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Spatio-temporal dynamics of soft-bottom fishes in the Professor Luiz Saldanha Marine Park:

Effectiveness of spatial protection and movement ecology of vulnerable species

UALG FCT
UNIVERSIDADE DO ALGARVE
FACULDADE DE CIÊNCIAS E TECNOLOGIA

2024

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Doutoramento em Ciências do Mar, especialidade em Ecologia Marinha

Degree in Doctor of Philosophy in Marine Sciences, specialty in Marine Ecology

Trabalho realizado sob a orientação de / Work carried out under the advisory of:

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2024

"The sea, the great unifier, is man's only hope. Now, as never before, the old phrase has a literal meaning: we are all in the same boat."

Jacques-Yves Cousteau

Título da Tese: Spatio-temporal dynamics of soft-bottom fishes in the Professor Luiz Saldanha Marine Park: Effectiveness of spatial protection and movement ecology of vulnerable species

Declaração de autoria de trabalho:

Declaro ser o(a) autor(a) deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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DATE: 2024

THESIS TITLE: Spatio-temporal dynamics of soft-bottom fishes in the Professor Luiz Saldanha Marine Park: Effectiveness of spatial protection and movement ecology of vulnerable species

Abstract

This thesis explores the effectiveness of the Professor Luiz Saldanha Marine Park (PLSMP) in Portugal, a coastal Marine Protected Area (MPA) in the north-eastern Atlantic, for conserving and managing soft-bottom fish species, including several elasmobranchs. The approach employed several methodologies, mainly experimental fishing and acoustic telemetry, to provide a comprehensive understanding of species' responses to spatial protection measures.

The work presented in Chapter I investigates the response of soft-bottom fishes to spatial protection within the PLSMP. Specifically, analyses of abundance, biomass, and total lengths of these fishes during and after the implementation of the MPA's management plan are presented. Results suggest that the community is primarily structured by depth and substrate, followed by protection level. Community composition analyses demonstrated significant differences between protection levels and over time. It is hypothesised that species-specific factors, such as habitat preferences or body size may influence the potential of benefiting from spatial protection. Overall, the chapter reports some evidence of the protection's effectiveness, based on the increased biomass in protected areas and the positive trends of certain species.

This thesis also dedicates particular focus on the study of skates, given their vulnerability and conservation concerns. Chapter III focuses on the thornback ray (*Raja clavata*). The PLSMP offered a valuable case study for assessing the effectiveness of spatial protection for this species. Using experimental fishing, conventional tagging, and acoustic telemetry, the study found that thornback rays are more abundant in the higher protection areas, and several individuals were classified as residents in the area (acoustic telemetry data).

Significant increases in adult and juvenile abundance over time were observed, although juveniles also exhibited a decrease in the later years of sampling. Acoustic telemetry data suggest seasonal trends, with higher residency in spring and summer and expanded utilization areas in autumn. Potential vulnerabilities are highlighted, such as the mature males preference for the offshore area of the marine reserve, and frequent excursions to outside the protected area, suggesting some susceptibility to fishing.

Chapter IV examines the spatial dynamics of the endangered undulate ray (*Raja undulata*) within the PLSMP. Acoustic telemetry data collected over three years indicated variability in residency among individuals. High site fidelity was observed in half of the tracked individuals, and the species seems to equally use both offshore and inshore areas within the MPA. Evidence is that both males and females may be recurrent users of this protected area. Compared with other ray species (Chapter III), the undulate ray seems to undertake less excursions to outside the MPA. These results suggest that the PLSMP may offer protection benefits for this species. However, adequate management measures for the conservation of the undulate ray are still depending on further research, to further understand its seasonal patterns and its patchy distribution.

Chapter V presents preliminary insights into the movement patterns of the regionally critically endangered white skate (*Rostroraja alba*). Acoustic telemetry was used to monitor three individuals in the PLSMP. The mature female exhibited high residency, spending over 70% of the time within the marine park, while the two immature individuals were detected for shorter periods. The study's findings, although limited by the small sample size, provide useful suggestions for future research on this poorly understood species.

In the current era of challenges for the marine environment, it is important to use MPAs as a tool to preserve marine biodiversity, including soft-substrate fish species. Overall, the findings presented in this thesis emphasize that the PLSMP has the potential to provide effective protection for the soft-bottom fish communities. The findings regarding the skates case studies also indicate that the PLSMP may offer protection benefits to these species. Nevertheless, the aspect of habitat continuity should be taken into account in the management of this and other MPAs aiming to protect soft-bottom communities. Furthermore, to apply fishing effort regulation measures in the adjacent areas of the marine reserve would likely enhance protection effectiveness. Other important factors for the success of the PLSMP and other MPAs include the integration of management institutions and stakeholders, as well as continuous monitoring of ecological and social indicators. The obtained data, along with previous work, underscore the importance of implementing measures to improve MPA effectiveness. To promote compliance through surveillance and stakeholder engagement is expected to contribute positively to the success of the PLSMP. This MPA is located in an important area to support connectivity of coastal habitats along the Portuguese coast and beyond. Adopting an adaptive approach for the management of the PLSMP would likely enhance its ability to contribute to the long-term sustainability and connectivity of marine ecosystems.

Keywords: Marine Protected Areas, reserve effect, Arrábida, soft-bottom fishes, Rajidae, experimental fishing, spatial ecology

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DATA: 2024

TÍTULO DA TESE: Dinâmica espaçotemporal dos peixes de substrato móvel no Parque Marinho Professor Luiz Saldanha: Efeitos da proteção espacial e estudo dos padrões de movimento de espécies vulneráveis

Resumo

Esta tese analisa a contribuição do Parque Marinho Professor Luiz Saldanha (PMPLS), uma Área Marinha Protegida (AMP) na costa oeste portuguesa, para a conservação e gestão de espécies de peixes de substratos móveis (e.g., areia e vasa), incluindo algumas espécies de elasmobrânquios. Nesta abordagem, foram usadas várias metodologias, nomeadamente pesca experimental e telemetria acústica, de modo a proporcionar um entendimento abrangente das respostas destas espécies às medidas de proteção espacial.

O trabalho apresentado no Capítulo I investiga a resposta da comunidade de peixes de substratos móveis à proteção espacial dentro do PMPLS. Especificamente, são apresentadas análises de abundância, biomassa e comprimentos médio das espécies, fazendo uma comparação entre o período de implementação do parque marinho com a fase após a sua implementação total. Os resultados sugerem que a comunidade é estruturada principalmente pela profundidade e substrato, sendo o nível de proteção das áreas amostradas também um fator significativo. As análises da composição da comunidade demonstraram diferenças significativas entre os níveis de proteção e ao longo do tempo. Coloca-se a hipótese de que fatores intrínsecos às espécies, como preferências de habitat ou tamanho máximo, possam influenciar seu potencial de beneficiar da proteção espacial. No geral, neste capítulo relatam-se algumas evidências da eficácia da proteção, com base no aumento da biomassa nas áreas de maior nível de proteção, e nas tendências positivas de certas espécies.

Esta tese dedica também particular foco ao estudo das raias, dada a sua vulnerabilidade aos impactos antropogénicos. O Capítulo III aborda o estudo da raia-lenga (*Raja clavata*). O PMPLS oferece as condições propícias para avaliar a eficácia da proteção espacial para

esta espécie. Utilizando pesca experimental, marcação convencional e telemetria acústica, verificou-se que esta espécie é mais abundante nas áreas de maior proteção, e vários indivíduos foram classificados como residentes na área (dados de telemetria acústica). Foram observados aumentos significativos da abundância de adultos e juvenis, embora os juvenis também tenham exibido uma diminuição nos últimos anos de amostragem. Os dados de telemetria acústica sugerem tendências sazonais, com maior residência na primavera e verão e maiores áreas de utilização no outono. Potenciais vulnerabilidades são identificadas, como a preferência dos machos maduros pela área exterior da reserva marinha, de onde fazem frequentes excursões para fora da área protegida. Este aspeto sugere alguma suscetibilidade à pesca.

O Capítulo IV examina a dinâmica espacial da raia-curva (*Raja undulata*) dentro do PMPLS. Os dados de telemetria acústica recolhidos ao longo de três anos indicaram variabilidade individual no grau de residência. Metade dos indivíduos monitorizados foram considerados residentes na zona de estudo, e a espécie parece usar de modo equitativo as áreas interiores e exteriores desta AMP. As evidências sugerem que tanto machos quanto fêmeas podem ser utilizadores recorrentes desta área protegida. Comparada com outras espécies de raias (Capítulo III), a raia-curva parece realizar menos excursões para fora da AMP. Estes resultados sugerem que o PMPLS pode oferecer benefícios de proteção para esta espécie. No entanto, medidas de gestão adequadas para a sua conservação dependem de mais estudos para entender os seus padrões sazonais e a sua distribuição.

O Capítulo V apresenta dados preliminares sobre os padrões de movimento da raia-tairoga (*Rostroraja alba*), uma espécie ameaçada. A telemetria acústica foi utilizada para monitorizar três indivíduos na área do PMPLS. A fêmea adulta exibiu elevada residência à zona de estudo, e passou mais de 70% do tempo de monitorização dentro da reserva marinha, enquanto os dois indivíduos imaturos foram detectados por períodos mais curtos. Os dados recolhidos, embora pouco conclusivos pelo pequeno tamanho da amostra, possibilitam deixar sugestões para pesquisas futuras sobre esta espécie pouco estudada.

Na atual era de desafios para o ambiente marinho, é importante usar as AMPs para a preservação da biodiversidade marinha, incluindo espécies de substratos móveis. No geral, as descobertas apresentadas nesta tese enfatizam que o PMPLS tem o potencial de fornecer proteção eficaz para as comunidades de peixes de substratos móveis. As descobertas nos casos de estudo das raias também indicam que o PMPLS pode oferecer benefícios de proteção para estas espécies. No entanto, a continuidade do habitat é um aspeto a ser tido em conta na gestão desta e de outras AMPs que visem proteger

comunidades de substratos móveis. Além disso, a aplicação de medidas de regulamentação do esforço de pesca nas áreas adjacentes à reserva marinha provavelmente aumentaria a eficácia da proteção. Outros fatores importantes para o sucesso do PMPLS e de outras AMPs incluem a integração das instituições de gestão e dos utilizadores, sendo também importante a monitorização contínua de indicadores ecológicos e sociais. Os dados obtidos, juntamente com trabalhos anteriores, sublinham a importância de implementar medidas para melhorar a eficácia desta AMP. Promover o cumprimento das medidas de gestão através da vigilância adequada e do envolvimento dos utilizadores iria contribuir positivamente para o sucesso do PMPLS. Esta AMP está localizada numa área importante para a conectividade dos habitats costeiros ao longo da costa portuguesa. Adotar uma abordagem adaptativa para a gestão do PMPLS iria provavelmente ser útil para aumentar a sua contribuição para a sustentabilidade e conectividade dos ecossistemas marinhos.

Palavras-chave: Áreas Marinhas Protegidas, efeito de reserva, Arrábida, peixes de substratos móveis, Rajidae, pesca experimental, ecologia espacial

Support

This study was funded by the Foundation for Science and Technology – Portugal (FCT): PhD grant (SFRH/BD/80771/2011). Part of the data used in the study was collected during the projects LIFE-BIOMARES (LIFE06 NAT/P/000192) and BUFFER (ERA-Net BiodivERsA). Another part of the data was obtained within the Raias Arrábida project. The Norwegian Institute of Marine Research kindly provided equipment and advice (Dr. Esben Moland Olsen and Dr. Even Moland). Arrábida Natural Park offered logistic support.

Apoio

Este estudo foi suportado através de uma bolsa de Doutoramento concedida pela Fundação para a Ciência e Tecnologia (FCT): Bolsa de Doutoramento (SFRH/BD/80771/2011). Parte dos dados utilizados neste estudo foram obtidos no âmbito dos projetos LIFE-BIOMARES (LIFE06 NAT/P/000192) e BUFFER (ERA-Net BiodivERsA). Outros dados foram obtidos durante o projeto Raias Arrábida. O Instituto Norueguês de Investigação Marinha gentilmente forneceu equipamento e orientações (Dr. Esben Moland Olsen e Dr. Even Moland). O Parque Natural da Arrábida ofereceu apoio logístico.

Acknowledgements

This thesis would not have been possible without the invaluable contributions of many individuals and institutions.

I am particularly grateful to my PhD advisors, Dr. Karim Erzini, Dr. Jorge Gonçalves and Dr. Emanuel Gonçalves, for accepting to be my advisors, for all the support and valuable advice provided throughout the course of this thesis.

I am grateful for the numerous learning opportunities I have had during my work and academic journey. Among others, I'm thankful to Joachim Claudet, Esben M. Olsen, Even Moland and Alexandra Cunha, for providing learning opportunities and for their willingness to share knowledge. I would also like to thank Pedro Afonso for the valuable advice in the early stages of fieldwork.

A big thanks goes to my colleagues from the Coastal Fisheries Research Group (CFRG), for all their support and assistance: Frederico Oliveira, Pedro Monteiro, Luís Bentes, Carlos Afonso, Rui Coelho, Pedro Veiga, Adela Belachova, Nuno Sales Henriques, Samira, João Pontes, Miguel Mateus, Ana Marçalo, Isidoro Costa, among others. Thank you all for being good colleagues but also wonderful friends. I am also grateful to Bárbara Horta e Costa for the enriching collaborative experiences. And Mafalda, thank you for your willingness to listen during difficult times.

In Arrábida, I owe a special thanks to Dr. Miguel Henriques from ICNF, always available to provide advice and logistical support. Among the Arrábida Natural Park staff, I am also particularly grateful to Carlos Silva, who was always in good mood and available to help.

I am particularly grateful to the juries of the Sesimbra Scientific Prize (2012 and 2019) for their recognition, and to Câmara Municipal de Sesimbra and Turifórum for their support and funding.

A special mention goes to Mestre Quinel, a passionate fisherman with a remarkable hospitality. I would also like to thank the students and colleagues who participated in the experimental fishing surveys: Daniela Rosa, João Rodrigues, Marie Renwart, Ana Salomé, Francisco Pires, Joana Dias, Sarah Laura Simons, Elisabeth Debusschere, Leonel Gonçalves, Rita Abecasis, David Abecasis, Sandra Fernandes, Pedro Vieira, Ana Coelho, Leila Pereira, Diana Minguéns, Sara Pereira, Maria Teresa Dias, Carina Caldeira, Catarina Português, Helena Abreu, Elena Troncoso, Miso Pavicic, Ivana Juric, Filipe Henriques, Rodrigo Sengo, Alejandro Usategui, Linn Sekund, Katja Geiger, among others. The ISPA

team was also often supportive: Diana Rodrigues, Sandra Fernandes, Gustavo Franco, Henrique Folhas and Pedro Coelho.

I am also thankful to all those who assisted in the tagging campaigns and acoustic range tests: Sara Cabral, Susana Santos, Joffrey Baeyaert, Gonçalo Silva, Sofia Tristancho, Laura Muñoz, Claudia Cores, Mauro Hilário, Virginia Reina, Lina Kabbadj, Rodrigo Delgado, Nestor Guerra, Lier Yeo, Silvia Tavares, and David Milla.

A group of kind and skilled divers helped me with acoustic data retrieval, to whom I am grateful: Diogo Paulo, Paulo Vilarinho, Sérgio Pedro, Fernando Agapito, Pedro do Canto, João Holtremann, Joaquim Alves and Frederico Almada. To Mário Rolim, a huge thank you for all your support! Your skills to help are remarkable, and you're a great friend to have around either below or above water!

I must acknowledge all my friends scattered in places where I've been happy, mostly Algarve, Lisbon, Porto and Azores. Thank you for guiding me to learn in hard times and for the shared joy during good times. I must also mention my furry friends, whose companionship has been crucial in making my life lighter.

I am very thankful to my family, my brothers who are great companions. I am especially grateful to my mum and dad, for being tireless in their love and for unconditionally believing in me. And to my grandmother Guilhermina, for being a role model of strength, caring and loving.

Finally, I am truly grateful to Nuno. I could not ever thank you enough for the sharing of kindness and craziness in the perfect amounts. Thank you for making my life brighter in every way.

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CHAPTER I

General Introduction

1. General Introduction

The oceans play a critical role in supporting life on Earth. They provide a wide range of essential goods and services that sustain human life on our planet, from producing approximately 40% of the oxygen, to absorbing and storing carbon dioxide, while regulating climate and providing food resources (UNESCO 2017). Over the last decades, it has become common knowledge that human impacts can cause drastic degradation of marine ecosystems, and that these impacts have been increasing in magnitude, diversity and rates of change (Jackson et al. 2001, Palumbi et al. 2008). Worldwide, scientists have documented the devastating effects of overexploitation (Pauly et al. 2005), habitat loss (Gray 1997), pollution (Jackson et al. 2001), and invasive species, among others. Moreover, it has become apparent that the marine environment must now face climate change as an additional stressor (Rau et al. 2012, Cheung et al. 2013, Jacquemont et al. 2022). Regarding overfishing, it has been a prominent issue since the mid-twentieth century (Alverson & Paulik 1973, Alverson & Dunlop 1998, Pauly et al. 2000). According to the FAO's latest report, the percentage of overfished stocks has been increasing since the 1970s, from 10% in 1974 to 35.4% in 2019 (FAO 2022). The concerns raised by these factors have prompted calls for the implementation of measures to protect, preserve and restore marine ecosystems (Lubchenco et al. 2003).

In the recent decades, there has been an increase in the urge to turn fishing into a sustainable activity, in order not only to ensure the prosperity of this resource as a food supply, but also to protect biodiversity and consequently allow for the recovery of several species from extremely low levels of abundance and potential extinction (FAO 2012). Intending to achieve these goals, different options are under discussion and test, among which the implementation of Marine Reserves and Marine Protected Areas (MPAs) is one of the most common options (Glenn et al. 2010).

1.1. Marine protected areas

Marine Protected Areas (MPAs) are presently among the main tools used for conservation and spatial management (Claudet et al. 2006, Guidetti et al. 2008, Lester et al. 2009). These are areas that are primarily designated to preserve marine biodiversity and ecosystems, and they often seek the recovery of threatened or overfished species, by restoring and maintaining their natural balance (Glenn et al. 2010). According to the IUCN (International

Union for Conservation of Nature) definition (Dudley 2008), a protected area is “a clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values. In light of this definition, a marine protected area should have nature conservation as its primordial goal. Specifically, MPAs are frequently established with the purpose of preserving and restoring marine biodiversity and habitats, often while also aiming to contribute to the recovery of fish stocks (Pauly et al. 2002, Russ & Alcala 2004, Batista et al. 2011). However, areas with the primary goal of managing human activities should not be classified as MPAs (Dudley 2008, Grorud-Colvert et al. 2021).

The services provided by MPAs, such as ensuring balanced natural systems and assuring livelihoods, have become increasingly important in the context of a changing climate (Predragovic et al. 2024). Well-managed MPAs may help mitigate the adverse effects of climate change by providing refuges for marine species, enhancing biodiversity, and increasing the resilience of populations to climate-induced stressors (Roberts et al. 2017). Additionally, MPAs can serve as natural laboratories for studying climate impacts and testing adaptive management strategies (Zentner et al. 2023).

It is now broadly accepted that strategies need to be adopted to increase the oceans’ resilience to the cumulative challenges they are confronting today (Gallo et al. 2017, IPCC 2022, Jacquemont et al. 2022). Within the variety of area-based management strategies, recent debate has evolved around other tools. Even though not regarded as MPAs, fisheries management areas and other effective area-based conservation measures (OECMs) may confer some protection benefits due to implemented restrictions on human uses (Laffoley et al. 2017, Vilas et al. 2021, Grorud-Colvert et al. 2021). There is presently some debate surrounding OECMs, often around their definitions, effectiveness, and the extent to which they contribute to global conservation targets (Reimer et al. 2020). OECMs should be regarded carefully in the achievement of global conservation goals because their effective management vary widely, often leading to overestimations of protected area coverage without ensuring long-term conservation outcomes (Grorud-Colvert et al. 2021, Garcia et al. 2022).

In addition, there is also a recognized need to evaluate the effectiveness of MPAs beyond simply assessing their primary objectives or measuring spatial coverage (Horta e Costa et al. 2016, 2019, Gill et al. 2017). The design, management and protection level of MPAs can vary significantly depending on the conservation goals they aim to accomplish, and these are often conditioned by the biological, physical and social characteristics of the target area (Day et al. 2019). MPAs can be broadly categorized into two groups (Sala & Giakoumi 2018). The first group is referred to as ‘no-take MPAs’, also known as marine reserves or sanctuary zones, and includes areas where all types of extractive activities such as fishing, harvesting, mining, and habitat destruction are completely prohibited. The second group, known as ‘partially protected areas (PPAs)’, includes areas with less restrictive rules where some human activities are allowed, typically some forms of resource extraction. MPAs can either be entirely no-take or completely PPA, or they can include multiple management zones, with various levels of protection and regulations (Sala & Giakoumi 2018).

Worldwide research on MPAs supports that fully protected or highly regulated areas provide greater effects on the replenishment of fish populations (e.g,Tetreault & Ambrose 2007, Caselle et al. 2015). However, many existing MPAs still allow damaging activities that undermine conservation goals (Sciberras et al. 2015, Horta e Costa et al. 2016, Zupan et al. 2018). For MPAs to be effective, several aspects must be met. They need detailed objectives and management plans (Pomeroy et al. 2004, Batista et al. 2014), monitoring and evaluation of relevant indicators (Batista et al. 2011, Leleu et al. 2012, Cardoso-Andrade et al. 2022, Gonçalves 2023), and effective enforcement (Claudet et al. 2008, Campbell et al. 2012, Edgar et al. 2014, Bergseth et al. 2023). The engagement of local communities in participation and support from early stages is also a key aspect (Vasconcelos et al. 2013, Horta e Costa et al. 2022, Guimarães et al. 2023, Rangel et al. submitted). Furthermore, information should be continuously incorporated back into the planning process to refine objectives, plans and outcomes if needed (Day 2008, Castro & Cruz 2009, Batista et al. 2014).

Research has also shown that the variability of ecosystem responses plays a crucial role in determining the effectiveness of MPAs. Ecological traits like longevity, size, growth rate, and reproductive potential influence how species respond to exploitation pressures, and additionally, they also play a role in species recovery potential (Claudet et al. 2010, Batista et al. 2015a, Audzijonyte & Kuparinen 2016).

1.2. MPAs and soft-bottom fishes

Although the number of studies focusing on the effects of MPAs as tools for conservation and fisheries management has increased in the past decade, there is still a gap of knowledge regarding its effectiveness regarding soft-bottom species. Among the species that inhabit soft substrates, many have high commercial value such as soles and other flatfish. Other species are vulnerable and any further information on effective conservation strategies is highly valuable.

The response of soft-bottom fish communities to spatial protection has been relatively underexplored, possibly due to the fact that most MPAs focus on reef-associated species and the unsuitability of standard sampling methods, such as underwater visual census, for soft bottom habitats where fish densities tend to be low (Giakoumi & Kokkoris 2013).

Soft-bottom species have been assessed in some general evaluations of marine protected areas, such as those conducted by Claudet et al. (2008), Guidetti & Claudet (2010), and Fetterplace (2017), while others have focused on individual species, including Hunter et al. (2005, 2006), Wiegand et al. (2011), Silva et al. (2013), Abecasis et al. (2014) and Fetterplace et al. (2016). Piet & Rijnsdorp (1998) found that the "plaice box," an area in the North Sea with seasonal fishing restrictions, led to larger fish populations. Similarly, fishing closures in Georges Bank and southern New England were beneficial to several species of flounders and skates in the Western Atlantic (Murawski et al. 2000), while a trawling closure in the Scotian Shelf resulted in significant increases in the abundance of winter flounder (*Pseudopleuronectes americanus*) and other fishes (Fisher & Frank 2002). In a Mediterranean marine protected area, Pipitone et al. (2000) reported increases in the abundance and biomass of some soft-bottom fish species (e.g., *Mullus barbatus* and *Lepidotrigla cavillone*) after a trawl ban.

A specific group of soft-bottom fishes that raises conservation concerns is the order Rajiformes. Broadly known as rays, Rajiformes are a group of cartilaginous fish with a flattened disc-shaped body. The order includes around 500 species, making it one of the largest groups of cartilaginous fish (Ebert & Sulikowski 2009, Siskey et al. 2019). Rays are present worldwide, from abyssal regions to coastal waters and depending on the species, can live on soft bottoms, coarser substrata and either on the continental shelf or the upper slope (Ebert & Sulikowski 2009, Serra-Pereira 2010). As elasmobranchs, their life-history traits, such as late maturation, low fecundity, slow growth rates, and long life spans, make them particularly vulnerable to over exploitation (Frisk et al. 2005, Dulvy et al. 2014, Liu et al. 2021).

Within the order Rajiformes, the family Rajidae encompasses 313 recognized species, and new species are still being discovered. Commonly known as skates, they are marine mesopredators, highly adapted to the benthic environment (Ebert & Sulikowski 2009). Many species are commercially important, mostly as food, but some species are also popular in the aquarium trade. Despite their commercial importance, skates have received limited research attention, and the status of populations remains largely unknown in most areas of the world (Dulvy & Forrest 2010, Siskey et al. 2019). The IUCN reports that at present, skate species face significant threats, with around 14% of the species classified as Threatened (Critically Endangered, Endangered or Vulnerable), 8.5% categorized as Near Threatened, and 29.2% of Least Concern. Some species are considered critically endangered in the Northeastern Atlantic (e.g., *Dipturus batis* complex, *Rostroraja alba*) (Dulvy et al. 2006, IUCN 2024). A staggering 48% of the existing Rajidae species are either Not Evaluated or listed as Data Deficient, highlighting the lack of critical knowledge regarding this group (Siskey et al. 2019, IUCN 2024). This knowledge gap can hinder efficient conservation and protection efforts, particularly for skates impacted by fishing.

In Portugal, the Rajidae group is commercially important and has been the dominant elasmobranch group in landings since at least 1986 (Alves et al. 2020). The official landings have been commonly registered under the common category ‘rays’ (raias, *Raja* spp.) (Figueiredo et al. 2007). Research showed that this generic terminology includes at least eight different species: *Leucoraja naevus*, *Raja brachyura*, *Raja clavata*, *Raja microocellata*, *Raja miraletus*, *Raja montagui*, *Raja undulata* and *Rostroraja alba* (Bordalo-Machado et al. 2004). To properly inform managers, some efforts have been made to report data at species level, but considerable levels of misidentification still occur (Figueiredo et al. 2020). Furthermore, the European Union (EU) applies a generic Total Allowable Catch (TAC) for all Rajiformes combined, even though scientific advice is generally provided at species level (European Commission. Joint Research Centre. 2017). Concerns about the exploitation status led the EU to include in 2009 some species in the list of prohibited species: *R. undulata* (undulate ray), *Dipturus batis* (common skate), and *Rostroraja alba* (white skate). These are species that cannot be landed or retained on board and should be returned to the sea immediately after capture. Additional national measures adopted for this group include a fishing ban during May and June, and a minimum landing size of 52 cm (all species of the genera *Raja* and *Leucoraja*) (Serra-Pereira et al. 2018). Ultimately, it is widely acknowledged that appropriate information and legislation to support management at species level is largely lacking.

1.3. Assessing the effectiveness of MPAs

1.3.1. Experimental fishing

The response of soft-bottom fish communities to protection efforts has been relatively understudied. This lack of research is likely due to two main factors: the fact that many MPAs focus on reef-associated species; and the unsuitability of standard underwater visual census methods for surveying these low-density habitats. As an alternative to standard census techniques, experimental fishing may provide useful insights into fish communities in soft-bottom environments. This methodology relies on the use of conventional fishing gear implemented through a standardized experimental design (Sánchez-Lizaso & Bayle 2000). It enables the estimation of catch per unit effort (CPUE), which has been widely used as an index of relative abundance (Sánchez-Lizaso & Bayle 2000). Biomass estimates can also be obtained by weighing the catch or by measuring fish length and applying established length-weight relationships available in literature. In the context of MPA monitoring, it may be applied to test expected gradients of abundance in time and space, as it is done using the before-after-control-impact (BACI) design (Underwood 1992, 1994). Samples from control zones in fished areas are collected to enable comparisons with the protected zone, and temporal replicates are also obtained (Sánchez-Lizaso & Bayle 2000, Quintella et al. 2019). Both passive (e.g., nets, traps, longline) or active gear (e.g., trawl, purse seine) may be used. However, experimental fishing carries certain limitations, such as the potential mortality, making it unsuitable to sample frequently or in specific areas. Catch-and-release methods may be employed, and mortality rates significantly differ depending on the fishing gear.

The use of trammel nets has demonstrated its suitability for capturing benthic and demersal fish (Erzini et al. 2006). The catch is typically composed of a considerable diversity of species (Gonçalves et al. 2007), with a predominance of adult individuals (Salvanes 1991). If used on soft substrate, this fishing gear may have low impact on the bottom (Depestele et al. 2012). It has a moderate mortality rate, but if used appropriately, this impact may be mainly on small individuals of low commercial value (Priester et al. 2021). On-board sampling is common, which allows catch-and-release practices (Sousa et al. 2018). Mortality depends on the species (e.g., pelagic species are more sensitive), depth (probability of barotrauma), hauling time (sunrise hauling can reduce the time fish remained entangled), and soak time (should not exceed 24 hours) (Castro et al. 2021). Another factor to highlight is the impact of necrophagous organisms, often amphipods, isopods or crabs, which can feed on the entangled fish. These factors should be considered in order to minimize the associated mortality (Castro et al. 2021). Overall, it is an efficient technique for studying areas with low organism density, as often occurs in soft substrate habitats.

1.3.2. Passive acoustic telemetry

The role of mobility has proven to be a relevant factor in the potential for species to benefit from spatial protection (Finger et al. 2017, Villegas-Ríos et al. 2018, Thorbjørnsen et al. 2021). Understanding which areas are consistently used, the range of displacement and migratory patterns helps in identifying habitat preferences and vital areas for the species' life cycle (essential fish habitat) (Abecasis et al. 2014, Brownscombe et al. 2022). Consequently, spatial and movement ecology of fish species is a fundamental component of MPA design (Afonso et al. 2008, Moffitt et al. 2009, Abecasis et al. 2014). Over the past century, mark-recapture methods provided the first systematic approach to understanding the movement patterns of fish (Borley 1916, Walker 1997). The method involves attaching a numbered tag to the individuals caught and then returning them to the water. This enables to infer movements of tagged animals based on reports of their recaptures. However, the information provided has limited detail and relies on fishermen to report recaptures, thereby neglecting areas with less fishing activity (Bolle et al. 2005).

During the last decades, aquatic telemetry has emerged as a prominent approach for advancing the study of spatial and movement ecology. The technique involves tagging animals with electronic devices ('transmitters' or 'tags') that transmit data to remote stations. These differ from tags that only record data (biologgers; e.g., DST - data storage tags) (Rutz & Hays 2009). Tags may be attached externally, or they can be surgically inserted into the abdominal cavity. In the marine environment, telemetry is primarily based on two main approaches: satellite telemetry and acoustic telemetry (Hussey et al. 2015). Satellite telemetry uses satellite networks to track animals over vast distances and is particularly useful to track large megafauna known to migrate long distances (e.g., tunas, sharks, marine mammals). Satellite tags are prepared to log data, which is transmitted to the satellites when either the animal or the tag surfaces (Hussey et al. 2015). The technology involved in these tags results in them being expensive. Acoustic tags are programmed to emit sound pulses (acoustic signals) at specific frequencies, which are detected and recorded by acoustic receivers. The transmitted signal consists of a unique code that allows for the identification of the individual (Hussey et al. 2015). Tracking may occur manually by using a vessel to follow and locate a tagged animal (Stasko & Pincock 1977), which is commonly called active acoustic telemetry. Conversely, in passive acoustic telemetry, tag transmissions are detected by submerged omnidirectional receivers moored at fixed locations. The detection logs are stored by receivers and retrieved periodically (Heupel et al. 2006). The analysis of collected data is often used to estimate the most frequented areas and levels of site fidelity, i.e., the tendency of fish to return and remain in particular sites over time (e.g, Afonso et al. 2011, Espinoza et al. 2015, Abecasis et al. 2015a). Some of the other many applications are the assessment of activity rates (e.g, Almeida et al. 2013, Wright et al. 2014, Kneebone et al. 2018), social dynamics (e.g, Mourier et al. 2017, Aspillaga et al. 2021), and behavioural traits (e.g, Aspillaga et al. 2021, Villegas-Ríos et al. 2021).

1.4. Study area: the Prof. Luiz Saldanha Marine Park

The study area is located on the Portuguese coast, between 38° and 39° North and 8° and 10° West (Figure 1.1). Most of this coastal area faces south and is sheltered from prevailing winds by the Arrábida mountain chain (Figure 1.2). Steep rocky cliffs dominate the coastline, and areas with boulders and small beaches are also found (Henriques et al. 1999). The rocky substrate extends into the subtidal zone, transitioning to soft sediments at depths greater than 15-20m (Henriques et al. 1999, 2015). At the western zone near Cape Espichel, the rocky bottom extends up to 40m depth. The area is influenced by upwelling events (Wooster et al. 1976), and water temperature commonly ranges from about 13°C in January to 21°C in September (Almada et al. 1990). Located in a transition zone where cold and warm water currents converge, the area is considered an important ‘barometer’ for studying the effects of climate change (Horta e Costa et al. 2017). The occurrence of species of different temperature affinities (Henriques et al. 1999, Gonçalves et al. 2003), the wide variety of habitats and benthic communities (Henriques et al. 2015), and the proximity of the Sado and Tejo estuaries, all contribute to enhancing the local marine biodiversity (Saldanha 1974, Henriques et al. 1999, 2007, Gonçalves et al. 2003, Cunha et al. 2014) Fisheries represent a vital economic activity in the region (Horta e Costa et al. 2013a c). Two important fishing harbours are located in this region: the port of Sesimbra, located within the MPA area, and the nearby port of Setúbal. The area is also used for other human activities, such as recreational fishing and scuba diving.

The Prof. Luiz Saldanha Marine Park (PLSMP) was created in 1998 in this region. This marine protected area, also known as Arrábida Marine Park, encompasses 38km of coastline and 53km^2 of area. It is located adjacently to the terrestrial Arrábida Natural Park created in 1976. The marine park was established to protect the existing high natural values and to promote sustainable local activities. The management plan was only implemented in August 2005 (RCM 2005), establishing three types of zones with distinct protection levels (Figure 1.1). The designated zones include: a fully protected area (FPA, total area=4.3km^2), which is a no-take/no-go area, with the exception of monitoring, surveillance and education activities; four partially protected areas (PPAs, total area=21km^2) allowing non-extractive recreational activities and licensed commercial fishing using traps and jigs only beyond 200m from coast; and three buffer areas (BAs, total area=27.5km^2), where recreational activities and licensed commercial fishing are allowed, with the exception of commercial fishing vessels larger than 7m, spearfishing, purse-seining, trawling and dredging. Zones and specific regulations for fisheries were sequentially implemented during a transition period, being fully implemented only in August 2009 (Pita et al. 2020). According to the regulation-based classification system for MPAs developed by (Horta e Costa et al. 2016), the area is considered ‘highly protected' (score of 4.5, scale 1 to 8; 1 - fully protected; 8 - unprotected) (Horta e Costa et al. 2017).

Over the past thirty years, the marine biodiversity in the region has been studied both at the community level (e.g, Henriques et al. 1999, Gonçalves et al. 2003, Horta e Costa et al. 2013b, Batista et al. 2015a) and at the individual species level (e.g, Abecasis et al. 2013, 2015b, Rodrigues et al. 2015). The fisheries activities in the area have also been the focus of studies (e.g, Batista et al. 2011, 2015b, Horta e Costa et al. 2013c), along with the assessment of the social aspects related with conservation (e.g, Vasconcelos et al. 2012, Pita et al. 2020). Together with other studies, this research has contributed valuable insights to the effective management of this area, and globally highlighted the significant social and ecological importance of the region.

Besides its local importance, this area is also relevant at the national and global scale. It is classified as a Natura 2000 site and as OSPAR MPA, and thus it integrates the list of areas considered relevant for marine conservation at the European level (EC 2008). Occupying a central location on the Portuguese coast, this area is vital for sustaining ecological connectivity throughout the Iberian coastal environments. The Portuguese Ministry of the Sea recently translated its recommendations on spatial protection into a legal resolution outlining the guidelines to achieve an effective National Network of Marine Protected Areas (*Rede Nacional de Áreas Marinhas Protegidas* - RNAMP) (RCM 2019). Furthermore, the United Nations Convention on Biological Diversity recently adopted a framework, the Kunming-Montreal Global Biodiversity Framework (GBF), setting as a target that the MPA coverage should be at least 30% of the world's oceans by 2030. They also underline that in addition to areal coverage, these areas need to be "effectively conserved and managed" (CBD 2022). Given the pressing need to safeguard the oceans' environment at local and global levels, the contribution of the PLSMP is highly important.

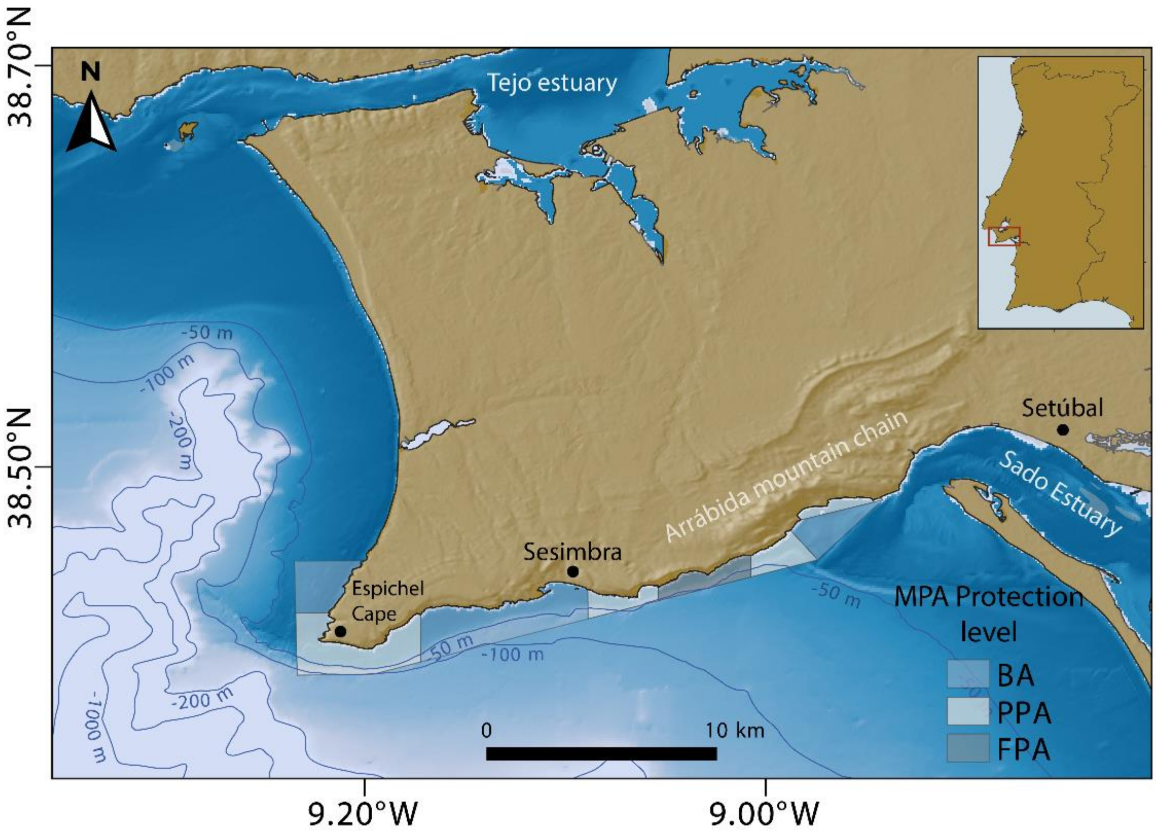

Figure 1.1. Location of the study area in the western coast of Portugal: the Prof. Luiz Saldanha Marine Park and its three protection levels - buffer area (BA), partially protected areas (PPA) and fully protected area (FPA).

Figure 1.2. View of the coastline in the study area (east of Sesimbra). The high cliffs are dominant along the shoreline in this region.

1.5. Objectives and thesis structure

The research outlined in this thesis was driven by the limited knowledge of how spatial management affects soft-bottom fish species. Hence, the primary aim of this study was to examine the effectiveness of spatial protection for soft-bottom fish species within this coastal MPA. For a comprehensive approach, this was addressed at two levels: through a broad assessment of the spatio-temporal abundance patterns of the fish community; and by studying spatial ecology with a focus on vulnerable species.

The specific aims of the thesis are as follows:

- Chapter II: Evaluate the effectiveness of protection of the PLSMP for the soft sediment fish community by analysing trends in abundance, biomass, and mean total length at different protection levels, from before to after the full implementation of the MPA (BACI experimental design);

- Chapter III: Investigate the potential of protection provided by the PLSMP for the thornback ray (*Raja clavata*; IUCN category 'Near Threatened'); specifically, by analysing if its abundance has increased over time within the MPA boundaries, whether globally or for different ontogenic groups (juveniles and adults); and also by examining the species' site fidelity to this protected area, its main utilization areas, dispersal patterns and seasonal dynamics;

- Chapter IV: Evaluate the potential of the undulate ray (*Raja undulata*; IUCN category 'Endangered') to benefit from protection within the PLSMP, by quantifying its site fidelity, utilization areas, and determining if these indicators of its spatial ecology vary according to season or sex;

- Chapter V: Obtain preliminary insights into the spatial ecology of the white skate (*Rostroraja alba*; IUCN global category 'Endangered', regional category Northeastern Atlantic 'Critically endangered'), focusing on its site fidelity and main utilization areas on this coastal MPA, in order to outline some future research directions on this species.

This thesis is structured in six chapters: the present chapter serves as the General Introduction (Chapter I), providing the background on the relevant themes and addressing the importance of the examined research questions; the four chapters in paper-style format (Chapters II, III, IV and V), which focus on the specific objectives detailed above; and the General Discussion (Chapter VI), the final chapter in which the main findings are revisited, some limitations of the study are identified, and possible future research directions are outlined.

1.6. List of manuscripts

Chapter II	Sousa, I., Gonçalves, J.M.S., Claudet, J., Coelho, R., Gonçalves, E.J., Erzini, K. 2018. Soft bottom fishes and spatial protection: findings from a temperate marine protected area. PeerJ 6:e4653
Chapter III	Spatial protection for the management of the thornback ray (*Raja clavata*): evidence from a coastal marine protected area. Sousa, I., Olsen, E.M., Moland, E., Gonçalves, J.M.S., Freitas, C., Bentes, L., Cabral, S., Gonçalves, E.J., Erzini, K.
Chapter IV	Spatial ecology and the suitability of a coastal marine protected area for the recovery of the endangered undulate ray (*Raja undulata*). Sousa, I., Moland, E., Olsen, E.M., Gonçalves, J.M.S., Villegas-Rios, D., Bentes, L., Gonçalves, E.J., Erzini, K.
Chapter V	Sousa, I., Baeyaert, J., Gonçalves, J.M.S., Erzini, K. 2019. Preliminary insights into the spatial ecology and movement patterns of a regionally critically endangered skate (*Rostroraja alba*) associated with a marine protected area. Marine and Freshwater Behaviour and Physiology, 52:6, 283-299

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CHAPTER II

Soft-bottom fishes and spatial protection: findings from a temperate marine protected area

Photo: Sara Pereira

Published in PeerJ, Aquatic Biology

Sousa, I., Gonçalves, J.M.S., Claudet, J., Coelho, R., Gonçalves, E.J., Erzini, K. 2018. Soft--bottom fishes and spatial protection: findings from a temperate marine protected area. PeerJ 6:e4653

2. Soft-bottom fishes and spatial protection: findings from a temperate marine protected area

2.1. Abstract

Numerous studies over the last decades have focused on marine protected areas (MPAs) and their effects on fish communities. However, there is a knowledge gap regarding how species that live associated with soft substrates (e.g., sand, mud) respond to spatial protection. We analysed abundance, biomass and total lengths of the soft-bottom fishes in a multiple-use MPA in the north-eastern Atlantic, the Prof. Luiz Saldanha Marine Park (Portugal), during and after the implementation of its management plan. Data were collected by experimental fishing in areas with three different levels of protection, during the implementation period and for three years after full implementation of the MPA. Univariate analysis detected significant biomass increases between the two periods. Fish assemblages were mainly structured by depth and substrate, followed by protection level. Community composition analyses revealed significant differences between protection levels and between the two periods. Species exhibited a broad variation in their response to protection, and we hypothesize that factors such as species habitat preferences, body size and late maturity might be underlying determinants. Overall, this study provides some evidence of protection effectiveness in soft-bottom fish communities, supported by the significant increase in biomass in the protected areas and the positive trends of some species.

Keywords: soft-bottom fishes, marine protected areas, reserve effect, multivariate regression trees, catch per unit effort

2.2. Introduction

The marine environment is nowadays increasingly impacted by human activities, with overfishing reported worldwide (Myers & Worm 2003, Pauly & Palomares 2005). As a consequence, conservation of marine fishes currently holds unique challenges for fisheries managers (Vincent & Hall 1996, Roberts 1997). Marine protected areas (MPAs) are one of the key tools to implement ecosystem-based management and marine spatial planning (Gaines et al. 2010, Halpern et al. 2010). As areas with restrictions to human uses, MPAs can promote the preservation and recovery of habitats and marine species, and are increasingly being used as tools for conservation and fisheries management (Roberts & Polunin 1993, Gaines et al. 2010). Through the reduction of fishing mortality, these areas can enhance density, biomass, fish size and species diversity within their boundaries (Osenberg et al. 2011), which is often termed the ‘reserve effect’. Moreover, the transport of larvae (‘recruitment subsidy’) and movement of juveniles and adults (‘spillover’) to the outside areas are expected to increase fisheries yields (Gell & Roberts 2003, Grüss et al. 2011).

MPAs include marine reserves or fully protected areas (FPAs), where extractive activities are prohibited (no-take zones), and partially protected areas (PPAs), which allow multiple uses (Lubchenco et al. 2003). PPAs offer a broad spectrum of protection levels, allowing either many human activities or just a few and like no-take zones, they have the potential to enhance social and ecological benefits in coastal areas (Guidetti & Claudet 2010, Claudet & Guidetti 2010, Sciberras et al. 2013, 2015, Horta e Costa et al. 2016).

For the achievement of conservation and management goals, it is important to understand the ecological effects of MPAs and the factors underlying these effects. The effectiveness of MPAs for the recovery of fish communities is affected by spatial design (Halpern & Warner 2002, Claudet et al. 2008), enforcement (Byers & Noonburg 2007, Campbell et al. 2012, Bergseth et al. 2015), habitat heterogeneity (García-Charton & Pérez-Ruzafa 1999), species movement ecology (Chapman et al. 2005; Villegas-Ríos et al. 2017) and life history traits (Jennings et al. 1999, Claudet et al. 2010, Hutchings et al. 2012). Life history traits, such as longevity, maximum size, growth rate and reproductive potential, are important components of species responses to environmental stressors (e.g., temperature, toxicants, food availability) and fishing exploitation (Jennings et al. 1999, Denney et al. 2002, Hutchings et al. 2012, Audzijonyte & Kuparinen 2016). Species habitat preferences and inter-specific relations are acknowledged as important sources of complexity in spatial protection effectiveness, alongside with movement ecology (García-Charton & Pérez-Ruzafa 1999, Claudet et al. 2010). The fact that there is still no clear consensus on the influence of migratory behaviour as a determinant of protection effectiveness (Blyth-Skyrme et al. 2006; Claudet et al. 2010) illustrates this complexity.

Despite the challenges posed by the study of spatial management, MPAs have been shown to enhance the recovery and resilience of rocky (Claudet et al. 2006, Tetreault & Ambrose 2007, Sala et al. 2012) and coral reef fish (Russ & Alcala 2004, Bruce et al. 2012). However, there has been relatively little research on the response to protection of soft-bottom fish communities, probably because most MPAs target reef-associated species and because standard survey methods such as underwater visual census are regarded as not suitable for soft-bottom habitats, where fish densities are generally low (Giakoumi & Kokkoris 2013).

Some MPA assessments have included multiple soft-bottom fish species among extensive community studies (e.g. Dimech et al. 2008; Guidetti & Claudet 2010), and others addressed single soft-bottom species (e.g. Hunter et al. 2006, Wiegand et al. 2011; Silva et al. 2013, Abecasis et al. 2014). Piet & Rijnsdorp (1998) reported that the ‘plaice box’, an area in the North Sea with seasonal fishing restrictions, favored the occurrence of larger fish. In the Western Atlantic, Georges Bank and southern New England fishing closures proved beneficial to several species of flounders and skates (Murawski et al. 2000), and significant increases in the abundance of winter flounder (*Pseudopleuronectes americanus*) and other fishes were reported after implementation of a trawling closure on the Scotian Shelf (Fisher & Frank 2002). Pipitone et al. (2000) also described increases in the abundance and biomass of some soft-bottom fishes (e.g. *Mullus barbatus* and *Lepidotrigla cavillone*) after a trawling ban in a Mediterranean MPA. More recently, Donovan et al. (2016) reported the effects of a gillnet ban in a sandy coastal area of Hawai‘i, and highlighted the variability of response according to species group, as only bonefishes (*Albula* spp.) exhibited a positive trend.

In this study we assessed the response to protection at the community and species level of the soft-bottom fish assemblage in the Prof. Luiz Saldanha Marine Park (PLSMP), a coastal MPA in Portugal with predominantly soft-substrates (Henriques et al. 2014). Data were collected using standardized fishing procedures (trammel nets) inside and outside the marine reserve, over the implementation period and after the MPA was fully established. We used univariate and multivariate methods to analyze trends in community composition, fish abundance and biomass, and mean total length per species according to protection level, from before to after the full implementation of the MPA.

2.3. Methods

2.3.1. Study area

Located on the western coast of Portugal, the Prof. Luiz Saldanha Marine Park (PLSMP) comprises 38km of coastline characterized by high rocky cliffs alternated by sheltered bays. With an area of 53km^2, the park includes many sublittoral habitat types, including hard and soft substrates. Soft bottoms replace the coastal rocky reefs in most of the area, at depths greater than 15-20m. A substantial mud component is found in the sediment at depths greater than 30m, while at shallower depths the dominant substrate is sand (medium, coarse and gravely sand) (Henriques et al. 2014). One interesting aspect to point out is the wide variety of soft-substrate habitats and benthic communities found in this area, as described by Henriques et al. (2014). The area has two nearby estuaries (Sado and Tejo) and is next to the Arrábida mountain chain, which shelters it from the dominant north winds. This region is also a biogeographic and oceanographic transition zone between warm and cold temperate waters (Henriques et al. 1999, Lima et al. 2007) which, along with the variety of available habitats (Henriques et al. 2014), makes it an important biodiversity hotspot (Henriques et al. 1999, Gonçalves et al. 2002).

The MPA was created in 1998 as part of the Arrábida Natural Park, but it was only in August 2005 that the management plan started to be implemented. The management regulation main goals are to enhance the conservation of marine species and promote sustainable fisheries management (Gonçalves et al. 2002). Given the diversity of human activities, including recreational fishing, scuba diving and commercial fisheries, and the presence of two important fishing harbours: the port of Sesimbra, located within the MPA area, and the nearby port of Setúbal, spatial management was crucial. To mitigate conflicts related to multiple stakeholders and their divergent concerns (Vasconcelos et al., 2013), the PLSMP regulations were established gradually over a four-year period and full implementation was achieved in August 2009 (implementation: 23rd Aug. 2005 - 23rd Aug. 2009; Year1: PLSMP area under BA protection level; Year 2: two PPAs were implemented; Year 3: two more PPAs were created, sampling started; Year 4: half of the FPA implemented; 23rd Aug. 2009 - the FPA is fully implemented). The regulations define different zones and levels of usage (Figure

2.1). Trawling, dredging, purse seining and spearfishing are prohibited in the entire area, and only licensed commercial vessels less than 7m long are allowed. The fully protected area (FPA) ($4km^2$) excludes all human activities, with the exception of research and monitoring. The four partially protected areas (PPA), totalling $21km^2$, allow some recreational activities, including recreational diving, and commercial fishing for cephalopods with octopus traps and jigs, although fishing is not allowed at distances less than 200 m from the shore. The three buffer areas (BA), totalling 28 km^2, have the lowest level of protection. In addition to octopus traps and jigs (here permitted close to the shore), longlines, trammel nets, gill nets and recreational fishing are allowed (nets at a distance beyond 463 m from the shoreline).

2.3.2. Data collection

Data were collected between November 2007 and May 2012 over a five years period: the first two years during the management plan implementation (period 1 - implementation period: August 2007-August 2009) and three years after the MPA was fully implemented (period 2 - after MPA full implementation: August 2009-August 2012). Sampling of the three protection levels occurred seasonally, each spring and autumn. Ten sampling campaigns (experimental fishing) and 183 trammel net sets were undertaken (Figure 2.1) (total number of samples: 179; four samples were excluded from the analysis due to problems with the fishing procedure). Sampling area covered the FPA (n=63), two adjacent PPAs (n=61) and the larger BA (n=59). To avoid spatial heterogeneity, areas in the eastern and western edges of the MPA were not included (west: proximity to cape; east: proximity to nearby estuary). Four sampling campaigns were undertaken during period 1 (n=57) and six during period 2 (n=126) (first three campaigns - 4 days each, 36 samples; following campaigns - 7 days each, total of 147 samples; three samples per day). Two substrate types were sampled: sandy bottom, at 10-20m (n=112), and muddy bottom, at 30-45m (n=71) (samples: sand - BA: 36, PPA: 38, FPA: 38; mud - BA: 23, PPA: 23, FPA: 25). The BA was considered as the control in the spatial analysis of protection effect due to the allowed use of small-scale fishing gear: gill nets, trammel nets, longlines, jigs, pots and traps. The area surrounding the MPA was not chosen as a control because of greater depths and differences in bottom type. Each sample consisted of the catch of one monofilament trammel net (500m long; 1.6m high; inner panel stretched mesh of 100mm; outer panel stretched mesh of 600mm; soak time 22-24h). Nets were set after sunrise and hauled about 24h later. Each fish was identified on board to the species level and total length (TL) was measured to the nearest millimetre. Specimens were released afterwards, following 'catch and release' practice (The Portuguese Institute for Nature Conservation and Forests - ICNF approved the field surveys for this study; ICNF reference nº 00540 140307). Biomass was estimated using published length-weight relations (Table S2.1 - list of species with length-weight references, environment, maximum size and fisheries category). Catch in number per unit effort (CPUE) and biomass per unit effort (BPUE) were calculated considering the unit effort as 500m of trammel net and a soak time of 24h. This provided average values (abundance and biomass) per sample that enabled comparisons of the three protection levels over time.

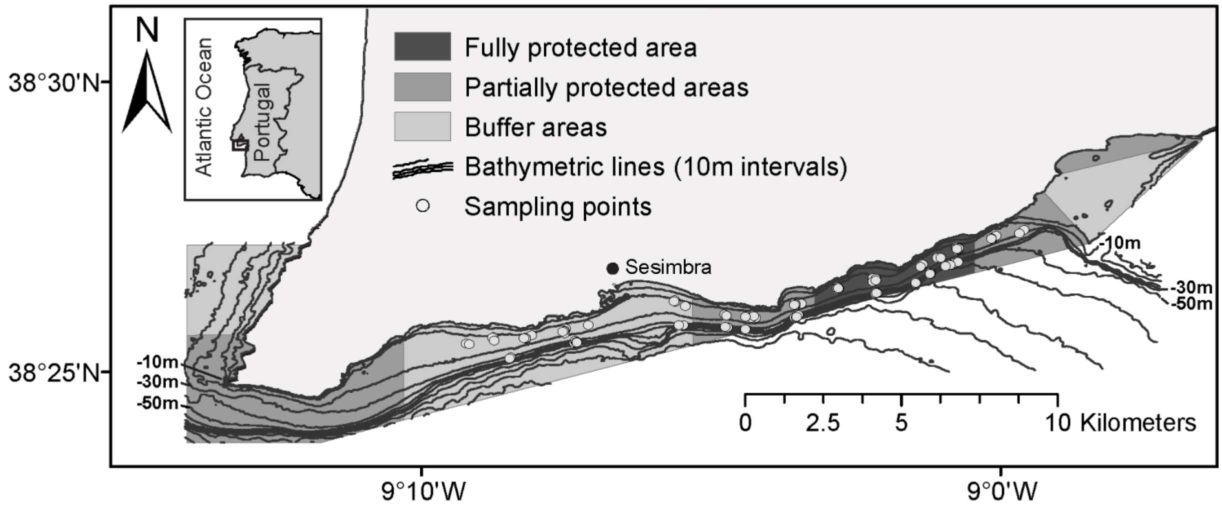


Figure 2.1. Map of the Prof. Luiz Saldanha Marine Park (PLSMP) zoning. The light grey dots indicate sampling points (experimental fishing with trammel net).

2.3.3. Data analysis

CPUE expressed as number of fish per 500 m of net (n·500m^{-1}) was considered as an index of relative abundance and used along with biomass (BPUE - kg·500m^{-1}) to evaluate differences between protection levels and trends over time (before and after full MPA implementation). Prior to data analysis, pelagic species (*Auxis rochei*, *Boops boops*, *Sarda sarda*, *Sardina pilchardus*, *Scomber* spp., *Trachurus* spp.) were excluded from the dataset. Pelagic species are expected to be less meaningful in habitat characterization (Fréon & Misund, 1999) and therefore were not analysed.

We used generalized linear mixed models (GLMM) (Breslow & Clayton 1993) to test significance of protection level (BA, PPA, FPA), substrate (sand, mud) and period (period 1, period 2) as predictors of abundance and biomass. The interaction of protection level and substrate was included in the models to assess whether the effect of protection level was different in each substrate type. Sampling campaign (n = 10) was included as random variable to deal with correlation between observations and with the variability related with campaigns (Zuur et al. 2009). Models were fitted by maximum likelihood with Laplace approximation. Poisson and Gamma distributions (log link) were used to fit, respectively, abundance (discrete) and biomass (continuous) data. Variable selection was carried out by likelihood ratio tests between nested models and by looking the deviance explained by each variable, while model validation was carried out with visual inspection of the residuals (Zuur et al. 2009).

To analyse species spatial and temporal patterns, ratios of abundance, biomass and total length per species (for stingrays - *Myliobatis aquila* - dimension used was disc width) were also estimated and applied in accordance with a Before-After-Control-Impact (BACI) analysis approach (Underwood, 1992, 1994). Comparisons were made between the buffer area (BA) and the two higher protection levels combined (PPA and FPA: PA - protected area), and between period 1 (implementation period) and period 2 (after full implementation) (protection level ratios included data from both periods; period ratios included data of the three protection levels). This specific analysis was done for the species with frequency of occurrence higher than 15% (Table S2.2). The average ratios (abundance, biomass and total length), standard errors (SE) and 95% confidence intervals (CI) were obtained by bootstrapping (9999 permutations). The 95% confidence intervals were calculated using the adjusted bootstrap percentile method and used to check for significance (Payton et al. 2003). To avoid problems resulting from small sample size, bootstrapping of total length was only performed for species with more than ten individuals per treatment (protection level or period). Hence, for total length comparisons, the species *Citharus linguatula*, *Raja undulata* and *Solea solea* were excluded from the protection level ratios, and *C. linguatula*, *R. undulata* and *Rostroraja alba* were excluded from the period ratios.

Multivariate procedures were applied to examine differences in fish assemblage according to protection level (PL) and time (period and year). The Hellinger transformation was used for abundance and biomass in order to overcome the problem of zero inflation (Legendre & Gallagher 2001). Permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) was performed to test significance of protection level, period and year as factors for community differences. Each constraint factor was assessed individually and then under interaction models: ‘protection level x period’ and ‘protection level x year’. Pairwise analyses were also obtained by comparing the protection levels within each period and comparing periods of each protection level.

The Euclidean distance matrix of transformed abundances was used in a principal components analysis (PCA). This technique provides visualization of multivariate data structure. It was used to explore the similarity between samples according to protection level and period. In order to compare samples, centroids per protection level and period, and dispersion ordiellipses (95% confidence interval) according to the weighted average scores of each protection level, were plotted. A second graphical output was also obtained with the species vectors in order to assess species contributions for community differences. Given the similarity of results obtained for abundance and biomass, the multivariate visualizations based on biomass are not presented.

To explore the main factors structuring fish assemblages, multivariate regression tree (MRT) analysis (De’Ath, 2002) was carried out using the Hellinger transformed abundances. The factors ‘protection level’ and ‘period’, and two environmental variables - depth and temperature were included. Substrate type was not included because it is correlated with depth, and priority was given to the variable that incorporated more information (depth). This analysis creates clusters of sites by splitting the data according to explanatory variable threshold values and site similarity. The clusters and their dependence on the environmental data are represented graphically by a tree. Through this procedure, each cluster defines a species assemblage, and its environment type is described by the associated environmental values. MRT was performed given its ability to deal with discrete variables (in our case, protection level and period), non-linearity, and higher-order interactions among explanatory variables (De’Ath, 2002). The criterion for final model selection was minimization of cross-validated relative error (CVRE).

Indicator values (IndVal) (Dufrêne & Legendre, 1997) were calculated for each species in each node and terminal node (leaf). With this index, species are considered as indicators of a certain cluster of sites according to their relative abundance and relative frequency of occurrence within that group. Specifically, the product of relative abundance and relative frequency of occurrence of the species within a group is calculated and then multiplied by by 100. The index takes the value of zero if there is no occurrence of the species within a group, increasing to 100 if the species occurs at all sites within a group and does not occur in any other group. A minimum of 10% contribution to the explained variance was required for a species to be considered as discriminant at a particular node and permutations were used to test for significance. Permutations were also used to assign significant discriminant species for each terminal node. A species was considered an indicator of a certain assemblage according to the cluster for which it had the highest IndVal. All analyses were done using the R statistical software version 2.15.2 (R Core Team, 2014). The package vegan (Oksanen et al., 2014) was used for the multivariate analysis and the MRT was built with the package mvpart (Therneau & Atkinson, 2012).

2.4. Results

In total, 8820 fish from 70 species were recorded in the trammel net catches, of which 7533 fish of 62 species were included in the analyses (eight pelagic species not included). The bony fishes were represented by 49 species and the elasmobranchs by 13 species. The list of species and their frequency of occurrence is shown in Table S2.2. Overall, the local soft-bottom fish community was dominated by three families of benthic fish, namely Soleidae (*Solea* and *Pegusa*), *Triglidae* (*Chelidonichthys* and *Trigloporus*) and *Bothidae* (*Bothus* and *Arnoglossus*). *Soleidae* was the most abundant family (42%), followed by *Triglidae* (22%) and *Bothidae* (7%). In relation to biomass, *Soleidae* was still the dominant family (30%), although *Rajidae* was the second most important (28%), followed by *Triglidae* (12%). Details of average abundance, biomass and total length per species are shown in Tables S3 and S4.

GLMM outputs (Table 2.1) revealed protection level and period as significant variables in the biomass model (protection level: $p < 0.001$; period: $p = 0.03$), while protection level and substrate were the significant variables in the abundance model (protection level: $p<0.001$; substrate: $p < 0.001$). These results are supported by the boxplots shown in Figure 2.2. Overall, this figure shows that abundance and biomass were higher in the PPA and FPA than in the BA, and that increases between period 1 – period 2 are only apparent in biomass. The interaction of protection level and substrate was significant in both models (abundance: $p < 0.001$; biomass: $p = 0.037$), indicating a different effect of protection level according to substrate type. The coefficients indicate that mud had higher average values than sand (abundance, coef. sand = -0.15; biomass, coef. sand = -0.25), and that the sandy PPA had higher abundances in comparison to the sandy FPA (coef. FPA sand = -0.17) (Figure S2.1). This pattern is different in the muddy substrate, with higher values in the FPA.

Plots of abundance and biomass per protection level and substrate are shown in Figure S2.1.

Table 2.1. Generalized linear mixed models of fish abundance and biomass: Results of the general linear mixed models (GLMMs) of fish abundance (CPUE – n·500 m^{-1}) and biomass (BPUE – kg·500m^{-1}) according to protection level, period and substrate. The interaction ‘protection level x substrate’ was included and sampling campaign (n = 10) was considered as a random variable. GLMM of abundance and biomass used respectively, Poisson and Gamma distributions (log link).

		Fixed effect					Random effect
Response	**Predictor**	**Level**	**Coef.**	**St. Error**	**χ^2**	**p**	**St. Dev.**
Abundance	Intercept (BA/Mud/Period 1)		3.33	0.15			
	Protection level (PL)	PPA	0.42	0.05	366.59	**<0.001**	
		FPA	0.57	0.05			
	Substrate	Sand	-0.15	0.05	28.25	**<0.001**	
	Period	Period 2	0.06	0.19	0.12	0.731	
	PL * Substrate	PPA-Sand	0.26	0.07	64.33	**<0.001**	
		FPA-Sand	-0.17	0.06			
	Sampling campaign						0.29
Biomass	Intercept (BA/Mud/Period 1)		1.89	0.16			
	Protection level (PL)	PPA	0.49	0.16	100.39	**<0.001**	
		FPA	0.76	0.16			
	Substrate	Sand	-0.25	0.14	0.32	0.569	
	Period	Period 2	0.33	0.15	4.71	**0.030**	
	PL * Substrate	PPA-Sand	0.50	0.20	6.61	**0.037**	
		FPA-Sand	0.13	0.19			
	Sampling campaign						0.13

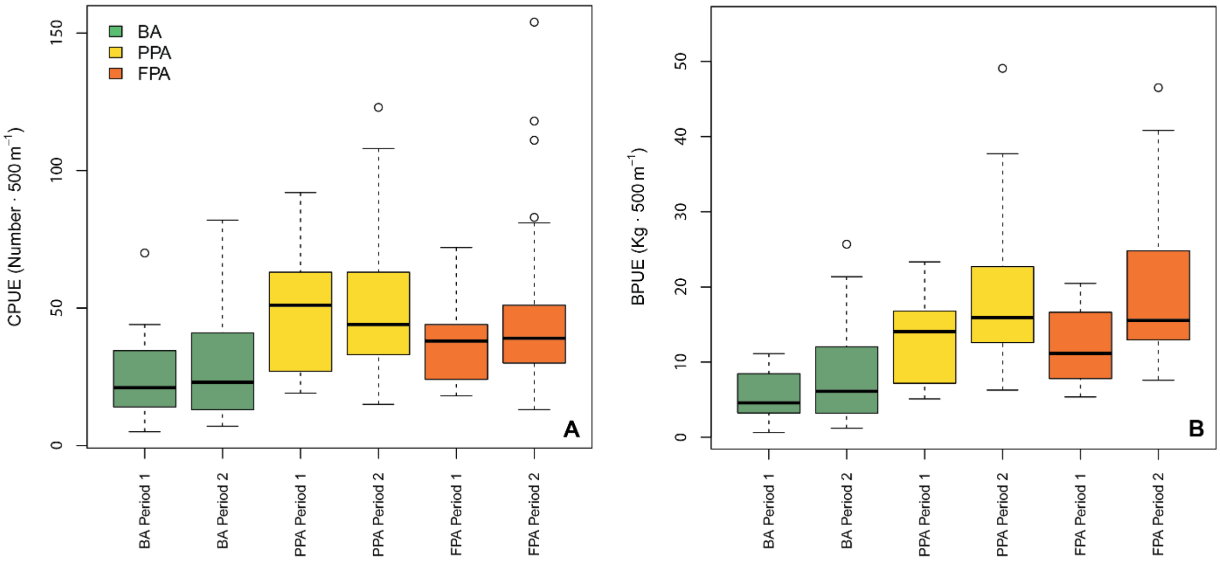


Figure 2.2. Boxplots of fish abundance and biomass: Boxplots of fish abundance (A, CPUE – n·500m^{-1}) and biomass (B, BPUE – kg·500m^{-1}) according to protection level (BA, buffer area; PPA, partially protected area; FPA, fully protected area) and period (Period 1, Before MPA Full Implementation; Period 2, After MPA Full Implementation) (Box extent, 25th to 75th percentile; Band near the middle of

the box, 50th percentile/median; Whiskers range, lowest to highest datum within the 1.5 x inter-quartile range interval).

Examining abundance and biomass ratios between the BA and higher protection levels (Figure 2.3 and 2.4), four flatfishes (*Solea senegalensis*, *S. solea*, *C. linguatula*, *Microchirus azevia*), four elasmobranchs (*Torpedo torpedo*, *M. aquila, R. alba, Raja clavata*,) two gurnards (*Chelidonichthys lastoviza* and *Chelidonichthys lucerna*), and the Lusitanian toadfish (*Halobatrachus didactylus*) were found to be significantly more abundant in the PPA and FPA. Of these, *R. clavata* and *S. solea* were the ones with the greatest differences. All of these species also had significantly higher biomasses in the PPA and FPA. Regarding biomass, one more species, *Mullus surmuletus,* had higher values in the PPA and FPA.

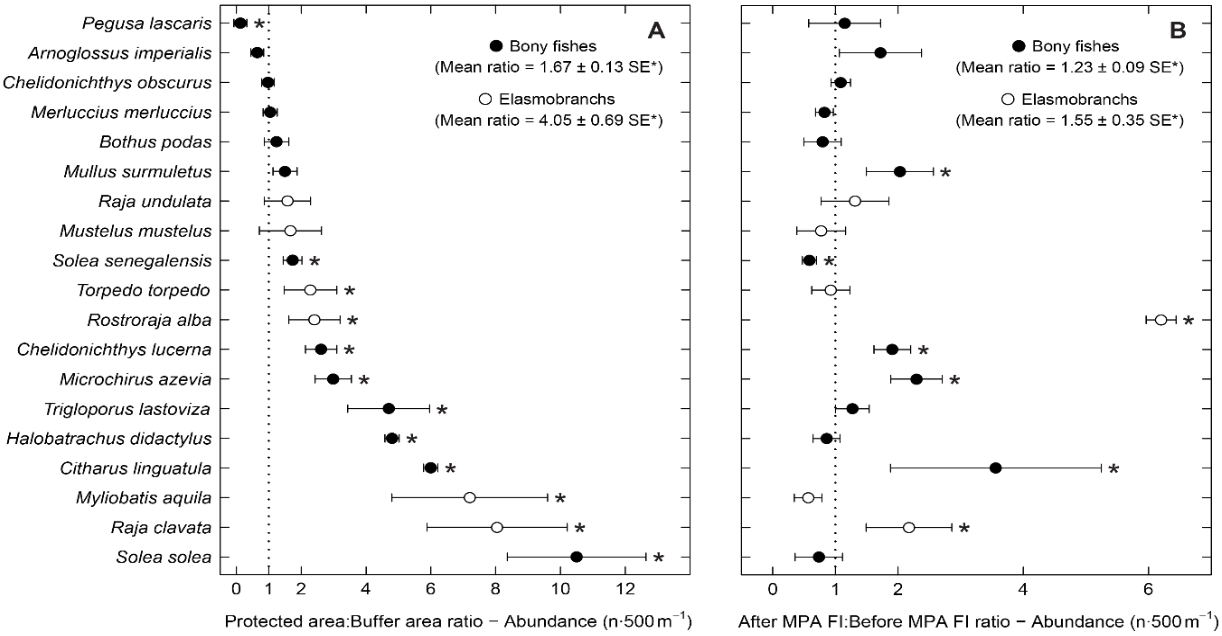


Figure 2.3. Abundance response ratio per species: Response ratios (± standard error: SE) of abundance per species for (A) Control-Impact (Protected area : Buffer area) and (B) Before-After (After MPA Full Implementation : Before MPA Full Implementation) comparisons (Protected area - fully protected area and partially protected area; Before MPA Full Implementation – Period 1; After MPA Full Implementation – Period 2; FI - Full Implementation). Protection level ratios included data from both periods and period ratios included data from the three protection levels. Ratios > 1 indicate positive responses and significant ratios (according to 95% confidence interval) are marked with '★'. Species are ordered according to increasing Control-Impact abundance response ratio. Bony fish species are marked with '•' and elasmobranch species are marked with '○'.

Of the eleven species with higher abundance in the higher protection levels, five also had significant increases in abundance and biomass from period 1 to period 2, namely *C.*

linguatula, *R. alba*, *R. clavata*, *C. lucerna* and *M. azevia*. (Figures 2.3 and 2.4). Other species showing positive changes over time were *M. surmuletus*, which showed an increase in abundance, and *R. undulata*, which had higher biomass in period 2. Other interesting results were the higher numbers of *Pegusa lascaris* in the BA. *Arnoglossus imperialis* showed a non-significantly higher abundance in the BA. Regarding time trends, the sole *S. senegalensis* exhibited a significant decrease in both abundance and biomass.

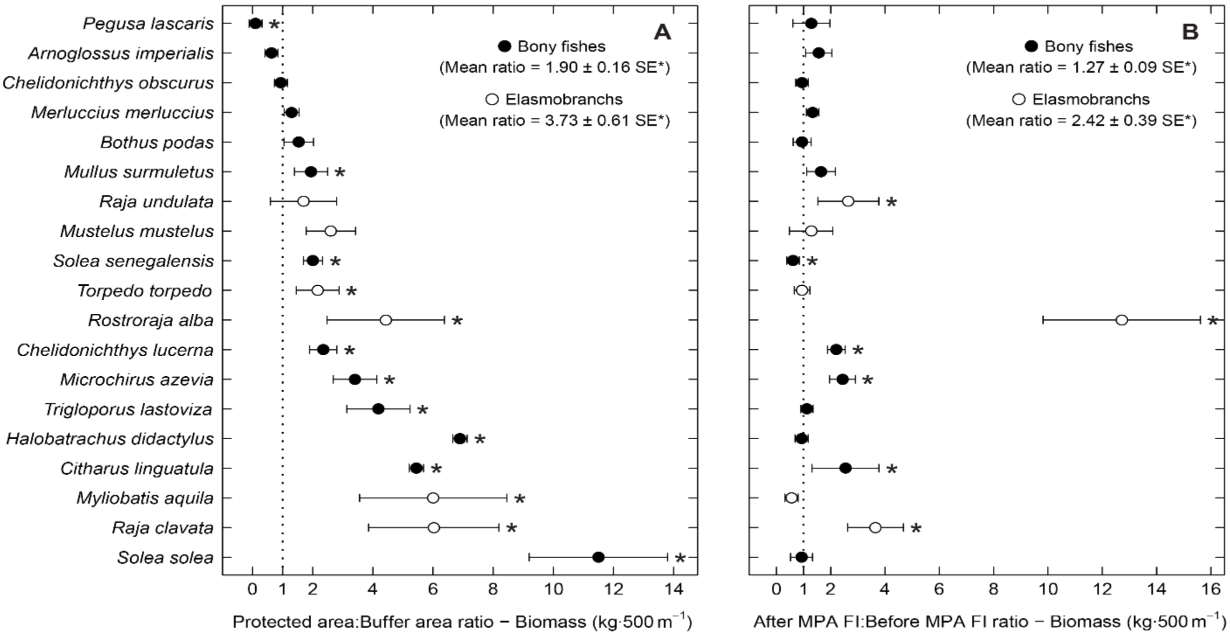


Figure 2.4. Biomass response ratio per species: Response ratios (± standard error: SE) of biomass per species for (A) Control-Impact (Protected area : Buffer area) and (B) Before-After (After MPA Full Implementation : Before MPA Full Implementation) comparisons (Protected area - fully protected area and partially protected area; Before MPA Full Implementation – Period 1; After MPA Full Implementation – Period 2; FI - Full Implementation). Protection level ratios included data from both periods and period ratios included data from the three protection levels. Ratios > 1 indicate positive responses and significant ratios (according to 95% confidence interval) are marked with ‘’. Species ordered according to increasing Control-Impact abundance response ratio. Bony fish species are marked with ‘●’ and elasmobranch species are marked with ‘○’.

Some species with positive trends in abundance and biomass also increased in average total length, including *R. alba*, *R. clavata*, *C. lucerna* and *M. azevia* (Figure 2.5). Other species, with no significant trends of abundance or biomass, also showed an increase in average size, specifically *Merluccius merluccius*, *Bothus podas*, *Mustelus mustelus* and *S. solea*. *S. senegalensis*, which decreased significantly in abundance, was another species with larger average sizes in the PA and in period 2. Conversely, both *A. imperialis* and

Chelidonichthys obscurus exhibited a decrease in average size from the first to the second period.

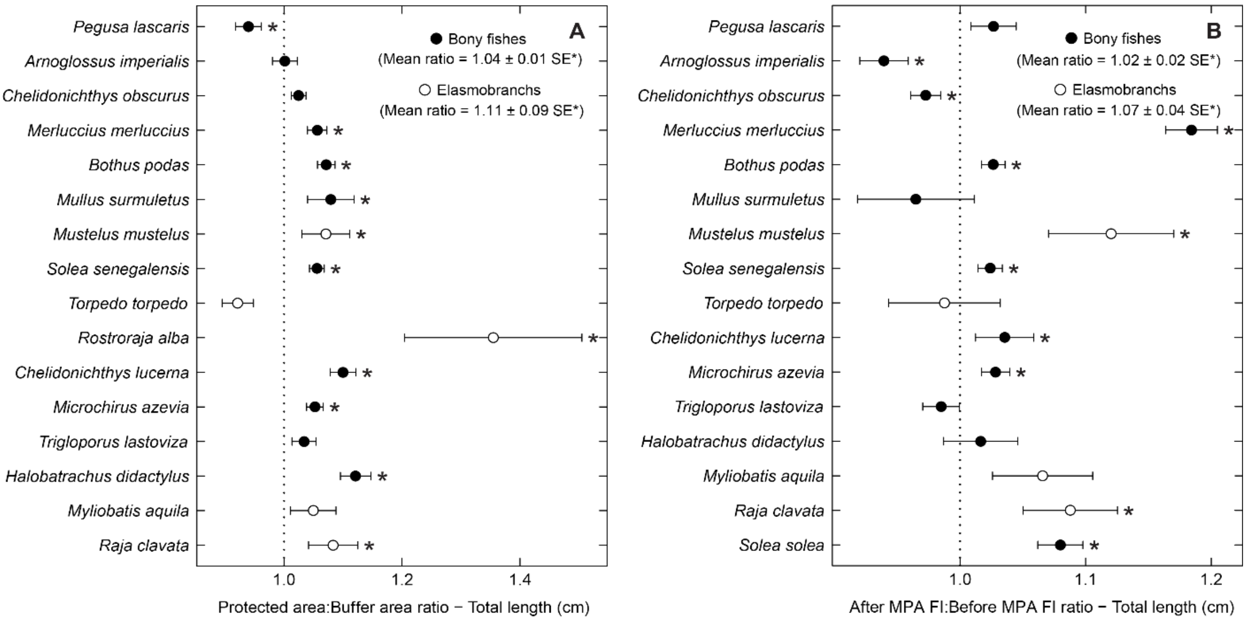
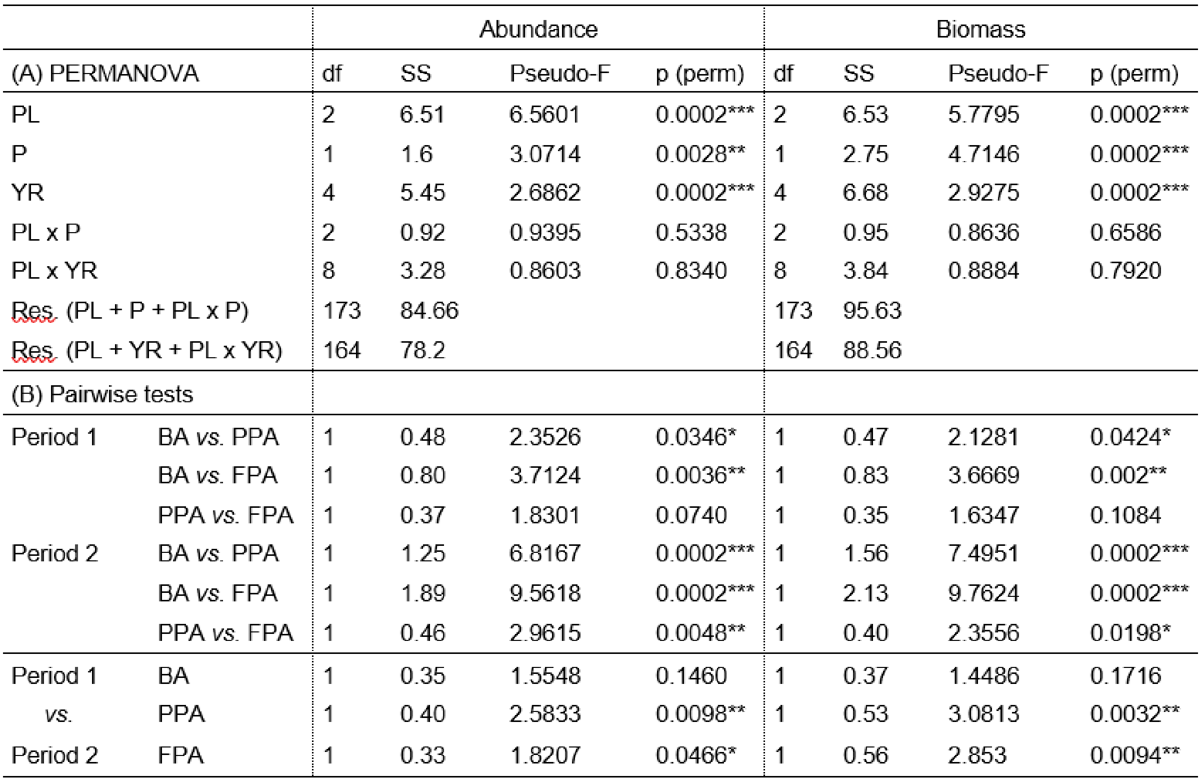


Figure 2.5. Total length response ratio per species: Response ratios (± standard error: SE) of total length per species for (A) Control-Impact (Protected area : Buffer area) and (B) Before-After (After MPA Full Implementation : Before MPA Full Implementation) comparisons (Protected area - fully protected area and partially protected area; Before MPA Full Implementation – Period 1; After MPA Full Implementation – Period 2; FI - Full Implementation). Protection level ratios included data from both periods and period ratios included data from the three protection levels. Ratios > 1 indicate positive responses and significant ratios (according to 95% confidence interval) are marked with '⋆'. Species ordered according to increasing Control-Impact abundance response ratio. Bony fish species are marked with '●' and elasmobranch species are marked with '○'.

PERMANOVA on fish abundance and biomass (Table 2.2) revealed significant differences for protection level (PL) and temporal factors (period and year). The effect of protection was not demonstrated by the interactions: the terms 'PL × period' and 'PL × year' were non-significant. However, pairwise comparisons detected some differences. Specifically, the FPA became significantly different from the PPA only in period 2, and when comparing within protection level, both the PPA and FPA communities showed significant differences between periods.

Table 2.2. PERMANOVA results obtained with fish abundance and biomass: (A) PERMANOVA outputs obtained with fish abundance and biomass (Hellinger transformation), with protection level (PL: BA - buffer area; PPA - partially protected area; FPA - fully protected area), period (P: Period 1 - MPA implementation period; Period 2 - after MPA implementation) and year (YR) as factors (Res. - Residuals). (B) Pairwise tests made per protection level within each period, and per period within each protection level. * p<0.05, ** p<0.01, *** p<0.001.



		Abundance				Biomass			
(A) PERMANOVA		df	SS	Pseudo-F	p (perm)	df	SS	Pseudo-F	p (perm)
PL		2	6.51	6.5601	0.0002***	2	6.53	5.7795	0.0002***
P		1	1.6	3.0714	0.0028**	1	2.75	4.7146	0.0002***
YR		4	5.45	2.6862	0.0002***	4	6.68	2.9275	0.0002***
PL x P		2	0.92	0.9395	0.5338	2	0.95	0.8636	0.6586
PL x YR		8	3.28	0.8603	0.8340	8	3.84	0.8884	0.7920
Res. (PL + P + PL x P)		173	84.66			173	95.63		
Res. (PL + YR + PL x YR)		164	78.2			164	88.56		
(B) Pairwise tests									
Period 1	BA *vs.* PPA	1	0.48	2.3526	0.0346*	1	0.47	2.1281	0.0424*
	BA *vs.* FPA	1	0.80	3.7124	0.0036**	1	0.83	3.6669	0.002**
	PPA *vs.* FPA	1	0.37	1.8301	0.0740	1	0.35	1.6347	0.1084
Period 2	BA *vs.* PPA	1	1.25	6.8167	0.0002***	1	1.56	7.4951	0.0002***
	BA *vs.* FPA	1	1.89	9.5618	0.0002***	1	2.13	9.7624	0.0002***
	PPA *vs.* FPA	1	0.46	2.9615	0.0048**	1	0.40	2.3556	0.0198*
Period 1	BA	1	0.35	1.5548	0.1460	1	0.37	1.4486	0.1716
vs.	PPA	1	0.40	2.5833	0.0098**	1	0.53	3.0813	0.0032**
Period 2	FPA	1	0.33	1.8207	0.0466*	1	0.56	2.853	0.0094**

The PCA ordination confirmed the results of the protection level comparison (Figure 2.6A), with the BA centroids constituting a distinct group, and the PPA and FPA showing some overlap. The first axis explained 23.9% of the variation, with 34.9% of the variation explained cumulatively with the inclusion of the second axis. This analysis provides information on inter-period variation and a progression along the first axis is evident for the PPA and FPA. In both these areas, period 1 is closer to the BA group. Species vectors are shown in the second biplot (Figure 2.6B). *M. azevia* (Maze), the most abundant species in our dataset, was highly related to the first component (PC1), while *M. merluccius* (Mmer) was the most correlated with the second component (PC2). Both *M. azevia* and *M. merluccius* were more abundant in the muddy substrate, and this was also the case for *S. solea* (Ssol) and *C. linguatula* (Clin). The latter two species exhibited higher abundances in the FPA, along with *R. clavata* (Rcla). *P. lascaris* (Plas), which has its vector isolated in the top-right of the plot, was more abundant in the BA and over sandy bottoms. Another species with affinity with sand was *S. senegalensis*, shown in the bottom-right. It was found in higher numbers in the

PPA (Table S2.3 - species abundance and biomass per protection level; Table S2.5 - species abundance per substrate).

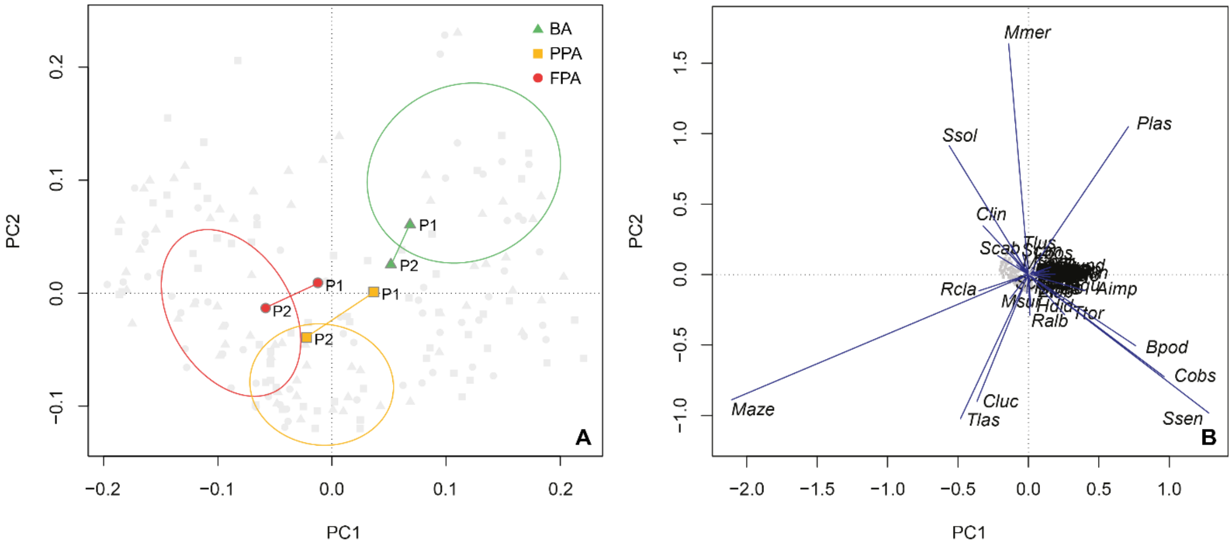


Figure 2.6. Principal components analysis of species abundance: Principal components analysis (PCA) of sites (light grey points) with transformed (Hellinger) species abundance. (A) Biplot (scaling 2) with centroids per period (period 1, period 2) and protection level (BA - buffer area; PPA - partially protected area; FPA - fully protected area), and dispersion ordiellipses (95% confidence interval) for each protection level; (B) Biplot (scaling 1) with the species vectors (see Table S2.2 for species codes).

A multivariate regression tree (MRT) with four nodes and five leaves (terminal nodes) was considered the most parsimonious to represent community structure (Figure 2.7). This model explained 32.2% of the species abundance variation and had an accuracy of 29% in assemblage association predictions. The primary split in the tree explained 19.6% of the species variation and separated fish assemblages according to depth, specifically depths less and greater than 19m. These two depth intervals suggested by the primary split have a correspondence with the two sampled substrates (sand and mud). After the main distinction according to depth strata/substrate, the protection level enabled the discrimination of four assemblages, two in each substrate. In the shallow sandy area, the BA species assemblage was found to be different from the one in the PPA and FPA (7.3% of variation), while in the muddy substrate, the PPA and BA constituted a group apart from the FPA (3.1% of variation). No more nodes were created on the muddy bottom assemblages. The sandy PPA and FPA was split according to period (2.2% of variance explained).

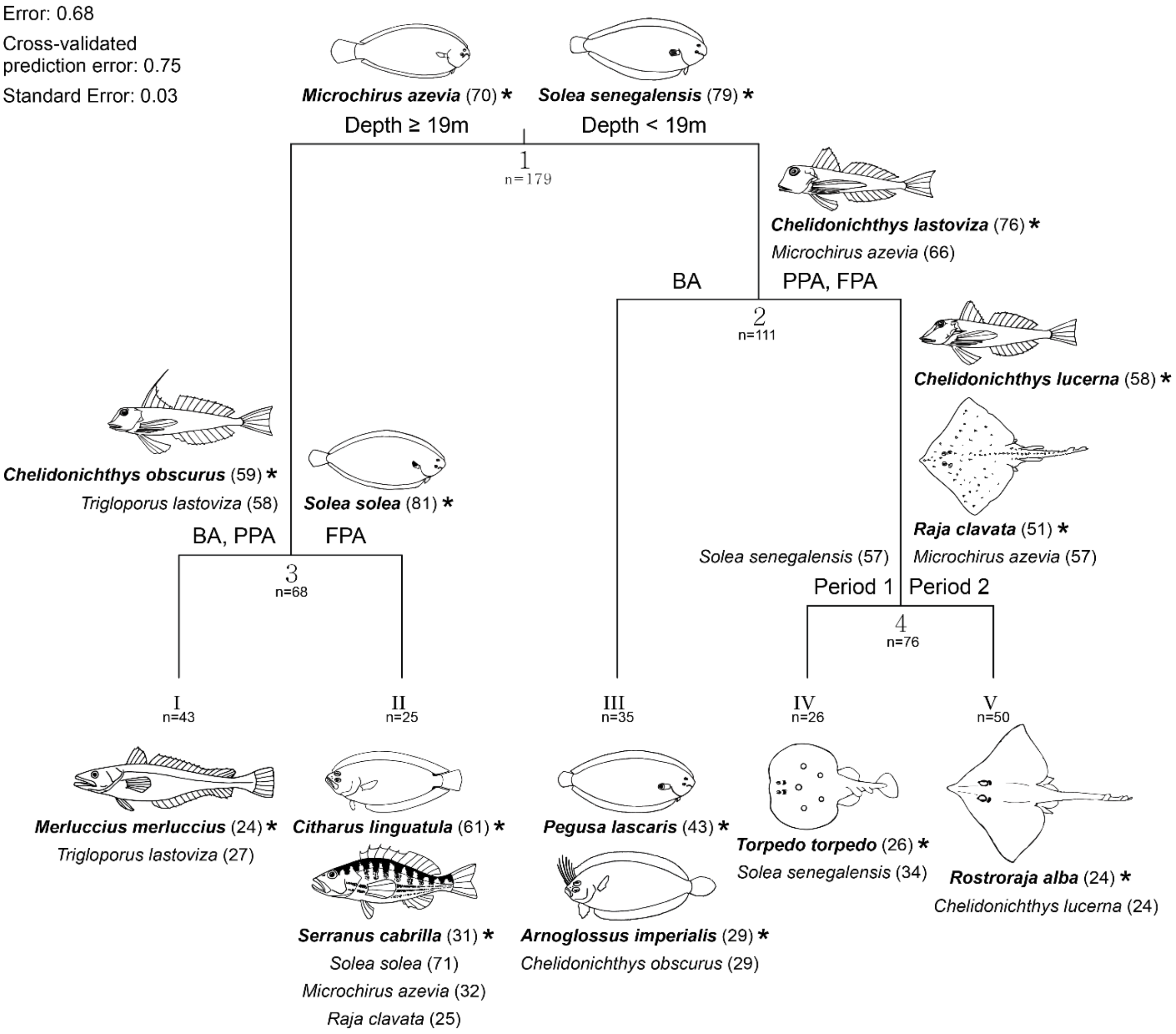


Figure 2.7. Multivariate regression tree with species abundance: Multivariate regression tree of transformed (Hellinger) fish species abundance and constrained by depth, temperature, protection level (BA - buffer area; PPA - partially protected area; FPA - fully protected area) and period (Period 1 - Before MPA Full Implementation; Period 2 - After MPA Full Implementation). Discriminant species (contribution to the explained variance ≥ 10%) with indicator values (IndVal) (Dufrêne & Legendre 1997) ≥ 20 listed at each split (Arabic numbers) above the cluster to which they were allocated, and below each leaf (Roman numbers). Corresponding indicator values are reported between parentheses. Species were considered as indicator species in the cluster where they had the highest IndVal. Indicator species (bold letters) are marked with '⋆' and illustrated (fish illustrations: FAO).

For each split, discriminant species were identified. Significant species were allocated as indicators in the cluster for which they had the highest indicator value (IndVal) (Figure 2.7). The fourteen indicator species shown in Figure 2.7 were considered consistent given their high (≥ 50; eight species) or moderately high (≥ 20 and < 50; six species) IndVal. Values of species mean abundance at each terminal node (leaf) are shown in Table S2.6. Species assigned to terminal nodes with IndVal lower than 20 (indicating some inconsistency in species allocation) are not shown in Figure 2.7 and are listed in Table S2.7.

By taking into account the clusters with consistent indicator species (IndVal ≥ 20), several distinct assemblages can be described (Figure 2.7). The main split, established by depth and substrate, separates the cluster of muddy bottom, with *M. azevia* as the most abundant species, from the sandy bottom assemblage, for which *S. senegalensis* and *C. obscurus* were assigned as indicator species. *S. solea* was considered as indicator at the FPA muddy substrate node, and the gurnards *C. lastoviza* and *C. obscurus* were discriminants of the muddy BA and PPA. The assemblage assigned for the muddy FPA terminal node included *C. linguatula*, *Serranus cabrilla* (both classified as indicators of this cluster), *S. solea*, *M. azevia* and *R. clavata*. The muddy BA and PPA community included *M. merluccius* (indicator species for this cluster) and *C. lastoviza*. With reference to the sandy stratum, two species were particularly frequent in the BA: *P. lascaris* and *A. imperialis*. *C. obscurus* was also a significant discriminant species of this group. Three other assemblages were related to sandy substrate. Firstly, *C. lastoviza* (indicator species at this cluster) and *M. azevia* appeared as discriminant species for the PPA and FPA sandy bottom assemblage. Within this cluster, *C. lucerna*, *R. clavata* (both classified as indicators of this cluster) and *M. azevia* allowed period 2 to be distinguished from period 1. *T. torpedo* and *S. senegalensis* were allocated to period 1, while *R. alba* and *C. lucerna* were assigned to period 2. It is also noteworthy that some species were discriminant for both sandy and muddy assemblages, indicating that they were not strictly associated with one habitat type. This was the case for *M. azevia*, *C. obscurus* and *C. lastoviza*.

A summary of the species ratios (abundance, biomass and size ratios according to protection and period) and MRT results is shown in Table S2.8. Considering the 20 species highlighted in these analyses (19 from the ratio analysis: frequency of occurrence > 15%; and *S. cabrilla* from the MRT output) and their abundance and biomass time trends, three response categories can be distinguished: species with positive signs (*M. surmuletus*, *R. undulata*, *C. linguatula*, *R. alba*, *R. clavata*, *C. lucerna* and *M. azevia*), species with no detected trends (*P. lascaris*, *A. imperialis*, *C. obscurus*, *M. merluccius*, *B. podas*, *M. mustelus*, *T. torpedo*, *M. aquila*, *C. lastoviza*, *H. didactylus*, *S. cabrilla* and *S. solea*), and species with negative trends (*S. senegalensis*). To overcome the problem of habitat heterogeneity, priority was given to time trends. Additional information of life history traits (e.g., length and age at maturity, longevity), movement pattern and commercial value of these 20 species is provided in Tables S9 and S10.

2.5. Discussion

2.5.1. The soft-bottom fish community and the MPA effect

In general, our study showed spatial heterogeneity in fish assemblages. Fish abundance and biomass differed according to protection level, with higher values in the PPA and FPA in comparison with the BA. Our results also point out to the importance of substrate, which in association with depth, plays a major role structuring the communities. This is common in coastal assemblages (Claudet et al. 2006, Claudet et al. 2011, Pais et al., 2014). It is worth noting that not all spatial complexity was incorporated by depth, substrate and protection level, as these variables do not explain the uneven distribution of some species. One outcome of this is that in the sandy substrate, the PPA had higher abundances than the FPA, while in the muddy bottom, the FPA had higher values than the PPA. This pattern is different regarding biomass, with the sandy FPA exhibiting values similar to the PPA. *S. senegalensis*, which was more abundant in the PPA, and *R. clavata* (large species with significant contribution in biomass), commonly found in the FPA, are among the species that contributed to these trends (next section moves on to discuss species patterns). Overall, fish assemblages were mainly structured by depth and substrate, followed by protection level. With respect to protection level, some differences in abundance and biomass were found between the PPA and FPA, and the BA was noticeably a distinct group with lower values.

Examining community structure, the two soft-bottom habitats showed different patterns according to protection level. In the sandy habitat, the BA assemblage was found to be distinct from the PPA and FPA, while in the muddy substrate, the PPA and BA constituted a group apart from the FPA. Many studies have demonstrated that partial protection generally results in different communities in relation to no-take areas (Denny & Babcock 2004, Lester & Halpern 2008, Guidetti et al. 2014), and this was also what we observed for the muddy stratum. Habitat heterogeneity (Henriques et al. 2014) is probably related with these differences. Another possible factor is the occurrence of illegal fishing, which was reported by Cunha et al. (2011) for this area. The MPA design is another possible cause for the similarity between the muddy PPA and the BA. Because the central PPA corresponds to the area where the marine park is narrowest, about one third of this area does not include depths greater than 30m, and the corresponding section of muddy bottom is found outside the park's border. This discontinuity of habitat protection is expected to affect longitudinal migrations of individuals and consequently, the increase of exposure to fishing is likely to prevent effective protection benefits (Gaines et al. 2010).

Conversely, the different pattern that we found in the sandy habitat, with the PPA similar to the no-take community (also regarding abundance and biomass), might be reflecting the effectiveness of the restrictions prevailing in the PPA, meaning that the prohibitions of fishing with longlines, gill nets and trammel nets, i.e. static gear with moderate to low selectivity (Erzini et al. 2003, 2006, Gonçalves et al. 2007), are effective management measures. This supports the idea that partial protection may offer effective benefits for soft-bottom fish species. This is clearly conditional on the implemented restrictions (Sciberras et al. 2013, Horta e Costa et al. 2016), and fishing gear that are generally regarded as artisanal might also have considerable impact (longlines, gillnets, trammel nets). Other factors possibly contributing to the similarity between partially protected areas and the reserve are related to geographical proximity (favoring potential movement of individuals between these areas - spillover) and site-specific effects (habitat heterogeneity; fishing pressure not equally distributed before the MPA establishment).

To surpass the complexity related to habitat differences and other site-specific effects, additional attention was given to time series analysis. Comparisons between periods showed that although no significant abundance differences were observed, a biomass increase occurred in the PPA and FPA, with higher values after full implementation of the MPA. Fish biomass increase was the first positive effect observed in many marine reserves (García-Charton et al. 2008, Watson et al. 2009, Di Franco et al. 2012), and it was recently detected in the rocky reef fish community of this particular MPA (Horta e Costa et al. 2013). This trend is possibly related to the decrease in fishing mortality and increase in longevity, allowing fish to grow to larger sizes. Fish assemblages were also analysed per period. Even though the interaction term of protection and time was not significant, dissimilarities between periods were found both in the PPA and FPA, suggesting that assemblages are possibly being influenced by management measures. Optimal design should have included sampling prior to the management plan implementation (Claudet & Guidetti 2010, Donovan et al. 2016). First samples were collected with some regulations already in place and protection effect may have been larger than what our data suggests.

2.5.2. The MPA effect at the species level

Overall, a wide variety of species trends and responses to spatial closure was found. Examining the three abundance and biomass response categories (positive, neutral, negative), two subgroups might be considered within the category with positive signs: species with a clear positive response (increase both in abundance and biomass) and species with only one positive indicator (increase only in abundance or biomass). The latter includes *M. surmuletus*, which increased only in abundance and was not significantly more abundant in the higher protection levels, and *R. undulata*, which showed a biomass increase but no significant abundance trend. The lack of consistency in these results is likely related to the overall low abundance of these species (*R. undulata* is currently listed as endangered; IUCN 2017), and a longer period of protection could potentially reveal more information of their trends. In this sense, they are similar to some neutral species, as some showed size increases (*M. merluccius*, *B. podas* and *M. mustelus*), others were more abundant in the PPA and FPA (*T. torpedo*, *M. aquila*, *C. lastoviza*, *H. didactylus* and *S. cabrilla*), and others were both more abundant in the higher protection levels and showed increases in size (*S. solea*).

The group with consistent positive indicators (abundance, biomass and size) includes two rays; *R. alba* and *R. clavata*; one gurnard: *C. lucerna*; and two flatfishes: *M. azevia* and *C. linguatula*. For *R. alba,* this study is the first to confirm spatial protection benefits. This is of particular importance given that this species is classified as endangered (IUCN 2017). Spatial management seems to be a useful tool for the recovery of this species, as well as for *R. clavata*, another elasmobranch with conservation concerns, currently classified as near threatened (IUCN 2017). Positive responses to spatial management were previously reported for *R. clavata* in the Mediterranean by Dimech et al. (2008). Hunter et al. (2006) and Wiegand et al. (2011) also concluded that spatial closures were an appropriate approach for this species in the North Sea. Although the distribution of the local populations is probably wider than the MPA, many individuals might be using the area on a regular basis, given the known tendency to repeatedly return to specific locations (Hunter et al. 2005, Ellis et al. 2011).

Concerning *C. lucerna*, few studies have addressed its abundance in protected areas, despite its commercial importance. Piet & Rijnsdorp (1998) reported an increase in abundance of this species after the establishment of the “plaice box” in the North Sea, while Guidetti & Claudet (2010) found that it occurred in lower abundances inside the Torre

Guaceto MPA (Italy) in comparison to the outside area. Our results show that this species can benefit from spatial closures. Within the Soleidae species (represented by nine species, four of which with frequency of occurrence > 15%), *M. azevia* was the one presenting more positive indicators. It showed increases in abundance, biomass and size, and these trends may be related to the implemented regulations. As for the flounder *C. linguatula*, no trends over time were found in the study by Iannibelli & Musmarra (2008) in an area with trawling restrictions, while in our results, this was the only non-target species with consistent positive indicators. Claudet et al. (2010) pointed out that bycatch species may also be affected by fishing pressure or habitat degradation, which seems to be the case for this species.

The only species that exhibited a decrease in abundance was *S. senegalensis*, even though it was more abundant in the protected area and showed a size increase. This abundance decrease might be related with migrations to nearby estuaries (Andrade 1990, Vinagre et al. 2007) or to fishing grounds outside the reserve, with increasing exposure and vulnerability to gillnets and trammel nets. This phenomenon was hypothesized by Abecasis et al. (2014) for this species in this same MPA (acoustic telemetry data).

It is also noteworthy that some species showed affinity to the BA, particularly *P. lascaris* and *A imperialis*. Both species had low frequency of occurrence and they exhibited restricted distributions in the study area. Similarly, *C. linguatula* was also unevenly distributed, but it showed affinity with the muddy bottoms in the FPA. This is in accordance with previous studies, that identified species with pronounced site-specific responses within MPAs (Claudet et al. 2011, Eddy et al. 2014). One possibility is that their patchy distributions are related to biotic factors such as niche breadth, perhaps habitat preferences and/or prey availability. Studies such as the conducted by Ross (1986) have shown that among fish assemblages, the availability of food items is commonly an important factor for habitat discrimination. Moreover, it is argued that specialist species are more vulnerable to environmental stressors and exploitation, and that their dependence on habitat heterogeneity is also higher (Wilson et al. 2008, Slatyer et al. 2013).

As with rocky reef fish assemblages (Micheli et al. 2004, Ashworth & Ormond 2005, Blyth-Skyrme et al. 2006), soft-bottom fishes exhibit a wide diversity of responses to spatial regulations. Our results suggest that part of this variability is connected with habitat heterogeneity. Still, the cause of site-specific preferences of some of the studied species is not fully understood. Studies of species movement ecology would provide insights for both habitat preferences and migratory behaviour. Our data illustrates this gap of knowledge, as some mismatches related with migratory species were found. For instance, both *R. clavata* and *R. alba* demonstrate that species with yearly migrations can benefit from spatial measures, in accordance with the results of other studies (e.g., Claudet et al. 2010). However, it would be simplistic to infer that home range does not play a role in the effectiveness of spatial protection. Instead, possible connections are probably more complex, as illustrated by the species *M. mustelus*, a species that shares life history traits with the rays. This species did not exhibit consistent positive response to protection, and its migratory habits might contribute for this (strictly migrant *vs* seasonally migrant). The complexity of the effect of movement patterns in species response to protection was pointed out in previous studies (Claudet et al. 2010, Villegas-Ríos et al. 2017).

In relation to species traits and their role in the response to protection, our most compelling results point out to body size as a possible factor. In our study, this is supported by the two rays (*R. clavata* and *R. alba*), two elasmobranchs with large body size and late maturity. This is in accordance with the observations of other authors (Claudet et al. 2006, García-Charton et al. 2008, Claudet et al. 2010, Sciberras et al. 2015), who reported body size as an important trait related to spatial protection. This might be due to the fact that larger fish may be more easily caught by fishing gear, and also because large body size generally reflects specific life history traits such as late maturity (Hutchings et al. 2012).

2.5.3. Implications and suggestions for future research

In this study, there was some evidence of protection effectiveness, supported by the significant increase in biomass in the protected areas. However, like in rocky habitats, effectiveness is cross-linked with habitat and species characteristics. In relation to habitat, it is important to identify habitat requirements of species that are protection targets, and habitat mapping should be carried out before the management plan development. This was also emphasized by other authors for rocky bottoms (e.g., Gaines et al. 2010, Halpern et al. 2010). In addition, we recommend that habitat continuity should be taken into account in the design of MPAs aiming to protect soft-bottom fishes. Furthermore, we highlight the importance of enforcement measures, as compliance is essential for effective protection, long-term benefits and appropriate monitoring (e.g., Bergseth et al. 2015). With respect to species characteristics, we highlight body size as a possible factor of response to protection. The inclusion of longer time scales would provide information on this and other underlying factors, and also reveal if more species could respond positively to spatial regulations. Future research on fish movements (telemetry), habitat requirements and trophic ecology (stable isotopes) would also offer useful knowledge to better understand how spatial protection measures should be used in the management and conservation of soft-bottom fish species.

2.6. Acknowledgements

We thank all the colleagues and students who helped in the field work, especially Leonel Gonçalves and Rita Abecasis, for conducting the first campaigns. We also thank ICNF-PNA, particularly Miguel Henriques, for the support from the marine park. We are also grateful to Ester Serrão and Alexandra Cunha for coordination and support in the LIFE-BIOMARES project. We also thank the jury of Sesimbra Scientific Prize and Câmara Municipal de Sesimbra for their recognition of our work. A special thanks also to Bárbara Horta e Costa, Frederico Oliveira, Joaquim Silva, and Mafalda Rangel Oliveira for their help and useful suggestions.

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2.8. Supplemental information

Figure S2.1. Barplots of fish abundance and biomass per protection level and substrate: Barplots of mean (± standard error) fish abundance (A: CPUE n·500 m^{-1}) and biomass (B: BPUE kg·500 m^{-1}) per protection level and substrate (sand, mud).

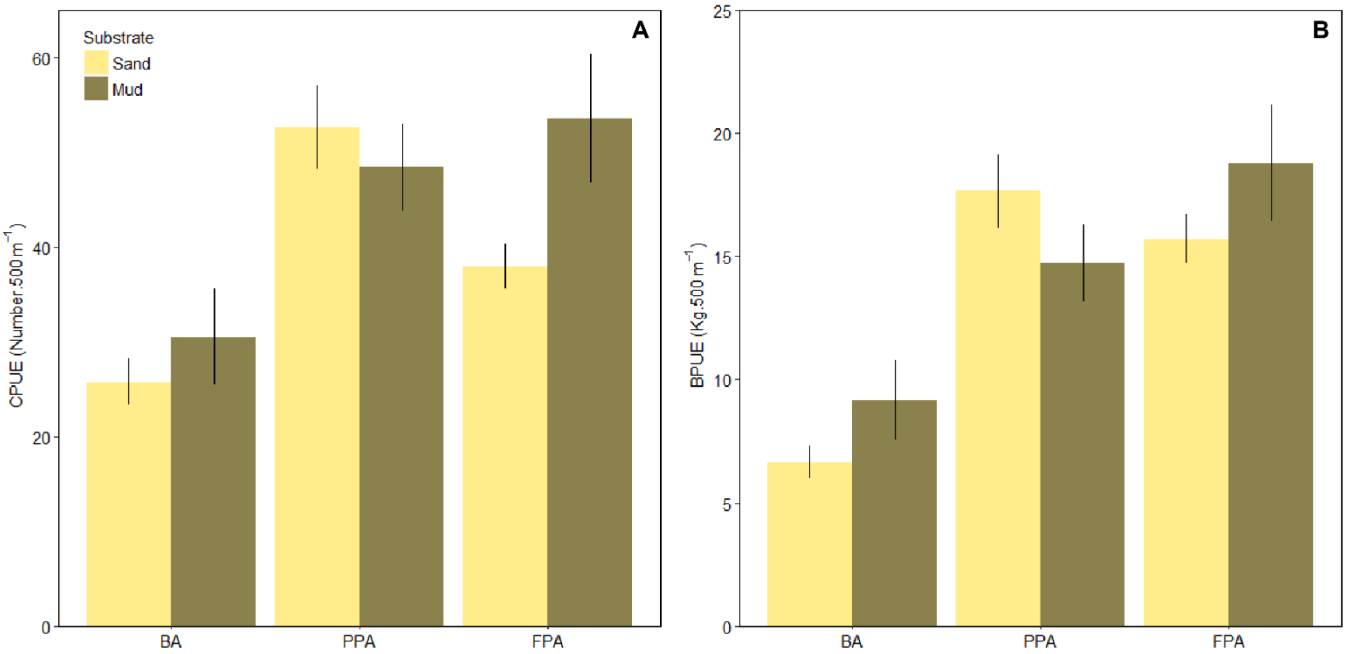

Table S2.1. List of fish species sampled during the experimental fishing surveys in the Prof. Luiz Saldanha Marine Park, and corresponding references (full references in File S1) used for length-weight (L-W) relationships (for all species except pelagics). The environment, maximum length reported (Froese and Pauly 2017, fishbase.org) and fisheries category (target, bycatch, non-commercial) of each species are also shown. (1) Dimension considered: disc width

Species	L-W reference	Environment	Maximum length (cm)	Fisheries category
Actinopterygii				
Argyrosomus regius	Santos et al. (2002)	Benthopelagic	230	Target
Arnoglossus imperialis	Borges et al. (2003)	Benthic	25	Bycatch
Arnoglossus laterna	Veiga et al. (2009)	Benthic	25	Bycatch
Arnoglossus thori	Santos et al. (2002)	Benthic	18	Bycatch
Auxis rochei	–	Pelagic	50	Target
Balistes capriscus	Santos et al. (2002)	Benthopelagic	60	Target
Boops boops	–	Pelagic	36	Non-commercial
Bothus podas	Morato et al. (2001)	Benthic	45	Bycatch
Chelidonichthys lastoviza	Santos et al. (2002)	Benthic	40	Bycatch
Chelidonichthys lucerna	Santos et al. (2002)	Benthic	75	Target
Chelidonichthys obscurus	Santos et al. (2002)	Benthic	34	Bycatch
Citharus linguatula	Santos et al. (2002)	Benthic	30	Bycatch
Conger conger	Gonçalves et al. (1997)	Benthic	300	Target
Dagetichthys lusitanicus	Cabral et al. (2003)	Benthic	40	Target
Dentex gibbosus	Mennes (1985)	Benthopelagic	106	Target
Dicentrarchus labrax	Gonçalves et al. (1997)	Benthopelagic	103	Target
Dicologlossa cuneata	Santos et al. (2002)	Benthic	30	Target
Diplodus sargus	Veiga et al. (2009)	Benthopelagic	45	Target
Diplodus vulgaris	Veiga et al. (2009)	Benthopelagic	45	Target
Halobatrachus didactylus	Veiga et al. (2009)	Benthic	50	Target
Labrus bergylta	Santos et al. (2002)	Benthopelagic	66	Bycatch
Lepidorhombus boscii	Mendes et al. (2004)	Benthic	40	Bycatch
Lophius budegassa	Morey et al. (2003)	Benthic	100	Target
Merluccius merluccius	Santos et al. (2002)	Benthopelagic	140	Target
Microchirus azevia	Veiga et al. (2009)	Benthic	40	Target
Microchirus ocellatus	Mendes et al. (2004)	Benthic	20	Target
Microchirus variegatus	Mendes et al. (2004)	Benthic	35	Target
Micromesistius poutassou	Santos et al. (2002)	Benthopelagic	50	Bycatch
Mugil cephalus	Koutrakis & Tsikliras (2003)	Benthopelagic	100	Bycatch
Mullus surmuletus	Veiga et al. (2009)	Benthic	40	Target
Pagellus acarne	Veiga et al. (2009)	Benthopelagic	26	Target
Pagellus bogaraveo	Veiga et al. (2009)	Benthopelagic	70	Target
Pagellus erythrinus	Gonçalves et al. (1997)	Benthopelagic	60	Target
Pagrus auriga	Pajuelo et al. (2006)	Benthopelagic	80	Target
Pagrus pagrus	Santos et al. (2002)	Benthopelagic	91	Target
Pegusa lascaris	Veiga et al. (2009)	Benthic	40	Target
Phycis phycis	Mendes et al. (2004)	Benthopelagic	65	Target
Sarda sarda	–	Pelagic	92	Target
Sardina pilchardus	–	Pelagic	28	Target
Scomber colias	–	Pelagic	64	Target
Scomber scombrus	–	Pelagic	60	Target

Table S2.1. Continuation.

Species	L-W reference	Environment	Maximum length (cm)	Fisheries category
Actinopterygii				
Scophthalmus maximus	Dorel (1986)	Benthic	100	Target
Scophthalmus rhombus	Veiga et al. (2009)	Benthic	75	Target
Scorpaena porcus	Petrakis & Stergiou (1995)	Benthic	41	Non-commercial
Scyliorhinus canicula	Santos et al. (2002)	Benthic	100	Target
Serranus cabrilla	Gonçalves et al. (1997)	Benthic	40	Target
Solea senegalensis	Veiga et al. (2009)	Benthic	60	Target
Solea solea	Veiga et al. (2009)	Benthic	70	Target
Sparus aurata	Veiga et al. (2009)	Benthopelagic	70	Target
Spicara maena	Valle et al. (2003)	Benthopelagic	25	Bycatch
Spondyliosoma cantharus	Veiga et al. (2009)	Benthopelagic	60	Target
Synapturichthys kleinii	Dulcic & Kraljevic (1996)	Benthic	40	Target
Trachinus draco	Santos et al. (2002)	Benthic	53	Non-commercial
Trachurus picturatus	–	Pelagic	60	Target
Trachurus trachurus	–	Pelagic	70	Target
Trisopterus luscus	Santos et al. (2002)	Benthopelagic	46	Target
Uranoscopus scaber	Mendes et al. (2004)	Benthic	40	Bycatch
Zeus faber	Morey et al. (2003)	Benthopelagic	90	Bycatch
Elasmobranchii				
Aetomylaeus bovinus	Basusta et al. (2012)	Benthopelagic	222 (1)	Non-commercial
Dasyatis pastinaca	Filiz & Bilge (2004)	Benthic	57 (1)	Bycatch
Mustelus mustelus	Filiz & Bilge (2004)	Benthopelagic	200	Target
Myliobatis aquila	Filiz & Bilge (2004)	Benthopelagic	183 (1)	Non-commercial
Raja brachyura	Dorel (1986)	Benthic	125	Target
Raja clavata	Mendes et al. (2004)	Benthic	139	Target
Raja miraletus	Mendes et al. (2004)	Benthic	63	Target
Raja undulata	Veiga et al. (2009)	Benthic	100	Target
Rostroraja alba	Ismen et al. (2007)	Benthic	240	Target
Torpedo cf. *mackayana*	Veiga et al. (2009)	Benthic	39	Target
Torpedo marmorata	Filiz & Bilge (2004)	Benthic	100	Target
Torpedo torpedo	Veiga et al. (2009)	Benthic	60	Target

Table S2.2. Table with frequency of occurrence of the species included in the analyses (FO%; percentage of samples with presence of each species). Species code (used in Figure 2.6) and family are also shown (species ordered according to decreasing FO%).

Species	Code	Family	FO%
Actinopterygii			
Chelidonichthys obscurus	Cobs	Triglidae	78.8
Microchirus azevia	Maze	Soleidae	77.1
Solea senegalensis	Ssen	Soleidae	72.6
Chelidonichthys lucerna	Cluc	Triglidae	68.2
Merluccius merluccius	Mmer	Merlucciidae	68.2
Chelidonichthys lastoviza	Clas	Triglidae	60.3
Halobatrachus didactylus	Hdid	Batrachoididae	36.3
Mullus surmuletus	Msur	Mullidae	35.2
Bothus podas	Bpod	Bothidae	31.8
Arnoglossus imperialis	Aimp	Bothidae	26.3
Pegusa lascaris	Plas	Soleidae	22.3
Solea solea	Ssol	Soleidae	22.3
Citharus linguatula	Clin	Citharidae	17.9
Balistes capriscus	Bcap	Balistidae	14.5
Spondyliosoma cantharus	Scant	Sparidae	14.0
Pagellus acarne	Paca	Sparidae	12.8
Scophthalmus rhombus	Srho	Scophthalmidae	11.7
Scyliorhinus canicula	Scan	Scyliorhinidae	11.2
Trisopterus luscus	Tlus	Gadidae	11.2
Serranus cabrilla	Scab	Serranidae	10.1
Trachinus draco	Tdra	Trachinidae	10.1
Dicologlossa cuneata	Dcun	Soleidae	9.5
Pagrus pagrus	Ppag	Sparidae	9.5
Diplodus vulgaris	Dvul	Sparidae	8.9
Zeus faber	Zfab	Zeidae	7.8
Conger conger	Ccon	Congridae	7.3
Microchirus variegatus	Mvar	Soleidae	5.0
Mugil cephalus	Mcep	Mugilidae	5.0
Pagellus erythrinus	Pery	Sparidae	5.0
Phycis phycis	Pphy	Phycidae	5.0
Scophthalmus maximus	Smax	Scophthalmidae	3.9
Diplodus sargus	Dsar	Sparidae	3.4
Scorpaena porcus	Spor	Scorpaenidae	3.4
Arnoglossus thori	Atho	Bothidae	2.8
Micromesistius poutassou	Mpou	Gadidae	2.8
Dicentrarchus labrax	Dlab	Moronidae	2.2
Microchirus ocellatus	Moce	Soleidae	2.2
Argyrosomus regius	Areg	Sciaenidae	1.7
Lepidorhombus boscii	Lbos	Scophthalmidae	1.7
Pagellus bogaraveo	Pbog	Sparidae	1.7
Dagetichthys lusitanicus	Dlus	Soleidae	1.7
Synapturichthys kleinii	Skle	Soleidae	1.7
Labrus bergylta	Lber	Labridae	1.1
Lophius budegassa	Lbud	Lophiidae	1.1
Pagrus auriga	Paur	Sparidae	1.1
Sparus aurata	Saur	Sparidae	1.1
Arnoglossus laterna	Alat	Bothidae	0.6
Dentex gibbosus	Dgib	Sparidae	0.6
Spicara maena	Smae	Centracanthidae	0.6
Uranoscopus scaber	Usca	Uranoscopidae	0.6

Species	Code	Family	FO%
Elasmobranchii			
Raja clavata	Rcla	Rajidae	49.2
Myliobatis aquila	Maqu	Myliobatidae	29.1
Torpedo torpedo	Ttor	Torpedinidae	26.8
Rostroraja alba	Ralb	Rajidae	25.1
Mustelus mustelus	Mmus	Triakidae	15.1
Raja undulata	Rund	Rajidae	15.1
Dasyatis pastinaca	Dpas	Dasyatidae	6.7
Aetomylaeus bovinus	Abov	Myliobatidae	2.2
Raja brachyura	Rbra	Rajidae	2.2
Raja miraletus	Rmir	Rajidae	1.7
Torpedo cf. *mackayana*	Tmac	Torpedinidae	0.6
Torpedo marmorata	Tmar	Torpedinidae	0.6

Table S2.3. Mean species abundance and biomass for each protection level and period. Mean (and standard error - SE) species abundance (CPUE - n·500m^{-1}) and biomass (BPUE - kg·500m^{-1}) according to the period (Period 1, Period 2) and the protection level (BA - buffer area; PPA - partially protected area; FPA - fully protected area). Only species with frequency of occurrence higher than 15% are shown.

	Buffer area (BA)				Partially protected area (PPA)				Fully protected area (FPA)			
	CPUE n·500 m^{-1}		BPUE kg·500 m^{-1}		CPUE n·500 m^{-1}		BPUE kg·500 m^{-1}		CPUE n·500 m^{-1}		BPUE kg·500 m^{-1}	
Taxa/Species	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
					Period 1 (Before MPA Full Implementation)							
Actinopterygii	24.9	4.5	5.0	0.8	42.2	5.0	8.9	0.8	35.1	3.3	9.4	0.9
Arnoglossus imperialis	0.40	0.16	0.02	0.01	0.24	0.11	0.01	0.01	0.05	0.05	0	0
Bothus podas	1.40	0.55	0.17	0.07	6.35	2.56	0.76	0.28	0.62	0.29	0.07	0.03
Chelidonichthys lastoviza	0.20	0.14	0.03	0.03	4.06	1.20	0.64	0.18	2.76	0.68	0.54	0.14
Chelidonichthys lucerna	1.33	0.56	0.32	0.13	1.41	0.41	0.40	0.10	2.10	0.50	0.55	0.13
Chelidonichthys obscurus	3.13	0.70	0.35	0.08	5.71	1.84	0.67	0.22	3.81	1.01	0.49	0.13
Citharus linguatula	0	0	0	0	0.06	0.06	0	0	0.33	0.13	0.03	0.01
Halobatrachus didactylus	0.13	0.13	0.04	0.04	0.88	0.26	0.33	0.10	2.24	0.48	0.97	0.21
Merluccius merluccius	3.27	0.92	0.74	0.18	2.41	0.59	0.77	0.21	2.14	0.55	0.71	0.17
Microchirus azevia	3.07	1.22	0.47	0.19	9.12	2.66	1.41	0.38	7.14	1.94	1.37	0.40
Mullus surmuletus	0.27	0.15	0.07	0.04	0.59	0.17	0.25	0.10	0.14	0.08	0.06	0.04
Pegusa lascaris	1.60	1.01	0.31	0.21	0.71	0.34	0.12	0.06	0.19	0.15	0.03	0.03
Solea senegalensis	5.33	1.43	1.64	0.45	7.88	1.79	2.73	0.63	5.76	1.35	2.16	0.52
Solea solea	0.40	0.40	0.07	0.07	0.47	0.29	0.13	0.07	3.76	1.69	1.34	0.45
Elasmobranchii	0.9	0.4	0.9	0.5	9.8	2.7	4.7	1.3	5.2	1.7	3.4	0.8
Mustelus mustelus	0.47	0.35	0.58	0.44	0.24	0.18	0.32	0.26	0.33	0.21	0.45	0.28
Myliobatis aquila	0.07	0.07	0.01	0.01	6.18	2.55	0.55	0.23	2.48	1.42	0.25	0.15
Raja clavata	0.07	0.07	0.06	0.06	0.94	0.64	2.01	1.21	0.95	0.28	1.67	0.71
Raja undulata	0	0	0	0	0.29	0.11	0.25	0.10	0.14	0.08	0.09	0.05
Rostroraja alba	0.07	0.07	0.04	0.04	0.18	0.13	0.29	0.20	0	0	0	0
Torpedo torpedo	0.20	0.14	0.14	0.10	1.00	0.34	0.85	0.31	0.81	0.31	0.57	0.23
TOTAL Period 1	25.9	2.4	5.9	0.6	51.9	4.9	13.6	1.3	40.3	2.8	12.8	1.0
					Period 2 (After MPA Full Implementation)							
Actinopterygii	26.4	2.7	5.7	0.6	45.4	3.5	9.9	0.6	43.0	4.0	11.5	1.1
Arnoglossus imperialis	0.48	0.10	0.02	0.00	0.51	0.13	0.02	0.00	0.20	0.09	0.01	0.00
Bothus podas	2.30	0.82	0.25	0.08	5.63	2.37	0.79	0.31	0.18	0.10	0.04	0.02
Chelidonichthys lastoviza	1.00	0.31	0.16	0.05	4.10	0.75	0.65	0.11	2.82	0.45	0.51	0.09
Chelidonichthys lucerna	2.20	0.66	0.54	0.16	3.44	0.60	0.90	0.13	3.53	0.51	1.13	0.16
Chelidonichthys obscurus	5.05	0.87	0.56	0.09	4.93	0.55	0.52	0.07	2.71	0.50	0.32	0.06
Citharus linguatula	0.13	0.10	0.01	0.01	0.32	0.11	0.02	0.01	0.87	0.27	0.05	0.01
Halobatrachus didactylus	0.13	0.08	0.04	0.02	1.17	0.38	0.51	0.16	1.31	0.25	0.61	0.12
Merluccius merluccius	1.73	0.34	0.76	0.19	2.12	0.53	1.21	0.34	2.00	0.43	0.85	0.18
Microchirus azevia	5.50	1.39	0.88	0.23	14.80	2.11	2.53	0.34	17.47	3.09	3.61	0.70
Mullus surmuletus	0.45	0.12	0.12	0.04	0.83	0.22	0.24	0.07	0.56	0.13	0.22	0.06
Pegusa lascaris	2.38	0.88	0.49	0.18	0.17	0.08	0.03	0.02	0.13	0.06	0.02	0.01
Solea senegalensis	2.10	0.40	0.67	0.13	4.32	0.70	1.66	0.27	3.64	0.53	1.39	0.20
Solea solea	0.05	0.05	0.02	0.02	0.17	0.06	0.05	0.02	2.84	0.86	1.23	0.33
Elasmobranchii	1.6	0.3	2.4	0.5	6.4	0.9	8.0	1.1	5.3	0.8	7.9	0.8
Mustelus mustelus	0.08	0.04	0.13	0.07	0.54	0.20	1.14	0.56	0.18	0.07	0.26	0.10
Myliobatis aquila	0.20	0.09	0.02	0.01	1.85	0.56	0.19	0.06	1.58	0.68	0.14	0.06
Raja clavata	0.30	0.10	0.93	0.36	1.63	0.23	3.17	0.49	1.82	0.26	4.26	0.69
Raja undulata	0.18	0.07	0.22	0.11	0.10	0.05	0.22	0.11	0.24	0.08	0.36	0.12
Rostroraja alba	0.38	0.12	0.66	0.31	0.83	0.19	2.44	0.59	0.62	0.17	2.26	0.61
Torpedo torpedo	0.40	0.15	0.35	0.14	1.07	0.44	0.63	0.22	0.44	0.15	0.43	0.15
TOTAL Period 2	**28.0**	**2.6**	**8.1**	**0.8**	**51.8**	**3.5**	**17.9**	**1.1**	**48.3**	**3.6**	**19.4**	**1.3**

Table S2.4. Mean species total length for each protection level and period: Mean total length (TL - cm) per species according to period (Period 1, Period 2) and protection level (BA - buffer area; PPA - partially protected area; FPA - fully protected area). Only species with frequency of occurrence higher than 15% are shown.

	Total length (cm)					
	Buffer area (BA)		Partially protected area (PPA)		Fully protected area (FPA)	
Taxa/Species	Mean	SE	Mean	SE	Mean	SE
	Period 1 (Before MPA Full Implementation)					
Actinopterygii	**27.1**	**0.3**	**26.3**	**0.2**	**28.6**	**0.2**
Arnoglossus imperialis	17.90	0.50	18.50	0.26	17.50	NA
Bothus podas	21.19	0.54	21.28	0.20	21.18	0.49
Chelidonichthys lastoviza	24.60	1.25	23.99	0.29	25.62	0.39
Chelidonichthys lucerna	29.50	0.86	31.21	0.90	30.34	0.61
Chelidonichthys obscurus	23.12	0.37	23.50	0.28	24.48	0.20
Citharus linguatula	NA	NA	21.20	NA	21.43	1.60
Halobatrachus didactylus	25.85	0.99	27.85	0.47	28.42	0.29
Merluccius merluccius	29.60	0.48	32.22	0.78	32.07	0.76
Microchirus azevia	23.43	0.42	23.39	0.25	25.07	0.29
Mullus surmuletus	24.28	0.82	27.87	1.38	26.17	4.86
Pegusa lascaris	24.37	0.47	23.58	0.56	23.68	1.40
Solea senegalensis	31.75	0.31	32.78	0.35	33.81	0.33
Solea solea	26.37	0.96	29.66	1.59	31.89	0.69
Elasmobranchii	**56.6**	**5.3**	**36.9**	**0.9**	**39.8**	**1.3**
Mustelus mustelus	72.93	0.69	74.75	2.10	74.43	1.48
Myliobatis aquila	33.30	NA	32.10	0.27	33.43	0.45
Raja clavata	53.00	NA	66.16	2.97	59.86	3.86
Raja undulata	NA	NA	58.02	4.60	53.00	4.04
Rostroraja alba	35.50	NA	46.63	5.96	NA	NA
Torpedo torpedo	34.33	1.69	36.24	1.49	33.68	1.68
TOTAL Period 1	**42.8**	**2.8**	**39.8**	**1.4**	**40.4**	**1.5**
	Period 2 (After MPA Full Implementation)					
Actinopterygii	**25.9**	**0.2**	**26.5**	**0.1**	**28.3**	**0.1**
Arnoglossus imperialis	16.84	0.26	16.60	0.41	18.31	0.18
Bothus podas	20.44	0.28	22.33	0.18	25.14	0.77
Chelidonichthys lastoviza	23.67	0.51	23.88	0.23	24.98	0.24
Chelidonichthys lucerna	29.73	0.31	30.10	0.40	32.50	0.36
Chelidonichthys obscurus	22.87	0.24	22.90	0.19	23.69	0.28
Citharus linguatula	20.46	0.56	20.08	0.97	19.12	0.30
Halobatrachus didactylus	27.49	0.63	28.49	0.27	29.40	0.23
Merluccius merluccius	36.46	0.68	39.18	0.57	35.27	0.67
Microchirus azevia	23.64	0.24	24.05	0.15	25.64	0.14
Mullus surmuletus	24.75	0.76	25.27	0.38	27.93	0.83
Pegusa lascaris	24.93	0.26	24.06	0.26	21.90	0.79
Solea senegalensis	32.07	0.36	34.03	0.31	34.02	0.29
Solea solea	32.30	1.10	30.46	0.68	34.10	0.53
Elasmobranchii	**50.3**	**2.7**	**49.8**	**1.3**	**53.3**	**1.3**
Mustelus mustelus	80.10	1.69	83.03	2.99	76.60	0.64
Myliobatis aquila	30.74	1.99	33.12	0.59	32.13	0.47
Raja clavata	74.12	4.39	64.43	1.37	69.00	1.18
Raja undulata	64.56	7.76	84.83	5.43	71.49	4.08
Rostroraja alba	43.09	4.15	56.00	1.79	58.11	2.85
Torpedo torpedo	37.29	1.02	31.65	0.99	37.83	1.42
TOTAL Period 2	**39.6**	**1.5**	**41.7**	**0.8**	**45.7**	**0.7**

Table S2.5. Mean species abundance for each substrate type: Mean (and standard error - SE) species abundance (CPUE - n·500m^{-1}) per substrate type. Only species with frequency of occurrence higher than 15% are shown.

Abundance (CPUE - n·500 m^{-1}) per substrate				
	Sand		Mud	
Taxa/Species	Mean	SE	Mean	SE
Actinopterygii	34.60	1.80	42.22	3.37
Arnoglossus imperialis	0.50	0.07	0.03	0.02
Bothus podas	3.97	0.97	0.27	0.20
Chelidonichthys lucerna	1.93	0.20	3.97	0.59
Chelidonichthys obscurus	5.34	0.47	2.13	0.35
Citharus linguatula	0.08	0.04	0.88	0.20
Halobatrachus didactylus	1.18	0.18	0.63	0.18
Merluccius merluccius	1.34	0.18	3.55	0.45
Microchirus azevia	6.55	0.90	18.92	2.29
Mullus surmuletus	0.43	0.07	0.69	0.15
Pegusa lascaris	1.27	0.35	0.03	0.02
Solea senegalensis	6.10	0.46	0.91	0.27
Solea solea	0.15	0.06	3.33	0.81
Trigloporus lastoviza	2.26	0.28	3.20	0.58
Elasmobranchii	5.93	0.70	2.70	0.30
Mustelus mustelus	0.23	0.08	0.38	0.12
Myliobatis aquila	2.66	0.57	0.11	0.07
Raja clavata	0.92	0.15	1.44	0.20
Raja undulata	0.24	0.05	0.03	0.02
Rostroraja alba	0.55	0.10	0.28	0.09
Torpedo torpedo	1.02	0.18	0	0
TOTAL	40.53	1.70	44.92	3.34

Table S2.6. Mean species abundance in each leaf - multivariate regression tree: Mean (and standard error - SE) species abundance (CPUE - n·500 m^{-1}) in each leaf of the multivariate regression tree analysis (MRT) (Leaf I - Muddy BA & PPA; Leaf II - Muddy FPA; Leaf III - Sandy BA; Leaf IV - Sandy PPA & FPA, Period 1; Leaf V - Sandy PPA & FPA, Period 2).

	Abundance (CPUE - n·500 m^{-1}) per leaf (MRT)									
	Leaf I		Leaf II		Leaf III		Leaf IV		Leaf V	
Species	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Aetomylaeus bovinus	0	0	0	0	0	0	0.22	0.13	0.02	0.02
Argyrosomus regius	0	0	0	0	0.09	0.06	0.04	0.04	0	0
Arnoglossus imperialis	0.05	0.03	0	0	0.66	0.12	0.22	0.09	0.55	0.11
Arnoglossus laterna	0	0	0	0	0	0	0.04	0.04	0	0
Arnoglossus thori	0	0	0	0	0	0	0.30	0.13	0	0
Auxis rochei	0	0	0	0	0	0	0	0	0.02	0.02
Balistes capriscus	0.10	0.06	0.08	0.08	1.14	0.52	0.35	0.20	0.53	0.22
Bothus podas	0.43	0.31	0	0	2.74	0.88	5.26	1.92	4.35	1.79
Chelidonichthys lastoviza	4.45	0.84	1.42	0.40	0.06	0.04	2.83	0.62	3.33	0.43
Chelidonichthys lucerna	4.33	0.82	3.23	0.74	0.89	0.22	1.39	0.38	2.84	0.33
Chelidonichthys obscurus	3.23	0.49	0.54	0.28	5.43	0.94	6.61	1.45	4.82	0.47
Citharus linguatula	0.28	0.12	1.77	0.41	0	0	0.04	0.04	0.13	0.07
Conger conger	0.05	0.03	0.12	0.06	0.03	0.03	0.04	0.04	0.13	0.05
Dagetichthys lusitanicus	0	0	0	0	0	0	0.04	0.04	0.04	0.03
Dasyatis pastinaca	0	0	0.15	0.15	0	0	0.22	0.14	0.27	0.14
Dentex gibbosus	0.03	0.03	0	0	0	0	0	0	0	0
Dicentrarchus labrax	0	0	0	0	0.03	0.03	0	0	0.05	0.03
Dicologlossa cuneata	0	0	0	0	0.26	0.11	0.35	0.13	0.18	0.09
Diplodus sargus	0	0	0	0	0.06	0.04	0.04	0.04	0.05	0.03
Diplodus vulgaris	0.05	0.03	0.19	0.10	0.03	0.03	0.13	0.13	0.33	0.16
Halobatrachus didactylus	0.05	0.03	1.62	0.39	0.20	0.11	2.13	0.40	1.38	0.30
Labrus bergylta	0	0	0	0	0.09	0.06	0	0	0	0
Lepidorhombus boscii	0	0	0.62	0.50	0	0	0	0	0	0
Lophius budegassa	0.08	0.06	0	0	0	0	0	0	0	0
Merluccius merluccius	3.40	0.58	3.50	0.68	1.74	0.40	1.83	0.51	0.93	0.16
Microchirus azevia	14.73	1.85	24.23	4.76	0.97	0.49	4.96	1.58	10.85	1.52
Microchirus ocellatus	0.10	0.06	0.04	0.04	0	0	0	0	0	0
Microchirus variegatus	0.10	0.05	0.31	0.14	0	0	0	0	0	0
Micromesistius poutassou	0.05	0.03	0	0	0.06	0.04	0.04	0.04	0	0
Mugil cephalus	0.05	0.05	0.08	0.05	0.06	0.04	0.26	0.14	0	0
Mullus surmuletus	0.88	0.22	0.35	0.11	0.26	0.12	0.39	0.12	0.58	0.12
Mustelus mustelus	0.40	0.17	0.31	0.12	0.09	0.06	0.22	0.18	0.35	0.14
Myliobatis aquila	0.05	0.03	0.19	0.16	0.26	0.10	6.65	2.16	2.62	0.66
Pagellus acarne	0.70	0.58	0	0	0.54	0.30	1.52	0.86	0.40	0.14
Pagellus bogaraveo	0.05	0.03	0.04	0.04	0	0	0	0	0	0
Pagellus erythrinus	0	0	0.08	0.05	0.06	0.04	0	0	0.09	0.04
Pagrus auriga	0	0	0	0	0.03	0.03	0	0	0.02	0.02
Pagrus pagrus	0.13	0.06	0.04	0.04	0.03	0.03	0.26	0.26	0.29	0.10
Pegusa lascaris	0.05	0.03	0.00	0.00	3.34	1.04	0.70	0.28	0.24	0.08
Phycis phycis	0.03	0.03	0	0	0	0	0.13	0.07	0.02	0.02

Table S2.6. Continuation.

Species	Abundance (CPUE - n·500 m^{-1}) per leaf (MRT) Leaf I Mean	 Leaf I SE	 Leaf II Mean	 Leaf II SE	 Leaf III Mean	 Leaf III SE	 Leaf IV Mean	 Leaf IV SE	 Leaf V Mean	 Leaf V SE
Raja brachyura	0.03	0.03	0	0	0	0	0.09	0.09	0.04	0.03
Raja clavata	1.38	0.32	2.00	0.36	0.06	0.04	0.35	0.12	1.47	0.21
Raja miraletus	0.10	0.06	0	0	0	0	0	0	0	0
Raja undulata	0.03	0.03	0.04	0.04	0.20	0.08	0.35	0.10	0.24	0.07
Rostroraja alba	0.35	0.13	0.15	0.09	0.20	0.10	0.13	0.10	0.96	0.17
Sarda sarda	0.03	0.03	0	0	0.03	0.03	0	0	0	0
Scophthalmus maximus	0.00	0.00	0.00	0.00	0.09	0.05	0.00	0.00	0.07	0.04
Scophthalmus rhombus	0	0	0.04	0.04	0.20	0.08	0.30	0.12	0.15	0.05
Scorpaena porcus	0.05	0.05	0.12	0.06	0.03	0.03	0.04	0.04	0	0
Scyliorhinus canicula	0.28	0.10	0.42	0.11	0	0	0.04	0.04	0.02	0.02
Serranus cabrilla	0.28	0.11	0.96	0.28	0	0	0	0	0	0
Solea senegalensis	0.88	0.22	1.00	0.57	4.43	0.69	10.00	1.31	5.71	0.53
Solea solea	0.38	0.18	7.62	1.66	0	0	0.48	0.25	0.11	0.04
Sparus aurata	0.03	0.03	0	0	0.06	0.06	0	0	0	0
Spicara maena	0	0	0	0	0	0	0	0	0.02	0.02
Spondyliosoma cantharus	0.25	0.15	0.69	0.31	0.17	0.14	0.09	0.09	0.33	0.11
Synapturichthys kleinii	0.03	0.03	0	0	0.03	0.03	0	0	0.02	0.02
Torpedo cf. *mackayana*	0	0	0	0	0	0	0	0	0.02	0.02
Torpedo marmorata	0.03	0.03	0	0	0	0	0	0	0	0
Torpedo torpedo	0	0	0	0	0.54	0.17	1.48	0.32	1.16	0.34
Trachinus draco	0.03	0.03	0	0	0.20	0.10	0.09	0.09	0.36	0.14
Trisopterus luscus	0.80	0.34	0.92	0.44	0.06	0.06	0.09	0.06	0.09	0.05
Uranoscopus scaber	0	0	0	0	0.03	0.03	0	0	0	0
Zeus faber	0.08	0.04	0.04	0.04	0.11	0.07	0.04	0.04	0.11	0.04

Table S2.7. Discriminant species at each leaf of the multivariate regression tree: Discriminant species at each leaf (terminal node) of the multivariate regression tree analysis (Leaf I - Muddy BA & PPA; Leaf II - Muddy FPA; Leaf III - Sandy BA; Leaf IV - Sandy PPA & FPA, Period 1; Leaf V - Sandy PPA & FPA, Period 2). Indicator values (IndVal) are shown (species ordered according to decreasing IndVal) and the symbol '⋆' signalizes the indicator species (highest IndVal obtained for that species was in that node) (note: discriminant species with IndVal ≥ 20 are shown in Figure 2.7).

Discriminant species for each leaf (terminal node)	
Species	IndVal
Leaf I	
Chelidonichthys lastoviza	27
Merluccius merluccius	24*
Mullus surmuletus	19*
Raja miraletus	8*
Leaf II	
Solea solea	71
Citharus linguatula	61*
Microchirus azevia	32
Serranus cabrilla	31*
Raja clavata	25
Scyliorhinus canicula	19*
Lepidorhombus boscii	12*
Microchirus variegatus	11*
Leaf III	
Pegusa lascaris	43*
Arnoglossus imperialis	29*
Chelidonichthys obscurus	29
Bothus podas	19*
Balistes capriscus	13*
Leaf IV	
Solea senegalensis	34
Torpedo torpedo	26*
Arnoglossus thori	19*
Halobatrachus didactylus	19*
Myliobatis aquila	19*
Aetomylaeus bovinus	12*
Raja undulata	12*
Dicologlossa cuneata	11*
Scophthalmus rhombus	11*
Leaf V	
Chelidonichthys lucerna	24
Rostroraja alba	24*

Table S2.8. Summary table of the ratios analyses and multivariate regression tree: Summary table of the ratio (Protected area : Buffer area; After MPA Full Implementation : Before MPA Full Implementation) analyses (N - abundance, B - biomass, L - total length) and multivariate regression tree (MRT) (MRT Ind Spp - cluster where the species was assigned as indicator) (BA - buffer area; PPA - partially protected area; FPA - fully protected area; PA - protected area: PPA&FPA; Period 1 - Before MPA Full Implementation; Period 2 - After MPA Full Implementation) (↑ - Increase; ↓ - Decrease). Species included in the ratios analyses and obtained in the MRT output with IndVal ≥ 20 are shown. (1) Species not included in the ratios analyses; (2) Species with less than 10 individuals in the BA; (3) Species with less than 10 individuals in Period 1; (4) Species with indicator value (IndVal) < 20.

Species	Abundance Ratio Significance		Biomass Ratio Significance		Length Ratio Significance		Ratios - Observations		MRT	N / B time trend
	PA : BA	P2 : P1	PA : BA	P2 : P1	PA : BA	P2 : P1	PA : BA	P2 : P1	Ind Spp	category
Solea senegalensis	> 1	< 1	> 1	< 1	> 1	> 1	N, B, L > PA	N, B ↓, L ↑	Sand	Negative
Pegusa lascaris	< 1	-	< 1	-	< 1	-	N, B, L < PA	-	Sand, BA	Neutral
Arnoglossus imperialis	-	-	-	-	-	< 1	-	L ↓	Sand, BA	
Chelidonichthys obscurus	-	-	-	-	-	< 1	-	L ↓	Mud, BA&PPA	
Torpedo torpedo	> 1	-	> 1	-	-	-	N, B > PA	-	Sand, PA, P1 (4)	
Myliobatis aquila	> 1	-	> 1	-	-	-	N, B > PA	-	Sand, PA, P1 (4)	
Chelidonichthys lastoviza	> 1	-	> 1	-	-	-	N, B > PA	-	Sand PA	
Halobatrachus didactylus	> 1	-	> 1	-	> 1	-	N, B, L > PA	-	Sand, PA, P1 (4)	
Serranus cabrilla	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Mud, FPA	
Merluccius merluccius	-	-	-	-	> 1	> 1	L > PA	L ↑	Mud, BA&PPA	
Bothus podas	-	-	-	-	> 1	> 1	L > PA	L ↑	Sand, BA	
Mustelus mustelus	-	-	-	-	> 1	> 1	L > PA	L ↑	-	
Solea solea	> 1	-	> 1	-	(2)	> 1	N, B > PA	L ↑	Mud, FPA	
Mullus surmuletus	-	> 1	> 1	-	> 1	-	B, L > PA	N ↑	Mud, BA&PPA (4)	Positive
Raja undulata	-	-	-	> 1	(2)	(3)	-	B ↑	Sand, PA, P1 (4)	
Citharus linguatula	> 1	> 1	> 1	> 1	(2)	(3)	N, B > PA	N, B ↑	Mud, FPA	
Microchirus azevia	> 1	> 1	> 1	> 1	> 1	> 1	N, B, L > PA	N, B, L ↑	Mud	
Rostroraja alba	> 1	> 1	> 1	> 1	> 1	(3)	N, B, L > PA	N, B ↑	Sand, PA, P2	
Raja clavata	> 1	> 1	> 1	> 1	> 1	> 1	N, B, L > PA	N, B, L ↑	Sand, PA, P2	
Chelidonichthys lucerna	> 1	> 1	> 1	> 1	> 1	> 1	N, B, L > PA	N, B, L ↑	Sand, PA, P2	

Table S2.9. Table of additional information of species traits: fisheries category and reproduction. Additional information of species traits: taxonomic family, environment, maximum length, market price and reproduction (mode, length and age at maturity) (references of length/age at maturity listed). Fisheries category is shown in the 'price per kg' column: T - target, B - bycatch, NC - non-commercial. Species ranked according to abundance (N) and biomass (B) time trend category (2nd column; see Table S8). (1) Information not found; (2) Disc width; (3) Hermaphroditic.

Species	N / B time trend category	Family	Environment	Reproduction mode	Price per kg (€)	Maximum length (cm)	Length at maturity (cm)	Age at maturity (yrs)	Reference
Solea senegalensis	Negative	Soleidae	Benthic	Ovuliparous	14.43 (T)	60	32	3	Dinis (1986)
Pegusa lascaris	Neutral	Soleidae	Benthic	Ovuliparous	9.22 (T)	40	23	3	Andrade (1990)
Arnoglossus imperialis		Bothidae	Benthic	Ovuliparous	3.31 (B)	25	(1)	(1)	–
Chelidonichthys obscurus		Triglidae	Benthic	Ovuliparous	1.28 (B)	34	13	0	Muñoz et al. (2003)
Torpedo torpedo		Torpedinidae	Benthic	Ovoviviparous	2.22 (T)	60	26	3	Consalvo et al. (2007)
Myliobatis aquila		Myliobatidae	Benthopelagic	Ovoviviparous	– (NC)	183 (2)	60 (2)	4	Fischer et al. (1987)
Chelidonichthys lastoviza		Triglidae	Benthic	Ovuliparous	1.28 (B)	40	16	3	Jrad et al. (2010)
Halobatrachus didactylus		Batrachoididae	Benthic	Ovuliparous	3.54 (T)	50	30	5	Palazón-Fernández et al. (2001)
Serranus cabrilla		Serranidae	Benthic	Ovuliparous (3)	3.25 (T)	40	15	3	García-Díaz et al. (1997)
Merluccius merluccius		Merlucciidae	Benthopelagic	Ovuliparous	4.19 (T)	140	45	4	Piñeiro & Saínza (2003)
Bothus podas		Bothidae	Benthic	Ovuliparous	3.31 (B)	45	14	2	Morato et al. (2007)
Mustelus mustelus		Triakidae	Benthopelagic	Viviparous	2.69 (T)	200	140	15	Smale & Compagno (1997)
Solea solea		Soleidae	Benthic	Ovuliparous	14.43 (T)	70	30	4	Dinis (1986)
Mullus surmuletus	Positive	Mullidae	Benthic	Ovuliparous	10.94 (T)	40	15	2	Morales-Nin (1991)
Raja undulata		Rajidae	Benthic	Oviparous	2.60 (T)	100	76	8	Coelho & Erzini (2006)
Citharus linguatula		Citharidae	Benthic	Ovuliparous	3.31 (B)	30	19	5	Teixeira et al. (2010)
Microchirus azevia		Soleidae	Benthic	Ovuliparous	9.22 (T)	40	23	3	Afonso-Dias et al. (2005)
Rostroraja alba		Rajidae	Benthic	Oviparous	2.60 (T)	240	129	27	Kadri et al. (2014a)
Raja clavata		Rajidae	Benthic	Oviparous	2.60 (T)	139	78	7	Serra-Pereira et al. (2011)
Chelidonichthys lucerna		Triglidae	Benthic	Ovuliparous	1.28 (T)	75	25	3	Vallisneri et al. (2011)

Table S2.10. Table of additional information of species traits: longevity and movement pattern. Additional information of species traits: maximum reported age, theoretical longevity (based on Von Bertalanffy Growth Function - VBGF) and movement pattern. Information on the type of study reporting movement pattern data is shown: FS - fishing surveys, MR - mark-recapture, OC - otolith chemistry, SI - stable isotopes, T - telemetry, UVC - underwater visual census, NS - not specified. Species ranked according to abundance (N) and biomass (B) time trend category (2nd column; see Table S2.8). (1) Information not found; (2) Based on congener species

Species	N / B time trend category	Max. reported age (yrs)	VBGF theoretical longevity (yrs)	Reference
Solea senegalensis	Negative	11	15	Andrade (1990)
Pegusa lascaris	Neutral	11	11	Dinis (1986)
Arnoglossus imperialis		(1)	11 (2)	Ilkyaz et al. (2017)
Chelidonichthys obscurus		(1)	11 (2)	Akalin & Ilhan (2013)
Torpedo torpedo		(1)	16 (2)	Duman & Basusta (2013)
Myliobatis aquila		(1)	27 (2)	Martin & Cailliet (1988)
Chelidonichthys lastoviza		18	11	Baron (1985) Akalin & Ilhan (2013)
Halobatrachus didactylus		12	16	Palazón-Fernández et al. (2010)
Serranus cabrilla		11	16	Gordo et al. (2016)
Merluccius merluccius		20	22	Muus & Nielsen (1999) Piñeiro & Saínza (2003)
Bothus podas		6	5	Morato et al. (2007)
Mustelus mustelus		24	46	Goosen & Smale (1997)
Solea solea		27	14	Fischer et al. (1987) Dinis (1986)
Mullus surmuletus	Positive	11	13	ICES (2012) Arslan & Ismen (2013)
Raja undulata		14	26	Moura et al. (2007)
Citharus linguatula		7	10	Vassilopoulou & Papaconstantinou (1994)
Microchirus azevia		8	9	Andrade (1998)
Rostroraja alba		35	77	Kadri et al. (2014b)
Raja clavata		15	30	Serra-Pereira et al. (2008)
Chelidonichthys lucerna		15	13	Baron (1985) Ismen et al. (2004)
		Movement pattern	Information source	Reference
Solea senegalensis	Negative	seasonal migrations home range: 1.18 km^2	FS, T	Andrade (1990) Abecasis et al. (2014)
Pegusa lascaris	Neutral	(1)	–	–
Arnoglossus imperialis		resident	SI	Vinagre et al. (2011)
Chelidonichthys obscurus		(1)	–	–
Torpedo torpedo		(1)	–	–
Myliobatis aquila		seasonal migrations	MR	Barreiros & Rodeia (2004)
Chelidonichthys lastoviza		seasonal migrations	FS	Tsimenides et al. (1992)
Halobatrachus didactylus		resident	NS	Palazón-Fernández et al. (2001)
Serranus cabrilla		resident home range: 0.77 km^2	T	Alós et al. (2011)
Merluccius merluccius		seasonal migrations	FS	Korta et al. (2015)
Bothus podas		resident	UVC	Carvalho et al. (2003)
Mustelus mustelus		migratory	MR	ICES (2013)
Solea solea		seasonal migrations	OC	Tanner et al. (2013)
Mullus surmuletus	Positive	seasonal migrations	NS	Gharbi & Ktari (1981)
Raja undulata		short-distance migrations (usually less than 13 km)	MR	Biais et al. (2014)
Citharus linguatula		(1)	–	–
Microchirus azevia		(1)	–	–
Rostroraja alba		seasonal migrations	FS	Quigley (1984)
Raja clavata		seasonal migrations home range: 50-60 km^2	MR, T	Walker et al. (1997) Hunter et al. (2005)
Chelidonichthys lucerna		seasonal migrations	FS	Tsimenides et al. (1992)

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CHAPTER III

Spatial protection for the management of the thornback ray (*Raja clavata*): evidence from a coastal marine protected area

Sousa, I., Olsen, E. M., Moland, E., Gonçalves, J. M. S., Freitas, C., Bentes, L., Cabral, S., Gonçalves, E. J., Erzini, K. Spatial protection for the management of the thornback ray (*Raja clavata*): evidence from a coastal marine protected area

3. Spatial protection for the management of the thornback ray (*Raja clavata*): evidence from a coastal marine protected area

3.1. Abstract

Marine protected areas (MPAs) are among the most promising solutions for meeting conservation and fisheries management goals in coastal areas. Elasmobranchs are recognized as a group that raises conservation concerns, given their low resilience to fishing pressure. Using MPAs as a tool for the recovery of elasmobranch populations is a promising approach that requires assessment of its effectiveness. Although the thornback ray (*Raja clavata*) is one of the most widespread skates in the Northeastern Atlantic, declines in abundance and biomass have been reported in many regions. The Prof. Luiz Saldanha Marine Park (PLSMP) is a coastal MPA in Portugal where it is possible to study populations living in temperate waters. We used experimental fishing, conventional tagging and acoustic telemetry to assess whether spatial protection measures were benefiting this species. The species was significantly more abundant in areas with higher protection levels, and tracking data confirmed regular use of these areas, as several individuals were classified as residents. We also observed significant increases over time in the protected area for both adults and juveniles. However, while adults maintained these higher levels, juveniles experienced a subsequent significant decrease. The collected tracking data on adult males indicated some seasonal trends. Utilization areas seemed to expand during autumn, and residency was higher in spring and summer. It also demonstrated, along with the abundance data, that adults preferably use the offshore section of the protected area, at depths greater than 30m. Overall, this coastal MPA shows potential for protecting both juvenile and adult thornback rays, particularly males. Nonetheless, these two groups have also shown some aspects of vulnerability. The reasons for the observed declining trend of juveniles need to be further investigated. There is evidence that adult males may often be susceptible to fishing, given their preference for depths greater than 30m and their high frequency of excursions to outside the protected area. These findings contribute valuable insights for the conservation of thornback rays and the broader study of Rajidae species' ecology.

3.2. Introduction

Over the last few decades, there has been an increasing interest in the field of movement ecology of aquatic organisms (Lennox et al. 2017, Lowerre-Barbieri et al. 2019). Understanding the spatial behaviour of individuals and communities is crucial for predicting the distribution dynamics of species in a changing environment (Hussey et al. 2015). Ultimately, this knowledge enables the adoption of species conservation measures, and allows the assessment of the effectiveness of spatial management strategies (Hays et al. 2019). As marine protected areas (MPAs) become one of the most prominent solutions to meet conservation and fisheries management goals in the marine environment, there is an increasing advocacy for MPA designs to consider the movement patterns of ecologically important species (D'Aloia et al. 2017).

In efforts of implementing effective MPAs, considerable discussion has focused on successful MPA attributes, such as size, spatial connectivity (network of MPAs), and level of protection and enforcement (Claudet et al. 2008, Campbell et al. 2012). Additionally, there has also been substantial attention given to how life history traits impact the efficiency of spatial protection measures (Claudet et al. 2010, Hutchings et al. 2012). More recently, studies have emphasized the role of individual behaviour in the potential benefits of marine reserves (Finger et al. 2017, Villegas-Rios et al. 2018, Villegas-Rios et al. 2021). The degree of mobility seems to be a particularly complex aspect, as it has inter- and intraspecific variability, and may also change across ontogenic stages (Chapman et al. 2012, Secor 2015). Research has revealed that, although resident species are expected to benefit more from MPAs, positive impacts may also occur in wide-ranging species (Knip et al. 2012).

As bottom dwelling predators, skates and rays (infraclass Batoidea) play a critical role for the maintenance of ecosystem balance (Saglam et al. 2012). Their life traits include low fecundity and late maturity, which translate into low recovery potential when populations are impacted by fishing exploitation and other anthropogenic pressures (Frisk et al. 2005, Dulvy et al. 2014). The batoid group (order Rajiformes) holds a remarkably high diversity, as it includes more valid species (n = 574) than the other nine orders of Chondrichthyes (sharks and rays) combined (n = 528) (Ebert & Compagno 2007). Despite its high diversity and recognition of conservation concerns, this group remains relatively understudied.

The thornback ray, *Raja clavata*, is one of the most frequently landed skate species in the Northeast Atlantic, both in the north (e.g. Walker et al. 1997; Dulvy et al. 2000) and southern Europe (e.g. Machado et al. 2004, Figueiredo et al. 2007). This coastal species ranges throughout the eastern Atlantic, from Iceland to South Africa, occurs in the Mediterranean and Black Seas, and can also be found in the Indian Ocean between Southern Africa and Madagascar (Froese & Pauly 2024). It occurs from shallow waters down to 700 m depth on a variety of substrata (Stehmann & Bürkel 1984), but it is usually found up to 250 m deep (Last et al. 2016, Santos et al. 2021). The estimated age at first maturity ranges from 3.5 to 6.5 years in males, and 5 to 6.5 years in females (Ryland & Ajayi 1984, Whittamore & McCarthy 2005). Reproductive season duration appears to differ between regions, as described, for example, between March and September for UK coastal waters (Ryland & Ajayi 1984), while in southern areas it seems to occur over a more extended period. Serra-Pereira et al. (2011) refer that spawning occurs mainly between May and January in Portuguese mainland coastal waters, although individuals in spawning conditions are found throughout the entire year. Although the thornback ray is one of the most widespread skates in the Northeastern Atlantic, declines in abundance and biomass have been reported for many regions (e.g., Fock 2014, Ferragut-Perello et al. 2023). It is presently classified as 'Near Threatened' by the IUCN (Ellis et al. 2016, IUCN 2024).

The movement ecology of thornback rays has been the focus of some studies. Adults are known to form local subpopulations (Walker et al. 1997). The species has been regarded as relatively sedentary and generally does not perform long-range migrations (Walker et al. 1997, Ellis et al. 2011). Several studies in the past decades have highlighted that thornback rays are capable of long-distance movements, even reaching hundreds of kilometres (Hunter et al. 2005a, Hunter et al. 2005b, Bird et al. 2019). Some of this research has also described a seasonal migration of mature individuals from deep to shallow waters in spring and summer, and relate this migration with reproductive processes, like mating and egg-laying, or species preferences (Hunter et al. 2005a, Ellis et al. 2005, Humphries et al. 2016). Additionally, juveniles have been described to remain close to the shore during their first years (Walker et al. 1997, Martin et al. 2012, Serra-Pereira et al. 2014, McAllister et al. 2023). The use of biologging has enabled advances in the study of this species' spatial ecology. Humphries et al. (2016) used data storage tags and reported that most displacements were under 30 km, specifically for the population in the southwest of the British Isles. Subsequent work by Simpson et al. (2021) using acoustic telemetry confirmed that the population showed site fidelity to relatively shallow areas (19-25m deep). Recent studies in the Iberian Peninsula highlighted the diverse behaviour of this species. Papadopoulo et al. (2023) reported low residency in a coastal bay within a protected area, while Kraft et al. (2024) described both transient and resident behaviours.

This work investigates the potential of protection that a relatively small coastal MPA can provide for the thornback ray. The Prof. Luiz Saldanha Marine Park, located in the western coast of Portugal, constituted the case study in our research. Some studies have already been undertaken in the area aiming to assess its potential of protection for elasmobranchs (Sousa et al. 2019, Kraft et al. 2023, Kraft et al. 2024) and other soft-bottom species (Abecasis et al. 2014, Sousa et al. 2018, Martínez-Ramírez et al. 2021). To examine if the abundance of thornback ray has increased overtime within the MPA boundaries, experimental fishing data collected during seven years was analysed. Furthermore, we used acoustic telemetry and conventional tagging to study the spatial ecology of this species. Specifically, we estimated site fidelity, spatial utilization and dispersal patterns, and also examined whether these indicators displayed seasonal trends.

3.3. Methods

3.3.1. Study area

The study was conducted in a MPA located in the western coast of Portugal (38º26’N, 9º1’W), the Prof. Luiz Saldanha Marine Park (PLSMP). Extending along 38 km of coast, this coastal MPA has an area of 53 km^2 that encompasses depths from 0 to 100m. A wide variety of habitats and benthic communities (Henriques et al. 2014), the influence of both cold and warm temperate waters, and two nearby estuaries (Sado and Tejo), contribute for the local marine biodiversity (Henriques et al. 1999, Gonçalves et al. 2002, Cunha et al. 2014). The area includes rocky shores and a wide extent of soft sediments, which are dominant at depths greater than 15-20m (Henriques et al. 2014). In most of its extent, sand is replaced by substrates with higher mud content at bottoms beyond 30m deep (Henriques et al. 2014). Two important fishing harbours are located in this region: the port of Sesimbra, located within the MPA area, and the nearby port of Setúbal. The area is also used for other human activities such as recreational fishing and scuba diving. The MPA was designated in 1998, yet the management plan was only established in 2005 (Pita et al. 2020). Restrictions were implemented gradually, and full implementation was achieved in August 2009 (PPA protection fully implemented since August 2007). The MPA includes three protection levels

(Figure 3.1): fully protected (FPA, one area - 4 km^2), partially protected (PPA, four areas - 21 km^2), and buffer (BA, three areas - 28 km^2). Within the park limits, purse-seining, trawling, dredging and spearfishing are forbidden. Commercial fishing boats need a license to operate in the area, and only boats <7m long are allowed. The FPA excludes all human activities (no-take, no-go area; exception for research). In the PPAs, commercial fishing with octopus traps and jigs is permitted, and in the BAs, longline, gill nets and trammel nets are additionally allowed. Recreational fishing is permitted only in the BAs.

3.3.2. Abundance analysis

To estimate the relative abundance of the thornback ray in the marine park, experimental fishing with trammel nets was conducted twice a year, each spring and autumn. This was undertaken under a broader monitoring of the fish community (Sousa et al. 2018, Martínez-Ramírez et al. 2021). Sampling occurred between 2007-2014: two years during the implementation period, and five years after full implementation (November 2007 - May 2014). In each campaign, at least two locations within each protection level were sampled in triplicate. The sampling area comprised the large BA, which included Sesimbra bay, the FPA and adjacent PPAs (Figure 3.1). A total of 260 samples were obtained, with each sample consisting of the catch of 500m of trammel net (soak time: ~24h). Nets were set at two different depth intervals, where distinct substrate types were present: at 10-20m, over sandy bottom, and at 30-45m deep, on muddy bottom. Rays were measured (total length, precaudal length, disc width), sex was determined, and they were subsequently released. Size was used to classify individuals as mature or immature, using the total length at first maturity reported by Serra-Pereira et al. (2011) as a reference (TL_{50} - total length size at 50% maturity; TL_{50} males = 67.6cm; TL_{50} females = 78.4cm). Total length was also used for weight estimation, according to the length-weight relationship described in literature (Mendes et al. 2004). Catch per unit effort (CPUE) was considered a proxy of abundance, and yearly averages enabled the analysis of temporal trends. As sampling started in autumn 2007, year 1 includes data from autumn 2007 and spring 2008; yearly data were obtained until year 7 (autumn 2013–spring 2014). As full implementation was achieved in August 2009, years 1 and 2 correspond to the MPA implementation period. For statistical comparisons, the PPA and FPA were joined under the category ‘protected area’ (PA), as fishing gear allowed to operate in the PPA (octopus traps and jigs) do not catch rays.

Two-proportion z-tests were applied to analyse the statistical significance of ratios (α=0.05) according to groups (males *vs* females, immature *vs* mature). In the graphical representations of sex groups, only mature individuals were depicted. To examine patterns in species abundance, model fitting was performed using generalized additive models (GAM). This approach accommodated the non-normality of data and nonlinear impacts of explanatory variables. The R package mgcv (Wood, 2017) was used for this process. A negative binomial distribution was selected to account for the overdispersed nature typical of count data. Models were applied to three different groups: all individuals, mature individuals, and immature individuals. Five explanatory variables were tested: protection level (PA and BA), depth interval (10-20m and 30-45m), and season (Spring and Autumn), which were modelled as parametric terms, and sea surface temperature (SST) and year (y1 to y7; y1: August 2007 - August 2008; y7: August 2013 - August 2014), for which a smooth function was fitted using thin plate regression spline. High resolution SST data were obtained from NOAA (https://psl.noaa.gov/data/). Significance of variables was first assessed individually, and significant variables were incorporated into an initial model, then used in backward stepwise model selection. Comparison of models was done using Akaike Information Criterion (AIC) and performing Chi-squared tests. The covariance between variables was also examined. The option of including 'Campaign' as random effect was also explored. The models without this component were selected based on the AIC and the homogeneity of residuals. The final model validation was performed by examining overdispersion, testing for zero-inflation, and visually inspecting the residual plots.

3.3.3. Conventional tagging and acoustic telemetry

During the experimental fishing campaigns of 2011-2013, a total of 91 specimens were tagged using traditional T-bar tags. These were then inserted into the musculature at the base of the tail. The tags exhibited the information of a unique identification number and telephone for contact.

Specimens used for telemetry tracking were captured in the MPA area during the November 2013 experimental fishing surveys (trammel net) and in three other fishing campaigns in 2015 using longline (soak time 3-12h; monofilament; hooks nº 3/0 baited with sardine and mackerel). Each individual was tagged with an ultrasonic transmitter (Innovasea V13-1L, 36 x 13mm, weight in water 6g) programmed to emit a unique identification code at random intervals of 40–80sec (expected battery life 600 days). The transmitter was surgically implanted in the abdominal cavity through a small incision, which was then closed with absorbable suture and surgical glue. The specimens were kept in a basin with saltwater while onboard and positioned with the ventral side up during tagging, which induced tonic immobility. Information of body measures (total length, precaudal length, disc width) and sex were registered. Individuals over the total length size at 50% maturity reported by Serra-Pereira et al. (2011) were considered mature. Before release, an external tag (T-bar tags or Petersen disc, with unique number and telephone information) was also placed in the posterior part of the body (T-bar: base of caudal fin; Petersen disc: posterior part of pectoral fin), allowing visual identification of individuals with electronic tags. After assessing the activity level (responsive to touch), individuals were released at the capture site (Figure 3.1). All rays immediately swam toward the bottom when released. Specimens were handled following standard procedures and were licensed by the National Nature Conservation Institute (ICNF, license no. 245/2013/CAPT).

An acoustic monitoring design with sixteen ultrasonic receivers (Innovasea VR2W, 69kHz) was set in the FPA and adjacent PPA in early 2014 (14 receivers in the FPA, two receivers in the PPA) (Figure 3.1). Receivers were positioned considering preliminary range tests results, which showed an average detection range of 475m (distance of 50% detection of tag transmissions). They were placed differently depending on their location relative to the coast. At inshore listening stations (LS), receivers were placed in concrete moorings on the bottom pointing upwards. In offshore stations, they were attached to a rope pointing downwards; this rope was fixed to a bottom mooring and was kept vertical by a submerged buoy (~5m below surface). Three stations were placed at three navigational buoys within the FPA (LS9, LD12, LS15). To confirm that detection efficacy was unaffected by environmental variables, three sentinel tags were attached to the three buoys. Receivers at LS10 and LS14 were removed from the array after being retrieved by fishermen due to entanglement with their gear (octopus traps). These stations collected data for eight and eleven months, respectively. The receiver at LS16 was lost, probably because of contact with fishing gear. The data retrieved from LS16 corresponded to approximately one month of monitoring. The stations were classified as inshore or offshore, according to their distance to the shore (Figure 3.1). Monitoring occurred over three years, between 25th of April 2014 and 13th of June 2017.

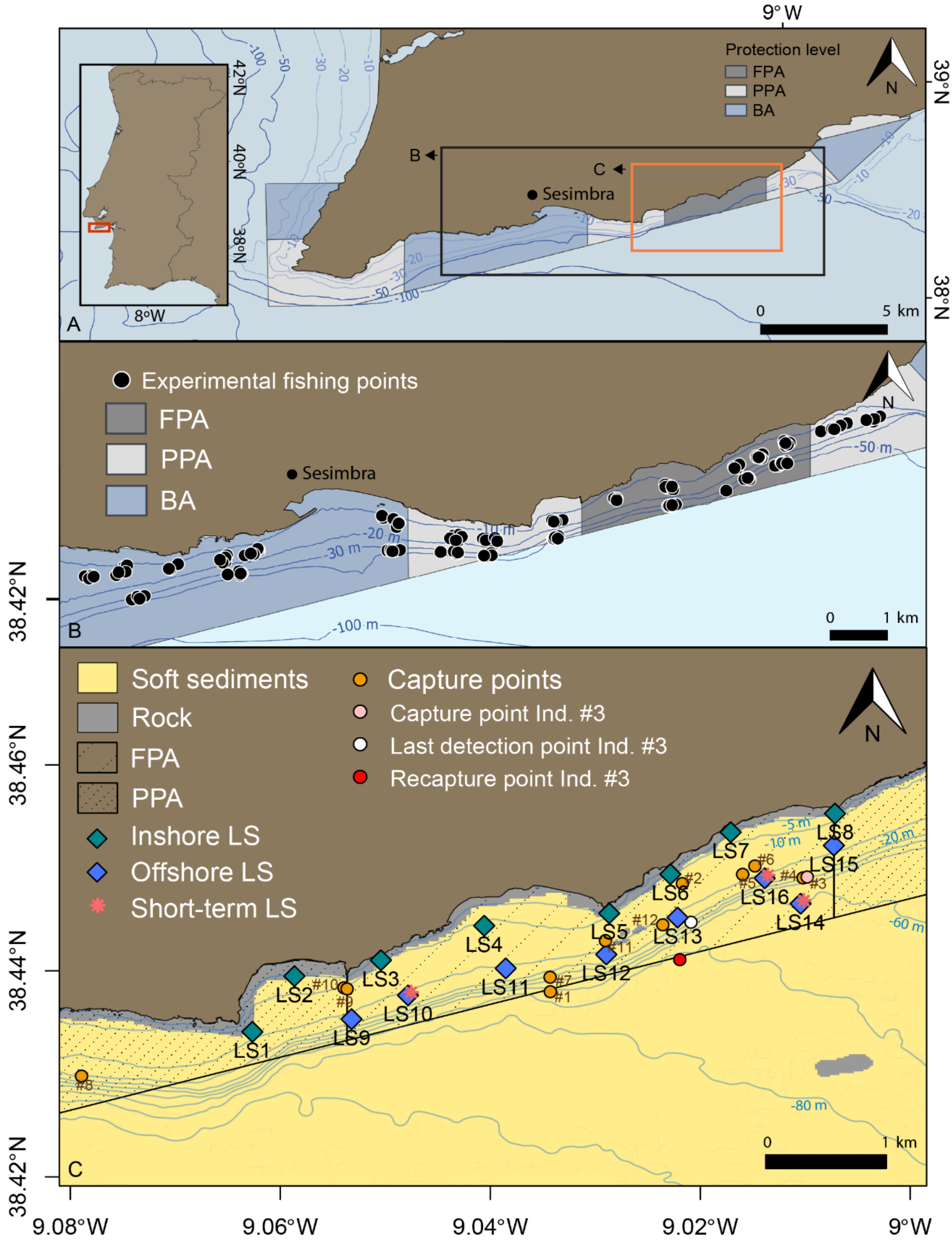

Figure 3.1. Map of the study area. A: The Prof. Luiz Saldanha Marine Park location in the Portuguese coast and its three protection levels: fully protected area (FPA), partially protected area (PPA) and buffer area (BA) (bathymetric values in meters). B: Map of locations of experimental fishing (2007-2014; central points of 500m of net). C: Map of the listening stations (LS) set in the study area, with information of position category (inshore, offshore). Red asterisks indicate stations that monitored for less than one year (Short-term listening stations - LS10: 8 months; LS14: 11 months; LS16: 1 month). Orange dots represent capture sites (numbers with hashes: ID of individuals). Individual #3 was caught by a fisherman approximately 11 months after tagging (details in Results section). Points of its capture, last detection and reported recapture location are represented by pink, white and red dots, respectively.

3.3.4. Telemetry data processing

Telemetry data obtained from the ultrasonic receivers were exported from Innovasea VUE software into R statistical software (R Core Team 2021). Isolated detections occurring in a 24h period were considered potentially spurious and removed (Heupel et al. 2006, Simpfendorfer et al. 2015). For an individual to be classified as present (also referred to as 'detected'), a minimum of two transmissions had to be logged within 24h.

Site fidelity of each individual was assessed using residence indices. The residence index (RI) (Afonso et al. 2008) was estimated by dividing the total number of days with detections (DD) by the number of days from the first to last detection (or tracking period, TP). The absolute residence index (AR) was based on the ratio of the number of days with detections (DD) to the number of days of potential detection (PDD) (Spares et al. 2015). This period spanned from first detection to the expected tag battery expiration, the last data download or receiver removal, or even a reported catch event. A third index, the weighted residence index (Iwr) (Lino 2012), was also determined. This was calculated as the ratio of the total number of days with detections to the total number of potential detection days, multiplied by the ratio of the interval between the first and last detection to the total of potential detection days. These indices were used because they weight presence differently. Because fish mortality can occur after the last detection, this period may be excluded from calculation (as in RI), or it can indicate a true absence from the study area due to animal movements (as in AR). As for the weighted residence index (Iwr), it compares both the tracking period and number of days with detections to the potential detection days, leading to a more conservative estimate of presence. All of these indices vary between 0 (no residency) and 1 (full time residency). They were applied to estimate overall fidelity to the study area, to assess residency per listening station, and to analyse residency by station category (inshore, offshore). In these analyses, the individual-level metrics enabled estimating average values representative of the full dataset (immature excluded). Site fidelity profile categories were defined based on RI and AR: individuals with RI ≥ 0.50 and AR ≥ 0.40 were considered high site-attached or residents, and conversely, low site-attached if their estimated indices were lower.

In order to calculate time spent inside the study area and absence periods, detection data of each individual was used for estimating a minimum threshold time interval with no detections that would define whether individual was present or absent from the study area. This was done following the method described by Knip et al. (2012). A mean exit time weighted by the number of data points was calculated for the sample. This value was used for classifying intervals between detections as presence or absence. Considering that certain regions may fall beyond the range of the acoustic monitoring array, an additional criterion was employed for classification of time intervals: if the last detection was at an inner listening station (LS2, LS4, LS5, LS6; LS3 and LS7 also, but only prior to the removal of adjacent outer stations - LS10, LS14 and LS16), only intervals longer than 8h were categorized as absence. This enabled estimating the total time within the study area and mean excursion duration. The total time inside was divided by the tracking period (time between first and last detection) and by the total potential detection days (first detection till expired battery or catch) to calculate respectively the proportion of tracking time inside (PTIT) and the proportion of study time inside (PTIS). The total potential detection days is here referred to as study time. Both proportions vary between 0 and 100 (percentage). The total time inside was also applied to estimate the proportion of time at each listening station. Furthermore, for absence intervals, the location of exit and enter was assessed based on the positioning average of the 60min of detections before the event (exit, enter). Directionality of these events was inferred based on the position of listening stations: if detections before event were at LS1, LS8 or LS15, direction was classified as coastwise. Offshore directionality was considered for exits and enters at the southern edge of the monitored area (Figure 3.1). The counts of classified events of each individual were used to estimate class averages for all the data (immature excluded).

To examine the spatial dispersal of individuals, two different methods were applied: minimum convex polygons (MCP) and kernel utilization distributions (KUD). Minimum convex polygons were obtained from the minimum area that encompassed all detections. The KUD areas were estimated based on position averaging calculations (Simpfendorfer et al. 2002). Position averaging (PAV) was performed for each ray for 60min periods. This method uses detections from multiple receivers and converts them to position estimates based on weighted means of the number of detections at each receiver during a particular time period. The choice of interval was done in order to optimize the trade-off between the number of receivers used to calculate PAV (avoid averaging with only one receiver) and position resolution (avoid combining together many detections). Calculation of KUD50 (area including 50% of PAV points) was used as a measure of the core activity area, and KUD95 (area including 95% of PAV points) was used as an estimate of home range. Data exploration enabled to choose the smoothing factor (*h*=350) for KUD modelling, by tuning the spread of positions density and taking into account the number of detections at each receiver. Overall and seasonal average values were derived from individual estimates (immature excluded).

Linear mixed-effects models were used to examine the effect of season on MCP area, home range (KUD95), RI, AR, Iwr and PTIS, with season as the fixed effect and individual ID as a random effect. Given the relevancy of ontogeny, the immature individual was excluded from these analyses. This type of model was also applied to assess the residence index according to the distance to the coast (inshore, offshore). Model fitting was performed by using the R package lme4. A generalized linear mixed model (GLMM) with a negative binomial distribution was used to analyse differences in the number of exits regarding directionality (offshore, coastwise). This model also incorporated individual ID as a random effect. Model fitting was performed by using the R package lme4. The significance of the fixed effects was tested using an analysis of variance (ANOVA) with a likelihood ratio test, which was used to compare models with the null model and estimate the significance of the fixed effect.

3.4. Results

3.4.1. Species abundance

A total of 238 individuals were caught, ranging from 30.6cm to 92.5cm, with an average total length of 67.4cm. The sample consisted of 123 immature and 115 mature individuals, representing 52% and 48%, respectively. A proportion test did not reveal a significant difference in this ratio of 1.1 immature individuals to 1 mature individual (p=0.648). In reference to sex composition, 130 were males and 98 were females, constituting 57% and 43%, respectively. This uneven ratio, with a dominance of males (1.3:1), was significantly different according to the applied proportion test (p=0.003). It should be noted that 10 individuals had no registered sex information. The male dominance was also significant within the mature group, being more pronounced, specifically at a ratio of 2.3:1 (80 males to 35 females; p<0.001). Among the immature individuals, there were more females, but this difference was not statistically significant (1.3:1; 50 males to 63 females; p=0.121). Within the male subgroup, the majority were mature, at a ratio of 1.6:1 (80 mature to 50 immature; p<0.001). Conversely, within the female subgroup, the majority were immature, at a ratio of 1.8:1 (63 immature to 35 mature; p<0.001). Of the total individuals caught (238), fifteen had no recorded status if they were release dead or alive. Of the remaining, 96% (217), were released alive following sampling, and 4% (8) were dead.

The yearly means of abundance, CPUE (n.500m^{-1}) (Figure 3.2), revealed distinct trends according to protection level and group (Figure 3.2). Additional graphical representations of CPUE temporal trends can be found in Supplemental information (Figures S3.1 and S3.2). Specifically, one of the auxiliary figures depicts the patterns in each of the three protection levels (FPA, PPA and BA), and also the biomass estimates (biomass per unit effort (BPUE) (Figure S3.1). A second figure exhibits CPUE per campaign (each spring and autumn throughout the study), with the data represented by ontogenic group, sex and depth interval (Figure S3.2).

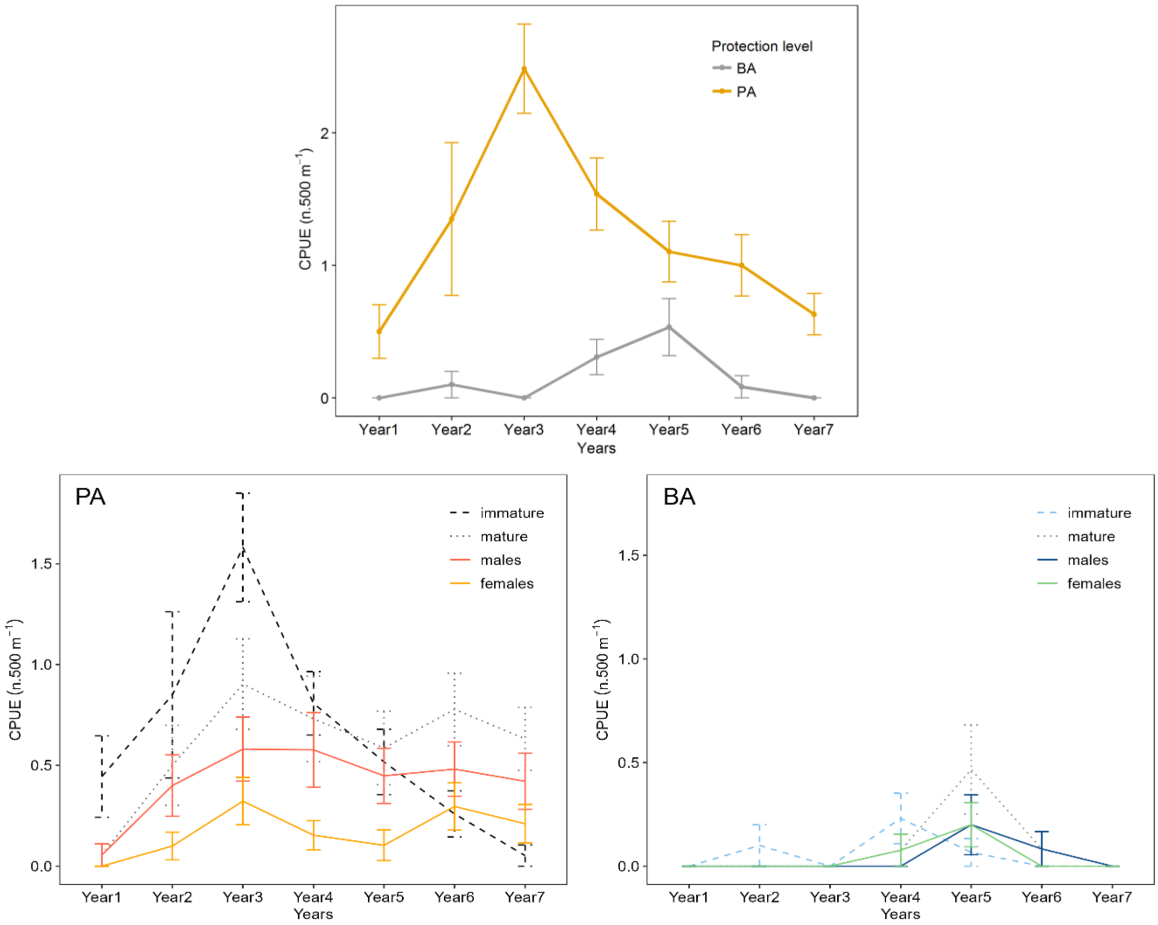

Figure 3.2. Yearly means of catch (*Raja clavata*) per unit effort (CPUE, n.500m-1) ± SE in each protection level: protected area - PA (PPA + FPA); buffer area (BA). Top plot shows the overall trends (all individuals) and bottom plots (left: PA; right: BA) display the trends of the maturity (immature and mature) and sex groups (mature males and mature females).

Overall, both the global values (Figure 3.2, top plot) and group trends (Figure 3.2, bottom plots) display a noticeably higher abundance in the PA. In this protection level (Figure 3.2), an increase in both mature and immature individuals was observed during the implementation period (years 1 and 2), with a more pronounced increase in the immature group. This was followed by a substantial decrease in the immature group over the subsequent four years of data. The main trend observed in the PA was thus predominantly driven by this ontogenic group. For mature individuals, the initial increase was followed by a relatively stable period with some fluctuations. This stability was driven largely by the abundance of males, which were more numerous than females. The initial increase in female abundance was less noticeable, and their fluctuations were more pronounced. With reference to the BA, the most notable detail is regarding year 5. According to Figure S3.2 (Supplemental information), the observed mean abundance in this year is attributable to the presence of both mature males and females in the deeper depth interval (30-45m), specifically in autumn.

The GAM model fitting demonstrated that season and sea surface temperature (SST) were not significant predictors of abundance across all groups (all individuals, mature and immature). Details of selected models are presented in Table 3.1, and Figure 3.3 displays the corresponding smoothing functions for the year variable. The analysis indicated that protection level significantly impacted the overall abundance, with higher values in the PA (all ind.: estimate = 2.1946 ± 0.4769 SE, $p<0.001$; mature: estimate = 1.6693 ± 0.3878 SE, $p<0.001$; immature: estimate = 2.0322 ± 0.4603 SE, $p<0.001$). Yearly trends also showed significant smooth terms. However, these trends diverged according to protection level, as demonstrated by the interaction of these terms. The plots of parametric terms (protection level and depth interval) and of model validation are shown in Supplemental information (Figures S3.3 and S3.4).

Table 3.1. Summary of the Generalized Additive Models (GAM) fitted for predicting abundance of *Raja clavata* (catch per unit effort) across different groups: all individuals, mature, and immature. Parametric coefficients (protection level - BA: buffer area; PA: protected area; depth interval - 10-20m, 30-45m) and the smooth terms for year in each model are shown.

Group	Final model				Deviance Explained
All Individuals	~ s(year) + Protection level + s(year):Protection level + Depth interval				32.8%
Mature	~ s(year) + Protection level + Depth interval				23.5%
Immature	~ s(year) + Protection level				31.4%
Parametric coefficients					
	Variable	Estimate	SE	z-value	P-value
All Individuals	Intercept	-2.1581	0.4764	-4.53	< 0.001
	Protection Level - PA	2.1946	0.4769	4.601	< 0.001
	Depth interval - 10-20 m	-0.4188	0.1602	-2.615	0.009
Mature	Intercept	-2.1826	0.3895	-5.603	< 0.001
	Protection Level - PA	1.6693	0.3878	4.305	< 0.001
	Depth interval - 10-20 m	-0.4789	0.2121	-2.258	0.024
Immature	Intercept	-2.9736	0.4564	-6.515	< 0.001
	Protection Level - PA	2.0322	0.4603	4.415	< 0.001
Smooth terms					
		edf	Ref.df	Chi.sq	P-value
All Individuals	s(year)	1.002	1.002	30.5	< 0.001
	s(year):Protection level - BA	2.465	2.81	21.05	< 0.001
	s(year):Protection level - PA	1.624	1.898	42.09	< 0.001
Mature	s(year)	2.339	2.71	8.541	0.044
Immature	s(year)	2.591	2.882	23.85	< 0.001

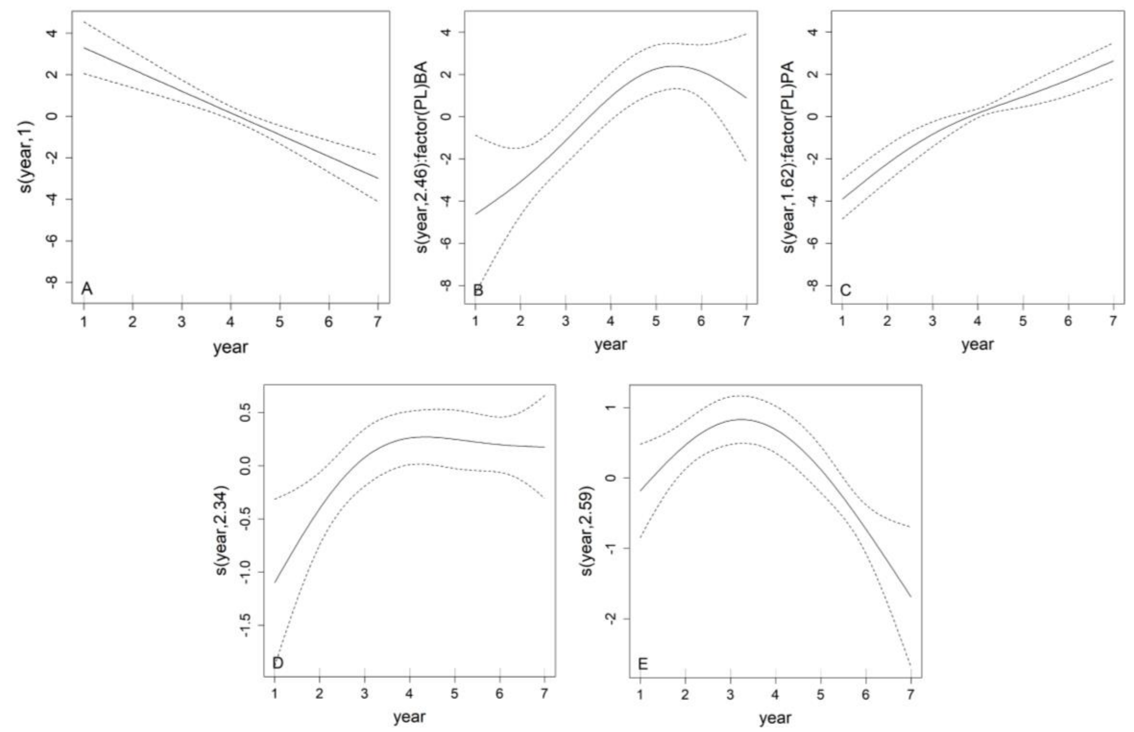

Figure 3.3. Generalized additive model (GAM) plots showing the partial effects of year in the abundance of *Raja clavata* (catch per unit effort). A: General smooth for year in the global model (all individuals). B: Smooth for year within protection level (PL) BA (buffer area). C: Smooth for year within protection level PA (protected area). D: Smooth for year in the model for mature individuals. E: Smooth for year in the model for immature individuals.

In addition to protection level, the global model included the interaction of year with protection level, demonstrating that time trends significantly differed between the BA and PA (Figure 3.3). The smooth plot for the general effect of year shows a significant overall decline (edf=1.002, Chi-sq=30.5, p<0.001). Within the BA, the smooth curve incorporated a peak in year 5 (edf=2.465, Chi-sq=21.05, p<0.001), matching the pattern seen in the yearly means (Figure 3.2). Conversely, in the PA, the exhibited trend showed a consistent increase over time (edf=1.624, Chi-sq=42.09, p<0.001). In this model, depth interval was also significant, with more individuals at the 30-45m interval (estimate 10-20m = -0.4188 ± 0.1602 SE, p=0.009). This model explained 32.8% of the observed deviance.

For mature individuals, the smooth plot of the year effect indicated a significant non-linear trend (edf=2.339, Chi-sq=8.541, p=0.044), with abundance increasing in the initial two-year period and followed by stabilization (Figure 3.3). Depth interval also significantly influenced the abundance of this group, mirroring the trend observed for all individuals (estimate 10-20m = -0.4789 ± 0.2121 SE, p=0.024). The deviance explained by this model was 23.5%.

The model for immature individuals (Figure 3.3) showed a significant non-linear temporal trend (edf=2.591, Chi-sq=23.85, p<0.001). The projected smooth increases to a maximum in year 3 and then gradually decreases. The effect of depth interval was not considered significant for this group. This particular model captured 31.4% of the observed variability.

3.4.2. Conventional tagging

A total of 55 of individuals tagged with T-bar tags were males (60%) and 36 (40%) were females. Regarding maturity, 43 individuals were immature (47%) and 48 (53%) were immature. Within males, most individuals were mature (33 males, 60%; 22 immature males, 40%), while most females were immature (21 females, 58%; 15 mature females, 42%). Six individuals were tagged in the BA near Sesimbra, 40 in the FPA, and 45 in the PPAs (25 and 20, respectively in the western and eastern PPA adjacent to the FPA). Individuals were released at the site of capture immediately after being measured and tagged. Of the total 91 individuals tagged with conventional T-bar tags, only two individuals (2%) were reported to be caught by fishermen. Tagging and recapture points of these two specimens are shown in Figure 3.4. One individual (Ind. A, mature male) was tagged on 14/05/2011 in the PPA (depth of 32m) and reported to be recaptured on 23/11/2011 (~6.5 months later) near the park border, at a point 500m to southeast of tagging location (reported depth of capture: 70m). The other individual (Ind. B, mature male) was tagged on 19/10/2011 in the FPA (depth of 17m) and recaptured on 19/01/2012 (3 months later) at a location approximately 40km distant, to southeast (near St. André coastal lagoon) of tagging point (reported depth of capture: 25m).

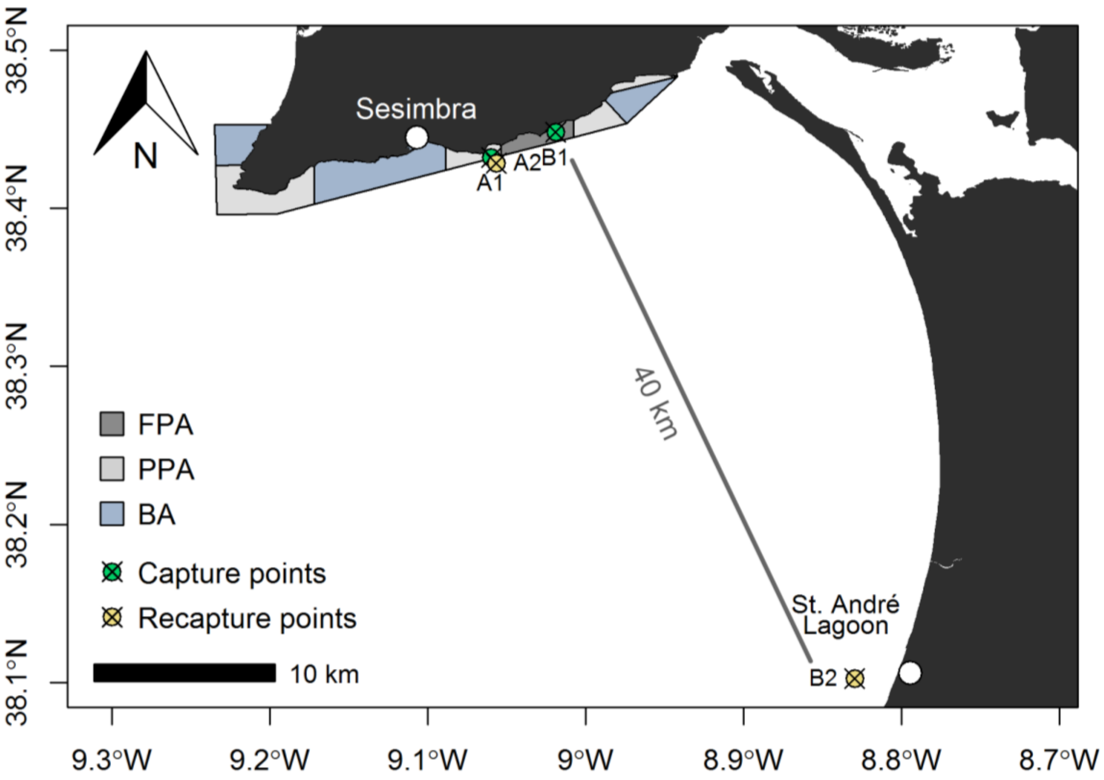

Figure 3.4. Map with capture (conventional tagging) and recapture points of the two rays (Ind. A, Ind. B) reported caught by fishermen (1 - capture point; 2 - recapture point). Ind. B was recaptured offshore St. André Lagoon as reported by a fisherman (~40km to southeast of the study area).

3.4.3. Acoustic telemetry

A total of twelve individuals were tagged with acoustic transmitters: ten mature males, one mature female, and one immature male (Table 3.2). Average total length of individuals (±SE) was 79.1cm ± 2.0cm, ranging from 70.0cm to 88.0cm. Three specimens did not have their total length registered, as their tails were incomplete due to prior damage (including the immature individual). The precaudal length averaged 45.0cm ± 1.5cm, with a minimum value of 31.5cm and a maximum of 50.5cm. For disc width, the mean was 51.5cm ± 1.6cm, with values ranging from 43.0cm to 64.0cm. Of the twelve tagged specimens, one was never detected by the acoustic array, specifically Ind. #2, the only tagged female (tag pinging was confirmed onboard, prior to release). It was tagged and released in the proximity of LS6 (depth of 15m). Capture and release points of all twelve individuals are shown in Figure 3.1. Telemetry data were thus obtained from eleven individuals (all males, one immature) (total study time: ~3 years).

Table 3.2. Details of rays tagged with ultrasonic transmitters: morphometrics (TL – Total length; PRC – Precaudal length; DW – Disc width), sex, maturity, tagging date, study start and end dates. Observations (Obs.) field includes additional information: Ind. #3 was reported captured by a fisherman. (a): damaged tail.

Fish ID	TL (cm)	PRC (cm)	DW (cm)	Sex	Mature	Tagging Date	Study Start	Study End	Obs.
#1	79	45	52	male	yes	27 Nov-13	31 May-14	20 Jul-15	
#2	88	48	64	female	yes	27 Nov-13	31 May-14	20 Jul-15	Never detected
#3	73	42	48	male	yes	28 Nov-13	31 May-14	25 Oct-14	Captured
#4	70	41	47	male	yes	28 Nov-13	31 May-14	21 Jul-15	
#5	(a)	43	48	male	yes	26 May-15	26 May-15	15 Jan-17	
#6	79	46	50	male	yes	26 May-15	26 May-15	15 Jan-17	
#7	(a)	31.5	43	male	no	28 May-15	28 May-15	17 Jan-17	
#8	(a)	48	55	male	yes	29 May-15	29 May-15	18 Jan-17	
#9	79	46	48.5	male	yes	12 Aug-15	12 Aug-15	3 Apr-17	
#10	86	50.5	55	male	yes	12 Aug-15	12 Aug-15	3 Apr-17	
#11	82.8	48.5	55	male	yes	22 Oct-15	22 Oct-15	13 Jun-17	
#12	75	50	53	male	yes	22 Oct-15	22 Oct-15	13 Jun-17	

3.4.3.1 Site fidelity

A total of 775 383 detections were considered valid for analyses (average of 70 489 ± 17 410 detections per individual). For eight individuals, study periods (total of potential days of detection - PDD) were considered to be equal to the maximum tag life expectancy of 600 days (20 months). Two individuals had study periods of approximately 15 months (Ind.: #1, #4), as they were tagged before the complete receiver array was implemented (Table 3.3).

Table 3.3. Summary of detections, total of study days (PDD, potential detection days), tracking period in days (TP; from first to last detection), and days with detections (DD) of the rays tagged with acoustic transmitters.

Fish ID	*N* detections	PDD	TP	DD	RI	AR	Iwr
#1	23 811	450	364	157	0.43	0.35	0.28
#3	71 164	182	182	173	0.95	0.95	0.95
#4	35 978	451	422	167	0.39	0.37	0.35
#5	214 986	600	369	369	1.00	0.62	0.38
#6	27 889	600	492	311	0.63	0.52	0.43
#7	17 918	600	102	34	0.33	0.06	0.01
#8	63 230	600	579	299	0.52	0.50	0.48
#9	46 125	600	600	139	0.23	0.23	0.23
#10	51 600	600	432	206	0.48	0.34	0.25
#11	120 650	600	417	352	0.84	0.59	0.41
#12	102 032	600	336	259	0.77	0.43	0.24

One ray with an acoustic tag (also tagged prior to full receivers' array implementation, on November 28, 2013) was reported caught by a fisherman, specifically Ind. #3. Therefore, its study period was limited to around six months (182 days). Catch occurred eleven months

(331 days) after tagging, on October 25, 2014, at a location adjacent to the FPA boundary, 1300m distant from tagging point and 300m from the last recorded detection (in LS13; Figure 3.1). According to the information provided by the fisherman, time of last detection roughly corresponds to the catching event (5am). A map with last estimated locations (positioning averaging, 60min) occurring either before dispersal, tag expiry or catch event is presented in Supplemental information (Figure S3.5). The mean tracking period per individual was 390 ± 45 days, with a minimum of 102 days and a maximum period of 600 days (tag life expectancy). The mean number of days with detections was 224 ± 31, ranging from a minimum of 34 to a maximum of 369 days (Table 3.3). Many individuals had relatively long tracking periods. Individuals #8 and #9 were detected until the end of the study period, indicating their survival. Individual #8 was tagged outside the monitored area, 1.5km west of LS1, within the PPA. It was not detected in the first 20 days post-release, whereas all other individuals, except for the dispersed female, were detected on the release day. Individuals #4, #6, #10, and #11 also had considerable tracking periods, ranging from 400 to 500 days. Only individuals #3 and #7 had tracking periods shorter than 200 days; Ind. #3 was the specimen reported caught after 182 days, and Ind. #7, the immature individual, was tracked for 102 days. Of the total of individuals tagged, seven (64%) apparently dispersed from the area at some moment, one was caught (9%), and two (20%) survived and remained in the area throughout the study period.

With no detections logged in the array of receivers, the female had an estimated residency of 0 in all indices (RI, AR, Iwr). For males, average residence index (RI) was 0.60, with range between 0.23 and 1. The other two indices registered lower average values, specifically 0.45 (range 0.06 - 0.95) for the absolute residency (AR), and 0.36 (range 0.01 - 0.95) for the weighted residence index (Iwr). The immature individual (Ind. #7) exhibited the second lowest residency (RI 0.33, AR 0.06, Iwr 0.01) (Table 3.3), as it permanently exited the array shortly after being tagged. Its last detections were in LS1.

Timelines with total daily detections of each individual throughout the overall study period are shown in Figure 3.5. Data exploration confirmed that the decrease in the number of detections after the first 20 days of monitoring was due to the loss of LS16. The timelines allowed identifying several site fidelity profiles. Individuals #3, #5, #11, and #12 exhibited the highest RI values (between 0.77 and 1) and remained consistently present in the study area for substantial periods.

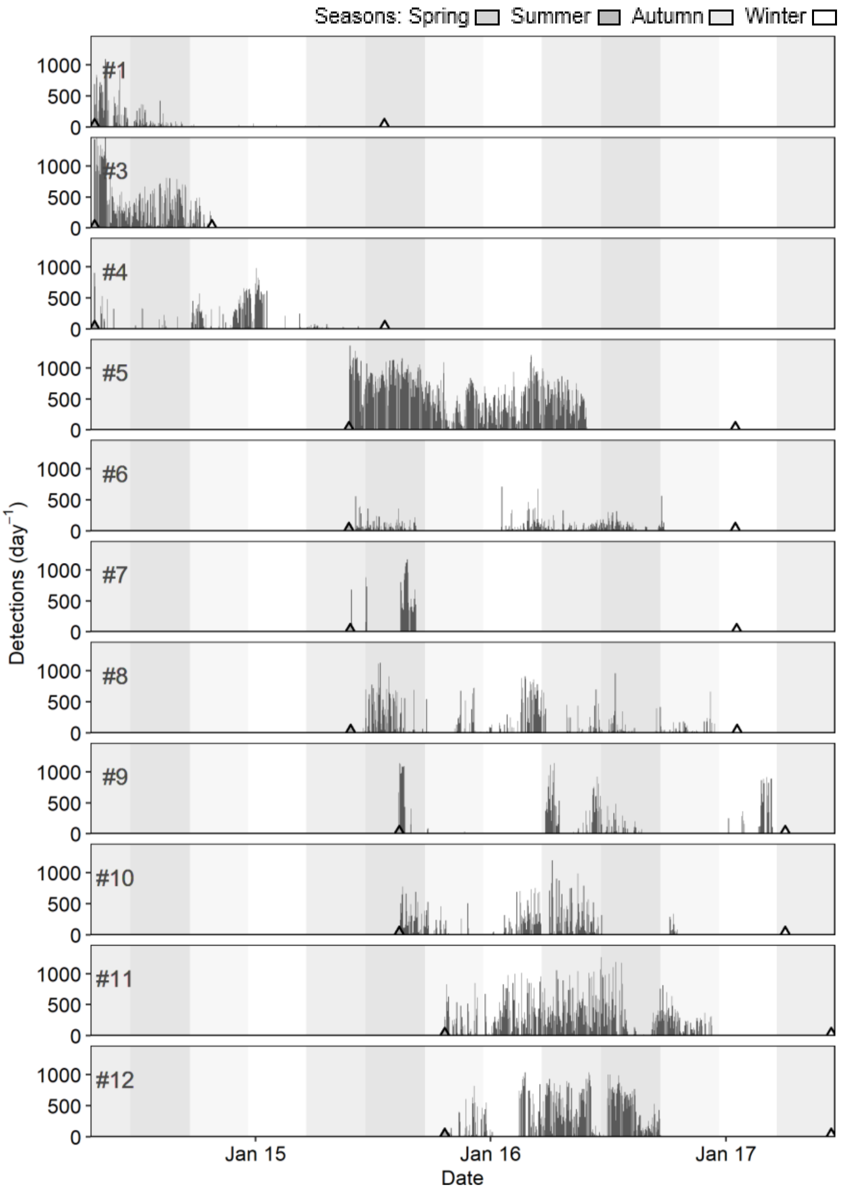

Figure 3.5. Timelines of daily detections of tagged rays (*Raja clavata*, males) (▲: study start; △: study end). Vertical stripes with different grey shadings indicate seasons. Ind. #3 was reported captured by a fisherman (end of monitoring due to fishing). Ind. #7: immature male.

Residency (RI) was particularly high for Ind #3, which remained continuously present until being captured (RI=0.95, AR=0.95, Iwr=0.95), and Ind. #5, which was uninterruptedly detected for over one year before dispersing (369 days; RI=1, AR=0.62, Iwr=0.38). Although with less daily detections, individuals #6 and #8 had around 300 days with detections, scoring high AR and IR (Ind. #6: RI=0.63, AR=0.52, Iwr=0.43; Ind. #8: RI=0.52, AR=0.50, Iwr=0.48). Their detections patterns and utilization areas (Figures S3.9 and S3.10) suggests that these individuals were often in zones where receivers' coverage might not have been optimal. Ind. #6 predominantly used the eastern site, where two receivers were removed

(LS14 and LS16), and Ind. #8 often used the PPA to the west. Despite this, they were recurrently detected and their estimated indices classify them as residents to the monitored area. Of the total tagged rays (N=12), six were classified as residents in the study area (50%; 60% of mature males; Ind.: #3, #5, #6, #8, #11 and #12). Other individuals exhibited intermittent presence. Detection data of individuals #1, #4, #9, and #10 shows that they were regularly absent during one (e.g., Ind. #10) to two months (e.g., Ind. #9). Ind. #1 kept being detected sporadically throughout one year (till 24th April 2015), although with few detections per day. These four (33%) individuals were considered periodic visitors. The immature individual and the female were considered occasional visitors to the monitored area (17%). The residency plot with presence-absence of tracked rays is shown in Figure S3.6. Some seasonal patterns were displayed in the daily detections data of the tagged rays. Several individuals (Ind.: #1, #3, #5, #9, #11, #12) exhibit a notable decrease in detections during the summer to autumn period. Conversely, other rays showed a marked increase in detections during the winter to spring period (Ind.: #5, #8, #9, #10, #11, #12), often with absolute absence in early winter.

Results of individuals' total time inside the study area, proportion of time inside (PTIT and PTIS), proportion of time according to receivers' protection level location (pti FPA, pti PPA), and also according to distance to the coast (pti Inshore, pti Offshore) are summarized in Table 3.4. Individuals with the highest PTIS scores are coincident with those with higher RI (Ind.: #3, #5, #11, #12). Several individuals spent a substantial proportion of their time in the PPA, in particular Ind. #7 (immature), #8 and #9. All these three specimens spent over 20% of their time being detected by inshore receivers. Two other individuals, Ind. #6 and #10, were detected inshore for over 30% of their time inside the study area.

Table 3.4. Values per individual of total estimated time inside the study area (h), proportion (%) of tracking time inside (PTIT), and proportion (%) of study time inside (PTIS). Regarding the time spent inside, the proportion (%) of time per protection level (pti FPA, pti PPA), and per distance to the shore (pti Inshore, pti Offshore) according to receivers' location is also shown.

Fish ID	Time In (h)	PTIT	PTIS	pti FPA	pti PPA	pti Inshore	pti Offsore
#1	761	9.1	7.6	100	0	0.7	99.3
#3	2273	64.3	64	100	0	0	100
#4	1483	16.5	14.8	100	0	0.2	99.8
#5	7334	82.6	76	100	0	17.9	82.1
#6	2384	24.9	24.7	100	0	35.7	64.3
#7	692	28.1	7.2	71.6	28.4	28.7	71.3
#8	2293	24.7	23.2	76.2	23.8	27.3	72.7
#9	1004	13	12.9	78	22	24.3	75.7
#10	2745	36.3	35.3	87.9	12.1	39.6	60.4
#11	3816	63.5	62.8	99.3	0.7	1.1	98.9
#12	2576	42.9	42.4	99.9	0.1	2.4	97.6

The assessment of residency according to the position of receivers (inshore, offshore) confirmed that tracked individuals were predominantly detected by offshore receivers (Figure 3.6). The mean RI was 0.35 ± 0.08 inshore, and 0.60 ± 0.08 offshore, significantly higher according to the LMM outputs (Table 3.5) (χ^2=4.40, p=0.036). Other spatial trends regarding site fidelity, specifically, average Iwr and proportion of total time inside at each receiver can be seen in Supplemental information (Figures S3.7 and S3.8).

The analysis of exits directionality highlights that individuals often moved away from the study area, predominantly in the offshore direction. Mean number of exits towards offshore was 268 ± 46, and the mean number of coastwise exits was 85 ± 32 (Figure 3.7). This average of number of offshore exits was over three-fold higher than the number of coastwise exits, and consisted of 76% of the total registered exit events. GLMM output (Table 3.5) indicates significance of the observed difference (χ^2=5.85, p=0.016). Additional analyses of exit and enter events can be found in Supplemental information: number of exits and enters of each individual (Table S3.1), and mean excursion duration per individual (Figure S3.9).

Table 3.5. Outputs of mixed models to test residence index (RI) in relation to distance to the coast (Linear mixed model), and number of exits per direction (Generalized linear mixed model, family binomial).

	Estimate	SE	t-value	P-value
RI ~ Distance to coast *				
Intercept	0.42	0.09	4.72	< 0.001 *
distance(offshore)	0.18	0.08	2.17	0.04 *
	Estimate	**SE**	**z-value**	**P-value**
N exits ~ Direction *				
Intercept	4.45	0.31	14.44	< 2e-16 *
direction(offshore)	1.15	0.43	2.64	0.01 *

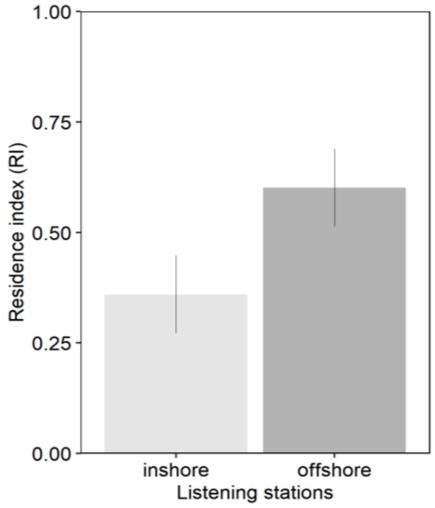

Figure 3.7. Mean residence index (RI) ± standard error estimated for *Raja clavata* according to the two listening stations categories based on distance to the coast: inshore, offshore.

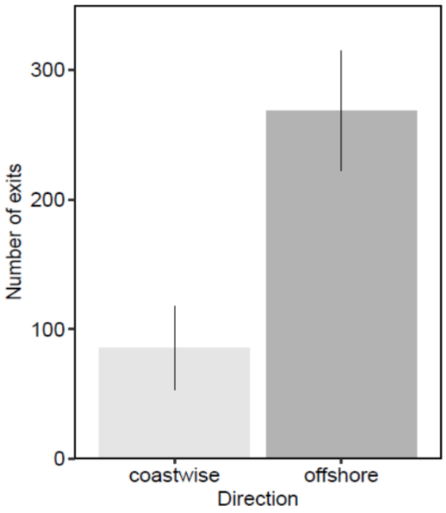

Figure 3.6. Mean number of exits from the study area ± standard error estimated for *Raja clavata*, according to directionality: coastwise, offshore. Direction was inferred from the position of the listening station that logged detections prior to absence.

Regarding the analysis at the individual level (Table S3.1), it is worth mentioning that only two individuals exhibited more coastwise exits, Ind. 8# and Ind. #9. They both exhibited utilization distributions (Figures S3.10 and S3.11) predominantly in the western side of the monitored area (PPA).

3.4.3.2 Area usage

The spatial utilization of the tagged rays was analysed using Kernel and MCP areas. The map with their projected home range (KUD95), obtained by positioning averaging of all tagged individuals (Figure 3.8), indicates that they used the full width of the monitored area, including the PPA. The core activity areas (KUD50) highlight that they frequently used the outer region of the protected area, furthest from the shore. Maps obtained for each individual are presented in Supplemental information (Figures S3.10 and S3.11). Of the eleven rays with tracking data, seven (64%) were detected predominantly in one of the sides of the monitored area (east side: Ind. #1, #3, #5, #6; west side: #7, #8, #9), and four (36%) presented patches of positions spread throughout the area (Ind. #4, #10, #11, #12).

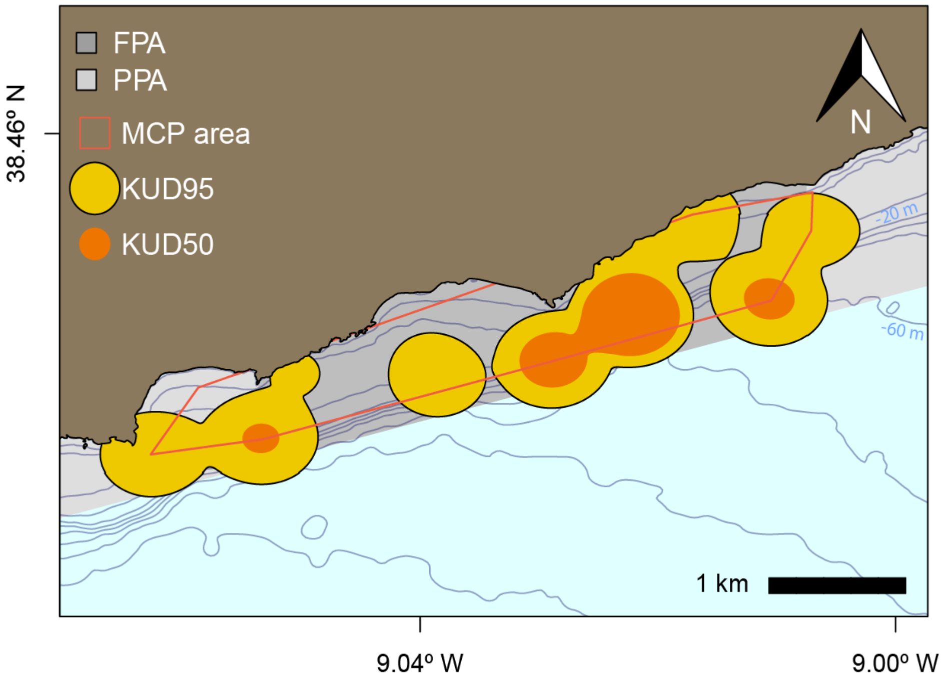

Figure 3.8. Map with home range (KUD95 - yellow), core activity area (KUD50 - orange) and Minimum Convex Polygon (MCP – red line) projected for *Raja clavata* in the study site, as determined from the positioning averaging (60 min intervals) of all individuals (n = 11 : 10 mature males, 1 immature male; three years of data collection).

Estimated utilization areas for each individual are shown in Table 3.6. Home range estimates (KUD95) ranged between 1.10km^2 (0km^2 if the single female is also considered) to 4.13km^2, with a mean of 1.85 ± 0.24km^2. Similar area sizes were obtained with the MCP area, the mean was 1.71 ± 0.23km^2, and values differed between of 0.97km^2 and 3.15km^2. Mean core activity area was 0.3 ± 0.07km^2 on average, ranging from 0.22km^2 to 0.99km^2

Table 3.6. Minimum convex polygon areas (MCP) and Kernel utilization distributions (KUD; KUD50 - core activity area; KUD95 - home range, KUD100) of each tracked ray. Areas (MCP, KUD50, KUD95, KUD100) are shown in km^2.

Fish ID	MCP area (km^2)	KUD50 (km^2)	KUD95 (km^2) (Home range)	KUD100 (km^2)
#1	1.49	0.36	1.83	4.34
#3	0.97	0.21	1.21	4.04
#4	1.58	0.29	1.83	4.73
#5	1.76	0.20	1.10	3.71
#6	1.20	0.55	1.75	4.11
#7	1.02	0.40	1.93	4.26
#8	2.56	0.40	2.00	6.47
#9	1.30	0.32	1.47	5.09
#10	3.15	0.99	4.13	4.53
#11	2.68	0.32	1.61	4.57
#12	1.10	0.23	1.51	1.53

3.4.3.3 Seasonal patterns

The residency data demonstrated seasonal variability, as illustrated in Figure 3.9. All three residence indices (RI, AR, Iwr) exhibited a similar pattern. The highest values were observed during the summer and spring seasons, and a marked decrease was registered in winter and autumn. The other site fidelity indicator, the proportion of study time inside (PTIS), was highest in spring. Summer average values were also high, contrasting with the lower averages registered for cold seasons. Autumn estimates of proportion of time inside the monitored area were the lowest. The linear mixed-effects modelling results (Table 3.7) confirmed these seasonal differences in RI, AR and PTIS (RI: χ^2=13.25, p=0.004; AR: χ^2=8.21, p=0.04; PTIS: χ^2=11.96, p=0.008), with significant differences detected between the spring-summer and autumn-winter groups ($p < 0.05$). The Iwr model replicated the same pattern, but the comparison against the null model did not reveal a significant model improvement by including the season variable (χ^2=7.13, p=0.068).

The seasonal utilization area averages (Figure 3.10) were higher during summer and autumn compared to winter and spring, and this was observed in both the MCP area and home range (KUD95). MCP area exhibited a higher mean in autumn, marginally higher in comparison to summer. However, the linear mixed-effects models (Table 3.7) did not find statistically significant differences (MCP: χ^2=4.72, p=0.194; KUD95: χ^2=4.45, p=0.217).

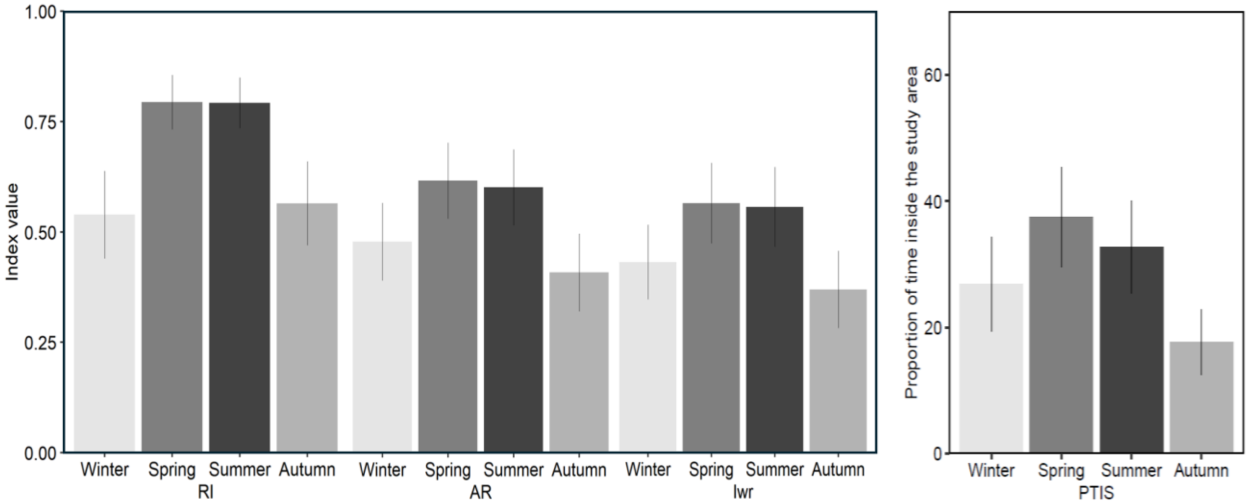

Figure 3.9. Mean residence index (RI), absolute residence index (AR), weighted residence index (Iwr), and proportion of study time inside (PTIS) ± SE per season. Values obtained with data of all mature rays (n = 10).

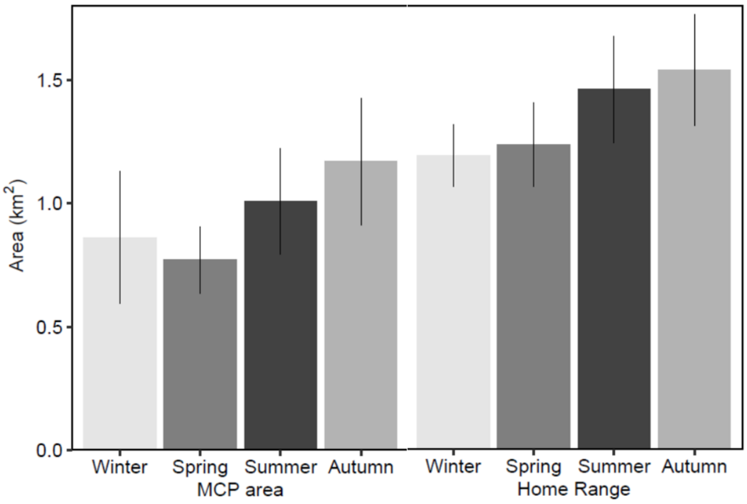

Figure 3.10. Mean MCP area and home range (KUD95) ± SE per season obtained with data of all mature rays (n = 10).

Table 3.7. Linear mixed model (LMM) results: estimates ± standard error, t-values and p-values obtained with season as a fixed effect for the following variables: MCP area (minimum convex polygon), KUD95 area (home range), residence index (RI), weighted residence index (Iwr) and proportion of study time inside the study area (PTIS). Fish ID was used as a random factor. The significance of factor 'season' by comparison with the null model (Chi-squared tests) is marked with '∗'.

	Estimate	SE	t-value	P-value
MCP area				
Intercept	1.18	0.23	5.04	< 0.001 *
season(spring)	-0.36	0.20	-1.76	0.09
season(summer)	-0.07	0.20	-0.34	0.74
season(winter)	-0.32	0.21	-1.52	0.14
KUD95 area				
Intercept	1.56	0.21	7.38	< 0.001 *
season(spring)	-0.18	0.20	-0.90	0.38
season(summer)	0.04	0.20	0.23	0.82
season(winter)	-0.34	0.20	-1.64	0.11
RI *				
Intercept	0.57	0.08	6.83	< 0.001 *
season(spring)	0.25	0.09	2.88	0.01 *
season(summer)	0.25	0.09	2.81	0.01 *
season(winter)	0.01	0.10	0.12	0.90
AR *				
Intercept	0.43	0.10	4.38	< 0.001 *
season(spring)	0.22	0.09	2.50	0.02 *
season(summer)	0.22	0.09	2.50	0.02 *
season(winter)	0.11	0.10	1.14	0.26
Iwr				
Intercept	0.39	0.10	3.90	0.001 *
season(spring)	0.21	0.09	2.27	0.03 *
season(summer)	0.22	0.09	2.34	0.02 *
season(winter)	0.10	0.10	1.01	0.32
PTIS *				
Intercept	21.28	8.40	2.54	0.02 *
season(spring)	19.27	5.81	3.32	0.002 *
season(summer)	16.63	5.86	2.84	0.01 *
season(winter)	11.28	6.39	1.76	0.08

3.5. Discussion

This study used complementary methods to assess the effectiveness of protection provided by a coastal marine protected area for the thornback ray (R. clavata). By identifying the relevance of ontogeny on abundance trends and examining the residency and dispersal patterns of this species within a coastal MPA, our findings offer valuable insights for managers aiming to protect this species in southern Europe and the broader Northeastern Atlantic region. Specifically, our results highlight the importance of ensuring connectivity between coastal areas and adjacent deeper waters, and identify the need to further investigate the factors affecting juvenile abundance variability.

3.5.1. Population structure

Our results show that the population of thornback ray found within the MPA is composed of both mature and immature individuals, with the immature being of both sexes, and the mature having significant male dominance. The less represented group were the mature females. Sexual segregation with dominance of males in coastal regions has been reported previously. This was noticed for instance by Rousset (1990) in the northern coast of France. However, in the nearby area of Peniche (~70km north from Lisbon), Serra-Pereira et al. (2014) reported the inverse proportion, with dominance of females close to the shore and male dominance at depths greater than 100m. The higher number of females was also reported for the Azores region (Santos et al., 2021) and Whitsand Bay in southern British Isles (Simpson et al. 2021). Authors have hypothesised that the phenomenon of sexual segregation might be related either with female avoidance of males or with different habitat requirements (Simpson et al. 2021). The drivers of sexual segregation have been subject of debate, and the theme is considered rather complex (Wearmouth & Sims 2008).

3.5.2. Depth preferences

Evidence from the models of species trends showed that the depth interval was not a significant variable influencing the abundance of immature specimens, as their presence was similar in both studied intervals (10-20m, 30-45m). Juveniles of this species have been reported to prefer coastal areas (Serra-Pereira et al. 2014, McAllister et al. 2023). Being a coastal zone, this is in accordance with the reported preference of juveniles for nearshore areas (Serra-Pereira et al. 2014, McAllister et al. 2023). We have observed that in the study area, juveniles occupy both sandy and muddy bottoms. This depth range is coincident with the one described by McAllister et al. (2023), that reported immature individuals preferably occurring down to 50m deep. Given this, our results imply that juvenile thornback rays may use most of the area contained within the MPA limits, at least down to 45 meters in depth.

The mature individuals were more abundant at 30-45m depth compared with shallower waters. For mature males, telemetry data confirmed their preference for this depth range over more shallow areas. While in the monitored area, individuals showed higher residency to offshore receivers. Furthermore, the dispersal patterns showing a predominant offshore direction imply the species' usage of deeper areas outside the MPA boundaries. The observations made by Silva et al. (2023) support that in the Portuguese coast, this species is most commonly found down to approximately 70m, but also at depths exceeding 175m. Conversely, the research undertaken in the south British Isles (Humphries et al. 2016) has detected its preference for shallow water down to 20m depth. The authors highlight that depth effect is often entangled with substrate type, and also with availability of prey species (Humphries et al. 2016, Simpson et al. 2021). In our study area, sandy substrates occur in the nearshore zone and muddy sediments predominate at depths greater than 30m. It is possible that seafloor type is influencing the depth preferences herein observed. This is further supported by the results obtained by Silva et al. (2023), that reported that the probability of occurrence was lower on sand in relation to other soft sediments. Simpson et al. (2021), also observed active selection of areas of fine sediments over coarse sediment zones. It is worth mentioning that depth usage by this species is likely driven by more than one factor. The seasonal migration to deeper waters which is consistent across regions (Rousset 1990, Hunter et al. 2006, Humphries et al. 2016) indicates that other factors, probably related with their life cycle, are likely involved.

3.5.3. Spatial-temporal patterns

An important finding from the species abundance analysis is the significance of protection level and year. Thornback rays were significantly more abundant in the higher protection levels (FPA and PPA), confirming previous observations (Sousa et al. 2018, Martínez-Ramírez et al. 2021). Tracking data also confirmed the regular use of these areas by this species, as indicated by the several individuals that were classified as residents. Average residency estimates obtained in our study were similar to those described by Kraft et al. (2024), confirming the regular use of this region by this species, particularly males. In relation to the residency described by Papadopoulo et al. (2023) for juvenile thornback rays in the Cíes Islands off northern Spain, our estimates were considerably higher.

Examining the abundance data by ontogenic group proved to be crucial to properly interpret the displayed trends. The use of non-linear modelling was also useful to assess the irregular time trends taking place. Hence, our results add valuable insights into the existing knowledge of this species in this area (Sousa et al. 2018, Martínez-Ramírez et al. 2021). Specifically, it was confirmed that mature and immature individuals undergo distinct temporal patterns. Although both groups showed an increase in abundance in the first two years, their response diverged according to ontogeny in the remaining period. Specifically, mature individuals were present in relatively stable numbers from the third year onwards, while immature individuals exhibited a significant decrease. According to Serra-Pereira et al. (2014), juveniles display seasonal fluctuations in abundance, mainly due to the contribution of newly hatched individuals. However, our sample was composed of older juveniles (minimum TL 30.6cm). Research has established that environmental variables may impact the population levels of Rajidae species (Rousset 1990, Ferrà et al. 2016). Rousset (1990) has observed fluctuations of abundance of thornback ray juveniles due to seasonal small-scale migrations. Overall, it is reasonable to propose that multiple factors have intervened, and the potential influence of fishing mortality should not be dismissed.

Evaluating seasonal trends, the abundance analysis did not reveal any statistically significant effects. This may be attributed to the limitations of sampling, not enabling enough resolution to reveal seasonality. However, telemetry data provided insights into seasonal trends. Utilization areas expanded during autumn, indicating an increase in displacement during this season. Residence ratios showed that individuals were more prevalent in the area during spring, followed by summer. Conversely, presence was lower during the colder seasons, particularly in autumn, and these findings aligns with the trends reported by Kraft et al. (2024). Migration to deeper waters in autumn has been previously described for this species (e.g., Hunter et al. 2006, Humphries et al. 2016). Also in our case study, it is likely that rays are predominantly moving to deeper areas when they leave the study site.

3.5.4. Individual movement patterns

Acoustic telemetry data also offered valuable insights when examined at the individual level. Two of the twelve tagged individuals were considered occasional visitors, specifically the female and the immature individual. The results obtained by Kraft et al. (2024) highlight that females have significantly lower residency in this area in comparison to males. They also studied juvenile specimens and observed both resident and transient individuals, illustrating the variability within this ontogenic group. In our results of movement patterns, variability was also observed. Both resident (six ind.) and transient (four ind.) mature males were found. Within the residents, one individual was caught while being detected. Conventional tagging also provided the report of a catch adjacent to the protected area. This supports that fishing effort near the MPA boundaries ('fishing the line') is taking place and may impact the local population. Regarding the importance of individual data, a third individual is worth mentioning, specifically the mature male that was caught 40km to the south (conventional tagging, Ind. B). Other displacements of this species to outside the marine reserve (FPA, PPA) were observed by Kraft et al. (2024). They reported a female that entered Sado estuary, and five males that visited Sesimbra bay, within the BA. Other locations that are potentially visited by rays that exit the area are the ones reported by Silva et al. (2023). They found peaks of biomass of thornback rays in the vicinity of Lisbon and south of Setúbal during fisheries research surveys. In addition to the insights obtained by animal tagging, these findings suggest locations that could be further investigated as possibly relevant for the local population.

3.5.5. Implications for conservation efforts

By including fishery-independent surveys and animal tagging techniques (electronic and conventional), we provide a comprehensive case study on the efficacy of spatial management in protecting the thornback ray. Our findings collectively demonstrate that a coastal MPA may offer moderate protection to this species. We have assessed that in the PLSMP, the abundance of this species was higher in the PPA and FPA (i.e., protected area), and a significant increase in abundance for both adults and juveniles was observed. Moreover, telemetry results suggest that adult males commonly exhibit moderate to high residency in the protected area. However, other aspects suggest that the effective protection conferred by this MPA to this species presents some constraints. The observed variability in the abundance of juveniles illustrates that the drivers of abundance are complex and need to be further investigated. Within the adult group, males are more likely to be provided protection by this MPA in comparison to females. Moreover, the fact that adult males preferably use the offshore section of the protected area suggests that the practice of fishing near the MPA border (‘fishing the line’) may be particularly detrimental for this species. The reported catches indicated that this practice occurs in the area, and we also confirmed that rays often exit the protected area towards deeper waters, thus becoming vulnerable to fishing. Regarding vulnerability to fishing, it is also worth mentioning that in the studied PPA, about one third of the area does not include depths greater than 30m, which are preferred by this species. The aspect of habitat continuity is relevant in MPA design (Gaines et al. 2010), and soft bottom substrates should also be considered. We highlight that this aspect is fundamental when establishing MPAs aimed at protecting Rajidae species, and thus recommend the study of an extension of this MPA into deeper waters.

Two main questions that emerge as important for future research are the study of the environmental variables that may drive fluctuations in juvenile abundance, and to identify other areas this population uses during its life cycle. In this regard, efforts should be directed to tag mature females using both data storage tags (DSTs) and pop-up satellite archival tags (PSATs), which would allow monitoring outside the study area. Besides bio-logging, other approaches like assessing fishers’ knowledge could be used (Serra-Pereira et al. 2014). Furthermore, genetic analysis and stable isotopes techniques could also be applied to address the connectivity between populations and with other areas. The insights from our research suggest that the study area has the potential of protecting both juvenile and adult thornback rays. Regarding sex, there is evidence that the area might offer limited protection for females (Kraft et al. 2024). The collected pool of findings herein presented provide valuable contributions for the implementation of conservation strategies for this species and are also a relevant contribution to the study of Rajidae species’ movement ecology.

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3.7. Supplemental information

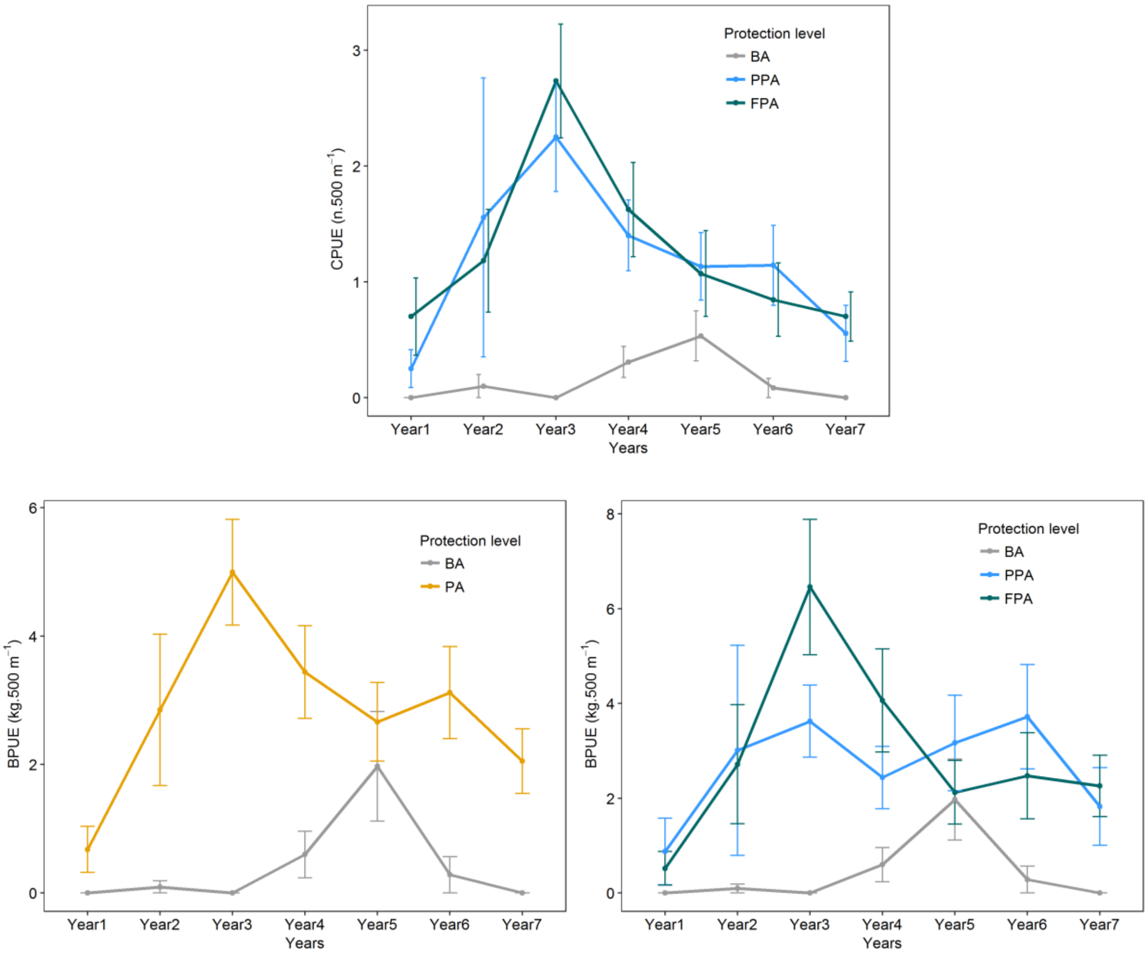

Figure S3.1. Yearly means of *lavata* catch per unit effort (CPUE, n.500m^{-1}) ± SE and biomass per unit effort (BPUE, kg.500m^{-1}) ± SE according to protection level. Top plot exhibits CPUE corresponding to the three protection levels: buffer area (BA), partially protected area (PPA), and fully protected area (FPA). Bottom plots display BPUE values - left plot presents data considering two protection levels: protected area - PA (PPA+FPA), and buffer area (BA); right plot presents data according to the three protection levels (BA, PPA, FPA).

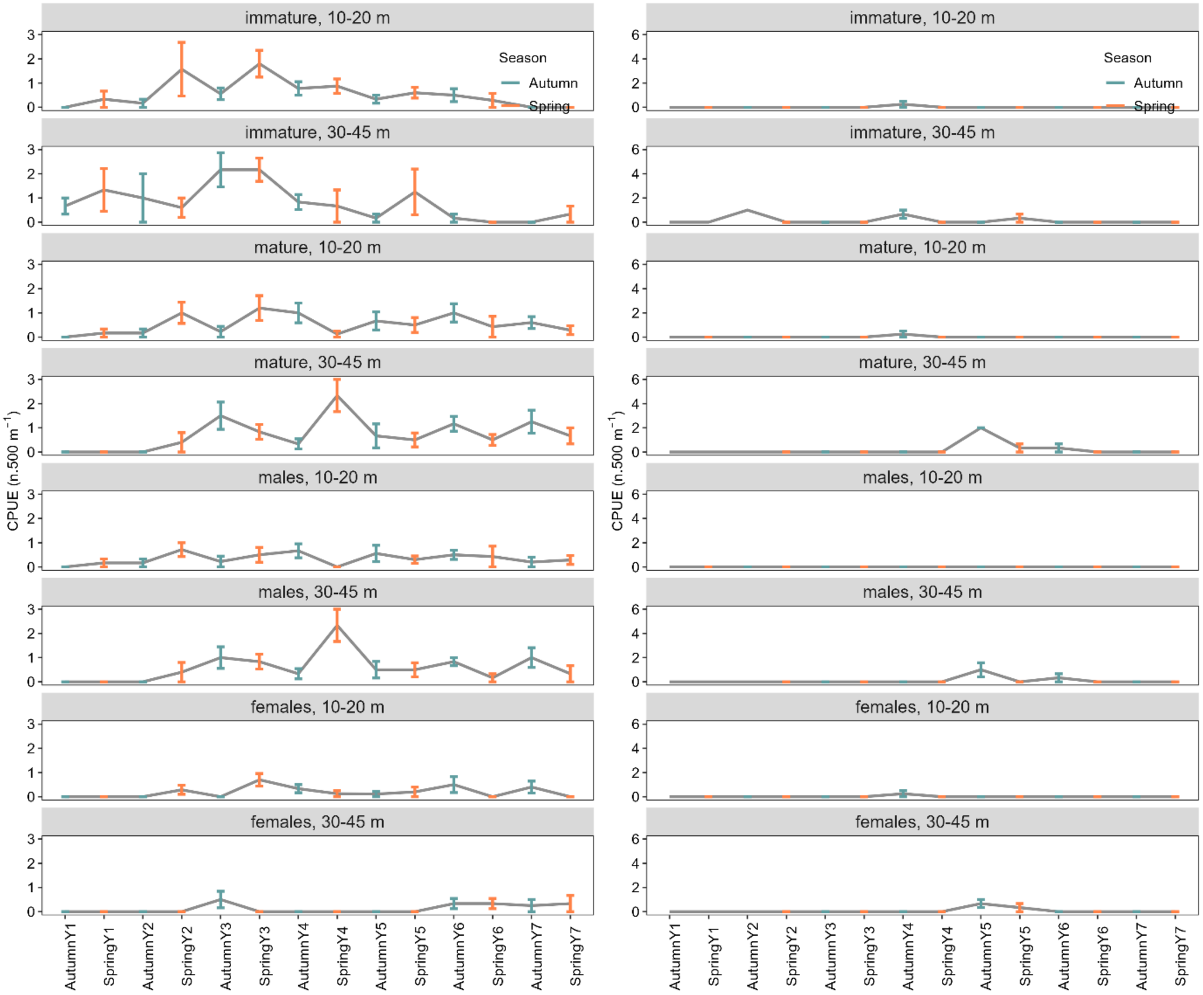

Figure S3.2. Mean catch per unit effort (CPUE, n.500m^{-1}) ± SE of *Raja clavata* per campaign (each autumn and spring over seven years) and in the two protection levels: protected area (PA) on the left, and buffer area (BA) on the right. Data is shown categorized by ontogenic group (immature and mature), sex (males and females, all mature), and depth interval (10-20m and 30-45m).

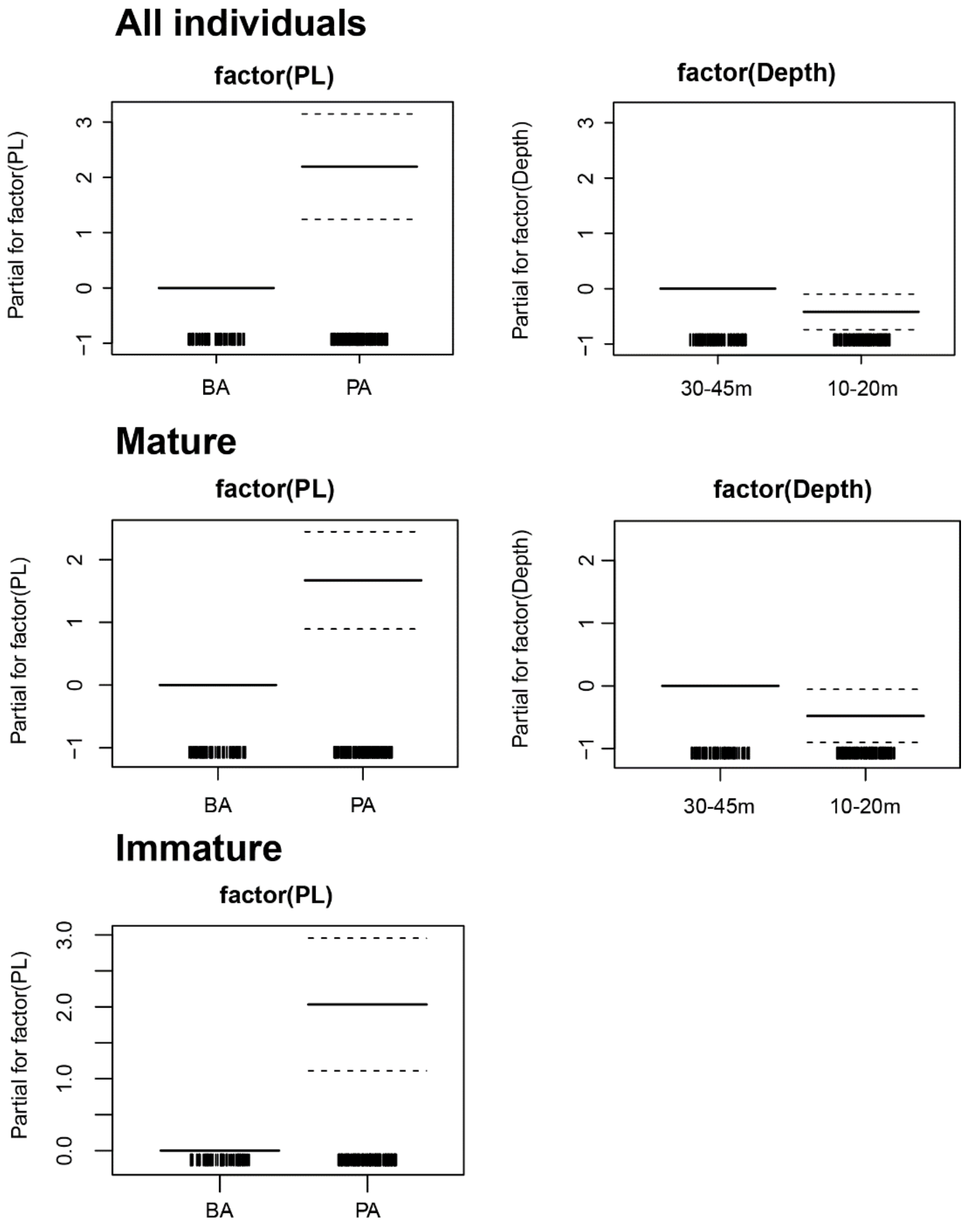

Figure S3.3. Partial effects of the parametric terms included in the models (GAM) fitted to abundance of *Raja clavata* (CPUE, n.500m^{-1}): PL - protection level (PA, protected area; BA, buffer area); Depth - depth interval (10-20m, 30-45m). Top plots: global model for all individuals; Central plots: model for mature individuals; Bottom plots: model for immature individuals.

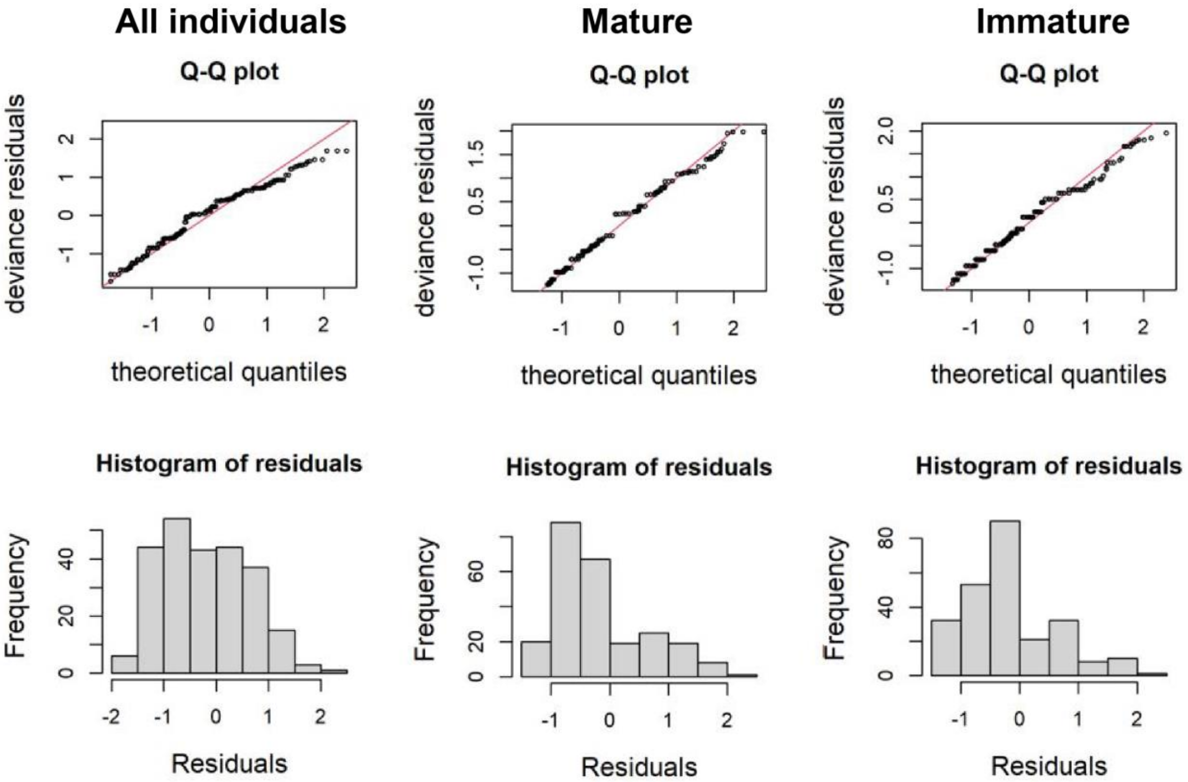

Figure S3.4. Diagnostic plots for Generalized Additive Models (GAMs) predicting abundance (CPUE, n.500m-1) of *Raja clavata*. Three models were considered for three different groups: all individuals, mature, and immature. The top row displays Q-Q plots, and the bottom row shows histograms of the residuals, illustrating the distribution and frequency of residuals for each group.

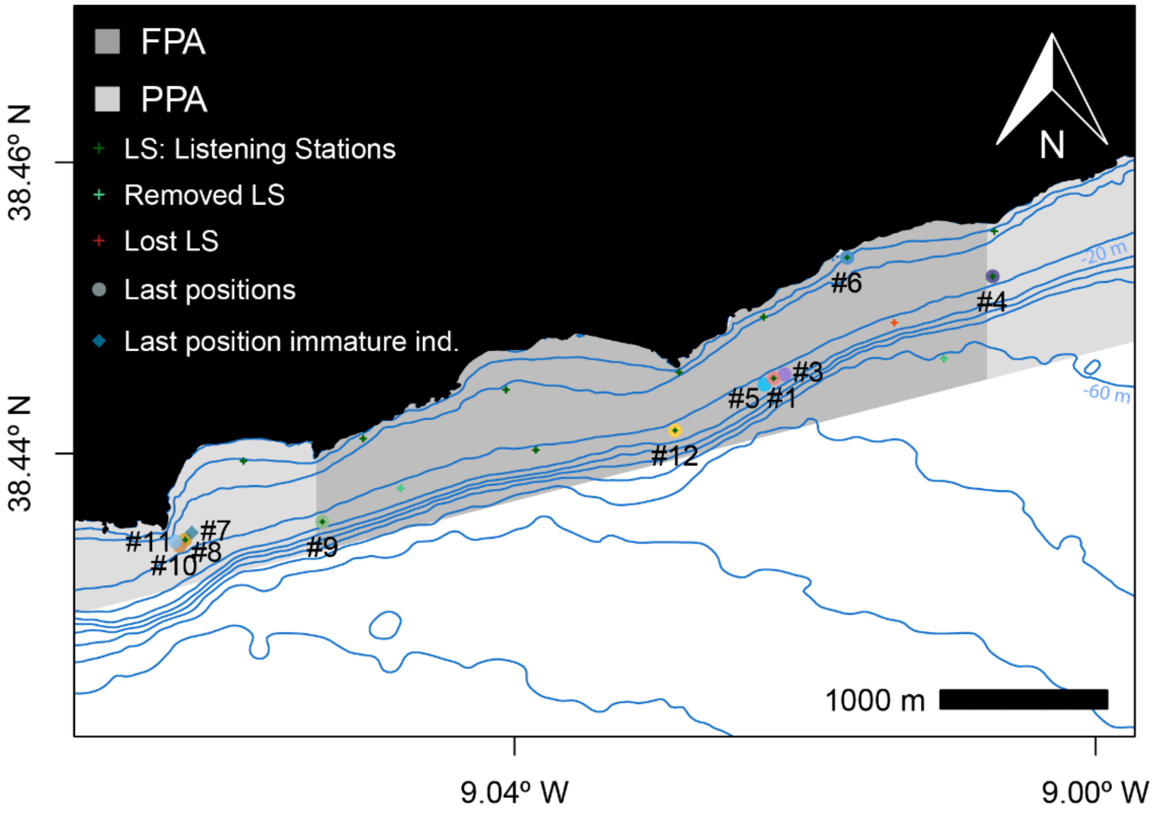

Figure S3.5. Locations of last positioning averaging according to last detections (60min) of the eleven thornback rays (*Raja clavata*; 10 mature males, 1 immature male - #7) tracked with passive acoustic telemetry. Only Ind. #8 and #9 were still being detected at the time of tag battery expiration. Ind. #3 was reported caught by a fisherman eleven months after tagging (at ~350m offshore from last detection position). Removed listening stations (light green crosses) monitored during eight (left) and eleven months (right). Lost listening station (red cross) only monitored during 21 days

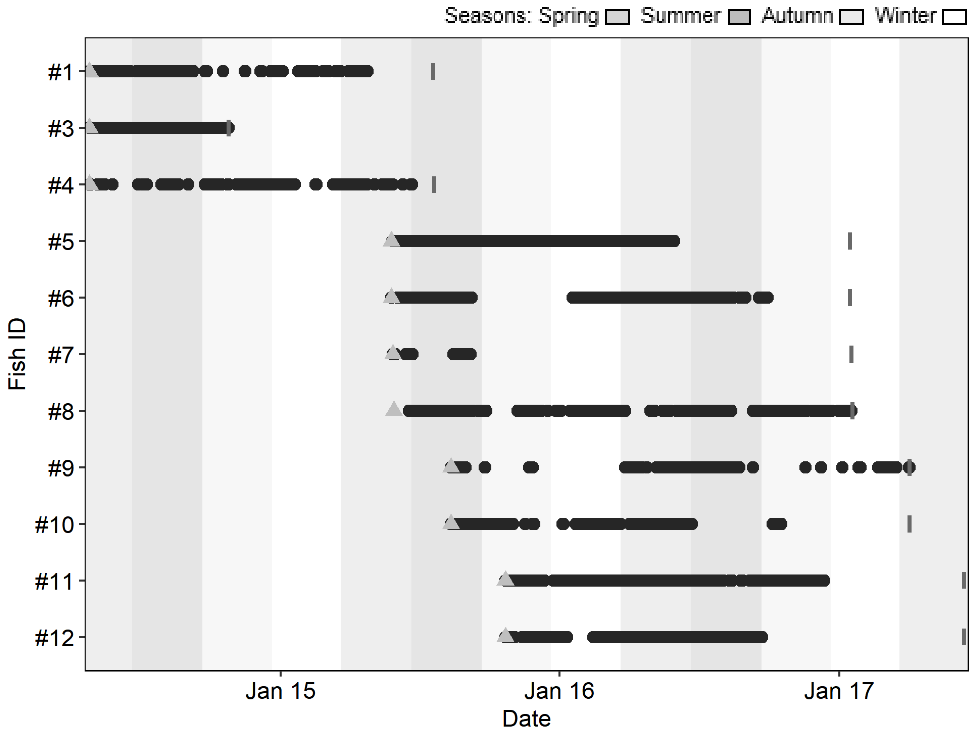

Figure S3.6. Residency plot of presence-absence data for each tagged *Raja clavata* detected within the study area over the entire study period. Each data point represents a day during which the individual was detected within the array. Grey triangle indicates study start, and the grey vertical line indicates study end (expected tag battery expiration; Ind. #3 reported caught by fisherman).

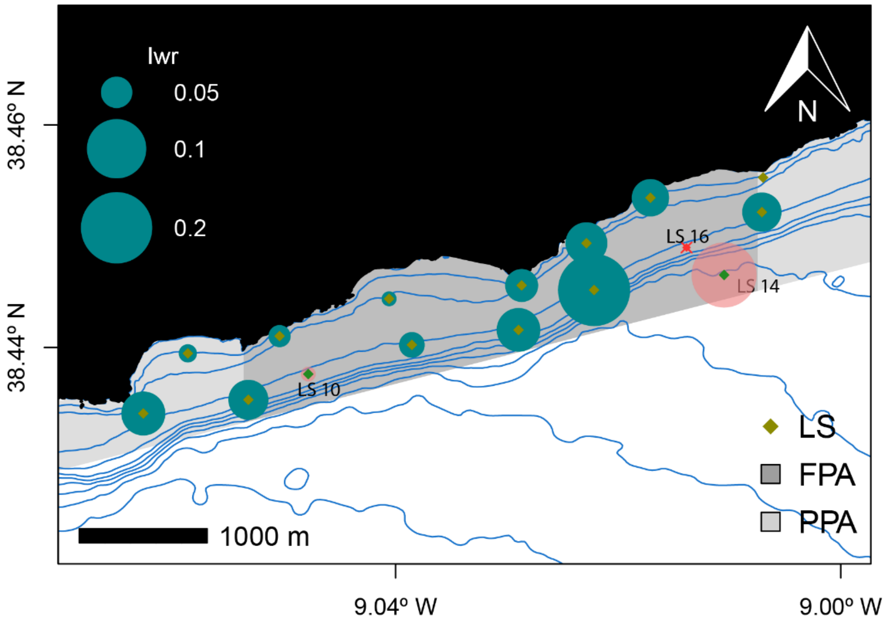

Figure S3.7. Spatial variation of weighted residence index (Iwr). The relative size of circles indicates the average Iwr at a given listening station (LS) obtained from the values of all tagged rays (*Raja clavata*, N=12). Values of LS10 and LS14 (pink circles) are regarding a shorter period (8 and 11 months, respectively), during which only three individuals were tagged (stations were removed after interaction with fishing gear). Crossed red dot: lost station (only 21 days of data collected).

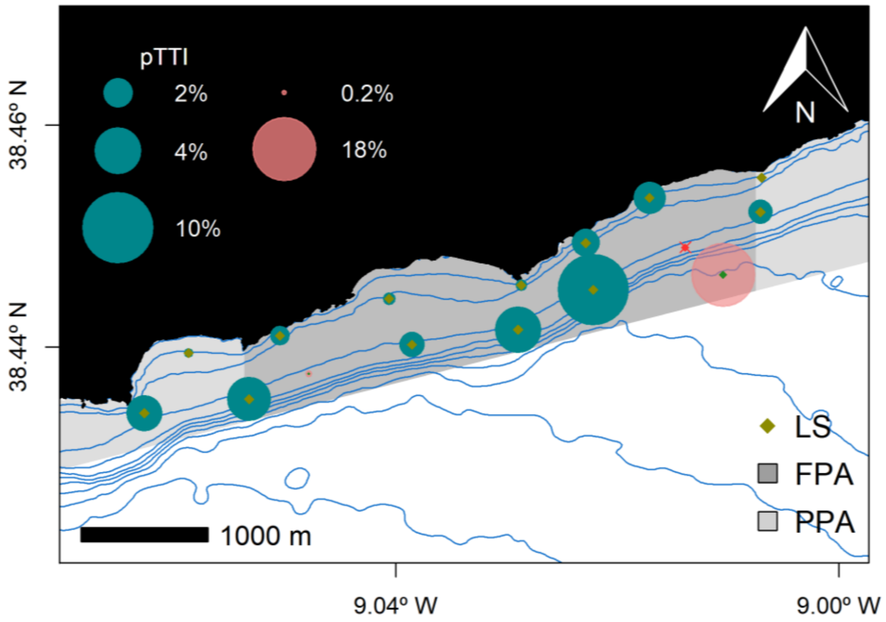

Figure S3.8. Spatial variation of proportion of total time inside de study area (pTTI), relative to the total study time (tagging to expired battery / reported catch / station removal). The relative size of circles indicates the total pTTI at a given receiver obtained from the values of all tagged rays (*Raja clavata*, N=12). Values of two LS (pink circles) are regarding a shorter period (8 and 11 months, respectively, during which only three individuals were tagged); these values are represented in a different scale (stations were removed after interaction with fishing gear). Crossed red dot: lost station (only 21 days of data collected).

Table S3.1. Number and proportion (N%) of exits and enters from and to the study area at each direction category (according to location of receiver with last and first detection: LS1, LS8, LS15 - coastwise) of each tagged ray (*Raja clavata*).

Exits and enters directionality					
	Offshore		Coastwise		Coastwise exits/enters
	Exits				
Fish ID	N	N %	N	N %	N per direction
#1	266	92.7	21	7.3	east: 21
#3	206	98.6	3	1.4	east: 3
#4	375	97.7	9	2.3	east: 9
#5	200	68.3	93	31.7	east: 93
#6	504	72.2	194	27.8	east: 194
#7	8	88.9	1	11.1	west: 1
#8	92	22.3	321	77.7	east: 1, west: 320
#9	46	31.3	101	68.7	west: 101
#10	238	74.1	83	25.9	east: 15, west: 68
#11	457	94.6	26	5.4	east: 6, west: 20
#12	300	99.0	3	1.0	west: 3
	Enters				
#1	268	93.4	19	6.6	east: 19
#3	205	98.1	4	1.9	east: 4
#4	376	97.9	8	2.1	east: 8
#5	197	67.2	96	32.8	east: 96
#6	484	69.3	214	30.7	east: 214
#7	7	77.8	2	22.2	west: 2
#8	118	28.6	294	71.4	east: 2, west: 292
#9	57	38.8	90	61.2	west: 90
#10	232	75.8	74	24.2	east: 13, west: 61
#11	454	94.2	28	5.8	east: 8, west: 20
#12	295	98.7	4	1.3	west: 4

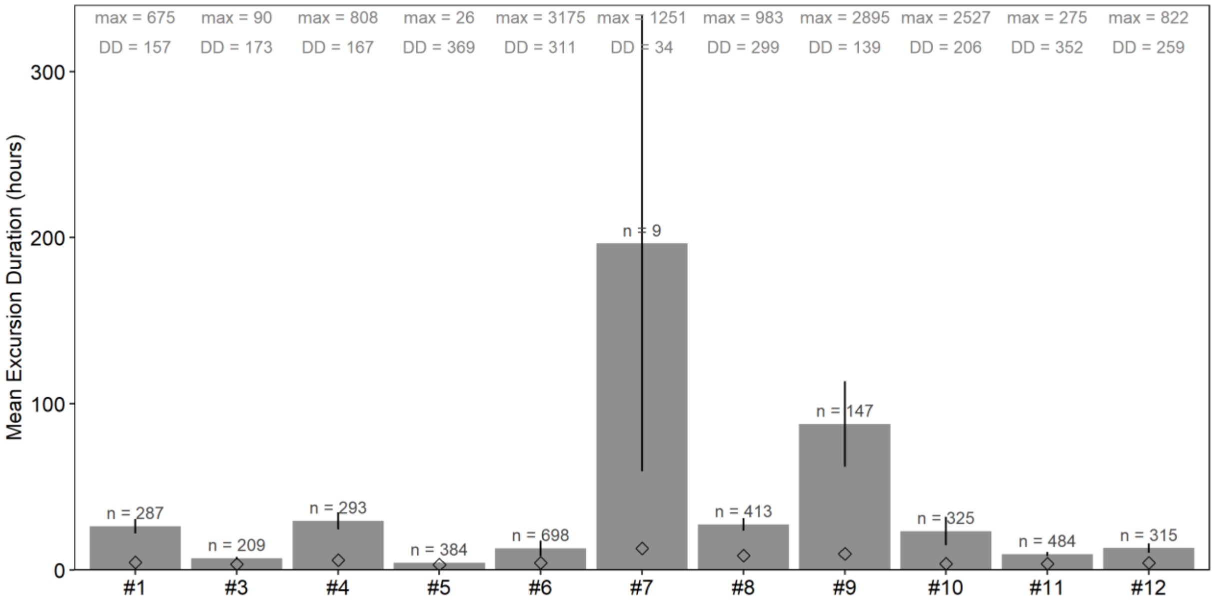

Figure S3.9. Mean excursions duration (hours) ± SE of each tagged ray (*Raja clavata*) (max - maximum excursion duration in hours; DD - days with detections). Median values are marked by '◊'.

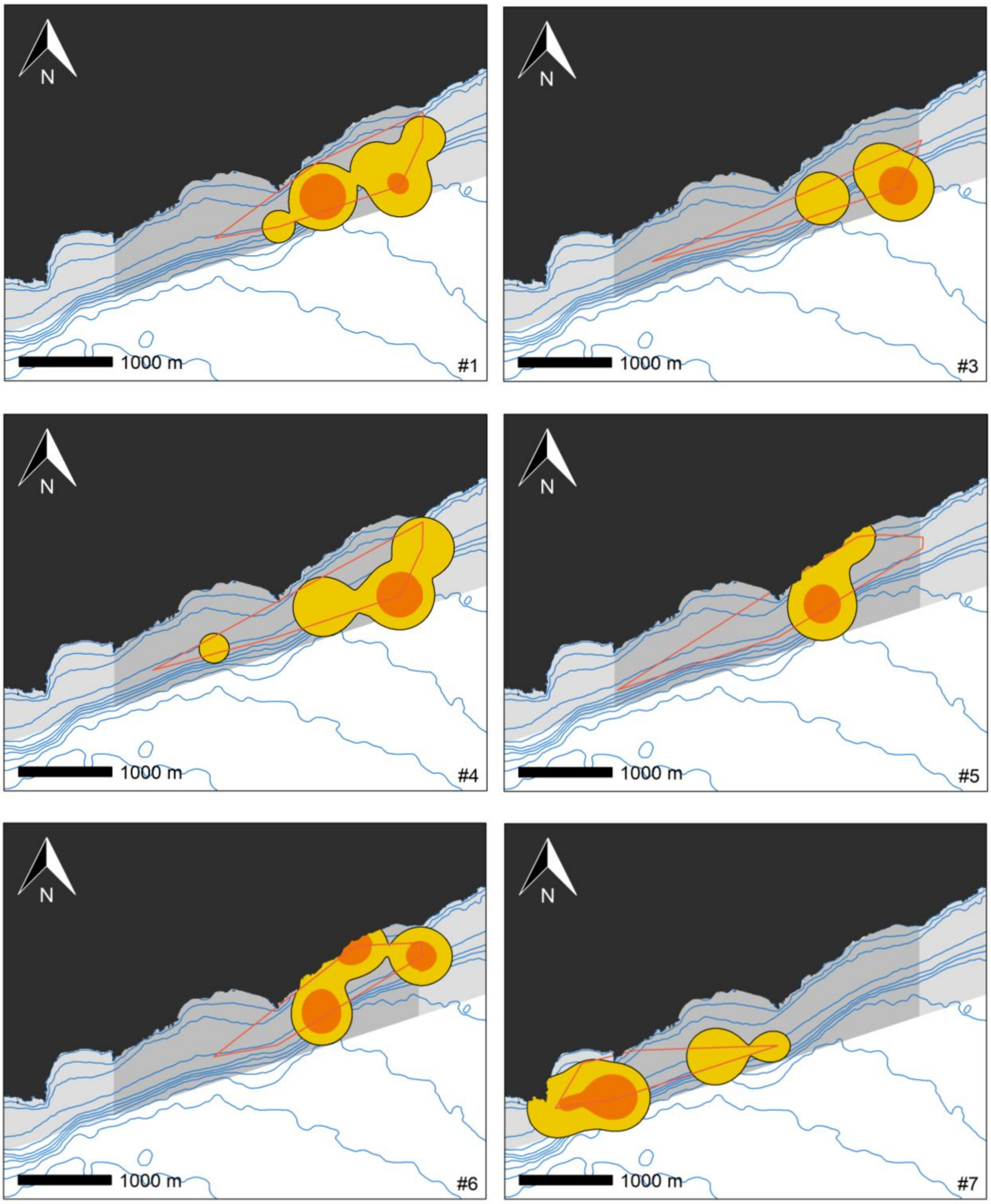

Figure S3.10. Maps of home range (KUD95, yellow) and core activity area (KUD50, orange) modelled for each tagged individual of *Raja clavata* (Ind. #1, Ind. #3, Ind. #4, Ind. #5, Ind. #6: mature males; Ind. #7: immature male) in the study site (positioning averaging interval: 60 min). Minimum convex polygon is also shown (red line). Ind. #3 was reported caught by a fisherman eleven months after tagging.

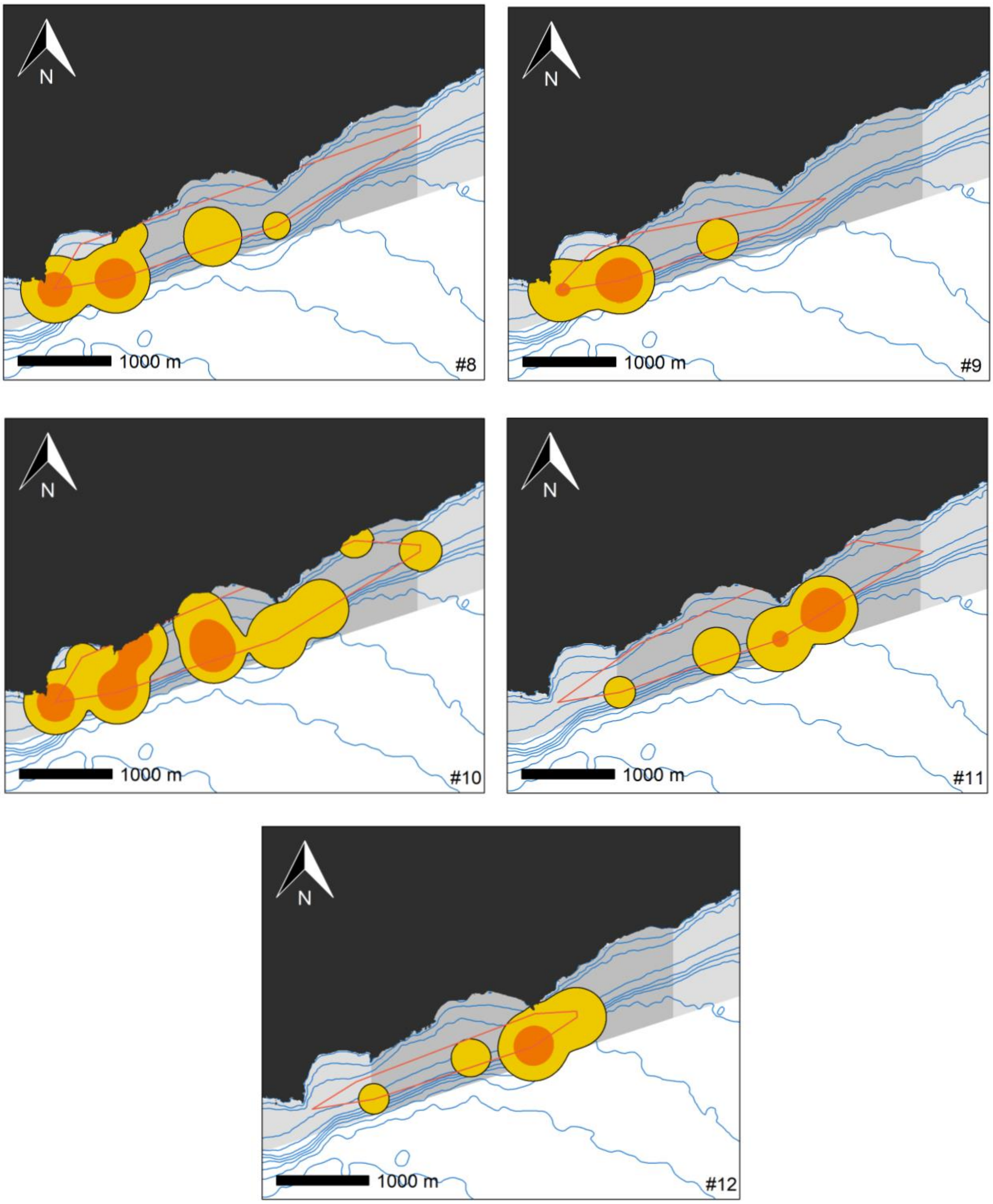

Figure S3.11. Maps of home range (KUD95, yellow) and core activity area (KUD50, orange) modeled for each tagged individual of *Raja clavata* (Ind. #8, Ind. #9, Ind. #10, Ind. #11, Ind. #12: mature males) in the study site (positioning averaging interval: 60 min). Minimum convex polygon is also shown (red line).

CHAPTER IV

Spatial ecology and the suitability of a coastal marine protected area for the recovery of the endangered undulate ray (*Raja undulata*)

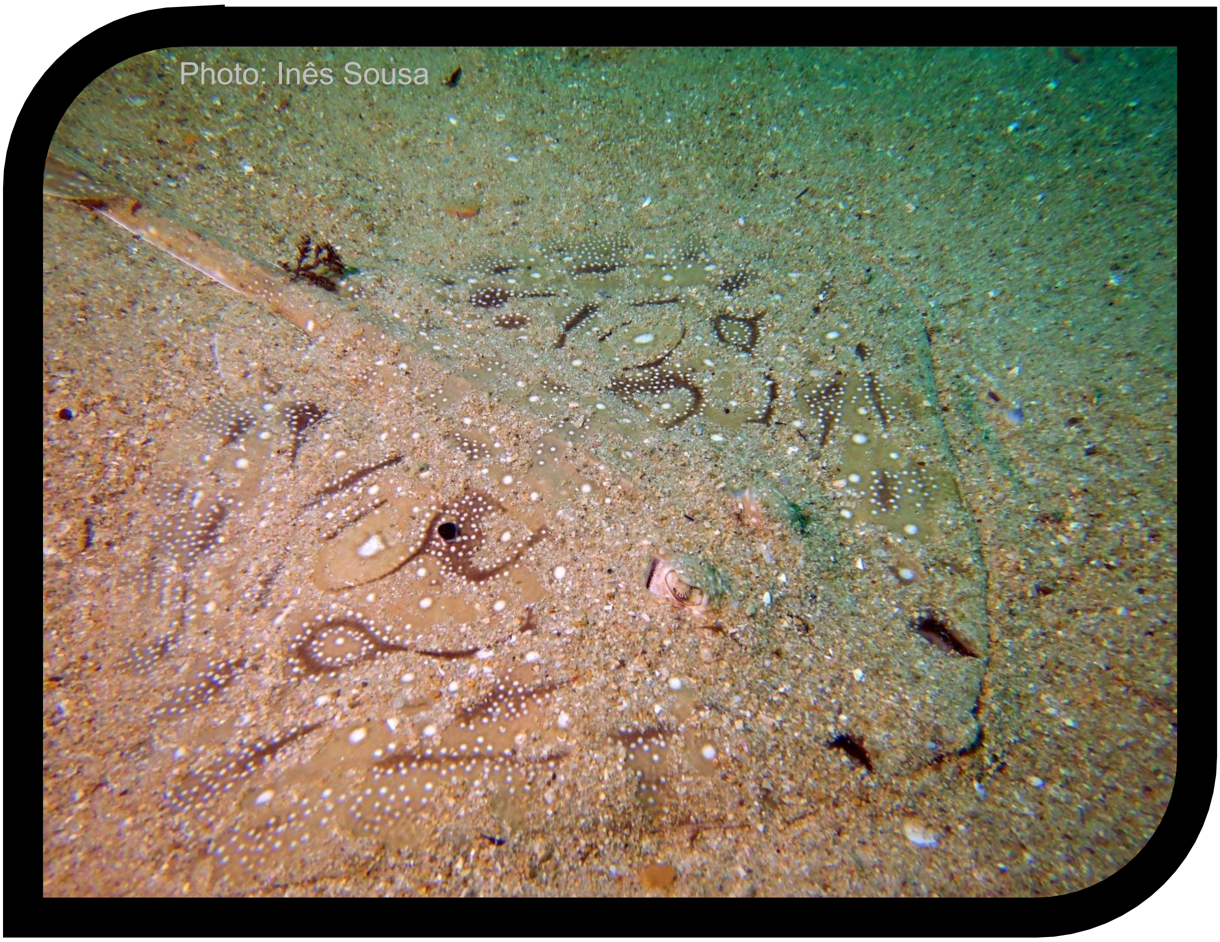

Photo: Inês Sousa

Sousa, I., Moland, E., Olsen, E. M., Gonçalves, J. M. S., Villegas-Rios, D., Bentes, L., Gonçalves, E. J., Erzini, K. Spatial ecology and the suitability of a coastal marine protected area for the recovery of the endangered undulate ray (*Raja undulata*)

4. Spatial ecology and the suitability of a coastal marine protected area for the recovery of the endangered undulate ray (*Raja undulata*)

4.1. Abstract

Sharks and rays (Elasmobranchii) are nowadays reported as the most threatened marine fishes in Europe, and shelf water species are considered potentially more vulnerable. The undulate ray (*Raja undulata*) is coastal species classified as endangered, and it is currently target of specific fisheries regulation (on-board retention of this species is restricted since 2009 by the European Union). Although some studies have focused on biological aspects of this species such as growth, reproduction and feeding, little is known about its movement patterns and response to spatial protection. We studied the spatial dynamics of this species in a coastal marine protected area located in the north-eastern Atlantic, the Prof. Luiz Saldanha Marine Park (Portugal). Acoustic telemetry data was collected during three years in the fully protected area and adjacent partially protected area. Data showed variability in residency. Some individuals exhibited year-round residency in the reserve, while others were not solely restricted to the fully protected area and migrated to the adjacent areas. Nevertheless, half of the tracked individuals exhibited high site fidelity. The data suggest that the species uses the width of this coastal area equally (similar residency in the offshore and inshore receivers). Our results suggest that spatial closures are an appropriate measure for the conservation of undulate ray populations, and that even small reserves can offer some protection to this species. However, the recovery of healthy populations regarding abundance and genetics would be favoured by MPAs enclosing extended sections of coastal habitats.

Keywords: marine protected areas, acoustic telemetry, spatial ecology, elasmobranchs, rays and skates, undulate ray

4.2. Introduction

Nowadays, marine protected areas are commonly advocated as one of the most promising tools to implement in coastal areas, for fishery management, biodiversity conservation, preservation of the stocks, enhancement of the sustainable yield, and for providing opportunities for scientific research and education, among others (Claudet et al. 2006). Therefore, marine protected areas are being created mainly with the purposes of (1) sheltering vulnerable and threatened species, (2) protecting habitats from overexploitation and reducing their degradation, (3) inducing the recovery of highly damaged areas, (4) preventing the loss of biodiversity, (5) making productivity sustainable by restocking commercial species, and finally (6), to promote tourism and recreational activities (Glenn et al. 2010). However, due to the social and governmental pressure to reach these goals in a short term, the implementation of marine protected areas often lacks prior scientific knowledge regarding biological and ecological facts (such as species' life cycle, habitat preferences, species' life span, among others), and the functioning of the MPA itself (Pike et al. 2024).

As cartilaginous fish, skates (family Rajidae) possess typical life traits that result in their low productivity, such as slow growth rates, late maturity and low fecundity. These characteristics are widely recognized as factors that increase their susceptibility to overexploitation and environmental changes (Dulvy et al. 2014). Predominantly benthic, skate species inhabit a wide range of marine environments, from coastal waters to deep-sea habitats (Ebert & Sulikowski 2009). It is usually presumed that as benthic species, they have relatively low activity rates (Walker 1997). Their foraging behaviour is not fully understood, with the prevailing assumption that they are predominantly opportunistic feeders, with activity levels dependent on prey distribution and availability (Walker 1997).

Raja undulata is one of several skates found in the Iberian Peninsula. The species is present in Eastern Atlantic, from southern England and Ireland to Senegal, including the Mediterranean and the Canary Islands (Froese & Pauly 2024). Since 2009, this species is classified as endangered in the Red List of the IUCN (Coelho et al. 2009). Despite being one of the most common ray species in the Portuguese coast (Prista et al. 2003, Machado et al. 2004, Coelho et al. 2005), its biology, life history and behaviour are still poorly understood. Although in Portugal the undulate ray used to be caught by commercial fishing vessels, with trammel nets, longline and trawls (Moura et al. 2008), nowadays there are no directed fisheries targeting this species due to the fact that the on board retention of this species became prohibited in 2009, under the European Union fishing regulations, forcing fishermen to promptly release unharmed all specimens (Serra-Pereira et al. 2018). However, *R. undulata* is still caught as bycatch, leading to a decline of its populations (Ellis et al. 2012). Expert advice is that unless exploitation rates become sustainable, fisheries targeting *R. undulata* should not be permitted (Ellis et al. 2012).

The species is a carnivore, feeding on benthic and semi-pelagic vertebrates and invertebrates, such as crabs, shrimps, fishes, among others (Moura et al. 2008, Ellis et al. 2012). When young, juveniles tend to be generalists, feeding upon crustaceans, molluscs and small fish, and when individuals become mature, they start to have a specialised diet in crabs, particularly a decapod, the Henslow swimming crab (*Polybius henslowi*) (Moura et al. 2008). Regarding the reproductive biology of this species, scientific data is scarce. Nevertheless, it is estimated that the age and length at first maturity are 8 years for males and 9 years for females and 0.74m of total length for males and 0.76m of total length for females, respectively (Coelho & Erzini 2006). The breeding is seasonal, since ovulation and mating occurs during the first half of the year, with the egg laying taking place from March to June (Moura et al. 2007). Notwithstanding, in Peniche (Portugal) spawning seems to occur during the whole year (Moura et al. 2007), whereas in the Algarve (south of Portugal), spawning only takes place during winter (Coelho & Erzini 2006). *R. undulata* is assumed to have a high site fidelity, with juveniles occurring in shallow and coastal waters, such as the Sado estuary (Moura et al. 2007), while adults tend to live in less shallow waters, yet close to the shore (Ellis et al. 2012). Prior studies using acoustic telemetry have provided some understanding of this species' spatial dynamics (e.g., Cabral 2014, Espasandín 2018, Leeb et al. 2021). There is evidence of individual heterogeneity in site fidelity, with some individuals exhibiting high residency, and others displaying higher dispersal patterns (Leeb et al 2021, Daban et al. 2024).

This study examines the protective benefits that a relatively small coastal MPA may offer to the undulate ray (*R. undulata*). We employed acoustic telemetry to investigate the spatial ecology of this species. In particular, we assessed site fidelity, spatial utilization, and dispersal patterns. We also analysed whether these indicators exhibited seasonal trends.

4.3. Methods

4.3.1. Study site

The study was performed in the Prof. Luiz Saldanha Marine Park (PLSMP), located in the Portuguese western coast (38º23' - 38º27'N; 8º56' - 9º14'W). This marine park has an extension of 38km and covers about 53km^2. It includes shallow coastal reefs and wide areas of soft bottoms down to ~100m deep. The area encompasses the Sesimbra fishing port and is close to the Setúbal fishing port, which is located in the nearby estuary (Sado river). Established in a region sheltered from the dominant northern winds and comprising a wide diversity of benthic habitats and communities (Henriques et al. 2014), the area is considered an important biodiversity hotspot (Henriques et al. 1999, Gonçalves et al. 2002). The marine park was created in 1998 and the management plan was implemented under a stepwise procedure between 2005 and 2009. It includes one fully protected area (FPA; 4km^2) where all human activities are forbidden; four partial protection areas (PPA; 21km^2) where some commercial fishing gears are allowed, specifically octopus traps and jigs; and three buffer areas (BA; 28km^2), in which octopus traps, jigs, longline and static nets (gill nets and trammel nets) can be used (Figure 4.1). Fishing by trawling, dredging, purse-seining and spearfishing are not allowed within the park. Recreational angling is only permitted in the BAs, and to perform commercial fishing, boats need to be <7m long and to have a permit.

4.3.1. Acoustic telemetry

The acoustic telemetry monitoring array was set in the FPA (fourteen stations) and in one adjacent PPA (two stations). Sixteen listening stations (LS) consisting of Innovasea VR2W receivers (69kHz) were set in place in May 2014, in an array composed by two lines: one inshore, with eight receivers, and the other offshore, with another eight receivers (Figure 4.1). Receivers at LS10 and LS14 were removed from the array after being retrieved by fishermen due to entanglement with their gear (octopus traps). These stations collected data for eight and eleven months, respectively. The receiver at LS16 was lost, probably because of contact with fishing gear. The data retrieved from LS16 corresponded to approximately one month of monitoring. Monitoring occurred over three years, between 25th April 2014 and 13th June 2017.

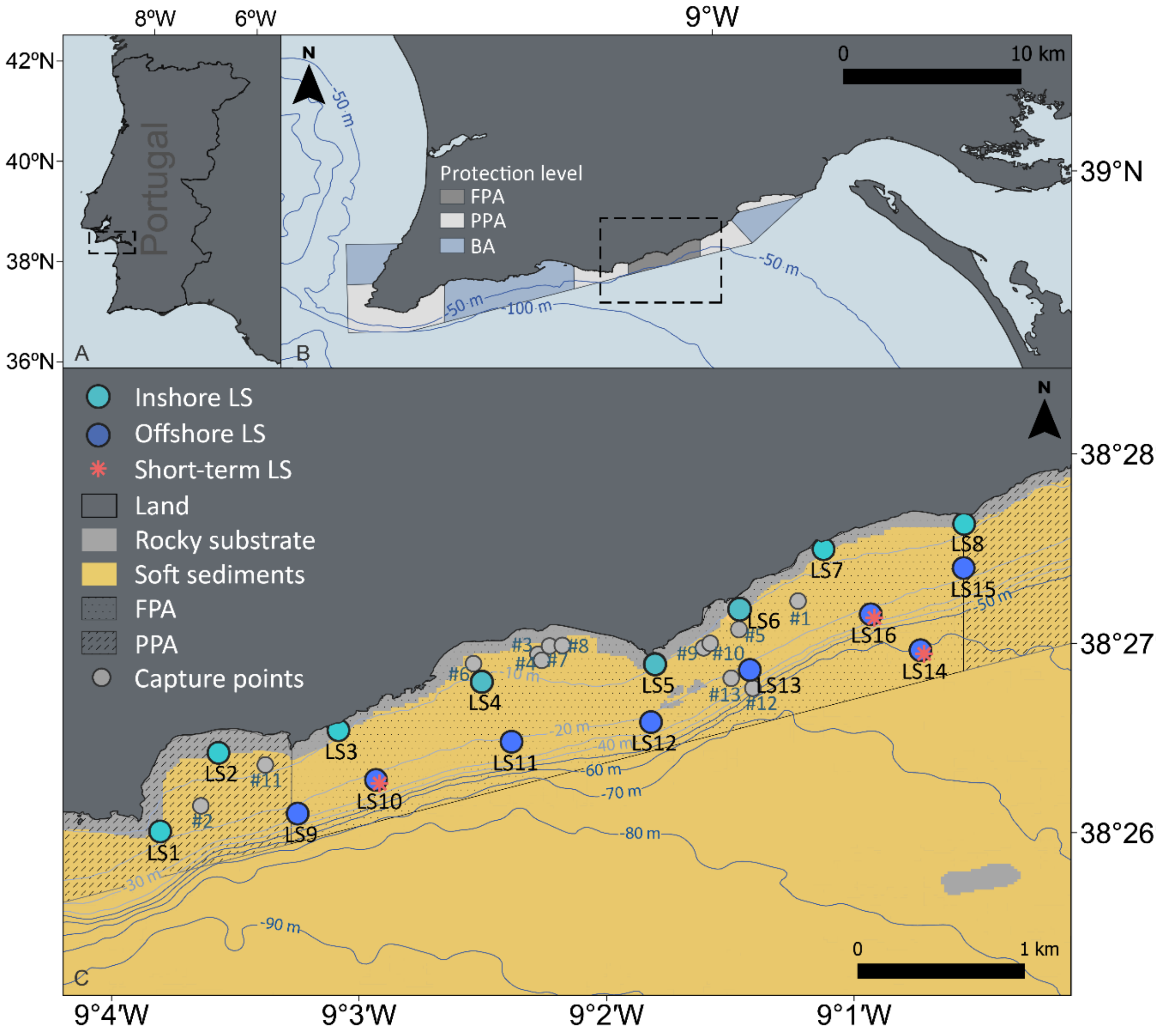

Figure 4.1. Map of the study area: the Prof. Luiz Saldanha Marine Park location in the Portuguese coast (A) and its three protection levels (B): fully protected area (FPA), partially protected area (PPA) and buffer area (BA) (bathymetric values in meters). C: Map of the listening stations (LS) set in the study area, with information of position category (inshore, offshore). Red asterisks indicate stations that monitored for less than one year (Short-term listening stations - LS10: 8 months; LS14: 11 months; LS16: 1 month). Grey dots represent capture sites (numbers with hashes: ID of individuals).

Rays were tagged with acoustic transmitters programmed to emit a unique coded signal at random intervals of 40–80sec (Innovasea V13-1L, 36 x 13mm, weight in water 6g; expected battery life 600 days). Tag performance tests were carried out before receivers' settlement and tagging campaigns. These tests showed detection ranges of approximately 475m (50% of emissions detected). Tagging occurred during six fishing campaigns (three days each) between November 2013 and October 2015: two with trammel net (November 2013 and November 2014; 500m long; soak time ~24h), and four with longline (May 2014, July 2014, August 2014 and October 2015; soak time ~3-12h). Undulate ray specimens were measured (total length, precaudal length, disc width), sex was registered, and an external tag (Petersen disc) with ID number and telephone number was attached in the posterior region of the pectoral fin. The acoustic tag (previously tested if pinging with VR100 receiver) was surgically placed internally in the abdominal cavity through a small incision, which was then closed with absorbable suture and surgical glue. Individuals were maintained in a basin with saltwater during all procedures except measurements. During tagging, rays were positioned with the ventral site up, which induced isotonic immobility. After checking stimuli response, specimens were then released approximately in the same point where they were caught.

4.3.1. Data analysis

Telemetry monitoring occurred throughout two years, between 1st of June 2014 and 28th June 2016. Data were retrieved from the acoustic receivers using Innovasea VUE software. The R statistical software (R Core Team 2021) was used for data processing. Isolated detections occurring in a 24h period were removed as considered potentially spurious. To examine the spatial dispersal of individuals, two different methods were used: minimum convex polygons (MCP) and kernel utilization distributions (KUD). Minimum convex polygons were obtained from the minimum area that encompassed all detections. The KUDs were estimated based on position averaging calculations (Simpfendorfer et al. 2002). Position averaging (PAV) was performed for each ray for 60min periods. This method uses detections from multiple receivers and converts them to position estimates based on weighted means of the number of detections at each receiver during a particular time period. Detections in a single receiver during the chosen interval will result in a PAV estimate corresponding to the exact location of the receiver. The choice of interval was done in order to optimize the trade-off between the number of receivers used to calculate PAV (avoid many detections in only one receiver) and position resolution (avoid combining together many detections). Calculation of KUD50 (area including 50% of PAV points) was used as measure of the core activity area, and KUD95 (area including 95% of PAV points) was used as an estimate of home range. Data exploration enabled to choose the smoothing factor (h=350) of KUD modelling, by tuning the spread of positions density and taking into account the number of detections at each receiver.

Site fidelity was assessed for each individual through three indexes: the residence index (RI), the absolute residence index (AR), and the weighted residence index (Iwr) (Lino 2012). The first index (RI) was obtained by dividing the number of days with detections by the number of days between first and last detection. The second index (AR) was determined by dividing the total number of days with detections (DD) by the number of study days (tagging till expired battery or data retrieval). The second index consisted of the ratio between the number of days with detections (DD) and the number of study days (RI) multiplied by the ratio between number of days from first to last detection (monitoring period) and the number of study days. All indexes vary between 0 (no residency) and 1 (full time residency).

In order to calculate time spent inside the study area and absence periods, detection data of each individual was used for estimating a minimum threshold time interval with no detections that would define whether individual was present or absent from the study area. This was done following the method described by Knip et al. (2012). A mean exit time weighted by the number of data points, was calculated for the species. This value was used for classifying intervals between detections as presence or absence and enabled estimating the total time within the study area and mean excursion duration. The total time inside was divided by the monitoring period (time between first and last detection) and by the study period (tagging till expired battery or data retrieval) to calculate respectively the proportion of monitoring time inside (PTIM) and the proportion of study time inside (PTIS). The total time inside was also used with the detections location data to compare the proportion of time in the FPA (pti FPA) and PPA (pti PPA), the proportion of time in the inshore (pti Inshore) and offshore (pti Offshore) receivers' areas, and the proportion of time at each receiver. Furthermore, for absence intervals, the location of exit and enter was assessed. Directionality of these events (exit, enter) was inferred based on the position of listening stations: if detections before event were at LS1, LS8 or LS15, direction was classified as coastwise. Offshore directionality was considered for exits and enters at the southern edge of the monitored area (Figure 4.1). The counts of classified events of each individual were used to estimate class averages for all the data.

Linear mixed-effects models were used to examine the effect of sex and season in MCP area, home range (KUD95), AR, Iwr and PTIS, with sex and season as the fixed effects and individual ID as a random effect. An analysis of variance (ANOVA) was used to compare models with the null model and estimate the significance of the fixed effect.

4.4. Results

A total of thirteen individuals were tagged with acoustic transmitters: five mature males, six mature females, and one immature male. Information of each individual is presented in Table 4.1. Average total length of individuals (±SE) was 84.6cm ± 1.2cm, ranging from 72.0cm to 90.0cm. Of the thirteen tagged individuals, one was tagged before the entire set of receivers was in place (Ind.#1, male; last detect at 11/12/2013). This individual was detected only during the preliminary phase and not during the actual monitoring period. The capture and release locations for all thirteen individuals are shown in Figure 4.1.

Table 4.1. Details of rays tagged with ultrasonic transmitters: morphometrics (TL – Total length; PRC – Precaudal length; DW – Disc width), sex, maturity, tagging date, study start and end dates. (a) Only detected during preliminary monitoring; (b) Tagged in the western PPA, adjacent to the FPA; study started when two listening stations were set in the area; (c) First week of data neglected, as individual was released 300m away from capture site.

Fish ID	Sex	Maturity	TL (cm)	PRC (cm)	DW (cm)	Tagging Date	Study Start	Study End	Obs.
#1	male	mature	86	51	53	27 Nov-13	26 Apr-14	20 Jul-15	(a)
#2	male	immature	72	45	46	5 May-14	9 Nov-14	26 Dec-15	(b)
#3	female	mature	85	55	55	6 May-14	6 May-14	27 Dec-15	
#4	female	mature	83	50	52	6 May-14	6 May-14	27 Dec-15	
#5	male	mature	88	52	52	7 May-14	7 May-14	28 Dec-15	
#6	female	mature	84	51	55	3 Jul-14	03 Jul-14	23 Feb-16	
#7	female	mature	84	55	57	3 Jul-14	03 Jul-14	23 Feb-16	
#8	male	mature	83	53.5	49	3 Jul-14	03 Jul-14	23 Feb-16	
#9	female	mature	85	56.5	56.5	19 Aug-14	19 Aug-14	10 Apr-16	
#10	male	mature	86	55	54.5	19 Aug-14	19 Aug-14	10 Apr-16	
#11	male	mature	85	57	57	1 Nov-14	1 Nov-14	23 Jun-16	
#12	female	mature	90	56.5	56	22 Oct-15	22 Oct-15	13 Jun-17	
#13	male	mature	89	53	53	22 Oct-15	28 Oct-15	13 Jun-17	(c)

4.4.1. Site fidelity

A total of 1 485 215 detections were considered valid for analyses (average of 123 786 ± 32 319 detections per individual). Table 4.2 summarizes total detections, number of potential detection days (study period), tracking period (number of days from first to last detection) and days with detections for each individual. For eleven individuals, study periods (total of potential days of detection - PDD) were considered to be equal to the maximum tag life expectancy of 600 days (20 months). Two other individuals had shorter study periods: Ind. #2(m) was tagged in the PPA prior to the settlement of acoustic receivers in this area; and for Ind. #13(m), the first week of data was excluded, as individual was released 300m away from capture site.

Males and females exhibited a wide range of residency values (Table 4.2). Average values of RI were 0.86 and 0.73 for males and females respectively. Mean AR was 0.37 for males and 0.54 for females, while Iwr was 0.25 and 0.51, for males and females respectively. Values for the immature male (Ind. #2) were 0.58, 0.56 and 0.53, for RI, AR and Iwr respectively. The values of individual residence indices (Table 4.2) enabled to classify six of the twelve tracked rays as residents (RI ≥ 0.50 and AR ≥ 0.40): three males (Ind. #10, #13, and #2; #2: immature male) and three females (Ind. #6, #7 and #9). The residency plot (Figure 4.2) shows that three of the twelve individuals were still being detected at the time their tag batteries were depleted. A map with last estimated locations (positioning averaging, 60min) occurring either before dispersal or tag expiry is presented in Supplemental information (Figure S4.1). Additionally, timelines of daily detections are also provided (Figure S4.2).

Table 4.2. Summary of detections, total of study days (PDD, potential detection days), tracking period in days (TP; from first to last detection), and days with detections (DD) of the rays tagged with acoustic transmitters.

Fish ID and sex	*N* detections	PDD	TP	DD	RI	AR	Iwr
#2 (m)	29 583	412	392	229	0.58	0.56	0.53
#3 (f)	23 171	600	82	63	0.76	0.11	0.01
#4 (f)	42 865	600	574	252	0.44	0.42	0.40
#5 (m)	83 069	600	108	108	0.99	0.18	0.03
#6 (f)	292 941	600	600	527	0.88	0.88	0.88
#7 (f)	249 411	600	599	475	0.79	0.79	0.79
#8 (m)	4 002	600	5	5	0.83	0.01	0.00
#9 (f)	250 854	600	600	599	1.00	1.00	1.00
#10 (m)	249 778	600	400	400	1.00	0.67	0.44
#11 (m)	79 115	600	140	138	0.98	0.23	0.05
#12 (f)	1 729	600	25	14	0.54	0.02	0.00
#13 (m)	178 697	594	451	341	0.75	0.57	0.44

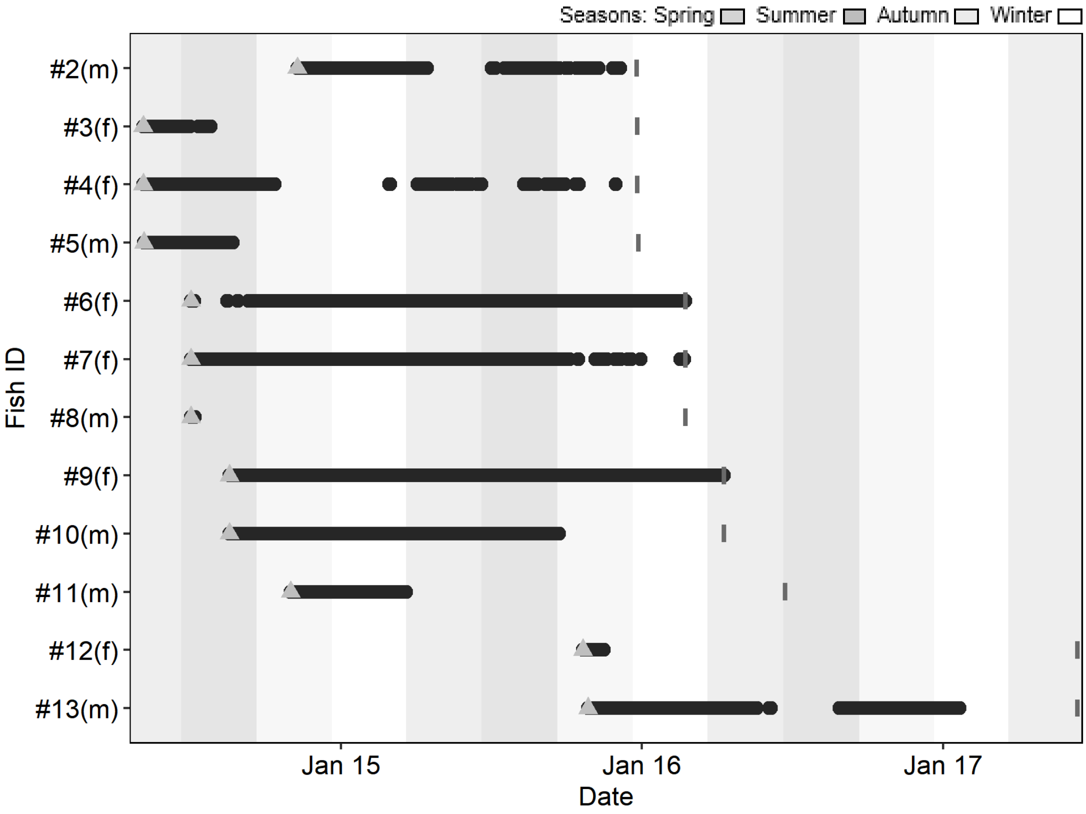

Figure 4.2. Residency plot with presence-absence data for each tagged *Raja undulata* detected within the study area over the entire study period. Each data point represents a day during which the individual was detected within the array. Grey triangle indicates study start (i.e., tagging). The grey vertical line indicates study end (expected tag battery expiration).

The values obtained of individuals' total time inside the study area, proportion of time inside (PTIT and PTIS), proportion of time according to receivers' protection level location (pti FPA, pti PPA), and also according to distance to the coast (pti Inshore, pti Offshore) are summarized in Table 4.3. Individuals exhibiting the greatest PTIS scores align with those demonstrating higher residency values (females: Ind. #6, #7 and #9; males: Ind. #2, #10, #13). The immature male (Ind. #2) was the one that predominantly used the PPA (98.7% of the time inside the study area), where it was initially captured and tagged.

Table 4.3. Values per individual of total estimated time inside the study area (h), proportion (%) of tracking time inside (PTIT), and proportion (%) of study time inside (PTIS). Regarding the time spent inside, the proportion (%) of time per protection level (pti FPA, pti PPA), and per distance to the shore (pti Inshore, pti Offshore) according to receivers' location is also shown. In the Supplemental information, the mean residency values and PTIS per sex are shown (Figure S4.3). Maps with Iwr values and PTIS per receiver are also provided (Figures S4.4 and S4.5).

Fish ID and sex	Time In (h)	PTIT	PTIS	pti FPA	pti PPA	pti Inshore	pti Offsore
#2 (m)	3092	32.9	31.2	1.3	98.7	31.0	0.3
#3 (f)	891	45.4	6.2	100	0	3.8	2.4
#4 (f)	2758	20.0	19.1	100	0	7.5	11.7
#5 (m)	2298	88.9	15.9	100	0	11.5	4.5
#6 (f)	11310	78.6	78.4	99.1	0.9	37.1	41.4
#7 (f)	8494	59.1	58.9	100	0	12.6	46.4
#8 (m)	109	100	0.8	100	0	0.5	0.2
#9 (f)	12072	83.9	83.7	99.8	0.2	41.6	42.3
#10 (m)	7639	79.7	53.0	100.0	0.0	19.5	33.6
#11 (m)	2652	78.7	18.4	90.1	9.9	8.6	9.8
#12 (f)	114	19.0	0.8	100	0	0.2	0.6
#13 (m)	6315	58.2	44.2	99.9	0.1	6.5	37.8

Residency assessment, based on receiver positions (inshore, offshore) reveals that tagged rays equally accessed both the inner and outer areas of the study site (Figure 4.3). Mean RI was 0.38 ± 0.18 inshore, and 0.43 ± 0.18 offshore. The Linear Mixed Model (LMM) output (Table 4.4) did not detect significant differences (χ^2=0.07, p=0.787).

The analysis of exit directionality (Figure 4.4) reveals that individuals were frequently absent from the study area, moving both towards offshore and in the coastwise direction. Mean number of exits per individual were 71 ± 23 in the offshore direction, and the mean number of coastwise exits was 63 ± 31. In the GLMM model, the significance of the fixed effects suggests some possible significant difference according to direction (Table 4.4). Conversely, the Chi-squared test of comparison with the null model was not significant (χ^2=3.62, p=0.057), leaving it unclear whether any of the perceived differences were statistically significant. Mean excursion durations per individual are provided in Figure S4.6, in Supplemental information. Individuals #4(f), #7(f), #9(f) and #13(m) predominantly moved away from the monitored area towards offshore, while individuals #2(m), #3(f), #10(m) and #12(f) mostly left the area through the coastwise direction.

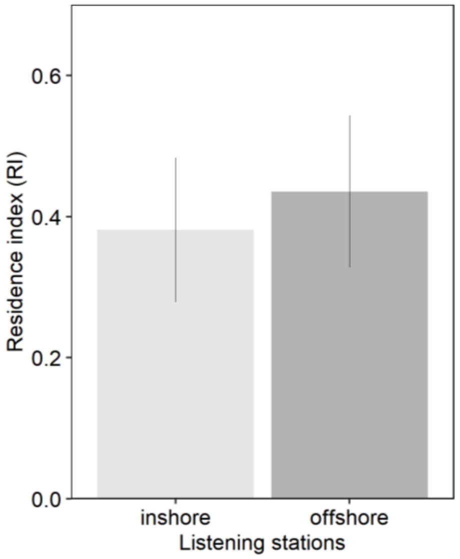

Figure 4.3. Mean residence index (RI) ± standard error estimated for *Raja undulata* according to the two listening stations categories based on distance to the coast: inshore, offshore.

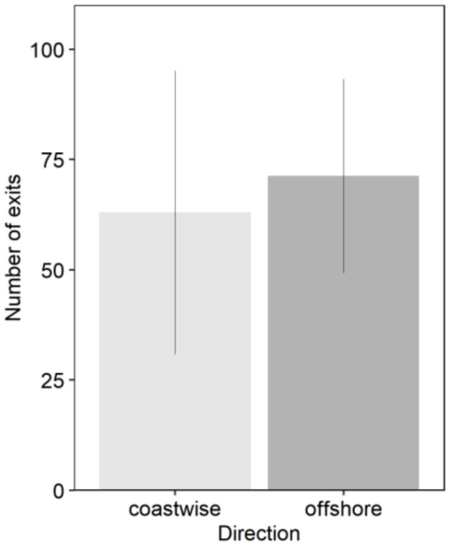

Figure 4.4. Mean number of exits from the study area ± standard error estimated for *Raja undulata*, according to directionality: coastwise, offshore. Direction was inferred from the position of the listening station that logged detections prior to absence.

Table 4.4. Outputs of mixed models to test residence index (RI) in relation to distance to the coast (Linear mixed model), and number of exits per direction (Generalized linear mixed model, family negative binomial).

	Estimate	SE	t-value	P-value
RI ~ Distance to coast				
Intercept	0.79	0.06	12.14	< 0.001 *
distance(offshore)	0.01	0.05	0.26	0.80
	Estimate	SE	z-value	P-value
N exits ~ Direction				
Intercept	3.77	0.28	13.37	< 2e-16 *
direction(offshore)	0.13	0.05	2.52	0.01 *

4.4.2. Area usage

The spatial utilization of the tagged rays was analysed using Kernel Utilization Distribution (KUD) and Minimum Convex Polygon (MCP) methods. The map depicting their projected home range (KUD95), generated by averaging the positions of all tagged individuals (Figure 4.5), indicates that they used the entire width of the monitored area, including the Partial Protection Area (PPA). Maps of utilization areas of each individual are presented in the Supplemental information (Figures S4.7 and S4.8).

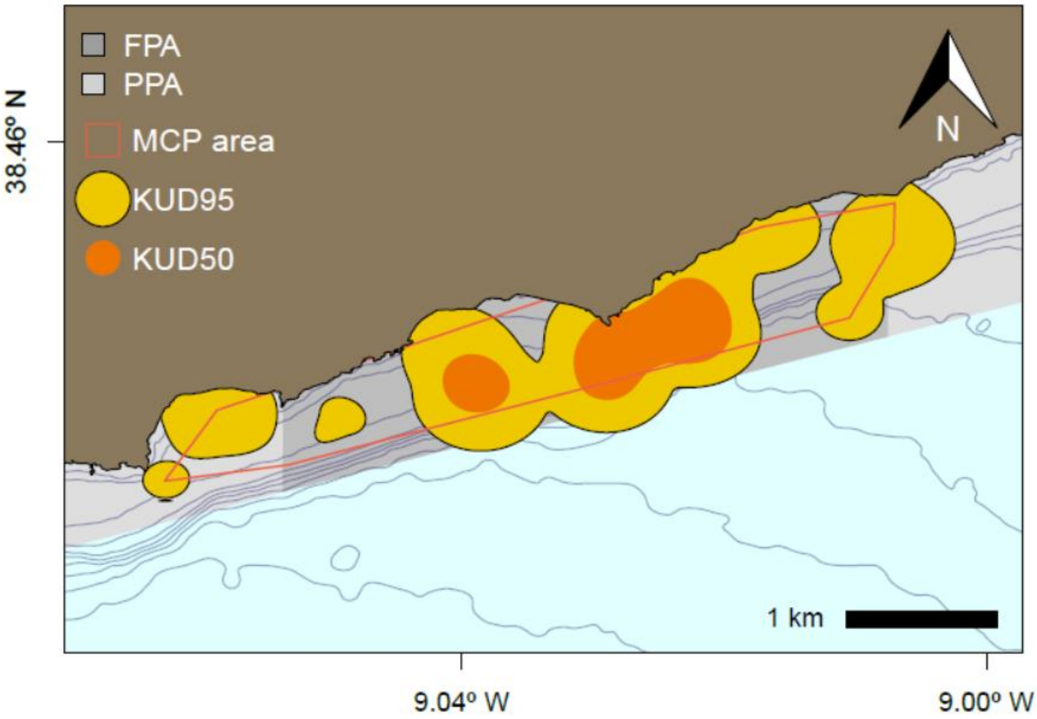

Figure 4.5. Map with home range (KUD95 - yellow), core activity area (KUD50 - orange) and Minimum Convex Polygon (MCP – red line) projected for *Raja undulata* in the study site, as determined from the positioning averaging (60 min intervals) of all individuals (n=12: 5 mature males, 6 mature females, 1 immature male; three years of data collection).

Table 4.5 displays the estimated utilization areas for each individual. The home range estimates (KUD95) varied from 0.86 km^2 to 3.63 km^2, with an average of 2.14 ± 0.25 km^2. Similar results were obtained using the Minimum Convex Polygon (MCP) method, with a mean area of 1.92 ± 0.32 km^2, with values ranging between 0.55 km^2 and 3.69 km^2. The mean core activity area was found to be 0.45 ± 0.06 km², with a range from 0.19 km^2 to 0.84 km^2.

Table 4.5. Minimum convex polygon areas (MCP) and Kernel utilization distributions (KUD; KUD50 - core activity area; KUD95 - home range, KUD100) of each tracked ray. Areas (MCP, KUD50, KUD95, KUD100) are shown in km^2.

Fish ID and sex	MCP area (km^2)	KUD50 (km^2)	KUD95 (km^2) (Home range)	KUD100 (km^2)
#2 (m)	0.55	0.19	0.86	2.85
#3 (f)	2.79	0.84	3.63	6.48
#4 (f)	0.69	0.48	1.57	3.31
#5 (m)	2.05	0.29	2.08	4.05
#6 (f)	3.69	0.51	3.19	4.10
#7 (f)	0.74	0.23	1.32	3.36
#8 (m)	1.35	0.34	1.62	3.89
#9 (f)	2.88	0.43	2.05	4.72
#10 (m)	3.05	0.74	2.77	7.15
#11 (m)	1.74	0.58	3.23	5.98
#12 (f)	0.72	0.34	1.41	2.51
#13 (m)	2.76	0.40	1.99	3.37

4.4.3. Seasonality

Some variability in the mean residency indices per season were observed, as illustrated in Figure 4.6. However, the linear mixed-effects modelling results, with individual as random factor, and sex and season as fixed effects (Table 3.7), did not confirm significant difference ($p>0.05$) in any of the indices (RI, AR, Iwr and PTIS).

The mean seasonal utilization area (Figure 4.7) was higher during winter, and this was observed in both the MCP area and home range (KUD95). However, in similarity to the residency outputs, no significance was detected in the linear mixed models ($p>0.05$). The summary of models outputs can be found in Table S4.2, available in the Supplemental information.

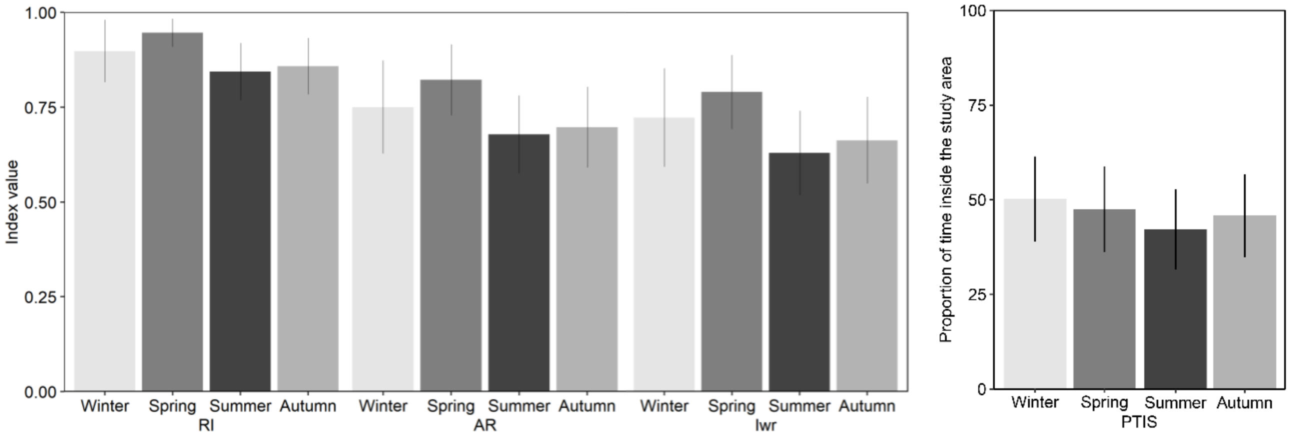

Figure 4.6. Mean residence index (RI), absolute residence index (AR), weighted residence index (Iwr), and proportion of study time inside (PTIS) ± SE per season. Values obtained with data of all mature rays (n=11).

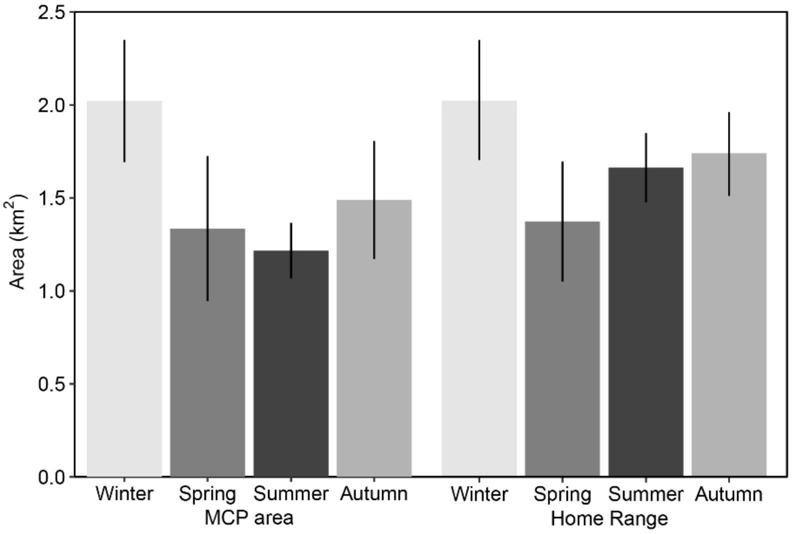

Figure 4.7. Mean MCP area and home range (KUD95) ± SE per season obtained with data of all mature rays (n=11).

4.5. Discussion

Through the data collected by passive acoustic telemetry, it was possible to obtain preliminary insights into the spatial ecology of the undulate ray in the study area. Our results offer valuable information for assessing the potential effectiveness of spatial protection measures for this species. Spatial ecology is key for species conservation, particularly for endangered coastal species like the undulate ray.

Our results show an overall variability in the spatial ecology characteristics of the monitored individuals. MCP and Kernel areas estimates exhibited some variability, and both resident and low site fidelity individuals were observed. Residency and home range variability within populations is relatively common. It has been described for this species in Northern Iberian Peninsula, in Galicia (Leeb et al. 2021), where more than half of the acoustically tracked specimens showed low site fidelity to a bay that is described as an aggregation site. Subsequent studies in that area suggest that the species might benefit from the diversity of movement strategies. Through their higher dispersal rates, individuals with wider home ranges may play an important role for population connectivity, enabling genetic transfers between sub-populations (Daban et al. 2024). However, the magnitude of dispersal of individuals with larger utilization areas is not fully understood. According to Figueiredo et al. (2020), the species tends to occur in localized preference sites, as evidenced by 75% of recaptured individuals being found within 10km of their initial tagging locations. To confirm this trend through acoustic telemetry would be relevant to understand the behaviour of individuals with higher dispersal rates. Both low and high residency behavioural traits of the population are important for the species adaptability (Daban et al. 2024), and both should be targeted by protection measures.

In relation to the species’ usage of both areas of the inshore and offshore receivers, this is in accordance with its substrate preferences, as the area primarily consists of sandy bottom. The preference of the undulate ray by sandy substrate over other soft-sediment types has already been described (Figueiredo et al. 2020). It is worth noting that the behaviour of leaving the monitored area towards offshore waters was also observed. According to Coelho et al. (2005), the species preferably occurs down to 50m deep. Investigating the species' depth preferences through fine-scale monitoring provided by acoustic telemetry would enhance our understanding of the depth limits that the species primarily uses.

It is worth noting that in comparison with other rays in the area (see Chapter III, *Raja clavata*), the species seems to undertake less dispersions to offshore waters, and globally less excursions to outside the area (mean number of exits per individual). These results, combined with the fact that it is likely that both males and females of the species are recurrent users of this area, lead us to hypothesize that the PLSMP may adequately provide protection benefits to this species. It is also likely that the area included by the FPA and adjacent PPAs combined (total 11.1 km^2) is large enough to protect a portion of the population (species mean home range estimated: 2.14 km^2). However, the apparent low density of the species in the area (Sousa et al. 2018) raises some concerns and should be further investigated.

Some trends related with season were observed, with larger utilization areas during winter. However, the linear mixed-effects models with season were found to be not significant. In Galicia, Leeb at al. (2021) described a marked seasonality in the presence of individuals in a relatively small bay in the Cíes MPA. They described higher number of individuals during late spring and summer. Moreover, the species is known to be a seasonal breeder (Moura et al. 2007, Coelho & Erzini 2006, Serra-Pereira et al. 2015). Having appropriate samples sizes is important for testing factors in circumstances with strong random effects (individual variability in this case). To appropriately address seasonality in our study area, further spatial ecology research needs to be undertaken.

Our results suggest that relatively small coastal MPAs may provide some conservation benefits to this species. However, the variability of behaviour (some individuals with high residence, others that frequently left the study area) takes us to consider that adequate protection would imply the protection of wider areas and/or a network of MPAs. The recovery of healthy populations regarding abundance and genetics would be favoured by MPAs enclosing extended sections of coastal habitats.

Further recommendations for future studies would be to use a larger number of acoustic receivers in order to monitor a larger area, as well as to tag more individuals. The questions on its maximum depth preference, and also the spatial behaviour of juveniles are particularly relevant. Furthermore, it would be interesting to use underwater video techniques, such as baited underwater video and submersible remote-controlled vehicles (ROV, AUV) to access the density of the species and eventually identify potential aggregation sites (Leeb et al. 2021). This would enhance our understanding of its habitat use, adding to the previously collected knowledge (e.g., Figueiredo et al. 2020) on this endangered species.

4.6. References

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4.7. Supplemental information

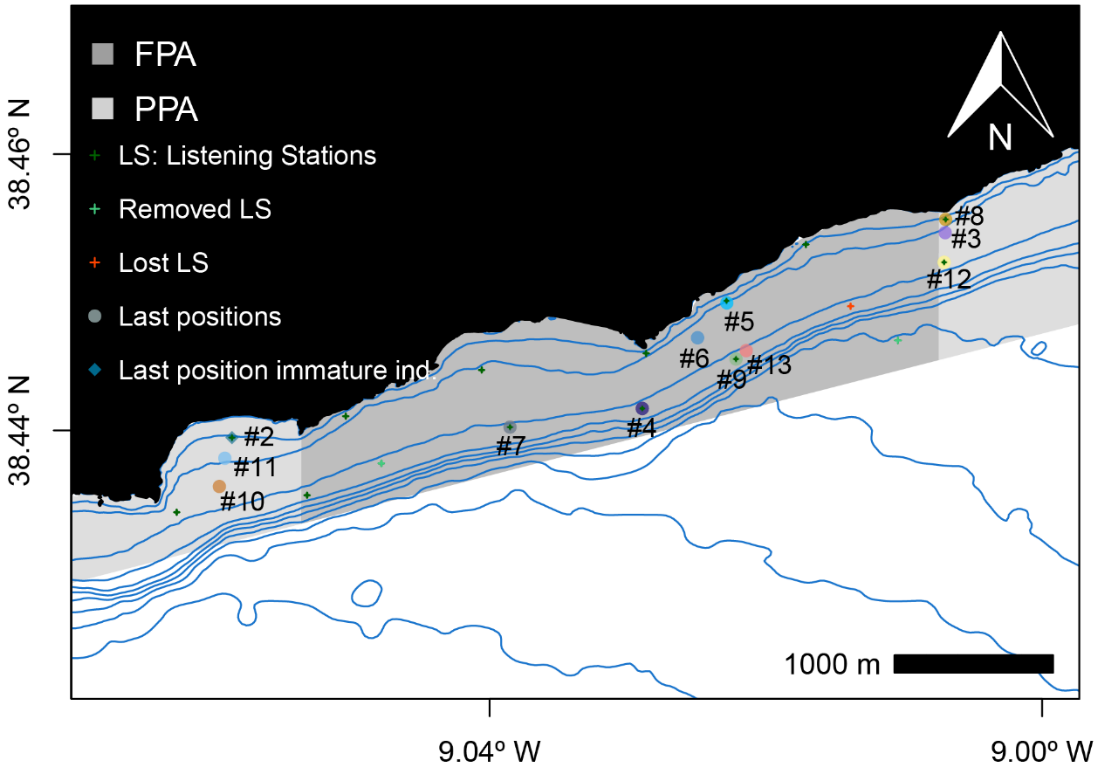

Figure S4.1. Locations of last positioning averaging according to last detections (60min) of the eleven thornback rays (*Raja undulata*; 5 mature males, 6 mature females, 1 immature male - #2) tracked with passive acoustic telemetry. Only Ind. #6, #7 and #9 were still being detected at the time of tag battery expiration. Removed listening stations (light green crosses) monitored during eight (left) and eleven months (right). Lost listening station (red cross) only monitored during 21 days.

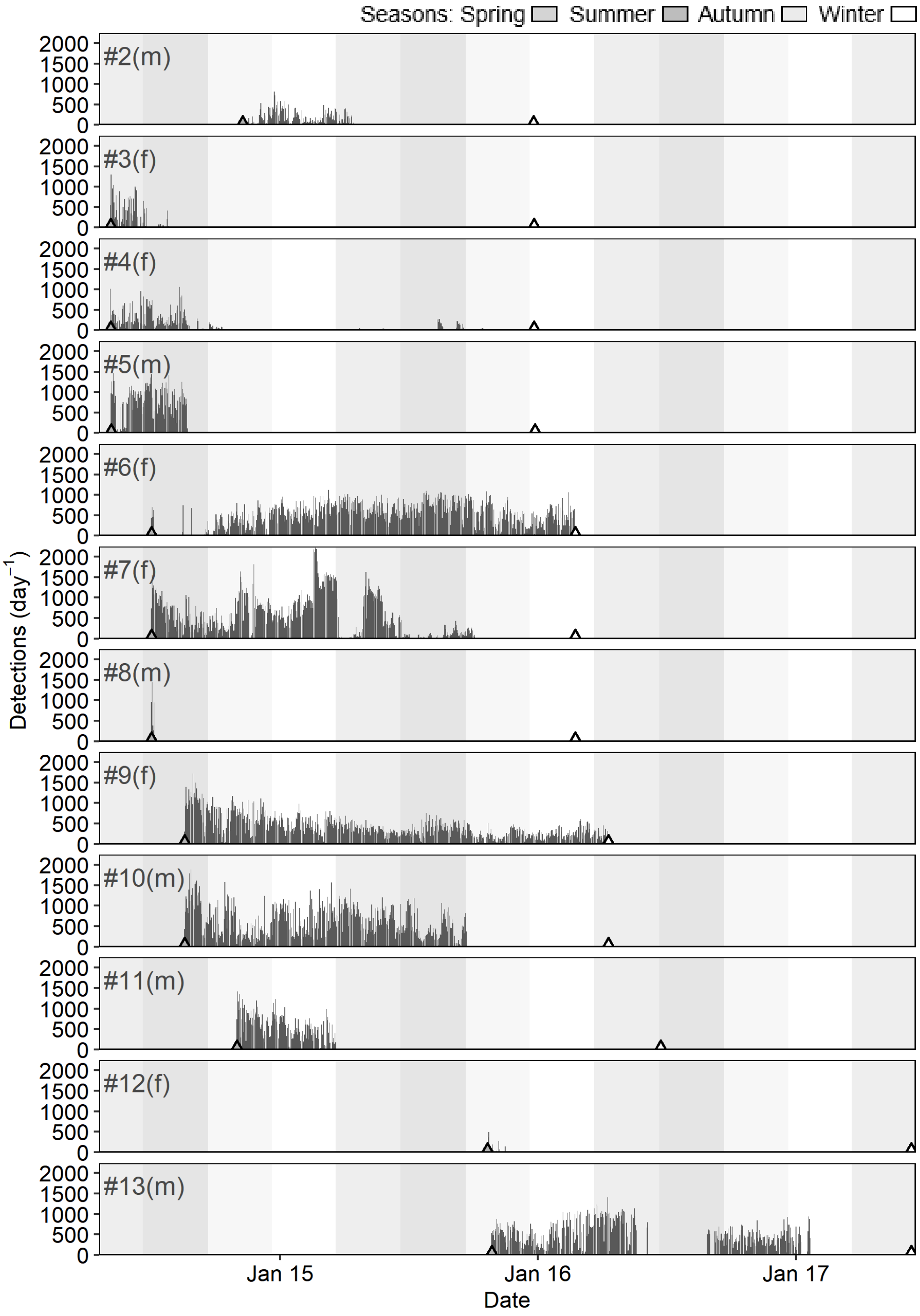

Figure S4.2. Timelines of daily detections of tagged rays (*Raja undulata*) (▲: study start; △: study end). Vertical stripes with different grey shadings indicate seasons. (m): male; (f): female. Ind. #2: immature male.

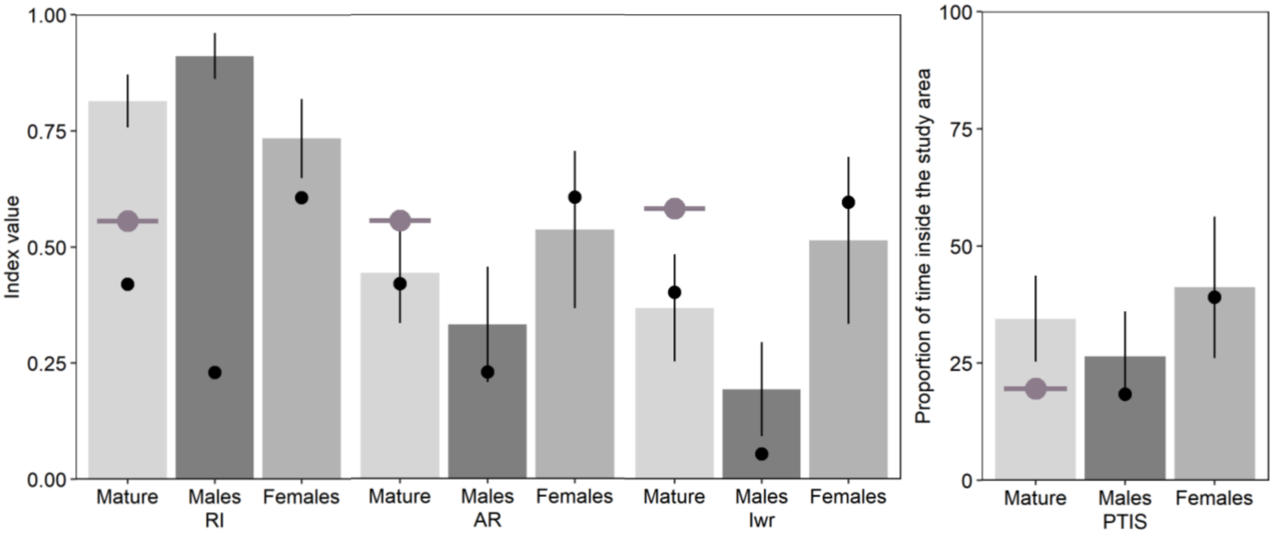

Figure S4.3. Mean residence index (RI), absolute residence index (AR), weighted residence index (Iwr), and proportion of study time inside (PTIS) ± SE per group: all mature individuals (n=11), mature males (n=5) and mature females (n=6). Values of the immature male (Ind. #2) are shown in grey (–●–).

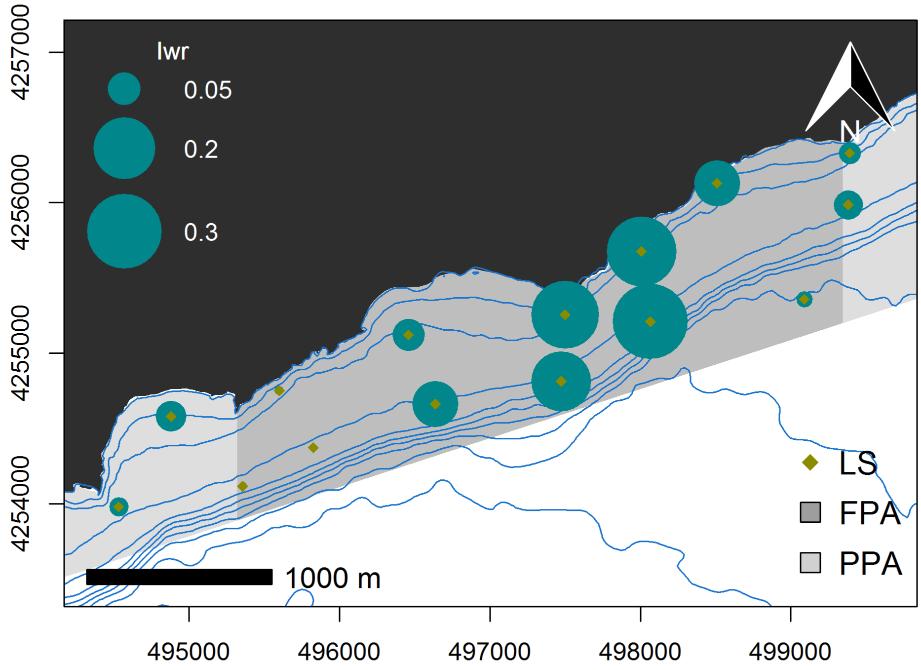

Figure S4.4. Spatial variation of weighted residence index (Iwr). The relative size of circles indicates the average Iwr at a given listening station (LS) obtained from the values of all tagged rays (*Raja undulata*, N=12).

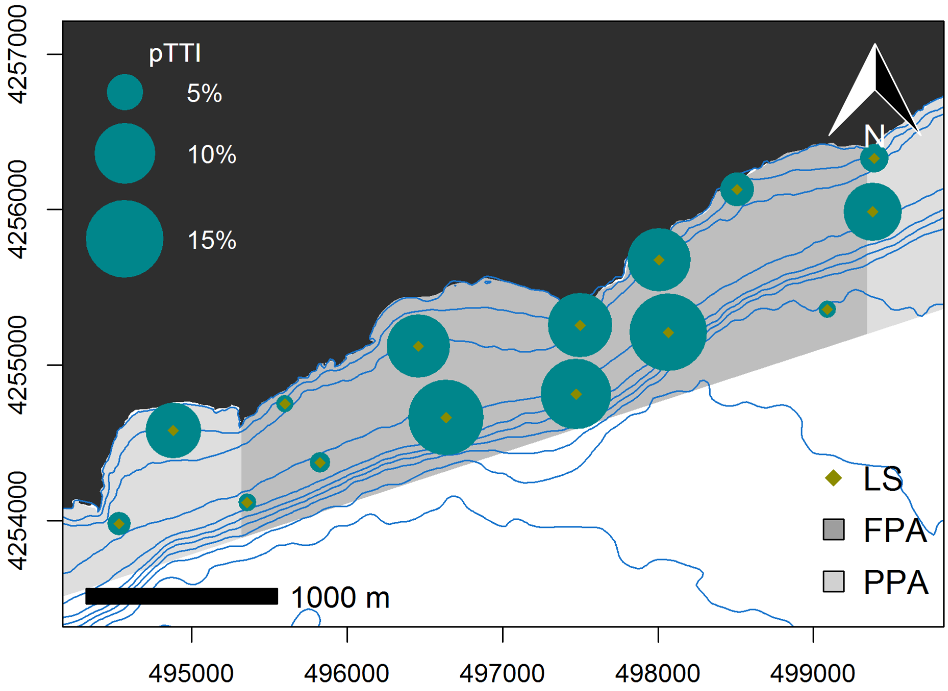

Figure S4.5. Spatial variation of proportion of total time inside de study area (pTTI), relative to the total study time (tagging to expired battery / reported catch / station removal). The relative size of circles indicates the total pTTI at a given receiver obtained from the values of all tagged rays (*Raja undulata*, N=12).

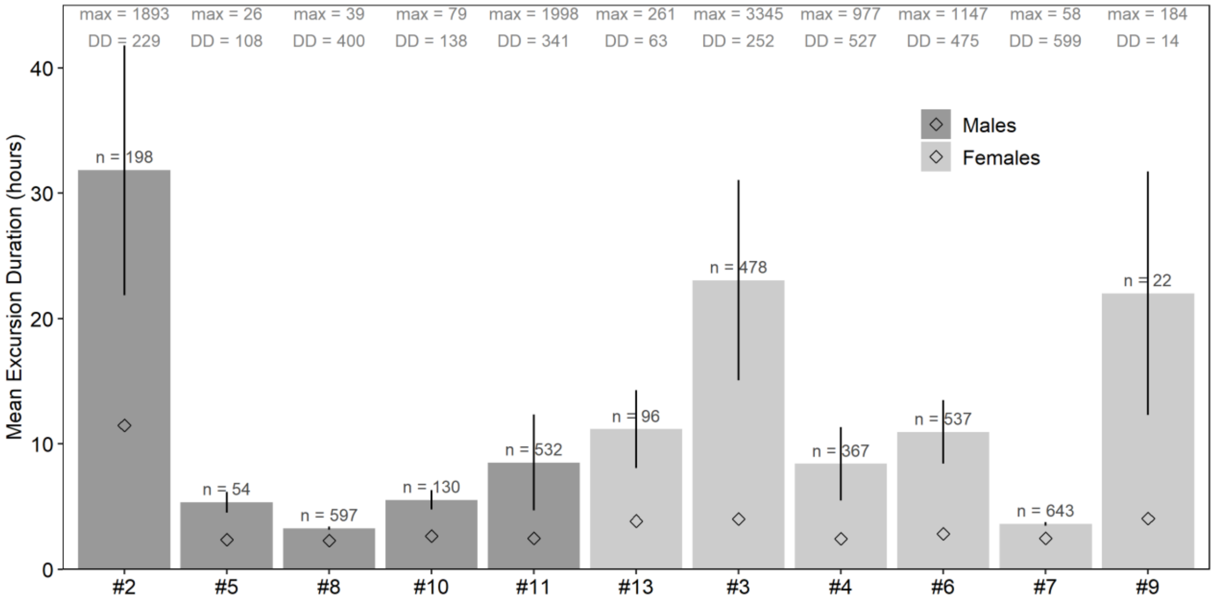

Figure S4.6. Mean excursions duration (hours) ± SE of each tagged ray (max - maximum excursion duration; DD - days with detections). Median values are marked by '◊'.

Table S4.1. Number and proportion (N%) of exits from the study area at each direction category (according to location of receiver with last and first detection: LS1, LS8, LS15 - coastwise) of each tagged ray (*Raja undulata*).

Exits directionality					
	Offshore		Coastwise		Coastwise exits/enters
Fish ID	N	N %	N	N %	N per direction
#2 (m)	3	4.5	64	95.5	east: 64
#3 (f)	7	11.3	55	88.7	east: 55
#4 (f)	192	100.0	0	0.0	
#5 (m)	16	88.9	2	11.1	east: 2
#6 (f)	57	44.5	71	55.5	east: 66, west: 5
#7 (f)	185	100.0	0	0.0	
#9 (f)	135	75.0	45	25.0	east: 38, west: 7
#10 (m)	48	11.3	375	88.7	east: 375
#11 (m)	22	44.0	28	56.0	west: 28
#12 (f)	3	14.3	18	85.7	east: 18
#13 (m)	116	76.8	35	23.2	east: 29, west: 6

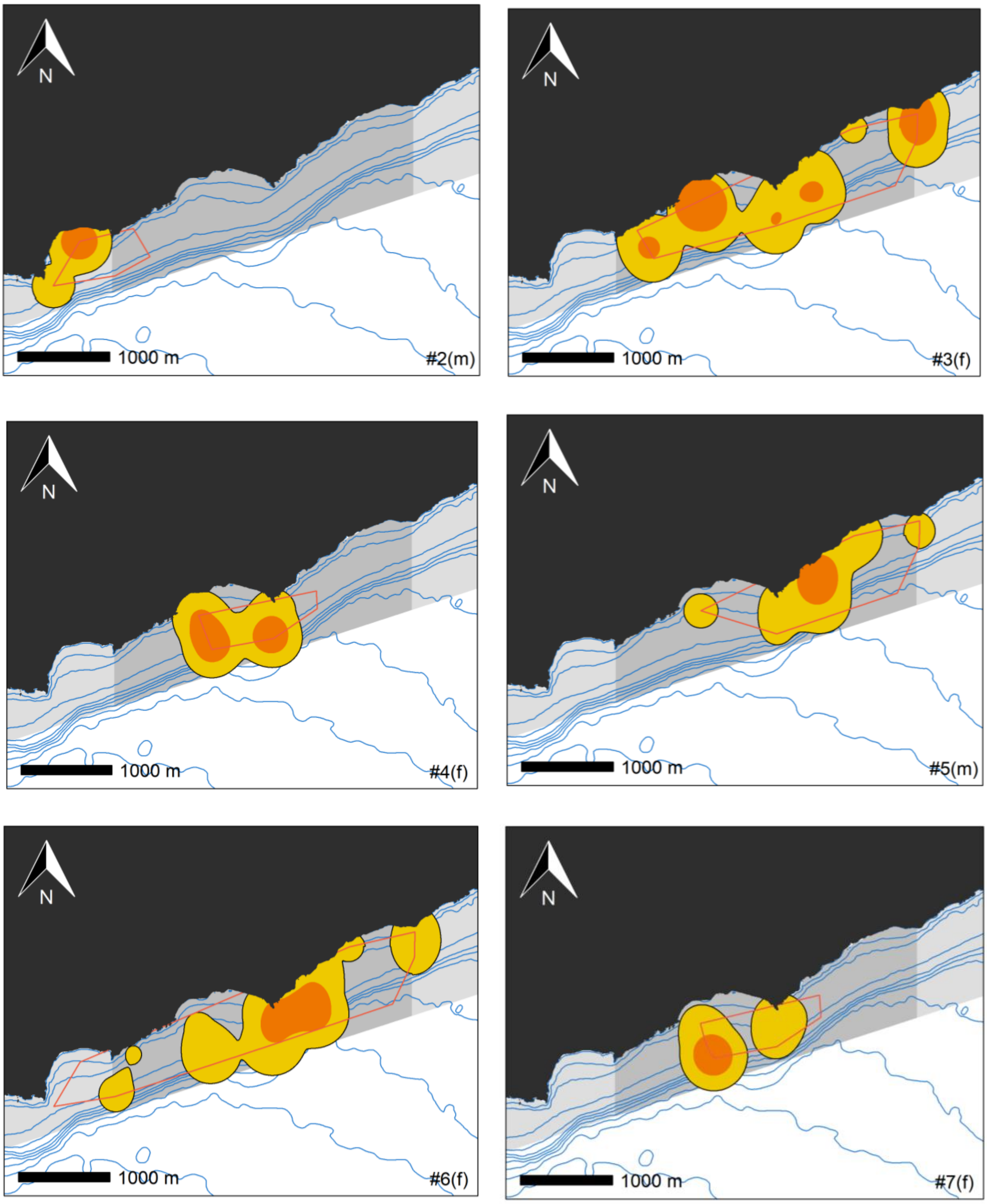

Figure S4.7. Maps of home range (KUD95, yellow) and core activity area (KUD50, orange) modelled for each tagged individual of *Raja undulata* in the study site (positioning averaging interval: 60 min). Minimum convex polygon is also shown (red line). (m): male; (f): female; Ind. #7: immature male.

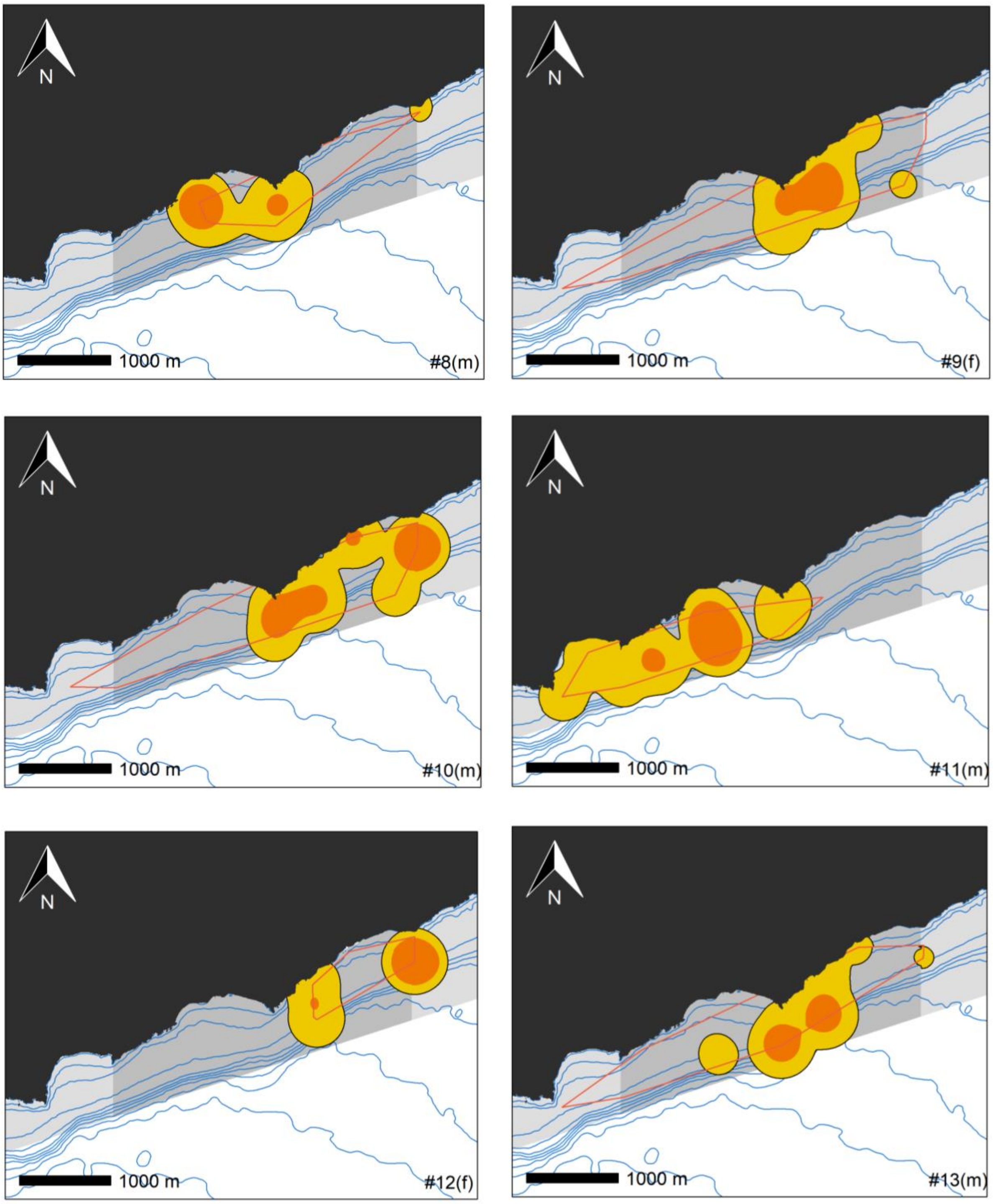

Figure S4.8. Maps of home range (KUD95, yellow) and core activity area (KUD50, orange) modelled for each tagged individual of *Raja undulata* in the study site (positioning averaging interval: 60 min). Minimum convex polygon is also shown (red line). (m): male; (f): female.

Table S4.2. Estimate ± SE and t-values obtained with sex and season as fixed effects in linear mixed models for the following variables: MCP area, home range (KUD95), absolute residence index (AR), weighted residence index (Iwr) and proportion of study time inside the study area (PTIS). Fish ID was used as a random factor.

			Estimate	SE	t-value
Response:	MCP area	Intercept	1.84	0.46	4.00
Parameter:	sex	sex(male)	0.22	0.68	0.32
Response:	Home Range	Intercept	0.73	0.13	5.51
Parameter:	sex	sex(male)	0.15	0.20	0.77
Response:	MCP area	Intercept	1.46	0.25	5.78
Parameter:	season	season(spring)	-0.19	0.36	-0.53
		season(summer)	-0.33	0.31	-1.07
		season(winter)	0.42	0.34	1.23
Response:	Home Range	Intercept	0.62	0.09	6.62
Parameter:	season	season(spring)	-0.15	0.11	-1.45
		season(summer)	-0.03	0.10	-0.28
		season(winter)	0.06	0.10	0.60
Response:	AR	Intercept	0.54	0.17	3.14
Parameter:	sex	sex(male)	0.02	0.25	0.07
Response:	Iwr	Intercept	0.51	0.18	2.85
Parameter:	sex	sex(male)	-0.06	0.27	-0.22
Response:	AR	Intercept	0.57	0.11	5.26
Parameter:	season	season(spring)	0.21	0.13	1.55
		season(summer)	0.13	0.13	1.02
		season(winter)	0.09	0.13	0.67
Response:	Iwr	Intercept	0.54	0.11	4.75
Parameter:	season	season(spring)	0.20	0.14	1.46
		season(summer)	0.13	0.13	1.00
		season(winter)	0.10	0.14	0.78
Response:	PTIS	Intercept	37.51	14.36	2.61
Parameter:	sex	sex(male)	5.89	21.30	0.28
Response:	PTIS	Intercept	38.18	10.40	3.67
Parameter:	season	season(spring)	5.85	4.87	1.20
		season(summer)	5.11	5.22	0.98
		season(winter)	1.35	4.93	0.28

CHAPTER V

Preliminary insights into the spatial ecology and movement patterns of a regionally critically endangered skate (*Rostroraja alba*) associated with a marine protected area

Published in Marine and Freshwater Behaviour and Physiology

Sousa, I., Baeyaert, J., Gonçalves, J.M.S., Erzini, K. 2019. Preliminary insights into the spatial ecology and movement patterns of a regionally critically endangered skate (*Rostroraja alba*) associated with a marine protected area. Marine and Freshwater Behaviour and Physiology, 52:6, 283-299

5. Preliminary insights into the spatial ecology and movement patterns of a regionally critically endangered skate (*Rostroraja alba*) associated with a marine protected area

5.1. Abstract

The implementation of spatial protection measures is currently a priority in batoid species' conservation strategies, but their spatial ecology remains largely unknown. We provide preliminary insights into the movement patterns of the white skate (*Rostroraja alba*), a batoid classified as Critically Endangered in European waters. Three individuals (two females: one mature, one immature; and one immature male) were monitored with acoustic telemetry in a marine protected area (Portugal). The mature female remained present in the study area throughout the whole monitoring period (20 months). Residency analyses revealed this specimen spent more than 70% of the time within this coastal marine park. The immature female and the immature male were only detected during three and four months, respectively. Whether the uncovered movement patterns are common within the population remains unclear, but the present study provides useful information to better plan future research on the movement ecology of a rather unstudied species.

Keywords: Rajidae, elasmobranchs, acoustic telemetry, fine-scale movement patterns, individual behaviour, MPA

5.2. Background

Over the last decades, skate populations (family Rajidae) have undergone significant worldwide decline (Dulvy et al. 2000, Stevens et al. 2000). Although the extent of population declines is still subject to debates, overexploitation, habitat alteration and other anthropogenic impacts have been identified as the main causes for critical losses, leading to many species being now of conservation concern (Siskey et al. 2019). Albeit generally not directly targeted, skates have a long history of exploitation and by-catch, globally associated with high discard mortality rates (Enever et al. 2009, Mandelman et al. 2013, Siskey et al. 2019). In spite of such circumstances, skates have received limited research attention and the status of populations remain largely unknown in most areas of the world (Dulvy & Forrest 2010, Siskey et al. 2019). The International Union for Conservation of Nature reports that presently, about 14% of skate species are Threatened (Critically Endangered, Endangered or Vulnerable), 8.5% are considered Near Threatened, and 29.2% are of Least Concern. Remarkably, about 48% of existing Rajidae species are either Not Evaluated or listed as Data Deficient (IUCN 2018, Siskey et al. 2019). This illustrates the lack of essential knowledge regarding this group, which is likely a barrier to efficient conservation and protection of skates, especially for those affected by fisheries (Dulvy et al. 2000, Siskey et al. 2019). This is the case for the white skate (*Rostroraja alba*, Lacepède 1803), a species belonging to the Rajidae family, currently registered by the IUCN Red List as Critically Endangered in the Northeast Atlantic and globally Endangered (IUCN 2018).

The white skate is a large benthic species with an original distribution that is thought to extend throughout the Eastern Atlantic (from the British Isles to South Africa), the Mediterranean and the southwestern Indian Ocean (Compagno et al. 1989; Ebert et al. 2007). The species occurs at depths ranging from 30m to 600m (Brito 1991; Sanches 1991), and it may also be found in shallow bays (Compagno & Ebert 2007). Like most chondrichthyans, the white skate is a K-selected reproducer, characterized by a late sexual maturity, low fecundity and overall slow life cycle (Ebert et al. 2007, Ellis et al. 2008). These distinctive life-history traits leave skates particularly vulnerable to anthropogenic pressure. Additionally, the large size of this batoid, up to 2.3m long (Stehmann 1995), along with its occurrence on the continental shelf and slope, make it highly susceptible to capture by fishing gear (Ellis et al. 2008). As a result of being an important bycatch of demersal fisheries in the Northeast Atlantic and the Mediterranean, and despite a conspicuous absence of scientific data, the species is believed to have undergone severe declines in recent decades and is now considered rare in most of its geographic range (Rogers & Ellis 2000, Dulvy et al. 2006). It is reported to have declined severely in the Brittany coast (ICES 2006), the British Isles (Rogers & Ellis 2000), and according to Dulvy et al. (2000), it has disappeared from the Irish Sea. There is evidence that related large bodied species, the common skate (*Dipturus batis*, now considered as two different species: *Dipturus intermedius* - *Dipturus batis* complex) and the barndoor skate (*Dipturus laevis*), have been driven to near-extinction in most of their geographic range (Brander 1981, Casey & Myers 1998).

In Portugal, while there have been some efforts to identify the skates and rays caught in commercial fisheries landings, the majority of records do not provide identification down to species level (Machado et al. 2004, Correia et al. 2016). Consequently, information on the distribution and abundance of skates and rays in Portugal, including the white skate, is largely lacking. However, from the intermittent data that have been obtained on the white skate, it was documented that the species occurs on the western coast of the country (Machado et al. 2004, Sousa et al. 2018). However, very little is known about its ecology or movement patterns.

Quigley (1984) examined catches from recreational fisheries in the western coast of Ireland and reported juvenile white skates to be common in coastal areas, mostly between October and April. Additionally, his results suggested seasonal movements of adults, inshore from spring to autumn and away from the coast during colder months, a pattern possibly related to the reproductive cycle.

Movement patterns have been documented in other skates, such as the thornback ray (*Raja clavata*). Based on depth data collected by electronic tags, Hunter et al. (2005) found that the southern North Sea population of thornback ray undertakes a seasonal migratory cycle with range contraction during spring. In western Scotland et al. (2009) found evidence that the common skate associates with specific sites, resulting in short-range horizontal movements. The results also revealed behavioural plasticity in the population, characterized by individual switching from high to low rates of vertical activity (Wearmouth & Sims 2009). Neat et al. (2015) further investigated the *Dipturus* genus, by using conventional mark-recapture and acoustic tagging to study the flapper skate (*D. intermedius*). These authors describe the South Western Scotland population of this species as a combination of site-attached and transient individuals.

Considering the Critically Endangered status of the white skate, it is essential to address knowledge gaps with regard to their ecology and movement patterns. It has been proposed that where such essential data are lacking, data from limited sample sizes are better than no data at all, and may provide valuable, albeit preliminary, insights into the ecology of species (Baeyaert et al. 2018). Furthermore, any information that is relevant, even if collected opportunistically or from small sample sizes may at the very least be useful for developing experimental designs for future studies. By addressing individual behavioural patterns, the outcomes contribute to the pool of individual variability data and to the formulation of questions regarding populational trends.

This investigation presents acoustic monitoring of three individuals of white skate, a regionally Critically Endangered species for which no movement ecology study has been conducted yet. Species movement patterns and spatial utilisation are important considerations for adequate design of MPAs or for monitoring the effectiveness of their protection. The tagging of the three white skate individuals was part of a monitoring program inside an MPA in Portugal, which aimed to investigate site fidelity, home range, diel and seasonal behaviour, and ultimately assess the suitability of small MPAs as tools to protect skate species. A decision to fit the three individuals with acoustic transmitters after they had been caught unexpectedly, was based on the poor conservation status of the species and the lack of knowledge on their movements. We aimed to provide preliminary data on whether these three animals remain in the marine reserve (i.e., residency), and if so, how they may be using the area, in relation to spatial and temporal patterns. Further, we planned to use the revealed individual behavioural patterns to frame some future research questions addressing the white skate population trends, and further contribute to the development of an adequate experimental design focusing on the effectiveness of the MPA for skate species protection.

5.3. Acoustic monitoring and data analyses

The present study was conducted in a coastal MPA in the North-eastern Atlantic: the Prof. Luiz Saldanha Marine Park (PLSMP, Portugal) (Figure 5.1). Comprising 53km^2 and extending to a maximum depth of 100m, this marine park covers a narrow stretch of rocky bottom, replaced by soft sediments at depths greater than 15m – 20m (Figure 5.1A & B).The marine park includes three protection levels: a fully protected area (FPA, 4.3km^2), four partially protected areas (PPA, encompassing 21km^2), and three buffer areas (BA, totalling

28km^2) (Figure 5.1A). The FPA is a no-take and no-access zone, thereby excluding all human activities, while the PPAs allow commercial fishing with traps and jigs only. Traditional small-scale fisheries (i.e. longlines, trammel nets and gill nets) and recreational fishing are authorized in the three BAs.

The passive acoustic monitoring that was undertaken relied on the experimental design for ongoing studies on other benthic species that were being conducted in the area. In May 2014, an original array of 15 underwater VEMCO acoustic receivers was deployed in the marine park (Vemco Ltd. Halifax, Nova Scotia, VR2W, 69kHz). Eleven receivers were deployed in the FPA, and four other receivers were placed in the two adjacent PPA areas (Figure 5.1B), ensuring acoustic coverage of the FPA and of the borders with adjacent PPAs (total of 15 listening stations - LS). The inner line was deployed at depths ranging from 7 m to 15 m and the outer line at depths between 17 and 25m deep (Figure 5.1B). The design took into account range tests, which showed a maximum detection range of 1330m. At this distance, less than 1% of transmissions were detected. Distances that enabled 50%, 85% and 100% of detections were 475m, 400m and 350m, respectively. Inferring daily behavioural patterns from acoustic telemetry should be performed carefully (Payne et al. 2010), and to rule out environmental effects in the number of detections, three sentinel tags were moored within the receiver array area (positioned in eastern, central and western points). The monitoring period took place between October 2015 and June 2017. Two receivers were lost before the first data download in January 2016, likely due to illegal fishing (Figure 5.1B).

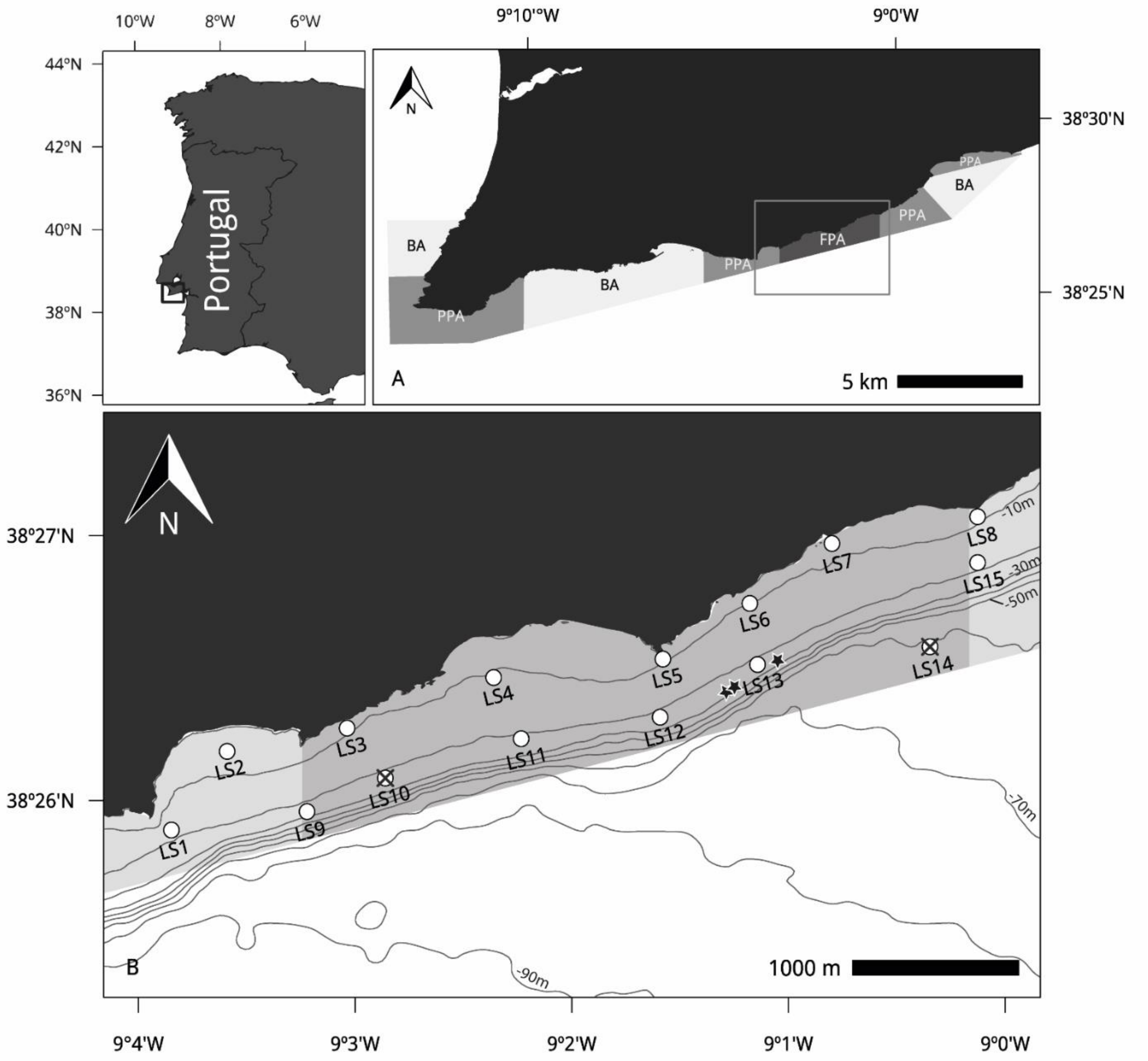

Figure 5.1. Map of the study area, the Prof. Luiz Saldanha Marine Park in Portugal (A), identifying the three protection levels (BA – buffer areas; PPA – partially protected areas; FPA – fully protected area). Initial location of acoustic receivers (listening stations – LS; plain white circles) and the two lost receivers (crossed white circles) are exhibited in panel B (bathymetric lines: 10m intervals). Black stars correspond to the capture/release sites of the three individuals.

Three white skate individuals, a male and two females, were caught inside the marine park in October 2015 using longline (three days fishing expedition; soaking time 3-12h; monofilament mainline and leaders; hooks size 3/0; capture events approved by ICNF - Instituto de Conservação da Natureza e Florestas). Upon capture, animals were brought onboard, sex and maturity level (according to size and claspers calcification in males) were determined, and morphometric measurements were taken (total length, precaudal length, disc width) (Table 5.1). Each individual was then fitted with an acoustic transmitter (Vemco

Ltd. Halifax, Nova Scotia, V13-1L, 36 x 13mm, weight in water 6g) inserted through a small incision in the peritoneal cavity, which was then closed with absorbable suture and surgical glue. Individuals were kept in a state of tonic immobility, inside a tub filled with seawater, during the tagging procedure. The tags were programmed to emit a random coded signal every 40 to 80 seconds at 69kHz (expected battery life 600 days). After ensuring post-surgery recovery (active response to touch and swimming ability), individuals were released at the capture location (Figure 5.1; Table 5.1).

In order to investigate temporal patterns, detections were refined by diel phases and seasons. Diel phases (i.e. day and night) were determined from the local times for sunset and sunrise, as estimated using the study area coordinates and the algorithms provided by the National Oceanic and Atmospheric Administration (NOAA, https://www.esrl.noaa.gov/). Seasons were defined based on the dates of solstices (i.e. summer and winter) and equinoxes (i.e. spring and autumn), available from the U.S. Naval Observatory Astronomical Applications Department (https://aa.usno.navy.mil).

Filtered data were preliminarily investigated through chronogram plots, which provides a visually interpretable timeline of the skate's occurrence within the marine park across the monitoring period (tagging date to transmitter expected battery life). Then, to quantify site fidelity of each individual inside the FPA, absolute (A_{Ri}) and relative (R_{Ri}) residency indexes were estimated (Baeyaert et al. 2018). The former index was based on the ratio of the total number of days the individual was detected by the array of receivers to the total number of days of monitoring, while the latter was obtained by dividing the number of days an animal was detected by the number of days between first and last detection. The indexes can take on values from 0, which indicates no residency, to 1, which suggests a full-time residency within the monitored area. Similarly, to better depict the temporal fluctuations, seasonal site fidelity was examined by estimating a third residency index (S_{Ri}) based on the ratio of the total number of days the individual was detected per season to the total number of days of monitoring per season (Baeyaert et al. 2018).

Spatial use was analysed through the estimation of centers of activity (COAs) for each individual (Simpfendorfer et al. 2002). This mean-position algorithm uses data from multiple receivers, performing a triangulation of the detections, and converting them to position estimates (i.e. relocation) based on weighed means of the number of detections at each receiver over a determined period of time – Δt. Using the arithmetic approach, a range of values for Δt was first tested and the appropriate time interval for COA computation for the white skate was determined to be 60 minutes. This decision resulted from a close inspection of the data, revealing limited changes in position with greater intervals. For each animal, a bivariate kernel utilization distribution (KUD) (Worton 1989) was applied to the relocations using the R package “adehabitatHR” (Calenge 2006). This probability density function assumes an individual uses a certain area over a given period of time. It is therefore acknowledged that the home range comprises 95 % of the positions of an individual (KUD 95%) while the core area encompasses 50% of them (KUD 50%) (Worton 1989; Seaman & Powell 1996). All distributions were estimated using a fixed kernel bandwidth (smoothing factor of 195), obtained from a rule-based approach as described in Gandra et al. (2018). For each individual, core activity areas (KUD 50%) and home range (KUD 95%) sizes were computed for the overall monitoring period as well as across seasons. As a proxy to understand the level of protection that the marine park provides to these individuals, total minimum convex polygons (MCPs) were estimated using the Δt 60-min COAs to quantify the extent of spatial use inside the FPA. Following the approach described in Knip et al. (2012), the MCP area was divided by the total FPA area ($4.3 km^2$) to determine the proportion of protected area space used by each individual (Knip et al. 2012).

Differences in detection frequency across daily phases were first inspected through boxplots. Then, for each individual, the number of hourly detections per phase was plotted for each season and for the overall monitoring period. Similarly, the proportions of hourly detections over a 24-h period were plotted. Diel activity patterns were further examined by individually pooling the total number of detections into diel phases and a weighted index was obtained based on the ratio of the latter value to the phase duration in minutes. This index, corresponding to the number of detections per minute per diel phase, was used as a mean to weight each phase duration and prevent over/under-estimation of the number of detections. Data were inspected through Q-plots and further tested for normality using the Shaphiro-Wilk test prior performance of analyses. Data were not normally distributed and could not be transformed accordingly, therefore differences between detection patterns per diel phases were statistically assessed through non-parametric Wilcoxon signed-rank tests for each individual.

To provide more insight into the temporal dimension of the movement patterns of the tagged skates, distances travelled were estimated for each 24-h period as a proxy for activity by dividing the sum of the linear distances between consecutive COAs by the duration of each diel phase. Cumulative distances covered per diel phase were then plotted across all seasons. To further examine diel activity patterns, hourly proportion of distances covered were mapped across 24-h. Overall intra-individual differences in movement patterns across diel phases and seasons were further investigated. As the data were not normally distributed, non-parametric statistical tests were used. Differences in activity patterns across diel phases were individually tested with Wilcoxon signed rank test, while intra-individual seasonal variations were examined through the Kruskal-Wallis rank sum test.

5.4. Movements and spatial ecology

Between October 2015 and June 2017, the array of receivers logged a total of 82 994 detections. Detections from two individuals, #2F and #3M, ceased to be recorded inside the monitored area in January and February 2016, respectively. Individual #1F remained for the entire study (600 days) and was detected on 440 days (Table 5.1; Figure 5.2). Overall, 73 826 transmissions of this individual were logged by 12 receivers, accounting for 89% of the total number of detections (Figure 5.2A; Table 5.1 & 5.2).

According to the available literature regarding female white skates in the Mediterranean region, individual #1F was determined to be mature, while individual #2F was considered to be immature (Capapé 1976; Bradai et al. 2012) (Table 5.1). Claspers calcification along with the examination of sizes at maturity (i.e. disc width and total length; Table 5.1) suggested that the male #3M was about to reach maturity at the time of tagging (Capapé 1976; Bradai et al. 2012).

Table 5.1. Summary data for the three white skates (*Rostroraja alba*) fitted with acoustic transmitters in the Prof. Luiz Saldanha Marine Park: TL – total length; DW – disc width; Dd – number of days with detections; Di – Total number of monitoring days (i.e. days between 1st and last detection); Total MCP - total minimum convex polygon; % FPA Use - percentage of fully protected area (FPA) used based on Knip et al. 2012.

Fish ID	**Sex**	**Maturity**	**TL** (cm)	**DW** (cm)	**Tagging Date**	**Tagging Location**	**Last detection**
#1F	Female	Mature	138	102	22/10/2015	LS13	13/06/2017
#2F	Female	Immature	95.3	72	22/10/2015	LS13	16/01/2016
#3M	Male	Immature	112	82	22/10/2015	LS13	13/02/2016

	Nº COAs	**Dd**	**Di**	**Total MCP** (km^2)	**% FPA Use**
#1F	7 108	440	600	2.83	66
#2F	190	18	86	1.12	26
#3M	588	62	114	2.46	57

Residency results revealed individual variability, i.e., absolute and relative residency indexes varied amongst individuals, displaying three patterns, from low to high site fidelity (Table 5.2). The detection frequency of the immature female showed occasional visits of this individual inside the monitored area, with most detections being concentrated over two months, October 2015 (58%) and January 2016 (28%) (Figure 5.2). The brief presence of this female inside the marine park was interspersed with long periods of absence, resulting in an extremely low degree of residency (R_{Ri} = 0.19; A_{Ri} = 0.02), while the male (#3M) frequented the marine park for half of its detection period (62 days) (Table 5.2). The latter exhibited moderate fidelity according to its relative residency index (R_{Ri} = 0.50; A_{Ri} = 0.1), contrasted by a high site attachment at one particular location, "LS15". This receiver, deployed at the boundary between the FPA and PPA, was the eastern-most receiver in the array, and accounted for 80% of the detections of the animal (Table 5.2; Figure 5.1 & 5.2). Seasonal residencies showed similar tendencies for both animals (Table 5.2). The mature female (#1F), however, presented the highest residency indexes, above 0.70 (0.73 for both R_{Ri} and A_{Ri}, Table 5.2), implying it spent more than 70% of its detection period within the marine park.

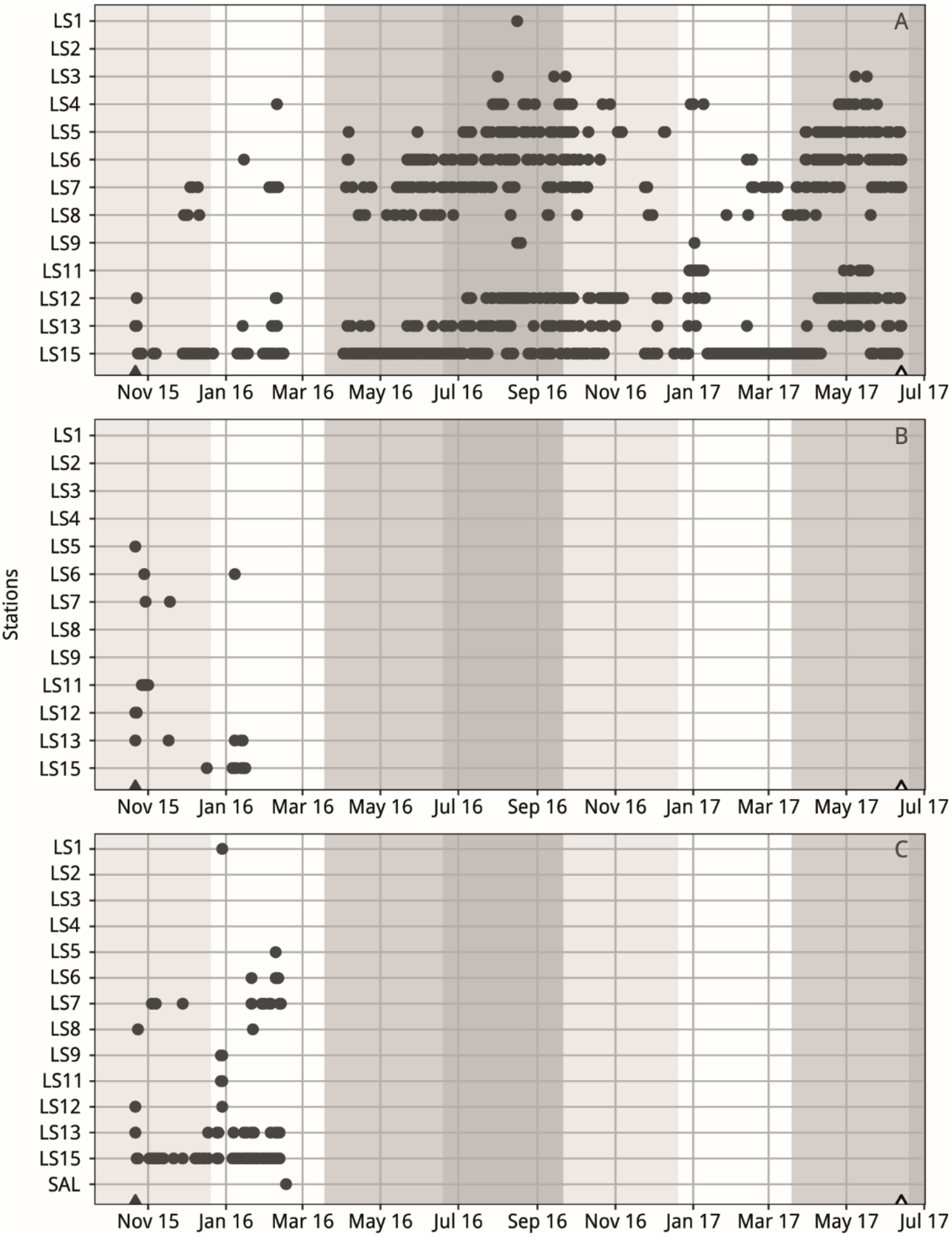

Figure 5.2. Calendar plots with detection patterns of the three white skates (*Rostroraja alba*) fitted with acoustic transmitters: detections through time and per listening station (LS). SAL: St. André Lagoon, station 43 km to the south. Shaded areas illustrate the seasons: Winter (white), Spring (grey), Summer (dark grey), Autumn (light grey). A - #1F, mature female; B – #2F, immature female; C - #3M, immature male; ▲ - tagging; △ - estimated battery life expiration of the transmitter).

In terms of spatial use, although the immature female (#2F) registered 190 COAs, due to a limited number of distinct relocations (i.e. less than 5) and the absence of the skate during the other seasons, KUD models could only be computed for autumn 2015 (Table 5.1 & 5.2; Figure 5.2B). The short-term spatial analysis of this individual disclosed the smallest areas. Its home range (KUD 95%) and core activity area (KUD 50%) were respectively 1.9km^2 and 0.2km^2, the latter being established around the deepest part of the study area (30 m to 70 m) (Table 5.2; Figure 5.3C). The MCP analysis for this individual revealed an estimate of 26% use of the FPA space (Table 5.1). The spatial use of the male presented similarities with the mature female, given that their detections were mostly concentrated on the eastern half of the FPA and neighbouring PPA (Figure 5.3A & C). However, the male's home range (2.59km^2) illustrated a relatively more scattered utilization of the protected area, concentrated along the deepest gradients of the FPA (Figure 5.3C; Table 5.2). The MCP area for this individual reached 2.46km^2, implying a 57% use of the FPA space (Table 5.1). The computation of the COA disclosed a total number of 7 108 relocations for the mature female #1F, allowing the estimation of KUDs across seven consecutive seasons (Table 5.1 & 5.2). The spatial analysis of this individual revealed an extended utilization of the marine park (Figure 5.3A). The size of the core activity areas varied seasonally from 0.2km^2 to 0.75km^2. Peaks were identified during two seasons, summer 2016 (0.75 km^2) and spring 2017 (0.63km^2), while winter and autumn recorded range contractions (Table 5.2). Similarly, the home range size fluctuated two-fold over the course of the study (Table 5.2), exhibiting temporal patterns concordant with the previous pattern. The largest KUD 95% areas were recorded in summer 2016 and spring 2017 (respectively 3.31km^2 and 3.4km^2) (Table 5.2). The MCP analysis revealed that this female used 66% of the FPA area (Table 5.1).

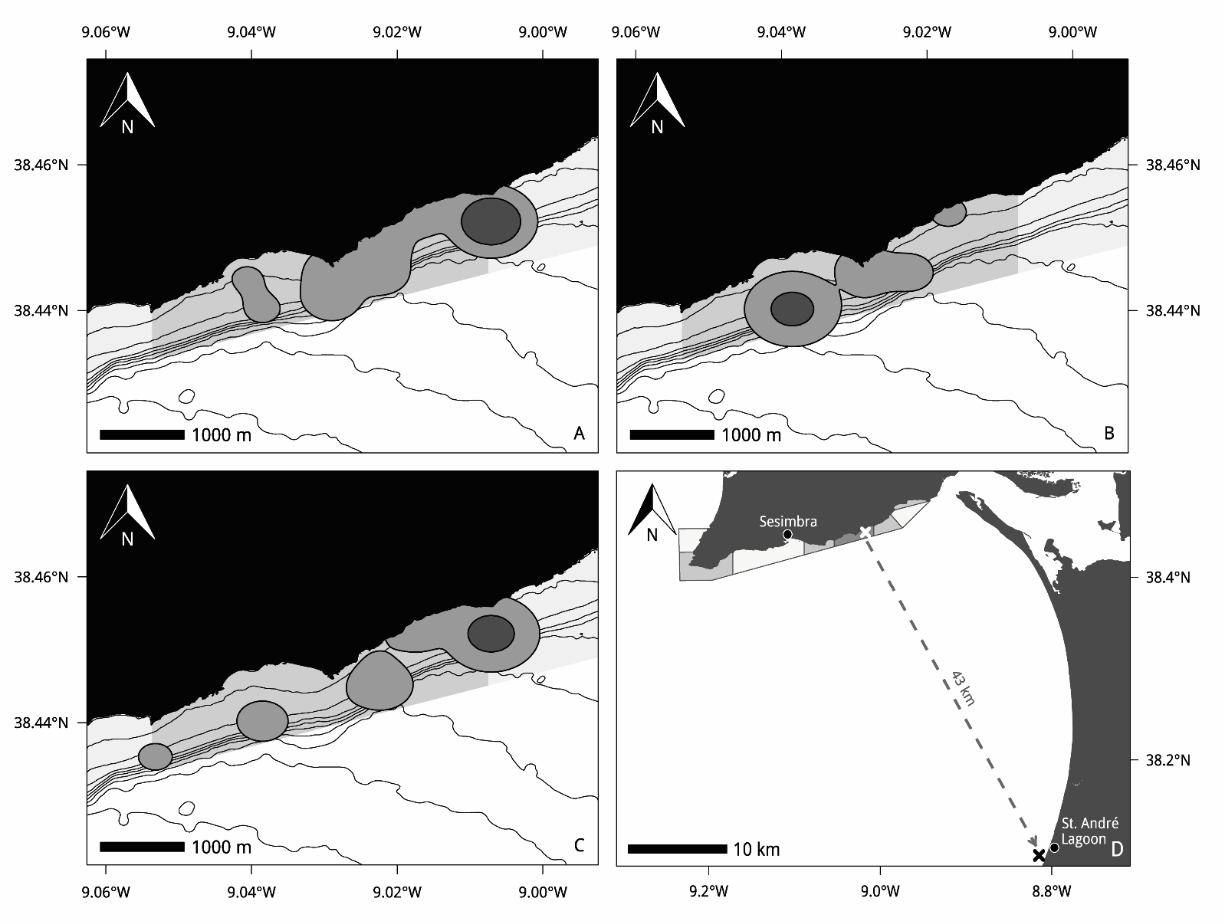

Figure 5.3. Home range (KUD 95%; grey area) and core activity areas (KUD 50%; dark grey area) of the three white skates (*Rostroraja alba*) fitted with acoustic transmitters. A – mature female #1F; B – immature female #2F; C – immature male #3M; D – Map illustrating the southward horizontal movement pattern expressed by individual #3M, depicting the location of the last detection inside the study area (white cross) and the detection off St. André Lagoon (black cross). The polygons with different grey shadings represent the three protection levels of the marine park: light grey - buffer areas; grey - partially protected areas; dark grey - fully protected area.

The analysis of the detection frequency over a 24-h period showed no significant difference between night and day for the immature female (#2F) and the male (#3M) ($p<0.05$). In contrast, the behaviour of the mature female was revealed to be significantly influenced by the diel phase, with a higher detection frequency being observed during the night ($p>0.05$). The proportion of detections reported in Figure 5.4A illustrates this relationship.

Table 5.2. Detailed results of the spatial ecology analyses for the three white skate (*Rostroraja alba*) individuals fitted with acoustic transmitters in the Prof. Luiz Saldanha Marine Park. A_{Ri} – absolute residency index; R_{Ri} – relative residency index; KUD 50% - Kernel utilization distribution corresponding to core activity area (km^2); KUD 95% - Kernel utilization distribution corresponding to home range area (km^2). Bold characters refer to total values while a dash indicates an absence of the individual.

		Ind. #1F	**Ind. #2F**	**Ind. #3M**
Residency				
	A_{Ri}	**0.73**	**0.02**	**0.10**
	R_{Ri}	0.73	0.19	0.54
	Autumn 2015	0.48	0.18	0.40
	Winter 2015	0.24	0.08	0.41
	Spring 2016	0.84	-	-
	Summer 2016	0.92	-	-
	Autumn 2016	0.63	-	-
	Winter 2016	0.92	-	-
	Spring 2017	0.98	-	-
Core activity area (KUD 50%) (km^2)	Total	**0.41**	**0.20**	**0.24**
	Autumn 2015	0.21	0.20	0.17
	Winter 2015	0.28	-	0.32
	Spring 2016	0.25	-	-
	Summer 2016	0.75	-	-
	Autumn 2016	0.42	-	-
	Winter 2016	0.20	-	-
	Spring 2017	0.63	-	-
Home range (KUD 95%) (km^2)	Total	**3.24**	**1.90**	**2.59**
	Autumn 2015	1.35	1.90	1.01
	Winter 2015	2.41	-	2.99
	Spring 2016	1.82	-	-
	Summer 2016	3.31	-	-
	Autumn 2016	3.11	-	-
	Winter 2016	1.63	-	-
	Spring 2017	3.40	-	-

Proportion of distances covered per 24-h as well as cumulative distances travelled indicated daily patterns of activity for all three individuals (p>0.05) (Figure 5.4B & 5.5). Both females (#1F & #2F) exhibited two distinct phases of activity, characterized by a more mobile phase close to sunset and sunrise hours, contrasting with noticeably reduced activity patterns during the day (Figure 5.4B). The male, however, displayed less explicit behaviour, defined by a strong peak of activity at sunset followed by relatively moderate movements interspersed with resting periods throughout the rest of the 24-h period (Figure 5.4B & 5.5). The investigation of the activity patterns across seasons revealed, for the latter individual, temporal variation between autumn and winter 2015, with the skate seemingly entering a more mobile phase during the latter season (p>0.05) (Figure 5.5). This individual showed excursions to areas adjacent to its core activity area, that were more frequent after mid-December 2015 (Figure 5.2). The last detection within the array, on 13th February 2016, was recorded inside the protected area (Receiver “LS7”). Intriguingly, the male was detected four days later by a receiver deployed off St. André Lagoon (Dr. B. Quintella, MARE Institute, pers, comm.), some ~43 kilometres south of the PLSMP (coordinates 38º5’44.016” N, 8º48’53.028” W) (Figure 5.2 & 5.3D).

Likewise, a seasonal dynamic was evident for the mature female (#1F). Not only were the distances travelled significantly different between night and day across all seasons (p>0.05; Figure 5.4B & 5.5), but the distances covered also varied significantly from one season to another. The patterns showed a period of increased activity and movements during spring and summer, with the female covering distances twice as large as the distances covered during the rest of the monitoring period (Figure 5.5). No difference across seasons (i.e. autumn and winter 2015) was detected in terms of distance travelled for the immature female (#2F).

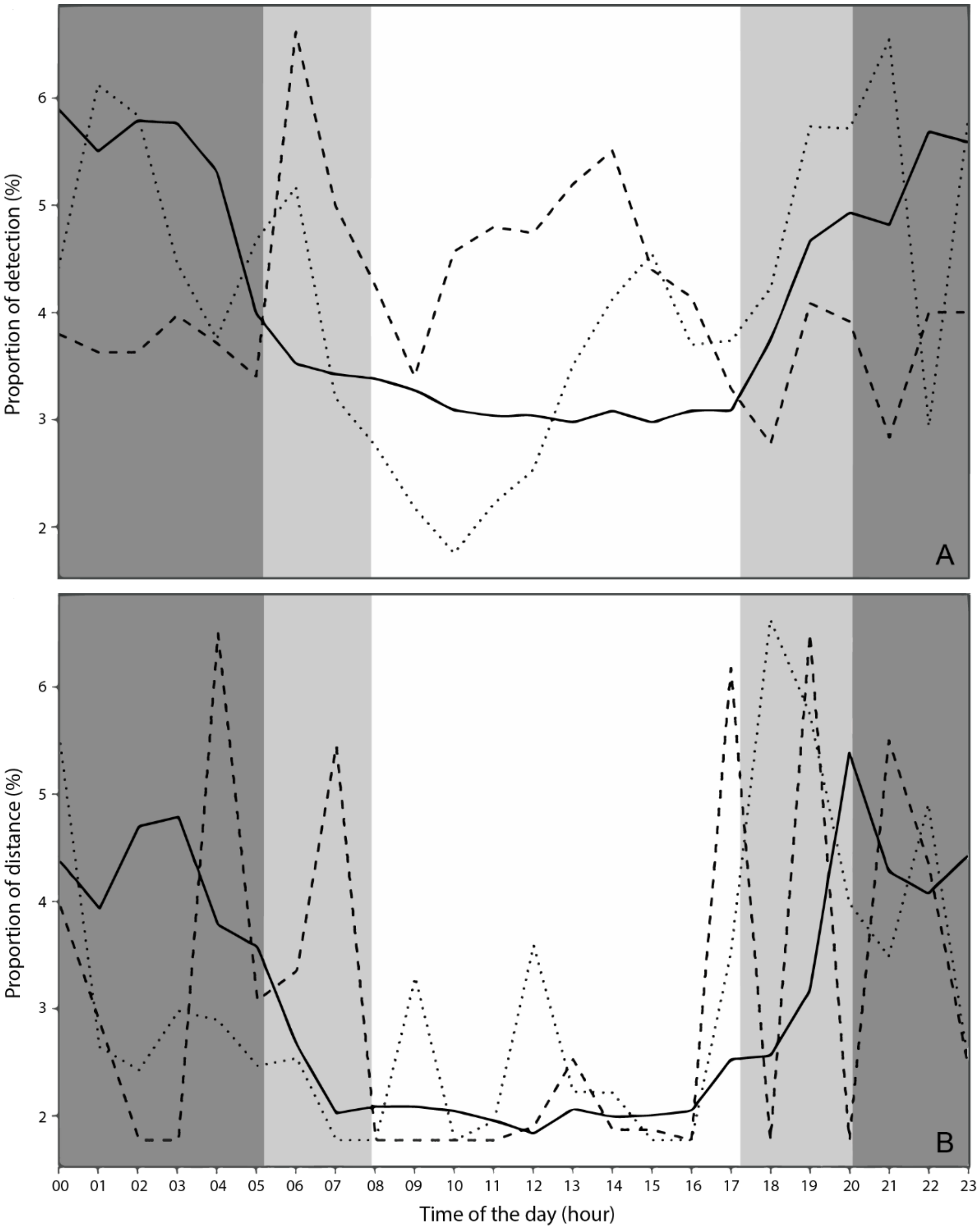

Figure 5.4. Proportion of the total number of detections (A) and proportion of the total distance travelled (B) for each of the three white skates (*Rostroraja alba*) fitted with acoustic transmitters, over the 24 hours of the day. Dark grey areas symbolize night time while light grey areas symbolize sunrise and sunset hours. Solid lines – mature female #1F; Dashed lines – immature female #2F; Dotted lines – immature male #3M.

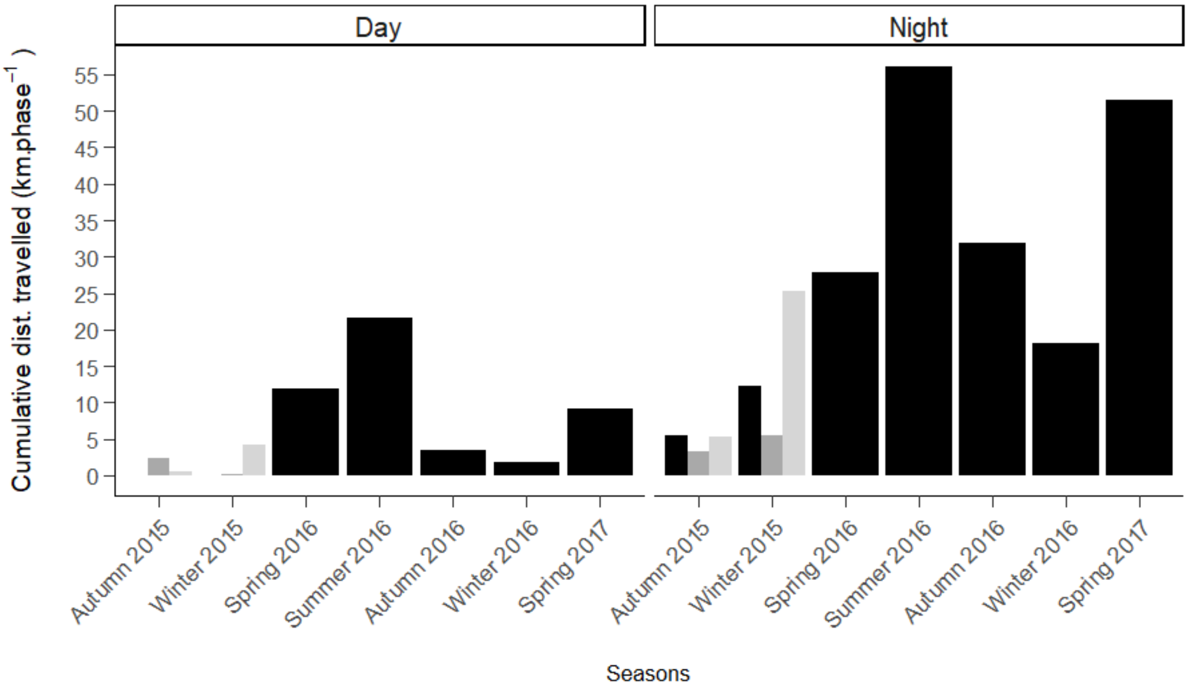

Figure 5.5. Cumulative distance covered (km) per diel phase (day VS night) across seasons of the three white skates (*Rostroraja alba*) fitted with acoustic transmitters. Black – mature female #1F; Dark grey – immature female #2F; Light grey – immature male #3M.

5.5. Horizontal movements, MPA usage and activity

To date, most research addressing the effectiveness of MPAs tended to focus on reef teleost species (e.g., Abecasis et al. 2015), and some studies have investigated charismatic elasmobranchs such as reef sharks (e.g., Knip et al. 2012; da Silva et al. 2013). Information on the spatial behaviour of skate species is either sparse or non-existent and only a few studies have addressed spatial protection effectiveness relative to this group (e.g., Wiegand et al. 2011; Neat et al. 2015). The present study documents the first species-specific movement ecology analysis of the poorly known and regionally Critically Endangered white skate. Our study draws on only three individual datasets, so that the interpretation of the findings and their implications cannot be seen as conclusive. While we are conscious of the level of uncertainty around the mechanisms underpinning the observed behaviours, we briefly present and discuss some possible scenarios.

Each individual exhibited a different degree of site fidelity. The seasonal indexes obtained for the mature female appeared to coincide with the finding of Quigley (1984), suggesting that the species uses inshore coastal areas during spring and summer while individuals move away towards deeper waters over the winter. A similar seasonal pattern of abundance of white skates was found by (Kadri et al. 2014) in the Gulf of Gabès (Tunisia). These authors also analysed the gonadosomatic index of white skate, reporting a higher reproductive condition during spring and summer, and proposing that the reproductive cycle is related with the seasonal use of inshore waters. If the inshore seasonal migration is prevalent among mature individuals, as suggested by Quigley (1984) and Kadri et al. (2014), then the use of coastal MPAs would be an appropriate approach for the conservation of this species. It is worth noting that the mature female in our case study exhibited high site fidelity to a coastal region, and further investigation could examine which depth strata are more extensively used by the species.

The immature female (#2F) showed the lowest site fidelity of all three individuals. The detection patterns and residency indexes (Figure 5.2) implied that the individual was visiting the FPA rather than using it as home range. Although the relatively small home-range size (1.9km^2) likely derived from its short-term spatial use analysis, the MCP results revealed a restricted use of the protected area (26%). This leads us to assume that the FPA offered a low level of protection to this individual. The last detection of the animal occurred at the easternmost station (LS15), implying the female left the study area. Cessation due to capture or transmitter defect (i.e. battery malfunction) are also possible.

The short-term spatial analysis of the male and mature female suggested a relatively high utilization of the protected area, concentrated mainly along the deeper gradients of both the FPA and the eastern adjacent PPA. The male exhibited relatively high site fidelity during two seasons, while the mature female spent a large portion of its time within the boundaries of the protected area, suggesting that the FPA afforded a high level of protection to this individual. The spatial patterns indicated that this individual associated with specific sites for long periods, concordant with the contracted range of horizontal movements described by Wearmouth & Sims (2009) for the common skate (*Dipturus batis*). However, the three observed site fidelity patterns suggest that both resident and transient individuals are present in the studied population, in similarity with the observations by Neat et al. (2015) for the Scottish flapper skate (*Dipturus intermedius*) population.

The results of spatial use inside the FPA indicate a preference for deeper areas of the marine park, a pattern common to all three individuals. This is unsurprising, given the depth range described for the species (Brito 1991, Sanches 1991), and it underlines the importance of protecting deeper habitats in this area to promote the recovery of the local white skate population. Moreover, even though the white skate is known to also occur in inshore waters and shallow bays (Quigley 1984, Compagno & Ebert 2007), the consistent presence of a mature individual at depths shallower than 80m deep is somewhat unexpected. This raises the question of the importance of inshore habitats for the overall population.

The fine-scale temporal analysis suggested an influence of the diel phase on the movement patterns of the tagged individuals. Preliminary assessment of sentinel tag performances did not indicate any major influence from environmental variables and/or background noises (Kessel et al. 2014). Thus, we assumed that the detection frequency obtained and the proportion of distance travelled, depicted activity patterns. The observed daily patterns, which showed more activity during the night period, could be attributed to feeding behaviour. It is conceivable that the white skate forages predominantly at night, as it is the case of other skates and various elasmobranchs (Ebert & Stehmann 2013, Humphries et al. 2017).

The seasonal differences in distances covered identified in the male's behaviour remain difficult to interpret, mostly due to the short monitoring period but also owing to the permanent departure of the individual, initiated by a southward movement. Horizontal movements of rather medium amplitude, i.e. ~20 km, have been documented in skates, such as the common skate (Little 1995, Little 1997, Wearmouth & Sims 2009) and the big skate (*Beringraja binoculata*) (King & McFarlane 2010). In some cases, while a high proportion of individuals showed moderate to high site attachment, some individuals exhibited long distance migrations, and this was documented for example in the common skate (Little 1995, Little 1997) and in the starry ray (*Amblyraja radiata*) (Templeman 1984). Despite a conspicuous need for further investigation of the mechanisms underpinning dispersal tendencies in our case study, a few hypotheses can be drawn around this observation. The movement of the male could have been related to an ontogenic shift, in that it may have left the area when about to reach maturity. Some authors, such as King & McFarlane (2010), suggest that migration ability and extension may depend on size or sex, and Wearmouth & Sims (2009) also suggest that body size may reflect different ontogenic foraging strategies (i.e., dietary preferences, swimming and preying capabilities). Ontogenic habitat change and behavioural shifts have been described for other skate species (Skjæraasen & Bergstad 2000, Bizzarro et al. 2014). It is also possible that the departure of the male in our study was related to sexual segregation. Such behaviour has been shown for the common skate by Little (1997), who described evidence for sexual disparity in space use. Both males and females were common during winter in the Sound of Mull (UK), with males leaving the area during warmer months. Other more incidental events may cause moderate amplitude movements in skates, such as following prey. In this study, the individual was never detected again after leaving the area, and mortality by fishing should not be ruled out.

5.6. Final remarks

The slow life cycle of the white skate coupled with intense fishing pressure and serious declines in abundance have been raising conservation concerns and led to this species being classified as Endangered (Siskey et al. 2019). Despite this, little is known about its behaviour and ecology. The preliminary insights provided in this study may guide future research. The results indicate some level of behavioural plasticity with regard to residency and horizontal displacement, which poses a challenge for spatial management. Of the three tagged animals, only the mature female seemed to consistently benefit from this coastal MPA, as the other two individuals seemed to be occasional or periodical visitors to the area. Several hypotheses and questions remain to be examined, such as an understanding of the proportion of resident and transient individuals, which would inform about the potential of spatial protection effectiveness. Also, information on whether the seasonality in movement patterns of mature females is consistent in the population would be useful, whether the species has nocturnal foraging habits, or knowing if there are differences of residency and home range according to sex or ontogeny. In particular, an interesting study could focus on determining whether the FPA can effectively protect mature females, or if immature males would benefit from protection over a more restricted period. Mating and egg laying related movements in mature individuals should also be targeted by future work. Investigation of the movement ecology across sex and ontogenic dynamics of the species would shed light on whether the patterns currently presented are representative features of the population. Besides addressing residency and migratory ranges, future studies should also provide information regarding trends in diel and seasonal usage of different habitats and for different biological processes (e.g., foraging, mating, resting, etc.). One of the most obvious questions regarding habitat is the relative importance of inshore and deeper habitats for the ecology of the species. Research should take advantage of the present availability of modern tracking technology, such as acoustic tags and DSTs, in order to address these knowledge gaps and contribute towards the conservation of an endangered and poorly studied species.

5.7. Geolocation information

This study took place in the North-eastern Atlantic, western coast of Portugal, specifically in the Prof. Luiz Saldanha Marine Park. The fully protected area where the monitoring was carried out is located at: 38.44387ºN 9.02858ºW.

5.8. Acknowledgements

The authors would like to thank all the colleagues and students who helped in the field work. We also thank ICNF-PNA for the support as the marine park administrative entity, especially Miguel Henriques e Carlos Silva. We are also grateful to Dr. B. Quintella, MARE Institute, project PELSA - Gestão sustentável da Pesca da Enguia na Lagoa de Santo André (PROMAR 31-03-02-FEP-4), for reporting the presence of one individual in their acoustic receivers array. The authors would like to thank to Dr. Richard Mace and Dr. David Abecasis for the constructive criticism and comments on the manuscript as well as for the advice regarding data analyses. We are also grateful to the editor and reviewers for careful reading and useful suggestions.

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CHAPTER VI

General Discussion

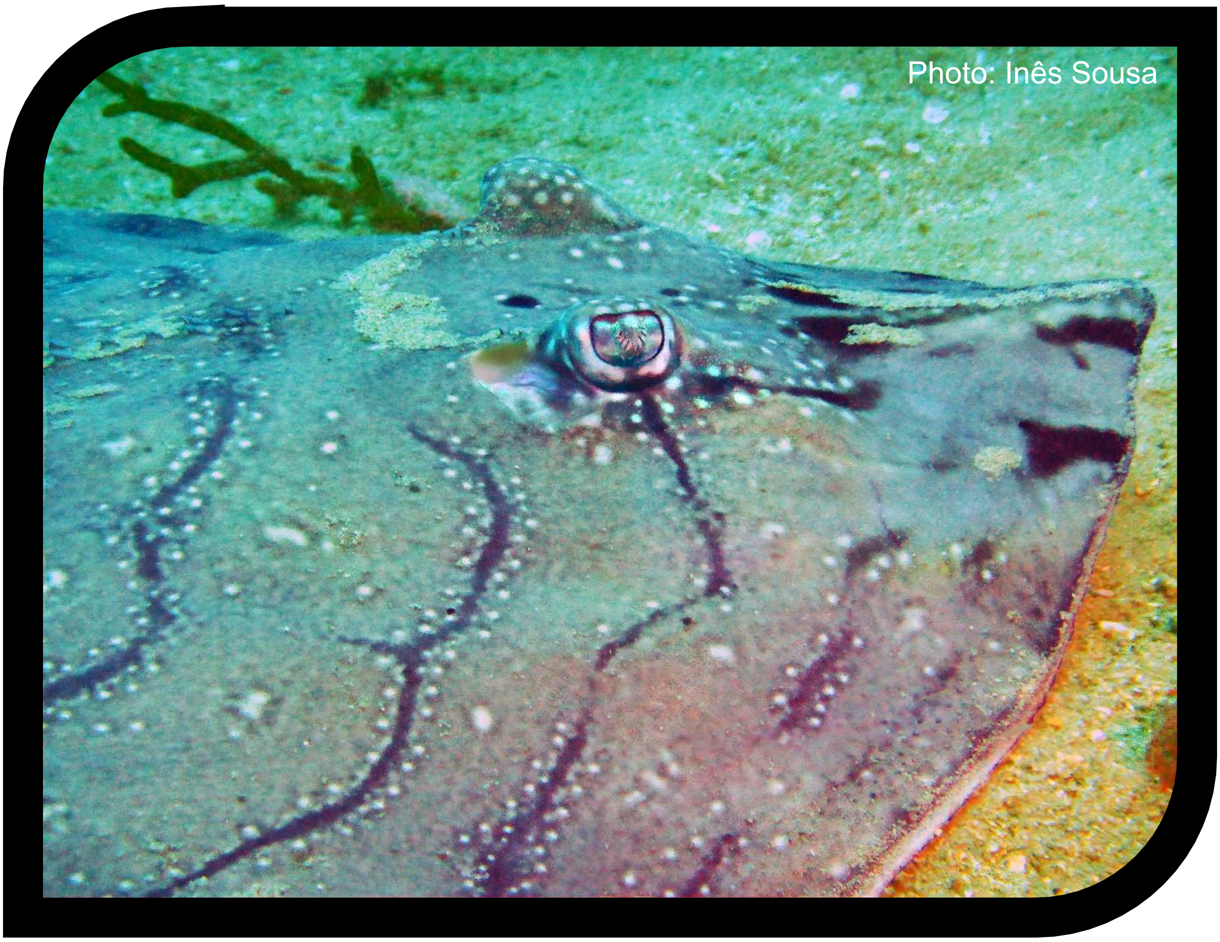
Photo: Inês Sousa

6. General Discussion

Despite the significance of coastal soft-bottom habitats as providers of fisheries resources, and their high exposure to human-induced pressures, many of their biological processes are still largely understudied (Lohrer & Whitlatch 2002). By using a suite of methodologies, the present study has provided some meaningful insights regarding the effects of spatial protection on soft-bottom fish assemblages. This general discussion integrates the main findings within a broader perspective and outlines the key management implications. Some of the limitations of the presented research and suggestions for future work are also discussed.

6.1. Implications for MPAs and soft-bottom fishes management

The community-level analysis presented in Chapter II allows to identify key inferences regarding the effectiveness of spatial protection for soft-bottom fishes. The study area demonstrated the wide diversity of responses that soft-bottom species may have within MPAs. Collectively, evidence suggests that the PLSMP is providing moderate protection for the local soft-sediment community, with some positive signals detected but also aspects suggesting limited protection efficacy. At the species level, several species exhibited size increases from before to after the full implementation of the MPA (e.g., several soles, *Solea senegalensis*, *Solea solea*, *Microchirus azevia*; and hake, *Merluccius merluccius*), and some exhibited positive trends of abundance and biomass (e.g., the bastard sole, *M. azevia*; the thornback ray, *Raja clavata*; the white skate, *Rostroraja alba*; the spotted flounder, *Citharus linguatula*; and the tub gurnard, *Chelidonichthys lucerna*). Subsequent surveys performed in this MPA (Martínez-Ramírez et al. 2021) enabled to obtain four more years of data (three more years, until mid-2016; and an additional year from mid-2018 to mid-2019). With this additional sampling, both skates and the bastard sole consistently exhibited higher abundance and biomass in the post-implementation period. It is worth mentioning the specific case of the Senegalese sole (*Solea senegalensis*), an important commercial species for the local artisanal fisheries (Batista et al. 2009). Both studies detected a global decrease in its abundance within the study area. Conversely, there was a significant increase in its mean size, and the species was more abundant in the FPA and PPA, where the decrease was less pronounced compared to the lower protection zone (BA). Passive acoustic monitoring data has suggested that at least part of the population may be resident in this area (Abecasis et al. 2014), pointing to potential protection benefits. However, its decreasing trend suggests that there are other factors affecting its abundance. It is possible that some adults may be undertaking migrations to nearby areas outside the reserve, with increasing exposure to fishing mortality (Abecasis et al. 2014). Furthermore, the recruitment of the species is acknowledged to be regulated by environmental conditions, in particularly river flow (Vinagre et al. 2007) and temperature (Vinagre et al. 2013). The species serves as an example of the diversity of factors affecting protection effectiveness.

At the community level, the increased biomass observed during the three years following the full establishment of the MPA (Chapter II) was no longer significant when analysing the data of ten years post-implementation (Martínez-Ramírez et al. 2021). It is hypothesized that this trend may be related with yearly climate phenomena, such as the North Atlantic Oscillation (NAO). However, the increase of illegal fishing may also be taking place. Poaching practices were documented by Cunha et al. (2011), but the frequency of these activities remains unknown. Globally, it is reasonable to consider that the absence of significant biomass increase in higher protection levels ten years after implementation is not the expected outcome for an effective MPA (e.g., Aburto-Oropeza et al. 2011, Di Franco et al. 2016, Rojo et al. 2021).

The collected body of evidence suggests that effectiveness of protection of soft-bottom fishes is intrinsically complex, cross-linked with species habitat preferences, spatial heterogeneity and environmental variability. Three main aspects should be considered when aiming for the recovery of soft-sediment fish populations. Firstly, estuaries are important for many of these species' life cycles, and connectivity between coastal areas and nursery grounds should be supported. To this end, measures to protect some nursery areas and to manage fishing effort should be considered (Roos et al. 2020). Secondly, heterogeneity of soft-bottom substrates and species' habitat preferences are important factors that should be further studied and integrated in MPA design. Habitat continuity should be ensured in order to protect individuals within their areas of preferable use (Gaines et al. 2010, Sousa et al. 2018, Martínez-Ramírez et al. 2021). Besides the physical characterization of substrates, soft-bottom habitats have the infauna community as an important component (Guille 1970), and the distribution of these communities should be considered also (Pinedo et al. 2022). A third aspect is that protection effectiveness is underpinned by compliance of implemented rules, and this should be insured by adequate surveillance (Guidetti et al. 2008, Giakoumi et al. 2018) and stakeholder engagement

(Batista & Cabral 2016, Giakoumi et al. 2018, Roos et al. 2020, Fidler et al. 2022, Horta e Costa et al. 2022). Overall, for successful MPA management, comprehensive ecological knowledge should be taken into account, and both users and decision-makers should be involved in the process and motivated for successful achievements (Batista et al. 2014, Giakoumi et al. 2018, Huang et al. 2024). At the regional level, the contribution of the PLSMP for the sustainability and connectivity of marine biodiversity (Batista & Cabral 2016, RCM 2019, Assis et al. 2021) can only be achieved by giving attention at the ecological, social and governmental dimensions. The present body of results, joined with previous work (e.g., Batista et al. 2011), highlights the need of taking measures that will improve the effectiveness of this MPA.

6.1.1. MPAs and skate species spatial ecology

Skates are recognized as important mesopredators that are particularly susceptible to overexploitation (Frisk et al. 2005, Ebert & Sulikowski 2009, Dulvy et al. 2014). Attention was given to this group in the present work (Chapters III, IV and V), particularly focusing on their spatial ecology. The studied skate species (Chapter III: *Raja clavata*, Chapter IV: *Raja undulata*, Chapter V: *Rostroraja alba*) also illustrate some aspects previously highlighted in Chapter II. Some diversity of recovery potential was observed, as our results provide evidence of higher protection effectiveness of the undulate ray (*R. undulata*) (Figure 6.1) afforded by this MPA, in comparison to the moderate protection provided to the thornback ray (*R. clavata*). The latter is considered to benefit from this coastal MPA because of its significant abundance increase and its patterns of residency. However, the density of mature females is low in the MPA area, and the results suggest that the species is vulnerable to the practice of 'fishing the line' due to its depth preferences.

This relates to another aspect highlighted by the skate case studies, which is the significance of habitat connectivity. The local population of thornback ray (*R. clavata*, Chapter III) has shown utilization areas and dispersal patterns that emphasize the importance of deeper muddy substrates for this species. Spatial protection should ensure longitudinal migrations within relevant depth strata (as highlighted in Chapter II), and additionally allow for some inshore-offshore migrations. This could be achieved either by enlarging the MPA boundaries or by implementing buffer zones with fishing effort regulation measures (Ohayon et al. 2021). In the specific case of this species, research should target the full depth preference of this species in this area, as it seems to vary considerably across regions (Humphries et al. 2016, Simpson et al. 2021, Kraft et al. 2024).

Furthermore, conservation measures tailored for these species have the challenge to incorporate the complexity of their ontogeny and behaviour. For instance, skates often present patchy distributions (e.g., Ellis et al. 2012). Whether this is an actual tendency, or a biased perception caused by fishing mortality is not fully understood (Fock 2014), but it poses a challenge for the study of their distribution. Additionally, habitat preferences may vary according to ontogeny and sex. Juveniles and adults often have different habitat requirements, and sexual segregation is common (e.g., Chapter III: juveniles and adults of *Raja clavata* exhibited different depth preferences; low abundance of mature females in the MPA). Areas of occurrence may also differ according to individual behaviour variability (Villegas-Ríos et al. 2017, 2021), and research has highlighted the importance of ensuring that a diverse array of behavioural traits is encompassed by MPA protection or targeted by other management measures (Villegas-Ríos et al. 2017, Villegas-Ríos et al. 2017). If, for instance, only resident individuals are protected, this may lead to a loss of behavioural traits in the population in the mid-term, due to different fishing mortality rates (Villegas-Ríos et al. 2017). The associated loss of genetic diversity would possibly be further aggravated by the fact that individuals with higher dispersal rates are likely important for population connectivity, enabling genetic transfers between sub-populations (Alves et al. 2019, Daban et al. 2024). Overall, given the complexity of their life cycles, MPAs alone may not be enough to protect skate species and their genetic diversity. But if effectively managed, they may be a valuable tool to use coupled with classical fisheries management (Wiegand et al. 2011, Simpson et al. 2020).

Figure 6.1. An undulate ray (*Raja undulata*) rests on the seafloor in the proximity of an acoustic listening station (LS1, visible in the top left corner of the image) (Photo: Ricardo Germano).

6.1.2. Integrated approach of MPA management

The current findings add to the existing body of knowledge regarding the importance of habitat and population connectivity. These aspects relate to the importance of the size of MPAs, which should ensure the representativeness of main habitat types (Rice & Houston 2011, Batista & Cabral 2016, Stratoudakis et al. 2019). A complementary approach to achieve this is to implement a set of interconnected MPAs that collectively contribute to promote ecological benefits, i.e., MPA networks. Conceptually, MPA networks can minimize the potential negative socioeconomic impact of a single large no-take reserve, while generating similar or even more significant ecological and economic benefits (Gaines et al. 2010, Grorud-Colvert et al. 2014, Meehan et al. 2020). If well managed, these type of MPA arrays may reduce the critical impacts that species face during their life cycle processes. For their success, there is the need for multidisciplinary knowledge (e.g. habitat mapping, spatial ecology of species, fishing effort distribution, social and economic data) and adequate monitoring (Cardoso-Andrade et al. 2022). This multidisciplinary knowledge should be used to inform managers and stakeholders, and thus enable adaptative management. This should include appropriate adjustments to legislation and targets as new information becomes available (Abecasis et al. 2015, 2017, Stratoudakis et al. 2019). As with individual MPAs, an integrated approach of ecological, social and governance dimensions is key for effective management of MPA networks (Brueckner-Irwin et al. 2019, Meehan et al. 2020, Gonçalves 2023).

6.2. Research challenges and limitations

The use of experimental fishing and passive acoustic telemetry as complementary techniques proved to be useful for assessing the potential of spatial protection effectiveness on soft-bottom fishes. Nevertheless, some challenges and limitations should be mentioned. Regarding experimental fishing, its main limitations are related to sampling occurring at very restricted moments in time. Surveys were carried out twice a year (each spring and autumn), which may not be frequent enough to identify relevant abundance patterns (e.g., seasonality). Furthermore, in our BACI design (before-after-control-impact), the lack of data prior to the establishment of any MPA regulations (some restrictions were already in place when experimental fishing surveys started) could lead to underestimate the MPA effects. Likewise, the area used as a control (buffer area, BA) for comparisons with the protected area, was within the MPA, corresponding to the lower protection level where fishing with static nets is allowed. Fishing in the area requires a specific permit, which itself regulates the fishing effort. However, it was the most suitable control area, as other options outside the MPA would have considerably different habitats.

Passive acoustic telemetry monitoring is an established method to estimate utilization areas and site fidelity (Donaldson et al. 2014). With the appropriate design, it enables fine scale estimates of space use. Nevertheless, one of the main limitations is that data collection is restricted to when tagged animals are within range of at least one acoustic receiver (Carlisle et al. 2019). Consequently, realistic home range estimates may be larger than the values provided by this methodology. However, in MPA monitoring, most research questions focus on specific areas, and passive acoustic telemetry is a reliable technique for this purpose, provided that tracking duration and receiver coverage are adequate (Heupel et al. 2006).

It is worth mentioning that, even though more affordable than satellite telemetry, resources may limit the number of receivers and tags available in a study (Heupel et al. 2006). The choice of the appropriate minimum sample size (number of tagged individuals) is intrinsically dependent on the research questions to address (Sequeira et al. 2019). In elasmobranch species such as skates, there is the added challenge related with marked ontogeny and sexual segregation, as individuals of different sex or life stage often have different spatial ecology (Ebert & Sulikowski 2009). In our study, the task of catching the target species presented significant challenges. This was particularly the case for the undulate ray (*R. undulata*). Either due to its lower densities, reduced tendency to move offshore (and lower probability of encountering our static fishing gear), or a combination of both factors, capturing and tagging this species proved difficult and resulted in a limited number of tracked individuals. Nonetheless, given the lack of knowledge of its spatial ecology, our study presents relevant insights that are useful in the initial phases of addressing the spatial behaviour of a given species (Sequeira et al. 2019). We provided long term tracking data of monitoring within a moderate size area (over 6km^2), which adds to previous research focusing this species in other areas (e.g., Leeb et al. 2021). The same applies to the data collected on the thornback ray (*R. clavata*), as this species shows significant regional differences in spatial behaviour (e.g., Rousset 1990, Simpson et al. 2021). The additional findings herein presented contribute to a more comprehensive picture of these skate species' spatial behaviour, especially considering their geographical and ontogenic complexity. While acknowledging that robust conclusions require tracking more individuals, this study still provides valuable contributions to the knowledge of their spatial ecology.

6.3. Future directions

One aspect that should be considered in the design and monitoring of MPAs is the importance and complexity of soft-bottom habitats and communities. Furthermore, to gain a better understanding of the protection effectiveness for soft-bottom fishes, it is crucial to continue monitoring efforts. Implementing comprehensive monitoring programs in the present study area and other MPAs globally, which are to include soft-bottom habitats, would improve our understanding of spatial protection effectiveness and contribute to global marine conservation goals. In the case of the PLSMP, the success of protection and research efforts depends significantly on improving the level of compliance of the management plan. This can be achieved through increased surveillance (including technological approaches, such as vessel tracking, drones, infrared cameras, artificial intelligence support) and active stakeholder engagement. Without these improvements, the adequate assessment of the effectiveness of spatial protection remains challenging.

Continuous monitoring, combined with better compliance, will provide more reliable data to evaluate the impact of MPAs on soft-bottom species and in general.

In relation to the spatial ecology of skates, further monitoring should be undertaken by expanding the monitored area (deploying additional receivers) and increasing the number of tagged individuals. To specifically monitor the movements of juvenile undulate ray (*R. undulata*) would also contribute to better understand possible differences according to ontogeny. Ideally, some individuals of thornback ray (*R. clavata*) and white skate (*Rostroraja alba*) should be fitted with Data Storage Tags (DSTs), in order to collect adequate depth data. Efforts should be made in particular to tag more mature females of these species. Given their low utilization of this coastal area, integrating pop-up satellite archival tags (PSATs) with DSTs would likely provide reliable data on their movement patterns (Fortuna et al. 2024). It is important to highlight that successful retrieval of DSTs will require outreach efforts directed at local fishermen. This is demonstrated by the low tag retrieval rate obtained from conventional tagging.

Complementary methodologies should also be employed to gain a broader understanding of the occurrence of soft-bottom fishes and spatial ecology of skates. These include the collection of Local Ecological Knowledge (LEK) and the use of baited remote underwater Video (BRUVs). There is research that has employed these techniques (e.g., Serra-Pereira et al. 2014, Fetterplace 2017, Silva et al. 2021, Batista et al. 2024), but they could be more extensively used given their relatively low costs. A particularly relevant question that a study on LEK could address would be to identify important areas for newborns (Serra-Pereira et al. 2014). Other typically employed techniques in the study of migrations and population connectivity are the use of stable isotopes and genetic analysis (Chevolot 2006, Marandel et al. 2018, Daban et al. 2024), and these could also be applied in this study area.

The use of complementary methodologies, alongside continued and improved monitoring efforts, will provide a broader perspective of the spatial protection effectiveness of soft-bottom fishes. Ultimately, this knowledge is needed to ensure the long-term sustainability of these species. These combined efforts will also provide a more comprehensive understanding of the spatial behaviour of skate species, which is essential for their effective management and conservation.

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