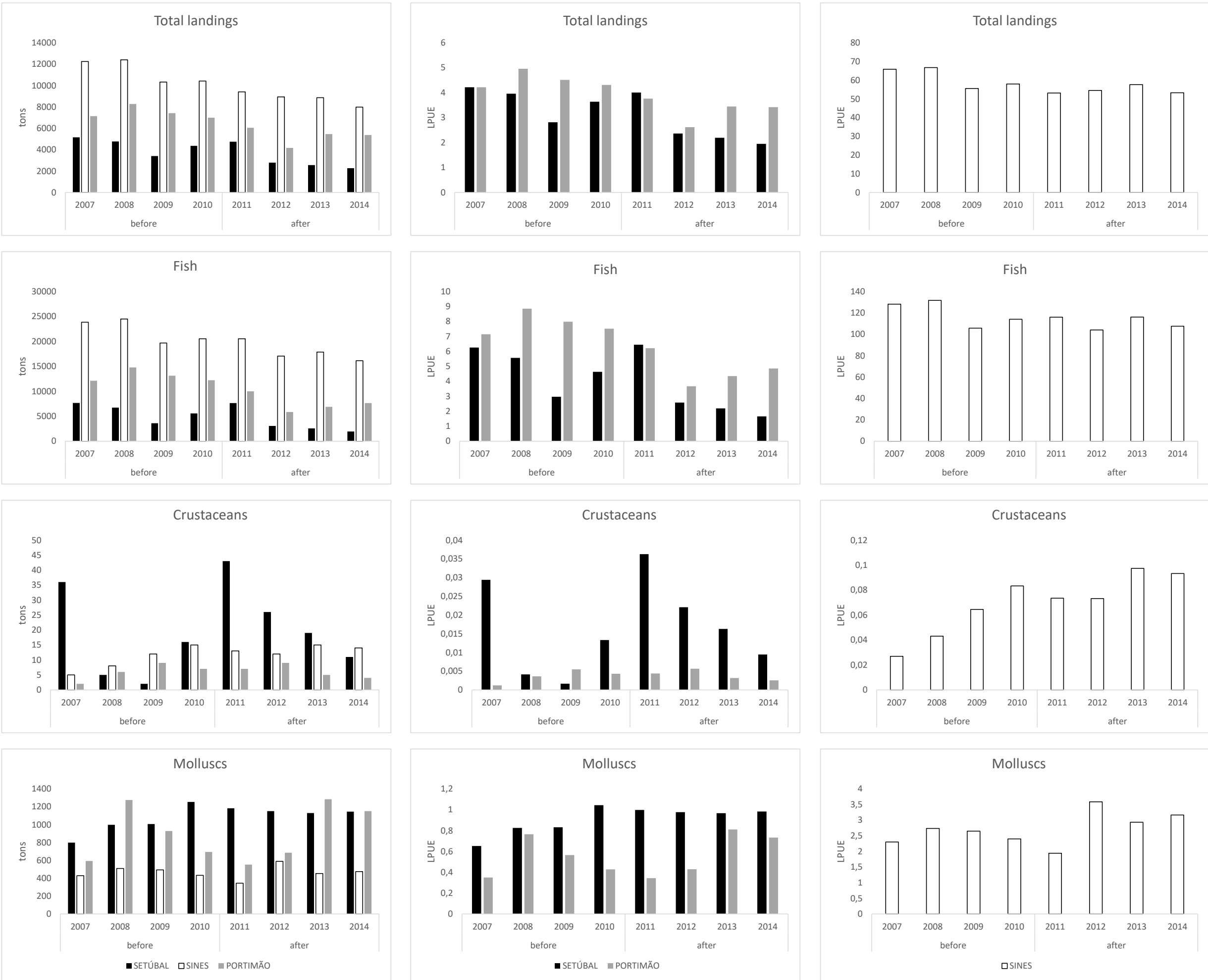

FIGURE 6.2 – Landings by gross tonnage and LPUE according to total captures, fish, crustaceans and molluscs before and after no take MPA designation inside PNSACV Marine Park and adjacent maritime jurisdictions.

Target species that contributed the most to differences in global landings were conger (SIMPER: %contribution = 4,8) and rays (SIMPER: %contribution = 3,72), captured in greater amounts before no take MPA designation, and octopus (SIMPER: %contribution = 7,9) and the forkbeard *Phycis phycis* (Linnaeus, 1766) (SIMPER: %contribution =) whose captures increased after protection.

TABLE 6.1 – Univariate ANOVA summary to compare fish landing by total tonnage and LPUE according total landings, fish, molluscs and crustaceans between before and after no take MPA designation inside PNSACV Marine Park maritime jurisdiction (Sines) and adjacent maritime jurisdictions

	Harbour	SS	df	MS	z	p
TOTAL						
Gross tonnage	Setúbal	3579150,125	1	3579150,125	3,902	0,096
	Sines	13058605,125	1	13058605,125	16,124	0,007
	Portimão	9658012,500	1	9658012,500	20,397	0,004
LPUE	Setúbal	2,113	1	2,113	3,397	0,115
	Sines	95,097	1	95,097	5,332	0,060
	Portimão	2,829	1	2,829	16,209	0,007
FISH						
Gross tonnage	Setúbal	8799012,500	1	8799012,500	1,785	0,230
	Sines	35832345,125	1	35832345,125	7,730	0,032
	Portimão	59677812,500	1	59677812,500	25,929	0,002
LPUE	Setúbal	5,384	1	5,384	1,584	0,255
	Sines	162,172	1	162,172	1,763	0,233
	Portimão	19,251	1	19,251	22,649	0,003
MOLLUSCS						
Gross tonnage	Setúbal	38088,000	1	38088,000	2,163	0,192
	Sines	1,125	1	1,125	0,000	0,989
	Portimão	4095,125	1	4095,125	0,038	0,853
LPUE	Setúbal	0,041	1	0,041	3,146	0,126
	Sines	0,297	1	0,297	1,127	0,329
	Portimão	,005	1	0,005	0,129	0,732
CRUSTACEANS						
Gross tonnage	Setúbal	200,000	1	200,000	0,947	0,368
	Sines	24,500	1	24,500	2,333	0,177
	Portimão	0,125	1	0,125	0,018	0,897
LPUE	Setúbal	0,000	1	0,000	1,098	0,335
	Sines	0,002	1	0,002	4,622	0,075
	Portimão	0,000	1	0,000	0,062	0,811

Considering the remaining target groups, mollusc landings were significantly different among harbours but not with no take MPA designation (TABLE 6.3), despite observed general the increase in landings in all harbours, except Sines (FIGURE 6.4). Crustacean landings, on the other hand, varied significantly among harbours but remained similar after protective measures (TABLE 6.3). However, interaction between factors was significant and pair-wise tests showed that before protective measures all harbours were significantly different in terms of crustacean landings but after implementation, Vila Nova de Milfontes and Zambujeira do Mar presented similar crustacean landings (pair-wise PERMANOVA: $p_{after,\ milfontes\ vs\ zambujeira}$ = 0,378).

Fish was the most representative group in landings from PNSACV Marine Park, with special emphasis for Sines harbour. Demersal fish revealed significant differences among harbours and between periods before and after protective measures, with landings decreasing in all harbours except Zambujeira do Mar (TABLE 6.4, FIGURE 6.5).

TABLE 6.2 – Univariate ANOVA summary comparing fish landings total tonnage and LPUE according to fish habitat (demersal and benthic) between before and after no take MPA designation inside PNSACV Marine Park maritime jurisdiction (Sines) and adjacen maritime t jurisdictions

	Harbour	SS	df	MS	z	p
DEMERSAL						
Gross tonnage	Setúbal	1352190,125	1	1352190,125	2,379	0,174
	Sines	7544670,125	1	7544670,125	12,079	0,013
	Portimão	6681340,125	1	6681340,125	10,990	0,016
LPUE	Setúbal	,850	1	0,850	2,135	0,194
	Sines	53,557	1	53,557	4,875	0,069
	Portimão	2,137	1	2,137	9,318	0,022
BENTHIC						
Gross tonnage	Setúbal	7381,125	1	7381,125	0,022	0,886
	Sines	209628,125	1	209628,125	0,310	0,598
	Portimão	4546620,125	1	4546620,125	47,508	0,000
LPUE	Setúbal	0,011	1	0,011	0,047	0,836
	Sines	2,029	1	2,029	0,106	0,756
	Portimão	1,588	1	1,588	45,213	0,001

Benthic fish also presented significant differences among harbours and between periods before and after no take MPA designation but landings increased both in Sines and, especially, in Zambujeira do Mar harbours (TABLE 6.4, FIGURE 6.5).

6.4. Discussion

Marine related activities play an important role in coastal communities along the Portuguese coast and the south western coast is no exception (Reis 2011). Along the Alentejo coast of the PNSACV Marine Park, where two no take MPAs were designated in 2011, several small fishing harbours exist, mainly serving small scale multi-gear fishing communities (Viegas 2013). When fishing restrictions are put in place, especially those creating areas where no fishing activities are allowed, available area decreases and fishing effort is redistributed (Stelzenmüller et al. 2008, Horta e Costa et al. 2013). Altogether, these factors are expected to cause a short term negative impact on local small scale fisheries, such as those from Alentejo coast, namely by reducing fish catches and consequently fish landings (Silvert and Moustakas 2011, Brown et al. 2014). Small multi gear vessels from the Alentejo region operate mainly near the coast inside the PNSACV Marine Park (Viegas 2013) and the implementation of no take MPAs, despite relatively small, may therefore cause an important impact on fishing strategies. Over the past decade global fish landings along the Portuguese coast showed a decreasing tendency and the Alentejo coast followed that tendency (Baeta et al. 2009, INE 2007-2015). At the same time, fishing fleet and registered professional fishermen have been also decreasing for the past 30 years, suggesting that the decrease in gross tonnage could result from fleet depletion (Teixeira et al. 2014).

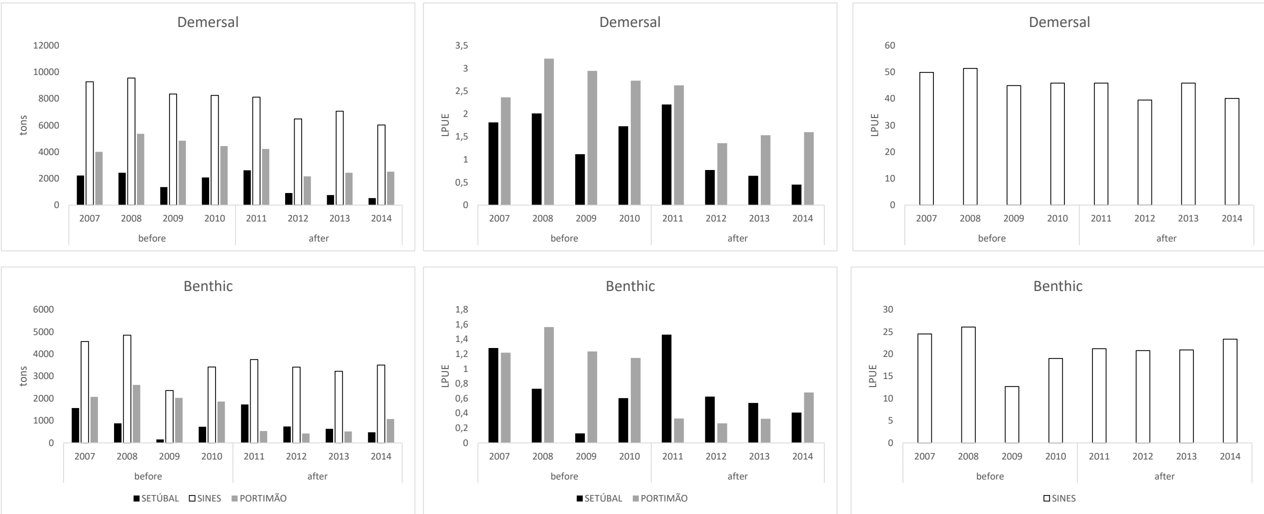

FIGURE 6.3 – Landings by gross tonnage and LPUE according to fish habitat (demersal and benthic) before and after no take MPA designation inside PNSACV Marine Park and adjacent maritime jurisdictions.

TABLE 6.3 – Results of PERMANOVA analysis to compare gross tonnage landings by total landings by total landings, fish, molluscs and crustaceans inside PNSACV Marine Park according to harbor and protection implementation

Factor	**df**	**SS**	**MS**	**Pseudo-F**	**P**
TOTAL LANDINGS					
Protection	1	1028,5	1028,5	7,4586	0,006
Harbour	3	41210	13737	99,62	0,001
Protection x harbour	3	1762,6	587,52	4,2607	0,009
FISH					
Protection	1	1341,7	1341,7	8,7541	0,001
Harbour	3	30506	10169	66,349	0,001
Protection x harbour	3	2340,8	780,28	5,0912	0,002
MOLLUSCS					
Protection	1	142,02	142,02	1,5901	0,211
Harbour	3	19770	6590	73,786	0,001
Protection x harbour	3	301,35	100,45	1,1247	0,325
CRUSTACEANS					
Protection	1	926,72	926,72	1,4351	0,205
Harbour	3	35888	11963	18,525	0,001
Protection x harbour	3	3970,9	1323,6	2,0498	0,036

However, the decrease in LPUE over the years, despite less evident, suggests that available resources may be decreasing too. In fact, over the time period considered for the present study, the fishing fleet from Alentejo coast did not present a marked decrease (Alentejo: 19% decrease from 186 in 2007 to 150 in 2014, at an average rate of 4,5 vessels/year), but global landings did (34% decrease, INE 2015) indicating a decrease in available resources.

TABLE 6.4 – Results of PERMANOVA analysis to compare gross tonnage landings inside PNSACV Marine Park by fish habitat (demersal and benthic) according to harbour and protection implementation

Factor	df	SS	MS	Pseudo-F	P
DEMERSAL					
Protection	1	1477,6	1477,6	8,1195	0,002
Harbour	3	29262	9753,9	53,597	0,001
Protection x harbour	3	2894,9	964,96	5,3024	0,001
BENTHIC					
Protection	1	1306,9	1306,9	11,355	0,001
Harbour	3	28633	9544,5	82,925	0,001
Protection x harbour	3	1955,2	651,73	5,6624	0,001

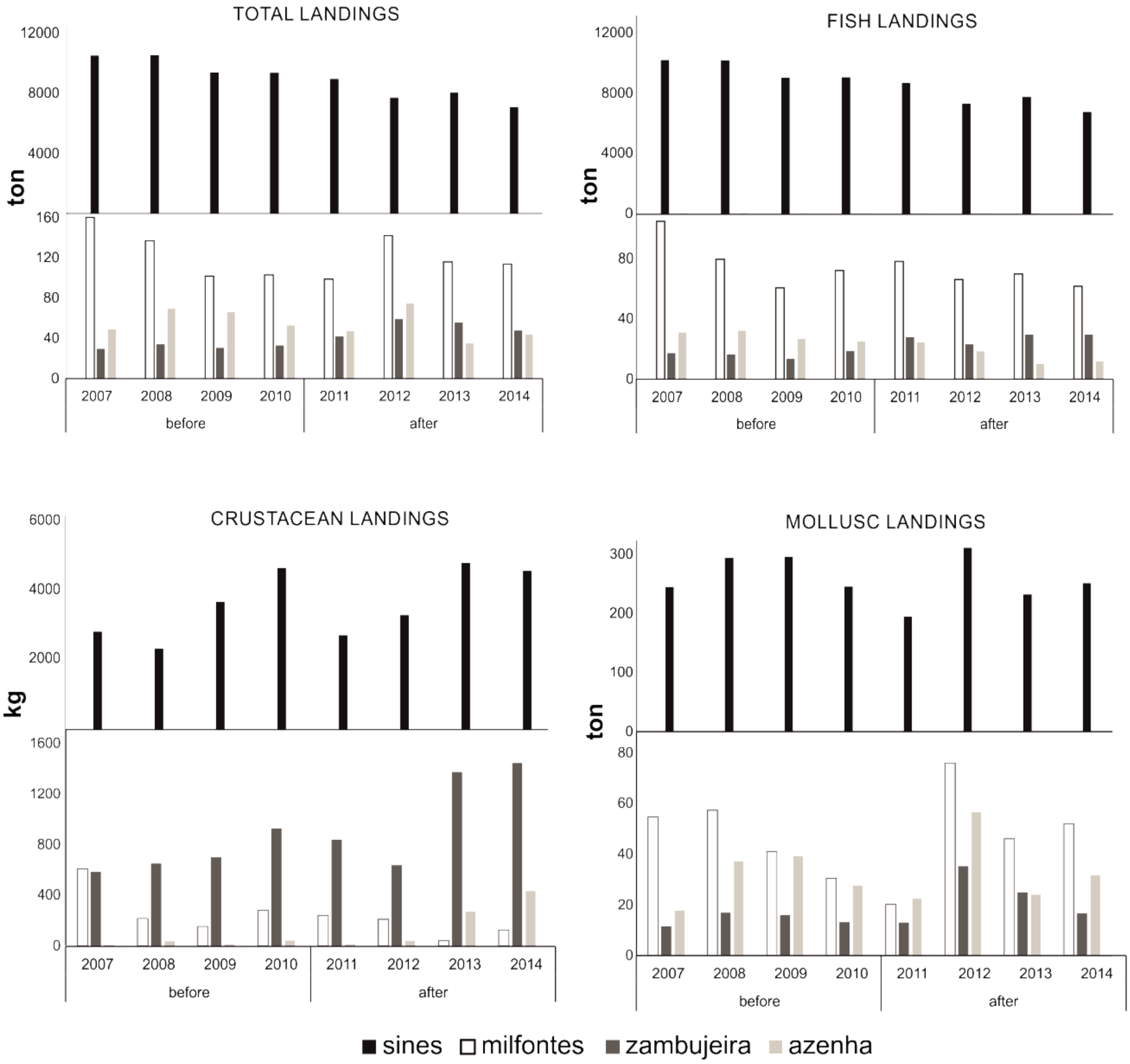

FIGURE 6.4 – Landings by gross tonnage according to total captures, fish, crustaceans and molluscs before and after no take MPA designation in the harbours from inside PNSACV Marine Park.

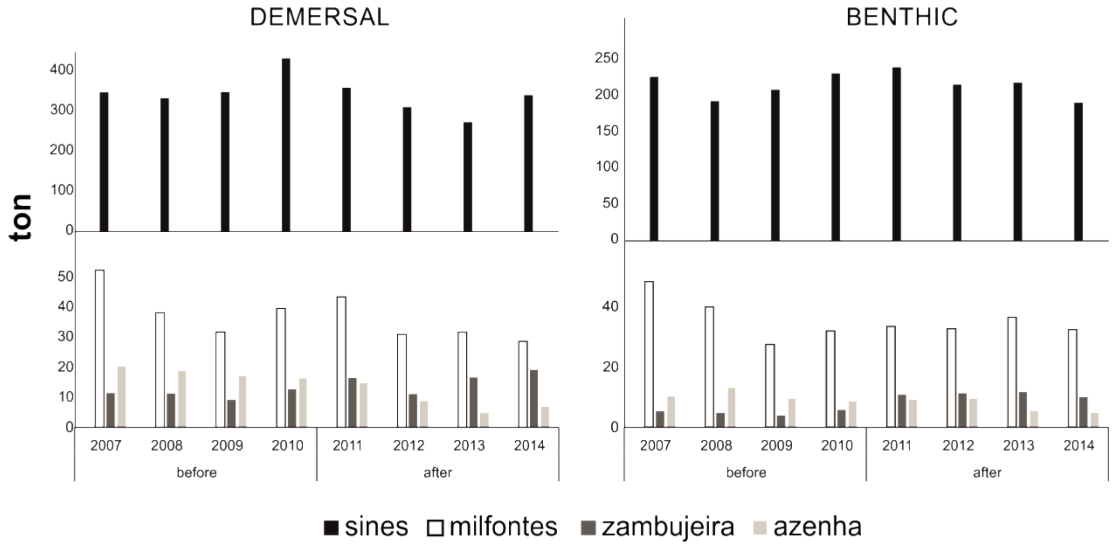

FIGURE 6.5 – Landings by gross tonnage according to fish habitat (demersal and benthic) before and after no take MPA designation in the harbours from inside PNSACV Marine Park.

The number of registered fishing vessels in the entire Alentejo region (which includes Sines) is relatively small when compared with its neighbouring regions [150 vessels in 2014 against 1166 in Lisboa e Vale do Tejo (which includes Setúbal) to the north, and 1572 in the Algarve (which includes Portimão) to the south] (INE 2015). Furthermore, the fleet decrease in neighbouring regions is also evident, with a loss of 59 (4,8%) fishing vessels to the north and 124 (7,3%) to the south between 2007 and 2014 (INE 2015), suggesting that the decrease in total and fish landings in these regions was also caused by the decrease in fishing effort as also described by Ferreira et al. (2013) and Teixeira et al. (2014). Note that data on the number of registered fishing vessels is only available by region and each region can include more than one maritime jurisdiction. Following the global decreasing tendency, it was possible to also observe a significant break in total landings from Alentejo (Sines maritime jurisdiction) between the periods before and after no take MPA designation in PNSACV Marine Park regarding gross tonnage. However, since this breakage had correspondence in the south jurisdiction (but not in the northern area), one could not relate this decrease with the protective measures implemented in 2011. Nevertheless, the implementation of protective measures may have boosted this tendency. Also, when considering LPUE data, an evident decrease was observed both in Sines and Portimão, suggesting that these measures inside the Marine Park may have had some influence on landings in the region but not in a relevant way. Silvert and Moustakas (2011) modelled the impact of no take MPA designation and concluded that the immediate consequence of these measures is a decrease in fish landings. However, at mid or long term, fish landing tend to return or even overcome the original landings, thus benefiting fisheries (Silvert and Moustakas 2011). It is widely accepted that MPAs lead to an increase in fish landings in mid to long term, with this pattern observed in several European reserves (e.g. Gell and Roberts 2003, Halpern et al. 2009), some of which after just four (Bastari et al. 2016) or six years (Alcala et al. 2005). It should therefore be expected that total fish landings increase in the Alentejo region in the upcoming years.

The network implemented inside this marine park comprises four small no take MPAs of around 6 km^2 each, making them too small for causing an immediate impact (positive or negative) on large scale fisheries (Gell and Roberts 2003, Halpern et al. 2009). Given this, small scale artisanal fisheries have the potential to be the most impacted by these protective measures on short term but also the ones that will probably benefit the most in the future. When designating a coastal no take MPA, such as those in the PNSACV Marine Park, besides the suppression of fishing ground, a no take barrier is created between the harbour and other coastal areas that will cause an increase in costs in terms of fuel and time. Since one of the most important factors influencing the deployment of fishing gears is the distance to the fishing harbour (Stelzenmüller et al. 2008, Horta e Costa et al. 2013), a redistribution of the fleet between the harbour and the boundaries of the no take MPAs may occur, which may lead to a concentration of vessels or an increase in fishing pressure in some areas (Stelzenmüller et al. 2008). However, given the relatively reduced amount of registered vessels in the Alentejo region, most of which are artisanal multi-gear vessels (INE 2015), and the distance between the harbours and the no take MPAs, this should not be an issue in this case. Nevertheless, it was possible to observe large amounts of static fishing gears deployed near the limits of the no take MPAs (N. Castro, pers. com.).

In the particular case of the no take MPAs of the Alentejo coast of the PNSACV Marine Park, the decreasing tendency on landings seemed to follow the global trend observed in the entire park as well as in the adjacent maritime jurisdictions. This further suggests that the implementation of protective measures in the area may not be entirely responsible for this decrease. However, despite this global decreasing tendency inside the park and neighbouring areas, the implementation of protective measures may have significantly impacted fish landings in each individual harbour from the marine park. While total landings followed the global decreasing tendency in all harbours, fish landings significantly increased in Zambujeira do Mar harbour after no take MPA designation, incidentally the smallest harbour in the region. Inversely, the most noticeable decrease was observed in Sines, the largest of the four harbours. In the remaining harbours, despite the significant decrease after 2011, it was possible to observe a tendency for fish landings to slightly increase over the years. This fact suggests that small artisanal fisheries may be the first to suffer from fishing prohibition but also the first to benefit, as previously described (Alcala et al. 2005, Silvert and

Moustakas 2011, Brown et al. 2014, Bastari et al. 2016). This may be due to the lower number of vessels fishing in the area, and the reduced fishing pressure on local resources, rather than from spill over effects, given that the small amount of time elapsed is likely not sufficient for these to become evident. In this particular scenario, creating no take MPAs does not seem to influence the availability of fishing grounds for the reduced amount of currently existing fishing vessels.

The impact of protective measures on small scale fisheries was evident not only on the smallest fishing communities but also on the targeted groups, which are a reflection of the most used gears in these communities. Most vessels operating in the region are small multi gear vessels targeting demersal and benthic fish species as well as crustaceans and molluscs (mainly octopus) (Viegas 2013). In Sines, demersal fish landings decreased after 2011 but seem to start to recover in 2014, while in the remaining harbours this increase appears to start a little earlier in time, c. 2013. Benthic species, on the other hand, revealed a consistently decreasing tendency in Sines while in the remaining smaller harbours, benthic fish landing seemed to stabilize after a small increase in 2011. Once again, these cases where a recovery is observed after an initial break in fish landings seem to evidence the short term impact of no take MPA designation on small scale fisheries where fishing ground limitation forces fishermen to relocate. However, in some cases (Zambujeira and Milfontes), landings started to increase shortly after protective measures took place indicating that the loss of access to fishing grounds in the closed area has been compensated by the remaining fishing grounds, as in other areas (Mangi et al. 2011). Nevertheless, this apparently rapid recovering in fish landings seems to be more evident in small scale fisheries and fishing communities given the fact that, despite the loss of fishing grounds, a large area remains available for the relatively small amount of fishing vessels operating locally. In these cases, it seems that the effects of spillover from no take MPAs are still negligible or inexistent given the small time elapsed since MPA designation and the relatively small size of closed areas (Vandeperre et al. 2011).

Despite the immediate impacts on fisheries and the apparent but slow recovery of fish landings, when implementing measures that affect fishing activities and impact fishermen livelihoods, special care must be taken into account. The success of a no take MPA can be influenced by how the perceptions and response of fishermen to these restrictive measures are understood (Lédée et al. 2012, Symes and Hoefnagel, 2010). Measures decided and applied with a “bottom up” approach, including the general public and the fishing community in the decision making process, will reduce conflicts resultant from prohibition (Ferreira et al. 2013) and an honest and transparent information on costs and benefits from MPA designation will contribute for an appropriate and efficient use of the protected area (Gall and

Radwell 2016). It is therefore important to analyse the trends in landings data in areas neighboring the no take MPAs before their implementation and continue to monitor catches and landings over time, before claiming that protective measures will benefit local fishing communities (Higgins et al. 2008).

This work contributed towards understanding how local fisheries were affected by no take MPA designation in the Alentejo coast of the PNSACV Marine Park and, globally, how the implementation of protective measures that include closed areas can impact fish landings at short term. At the same time, this study showed how small scale fisheries may recover relatively rapidly, with an increase in landings with time as a result of fleet relocation to available fishing grounds. However, direct contributions of these no take MPAs for fisheries, namely as a result of spillover effects are yet to be shown. For that purpose, a larger period of time must elapse and specific sampling surveys must be carried out, given the small size of these no take MPAs and the absence of official data that can be accurately used for that purpose. Nevertheless, recent works demonstrate the increase of fishing resources inside these protected areas (Pereira et al. 2017), confirming their adequacy for protection of commercial fish species and that they potentially act as nursery and export biomass to surrounding areas. Nevertheless, further monitoring of official landing data must be carried out in order to confirm these finding and follow the evolution of fishing trends.

6.5. References

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CHAPTER 7

FINAL REMARKS

7.1. Final remarks

Despite its small size, the designation of type I partial protection areas (no take MPAs) in the Alentejo coast of the Sudoeste Alentejo and Costa Vicentina Marine Park positively impacted local fisheries and local fish communities shortly after, proving to be adequate tools to protect local commercially important fish species.

For example, the implementation of the Pessegueiro Island no take MPA proved to be important and adequate for protecting some commercially important fish species which find in this area optimal feeding and sheltering areas. Such were the cases of *M. helena*, *C. conger* and *D. sargus*, whose potential feeding and refugee areas inside this no take MPA and movements between those were identified. Most of these species' individuals remain inside the same area during the summer period, displaying high spatial fidelity. Areas surrounding the island are very important for feeding purposes and possibly refuge. These conclusions are in agreement with other studies stating that small MPA's can provide efficient protection for reef species with small home ranges (Kerwarth et al. 2007a, Afonso et al. 2011, Alós et al. 2012, La Mesa et al. 2013, Taylor and Mills 2013). Consequently, the protection granted to the Pessegueiro Island with the creation of the no take MPA is an adequate and relevant measure for the protection and sustainable exploitation of these species inside the PNSACV.

Small and young MPAs can therefore provide short term effectiveness in protecting commercially important fish species. And if implemented as a network of small MPAs, such as those implemented inside the PNSACV Marine Park, the potential to be long term effective and to benefit larger geographic areas increases, especially if connectivity between MPAs occurs (Chateau and Wantiez 2009). Nevertheless, negative effects on small fish species caused by predator increase inside MPAs are possible and should be taken into account.

Local fish assemblages were also positively impacted, in this case by the designation of both Pessegueiro Island and Cape Sardão no take MPAs. Results showed a significant increase in fish abundance and significant differences in their structure (abundance and fish size) between protected and neighbouring areas. In addition, specimens of larger sizes started to occur more frequently within the northernmost no take MPA. This protected area also seemed to be adequate to protect juvenile fish and to protect commercially important species. This way, these two no take MPAs seem to have an appropriate size and location, making them effective and ecologically distinctive from adjacent areas.

Ideally, MPA design should be validated through preliminary studies to define their correct size and location thus ensuring its effectiveness and benefits from an early stage. The same way, the present work would have provide valuable additional information if started prior to protective measures had taken place. Nevertheless, works started at an initial phase of protection, allowing capturing an early picture of local communities before the main effects could start to be visible. However, only long term monitoring can provide answers regarding biodiversity evolution and determine additional measures necessary to achieve an adequate balance. Overall, MPA management should be flexible and fish assemblages status periodically assessed, in order to adapt protective measures accordingly, by maintaining or reinforcing current measures (adapt MPA size, ban all activities) or temporarily adopting less restrictive ones (open recreational and/or professional fishing seasons for certain species).

The first visible effects of MPA designation on fish assemblages are usually the increase in fish abundance and diversity, with effects on trophic ecology taking longer to occur (Edgar and Barrett 1999, Colléter et al. 2012, Seytre et al. 2013). These two no take MPAs were no exception. The observed increment in fish abundance (CHAPTER 4), and eventual increase in intraspecific competition for food resources, impacted the niche breadth of the most abundant species. Despite this, protective measures and consequent changes in fish assemblage structure did not directly impact diet composition. However, it may have contributed to reinforce the already naturally existent differences. Additionally, the increase in number of larger specimens inside one of the no take MPAs may have caused predatory pressure on smaller organisms. But, at some point in time, a more balanced ecosystem may be attained, given that protective measures are known to benefit all trophic levels with time (e.g. Soler et al. 2015). It is therefore key to investigate if the initial predatory pressure on small fish and other items continues over time as well as if predatory pressure causes impacts that can be negative to the entire assemblage in the long term.

Overall, small no take MPAs such as those implemented in the PNSACV Marine Park seem in fact to initially benefit fish assemblages, especially concerning commercially targeted fish species, but their small size may lead to rapid effects on the trophic chain by rapidly increasing the consumption of small organisms, both inside and in adjacent non protected areas. It is therefore important, when designing and implementing a no take MPA, to take into account not only short term impacts, usually positive and rapidly observable for commercially important species, but also mid and long term effects, namely on the assemblage of potential preys, that take into account fish assemblages life cycles and habitat requirements.

However, before any visible impacts on fish communities, the most immediate consequence of implementing restrictive measures that include no take areas is the loss of available fishing grounds, directly impacting local small scale multi gear fisheries. However, in the case of Alentejo coast MPAs, fleet relocation was an option and resulted in the increase in fish landings over time, showing that small scale fisheries may recover rapidly from fishing ground loss. This conclusion shows that fisheries and protective measures can cohabit as long as the size and location of the no take areas allows the occurrence of alternative fishing grounds, which seems to be the case of the PNSACV Marine Park. However, the direct contributions of these no take MPAs for fisheries, namely as a result of biomass spillover are yet to be effectively estimated. For that purpose, a larger period of time must elapse and specific sampling surveys must be carried out, given the small size of these no take MPAs and the absence of official data that can be accurately used for that purpose. Nevertheless, recent works demonstrate the increase of fishing resources inside these protected areas (CHAPTERS 4 AND 5), confirming their adequacy for protection of commercial fish species and potentially act as nursery and drive biomass export. Nevertheless, as in previous conclusions, further monitoring of official landing data must be carried in order to confirm these findings and follow the evolution of fishing trends.

Overall, this work validates the no take MPAs in the Alentejo coast of the PNSACV Marine Park as adequate and effective protection tools for marine conservation and sustainable resource exploitation. It also confirms and provides additional evidence that small no take MPAs can be short term effective and provide protection for commercially important fish species, thus contributing for the increase in biodiversity and biomass. However, three years of monitoring is evidently a short time window to draw definitive conclusions. This way, continuous monitoring of the local fish assemblages is of the uttermost importance to adapt protective measures according to their evolution and long term responses. Also, a wider spatial monitoring would help to better assess the impact of these measures on the fish assemblage of the entire Marine Park under the concept of the no take MPA network in place, namely by evaluating the connectivity between protected areas. Considering all the above, the permanence of the currently existing protective measures, in light of the positive results obtained, is advised. However, the long way that is still to come in order for all people impacted to fully recover and benefit from those measures has also to be pointed out.

Besides validating these two particular no take MPAs as effective, this work confirms small no take MPAs are useful tools for marine protection that can and should be used for marine resource management in other regions. Their size and location should be assessed through solid scientific evidence obtained prior to their implementation. An adequate monitoring program should also be put in place in order to evaluate their effectiveness over time, and adjusting protective measures accordingly if necessary.

7.2. References

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APPENDIX I

Appendix I

Supplementary material and added notes on data analysis for Chapter 2

I.1. Data exploration

Files downloaded from the VR2W receivers contain parameters that can be used to calculate the noise quotient and code collision rate. This allows assessing how these parameters affect receiver performance and detection range. Noise quotient was calculated as nq = P-(S.cl), where P is the number of pulses detected, S is the number of synchs, and cl is the number of pulses it takes to make a valid code. Positive values of nq indicate environmental noise and negative values indicate code collision (Simpfendorfer et al. 2008).

Data was sorted using simple Visual Basic routines in Microsoft Office Excel© for posterior analysis. Codes from the same transmitter detected by different receivers less than 30 s apart were filtered out. The first recorded code was kept with the average geographic coordinates of the multiple detecting receivers, except for network analysis. In this case only the first detecting receiver was taken into account.

Due to the low number of individuals captured outside the MPA and the short time of detection of conger C#5 and C#6, the analysis on these data was restricted to residency and activity indices, average daily distance covered and final displacement vector.

I.2. Residency, Activity and Covered Distance Index

The Residency Index (Ir) must be addressed with caution when applied to cryptic species, as tagged individuals may be present in the study area but concealed in crevices undetected by acoustic receivers (Almeida et al. 2013). For the present work, individuals were considered active when intervals between consecutive detections were less than 10 minutes. Above this interval, individuals were assumed to be stationary, hidden inside refuges, outside the no take MPA or out of the receivers' array range. The sum of activity intervals equals total activity time.

I.3. Kernel Density Estimation (KDE) analysis

KDE was calculated using raw data but multiple detections were also used to obtain an intermediate position (average coordinates) when multiple receivers detected the same code. This process increased the spatial definition in some areas. The smoothing factor (h) plays an important role on this analysis and the higher its value, the less detailed home range estimation is (Worton 1989). This parameter was calculated using the h_{ref} method (Worton 1989), resulting in h=90 for morays and h=135 for congers. Cell size of 10m x 10m was used in order to display adequate image resolution. The percentage of the protected area used by each individual was calculated as %MPA = [home or core range area (ha)/Pessegueiro Island no take MPA area (ha)]*100.

I.4. Network analysis (NA)

Network analysis was applied to understand which areas were more relevant for the tagged fish by analysing the movements between them. This method, based on graph theory, consists on a complex system of nodes connected by edges (Jacoby et al. 2012) described by metrics such as centrality. There are two types of centrality, both measuring the importance of a node to the network (Gómez et al. 2013): degree centrality (Cd), that takes into account the number of edges that connect to a node (Jacoby et al. 2012, Makagon et al. 2012), and betweenness centrality (Cb), which is based on the number of shortest paths between any two nodes which cross the focal node (Freeman 1977). High values of Cb indicate that the focal node has an intermediate location between several paths (Makagon et al. 2012). In diagrams node size is proportional to centrality values while edge thickness is proportional to the number of connections.

Directed and weighted networks were analysed by means of square matrices in which relative movement of an individual was regarded as the number of movements between two receivers divided by its total number of movements. Before this process, all networks were tested for non-random patterns. Random networks (n = 10 000) were generated by flipping the edges 100 times per permutation while keeping the degree distribution of the original network in order to get a biologically meaningful null model (Newman et al. 2001, Croft. et al. 2011). Random networks metrics [i.e. maximum modularity (Q) (Brandes et al. 2008, Jacoby & Freeman 2016)] were calculated and compared with metrics from the original networks using Wilcoxon Onesample Signed Ranks Test in IBM® SPSS® Statistics Version 23, assuming the maximum Q of the original networks as the hypothetical medians. Networks no different from random (n=5) were excluded from subsequent analyses.

I.5. Environmental parameters

Influence of circadian cycle (day vs night), lunar cycle (first quarter vs full moon vs third quarter vs new moon), tidal cycle (ebbing vs rising) and swell (low - <1 m height; high - >1 m height) on individual activity index (I_a) was also assessed. An I_a was determined and weighed against the total I_a for each individual and combination of factors. Activity indices were then compared by means of multivariate PERMANOVA (Anderson et al. 2008). Differences in movement patterns, concerning space use according to circadian, lunar cycle and swell were analysed by means of Mantel tests.

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APPENDIX II

TABLE II a - Summary table of degree and betweeness centrality (normalized and balanced by the total number of links per network) for morays and congers. For morays, global measures as well as analysed per environmental conditions are shown. Environmental parameters considered were: Circadian day (Day and Night), Swell (Low/Medium/High), and Lunar phase (New moon, First quarter, Full moon, Third quarter). Given the low sample, for congers centrality was calculated per tagged individual

		Receivers																			
MORAYS	**Centrality**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**mean**
Global	*Degree*	8	9	8	21	7	0	0	3	13	0	0	0	0	6	0	0	11	16	2	6
	Betweeness	203	164	54	408	233	0	0	165	41	2	0	0	0	1	0	0	78	78	0	43
Day	*Degree*	0	4	18	27	3	0	0	9	10	0	0	0	0	12	0	0	19	4	1	6
	Betweeness	34	45	45	72	69	0	0	52	5	0	0	0	0	0	0	0	55	24	24	22
Night	*Degree*	2	7	20	18	2	0	0	6	10	0	0	0	0	16	0	0	13	12	0	6
	Betweeness	4	7	15	42	11	0	0	47	19	0	0	0	0	2	0	0	32	37	0	11
Low	*Degree*	2	6	18	22	2	0	0	8	10	1	0	0	0	12	0	0	16	8	0	6
	Betweeness	9	4	11	2	19	0	0	34	19	0	0	0	0	2	0	0	34	18	5	8
Medium/High	*Degree*	1	6	22	17	2	0	0	5	8	1	0	0	0	18	0	1	10	14	0	6
	Betweeness	18	44	10	142	51	0	0	102	61	0	0	0	0	3	0	0	64	59	17	30
New Moon	*Degree*	2	8	20	16	2	0	0	8	9	0	0	0	0	16	0	4	14	10	0	6
	Betweeness	0	109	79	133	2	0	0	169	58	0	0	0	0	26	0	0	128	9	0	38
First Quarter	*Degree*	3	8	22	16	2	0	0	7	10	0	0	0	0	16	0	0	8	12	1	6
	Betweeness	30	12	26	19	36	0	0	50	19	0	0	0	0	4	0	0	55	34	14	16
Full Moon	*Degree*	0	6	18	22	2	0	0	4	8	0	0	0	0	14	0	0	14	17	1	6
	Betweeness	29	35	26	40	55	0	0	130	14	0	0	0	0	26	0	0	135	190	86	40
Third Quarter	*Degree*	0	4	20	26	1	0	0	6	10	0	0	0	0	12	0	0	20	6	0	6
	Betweeness	0	29	50	42	26	0	0	60	0	0	0	0	0	0	0	0	45	49	0	16
CONGERS																					
C2	*Degree*	0	0	0	26	8	0	0	4	20	2	0	0	0	0	0	0	18	24	0	5
	Betweeness	0	0	0	9	22	0	0	1	32	0	0	0	0	0	0	0	40	18	0	6
C4	*Degree*	0	0	30	0	0		0	32	0	0	0	0	0	40	0	0	2	0	0	6
	Betweeness	0	0	132	0	0	0	0	39	63	0	0	0	0	3	0	24	0	21	0	15

*all values in scientific notation (degree centrality x 10^{-3}, betweenness centrality x 10^{-6}).

TABLE II b - Results of pair-wise PERMANOVA tests between interacting factors regarding moray activity index I_a where df: degrees of freedom; SS: sum of squares; MS: mean squares; *t*: test statistic; *p*-value: calculated probability to significance level α=0.05; Perm: number of permutations

Factor 1	**Factor 2**				
Lunar phase	**Lunar phase**	**df**	***t***	***p*-value**	**Perm**
Full Moon	New Moon	115	0.56	0.74	999
Full Moon	First Quarter	122	1.73	0.06	999
Full Moon	Third Quarter	106	2.04	0.02	999
New Moon	First Quarter	127	1.55	0.09	999
New Moon	Third Quarter	111	2.76	0.00	996
First Quarter	Third Quarter	118	3.96	0.00	998
Lunar phase	**Time of day**				
Full Moon x New Moon	Day	47	0.58	0.71	997
Full Moon x First Quarter	Day	53	0.31	0.94	999
Full Moon x Third Quarter	Day	43	2.51	0.01	999
New Moon x First Quarter	Day	50	0.86	0.47	999
New Moon x Third Quarter	Day	40	2.40	0.02	999
First Quarter x Third Quarter	Day	46	2.80	0.00	999
Full Moon x New Moon	Night	68	1.40	0.15	999
Full Moon x First Quarter	Night	69	2.77	0.00	998
Full Moon x Third Quarter	Night	63	0.60	0.71	997
New Moon x First Quarter	Night	77	1.48	0.12	998
New Moon x Third Quarter	Night	71	1.13	0.24	999
First Quarter x Third Quarter	Night	72	2.68	0.00	999
Swell	**Lunar phase**				
Low x Medium/High	Full moon	55	0.97	0.39	998
Low x Medium/High	New moon	60	0.56	0.76	998
Low x Medium/High	First Quarter	67	1.14	0.25	998
Low x Medium/High	Third Quarter	51	3.51	0.00	999

APPENDIX III

Appendix III

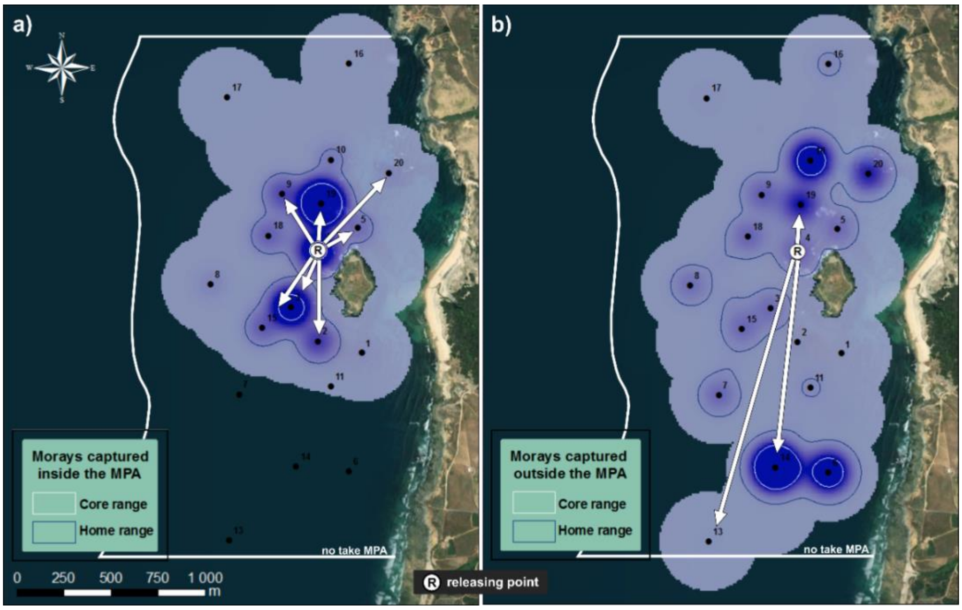

FIGURE III a - Final displacement vectors of tagged morays a) captured inside the MPA, b) captured outside the MPA in relation to releasing point.

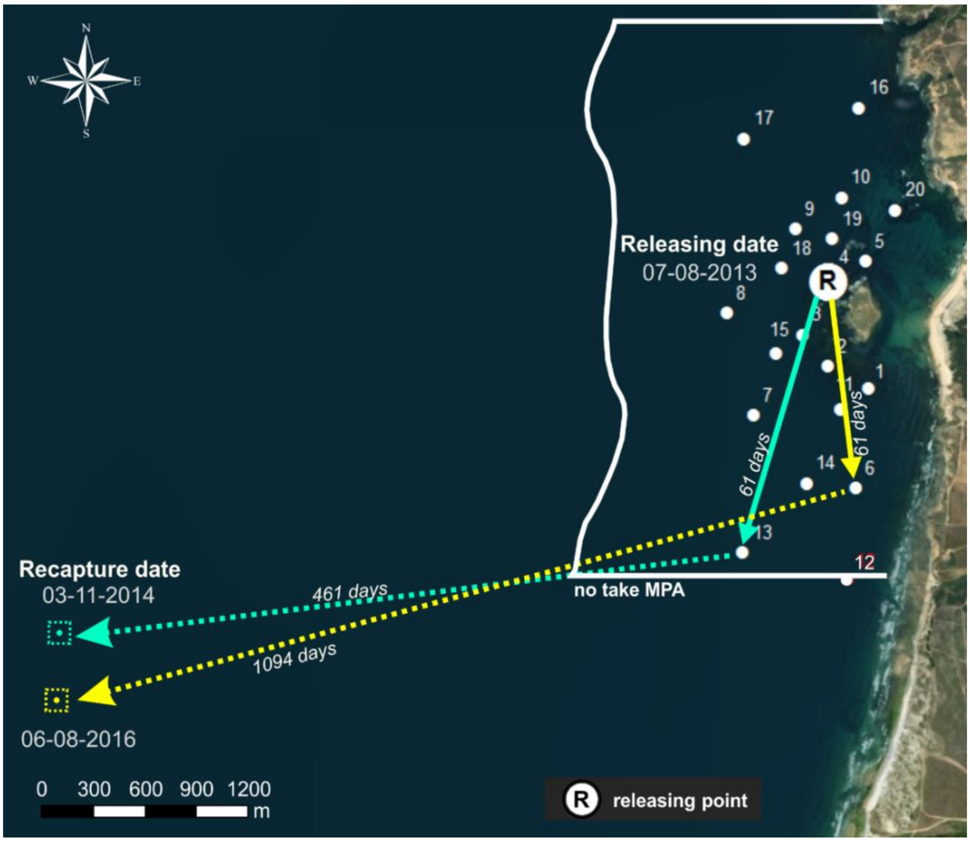

FIGURE III b -. Final displacement vectors and recapture locations of two morays captured outside the MPA (moray M#7 and M#11).

APPENDIX IV

TABLE IV a - Summary table of the centrality measures calculated for each *D. sargus*, by receiver, including indegree, outdegree centrality and betweenness

Specimen	Receiver	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
	Outdegree	3.00	6.00	4.00	7.00	6.00	0.00	2.00	3.00	5.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.90
#1	InDegree	3.00	4.00	4.00	7.00	8.00	0.00	1.00	4.00	6.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.90
	Betweeness	0.00	2.58	0.00	15.50	25.75	0.00	0.00	0.00	3.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.35
	Outdegree	6.00	6.00	9.00	9.00	7.00	1.00	10.00	10.00	7.00	5.00	1.00	4.00	9.00	0.00	4.00	0.00	1.00	2.00	0.00	0.00	4.55
# 2	InDegree	9.00	6.00	9.00	10.00	7.00	2.00	9.00	11.00	10.00	0.00	1.00	3.00	8.00	0.00	4.00	0.00	1.00	1.00	0.00	0.00	4.55
	Betweeness	7.00	0.00	8.05	10.38	22.03	0.00	37.02	38.37	20.94	0.00	0.00	14.50	26.34	0.00	2.03	0.00	0.00	0.00	0.00	0.00	8.00
	Outdegree	2.00	5.00	5.00	5.00	4.00	0.00	6.00	7.00	7.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
# 3	InDegree	3.00	4.00	6.00	6.00	3.00	0.00	5.00	8.00	7.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.15
	Betweeness	0.00	0.00	3.00	2.33	0.00	0.00	2.17	20.25	12.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00
	OutDegree	0.00	3.00	6.00	5.00	5.00	0.00	7.00	7.00	6.00	0.00	0.00	0.00	4.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	2.20
# 4	InDegree	0.00	3.00	7.00	5.00	5.00	0.00	7.00	6.00	6.00	0.00	0.00	0.00	4.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	2.20
	Betweeness	0.00	0.00	5.00	0.00	0.00	0.00	8.00	5.00	4.00	0.00	0.00	0.00	14.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80
	OutDegree	0.00	0.00	0.00	2.00	2.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30
# 5	InDegree	0.00	0.00	0.00	2.00	2.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30
	Betweeness	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	OutDegree	3.00	6.00	6.00	7.00	7.00	2.00	8.00	7.00	9.00	3.00	0.00	1.00	4.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	3.20
# 6	InDegree	7.00	6.00	7.00	6.00	8.00	1.00	8.00	7.00	9.00	0.00	0.00	1.00	3.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	3.20
	Betweeness	11.42	3.07	1.45	3.90	8.95	0.00	22.18	3.43	28.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.00
	OutDegree	4.00	6.00	8.00	6.00	7.00	0.00	7.00	3.00	11.00	4.00	0.00	0.00	4.00	0.00	0.00	2.00	3.00	2.00	0.00	0.00	3.35
# 7	InDegree	7.00	5.00	8.00	5.00	5.00	0.00	5.00	11.00	10.00	0.00	0.00	0.00	5.00	0.00	0.00	1.00	3.00	2.00	0.00	0.00	3.35
	Betweeness	5.10	1.37	6.77	2.12	4.57	0.00	0.00	9.00	49.72	0.00	0.00	0.00	2.43	0.00	0.00	0.00	1.25	1.87	0.00	0.00	4.00
	OutDegree	1.00	5.00	8.00	8.00	5.00	0.00	6.00	7.00	7.00	3.00	0.00	0.00	4.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	2.80
# 8	InDegree	2.00	5.00	8.00	8.00	5.00	0.00	6.00	7.00	8.00	0.00	0.00	0.00	5.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	2.80
	Betweeness	0.00	0.00	16.93	9.03	0.00	0.00	1.97	3.03	11.84	0.00	0.00	0.00	1.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00
	OutDegree	0.00	0.00	1.00	6.00	4.00	0.00	3.00	3.00	3.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.05
# 9	InDegree	0.00	0.00	1.00	6.00	4.00	0.00	2.00	3.00	4.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.05
	Betweeness	0.00	0.00	0.00	19.33	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	OutDegree	4.00	5.00	6.00	7.00	2.00	0.00	6.00	6.00	6.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.15
# 10	InDegree	5.00	5.00	5.00	7.00	1.00	0.00	6.00	6.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.15
	Betweeness	0.00	0.00	0.00	10.20	0.00	0.00	0.00	0.00	10.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.02

(continues)

TABLE IV a (continuation)

	OutDegree	5.00	7.00	7.00	6.00	2.00	1.00	8.00	7.00	7.00	1.00	0.00	4.00	3.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	2.95
# 11	InDegree	6.00	5.00	8.00	6.00	3.00	1.00	9.00	7.00	7.00	0.00	0.00	4.00	2.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	2.95
	Betweeness	0.00	2.03	8.90	0.00	0.00	0.00	33.97	13.73	23.47	0.00	0.00	23.17	3.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.45
	OutDegree	2.00	4.00	2.00	2.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55
# 12	InDegree	2.00	3.00	3.00	2.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55
	Betweeness	1.00	7.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48
	OutDegree	1.00	4.00	4.00	2.00	0.00	0.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
# 13	InDegree	1.00	4.00	4.00	2.00	0.00	0.00	3.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
	Betweeness	0.00	9.00	7.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85
	OutDegree	6.00	7.00	8.00	6.00	5.00	1.00	7.00	7.00	4.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.60
# 14	InDegree	7.00	7.00	7.00	6.00	5.00	1.00	7.00	7.00	4.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.60
	Betweeness	1.48	9.48	10.57	1.50	0.00	0.00	16.40	2.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.10
	OutDegree	6.00	6.00	7.00	5.00	5.00	1.00	5.00	7.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.30
# 15	InDegree	7.00	7.00	7.00	6.00	3.00	1.00	4.00	7.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.30
	Betweeness	9.25	3.25	4.75	1.00	0.00	0.00	7.00	4.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50
	OutDegree	6.00	3.00	3.00	6.00	3.00	4.00	9.00	6.00	2.00	0.00	3.00	4.00	12.00	3.00	4.00	0.00	0.00	0.00	0.00	0.00	3.40
# 16	InDegree	5.00	6.00	5.00	5.00	3.00	3.00	8.00	6.00	2.00	0.00	3.00	4.00	9.00	4.00	5.00	0.00	0.00	0.00	0.00	0.00	3.40
	Betweeness	6.02	1.62	2.50	5.12	0.00	11.15	21.83	6.20	0.00	0.00	4.54	9.38	58.02	3.59	9.07	0.00	0.00	0.00	0.00	0.00	7.00
	OutDegree	2.00	5.00	8.00	4.00	1.00	0.00	6.00	5.00	2.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.75
# 18	InDegree	2.00	5.00	9.00	4.00	0.00	1.00	6.00	4.00	1.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.75
	Betweeness	0.00	3.50	25.67	0.00	0.00	0.00	11.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00
	OutDegree	6.00	7.00	7.00	8.00	5.00	1.00	8.00	8.00	6.00	0.00	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00
# 19	InDegree	6.00	7.00	7.00	7.00	5.00	1.00	9.00	8.00	6.00	0.00	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00
	Betweeness	1.00	1.50	1.50	10.07	0.00	0.00	12.17	4.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.52
	OutDegree	1.79	2.26	2.42	2.05	1.11	0.42	2.37	2.26	1.32	0.05	0.16	0.42	1.16	0.21	0.21	0.00	0.00	0.00	0.00	0.00	
	Betweeness	0.99	1.97	3.28	0.93	0.00	0.59	5.45	1.65	1.24	0.00	0.24	1.71	3.25	0.19	0.48	0.00	0.00	0.00	0.00	0.00	

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