SOFIA GRAÇA ARANHA CARVALHO RAMOS

CONDITION AND MORTALITY RATES OF DEEP-SEA ELASMOBRANCHS FROM CRUSTACEAN BOTTOM TRAWL FISHERIES IN THE SOUTHERN PORTUGAL

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do Ambiente (ramo Ciências do Mar,
especialidade Ecologia Marinha)

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Declaration of authorship

This work has not previously been submitted for a degree in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Declaração de autoria de trabalho

Declaro ser a autora 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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ELASMOP

OUTPUTS

During the four/five years of this doctoral research, significant contributions were made in advancing our understanding of DSE, a largely understudied group. This research tackled critical questions regarding their ecology, biology, and conservation, contributing valuable knowledge to a field where data is scarce. Key outputs from the study are highlighted through articles and protocols that compose this thesis. They not only highlight the interactions between DSE and fisheries but also drive advancements in monitoring technologies and conservation efforts.

Two major research projects, initiated in 2020 and 2021, played a pivotal role in supporting the fieldwork and analyses required for this research, namely EMREP and Delasmop. The first project, *EMREP* (The Development of Electronic Monitoring and Reporting Technology for Fisheries in Portugal), funded by an EEA Grant (PT-Innovation-0007) focused on integrating video monitoring systems with existing electronic logbook solutions to develop an Integrated Electronic Monitoring and Reporting system (iEMR) for the Portuguese fishing fleet (Figure 0.1). The project aimed to improve the accuracy of reporting bycatch data, with DSE serving as a case study, thus enabling better management and conservation strategies. As co-Principal Investigator, I oversaw all tasks involving management, data analysis, and the actual tool development, ensuring the research objectives were met. This project not only provided the financial backbone for much of this doctoral research but also led to innovative and promising monitoring outcomes, such as the remote identification of DSE species and genus using iEMR technology, which is highlighted at the chapter 6, and the assessment of secondary stress responses in DSE blood plasma assessed at chapter 3. This project outputs, reports, pictures, videos and further materials were made available in the folder *EMREP* within the Open Science Framework (OSF) repository (Graça Aranha, 2024).

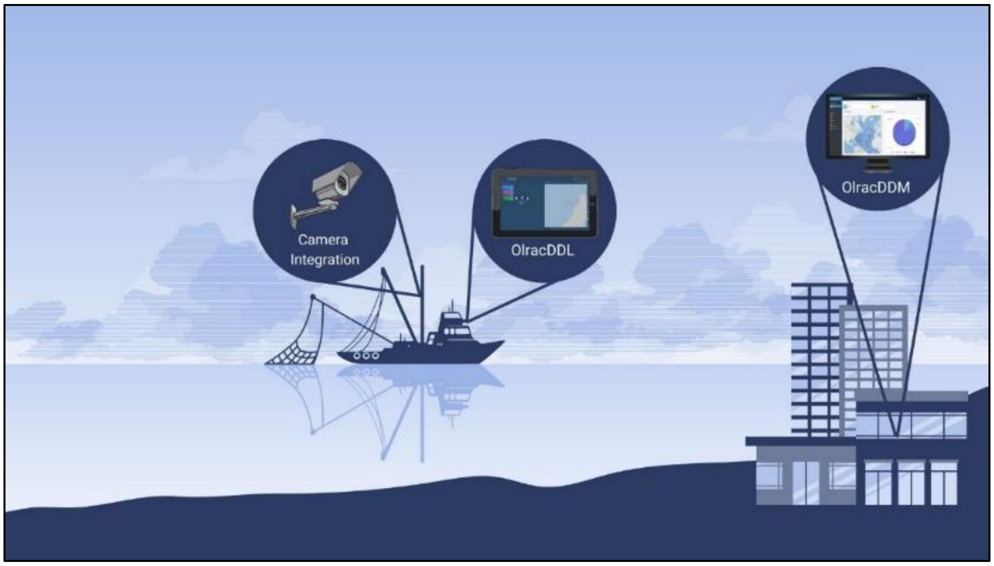

Figure 0.1 Scheme of the integrated electronic monitoring tool Olrac DDL ® developed by the project EMREP

The *Delasmop* project (Deep-Sea Elasmobranchs of Portugal), funded by a Keystone grant from the Save Our Seas Foundation (SOSF501), had the primary goal of exploring the interactions between DSE and crustacean bottom trawlers in southern Portugal. As a project leader, I oversaw all aspects of this research, including stable isotope analysis that are integrated in the chapter 3 and the development of a protocol for best practices in handling DSE onboard fishing vessels which was adapted to integrate this thesis in the chapter 7. In addition to contributing to the thesis results, this project produced other important outreach activities designed to engage a diverse range of stakeholders, including fishers, managers, researchers, children, and the general public. The main objective of these activities was to increase awareness and understanding of DSE, that remains largely unfamiliar to most people. These efforts were carried out through meetings, workshops, educational initiatives, national and international media campaigns and storytelling (e.g. social media, Tv shows, podcasts, webinars, videos, pictures), and presentations at scientific conferences, both in-person and online (Figure 0.2).

For fishers and managers, the focus was on fostering collaboration and promoting sustainable practices informed by scientific insights, in this regard meetings and workshops were conducted, and a questionnaire was developed to understand fishers' level of knowledge about several aspects relevant to their daily interactions with DSE such as best handling practices and existing regulations. Educational activities titled "*Elasmofixes: quem são os tubarões e raias da nossa costa Portuguesa"*, targeting children and the general public were developed in schools, and science centers namely "Centro de Ciência Viva" from the Algarve (South of Portugal) and Vila nova do Conde (North of Portugal), and included lectures, board games, videos, visualization under the magnifier of jaws and skins of real DSE, realistic small sculptures of five DSE and other materials to spark interest and inspire future generations. Furthermore, an exposition showcasing deep-sea researchers at the Dynamic Earth science center (Edinburgh) was exhibited during the summer of 2022, reaching over 9,000 visitors. Broader media campaigns and public engagements aimed to bridge the gap between science and society, bringing attention to the problematic DSE subjected in face of anthropogenic pressures. Finally, outreach of the results generated by this doctoral study, included open access publications within the chapters of this thesis, to make the findings freely available to everyone. Combined those activities reached over 10,000 people through in person interactions and several other through media and storytelling disseminations.

The combined outcomes of the *EMREP* and *Delasmop* projects may significantly contribute to fisheries science and policy. A 70-page report on the state of fisheries monitoring in Europe and Portugal, various scientific articles and reports, educational materials such as board games and videos all arose from those mentioned projects and can be find at the OSF repository folders Emrep, Delasmop and Articles within the OSF repository (Graça Aranha, 2024).

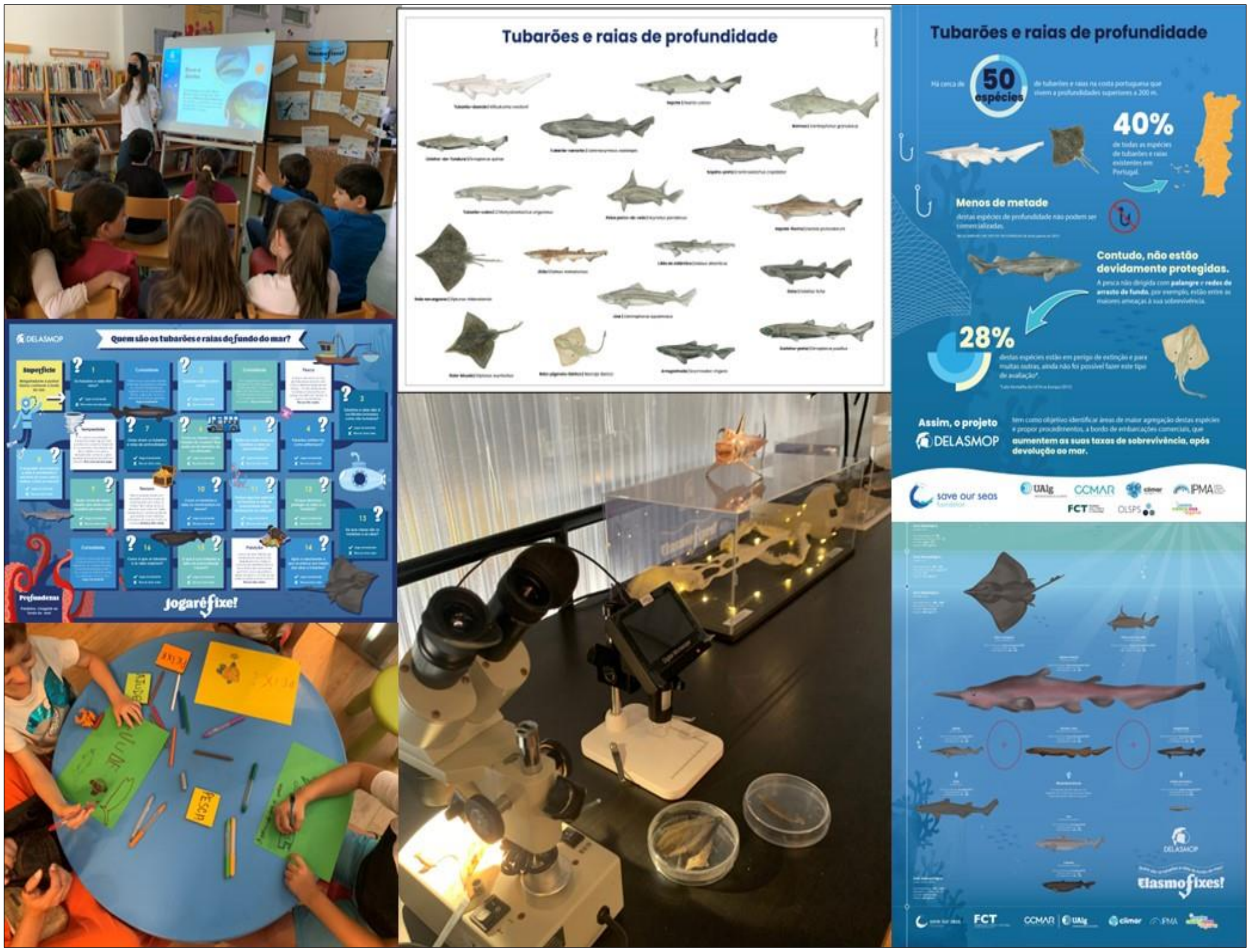

Figure 0.2 Educational activity "Elasmofixes" conducted by the project Delasmop in schools and *Centros de Ciência Viva* in the North and South of Portugal.

The data collected during this study was also shared with a broad network of collaborators across multiple research institutions, resulting in significant scientific outcomes, including publications (Chapters 9 and 10, with more in progress) and conference presentations where I contributed as a co-author. Furthermore, the supervision of undergraduate and master's students from various institutions (detailed in Table 0.1) facilitated the multidisciplinary exploration of the collected data. These efforts led to theses and reports addressing diverse topics such as surveys with fishers, DSE ecology and biology, DSE endoparasitic communities, and the scientific outreach of the Delasmop project. Collectively, these contributions have broadened the academic impact of this work, deepened the understanding of DSEs, and supported the development of sustainable management strategies for these vulnerable species.

Table 0.1 Reports titles from the students supervised by the doctoral candidate.

Type	Title	Degree	University	Role
Thesis	“Fishermen’s General Perception of Deep-Sea Elasmobranch Species on the South Coast of Portugal.”	BSc	University of Aveiro	Co-supervision
Internship report	“Evaluation of Chondrichthyan Stomach Content on the Southern Coast of Portugal.”	BSc	University of Porto	Co-supervision
Internship report	“Methods for Extracting Myxozoa Parasites from the Muscle of Teleosts and Selachians on the Portuguese Coast.”	BSc	University of Porto	Co-supervision
Internship report	“Search for Anisakis Parasites and Other Helminths in Chondrichthyan Species.”	BSc	University of Porto	Co-supervision
Internship report	“Assessment of Secondary Stress Responses in Deep-Sea Sharks on the South Coast of Portugal.”	MSc	University of La Rochelle	Supervision
Internship report	“Internship Report on the Scientific Communication of the Delasmop Project.”	MSc	University of Algarve	Supervision
Internship report	“Feeding Ecology and Vitality of Deep-Sea Elasmobranchs in the Algarve (South Portugal).”	MSc	University of Algarve	Supervision
Report Experimental Project in Marine Biology	“Assessment of Elasmobranch Bycatch from Crustacean Bottom-Trawlers in the Algarve, Portugal.”	MSc	University of Algarve	Supervision
Report Experimental Project in Marine Biology	“Ecobiological Traits of Elasmobranch Species from the South of Portugal.”	MSc	University of Algarve	Supervision

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RESUMO

O mar profundo compõe 90% dos oceanos e apesar de ainda muito desconhecido apresenta uma elevada biodiversidade, sendo extremamente vulnerável às ações antropogénicas, como a pesca de arrasto de fundo, especialmente para crustáceos. Esta prática apresenta altas taxas de capturas acessórias, incluindo elasmobrânquios de profundidade, que são particularmente sensíveis devido seu complexo ciclo de vida. Embora representem uma parte significativa das capturas acessórias nos arrastões de crustáceos, pouco se sabe sobre os impactos desta atividade e a biologia e ecologia destas espécies. Este estudo propôs-se a preencher algumas lacunas no conhecimento a este propósito, com base na avaliação de 1.559 espécimens pertencentes a 18 espécies de elasmobrânquios de profundidade nas costas Sul e Sudoeste de Portugal. Os resultados mostraram que estas espécies contribuem significativamente para as taxas de capturas acessórias, chegando a representar até 60% do peso total capturado em profundidades superiores às permitidas (> 800 m). Além disso, apresentaram elevados níveis de stress e mortalidade a bordo, com 95% dos espécimens encontrados mortos ou a morrer. A sobreposição das zonas de alimentação dos elasmobrânquios com áreas de pesca pode justificar estas elevadas taxas de captura, pois as espécies mais abundantes alimentam-se de diversas presas, bem como de crustáceos alvos desta pescaria. Importantes observações para a gestão pesqueira são apresentadas a destacar a necessidade de implementar medidas eficazes de monitorização e mitigação dos impactos. Para tal, foi desenvolvido um sistema de monitorização eletrónica que permitiu a identificação remota dos espécimens capturados. Além disso, foi criado um protocolo de boas práticas para o manuseamento de elasmobrânquios a bordo dos arrastões. Os resultados ampliam o conhecimento destas espécies e fornecem uma base sólida para políticas rigorosas, essenciais à conservação e à gestão sustentável das pescarias e das populações destes animais.

Palavras-chave: mortalidade a bordo, capturas acessórias, respostas secundárias ao stress, ecologia trófica, monitorização eletrónica

ABSTRACT

The deep-sea comprises 90% of the oceans and, despite being largely unexplored, harbours high biodiversity and is extremely vulnerable to anthropogenic activities, such as bottom trawl fishing, particularly for crustaceans. This practice results in high bycatch rates, including deep-sea elasmobranchs, which are markedly sensitive due to their complex life cycles. Although these species constitute a significant portion of bycatch in crustacean trawling, little is known about the impacts of this activity and the biology and ecology of these species. This study aimed to address some of these knowledge gaps by evaluating 1,559 specimens from 18 deep-sea elasmobranch species off the South and Southwest coasts of Portugal. Results showed that these species significantly contribute to bycatch rates, representing up to 60% of the total catch weight at depths exceeding the legal limit (>800 m). Additionally, high levels of stress and onboard mortality were observed, with 95% of specimens found dead or dying. The overlap between elasmobranch foraging areas and fishing zones likely explains these high capture rates, as the most abundant species feed on various prey, including the target crustaceans of this fishery. Key observations for fisheries management are presented, highlighting the urgent need to implement effective monitoring and mitigation measures. To this end, an electronic monitoring system was developed, enabling the remote identification of captured specimens. Furthermore, a best-practices protocol for handling elasmobranchs onboard trawlers was established. The findings expand the knowledge of these species and provide a solid foundation for stringent policies essential for the conservation and sustainable management of fisheries and elasmobranch populations.

Keywords: at-vessel mortality, bycatch, secondary stress responses, trophic ecology, electronic monitoring

RESUMO ALARGADO

Cobrindo profundidades superiores a 200 m e 90% do oceano, o mar profundo é um vasto e delicado ecossistema com alta biodiversidade, mas permanece em grande parte inexplorado e vulnerável a ameaças como a mineração, as mudanças climáticas e a pesca. As pescarias de profundidade, particularmente a pesca de arrasto de fundo, expandiram-se desde a década de 1960 devido ao aumento da procura por pescado e à sua diminuição nas zonas costeiras, causando danos significativos aos habitats e rapidamente esgotando as populações de peixes. As espécies de profundidade, incluindo elasmobrânquios como tubarões e raias, são especialmente vulneráveis devido ao seu crescimento lento, maturação tardia e baixas taxas reprodutivas, tornando a gestão sustentável de suas populações um grande desafio.

Nas costas Sul e Sudoeste de Portugal, os arrastões de crustáceos possuem grande expressão e contribuem para a liderança portuguesa no consumo *per capita* de pescado em face aos outros países europeus, direcionando a atividade a crustáceos economicamente importantes, como o lagostim (*Nephrops norvegicus*) e camarões de profundidade (*Aristeus antennatus* e *Aristaeomorpha foliacea*). As capturas acessórias de espécies que não são alvo da pesca, com a arte de arrasto pelo fundo podem ser significativas. Quando a arte de arrasto atua em áreas com profundidades superiores a 400 m as capturas de espécies de elasmobrânquios de profundidade podem ser significativas. A Comissão Europeia adotou medidas legislativas que têm impactos benéficos nas populações de elasmobrânquios, nomeadamente interdição de pesca de arrasto pelo fundo a profundidades superiores a 800 m e de interdição de redes de emalhar a profundidades superiores a 600 m. E ainda a inclusão de várias espécies de tubarões e raias de profundidade na lista de espécies proibidas da União Europeia. Estimativas das taxas de mortalidade de elasmobrânquios de profundidade capturados nos arrastões de crustáceos são deficientes e as existentes mostram que as taxas variam com a espécie e com o tamanho do espécime, com as características da arte, com as operações de pesca, profundidade de pesca, com variáveis ambientais (por exemplo a temperatura da água), entre outras. A relação entre a condição nutricional dos espécimes e a capacidade de sobrevivência é igualmente, pouco conhecida, mas são aspetos relevantes na apreciação da capacidade de sobrevivência à devolução ao mar de espécimes após captura. Em resumo a informação deficiente sobre a captura acessória, a ecologia

e biologia dos espécimes inviabiliza a proposta de medidas adequadas à conservação das populações de elasmobrânquios.

O objetivo do presente estudo doutoral foi contribuir para melhorar o conhecimento dos impactos da atividade de pesca do arrasto de crustáceos, que opera ao largo das costas sul e sudoeste de Portugal, sobre as populações de elasmobrânquios, com ênfase na determinação de taxas de captura e de mortalidade, e na avaliação dos níveis de stress e das condições ecofisiológicas dos espécimes de elasmobrânquios capturados nos lances de pesca. Foram ainda estudados outros aspetos como agregações de espécimes em função da profundidade, ecologia trófica e sobreposição espacial entre as zonas de alimentação e as zonas de atuação da pesca de arrasto. No âmbito do acompanhamento das operações de pesca foi ainda desenvolvido e ensaiado um software integrado de relatórios de pesca e monitorização eletrónica, que possibilita a identificação de elasmobrânquios de forma remota. De forma a se mitigarem os impactos da pesca nestes animais, foi desenvolvido um guia de melhores práticas de manuseamento a bordo, voltado para os pescadores que lidam diariamente com estes espécimes. Por fim, de maneira a se adquirir maiores conhecimentos sobre espécies pouco estudadas foram desenvolvidos estudos sobre a ecologia e genética de duas espécies de elasmobrânquios.

No período em que o estudo decorreu, foram recolhidos 1559 espécimes de elasmobrânquios de 18 diferentes espécies em lances de pesca a bordo de um arrastão comercial de crustáceos e do navio de investigação Mário Ruivo, do Instituto Português do Mar e da Atmosfera (IPMA). Foram recolhidas e analisadas em laboratório, amostras de tecidos biológicos com objetivo de estudar a ecologia trófica (análise de isótopos estáveis de azoto e carbono), avaliar o stress (avaliação de eletrólitos e metabolitos no plasma sanguíneo) e avaliar o estado nutricional dos espécimes (análise de rácios RNA/DNA), bem como também diversas informações técnicas da pesca e de variáveis ambientais.

A tese está organizada em três partes compostas por diversos capítulos que contemplam os objetivos supracitados. O capítulo 2 faz referência à ecologia dos elasmobrânquios, e sua distribuição preferencial em função de profundidade. No conjunto de espécies analisadas pôde certificar-se que algumas são consistentemente encontradas a profundidades inferiores a 400 m como no caso do *Etmopterus spinax* e *Galeus melastomus*, e outras em maiores profundidade (*ca.* 1000 m) como o *Deania calceus* e *Centroselachus crepidater*. No grupo de espécies/géneros em que foram capturados mais de três espécimes, *Galeus*, *Deania*, *Etmopterus*, e *Scymnodon ringens* foram os taxa com maior abundância enquanto *Centrophorus*, *Dalatias licha* e *Chlamydoselachus anguineus* foram os menos abundantes e apresentaram um comportamento mais individual. Foram documentadas elevadas taxas de captura acessória de elasmobrânquios principalmente a profundidades superiores a 800 m, com valores iguais ou inferiores a 60% da biomassa total da captura em alguns lances de pesca.

O capítulo 3 aborda os temas taxas de mortalidade e níveis de stress após a captura. A mortalidade a bordo após a captura é elevada, com 95% dos espécimes a chegarem a bordo mortos ou em condições muito debilitadas, com baixa probabilidade de sobrevivência após a devolução ao mar. Os espécimes da espécie *S. ringens* e de menor tamanho, tem maiores taxas de mortalidade. Também apresentaram maiores taxas de mortalidade espécimes capturados em lances onde a rede continha maior peso de pescado, e com arte de arrasto pelo fundo com rede de malhagem de 55mm (destinadas à captura de camarões e gambas) em comparação com os lances realizados com rede de malhagem 70mm (destinados ao lagostim). Os resultados obtidos também permitem concluir que as diferenças de temperatura entre a superfície e o fundo, têm grande influência nas taxas de mortalidade, onde maiores diferenças resultaram em maior mortalidade. Observou-se ainda sinais de elevado stress fisiológico nos espécimes capturados, conforme indicado pelas concentrações de potássio, ureia e magnésio no plasma. Os níveis elevados de lactato em espécies como o *G. melastomus* sugerem que mesmo os espécimes devolvidos ao mar em boas condições, têm um alto risco de mortalidade após a libertação.

Os capítulos 4 e 5 referem-se a estudos de ecologia trófica. Os resultados obtidos indicam que as espécies de elasmobrânquios de profundidade estudadas são, de forma geral, consumidores terciários e mesopredadores, alimentando-se de pequenas proporções de presas variadas, como crustáceos, teleósteos e cefalópodes, o que sugere um comportamento generalista. A sobreposição intra- e interespecífica dos nichos tróficos, e com as zonas de pesca (através dos rácios de RNA:DNA), assim como a presença de crustáceos de importância comercial na dieta destes animais sugere uma partilha de recursos entre os elasmobrânquios e as atividades pesqueiras nestas regiões. Entretanto a boa condição nutricional indica que, ou os recursos disponíveis estão a satisfazer as necessidades nutricionais destes animais ou ainda que estes encontraram estratégias para coabitar nas mesmas zonas sem comprometer as suas necessidades nutricionais. Para além disso, aparentes alterações de comportamento trófico intraespecíficas por zona, estágio de maturidade e sexo apontam para uma flexibilidade trófica, que pode refletir a adaptação às condições físicas e ambientais, ou a uma pressão antropogénica como a atividade pesqueira.

O capítulo 6 descreve um sistema integrado de monitorização e relatórios eletrónicos que foi desenvolvido para monitorizar remotamente as capturas acessórias, e os elasmobrânquios foram utilizados como caso de estudo. Foram identificados com sucesso 2.195 espécimes de 11 géneros/espécies através da análise de imagens de vídeos. O sistema mostrou-se eficaz e tem potencial para ser uma ferramenta a utilizar expandido para a implementação da lei da obrigação de descarga (Artigo 15.º do Regulamento 1380/2013). Contudo, ainda existem dificuldades na distinção entre taxa morfologicamente semelhantes como *Deania*, *Galeus,* e *Etmopterus*, por isso se o objetivo for a identificação a nível específico, outras formas de monitorização são necessárias e podem complementar este sistema como monitorização por observadores de bordo.

De forma a se mitigarem os impactos da pescaria de arrasto nos elasmobrânquios de profundidade, foi criado um protocolo de manuseamento que inclui técnicas para evitar lesões, minimizar a exposição ao ar e reduzir a mortalidade destes animas. Este protocolo está disponível abertamente para consulta online, mas foi devidamente adaptado para integrar o corpo desta tese através do capítulo 7, apoiando-se nos resultados obtidos por este estudo doutoral e também na literatura disponível. Este protocolo também serve como ferramenta educativa para os pescadores, promovendo práticas de pesca mais sustentáveis. Apesar de ter sido desenvolvido para pesca de arrasto, as técnicas de manipulação dos elasmobrânquios podem ser aplicadas em todas as pescarias.

Os capítulos 8, 9 e 10 ajudam a ampliar ainda mais o conhecimento de espécies pouco estudadas visto que incluem informações de ecologia e genética sobre espécies do tubarão *Oxynotus paradoxus* e da raia *Neoraja iberica*. O capítulo 8 inclui a referência de ocorrência do *O. Paradoxus* aos 1.238m, uma profundidade ainda não reportada para esta espécie, igualmente reportou-se pela primeira vez a informação sobre o tamanho de maturidade sexual de uma fêmea, que com 65 cm de tamanho total já se encontrava sexualmente ativa. Outras informações são fornecidas sobre a morfometria deste único espécime assim como o reporte de seu baixo estado nutricional. Já os capítulos 9 e 10 trazem a sequenciação de genomas mitocondriais completos para cada uma dessas espécies*, O. Paradoxus* e *N. iberica*, expandindo assim o conhecimento que se tem sobre estas espécies.

Em análise global, os resultados obtidos mostraram-se satisfatórios para se atingirem os objetivos propostos e fornecem informações cruciais para futuros esforços de conservação das populações de elasmobrânquios de profundidade em Portugal. Foram também identificadas outras necessidades de investigação, nomeadamente a avaliação das alterações sazonais de ecologia trófica e condição nutricional e melhoria do conhecimento de biologia e de ecologia de espécies menos abundantes, como *Mitsukurina owstoni, O. paradoxus* e mesmo de espécies abundantes, mas que se possui pouco conhecimento como *Etmopterus pusillus, Deania profundorum e S. ringens*. Os resultados obtidos poderão vir a contribuir para melhorar a gestão das pescas e proteger as espécies de elasmobrânquios de profundidade, garantindo a sustentabilidade dos ecossistemas marinhos e da indústria pesqueira para as gerações futuras.

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ABBREVIATIONS

AI	Artificial intelligence
AIC	Akaike Information Criterion
AVM	At-vessel mortality
BBNJ	Biodiversity Beyond National Jurisdiction
CCAMLR	Convention for the Conservation of Antartic Marine Livine Resources
CPUE	Capture per unit effort
DDL	Dynamic Data logger
Delasmop	Deep-sea elasmobranchs of Portugal
DGAV	Direção-Geral de Alimentação e Veterinária
DSCC	Deep Sea Conservation Coalition
DSE	Deep-sea elasmobranchs
eLog	Electronic Logbook
EM	Electronic monitoring
EMR	Electronic monitoring and reporting
EMREP	The Development of Electronic Monitoring and Reporting Technology for Fisheries in Portugal
ER	Electronic reporting
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GLM	General Linear Model
GSI	Gonadosomatic index
HSI	Hepatosomatic index
ICCAT	The International Commission for the Conservation of Atlantic Tunas
ICES	The International Council for the Exploration of the Sea
iEMR	Integrated electronic monitoring and reporting system
IPMA	Instituto Português do Mar e da Atmosfera
IPOA-Sharks	International Plan of Action for the Conservation and Management of Sharks
ISA	International Seabed Authority
IUCN	International Union for Conservation of Nature
IUU	Illegal, um
NAFO	Northwest Atlantic Fisheries Organization
NEAFC	Organizations such as the North-East Atlantic Fisheries Commission
RD	RNA/DNA ratios
RFMOs	Regional Fisheries Management Organizations
S.D.	Standard deviation
SEA	Standard ellipse areas
SEAc	Standard ellipse areas corrected
SEAFO	Southeast Atlantic Fisheries Organisation
SIA	Stable isotopes analyzes
sRD	Standardized RNA/DNA ratios
SW	Southwest

TAC	Total allowable catch
TL	Total length
TP	Trophic position
UNGA	United Nations General Assembly
VME	Vulnerable marine ecosystems

Chapter 1: GENERAL INTRODUCTION

1.1 Deep-sea ecosystems

The deep-sea represents the largest biome on Earth, accounting for approximately 90% of the ocean and spanning from 200 m to depths reaching around 11,000 m (Thiel, 2003; Ramírez-Llodra et al., 2010). It is generally defined by low light levels, cold temperatures, and scarce nutrient availability, and environmental conditions tend to remain relatively stable over time (Thurber et al., 2014). The abyssal plains, situated at depths of 3,000 to 6,000 m, are the most prevalent feature of the deep-sea, covering approximately 50% of the Earth's surface, making them the planet's largest habitat (Ramírez-Llodra and Billett, 2006). These plains consist of vast expanses of relatively uniform topography that, despite hosting high biodiversity, exhibit low biomass and productivity (Lambshead and Boucher, 2003; Smith et al., 2013; Amaro et al., 2019). Continental slopes, an extension of continental shelves, are characterized by their steep gradient, beginning at approximately 200 m and extending to around 4,000 m in depth (Levin and Dayton, 2009). Between the continental slopes and the abyssal plains lies the continental rises, which are more gradual slopes formed by sedimentary deposits (Shepard, 1972). Trenches, which are steep depressions formed in subduction zones, occur below abyssal depths greater than 6,000 m due to tectonic plate activity, are typically 50 to 100 km wide, and are found in all oceans (Ramírez-Llodra et al., 2010; Harris et al., 2014). These trenches represent some of the most extreme environments on Earth, characterized by high pressures, very low temperatures, and the absence of sunlight, where only specially adapted organisms such as invertebrates (e.g., sea stars and jellyfish) and certain gelatinous-bodied fish species can thrive (Ramírez-Llodra et al., 2010). In contrast, volcanic mountain ranges known as mid-oceanic ridges separate ocean basins. These are underwater chains of mountains that flank central valleys and are formed through plate tectonic activity, often featuring hydrothermal vents (Van Dover, 2000).

In addition, several smaller habitats contribute to the uniqueness and diversity of life in the deep-sea. Seamounts, which are submarine mountains rising at least 1,000 m from the seafloor without reaching the ocean surface, host a diverse ecosystem and are often referred to as “oases of life” due to their higher species diversity and biomass compared to the abyssal plains (Samadi et al., 2006, Hall-Spencer et al., 2007, McClain et al., 2009, Rowden et al., 2010). These features are the result of volcanic activity and are abundant in Earth’s oceans, also exhibiting a high rate of endemism. However, less than 0.1% of the world's seamounts have been studied (Koslow et al., 2001; Kitchingman and Lai, 2004). Chemosynthetic habitats, such as hydrothermal vents and cold seeps, also support unique ecosystems with high densities of endemic fauna (Levin et al., 2001). Hydrothermal vents are submarine hot springs where water emerges from the seafloor, sustaining a distinct ecosystem. These vents are located in tectonically active regions, often associated with volcanoes and seamounts, and can reach temperatures as high as 450°C, supporting various life forms in the deep-sea (De Angelis et al., 1993; Dick et al., 2009). One notable example is the giant tube worm *Riftia pachyptila* Jones, 1981, which thrives in this environment and is one of the fastest-growing organisms on Earth, reaching up to one meter in length within a year (Van Dover, 2000). Cold seeps, also found in tectonically active areas, contrast hydrothermal vents by maintaining much lower temperatures, usually similar to the surrounding seawater. These habitats release hydrocarbon-rich fluids such as methane or hydrogen sulphide, supporting long-lived, slow-growing organisms like the tubeworm *Lamellibrachia luymesi* van der Land & Nørrevang, 1975 (Wakeham et al., 2003; Tavormina et al., 2008).

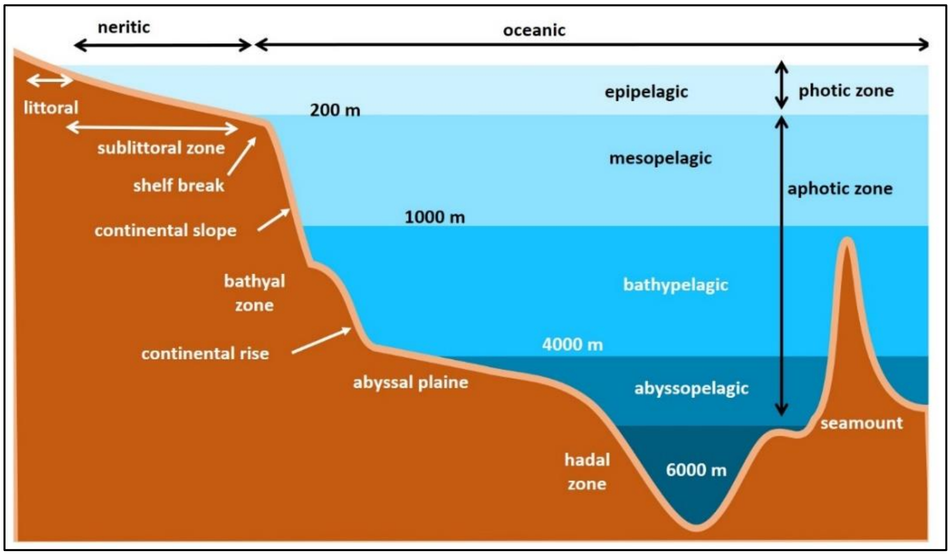

Figure 1.1 The deep-sea realm (Source: Dronkers J. https://www.coastalwiki.org/wiki/File:OceanZones.jpg)

Despite its vast geographical range, the deep-sea was historically believed to harbor little or no life (Tyler, 2003). This assumption arose primarily due to the extreme environmental conditions of the deep ocean, such as the absence of light, low temperatures, high pressure, and limited food availability (Gage and Tyler, 1992; Thistle, 2003). In this biome, pressure ranges from 20 to 1100 atmospheres, and temperatures can drop as low as -1.8°C in Antarctic waters or rise to 450°C near hydrothermal vents. Additionally, hypoxic and anoxic habitats add further complexity to this ecosystem. However, it is now understood that the deep-sea hosts some of the highest biodiversity on the planet, comparable to that of tropical rainforests, though distinct from shallower ocean layers, with a fauna predominantly composed of macro- and microfauna (Grassle and Maciolek, 1992; Van Dover, 2000). Biodiversity is widely recognized as the cornerstone of healthy ecosystems (Worm et al., 2006; Hector and Bagchi, 2007), making its conservation a key objective in environmental management (Brooks et al., 2006). It is estimated that the ocean contains around 2.2 million species, yet approximately 90% remain undescribed (Mora et al., 2011). In the deep-sea, taxa such as annelids, arthropods, and molluscs demonstrate notably high biodiversity, whereas megafauna, including vertebrates like elasmobranchs (sharks and skates), exhibit low diversity (Morrissey and Sumich, 2012; McClain and Schlacher, 2015). This immense biodiversity is enabled, in part, by the deep ocean's 22 distinct habitats and ecosystems, which are each physically, biogeochemically, and ecologically unique (Thistle, 2003; Tyler, 2003; Ramírez-Llodra et al., 2010).

Due to its unique habitats and biodiversity, the deep-sea plays a crucial role in sustaining essential ecosystem services that are vital for life on Earth. It provides society with key provisioning services, including food resources and hydrocarbons, as well as important regulating services such as temperature regulation, control of atmospheric greenhouse gases, and the absorption of waste and pollutants (Armstrong et al., 2010, 2012). Most notably, the deep-sea supports ocean life by cycling nutrients (Danovaro et al., 2003; Luna et al., 2012) and providing habitats for a wide range of species, many of which hold significant commercial value. These species include the *Hoplostethus atlanticus* and various species of *Sebastes* spp. (Norse et al., 2012), as well as valuable crustaceans such as *Aristaeomorpha foliacea* and *Aristaeopsis edwardsiana* (Figueiredo et al., 2001). These species are commercially exploited, primarily through bottom trawling, *A. foliacea* and *A. edwardsiana* command some of the highest market prices among crustaceans (Leocádio et al., 2012).

Despite its critical importance, the deep-sea remains the least explored biome on Earth, with only 0.0001% of it having been investigated, largely due to the extreme conditions that make exploration difficult (Danovaro et al., 2017). Deep-sea ecosystems are highly vulnerable to disturbances due to their unique characteristics. Many species inhabiting these ecosystems grow slowly, mature late, and have low reproductive rates, making them particularly susceptible to overexploitation (Koslow et al., 2000; Morato et al., 2006; Norse et al., 2012). Climate change further exacerbates these challenges, posing significant threats through rising ocean temperatures, acidification, and the expansion of oxygen minimum zones (Ramirez-Llodra et al., 2010). In fact, climate change is considered the greatest future challenge for deep-sea ecosystems, potentially impacting species in ways that are yet to be fully understood (Seibel et al., 2019). Human activities such as deep-sea fishing cause severe—and sometimes permanent—damage to benthic habitats and disrupt predator-prey relationships (Clark, et al., 2016). The growing threat of deep-sea mining raises additional concerns, not only for the waste it may generate but also for the potential irreversible and unknown damage it could inflict on these fragile ecosystems (Drazen et al., 2019). Still, among these threats, deep-sea fishing is recognized as having the most significant impact on deep-sea communities (Benn et al., 2010).

Global legislation governing deep-sea activities is evolving, with increasing recognition of the necessity for precautionary measures to protect vulnerable marine ecosystems. The regulation of deep-sea activities, particularly in areas beyond national jurisdiction, is primarily overseen by the International Seabed Authority (ISA), which manages the exploration and potential exploitation of marine resources, especially in the high seas. Recent legislative developments, including the signing of the ‘High Seas Treaty’—also known as the Biodiversity Beyond National Jurisdiction (BBNJ) agreement—in September 2023, are shaping the future of deep-sea mining and environmental protection. This treaty, a significant milestone in global ocean governance, aims to regulate nearly half of the Earth’s surface and 95% of its ocean volume. It emphasizes promoting equity, addressing environmental degradation, combating climate change, and preventing biodiversity loss in the high seas. Once ratified, the High Seas Treaty will enable the establishment of marine protected areas at a global scale, safeguarding oceans from the adverse impacts of human activities. This will significantly contribute to mitigating climate change, protecting biodiversity, and advancing the objective of conserving at least 30% of the planet’s ecosystems by 2030. The treaty addresses a critical gap in ocean protection, as currently only about 1% of the high seas is protected. Additionally, the treaty provides a framework for the fair and equitable sharing of both monetary and non-monetary benefits derived from marine genetic resources. It also promotes capacity building and the transfer of marine technologies to developing nations, supported by a voluntary fund aimed at helping these countries achieve the United Nations Sustainable Development Goal 14: "Life Below Water."

In response to these imminent threats, conservation measures are critical. Organizations like the Deep-Sea Conservation Coalition (DSCC) advocates for strong protective actions, including preventing destructive fishing practices and halting deep-sea mining. Conservation strategies should focus on establishing marine protected areas in vulnerable habitats, enforcing strict regulations on fishing and mining, conducting thorough environmental impact assessments before any exploitation, and investing in research to address knowledge gaps and improve understanding of deep-sea ecosystems. These are essential for guiding evidence-based conservation policies.

1.2 Fishing in the deep-sea

Since the 1960s, longliners, traps, gillnets and (mainly) bottom trawlers, have been forced to venture further from coastal waters into deeper ocean zones as global demand for fish has risen due to increasing population and because of the depletion of fish stocks on continental shelves and in the epipelagic zone (Koslow et al., 2000; Roberts, 2002; Morato et al., 2006). In fact, because of this depletion, 40% of the world’s trawling ground shifted to deeper areas by 1999 (Roberts, 2002) encouraged by government subsidies (Milazzo, 1998; Donnely, 1999; Sumaila and Pauly, 2006; Sharp and Sumaila, 2009). The International Council for the Exploration of the Sea (ICES) defines deepwater fisheries as those taking place in waters deeper than 400 m, while the Food and Agriculture Organization of the United Nations (FAO) defines them as those that occur beyond and below the continental-shelf break i.e., below 200 m (Clarke et al., 2003; FAO, 2011).

The access to deep-sea habitats is challenging, requiring large vessels, expensive equipment, and rigorous logistical protocols, which resulted in the development of new and robust fishing gear in order to reach species living at depths of as much as 2,000 m. Bottom trawling is employed by several deep-sea fisheries (Norse et al., 2012; Victorero et al., 2018) and consists of using heavy metal "doors" weighing up to 5,000 kg to ensure the net reaches the seafloor and remains open while being dragged across it (Morgan and Chuenpagdee, 2003; Stiles et al., 2007). These trawls can be expansive, with total widths—including sweeps, bridles, and ground gear—ranging from 80 to 200 m. The ground gear is outfitted with steel bobbins or stiff rubber discs, allowing the net to move over rough terrain without getting snagged (Clark and Koslow, 2007). This not only guarantee bottom trawling’s efficiency for deep-sea fishing, but also its harvesting potential to wipe out benthic habitats, often composed of long-lived, habitat-forming species like deep-sea corals and sponges (Clark et al., 2016). Additionally, deep-sea fish are more vulnerable to fisheries. Drazen and Haedrich (2012) observed 41 fish species living at varying depths and found a clear pattern of increased longevity, reduced fecundity, and a lower potential for population growth as depth increased. Adding to their vulnerability, deep-sea fish tend to aggregate around features such as seamounts, taking advantage of trapped vertically migrating nekton due to the seamount's topography (Morato and Clark, 2007). Consequently, deep-sea fisheries are likely to rapidly overexploit fish populations residing on seamounts, on ridges, or along continental slopes worldwide. While for some targeted species, population recruitment and restoration may occur if exploitation is significantly reduced, eliminated, or adjusted for a number of years, as seen in the case of the blue ling *Molva dypterygia* (Pennant, 1784) (Large et al., 2010), other species have been driven to critically low numbers, often within a decade or two as was the case of the *H. atlanticus* where fisheries have specifically targeted spawning and feeding aggregations to remain economically viable (Clark, 1999; Roberts, 2002). Likewise, slender armorhead *Pentaceros wheeleri* (Hardy, 1983) saw its population reduced to a fraction of its original biomass in just eight years. Other examples are of the longfin codling *Laemonema longipes* Schmidt, 1938 in which landings reached 200,000 tons in 1986 but dropped to 55,000 tons by 1994; and the groundnose grenadier *Coryphaenoides rupestris* Gunnerus, 1765 catches peaked at over 60,000 tons in 2001 but declined so rapidly that a moratorium was imposed in 2006.

Passive fishing gear, such as longlines, traps, and gillnets, tend to be less destructive and more selective than active gear like bottom trawls. However, concerns remain due to issues such as the loss of gillnets and traps, which can continue to "ghost fish" deep-sea species, the bycatch of species of conservation concern like deep-sea elasmobranchs (DSE), and the slow production rates of deep-sea fauna (Connolly and Kelly, 1996; Roberts, 2002). Active fishing gear, particularly bottom trawls, present additional direct and indirect consequences. Direct impacts include the physical modification of the seabed (Haedrich et al., 2001), removal of deep-sea communities, and high mortality rates for species caught. Indirect effects involve ghost fishing from lost nets, post-capture mortality of injured species, alterations to sediment biogeochemistry, and disruptions to local food webs (Jones, 1992; Jennings and Kaiser, 1998; Koslow et al., 2000; Wilson et al., 2015; Clark et al., 2015). The severity of these effects is influenced by the characteristics of the gear used, the frequency of fishing, and the ecological resilience of the habitats and fauna affected by such activities (Duplisea et al., 2001; Tillin et al., 2006).

The sustainability of deep-sea fisheries remains a subject of debate, with the answer dependent on a complex interaction of ecological, economic, and legal factors. The scarcity of reliable data on fish populations further complicates efforts to set appropriate catch limits and implement effective management strategies (Ramírez-Llodra et al., 2010). Additionally, deep-sea fishing threatens non-target species, including vulnerable deep-sea sharks, whose unintended capture can have long-lasting impacts on their populations (Norse et al., 2012).

From an economic perspective, deep-sea fishing faces significant challenges. High operational costs, combined with the low productivity of fish stocks, often create incentives for overexploitation rather than sustainable management (Sumaila et al., 2010). Government subsidies exacerbate this issue by promoting overcapacity in fishing fleets and unsustainable practices. It is estimated that subsidies for high seas bottom trawling fleets amount to $152 million annually, representing about 25% of the fleet's total catch value (Sumaila et al., 2010). In addition, profit margin for bottom trawlers is typically no more than 10% of the catch’s landed value, suggesting that their overall economic impact is limited (Sumaila et al., 2010). This implies that without governments’ subsidies, most bottom trawl fleets would be economically unviable, which could reduce pressure on deep-sea fish stocks (Norse et al., 2012). Despite their high environmental cost, these fleets contribute to less than 0.5% of the global marine catch, having a negligible impact on global food security (Giani, 2004; Sumaila et al., 2009; Victorero et al., 2018). Limited scientific knowledge of deep-sea ecosystems and species further hinders the development of sustainable and economically efficient fishing practices (Koslow et al., 2000).

Legally, enforcing regulations in remote deep-sea areas is difficult and costly (Gjerde et al., 2013). The absence of reliable data on deep-sea fish stocks complicates efforts to set appropriate catch limits, and many international waters lack comprehensive management frameworks to ensure sustainable fishing practices (Ardron et al., 2014). The sustainability of certain deep-sea fisheries is also linked to species' ability to inhabit shallower waters (< 200 m) and the use of non-trawl fishing methods. Bottom trawling, however, is universally considered unsustainable for deep-sea species (Merrett and Haedrich, 1997; Norse et al., 2012). Given these challenges, deep-sea fisheries, especially those employing bottom trawling, fail to meet the criteria for ecological, economic, and legal sustainability. This underscores the need for precautionary, science-based management approaches, improved data collection, and international cooperation to ensure the long-term sustainability of deep-sea fisheries.

Within the European Union (EU), deep-sea fisheries are subject to strict regulations designed to protect vulnerable marine ecosystems and ensure the sustainability of fish stocks. A landmark regulatory framework is the EU Deep-Sea Access Regulation (Regulation 2016/2336), introduced in 2016. This regulation restricts fishing for deep-sea species to areas with a historical fishing presence, known as "fishing footprints." It also bans bottom trawling below depths of 800 meters in EU waters of the Northeast Atlantic, protecting sensitive species such as long-lived sponges and corals. Fishers are required to report bycatch of vulnerable species and relocate if a certain threshold is exceeded. Scientific data collection has been enhanced through an observer scheme to improve monitoring and understanding of deep-sea fisheries. As of September 15th, 2022, the EU imposed further restrictions, banning bottom trawling in 87 designated areas, which cover 17% of the seafloor between 400-800 m in the Northeast Atlantic, offering vital protection to vulnerable marine ecosystems (VME). The EU has also implemented total allowable catches (TAC) for species such as skates, which are reviewed biannually to ensure sustainable fishing practices. These measures reflect a growing global concern over the environmental impacts of deep-sea exploitation, particularly as discussions surrounding deep-sea mining intensify.

1.3 Deep-sea elasmobranchs

Elasmobranchs, comprising sharks, rays, and skates, form a diverse group of cartilaginous fishes with over 1,200 known species. As one of the oldest vertebrate groups, dating back at least 420 million years, they have rapidly evolved to occupy upper levels of aquatic food webs (Compagno, 1990). Taxonomically, elasmobranchs are divided into two main subclasses: Selachii (sharks) and Batoidea (rays, skates, and sawfish), inhabiting ecosystems ranging from freshwater to marine environments. In marine ecosystems, DSE represents nearly half of all known species, with 283 species of sharks and 238 species of skates and rays inhabiting depths beyond 200 m (Compagno and Musick, 2005; Simpfendorfer and Kyne, 2007; Finucci et al., 2024). Some researchers argue that most “true” DSE are found at depths below 400 m (ICES, 2020; O’Hea et al., 2020) but rarely beyond 3000 m (Priede et al., 2006).

Deep-sea elasmobranchs exhibit unique life history traits, including slow growth, late maturation, high longevity, low fecundity, and very low productivity, making them particularly vulnerable to overexploitation (Garcia et al., 2008; Simpfendorfer and Kyne, 2009; Rigby and Simpfendorfer, 2015; Villagra et al., 2022). Their growth rates are typically half that of coastal sharks that exhibits a population doubling time of 2.6 years, while deep-sea species such as greenland shark *Somniosus microcephalus* (Bloch & Schneider, 1801) exhibit the lowest growth rates of any marine vertebrate, with a population doubling time of 31.8 years (Finucci et al., 2024). In the Azores, deep-sea sharks reach sexual maturity at lengths ranging from 56% to 89% of their maximum size for females, and 47% to 90% for males (Fauconnet et al., 2020). Reproductive strategies are diverse, but typically involve internal fertilization and specialized uterine gestation, including viviparous (pregnant females develop embryos internally and give birth to live young), oviparous (females lay fertilized eggs at the environment), and ovoviviparous (eggs hatch internally) modes (e.g., Wourms, 1977; Conrath and Musick, 2012; Litscher and Wassarman, 2018). Most deep-sea sharks, particularly those in the Squaliformes order (e.g., *Centrophorus* spp. Müller & Henle, 1837, *Deania* spp. Jordan & Snyder, 1902, *Etmopterus* spp. Rafinesque, 1810), are ovoviviparous (i.e., perform lecithotrophic viviparity), while others, such as the catsharks *Galeus* spp. Rafinesque, 1810 and *Apristurus* spp. Garman, 1913, are oviparous (e.g., Iglésias et al., 2002; Blackburn and Hughes, 2024). Deep-sea skates, particularly from the family Rajidae, are also oviparous (e.g., *Dipturus* spp. Rafinesque, 1810). Litter sizes are generally small, with most species producing between two and 20 pups, though the bluntnose sixgill shark *Hexanchus griseus* Bonnaterre, 1788 (Figure 1.2) can produce up to 78 pups per litter (Larson et al., 2011). Additionally, DSE exhibits high maternal investment, with gestation periods lasting from one to 3.5 years in species like the frilled shark *Chlamydoselachus anguineus* Garman, 1884 (Tanaka et al., 1990). These traits result in very low population growth rates, making DSE particularly sensitive to fishing pressures and slow to recover from overexploitation (Musick, 1999; Cortés, 2002; García et al., 2008).

Figure 1.2 Bluntnose sixgill shark *Hexanchus griseus*. Art by Luis Thiem.

The vulnerability of DSE is further compounded by their wide-ranging geographical distribution and long-distance migrations throughout their life cycle (Moura et al., 2014). For example, the Portuguese dogfish *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello, 1864 is thought to have nursery areas off the Mauritanian coast, with mature females undertaking large-scale migrations related to their reproductive cycles (Veríssimo et al., 2011). This extensive range complicates conservation efforts, as fishing operations in different regions may target different segments of the same population.

The ecological significance of elasmobranchs has been increasingly acknowledged given that, as top and mesopredators, they exert top-down regulation on marine ecosystems, influencing both the structure and functioning of food webs (e.g., Ruppert et al., 2013; Barley et al., 2017). In regards of DSE little is known about the role they exert on structuring and functioning of the deep-sea food webs, but they do promote energetic connectivity between neritic, oceanic and deep-sea ecosystems (e.g., Valls et al., 2017; Shipley et al., 2023) highlighting their importance in maintaining ecosystem health. These species possess unique combinations of physiological, morphological, and behavioural traits such as long longevity, slow metabolism, slow swimming speed, large livers, enlarged eyes, bioluminescence, which supports their existence in the deep-sea, contributing to the diversity of ecosystem functions (e.g., Wetherbee and Nichols, 2000; García et al., 2008; Pinte et al., 2020).

Deep-sea elasmobranchs face a range of threats, with overfishing being the primary concern. Of the species assessed, 99.3% are impacted by overfishing, primarily as bycatch in bottom trawling and longlining fisheries but may also be targeted for their liver oil and meat (Finucci et al., 2024). High at-vessel and post-release mortality rates further exacerbate fisheries impact (Brooks et al., 2015; Rodríguez-Cabello and Sánchez, 2017; Talwar et al., 2017; Scacco et al., 2023). Consequently, many populations have experienced catastrophic declines, with species like the spiny dogfish *Squalus acanthias* Linnaeus, 1758 in the Northeast Atlantic reduced to about 5% of its original biomass, and the blue skate *Dipturus batis* (Linnaeus, 1758) locally extinct in some regions (Abdulla, 2004; Hammond and Ellis, 2004). Climate change poses additional threats, as shifts in ocean temperatures and acidification force species to migrate to deeper waters or higher latitudes (Cheung et al., 2013; Nagelkerken and Munday, 2015; Polockzanska et al., 2016). The movement of species into deeper waters has reshaped communities, with low-latitude species now confined to small, deep-water regions (Coulon et al., 2024). These environmental changes are further exacerbating fisheries-related threats, increasing the frequency and severity of extreme events (Daw et al., 2009; Bindoff et al., 2019). Recent studies suggest that elasmobranchs are vulnerable to both ocean warming and acidification, contrary to earlier assumptions (Santos et al., 2021; Hasenei et al., 2023; Coulon et al., 2024). While not as significant as overfishing, pollution also contributes to DSE decline, with 2.5% of species affected by pollution (Finucci et al., 2024). Combined, these factors have driven many species toward extinction, with 37.5% of chondrichthyans currently threatened, including 14.1% of deep-sea species (Dulvy et al., 2021; Finucci et al., 2024). This dire situation underscores the urgent need for comprehensive conservation measures to protect these vulnerable deep-sea species.

1.4 Deep-sea elasmobranchs and interactions with fisheries

Deep-sea elasmobranchs, particularly sharks, have historically been targeted globally for their valuable liver oil, rich in squalene which is used by pharmaceutical and cosmetic industries, as well as for their meat and fins (Finucci et al., 2024). Although only around 12% of all DSE (51 out of 438 species) are specifically targeted in fisheries worldwide, about 35% (21 of 60) are threatened, mainly including species from families Centrophoridae, Squalidae, and Rajidae (Finucci et al., 2024). From the mid-20th century, species like *Centrophorus* spp. were heavily exploited, initially as bycatch in fisheries targeting other deep-sea species such as the grenadier *C. rupestris* and black scabbardfish *Aphanopus carbo* Lowe, 1839 (Clarke et al., 2005; Figueiredo et al., 2005; ICES, 2007) and later through targeted fishing (Gordon, 1999; Hareide et al., 2005). This exploitation was especially conducted by Portuguese and Spanish fleets in the Northeast Atlantic and driven by the commercial value of their squalene-rich liver (Gordon, 1999; Hareide et al., 2005; ICES, 2010). These slow-growing, late-maturing sharks with low reproductive rates are highly vulnerable to overfishing, and their extensive migratory behaviour complicates conservation efforts (Veríssimo et al., 2011; Moura et al., 2014).

As DSE populations began to decline, regulatory actions were introduced to protect these species. In the early 2000s, ICES raised concerns about the unsustainable fishing of deep-sea sharks in the North Atlantic. In response, the EU implemented a zero TAC for several deep-sea shark species in 2010 through the Regulation 1359/2008 aiming to halt targeted fishing and mitigate the population declines. Other regulations followed, reinforcing these protections by continuing the ban on targeted deep-sea shark fisheries and bycatch retention.

Despite the prohibition of targeted fisheries, DSE continues to suffer from bycatch, particularly in trawl and longline operations targeting teleosts and crustaceans such as shrimps and prawns. These fisheries indiscriminately catch non-target species, including DSE, which are consistently discarded, due to their low economic value or imposed prohibitions (Connolly and Kelly, 1996). Globally, half of the total chondrichthyan catch is bycatch, and this number may be even higher if unreported catches are considered (Bonfil, 1994; Stevens, 2000). For example, in some regions, like Ireland and Scotland, deep-sea sharks make up to 54% of the discards in weight in bottom trawls (Connolly and Kelly, 1996). A more recent study estimates that *ca*. 88% of DSE are taken as bycatch in deep-sea fisheries (Finucci et al., 2024).

For most DSE caught as bycatch, estimates on mortality rates are inexistent, but some of the available estimates vary greatly depending on the species, the fishing methods, and on the environmental conditions. A longline study on species like the *C. coelolepis* and *C. squamosus* showed relatively low at-vessel mortality rates ranging from 19% to 39%; however, this study presented low soaking times (2-3h) and the fishing gear was hauled in a much slower speed (0.5 m/s) than when in commercial operations (Rodríguez-Cabello and Sanchez, 2017). In deep-water trawlers DSE mortality may be particularly high with increasing haul duration, increasing codend weight, increasing air exposure (Rodríguez-Cabello et al., 2005; Enever et al., 2009; Heard et al., 2014), larger temperature changes between cold deep-sea habitats (~ 10°C) and warmer surface waters (~ 20°C), which induce thermal stress (Davis, 2002; Gale et al., 2013; Weltersbach and Strehlow, 2013).

Skates face similar threats and are heavily impacted by bottom trawling. Species such as *D. batis* (Figure 1.3) complex and the starry ray *Amblyraja radiata* (Donovan, 1808) and have suffered severe population declines locally or throughout their ranges (Brander, 1981; Casey and Myers, 1998; Walker and Hislop, 1998). For instance, in the Irish sea, *D. batis* has been "brought to the brink of extinction" by trawling (Brander, 1981) and in the North Atlantic, *A. radiata* populations have dwindled due to high bycatch mortality in trawl fisheries (Packer et al., 2003).

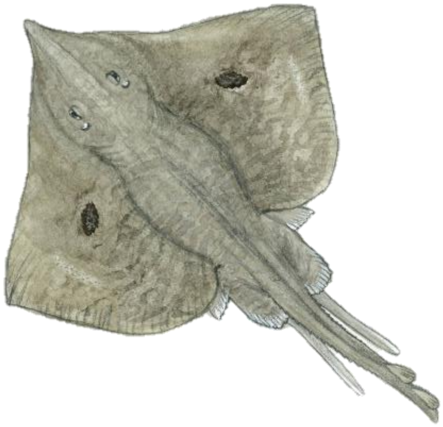

Figure 1.3 Blue skate *Dipturus batis*. Art by Luis Thiem.

Globally, the management of DSE fisheries is, in general, poor, and is primarily guided by international frameworks that promote sustainable fishing practices. One of the leading initiatives is the FAO International Plan of Action for the Conservation and Management of Sharks (IPOA-Sharks), which encourages countries to develop national action plans aimed at preventing the overexploitation of elasmobranchs, including deep-sea species like *Centrophorus spp.* and *C. coelolepis*. However, significantly less measures for deep-sea sharks (n° of measures = 4) are in force than for coastal and pelagic sharks (n° of measures = 102), and skates and rays (n° of measures = 106). Regional Fisheries Management Organizations (RFMOs) play a key role in regulating deep-sea fisheries that interact with DSE. The Convention for the Conservation of Antarctic Marine Livine Resources (CCAMLR) set skates bycatch to 120 tonnes (CCAMLR, 2023) and the Southeast Atlantic Fisheries Organization (SEAFO) have a recommendation on banning directed fisheries of deep-sea sharks until additional information is provided to support sustainable harvesting levels (SEAFO, 2008). Organizations such as the North-East Atlantic Fisheries Commission (NEAFC) and the Northwest Atlantic Fisheries Organization (NAFO) have established measures to protect vulnerable deep-sea species. These regulations include restricting catches, setting fishing limits, and prohibiting fishing in vulnerable habitats, such as seamounts, which are hotspots for DSE. The International Commission for the Conservation of Atlantic Tunas (ICCAT) also implements bycatch mitigation measures‒despite its primary focus on pelagic species ‒ which indirectly benefit deep-sea sharks in longline fisheries. Because bottom trawling is one of the most harmful fishing practices for deep-sea species and has been heavily criticized for its impact on both targeted and non-targeted species, the United Nations General Assembly (UNGA) has called for a reduction in bottom trawling in areas of deep-sea habitats, recognizing the destructive impact this practice has on deep-sea ecosystems and vulnerable species which includes DSE.

In addition to the zero TAC for deep-sea sharks, further European measures also helped reduce direct fishing pressure in DSE. Ban on bottom trawling at depths greater than 800 m since 2016 (Regulation 2016/2336) were set to reduce the impact on deep-sea habitats, hence also reducing the impacts on DSE populations.

Despite regulatory measures aimed at reducing bycatch and discards of DSE, bycatch remains a significant challenge, largely due to the non-selective nature of many fishing practices and non-compliance with regulations. Furthermore, high mortality rates are still observed for many DSE species caught as bycatch, particularly because of their vulnerability to stress, injury, and susceptibility to environmental changes. Therefore, bycatch mitigation measures and strategies to reduce mortality are crucial to protect these vulnerable species.

One of the most effective approaches to mitigate bycatch is to prevent the capture of DSE in the first place. Selective fishing gear plays a critical role in achieving this. For example, circle hooks in longline fisheries have been shown to reduce bycatch by decreasing the likelihood of elasmobranchs swallowing the hook, which in turn reduces injury (Gilman, 2011; Clarke et al., 2014; Gilman and Hall, 2015). Additionally, modified trawl nets with exclusion devices and escape panels allow non-target species, such as skates and sharks, to exit the nets before going to the codend and being hauled to the surface, significantly reducing their bycatch (Isaksen et al., 1992; Hall and Mainprize, 2005; Brewer et al., 2006). Another key strategy is the implementation of time-area closures, which restrict fishing activities in zones or during periods where bycatch rates for DSE are known to be high. This can help reduce encounters between fisheries and DSE populations during their peak activity or vulnerable life stages. Such closures have proven effective in other fisheries and can be tailored to deep-sea habitats where DSE gather (Finucci et al., 2024).

In addition to preventing bycatch, improving the survival rates of DSE once they are captured is crucial. Handling protocols on board fishing vessels are key to this, as proper handling can greatly increase the chances of survival upon release (Gilman et al., 2007; Benoît et al., 2010; Poisson et al., 2016). Best practices for handling DSE include minimizing the duration of the fishing activity and the time the animals are exposed to air, the use of de-hooking tools to safely release them and ensure that they are returned to the water as quickly and gently as possible (Olla et al., 1998; Davis et al., 2001, 2002; Mandelman and Farrington, 2007; Rulifson, 2007). Handling protocols should also focus on reducing physical injury, which is a common cause of mortality in bycaught DSE species (Rodríguez-Cabello and Sanchez, 2017). Crew training on these protocols is essential to ensure that these vulnerable species are handled with care and have the best chance of survival if incidentally caught.

Monitoring of the bycatch is important for improving compliance with mitigation regulations and is basically done by an at-sea observer or by electronic monitoring (EM) systems. Electronic monitoring is one of the most promising tools for monitoring bycatch. These systems are equipped with cameras and sensors, and can continuously record fishing activities on board vessels, providing valuable data on bycatch rates, species identification, and compliance with regulations. Electronic monitoring can complement traditional observer programs, especially in fisheries where human observers may be impractical or costly. By providing comprehensive coverage of fishing activities, EM helps to ensure that bycatch regulations are being followed and provides a reliable means of collecting data on bycatch incidents (EFCA, 2019).

1.5 The Portuguese case study

Portuguese fisheries have a long-standing tradition, deeply rooted in the country’s coastal economy and culture. In fact, according to EU and national estimates, Portugal stands out as the EU country with the highest *per capita* consumption of fishery and aquaculture products (EP, 2023; EUMOFA, 2023). While the global average fish consumption is around 21 kg per capita, Portugal far exceeds this figure, with a consumption rate of 57 kg per capita (Guillen et al., 2019; EP, 2023). This, in combination with the large size of the fishing fleet (3,728 vessels; EP, 2023), reflects the nation's deep dependence on and dedication to its maritime resources.

Portugal's unique geographical and oceanographic characteristics, particularly along the southern coasts, create ideal conditions for a variety of fishing activities, with the crustacean bottom trawling fishery being one of the most economically significant. This fishery is critical to national fisheries, ranking third in terms of national landings (EP, 2023). Crustacean trawlers primarily target high-value species such as the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758), the red shrimp *Aristeus antennatus* (Risso, 1816), and the giant red shrimp *A. foliacea* (Risso, 1827) (Campos et al., 2007). These vessels operate at depths between 200 and 700 m, primarily in the South and Southwest regions (Bueno-Pardo et al., 2017), where the continental shelf widens and slopes into deeper waters, providing favourable conditions for trawling. The South coast experiences higher fishing pressure than the Southwest, driven by its geomorphological advantages and proximity to key landing ports like *Olhão* and *Portimão*, which facilitate trade, particularly with Spain, further boosting the economic importance of this fishery (Bueno-Pardo et al., 2017; Campos et al., 2021).

The Portuguese crustacean bottom trawl fleet, currently consisting of around 25 vessels (data provided by DGRM from the period 2017-2021), benefits from seasonal upwelling, occurring from spring to late summer, which introduces nutrient-rich cold water that enhances marine productivity (Relvas et al., 2007). However, despite the fishery's economic value, bottom trawling is associated with significant environmental concerns, particularly related to bycatch, which in Portugal is generally around 70%, but can be as high as 90% of the total catch, with DSE comprising as much as 40% of the total catch in weight (Borges et al., 2001; Monteiro et al., 2001; Costa et al., 2008).

Bottom trawling scrapes the seafloor, leading to habitat destruction and the displacement of sediment. This process not only disrupts benthic communities but also causes sediment resuspension, which can alter local biogeochemistry and reduce water quality (Benn et al., 2010). Furthermore, submarine canyons and rocky outcrops, common along the West Iberian Margin, including South and Southwest of Portugal, are particularly sensitive to such disturbances, as they host diverse benthic habitats that are crucial for biodiversity (McClain and Barry, 2010; Fernandez-Arcaya et al., 2017). Hence, while the southern coast of Portugal remains a hub for bottom trawling due to its favourable conditions, it is also a hotspot for biodiversity (Gomes et al., 2018) and for that reason, the environmental costs of such impactful activity may be significant.

Despite the increase in studies on DSE over the years (e.g., Gordon 1999; Wetherbee, 2000; Graham et al., 2001; O'Hea et al., 2020; Besnard et al., 2022; Finucci et al., 2024) there are still several key gaps in knowledge surrounding DSE interactions with fisheries, which hinder effective management and conservation. Species-specific bycatch data are often insufficient, as many fisheries reports catches under broad categories like "sharks" or "rays", making it difficult to assess the true impact on DSE species. Because DSE are consistently discarded and given the lack of monitoring, bycatch is frequently underreported, and post-capture mortality data remain incomplete, particularly regarding how different fishing gear and practices affect survival rates (Rodríguez-Cabello and Sanchez, 2017). Another significant gap lies in our understanding of the movement patterns and habitat use of DSE. This lack of knowledge complicates efforts to design effective time-area closures or other habitat protection measures. Moreover, there is limited information on the long-term effects of fisheries on population dynamics, particularly for species with slow reproductive cycles, making stock assessments challenging. Research on the effectiveness of bycatch mitigation strategies for deep-sea species, such as gear modifications and time-area closures, is also lacking, as many of these measures have been developed for coastal or pelagic species. Finally, while EM is a promising tool for tracking bycatch and ensuring compliance with regulations, its implementation in deep-sea fisheries is still in the early stages, and more research is needed to assess its effectiveness in monitoring these vulnerable species.

1.6 Objectives and thesis structure

The main objective of this doctoral study was to assess the impact of crustacean bottom trawling on the ecophysiological conditions and mortality rates of DSE species off the southern coast of Portugal. Additionally, the study aimed to propose solutions for monitoring bycatch and mitigating the impacts of bottom trawling on DSE populations, while also providing a deeper understanding of their ecological and biological characteristics. It was hypothesized that DSE in these regions are negatively impacted by bottom trawling due to several interconnected factors. Firstly, the high intensity of trawling in the study areas (Bueno-Pardo et al., 2021; Campos et al., 2021) results in significant bycatch of DSE species (e.g., Borges et al., 2001; Monteiro et al., 2001; Costa et al., 2008). Secondly, the conservative life-history traits of DSE species make them particularly vulnerable to disturbances caused by trawling activities (e.g., García et al., 2008; Simpfendorfer and Kyne, 2009; Villagra et al., 2022). Finally, their dietary preferences, which include commercially valuable crustaceans (Neves et al., 2007; Ricci et al., 2021), likely increase spatial overlap with trawling operations. This overlap, in turn, justifies the persistent presence of DSE species in crustacean trawl bycatch and intensifies the negative interactions between trawling activities and DSE populations.

To address these objectives, a multidisciplinary approach involving complementary tasks was employed, combining data and insights from various aspects of DSE research. This thesis is structured into three thematic parts encompassing nine chapters (excluding the General Introduction and Final Conclusions). Each part focuses on a key area of investigation, integrating related chapters for a cohesive presentation of findings. **Part I** comprises four chapters (**Chapters**

2, 3, 4, and 5) that provide a comprehensive understanding of the bycatch composition of DSE, their mortality and stress rates, trophic ecology, and the overlap with bottom trawling activities. These chapters lay the groundwork by exposing the extent of the challenges faced by DSE in trawled areas. In response to the challenges identified in Part I, **Part II** includes two chapters (**Chapters 6 and 7**) that explore solutions to monitor DSE bycatch and mitigate the negative impacts of bottom trawling. These chapters emphasize practical approaches to reducing harm to DSE while supporting sustainable fishing practices. The final section, **Part III** (**Chapters 8, 9, and 10**) delves into the biology, ecology, and genetics of rare and poorly studied DSE. These chapters contribute novel insights into the life-history traits and genetic profiles of these species, filling critical knowledge gaps and advancing scientific understanding.

PART I - ECOLOGY OF DEEP-SEA ELASMOBRANCHS AND THEIR INTERACTIONS WITH BOTTOM TRAWL FISHERIES:

CHAPTER 2 is a scientific article that provides data on the composition of DSE in bycatch in the crustacean bottom trawl fisheries in the South and Southwest coasts of Portugal, including species richness, abundance and biomass. Using capture per unit of effort as a proxy, their catch rates were estimated for permitted fishing depths (above 800 m) and non-permitted fishing depths (below 800 m).

> **Graça Aranha, Sofia**; da Rocha, Pedro; Marsili, Tiago; Barkai, Amos; Queiroz, Nuno; Dias, Ester & Teodósio, Alexandra (2024). Unravelling the deep: assessing the bycatch of deep-sea elasmobranchs in crustacean bottom trawl fisheries in Portugal. **Under revision at *Marine Policy***

CHAPTER 3 is a scientific article that explored the impact of crustacean bottom trawling on deep-sea sharks' condition by evaluating their at-vessel mortality rates and stress levels using statistical models and biochemical markers in blood plasma, respectively.

> **Graça Aranha, Sofia**; Marsili, Tiago; da Rocha, Pedro; Modesto, Teresa; Guerreiro, Pedro Miguel; Tambutté, Aurélien; Alves, Alexandra; Teodósio, Alexandra & Dias, Ester (2024). Under Pressure: Deep-Sea Elasmobranchs' Experience High Mortality and Stress in a Crustacean Trawling Fishery. **Under revision at *Frontiers in Fish Science, Elasmobranch Science*, topic "*Women in Elasmobranchs Science"***

CHAPTER 4 is a scientific article that investigated some aspects of the trophic ecology of deep-sea sharks in the Southwest coast of Portugal. Potential groups of prey (including groups of crustaceans of economic interest) were identified using stable isotopes, and their nutritional condition and potential overlap between their foraging grounds and bottom trawling fishing areas was evaluated using RNA:DNA ratios.

Graça Aranha, Sofia; Teodósio, Alexandra; Baptista, Vânia; Erzini, Karim, & Dias, Ester (2023). A glimpse into the trophic ecology of deep-water sharks in an important crustacean fishing ground. *Journal of Fish Biology,* 102(3), 655-668. https://onlinelibrary.wiley.com/doi/10.1111/jfb.15306
****FSBI Huntingford medal Winner for best research article from an early career in 2023****

CHAPTER 5 is a follow-up scientific article of chapter 4. Information on the trophic position and trophic niche size of several DSE from the South and Southwest coasts of Portugal was obtained using stable isotopes. Niche overlap between species and within species (sex, maturity stage) was also evaluated according to the study area. The RNA:DNA ratios were used to determine DSE nutritional condition and the potential overlap between their foraging grounds and bottom trawling fishing areas.

Graça Aranha, Sofia; Teodósio, Alexandra; Marsili, Tiago; Pires Da Rocha, Pedro; Baptista, Vânia; Cruz, Joana; Figueiredo, Ivone & Dias, Ester (2024). Trophic ecology of deep-sea elasmobranchs and notes on the overlap with crustacean bottom trawl Fisheries. ** *In preparation***

PART II – MONITORING AND MITIGATION OF IMPACTS IN DEEP-SEA ELASMOBRANCHS:

CHAPTER 6 is a scientific article describing the usefulness of an integrated electronic reporting and monitoring tool (iEMR Olrac) to remotely identify DSE. For this purpose, video imagery collected onboard during the field surveys conducted for this doctoral study were analyzed. An extensive discussion on the strengths and constraints of this method is provided. The candidate is the senior author of this publication.

da Rocha, Pedro; Marsili, Tiago; Barkai, Amos; Figueiredo, Ivone; Dias, Ester; Modesto, Teresa; Relvas, Paulo; Teodósio, Alexandra & **Graça Aranha, Sofia**

(2023). Remote monitoring the bycatch of demersal elasmobranchs using video imagery: a case study from a deep-water crustacean trawler. **Under revision at *Marine Ecology Progress Series***

CHAPTER 7 is a protocol providing the best practices to handle DSE onboard crustacean bottom trawlers. This protocol not only provides graphic images on best handling practices but also highlights the main results from the project Delasmop - embedded in the articles that composes this thesis - which also suggest best practices to avoid DSE bycatch, and to minimize their at-vessel mortality. This protocol was adapted to fit this thesis layout, so text and figures are adapted accordingly. A more accessible form of communication was used in this protocol since the final product is intended for the general public, e.g., DSE are referred to according to the species common name and not the species scientific name. There is an online Portuguese version available for the general public, which also contains illustrations of all the DSE found in Portugal, which were not included in the body of this thesis. There is also the complete English version, which is available in the folder "Delasmop" of my Open Science Framework repository (Graça Aranha, 2024).

Graça Aranha, Sofia; Teodósio, Alexandra; & Dias, Ester (2024). Handling protocol for sharks and skates for bottom trawl fishing vessels: case of study on a crustacean trawler in southern Portugal within the scope of the Delasmop project "Deep-sea elasmobranchs of Portugal" [link online]

PART III – FURTHER INSIGHTS INTO THE KNOWLEDGE OF POORLY KNOWN DEEP-SEA ELASMOBRANCHS:

CHAPTER 8 is a scientific article providing biological and ecological information on a data-poor deep-sea shark, endemic of the northeast Atlantic, the *Oxynotus paradoxus,* where a new depth record was reported, and length-at-maturity of females was suggested for the first time.

Graça Aranha, Sofia; Dias, Ester; Marsili, Tiago; Pires Da Rocha, Pedro; Teodósio, Alexandra; & Figueiredo, Ivone (2024). New insights on the ecology and biology of the rare *Oxynotus paradoxus* from recent records. *Cybium,* 48(3): 211-217. https://doi.org/10.26028/CYBIUM/2024-013

CHAPTER 9 and **CHAPTER 10** are scientific articles resulting from collaborations with researchers from CIIMAR from the University of Porto, Portugal, in which the mitogenome of the shark *O. paradoxus* and of the skate *Neoraja iberica* were assessed for the first time.

> Matos, Ana; Gomes-dos-Santos, André; **Graça Aranha, Sofia**; Dias, Ester; Verissimo, Ana; Teodósio, Alexandra; Figueiredo, Ivone; C. Castro, L. Filipe; & Froufe, Elsa (2024). Dataset of the complete mitogenome of the deep-sea sailfin roughshark, *Oxynotus paradoxus* Frade, 1929. *Data in Brief*, 52, 109836. https://doi.org/10.1016/j.dib.2023.109836
>
> Gomes-dos-Santos, André; Machado, André; **Graça Aranha, Sofia**; Dias, Ester; Verissimo, Ana; C. Castro, L. Filipe, & Froufe, Elsa (2021). The complete mitochondrial genome of the endemic Iberian pygmy skate *Neoraja iberica* Stehmann, Séret, Costa & Baro 2008 (Elasmobranchii, Rajidae). *Mitochondrial DNA B Resour*ces, 6(3), 848-850 https://doi.org/10.1080/23802359.2021.1884030

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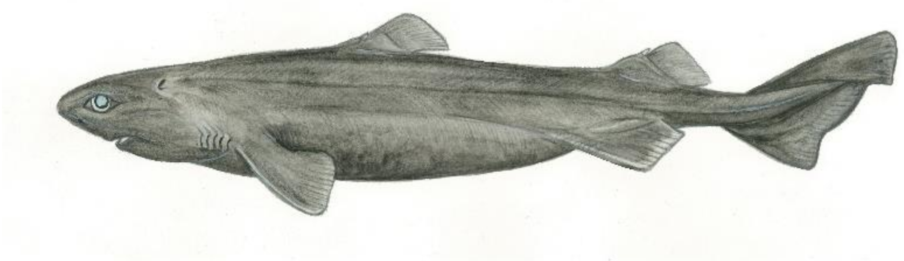
PART I - ECOLOGY OF DEEP-SEA ELASMOBRANCHS AND THEIR INTERACTIONS WITH BOTTOM TRAWL FISHERIES



Chapter 2: UNRAVELLING THE DEEP: ASSESSING THE BYCATCH OF DEEP-SEA ELASMOBRANCHS IN CRUSTACEAN BOTTOM TRAWL FISHERIES IN PORTUGAL

Graça Aranha, Sofia; da Rocha, Pedro; Marsili, Tiago; Barkai, Amos; Queiroz, Nuno; Dias, Ester & Teodósio, Alexandra. Unravelling the deep: assessing the bycatch of deep-sea elasmobranchs in crustacean bottom trawl fisheries in Portugal. ***Under revision at Marine Policy***

Abstract

Deep-sea elasmobranchs (DSE) play a crucial role in marine ecosystems. However, they are poorly studied and face threats from overfishing, emphasizing an urgent need for improved scientific information, monitoring, and management strategies to reduce their bycatch. This study aimed to assess DSE bycatch from crustacean bottom trawling in southern Portugal, considering depths above and below 800 m (Regulation 2016/2336). Potential bycatch of deep-sea sharks was evaluated using *in situ* observations from the months of February and March and extrapolated for the fishing ban period (2017-2022). A total of 1559 specimens belonging to 18 DSE species were collected from 77 hauls between June 2020 and May 2022. Despite trawlers preferences for fishing above 800 m in the South, fishing below 800 m in the Southwest resulted in increased bycatch of DSE, including protected, uncommon, and endangered species such as *Deania calceus, Mitsukurina owstoni*, and *Centroscymnus coelolepis*. Furthermore, the areas and depth strata occupied by species like *Galeus melastomus* and *Scymnodon ringens*, suggested habitat flexibility, while others showed an apparent preference for specific depths and areas. These findings highlight the complexity of managing DSE populations amidst fishing pressures and depth restrictions. Despite the ban imposed to fishing below 800 m, bottom trawling persisted, leading to a potential elevated bycatch of deep-sea sharks for the months of February and March. This study emphasizes the urgency for improved enforcement of regulations in Portuguese waters and calls for the implementation of effective bycatch mitigation and fisheries management practices to safeguard DSE populations.

Keywords: CPUE, sharks, skates, discards, SW Iberian

2.1 Introduction

Elasmobranchs (sharks, skates and rays) constitute an ancient group within marine ecosystems, boasting a remarkable evolutionary history spanning over 400 million years (Camhi et al., 2008). They play a pivotal role in shaping marine communities by influencing the mortality rates and behaviour of various organisms (e.g., Myers et al., 2007; Heithaus et al., 2007; Last et al., 2016). Their life history characteristics, including late maturity, long lifespans, extended gestation periods, and low offspring production, makes them highly susceptible to anthropogenic disturbances such as fishing pressure even at low levels (Agnew et al., 2000; Dulvy et al., 2008; Tiralongo et al., 2018; Jabado, 2019; D'Iglio et al., 2021). As a result, global elasmobranchs populations are experiencing fast declines (Pacoureau et al., 2021). A recent reassessment of 1,147 elasmobranch species (Dulvy et al., 2021) showed an alarming increase in the percentage of threatened species since the first assessment in 2014 (Dulvy et al., 2014). Overfishing is one of the main threats to elasmobranchs' populations, which includes industrial fishing bycatch (Dulvy et al., 2021; Juan-Jordá et al., 2022) − i.e., the unintentional catch of non-targeted species that is either unused or unmanaged (Davies et al., 2009). Industrial fisheries, such as bottom trawling, exert significant impacts by physically altering the seafloor, leading to habitat destruction, and sediment resuspension (e.g., Friedlander et al., 1999; Connolly and Kelly, 1996; Clark et al., 2015). These activities also affect sediment biogeochemistry (Borges et al., 2001) and result in the removal of substantial amounts of fish and biomass (Victorero et al., 2018; Tiralongo et al., 2021). Moreover, bottom trawling accounts for over a third of discarded bycatch worldwide (Lobo et al., 2010; Pérez Roda et al., 2019), with crustacean bottom trawling having the highest impact, followed by fish bottom trawling (Borges et al., 2001; Rijnsdorp et al., 2020). In the International Council for the Exploration of the Sea (ICES) ecoregions, bottom trawling stood out as the fishing activity responsible for some of the highest bycatch values of elasmobranch species (e.g., Yaglioglu et al., 2015; Carpentieri et al., 2021).

Elasmobranchs bycatch is a worrying issue in the bottom trawling fishery due to the net poor selectivity and high at-vessel and post-release mortality rates (Enever et al., 2009; Benoît et al., 2010; Rodríguez-Cabello et al., 2005; Scacco et al., 2023), which, associated with their low resilience to fishing pressures, presents conservation concerns. Since bottom trawlers do not specifically target elasmobranchs, the bycatch rate is anticipated to be irregular and variable over time, even when using the same fishing gear and within the same country and subregion

(Vannuccini, 1999; Serena et al., 2009; Bradai et al., 2012, 2018; Ramírez-Amaro et al., 2017). The bycatch of elasmobranchs, contrarily to that of seabirds, marine mammals, and chelonians (with some exceptions) may be retained if the captured species is of commercial interest and has no regulatory concern. However, in European Union waters, species that do not meet these criteria, such as deep-sea elasmobranchs (DSE), must be discarded back at sea (Regulation 2024/257). The DSE group, is characterized by sharks and skates that generally inhabit depths below 200 m, mainly below 500 m (e.g., M.W. Clarke et al., 2003; ICES, 2020; O'Hea et al., 2020) and are meso-top-predators, important for the balance of local food webs (Cortés, 1999; Churchill et al., 2015; Graça Aranha et al., 2023). According to the European legislation, deep-sea sharks are prohibited from being targeted by commercial fisheries (Regulation 2018/2025), and since 2010, their Total Allowable Catch (TAC) has been set to zero (Regulation 1225/2010). Despite these regulations, they continue to be caught as bycatch in significant numbers and discarded by various fisheries (Borges et al., 2001; Suuronen and Gilman, 2020), especially by bottom trawlers (Brčić et al., 2015; ICES, 2020), leading to a reduction in their abundance and diversity (Ferretti et al., 2013; Serrat et al., 2023). This highlights the need for improved monitoring and management strategies to reduce the bycatch and protect these species.

Regionally, at the southern of mainland Portugal, crustacean bottom trawling is a socioeconomic important fishery (Cascalho et al., 1984; Pestana, 1991; Borges et al., 2001; Pita et al., 2001; Bueno-Pardo et al., 2017). The southern coast, which includes the South and Southwest subareas, is a crucial area for the bottom trawl fishery due to widening of the continental shelf and more uniform bathymetry that increase the access to greater depths (M. J. Figueiredo, 1989). Since 1970s this region is the most important fishing ground for crustaceans in Portugal, mainly targeting the rose shrimp *Parapenaeus longirostris* and the Norway lobster *Nephrops norvegicus* which are found in areas on the continental shelf and upper slope down to 500-600 m (S.E.P., 1984). In the 1980s, technological advancements allowed expanding this fishery to greater depths, targeting species that were previously considered unimportant, such as the shrimps *Aristeus antennatus*, *Aristaeomorpha foliacea,* and *Aristeopsis edwardsiana* (Campos et al., 2021). However, a significant portion of the catch of *A. edwardsiana* (currently highly valued) originates from fishing grounds that are well beyond the 800 m depth permitted by law in EU waters where bottom trawlers are banned from fishing in the NE Atlantic since 2017 (Regulation 2016/2336; Campos et al., 2021). Furthermore, DSE, especially uncommon and endangered sharks, are often captured in waters surpassing 500 m depth (I. Figueiredo et al., 2005; Ramírez-Amaro et al., 2015; Moura et al., 2018; ICES, 2020). In fact, deep-sea sharks' species such as *Deania calceus*, *Centroselachus crepidater*, and *Centrophorus granulosus* rank among the top 20 most landed species associated with deep-sea trawling activities at depths above 800 m in southern Portugal (Campos et al., 2021). While several studies delved into the bycatch of DSE (e.g., Borges et al., 2001; Monteiro et al., 2001; Coelho et al., 2003; Coelho and Erzini, 2008; Costa et al., 2008; Moura et al., 2018; Campos et al., 2021), to our knowledge, none has provided information on the discarded bycatch of DSE by species and metier at depths below 800 m in this area. This hinders our understanding of the extent of its impact on DSE populations especially if fishers maintain this activity at non-permitted depths. Hence, the present study aimed to evaluate the fishing effort and bycatch of DSE in the crustacean bottom trawl fishery along the South (at depths above 800 m) and Southwest (at depths above and below 800 m) coasts of Portugal, between the period 2020-2022 (*in situ* data). This was achieved by determining the prevalence and diversity of DSE species in relation to the fishing effort among the studied subareas and depth stratum. It was also examined whether fishing activities conducted at depths exceeding the regulated limit (below 800 m), primarily targeting economically valuable deep-sea shrimps (i.e., *A. antennatus*, *A. foliacea*, *A. edwardsiana*), could result in increased DSE bycatch (which is compulsorily discarded) in relation to the total catch. Furthermore, the potential bycatch of deep-sea sharks below 800 m after the fishing ban (2017-2021) was estimated using extrapolated data from shark counts and weights in the Southwest subarea. This estimation focused on the months of February and March, aligning with the periods when *in situ* data was collected—specifically February 2018 (Graça Aranha et al., 2023) and March 2021. The analysis incorporated fishing effort data from bottom trawlers operating in the same subareas, obtained from the Global Fishing Watch website (GFW; https://globalfishingwatch.org), using the Automatic Identification System (AIS).

2.2 Materials and methods

2.2.1 *In situ* data collection

The study was conducted off the southern Portuguese coast and for the analysis's purposes, it was divided in two subareas: South (37°-36°N; 9°-7.5°W) and Southwest (39°-37° N; 9°-11° W, Figure 2). Detailed catch data was collected between June 2020 and May 2022 onboard a commercial crustacean bottom trawler. Ten fishing trips were opportunistically conducted, totalling 77 hauls, 351 h of fishing effort in approximately 35 days (Table 1). The vessel presents two bottom-trawl nets with a codend diamond mesh of 55 and 70 mm for targeting shrimps/prawns, and Norway lobster, respectively. The fishing speed varied between 1.5 and 3.7 nm/h and the duration of the fishing hauls varied between 2.3 and 8.6 h at depths of 96-810 m in the South and 403-1244 m in the Southwest (excluding depths of 800-1200 m where no sampling was conducted given the opportunistic nature of the data collection).

The fishing effort unit corresponds to the fishing haul duration (in hours), and it was calculated from the moment the net reached the bottom of the ocean until the moment it started to be lifted. The fishing effort and depth (m) were registered using the Electronic Logbook (eLog) Olrac Dynamic Data logger (Olrac DDL®) and a mini DST-CTD logic® Star-Oddi® attached to the net, respectively. Geographic coordinates were registered using a GPS at the beginning and end of each fishing haul. The target species were divided into the following categories: shrimp (*A. foliacea* and *A. antennatus* in the South and *A. foliacea, A. antennatus*, and *A. edwardsiana* in the Southwest), Norway lobster (*N. norvegicus*), prawn (*P. longirostris* and *P. monodon*). For each fishing haul, the total weight of the catch inside the net (in kg) was visually estimated by the skipper.

After the end of each fishing haul, DSE specimens were immediately collected from the sorting table, identified (Compagno et al., 2005; Last et al., 2016), measured [total length from the tip of the snout to the tip of the caudal fin (0.5 cm)], and weighed (to the nearest 0.5 kg). The information was registered in the Olrac DDL® eLog for further analysis. All the DSE were discarded by fishers.

2.2.2 Data analysis

To understand if the fishing occurring at depths below 800 m was contributing to increasing DSE bycatch, the proportion of DSE relatively to the total catch (in weight) was determined by subareas and depth stratum (i.e., at < 800 m and > 800 m) in the Southwest; in the South there was no bottom trawling conducted below 800 m depth.

The total DSE bycatch in number (n) and weight (kg) per fishing effort (hour) were calculated in each subarea and by depth stratum and are further denoted as CPUE n (given in n/h), CPUE kg (given in kg/h), respectively. The number of DSE species (diversity), and the CPUE n (abundance) and CPUE kg (biomass) by species, were also determined by subarea and depth stratum and were given for all the DSE species caught during this study.

To compare the CPUE n and CPUE kg between species in a given subarea, a Kruskal-Wallis followed by a Dunn-test (using p-adjusted Bonferroni) were conducted. The CPUE n and CPUE kg values were compared between subareas and within species at depths above 800 m using a Mann-Whitney test. Only species that presented five or more CPUE observations were included in these analyses. These tests were done after checking for the assumptions of normality and homoscedasticity of residuals.

2.2.3 Shark bycatch estimation below 800 m after the ban

To estimate the bycatch (counts and weight) of deep-sea sharks at depths below 800 m in the Southwest since the fishing ban in 2017, we followed the methodology outlined by Mucientes et al. (2022). The approach used *in situ* CPUE data on deep-sea sharks and fishing effort retrieved from the open-source GFW website. The *in situ* CPUE n and kg of deep-sea sharks, was collected in February 2018, where five hauls totalling *ca*. 25 h of fishing effort were conducted within the coordinates 37°50’45’’N to 38°9’44’’N and 9°35’12’’W to 9°23’15’’W (Graça Aranha et al., 2023), and in March 2021, with six hauls totalling *ca*. 47 h of fishing effort within the coordinates 37°12’41’’N to 37°41’28’’N and 9°35’21’’W to 9°28’26’’W (present study). The commercial vessel used for this purpose provides a good representation of the crustacean bottom trawlers that fish at depths below 800 m in the Southwest subarea. This is a small fleet of ten vessels (*ca*. 40% of the Portuguese crustacean bottom trawlers) with an average length of 22.5 ± 2.93 m and average gross tonnage of 145.5 ± 53.77 tons (GFW). To obtain fleetwide fishing effort for the same areas and months from the post-ban period (i.e. 2017-2021), AIS data was retrieved from the GFW website. First, the period was selected for February and March 2017- 2021. Second, to select only AIS data from Portuguese trawling vessels, the following filters were applied: “Flags = Portugal” and “Gear types = Trawlers”. Zooming in to the relevant location using the above mentioned *in situ* coordinates, fishing effort in hours and the number of vessels operating in the area were extracted from each cell. Then, the total hours (mean ± S.D.) of fishing effort were computed for each month. Finally, to estimate the potential bycatch of deep-sea sharks in numbers and weight for the studied subareas and the years following the prohibition, we multiplied the *in situ* CPUE n and kg by the corresponding fleetwide fishing effort total hours (mean ± S.D.) of the obtained from GFW.

All statistical analyses were conducted with the open-source statistical language R (R Development Core Team, 2023).

2.3 Results

2.3.1 *In situ* data

The fishing effort was approximately three times higher in the South (259 h) than in the Southwest (92 h), covering all seasons of the year in the South, whilst in the Southwest sampling was only conducted in the Summer (< 800 m) and Winter (< 800 m and > 800 m). A rough comparison of the fishing effort only at depths above 800 m, showed that effort was *ca*. six times higher in the South than in the Southwest (Table 1). Hauls at depths above 800 m primarily targeted Norway lobster (*N. norvegicus*), prawn (*P. longirostris* and *P. monodon)*, and shrimp (*A. foliacea* and *A. antennatus)*, while those at depths below 800 m focused primarily on shrimps (*A. foliacea*, *A. antennatus* and *A. edwardsiana;* Table 2.1).

Table 2.1 For each reported subarea (South and Southwest) and depths (< 800 m and > 800 m), the targets of the crustacean bottom trawl fishery were Norway lobster (NL), prawn (P) and shrimp (SR). The number of hauls is also given by subarea and depth stratum as well as the number of deep-sea elasmobranchs (DSE) species. The fishing effort in hours, and the capture per unit effort of the number and weight of DSE specimens (CPUE n and CPUE kg, respectively) are reported as the median with the range of values in parenthesis. No fishing activity occurred below 800 m in the South, during the sampling period.

Subarea	**South**	**Southwest**	
Depth (m)	< 800	< 800	> 800
Seasons	All	Summer, Winter	Winter
Targets	NL, P, SR	NL, P, SR	SR
Hauls (n)	61	10	6
DSE spp.	14	12	14
Effort (h)	4.0 (2.7 - 6.3)	4.7 (2.9 - 5.6)	7.7 (7.3 - 8.6)
CPUE n	0.9 (0 – 55.4)	2.6 (0.2 – 11.8)	3.3 (2.2 – 6.0)
CPUE kg	0.3 (0 – 11.6)	4.2 (0.3 – 9.3)	11.5 (6.2 – 18.5)

A total of 1559 specimens of DSE were caught, belonging to 18 species (5 orders and 9 families): 15 sharks and three skates. Most of the specimens were collected at depths below 500 m. Within the Southwest subarea, shallower depths (< 800 m) contained a lower diversity in relation to deeper areas (> 800 m, Table 1). The proportion of hauls catching DSE was 71% in the

South and 100% in the Southwest. The weight of discarded DSE in relation to the total catch varied between 0 – 47% (median 1.05) above 800 m in the South, whilst for the Southwest values varied between 1 – 34% (median 12.21) above 800 m and 25 – 58% (median 33.37) below 800 m (Figure 2.1).

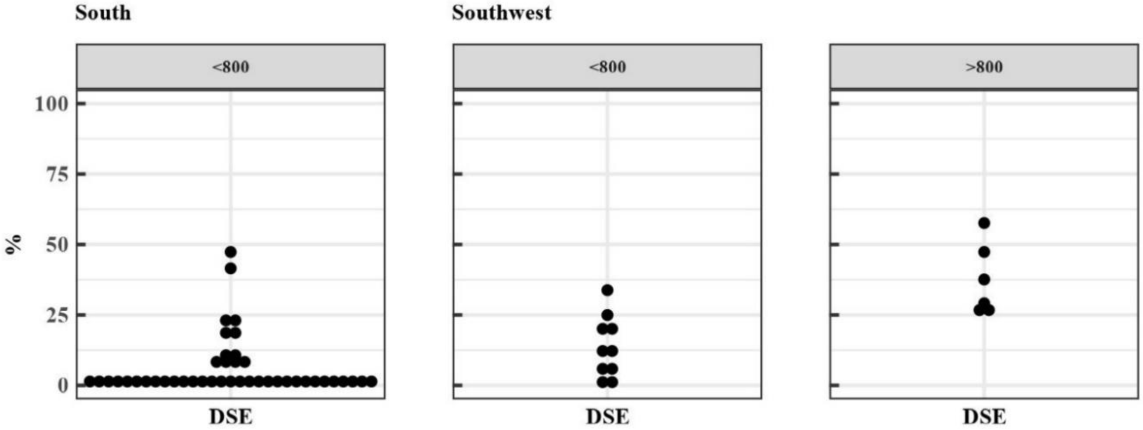


Figure 2.1 Proportion (%) of the discarded bycatch weight of deep-sea elasmobranchs (DSE) in relation to the total catch weight of a crustacean bottom trawler in the South and Southwest coasts of Portugal at different depths (< 800 m and > 800 m). Each point corresponds to a fishing haul.

In the South the highest CPUE n (median: 0.88, IQR: 3.32) was observed between 500-800 m depth mostly between the cities of Portimão and Sagres and within and surrounding the Portimão canyon (Figure 2.2a). The CPUE kg in the South (median: 0.25, IQR: 0.99) followed the overall pattern of the CPUE n. However, higher CPUE kg was found nearby the Portimão canyon (~800 m), when compared to the other depths above 800 m (Figure 2.2b). At the Southwest, CPUE n and CPUE kg values were the highest at depths below 800 m (median: 6.27, IQR: 5.36; Figure 2.2b).

Galeus melastomus and *S. ringens* are both small to medium-sized species of least concern conservation status (Table 2.2). *Galeus melastomus* showed the highest CPUE n and CPUE kg in the South and were commonly caught in groups across both subareas and depth stratum in the Southwest (Table 2.2). It exhibited peak CPUE n at 500-600 m in the South and higher CPUE kg above 800 m in the Southwest (Figure 2.2). In the South it presented significantly higher CPUE n (median: 1.2, IQR: 1.74) and CPUE kg (median: 0.19, IQR: 0.45) compared to *D. oxyrinchus* (median: 0.28, IQR: 0.25) and *E. spinax* (median: 0.07, IQR: 0.07) respectively (Appendix 2.2); however, the CPUE n and CPUE kg of this species between subareas at depths above 800 m were not significantly different (Appendix 2.1). *Scymnodon ringens* presented the highest CPUE n and second-highest CPUE kg in the Southwest, also commonly caught in groups across both subareas and depth stratum (Figure 2.2; Table 2.2). It showed peak CPUE n around 800 m in the Portimão canyon and below 800 m in the Southwest (Figure 2.2). Exhibited significantly greater CPUE kg (median: 0.32, IQR: 0.94) than *E. spinax* and *Galeus atlanticus* (median: 0.03, IQR: 0.06) (Appendix 2.2) in the Southwest. The other least concern species were exclusively caught in specific areas. The skate *N. iberica*, was exclusively caught in the South with some of the lowest CPUE n values (Table 2.2). *Centroselachus crepidater*, was exclusively caught in the Southwest, below 800 m depth and in groups. It presented high CPUE n values despite being limited to one subarea and depth stratum (Figure 2.2; Table 2.2).

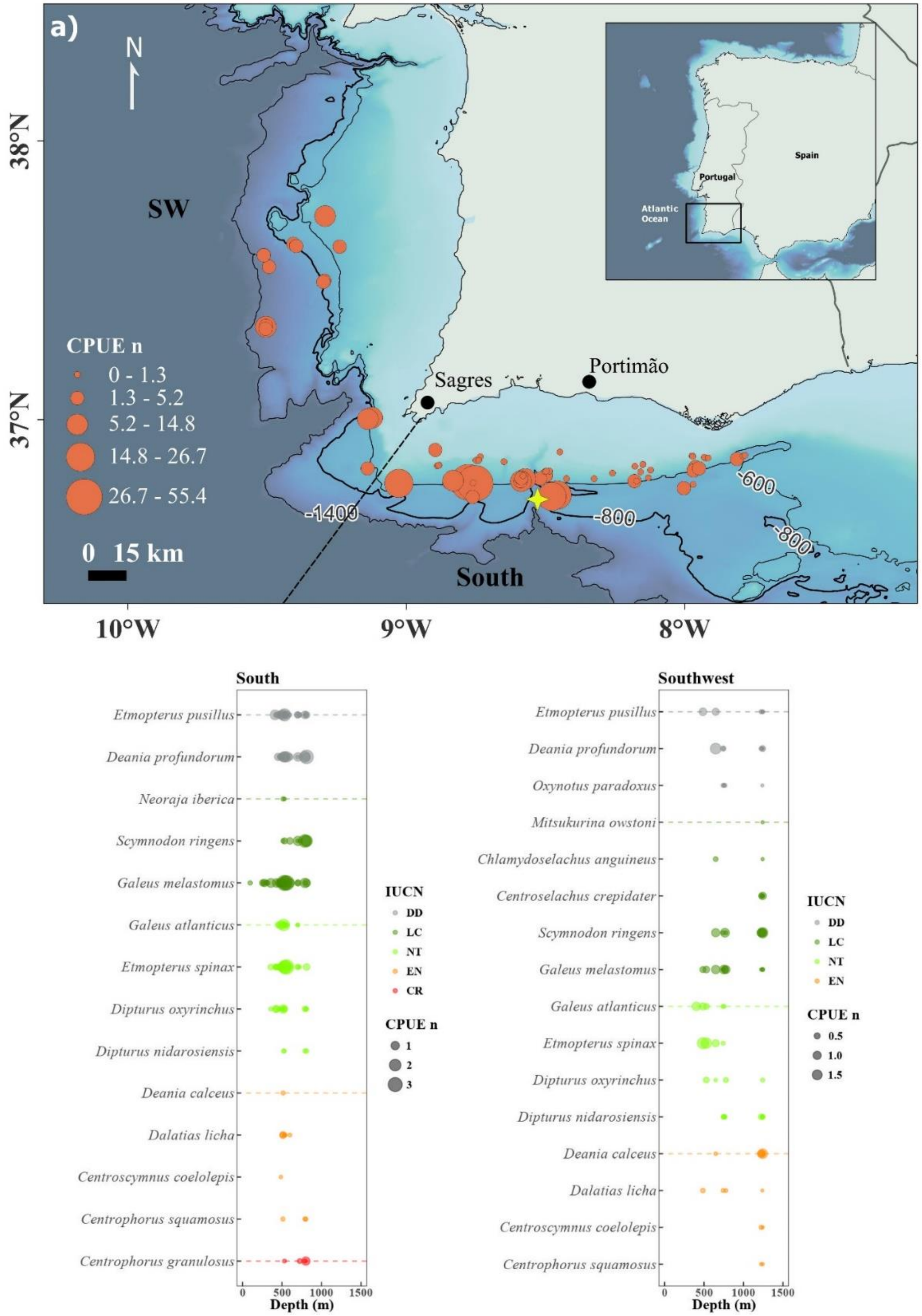
Etmopterus spinax, *D. nidarosiensis*, *D. oxyrinchus*, and *G. atlanticus* are classified as near threatened (Table 2.2). *Etmopterus spinax*, a small-sized species, showed the second-highest CPUE n in the South, peaking at 500-600 m depth (Figure 2.2; Table 2.2). It was commonly caught in groups, but only above 800 m at both subareas (Table 2.2). *Dipturus nidarosiensis*, a large-bodied skate, was caught individually, in pairs, or groups across both subareas and depth stratum in the Southwest (Table 2.2). It presented low CPUE n but higher CPUE kg at both subareas in comparison with the other species, with the highest CPUE kg in the Southwest (Figure 2.2; Table 2.2). In fact, in the Southwest, it presented the highest CPUE kg (median: 3.13, IQR: 0.72) among species, significantly higher than *D. profundorum* (median: 0.09, IQR: 0.03), *E. pusillus* (median: 0.08, IQR: 0.07), *G. atlanticus* (median: 0.04, IQR: 0.1), and *G. melastomus* (median: 0.17, IQR: 0.47) (Appendix 2.1). *Dipturus oxyrinchus*, a medium-sized skate, was caught in various group sizes across both subareas and depth stratum in the Southwest, primarily above 800 m (Table 2.2). *Galeus atlanticus*, a small-sized species, was commonly caught in groups at both subareas but it was not present below 800 m in the Southwest (Table 2.2). The statistical comparisons performed on the CPUE n and CPUE kg values of this species between the studied subareas at depths above 800 m did not show significant differences (Appendix 2.1).

Deania profundorum and *E. pusillus* are both data deficient, small-sized species (Table 2.2). *Deania profundorum* showed high CPUE n in both subareas and was commonly caught in groups (Table 2.2). In the South, its highest CPUE n was around 500-600 m and 800 m in the Portimão Canyon, with heavier specimens above 800 m in the Southwest (Figure 2.2). *Etmopterus pusillus* was also commonly caught in groups across both subareas and depth stratum in the

Southwest, primarily above 800 m with peak CPUE n around 500-600 m in the South (Figure 2.2; Table 2.2).

Deania calceus, *Centroscymnus coelolepis*, *Dalatias licha*, and *Centrophorus squamosus* are classified as endangered (Table 2.2). *Deania calceus* showed low CPUE n and CPUE kg in the South but high values in the Southwest and was commonly caught in groups below 800 m (Figure 2.2; Table 2.2). *Centroscymnus coelolepis* presented low CPUE n and CPUE kg, was caught individually or in pairs only below 800 m in the Southwest (Table 2.2). *Dalatias licha* was caught in groups with different sizes across both subareas and depth stratum in the Southwest, with higher CPUE kg around 500 m depth in the South and 800 m in the Southwest (Figure 2.2; Table 2.2). *Centrophorus squamosus* was caught individually at both subareas, appearing only below 800 m in the Southwest, with higher CPUE kg around 800 m depth in the South (Figure 2.2; Table 2.2). *Centrophorus granulosus*, a critically endangered species, was exclusively caught in the South (Table 2.2). Despite generally low CPUE n, it showed high CPUE n and some of the highest CPUE kg around 800 m depth in the Portimão canyon (Figure 2.2).

Chlamydoselachus anguineus, *M. owstoni*, and *O. paradoxus* are rare species however the first two are listed as least concern and *O. paradoxus* is data deficient (Table 2.2). They were exclusively caught in the Southwest, mainly or exclusively below 800 m depth, generally individually (Table 2.2). While they presented some of the lowest CPUE n and CPUE kg values, *M. owstoni* showed relatively high CPUE kg values (Figure 2.2; Table 2.2).


a)
N
38°N
37°N
10°W
9°W
8°W
SW
South
Sagres
Portimão
CPUE n
0 - 1.3
1.3 - 5.2
5.2 - 14.8
14.8 - 26.7
26.7 - 55.4
0 15 km
-1400
-800
-600
-800
Spain
Portugal
Atlantic Ocean
South
Etmopterus pusillus
Deania profundorum
Neoraja iberica
Scymnodon ringens
Galeus melastomus
Galeus atlanticus
Etmopterus spinax
Dipturus oxyrinchus
Dipturus nidarosiensis
Deania calceus
Dalatias licha
Centroscymnus coelolepis
Centrophorus squamosus
Centrophorus granulosus
0
500
1000
1500
Depth (m)
IUCN
DD
LC
NT
EN
CR
CPUE n
1
2
3
Southwest
Etmopterus pusillus
Deania profundorum
Oxynotus paradoxus
Mitsukurina owstoni
Chlamydoselachus anguineus
Centroselachus crepidater
Scymnodon ringens
Galeus melastomus
Galeus atlanticus
Etmopterus spinax
Dipturus oxyrinchus
Dipturus nidarosiensis
Deania calceus
Dalatias licha
Centroscymnus coelolepis
Centrophorus squamosus
0
500
1000
1500
Depth (m)
IUCN
DD
LC
NT
EN
CPUE n
0.5
1.0
1.5

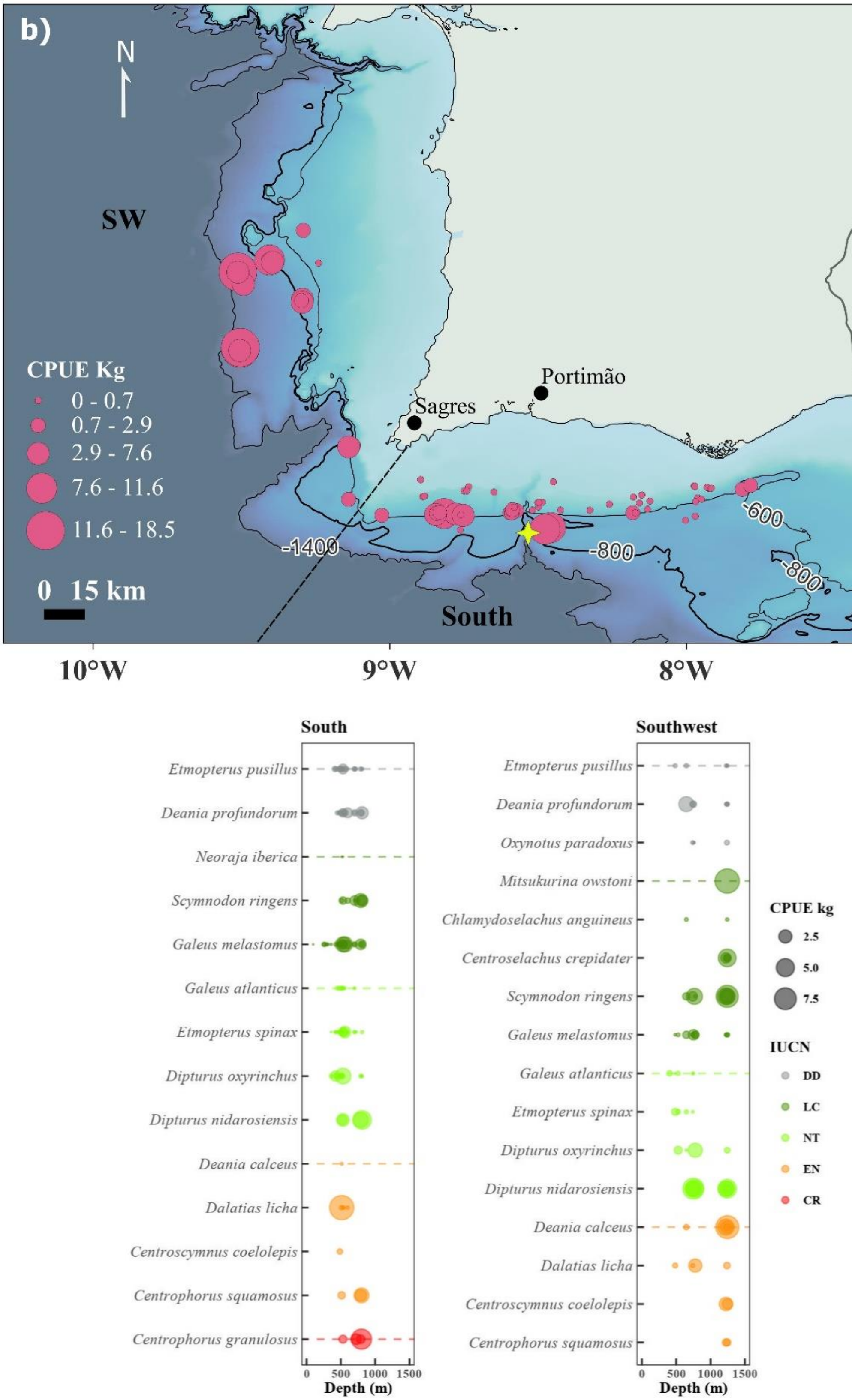

b)
N
SW
CPUE Kg
0 - 0.7
0.7 - 2.9
2.9 - 7.6
7.6 - 11.6
11.6 - 18.5
0 15 km
Portimão
Sagres
-1400
-600
-800
-800
South
38°N
37°N
10°W
9°W
8°W
South
Etmopterus pusillus
Deania profundorum
Neoraja iberica
Scymnodon ringens
Galeus melastomus
Galeus atlanticus
Etmopterus spinax
Dipturus oxyrinchus
Dipturus nidarosiensis
Deania calceus
Dalatias licha
Centroscymnus coelolepis
Centrophorus squamosus
Centrophorus granulosus
0 500 1000 1500
Depth (m)
Southwest
Etmopterus pusillus
Deania profundorum
Oxynotus paradoxus
Mitsukurina owstoni
Chlamydoselachus anguineus
Centroselachus crepidater
Scymnodon ringens
Galeus melastomus
Galeus atlanticus
Etmopterus spinax
Dipturus oxyrinchus
Dipturus nidarosiensis
Deania calceus
Dalatias licha
Centroscymnus coelolepis
Centrophorus squamosus
0 500 1000 1500
Depth (m)
CPUE kg
2.5
5.0
7.5
IUCN
DD
LC
NT
EN
CR

Figure 2.2 Capture per unit effort of the number of deep-sea elasmobranchs CPUE n (a) and weight CPUE kg (b) in the South and Southwest subareas of Portugal mainland. Each data point in the map represents the start of each fishing haul conducted by a crustacean bottom trawler. The dashed black line indicates the limits between the subareas South and Southwest. The yellow cross indicates the Portimão canyon. Isobaths lines represented in the map are of 600 m, 800 m (thicker line), and 1400 m depth. For each map, a density graph for the deep-sea elasmobranchs found in each subarea was also provided for the CPUE n (log-transformed values) and CPUE kg. Species are grouped according with the categories outlined by the International Union for Conservation of Nature and Natural Resources (IUCN; Nieto et al., 2015), where DD-data deficient, LC-least concern, NT-near threatened, EN-endangered, and CR-critically endangered. In the South, depth range of collection is from 96 to 810 m and in Southwest from 400 to 1400 m, but there is a data gap between 800 to 1200 m where no fishing was conducted

Table 2.2 Deep-sea elasmobranchs (DSE) species bycatch by subarea (South and Southwest), with the number of specimens per depth stratum (< 800 and > 800 m), total number of hauls (mean specimens per haul), total CPUE n and CPUE kg for each subarea and range of sizes (cm) for each species per subarea. European conservation status of the DSE is given in accordance with the European Red List from the International Union for Conservation of Nature and Natural Resources (Nieto et al., 2015) where DD-data deficient, LC-least concern, NT-near threatened, EN-endangered, and CR-critically endangered.

DSE Species	**Subarea**	**Depth (m)**		**Hauls (n)**	**CPUE n**	**CPUE kg**	**Size (cm)**	**IUCN Europe**
		< 800	**> 800**					
Etmopterus pusillus	South	115		25 (4.6)	0.44	0.07	19-50	DD
	Southwest	10	6	6 (2.7)	0.17	0.04	17.5-46	
Deania profundorum	South	167		18 (9.3)	0.64	0.12	23-92	DD
	Southwest	32	9	9 (4.6)	0.45	0.29	27-87	
Oxynotus paradoxus *	South							DD
	Southwest	3	1	4 (1)	0.04	0.03	24-65	
Mitsukurina owstoni	South							LC
	Southwest		1	1 (1)	0.01	0.77	250	
*Chlamydoselachus anguineus**	South							LC
	Southwest	2	1	2 (1.5)	0.03	0.01	55.5-65	
*Centroselachus crepidater**	South							LC
	Southwest		17	6 (2.8)	0.18	0.63	64-116	
Neoraja iberica	South	2		2 (1)	0.01	0.0001	18-19	LC
	Southwest							
Scymnodon ringens *	South	101		11 (9.18)	0.39	0.14	30.4-70	LC

	Southwest	22	75	12 (8.1)	1.05	1.81	32-100	
Galeus melastomus	South	450		28 (15.9)	1.74	0.24	12.4-65	LC
	Southwest	40	4	9 (4.4)	0.48	0.23	18-74	
Galeus atlanticus	South	47		10 (4.7)	0.18	0.01	14-44	NT
	Southwest	17		7 (2.4)	0.18	0.03	13-45	
*Etmopterus spinax**	South	264		23 (11.5)	1.02	0.08	7-43.5	NT
	Southwest	39		4 (9.8)	0.42	0.04	16.5-44	
Dipturus oxyrinchus	South	30		17 (1.77)	0.12	0.13	18.5-120	NT
	Southwest	5	2	4 (1.75)	0.08	0.22	38-113	
Dipturus nidarosiensis	South	4		4 (1)	0.02	0.20	99-163	NT
	Southwest	5	10	9 (1.7)	0.16	1.99	97-172	
*Deania calceus**	South	1		1 (1)	0.004	0.0008	40.5	EN
	Southwest	1	44	6 (7.5)	0.49	1.30	67-101	
*Dalatias licha**	South	9		5 (1.8)	0.03	0.16	37.5-145	EN
	Southwest	3	1	4 (1)	0.04	0.20	54-127	
*Centroscymnus coelolepis**	South	1		1 (1)	0.004	0.01	149	EN
	Southwest		3	2 (1.5)	0.03	0.34	100-112	
*Centrophorus squamosus**	South	3		3 (1)	0.01	0.09	80-126	EN
	Southwest		2	2 (1)	0.02	0.11	92-92.5	
*Centrophorus granulosus**	South	10		4 (2.5)	0.04	0.15	84-92	CR
	Southwest							

*TAC Zero European Union (EU) list of deep-water sharks (Regulation 2024/257; 2023/194)

2.3.2 Shark bycatch estimation below 800 m after the ban

In situ CPUE n and CPUE kg for deep-sea sharks in February 2018 were 1.58 and 2.43 respectively. For March 2021 *in situ* CPUE n and CPUE kg were 3.77 and 9.48 respectively. The fishing effort data collected using the GFW website, for the months of February 2017-2021 was conducted by one to three bottom trawlers at depths varying between 1132 and 1447 m. For the month of March only 2017, 2019, and 2021 presented fishing effort data below 800 m, with fishing conducted by one to three trawling vessels, at depths varying between 1236 and 1369 m. February presented the highest fishing effort in comparison with March, hence it also presented the highest estimates in numbers and weight of deep-sea sharks (Table 2.3).

Table 2.3 Data presented for the months of February and March (2017-2021) on the fishing effort in hours (mean ± S.D.) which was directly recovered from the Global Fishing Watch website from crustacean trawlers fishing below 800 m depth. Deep-sea sharks counts and weight were estimated using in situ capture per unit effort values.

	February (2017-2021)	**March (2017, 2019, 2021)**
Fishing effort (h)	813.4 (162.7 ± 199.2)	80.2 (26.8 ± 13.8)
Estimated counts (n)	1285.7 (256.3 ± 313.8)	302.6 (100.9 ± 52.1)
Estimated weight (kg)	1980.0 (396.0 ± 484.9)	760.9 (253.7 ± 131.0)

2.4 Discussion

2.4.1 *In situ* data

In the present study it was noted that a greater fishing effort was conducted in the South than in the Southwest and that, within the Southwest, the fishing effort was nearly equal between depth stratum (i.e. < 800 m and > 800 m). This does not necessarily indicate a preference for fishing in the South. Sampling was opportunistic and some impediments were imposed by Covid-19 lockdowns. It could simply indicate that when the researchers were allowed to board the vessel, the environmental conditions favoured fishing in the South rather than in the Southwest. However, studies conducted with the crustacean trawling fleet off southern Portugal indicate that crustacean bottom trawlers based in the South indeed often exploit nearby shrimp grounds at depths above 800 m targeting Norway lobsters (*N. norvegicus*) and prawns (*P. longirostris* and *P. monodon*) (Borges et al., 2001; Bueno-Pardo et al., 2017). Since prawns and Norway lobster are readily available near their home ports, trawlers based in the South venture to Southwest waters if the weather is favourable and if the economic benefits outweigh the costs of the longer journey, hence targeting more lucrative crustacean species like the scarlet shrimp *A. edwardsiana*. This species is responsible for 16% of the total sales for crustacean bottom trawlers reaching an average 1M € per year (Campos et al., 2021). Despite its great economic value, a significant portion of the catch of *A. edwardsiana* originates from a relatively narrow area parallel to the Southwest coast between 1100-1400 m depth (Campos et al., 2021), well below the 800 m depth line permitted by law in

EU waters (Regulation 2016/2336). Hence, the implementation of the depth ban in 2017, could have presented an economic impact for trawlers operating in the area, especially for those obtaining most of their revenues from this fishery (Campos et al., 2021). Nonetheless, when looking at AIS data on bottom trawlers' fishing effort on the website GFW (globalfishingwatch.org), for the years following the depth ban implementation (i.e. 2017-2021), it is notable that fishing below 800 m in the Southwest coast still occurred. Aside from the economic benefits, it is unclear why fishers were fishing at depths below 800 m during the studied period in opposition with the EU regulation in place. An extraordinary concession by the Portuguese Directorate-General for Natural Resources, Security and Maritime Services (Portuguese acronym DGRM) could have been conceded for some trawlers to operate in that area at those depths, however, no information is publicly available regarding this matter. The DGRM was contacted via email to clarify this situation but did not reply until the date of the publication of this study. According to results from the present study, fishing at depths below 800 m in the Southwest exhibited a notably higher proportion of DSE biomass, ranging from 25-58%, compared to hauls conducted above 800 m, which ranged from 0-47%. This highlights some of the highest bycatch values reported in the literature, which is predominantly focused on shallower Mediterranean depths. For example, around the Balearic Islands and in the Aegean Sea, elasmobranchs represent 5–8% and 14% respectively, of the total catch biomass (Carbonell et al., 2003; Damalas and Vassilopoulou, 2011) while specific hotspots like the Alboran Sea, recorded high biomass of species such as *G. melastomus* which exceeded the target species *A. antennatus* (Torres et al., 2001). Recently, in the Moroccan Mediterranean Sea (20 to *ca.* > 60 m depth), elasmobranchs constituted only 5-9% of the total catch biomass in trawlers targeting cephalopods, teleosts, and crustaceans (Keznine et al., 2024). Similarly, in the northeastern Mediterranean, demersal elasmobranchs accounted for 23% of the total fish biomass, with fishing efforts concentrated at depths from 0 to >100 m (Yaglioglu et al., 2015). On the Egyptian Mediterranean coast, species like *D. oxyrinchus* and *G. melastomus* were part of the bycatch retained by bottom trawlers, comprising up to 21% of the total catch biomass, with discards including species like *E. spinax* and *Centrophorus* sp., contributing to 9% of the biomass discarded at depths above 800 m (Farrag, 2022). Additionally, the current study reports DSE biomass values higher than those reported in earlier studies conducted in the same areas at depths above 800 m. For example, Monteiro et al. (2001) recorded 15% of chondrichthyan bycatch while Borges et al. (2001) reported chondrichthyan bycatch values as high as 29% for crustacean trawlers, which included elasmobranch species not typically classified as deep-sea (e.g., *Torpedo nobiliana, Raja clavata*, and *Scyliorhinus canicula*) but excluding DSE that were present in great proportions in the present study (e.g., *S. ringens*, and *D. profundorum*). This suggests that, if all chondrichthyans caught in the present study were considered—including those not frequently observed in deep-sea environments—the bycatch values to report would be even higher. The disparity between the bycatch values presented in this study and in those above-mentioned, indicates a potential increase in elasmobranchs' bycatch values over time and/or a possible shift in the composition of the species encountered.

In the South, the abundance (CPUE n) and biomass (CPUE kg) values were lower than in the Southwest for the same depths (i.e., < 800 m), which could be a consequence of elevated fishing activity in the South (Borges et al., 2001; Bueno-Pardo et al., 2017) since abundance and biomass are negatively correlated with fishing effort (Navarro et al., 2016; Peristeraki et al., 2020; Ruiz-García et al., 2023). This trend was also observed by Ferretti et al. (2013). Conversely, it could also be due to a patchy distribution of DSE in this subarea, but those assumptions require further investigations which were out of the scope of the present study, since the opportunistic nature of the sampling scheme did not allow to identify the drivers for the observed differences. Furthermore, in the South high CPUE n and CPUE kg of DSE were generally observed between 500-800 m depth, with variations among species. Species like *C. granulosus*, *D. profundorum*, and *S. ringens* exhibited higher values around 800 m depth in comparison with shallower areas. Notably, hauls in the vicinity and within the submarine Portimão Canyon (~800 m), revealed specimens of the critically endangered *C. granulosus,* endangered *C. squamosus,* and near-threatened *D. nidarosiensis*, along with a high CPUE n of smaller species like *D. profundorum* and *S. ringens* that were caught in groups. These findings underscore the ecological importance of the Portimão Canyon, a hotspot for biodiversity in the South subarea (Gomes et al., 2018), due to the unique physical and oceanographic features characteristic of submarine canyons (Fernandez-Arcaya et al., 2017; Santora et al., 2018). Morais et al. (2007) studied the substrate and fauna at the head of the Portimão canyon (~200 m), identifying a dynamic ecosystem with notable shifts in species composition and trophic structures, likely impacted by intense trawling activity (Borges et al., 2001). Although Scyliorhinidae sharks were observed, no other shark species were detected, potentially due to the relatively shallow depth at which images were captured (Morais et al., 2007).

Despite the fact that, in the present study, there were some hauls conducted within the canyon at greater depths than the ones addressed by Morais et al. (2007) which obtained species of conservation concern, it is unclear if bottom trawlers consistently operate within this canyon or if they 'fly' the nets over the canyon (Morais et al., 2007). A deeper understanding of the DSE assemblages that inhabit the canyon across various depths, and of the behaviour of trawling activities within this region, would help identify potential impacts of this activity within DSE inhabiting the Portimão canyon.

In the Southwest, high CPUE n but low CPUE kg were generally observed between 400-800 m. However, endangered species like *C. squamosus*, *C. coelolepis*, and *D. calceus*, along with the least concern species *C. crepidater* and *S. ringens,* were either found only at depths exceeding 1200 m or exhibited high CPUE n at those depths, in accordance with previous studies for the Portuguese coast (Moura et al., 2014; Moura et al., 2018; ICES, 2020). Likewise, higher CPUE kg and lower CPUE n was found at depths below 1200 m. This suggests that immature or small DSE specimens inhabit shallower depths (< 800 m), whereas larger or mature specimens of uncommon and endangered sharks inhabit depths below 1200 m, a trend also noted by Ruiz-García et al. (2023) in the western Mediterranean. It is known that depth plays a crucial role in shaping the composition of elasmobranch communities, with skates like *D. oxyrinchus* preferring the upper continental slope (200-500 m) and most sharks preferring the deepest part of the continental slope (> 500 m; Carbonell et al., 2003; Moura et al., 2018; Das et al., 2022; Ruiz-García et al., 2023). J. Clarke et al. (2015) recommended restricting bottom trawling to depths of 600 m instead of the current 800 m due to the potential negative ecological impacts on DSE which would outweigh the commercial benefits below 600 m. Because DSE exhibited higher CPUE n and CPUE kg starting around 500 m in the South and 400 m in the Southwest, consistent with the literature from the Northeast Atlantic (e.g., ICES, 2020; O'Hea et al., 2020), that could indicate that the depth limits for bottom trawlers could be even less than the suggested 600 m to increase DSE protection. Furthermore, avoidance of depths below 800 m by bottom trawlers, seems to be beneficial not only to the protection of DSE communities but might as well benefit fishers economically, since at depths above 800 m the retained catch weight might be greater than the discarded weight given that DSE weight presented a median of 1.1%. Yet, the assessment of the economic performance of this fishery was beyond the scope of the present study, but such analyses are encouraged for future research, considering the distribution of high valued shrimps at depths beyond the permitted by law. It is also important to recognize that the percentage of total weight attributed to DSE was calculated in relation to the total weight of the catch in the net codend, which includes both target and bycatch species, whether discarded or retained. The net codend weight was visually estimated by the skipper either when the net is lifted on top of deck, or when the catch is already inside the "pond" (an area below deck where the codend is offloaded). Visual estimate of the total catch is usually conducted by experienced observers or skippers, however, some bias could be introduced by under- or overestimating the weight, given that it is a visual estimation. Nonetheless, it is one of the recognized methodologies to estimate total catch weight and discards (e.g., Alaska Fisheries Science Center, 1997; MRAG Americas, 2019). The use of a codend weigher (which is basically a scale that weigh the codend as it is hauled aboard the vessel), along with information on the retained catch weight (that is daily recovered by fishers using onboard scales), could help estimate discards in a more precise way, hence assisting in reducing the level of discards in EU fisheries (Caslake, 2009).

Species of least concern (e.g., *G. melastomus* and *S. ringens*) generally exhibited higher CPUE values than the most imperilled, suggesting more stable and abundant populations. In contrast, the low CPUE values of endangered and critically endangered species (e.g., *C. coelolepis* and *C. granulosus*) reflect their high risk of population's depletion or even extinction. Near threatened and data deficient species (e.g. *D. oxyrinchus* and *E. pusillus*) exhibited intermediate CPUE values. Furthermore, the high abundance of *G. melastomus* could have masked the CPUE n contribution of other comparable species (i.e., species with number of specimens > 4; *D. oxyrinchus, D. licha, Etmopterus spp.,* and *G. atlanticus*) in the South subarea as previously seen in other studies (e.g., Serrat et al., 2023); however, statistical analysis (Appendix 2.2), showed that CPUE n of *G. melastomus* was not significantly different than the other evaluated species, except for *D. oxyrinchus* which presented much lower abundance comparatively with *G. melastomus* (Appendix 2.2). *Galeus melastomus* is a prolific species (Tursi et al., 1993; O'Hea et al., 2020), that seems to prefer depths above 800 m (e.g., ICES, 2013, O'Hea et al., 2020; Kelly and Gerritsen, 2022). It is probably the most well-known species among the studied DSE, with a fair number of studies in European waters, mostly for the Mediterranean Sea (e.g., Ramírez-Amaro et al., 2015, 2020; Follesa et al., 2019; D'Iglio et al., 2021; Ruiz-García et al., 2023; Serrat et al., 2023). In contrast, very little is known about *S. ringens* despite its common occurrence in the bycatch of deep-sea fisheries in Northeast Atlantic waters (Compagno, 1984; Gibson et al., 2006; ICES, 2020;

Kelly and Gerritsen, 2022); however, their presence seems to be more prominent at depths below 700 m (Moura et al., 2018; ICES, 2020; O'Hea et al., 2020). *Galeus melastomus*, *S. ringens, E. pusillus*, and *D. profundorum*, appeared generally in groups (> 3 specimens per haul) at both studied subareas and depth stratum, which suggests some habitat flexibility. Species that generally appeared individually in the hauls, were uncommon and endangered species that occurred in only one of the studied subareas (*M. owstoni, O. paradoxus*, *C. anguineus,* and *C. granulosus*) or that occurred both in South and Southwest but at different depth strata (*D. licha*, *C. squamosus*, and *C. coelolepis*). Indeed, it is known that deep-sea sharks segregate in habitat by age, sex, and reproductive states (e.g., Yano, 1995; Jakobsdottir, 2001; Kiraly et al., 2003; Bañon et al., 2006; Veríssimo et al., 2011). For example, *C. coelolepis* segregates by size, sex, and maturity stage (Veríssimo et al., 2011) while *D. licha* shows different depth preferences in different areas (Stefanescu et al., 1992; Baino et al., 2001; Ungaro et al., 2001). However, practically all deep-sea sharks are threatened by bycatch even at low levels, which may prevent recovery given their low resilience to exploitation (Dulvy et al., 2021; Finucci et al., 2024), especially those living at the deepest depths (Simpfendorfer and Kyne, 2009).

In EU waters, DSE are included in a few regulations like biannual quotas (skates from the order Rajiformes) and zero TAC (Regulation 2024/257). The zero TAC imposed on deep-sea shark species is important to avoid targeted fisheries, but at the same time, has precluded the development of comprehensive stock assessments and spatial distribution models. This is because sharks are promptly discarded upon capture, with no monitoring or records of their bycatch in deep-sea fisheries (ICES, 2020). Furthermore, this regulation does not avoid the bycatch of these species, as it was seen in the present study, where endangered species such as *C. squamosus, D. calceus, D. licha,* and *C. coelolepis*, exhibited some of the highest CPUE kg among species in the Southwest region below 800 m. Additionally, the Regulation 2024/257 does not ensure the survivorship of the specimens after discards. Mortality estimates of DSE are generally lacking for bottom trawling, but a recent study found that 85%, 91% and 88% of the specimens from the species *D. oxyrinchus, G. melastomus and E. spinax* respectively, were either dead or inactive (i.e., not responsive but still breathing hence with extremely low chances of survival after discards; Scacco et al., 2023). These numbers are extremely alarming and call for improved monitoring of the discards of the DSE in order to understand the impact of bottom trawling on the survivorship of DSE species of conservation concern.

The ecological implications of DSE elevated bycatch, combined with frequent discarding practices because of regulations or low commercial and with high mortality rates may be significant. Elasmobranchs, particularly deep-sea species, play essential roles in maintaining ecosystem balance, acting as apex or mesopredators (J. Clarke et al., 2015). Discarding these species without understanding their mortality rates and the potential consequences, can lead to disruptions in trophic dynamics and reduce biodiversity, impacting ecosystem health (Simpfendorfer and Kyne, 2009).

2.4.2 Recommendations for the monitoring of bycatch and mitigation of impacts

Given the important but concerning findings from the present study several approaches could be followed to monitor DSE bycatch and discards and to mitigate the impacts of bottom trawling on these animals. For the monitoring, efforts should focus on improving fisheries technology to remotely identify specimens and maintain records of their population status for stock assessments and distribution patterns. Emerging technologies, such as electronic monitoring, are promising in addressing this challenge by enabling remote identification of DSE using video imagery. For instance, in a study conducted simultaneously to the present study [da Rocha et al. (unpublished results)] several DSE specimens were successfully identified up to the species level using images from an onboard camera in a crustacean bottom trawler; however, the complexity of distinguishing between congeneric species, such as *Etmopterus* spp. and *Deania* spp., often complicates precise species-level identification. While many studies group all sharks and all skates, sometimes even grouping by order (GFCM, 2012; Bradai et al., 2012; Coll et al., 2013; Dulvy et al., 2014), it is important to recognize that even congeneric species can differ significantly in their ecology and biology, requiring distinct regulatory measures. For example, *E. spinax* and *E. princeps*, along with *D. calceus*, are designated as zero TAC species, whereas *E. pusillus* and *D. profundorum* are not, and this could lead to enforcement constrains given their notorious physical similarities with congeners. Hence, the importance in identifying specimens up to species level calls for improvement on such monitoring tools not only to properly identify species, but to speed up the identification process by developing AI tools integrated with electronic monitoring at a fishery/fleet basis. However, since congeneric species like *Deania* spp. and *Etmopterus* spp. cannot currently be reliably distinguished via video imagery, implementing supplementary strategies, such as amalgamating congeneric species in the deep-sea shark list (Regulation 2024/257), would, at least for the time being, streamline the utilization of this accessible electronic monitoring tool to identify DSE, albeit limited to the genus level, thereby contributing to a better understanding of data poor groups like DSE.

To mitigate the impacts of bottom trawling on DSE, a set of measures could be applied. First, efforts to reduce DSE bycatch should be prioritized. This can be achieved by identifying areas and seasons of high bycatch of DSE using fishery dependent or independent data (e.g. surveys conducted in fishing and in research vessels, or species distribution models), and limit fishing activities during a certain period or in certain areas. Additionally, testing the use of bycatch-reducing devices is crucial. Examples of such devices include the Nordmøre grid (Isaksen et al., 1992), which has been regarded as an efficient method to mitigate elasmobranch bycatch in various crustacean trawl fisheries globally, being mandatory in all shrimp fisheries inside the Norwegian EEZ. Furthermore, employing turtle excluder devices in nets (Hall and Mainprize, 2005; Brewer et al., 2006; Belcher and Jennings, 2011), as well as electromagnetic exclusion devices, acoustic or light-based deterrents, were all proven successful and should be explored (ICES, 2020). However, the effectiveness of such measures in reducing the bycatch of DSE specifically in the European crustacean bottom trawl fishery, requires further investigation. Second, if DSE's bycatch cannot be prevented, DSE should be immediately discarded and recorded in logbooks. This type of data is difficult to obtain but is to be done by skippers and onboard observers in deep-sea fisheries, ultimately with the aid of electronic monitoring by onboard video cameras, as previously explained. Recording of this data would allow collecting information on i) species composition, biomass and abundance; ii) spatial and temporal bycatch patterns and rates, which allows for comparisons among and within fishing gears and data reporting methods (Alverson et al., 1994; Ye et al., 2000); iii) potential breeding areas; iv) the effectiveness of different management measures, such as spatio-temporal closures, net restrictions, and the use of bycatch reduction devices for their discards. Third, best practices protocols for safe onboard handling and release of DSE should be developed to improve DSE discards survival (Gilman et al., 2007; Benoît et al., 2010).

2.4.3 Sharks bycatch estimation below 800 m after the ban

Estimates of deep-sea sharks’ bycatch in the Southwest, measured by both count and weight, suggest that substantial numbers of deep-sea sharks may have been caught, in the five years following the prohibition on bottom trawling at depths below 800 m in the Northeast Atlantic (Regulation 2016/2336). A comparison of February and March data provides insights into why shark capture estimates were higher in February than in March. Specifically, *in situ* data from February 2018 and March 2021, combined with GFW data for February and March across 2017–2021, indicate that seasonal fishing conditions and regulatory factors have influenced these estimates. A critical factor is the annual January fishing cessation mandated by Ordinance N° 43/2006, which requires crustacean trawlers with codend mesh sizes of 55–59 mm or larger to remain in port for that month. Because trawling ceases in the month preceding February’s sampling, fishers have observed increased catches of commercially valuable crustaceans in the weeks immediately following this break (personal communications, March 2021). This increased crustacean availability likely attracts more sharks to the area, leading to higher shark concentrations for feeding purposes. Sharks in this region are known to feed on crustaceans (Graça Aranha et al., 2023), a behaviour that could heighten their susceptibility to capture in February.

The periods of *in situ* data collection in February 2018 (the first year of the fishing prohibition) and March 2021 (four years into the prohibition, with Covid-19 lockdowns) may explain variations in fishing effort and shark capture rates. In February 2018, fishing effort may have remained consistent as fishers did not immediately alter their practices in response to the new depth restrictions. By March 2021, while the prohibition was well established, Covid-19 restrictions introduced additional complications. The pandemic led to disrupted supply chains, restricted movement, and reduced crew availability, which could have caused disruptions in fishing activities. These combined factors likely contributed to lower effort, hence lower shark captures in March 2021, complicating comparisons between February and March and adding a layer of variability to long-term bycatch estimates.

It is also important to consider limitations in AIS data, as some vessels capable of fishing at these depths may not use AIS tracking. While GFW data is reliable for global fishing activity monitoring, challenges like intentional AIS disabling and occasional inaccuracies in vessel identification still exist. Additionally, while GFW's “Gear type = Trawler” filter includes various types of trawling vessels, specific vessel reviews confirm that all identified vessels in this area engage in crustacean bottom trawling activities, confirming the reliability of the fishing effort data collected.

However, it is crucial to highlight that the estimates provided are underestimating actual numbers of deep-sea sharks caught below 800 m depth in the Southwest of Portugal, since data collection was limited to February and March and included only one to three vessels in a specific subarea. So, if we were to extrapolate the analyses to the entire fishing area below 800 m and extend it throughout the year this would significantly increase those estimates, ate least by tripling vessel counts to as many as ten according to GFW data - which corresponds to 40% of the total number of crustacean trawlers of Portugal (personal communications DGRM, October 2024). This expansion would provide a fuller picture of fishing effort and shark bycatch, underscoring the potential impact of trawling on deep-sea shark populations.

Considering all the limitations presented, these results should be viewed as exploratory; but given that fishing took place at prohibited depths and resulted in large estimates of deep-sea sharks bycatch, the compliance and enforcement of the 800 m depth restriction is essential to reduce DSE bycatch effectively. Further studies on the ecology and biology of DSE would enhance our understanding of their vulnerability to fisheries and their distribution in this region.

2.5 Conclusion

In the present study it was seen that DSE represents a high proportion of the total catch biomass reaching up to 60%, especially at depths below 800 m. Despite EU regulations restricting fishing below 800 m, bottom trawlers were observed operating at these depths during *in situ* data collection, corroborated by GFW data from 2017 to 2021. This activity led to some of the highest DSE bycatch rates recorded when compared to shallower depths within this study and to previous studies in the area and in EU waters. This is concerning given that they have very low chances of survival even if returned to the water alive, hence avoiding their capture in first place should be prioritized. Furthermore, it was highlighted the relevance of depth into shaping DSE communities in the study areas, with deeper areas hosting larger and endangered specimens. The presence of endangered and critically endangered DSE species in the submarine Portimão canyon is of concern and should be further investigated given the high trawling activity in the South of Portugal. Understanding long-term effects of trawling on DSE species composition and ecosystem dynamics is crucial for evaluating the ecological role of the studied areas and for shaping effective management strategies to protect biodiversity, especially of taxa of conservation concerns. Further studies are encouraged to help bring solutions tailored for crustacean trawlers operating in EU waters to avoid DSE bycatch. Nonetheless, the present study demonstrated that due to the high bycatch of DSE, immediate action is required to mitigate the impact of bottom trawling on DSE populations and fragile deep-sea ecosystems.

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Ethical standards – This study was conducted in accordance with the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of animals and enforced by CCMAR. CCMAR staff are certified to house and conduct experiments with live animals, and their facilities are also certified in accordance with the three "R" policy, national and European legislation, and with guidelines defined by the ethical committee ORBEA CCMAR-CBMR.

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2.7 Appendices

Appendix 2.1 Kruskal Wallis (KW) and Mann-Whitney (MW) test results among deep-sea elasmobranch species' capture per unit effort of specimens (CPUE n) and weight (CPUE kg) in the South and Southwest coasts of Portugal from a crustacean bottom trawler.

Subarea	Source	Test	Test Stat	df	p-value
South	Species x CPUE n	KW	17.25	7	0.016
Southwest	Species x CPUE n	KW	12.21	7	0.094
South	Species x CPUE kg	KW	22.44	7	0.002
Southwest	Species x CPUE kg	KW	36.38	7	< 0.0001
All	*Galeus atlanticus* x CPUE n	MW	23		0.6612
All	*G. atlanticus* x CPUE kg	MW	23		0.6612
All	*G. melastomus* x CPUE n	MW	97		0.9337
All	*G. atlanticus* x CPUE kg	MW	81		0.5877

Appendix 2. 1 *Post hoc* Dunn test results between deep-sea elasmobranch species' (with n > 4) and their designated capture per unit effort of specimens (CPUE n) and weight (CPUE kg) in the South and Southwest coasts of Portugal from the crustacean bottom trawl fishery. Results presented are only the ones which were significant, i.e. p-adjusted < 0.05, meaning that for the other pair-wise combinations, there were either no differences among the species, or the number of specimens was < 5 hence no analyses were conducted.

Subarea	Variable	Species 1	Species 2	n1	n2	statistic	p	p-adjusted
South	CPUE n	*Dalatias licha*	*Deania profundorum*	6	18	2.158	0.031	0.866
South	CPUE n	*Deania profundorum*	*Dipturus oxyrinchus*	18	17	-2.261	0.024	0.665
South	CPUE n	*Dalatias licha*	*Dipturus oxyrinchus*	6	17	0.532	0.595	1.000
South	CPUE n	*Dalatias licha*	*Etmopterus pusillus*	6	25	1.971	0.049	1.000
South	CPUE n	*Deania profundorum*	*Etmopterus pusillus*	18	25	-0.392	0.695	1.000
South	CPUE n	*Dipturus oxyrinchus*	*Etmopterus pusillus*	17	25	2.047	0.041	1.000
South	CPUE n	*Dipturus oxyrinchus*	*Etmopterus spinax*	17	24	2.310	0.021	0.585
South	CPUE n	*Dalatias licha*	*Etmopterus spinax*	6	24	2.158	0.031	0.866
South	CPUE n	*Deania profundorum*	*Etmopterus spinax*	18	24	-0.104	0.917	1.000
South	CPUE n	*Etmopterus pusillus*	*Etmopterus spinax*	25	24	0.311	0.756	1.000
South	CPUE n	*Dalatias licha*	*Galeus atlanticus*	6	11	1.267	0.205	1.000
South	CPUE n	*Deania profundorum*	*Galeus atlanticus*	18	11	-0.978	0.328	1.000
South	CPUE n	*Dipturus oxyrinchus*	*Galeus atlanticus*	17	11	1.009	0.313	1.000
South	CPUE n	*Etmopterus pusillus*	*Galeus atlanticus*	25	11	-0.700	0.484	1.000
South	CPUE n	*Etmopterus spinax*	*Galeus atlanticus*	24	11	-0.939	0.348	1.000
South	CPUE n	*Dipturus oxyrinchus*	*Galeus melastomus*	17	29	3.371	0.001	0.021*
South	CPUE n	*Dalatias licha*	*Galeus melastomus*	6	29	2.859	0.004	0.119
South	CPUE n	*Deania profundorum*	*Galeus melastomus*	18	29	0.883	0.377	1.000
South	CPUE n	*Etmopterus pusillus*	*Galeus melastomus*	25	29	1.415	0.157	1.000
South	CPUE n	*Etmopterus spinax*	*Galeus melastomus*	24	29	1.078	0.281	1.000
South	CPUE n	*Galeus atlanticus*	*Galeus melastomus*	11	29	1.805	0.071	1.000
South	CPUE n	*Dalatias licha*	*Scymnodon ringens*	6	11	1.778	0.075	1.000

South	CPUE n	*Deania profundorum*	*Scymnodon ringens*	18	11	-0.301	0.764	1.000
South	CPUE n	*Dipturus oxyrinchus*	*Scymnodon ringens*	17	11	1.679	0.093	1.000
South	CPUE n	*Etmopterus pusillus*	*Scymnodon ringens*	25	11	0.017	0.986	1.000
South	CPUE n	*Etmopterus spinax*	*Scymnodon ringens*	24	11	-0.227	0.821	1.000
South	CPUE n	*Galeus atlanticus*	*Scymnodon ringens*	11	11	0.608	0.543	1.000
South	CPUE n	*Galeus melastomus*	*Scymnodon ringens*	29	11	-1.073	0.283	1.000
South	CPUE kg	*Galeus atlanticus*	*Scymnodon ringens*	11	11	3.173	0.002	0.042*
South	CPUE kg	*Etmopterus spinax*	*Scymnodon ringens*	24	11	3.164	0.002	0.044*
South	CPUE kg	*Etmopterus spinax*	*Galeus melastomus*	24	29	3.158	0.002	0.044*
South	CPUE kg	*Galeus atlanticus*	*Galeus melastomus*	11	29	3.029	0.002	0.069
South	CPUE kg	*Deania profundorum*	*Galeus atlanticus*	18	11	-2.515	0.012	0.333
South	CPUE kg	*Deania profundorum*	*Etmopterus spinax*	18	24	-2.442	0.015	0.409
South	CPUE kg	*Dipturus oxyrinchus*	*Scymnodon ringens*	17	11	2.338	0.019	0.544
South	CPUE kg	*Dalatias licha*	*Deania profundorum*	6	18	0.401	0.688	1.000
South	CPUE kg	*Dalatias licha*	*Dipturus oxyrinchus*	6	17	-0.684	0.494	1.000
South	CPUE kg	*Dalatias licha*	*Etmopterus pusillus*	6	25	-0.244	0.807	1.000
South	CPUE kg	*Dalatias licha*	*Etmopterus spinax*	6	24	-1.254	0.210	1.000
South	CPUE kg	*Dalatias licha*	*Galeus atlanticus*	6	11	-1.524	0.128	1.000
South	CPUE kg	*Dalatias licha*	*Galeus melastomus*	6	29	0.667	0.505	1.000
South	CPUE kg	*Dalatias licha*	*Scymnodon ringens*	6	11	1.142	0.253	1.000
South	CPUE kg	*Deania profundorum*	*Dipturus oxyrinchus*	18	17	-1.519	0.129	1.000
South	CPUE kg	*Deania profundorum*	*Etmopterus pusillus*	18	25	-0.971	0.331	1.000
South	CPUE kg	*Deania profundorum*	*Galeus melastomus*	18	29	0.367	0.713	1.000
South	CPUE kg	*Deania profundorum*	*Scymnodon ringens*	18	11	1.021	0.307	1.000
South	CPUE kg	*Dipturus oxyrinchus*	*Etmopterus pusillus*	17	25	0.679	0.497	1.000
South	CPUE kg	*Dipturus oxyrinchus*	*Etmopterus spinax*	17	24	-0.781	0.435	1.000
South	CPUE kg	*Dipturus oxyrinchus*	*Galeus atlanticus*	17	11	-1.159	0.246	1.000
South	CPUE kg	*Dipturus oxyrinchus*	*Galeus melastomus*	17	29	2.043	0.041	1.000
South	CPUE kg	*Etmopterus pusillus*	*Etmopterus spinax*	25	24	-1.614	0.107	1.000
South	CPUE kg	*Etmopterus pusillus*	*Galeus atlanticus*	25	11	-1.830	0.067	1.000
South	CPUE kg	*Etmopterus pusillus*	*Galeus melastomus*	25	29	1.504	0.133	1.000
South	CPUE kg	*Etmopterus pusillus*	*Scymnodon ringens*	25	11	1.910	0.056	1.000
South	CPUE kg	*Etmopterus spinax*	*Galeus atlanticus*	24	11	-0.552	0.581	1.000
South	CPUE kg	*Galeus melastomus*	*Scymnodon ringens*	29	11	0.792	0.428	1.000
Southwest	CPUE kg	*Dipturus nidarosiensis*	*Etmopterus pusillus*	9	6	-4.020	0.000	0.002**
Southwest	CPUE kg	*Dipturus nidarosiensis*	*Galeus atlanticus*	9	5	-3.983	0.000	0.002**
Southwest	CPUE kg	*Deania profundorum*	*Dipturus nidarosiensis*	9	9	3.731	0.000	0.005**
Southwest	CPUE kg	*Etmopterus pusillus*	*Scymnodon ringens*	6	10	3.367	0.001	0.021*
Southwest	CPUE kg	*Galeus atlanticus*	*Scymnodon ringens*	5	10	3.363	0.001	0.022*
Southwest	CPUE kg	*Dipturus nidarosiensis*	*Galeus melastomus*	9	10	-3.303	0.001	0.027*
Southwest	CPUE kg	*Deania profundorum*	*Scymnodon ringens*	9	10	3.001	0.003	0.075
Southwest	CPUE kg	*Deania calceus*	*Galeus atlanticus*	6	5	-2.636	0.008	0.235
Southwest	CPUE kg	*Deania calceus*	*Etmopterus pusillus*	6	6	-2.585	0.010	0.272

Southwest	CPUE kg	*Galeus melastomus*	*Scymnodon ringens*	10	10	2.544	0.011	0.307
Southwest	CPUE kg	*Centroselachus crepidater*	*Galeus atlanticus*	6	5	-2.450	0.014	0.400
Southwest	CPUE kg	*Centroselachus crepidater*	*Etmopterus pusillus*	6	6	-2.390	0.017	0.471
Southwest	CPUE kg	*Deania calceus*	*Deania profundorum*	6	9	-2.149	0.032	0.885
Southwest	CPUE kg	*Centroselachus crepidater*	*Deania calceus*	6	6	0.195	0.845	1.000
Southwest	CPUE kg	*Centroselachus crepidater*	*Deania profundorum*	6	9	-1.936	0.053	1.000
Southwest	CPUE kg	*Centroselachus crepidater*	*Dipturus nidarosiensis*	6	9	1.401	0.161	1.000
Southwest	CPUE kg	*Centroselachus crepidater*	*Galeus melastomus*	6	10	-1.509	0.131	1.000
Southwest	CPUE kg	*Centroselachus crepidater*	*Scymnodon ringens*	6	10	0.694	0.487	1.000
Southwest	CPUE kg	*Deania calceus*	*Dipturus nidarosiensis*	6	9	1.188	0.235	1.000
Southwest	CPUE kg	*Deania calceus*	*Galeus melastomus*	6	10	-1.727	0.084	1.000
Southwest	CPUE kg	*Deania calceus*	*Scymnodon ringens*	6	10	0.476	0.634	1.000
Southwest	CPUE kg	*Deania profundorum*	*Etmopterus pusillus*	9	6	-0.683	0.495	1.000
Southwest	CPUE kg	*Deania profundorum*	*Galeus atlanticus*	9	5	-0.830	0.406	1.000
Southwest	CPUE kg	*Deania profundorum*	*Galeus melastomus*	9	10	0.524	0.600	1.000
Southwest	CPUE kg	*Dipturus nidarosiensis*	*Scymnodon ringens*	9	10	-0.827	0.408	1.000
Southwest	CPUE kg	*Etmopterus pusillus*	*Galeus atlanticus*	6	5	-0.171	0.865	1.000
Southwest	CPUE kg	*Etmopterus pusillus*	*Galeus melastomus*	6	10	1.164	0.245	1.000
Southwest	CPUE kg	*Galeus atlanticus*	*Galeus melastomus*	5	10	1.286	0.199	1.000

Chapter 3: UNDER PRESSURE: DEEP-SEA ELASMOBRANCHS EXPERIENCE HIGH MORTALITY AND STRESS IN A CRUSTACEAN TRAWLING FISHERY.

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Abstract

Crustacean bottom trawling in southern Portugal is a an economic and culturally important fishing activity but may result in considerable bycatch of deep-sea elasmobranchs (DSE). Due to DSE life-history strategies, at-vessel mortality (AVM) rates in crustacean bottom trawl fisheries are expectedly high but require further investigations. This study assessed the at-vessel condition of 18 species of DSE, and AVM rates and stress of four deep-sea shark species (*Etmopterus pusillus*, *E. spinax*, *Galeus melastomus*, and *Scymnodon ringens*), to understand the impact of bottom trawling on these animals. Opportunistic sampling on a crustacean trawler in the southern Portuguese coast, revealed that 95% of specimens were either dead (n=1258) or in poor condition (n=224) upon collection, underscoring their minimal chance of post-release survival. General linear model analyses showed that AVM was species-specific and highest in smaller sharks, as well as in those from hauls that exhibited larger temperature differences between bottom and surface waters, and those caught in hauls with heavier codend weight using a 55 mm codend mesh (targeting shrimp and prawns) instead of those caught in hauls using a 70 mm codend mesh (targeting Norway lobster). Stress, evaluated through metabolites and electrolytes levels in sharks' plasma, indicated significant differences in potassium, urea, and magnesium levels between live and deceased specimens of *E. pusillus* and *G. melastomus*, suggesting these as reliable mortality markers. Elevated lactate levels in *G. melastomus* further pointed to high post-release mortality risk. These findings highlight an urgent need to find solutions to mitigate the impacts of bottom trawling on those DSE, which are thoroughly discussed. A coordinated, multi-stakeholder approach involving researchers, the fishing industry, and regulatory bodies is crucial for

developing and implementing effective, and more sustainable fisheries management and protection of DSE populations.

Keywords: at-vessel mortality, condition, survival, plasma, secondary responses, Iberian Peninsula, Portugal

3.1 Introduction

Bottom trawling is an important fishing practice in Europe. In Portugal, trawl fleet landings rank third in national seafood landings (INE, 2023), with crustacean trawlers specifically targeting commercially valuable species such as *Nephrops norvegicus* (Linnaeus, 1758), *Aristeus antennatus* (Risso, 1816), and *Aristaeomorpha foliacea* (Risso, 1827) (Campos et al., 2007). This fleet primarily operates at depths of 200-800 m in the South and Southwest regions off Portugal (e.g., Bueno-Pardo et al., 2017; Campos et al., 2021). The proximity of southern Portuguese ports, like Olhão and Portimão, to Spain facilitates trade with Spanish buyers, which further enhances the economic importance of crustacean trawling in this area (Bueno-Pardo et al., 2017).

Trawl fisheries bycatch, particularly in deeper waters, includes deep-sea elasmobranchs (DSE; i.e., sharks and skates), which can constitute up to ca. 60% of the total catch in weight (Borges et al., 2001; Monteiro et al., 2001; Carbonell et al., 2003; Coelho et al., 2005; Costa et al., 2008; Graça Aranha et al., (unpublished results)]. Deep-sea elasmobranchs, area classified as meso- and top-predators (e.g., Cortés, 1999; Bizzarro et al., 2007; Churchill et al., 2015; Graça Aranha et al., 2023), and predominantly inhabit slopes at depths over 400 m (ICES, 2020; O' Hea et al., 2020), though it is generally conceived that deep-sea species are defined as those dwelling at depths greater than 200 m. They play a crucial role in maintaining ecosystem balance and thus, their population decline could impact other species' populations, potentially leading to structural shifts in these ecosystems (Ruppert et al., 2013; Barley et al., 2017; Valls et al., 2017; Shipley et al., 2023).

According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, one in seven (14%) DSE are threatened with an elevated risk of extinction globally, and 43 of the 283 (15%) deep-sea shark species are classified as threatened with a high risk of extinction (Finucci et al., 2024). This vulnerability primarily stems from their biological characteristics, including slow growth, late maturity, and low reproductive rates (Simpfendorfer and Kyne, 2009). The European Union has implemented legislation aimed at reducing the capture of these species in European waters. The EU Regulation 2023/194 acknowledges that even minimal fishing activity poses a serious conservation risk for several shark species. As a result, a ban was established that prohibits EU fishing vessels, as well as vessels from non-EU countries operating in EU waters, from fishing, retaining on board, transshipping, or landing certain prohibited shark species. Since 2010, a zero Total Allowable Catch (TAC) for deep-sea sharks has been enforced within the EU. In compliance with this regulation, any prohibited shark species caught must be promptly released unharmed, preventing their fins, meat, and liver oil from entering the market. However, despite these measures, the survival of released animals is not guaranteed.

For most deep-sea sharks, data on at-vessel mortality (AVM) rates are extremely rare. The few available estimates show considerable variability, ranging from 0-100% in longline fisheries (Brooks et al., 2015; Rodríguez-Cabello and Sánchez, 2017; Talwar et al., 2017) and from 85–91% in bottom trawl fisheries (Scacco et al., 2023). Information on post-release mortality (PRM) is even scarcer, mainly with estimates from longline fisheries, presenting great variation among the studies (14-83%; Daley et al., 2015; Brooks et al., 2015; Rodríguez-Cabello and Sánchez, 2017; Talwar et al., 2017). These rates indicate that mortality is likely both fishery- and species-specific, and that is generally positively correlated with depth and inversely correlated with body size (Rodríguez-Cabello and Sánchez, 2017; Scacco et al., 2023). When captured during fishing activities, deep-sea sharks are often exposed to various environmental stressors, including sudden changes in water temperature, pressure, and oxygen levels (Fauconnet et al., 2023), which frequently result in mortality. For instance, a mortality study conducted in the Mediterranean found that fishing on the slope increases deep-sea sharks' mortality rates compared to fishing for coastal species at shallower depths (Scacco et al., 2023). Additionally, the AVM of demersal shark species is affected by factors such as fishing effort, as well as the composition and weight of the catch (Broadhurst et al., 2006). Post-release mortality, on the other hand, is influenced by factors like the amount of time specimens spend on deck before being returned to the sea (Revill et al., 2005; Rodríguez-Cabello et al., 2005).

The evaluation of the at-vessel condition of a specimen using vitality scores (e.g. excellent, good, poor dead conditions) is a subjective but simple, quick, and cost-effective method, providing important estimates of AVM, though not reporting information on PRM which is generally assessed through tag-telemetry studies (Skomal, 2007; Braccini et al., 2012). However, some holding and tagging studies suggest that PRM could be somewhat predicted (e.g., Van Beek et al., 1990; Hueter and Manire, 1994; Richards et al., 1995). Specimens scored in poor condition are likely to die after being discarded due to several underlying mechanisms, including physiological stress that may further impact behaviour, and reproduction, and ultimately lead to PRM (Wedemeyer et al., 1990; Gallagher et al., 2014). Hence, the assessment of physiological stress parameters could inform about the fate of discarded animals.

Animal stress is generally defined as a disruption of an organism's homeostasis caused by internal or external *stimuli*, which triggers compensatory behavioural or physiological responses (Wendelaar Bonga, 1997). These responses often entail large metabolic costs, diverting energy away from growth and reproduction and toward respiration, movement, and tissue repair (Wendelaar Bonga, 1997), a shift which may potentially lead to mortality. Understanding the impacts on animal stress responses is essential for developing measures aimed at reducing mortality among specimens captured in fisheries and for informing species-specific management and conservation strategies (Ferguson and Tufts, 1992; Wikelski and Cooke, 2006; Young et al., 2006).

Stressful events such as those occurring during fishing activities, prompt a cascade of physiological responses, often due to exertion as animals struggle to escape the stressor (e.g., Barton, 2002). This triggers a first response through the rapid release of stress hormones, such as catecholamines and corticosteroids, along with a rise in blood sugar levels, which depletes the animal's energy reserves while it also leads to lactic acid accumulation in muscles and plasma (Mazeaud et al., 1977; Randall and Ferry, 1992). Such responses disrupt the balance of ions, water, and other essential substances in blood (Wendelaar Bonga, 1997). Thus, secondary stress responses in animals can be measured through plasma biomarkers, such as pH, pCO_2, lactate, glucose, haematocrit, and osmolality (Skomal and Bernal, 2010). These indicators help assess an animal's condition following exposure to stressors, like those encountered during capture (Cliff and Thurman, 1984; Wells et al., 1986; Harrenstien et al., 2005; Skomal, 2007). While the level of stress responses may be largely species-specific (e.g., Mandelman and Skomal, 2009; Marshall et al., 2012; Skomal and Mandelman, 2012; Gallagher et al., 2014), factors such as fishing gear type and duration, environmental conditions and the shark's respiratory mode (either buccal pumping or ram ventilation; Skomal and Mandelman, 2012; Dapp et al., 2016; Guida et al., 2016; Mohan et al., 2020) as well as the individual condition, will also determine an animal's response to stress and the variations in the above mentioned indicators.

To determine if an animal is experiencing stress or if a particular activity induces stress, baseline (or “stress-free”) values of secondary stress responses are essential. However, acquiring these baseline values is challenging, as sample collection itself can trigger stress responses in specimens, and this is further exacerbated in fisheries. Consequently, researchers often rely on data obtained from studies with animals in captivity (Frick et al., 2012; Skomal and Mandelman, 2012; Barragán-Méndez et al., 2019) and/or estimated using mathematical models (Skomal, 2007). Laboratory studies aim to minimize capture time (Marshall et al., 2012) and often include behavioural analyses (Manire et al., 2001; Skomal et al., 2007; Hyatt et al., 2016; Whitney et al., 2016). However, these methods are insufficient for studying free-ranging sharks, particularly deep-sea species, as they cannot be kept in captivity or sampled quickly enough to obtain true baseline values (Skomal and Bernal, 2010). The lack of established baseline parameters for sharks indicates that most of the stress studies lack proper control groups, often limiting findings to descriptive analyses that report plasma ion concentrations without definitive conclusions on whether animals were genuinely stressed. Utilizing data from deceased animals may provide valuable control for free-ranging sharks, as suggested by Wosnick et al. (2017) and discussed in prior studies (Moyes et al., 2006; Hight et al., 2007; Hutchinson et al., 2015). Blood analyses from deceased animals can help determine when animals approach critical conditions, potentially improving release protocols. This alternative approach would shift the point of view from “the closer to baseline, the less stressed” to “the closer to death-reference levels, the more stressed and vulnerable.”

This study aimed to evaluate the impact of bottom trawling on the at-vessel condition of DSE species. To achieve this, DSE arriving onboard a crustacean bottom trawler were classified as dead, or in poor, good, or excellent condition. The influence of environmental factors and fishing practices on AVM of deep-sea sharks was analysed using logistic regression models. Deep-sea sharks were further subjected to analyses of secondary stress responses concentrations assessed through metabolites (glucose, lactate, urea) and electrolytes (calcium, sodium, phosphorus, potassium, chloride, magnesium). Metabolites and electrolytes levels were compared between deceased and alive specimens to test the potential of using values from deceased animals as reference points (Wosnick et al., 2017). Our hypotheses were that: (1) DSE would primarily arrive onboard dead or in poor condition (Scacco et al., 2023); (2) AVM in deep-sea sharks would be influenced by factors such as depth, body size (Rodríguez-Cabello and Sánchez, 2017; Scacco et al., 2023), water temperature (Brooks et al., 2015; Rodríguez-Cabello and Sánchez, 2017; Talwar et al., 2017), species (Morgan and Carlson, 2010; Braccini and Waltrick, 2019; Scacco et al., 2023), and fishing effort (e.g., Díaz and Serafy, 2005; Morgan and Carlson, 2010); and (3) deep-sea sharks would exhibit elevated stress levels (Prohaska et al., 2021).

3.2 Materials and methods

3.2.1 Ethics statement

This study was conducted following the Guidelines of the European Union Council (2010/63/UE) and Portuguese legislation "The protection of Animals Used for Scientific Purposes" (DL 113/2013). All the procedures were approved by CCMAR Animal Welfare Committee (ORBEA CCMAR - Organization Responsible for Animal Welfare of CCMAR) and the *Direção-Geral de Alimentação e Veterinária* (DGAV) of the Portuguese Government. All animal protocols were performed under Group-C licenses from the DGAV, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, Portugal.

3.2.2 Field campaigns

This study was conducted on a commercial crustacean bottom trawler in the South (37°-36°N; 9°-7.5°W) and Southwest (37.9°-36.7° N; 7.7°-9.6° W) coasts of Portugal (Figure 3.1). Ten fishing trips with a total of 77 hauls were performed from June 2020 to May 2022, summing up to 351 h of fishing effort. The target species were Norway lobster (*Nephrops norvegicus*), shrimps and prawns [*Aristeus antennatus*, *Aristaeomorpha foliacea, Aristaeopsis edwardsiana* (Johnson, 1868), *Parapenaeus longirostris* (Lucas, 1846), and *Penaeus monodon,* Fabricius, 1798] using net codend (i.e., the terminal section of a trawling net) mesh sizes of 70 mm (mean depth of 483 m) and 55 mm (mean depth of 636 m) respectively.

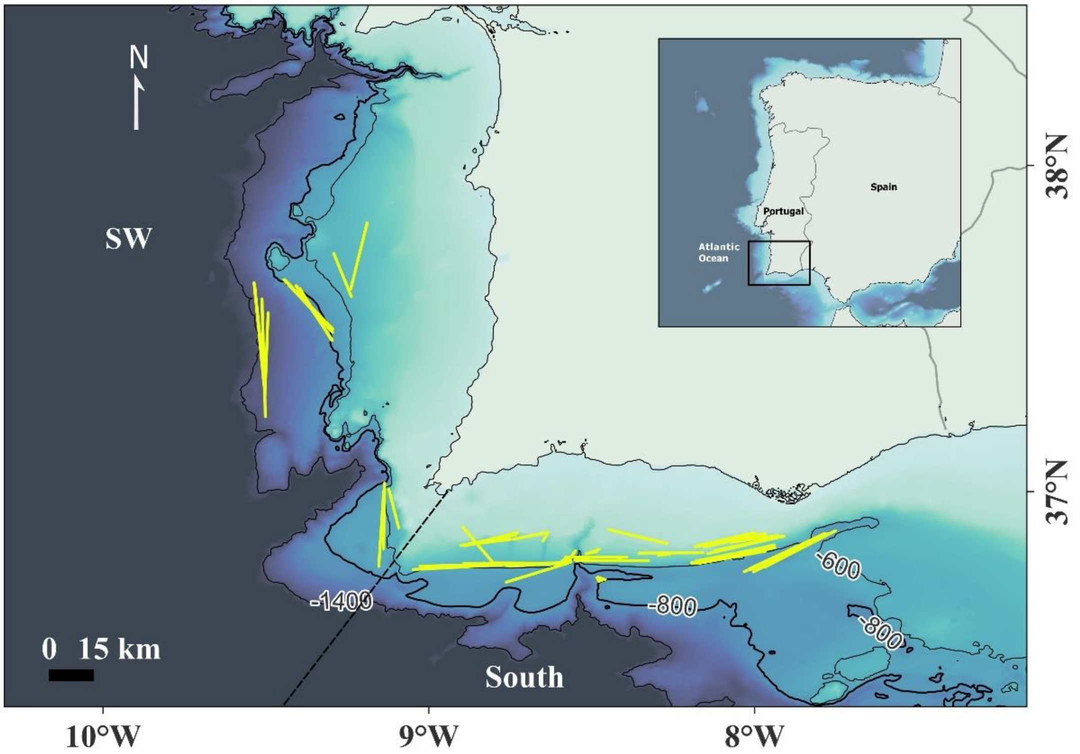

Figure 3.1 Study area in Portugal's South and Southwest (SW) coasts. The yellow lines represent the sites where hauls were conducted.

Fishing hauls lasted 2.3-9 h, with a velocity of 1.5-3.7 nm/h, and were conducted across all seasons of the year. Fishing effort was computed in hours from the moment the net reached the bottom of the ocean up to the moment it began to be hauled back to the vessel. Capture and handling time was computed in hours since the start of a haul until the start of the sorting of the catch by fishers. The weight of the net codend (kg) was a visual estimation given by the skipper when the net was lifted from the water at the end of every haul, or when the catch was already inside the "pond" (an area below deck where the codend is offloaded). Water temperature (°C) at the bottom/fishing depth was either collected by a Scanmar® or by a mini DST-CTD logic® Star-Oddi® attached to the net (temperature was recorded at every 5 min for hauls < 1000 m and every 10 min for hauls > 1000 m). Surface water temperature was collected by the DST-CTD whenever a reading was performed near the surface (<1m), otherwise, data from the Copernicus website on the sea surface temperature for the sampled regions, dates and hour of each haul (to ensure the precision of the estimate), was used instead. Technical (i.e., codend mesh size, codend weight, and fishing effort), environmental (i.e., water temperature, and fishing depth), and biological data (i.e., sharks' total length and maturity stage) were registered into the Electronic Logbook (eLog) Olrac

Dynamic Data logger (Olrac DDL®) where information per specimen was inserted. Capture and handling duration (h) was assessed from the moment each haul started up to the moment the sorting of the catch started.

3.2.3 At-vessel condition

The DSE caught during the fishing events, were sorted by species. Each specimen was identified following Ebert et al. (2013) and Last et al. (2016) and measured [total length (TL) to the nearest 0.5 cm: from the tip of snout to the tip of the caudal fin]. DSE's at-vessel condition was assessed following the criteria of Benoît et al. (2010) and Catchpole et al. (2017). Both methods include a rapid observation of possible injuries and reflex impairments (i.e., reduction or loss of responses to stimuli) to classify specimens into a small number of ordinal vitality categories (Table 1). After this evaluation, the alive specimens were kept inside acclimatized holding tanks until the moment they were discarded, i.e., after the end of all the analyses with the DSE from the same haul, which took up to 1h in some instances.

Table 3.1 At-vessel condition of deep-sea elasmobranchs' specimens, assessed through vitality categories.

State	Description
Excellent	Vigorous body movement. No or minor external injuries only.
Good	Weak body movement. Responds to touching and prodding. Have minor external injuries.
Poor	No body movement but can move spiracles. Minor or major external injuries.
Dead	No body or spiracle movement. No response to touching or probing.

3.2.4 At-vessel mortality

The most frequently caught deep-sea shark species (hereafter referred to as "sharks") — comprising three species from the order Squaliformes [*Etmopterus pusillus* (Lowe, 1839), *E. spinax* (Linnaeus, 1758), and *Scymnodon ringens* Barbosa du Bocage & de Brito Capello, 1864] and one species from the order Carcharhiniformes (*Galeus melastomus* Rafinesque, 1810; Table 2) — were selected for modeling the influences of variables on their AVM rates. At-vessel mortality rates were based on the assigned vitality category of each specimen (Table 3.1). Specimens categorized as dead were from the vitality category 'dead', while those categorized as alive were from the vitality categories 'poor', 'good', or 'excellent' (Table 3.1).

3.2.5 Capture and handling stress

Blood sampling was conducted over four field trips between May and August 2021 and February and April 2022 along the South coast of Portugal. Hauls were randomly selected for sampling, with five alive and five dead sharks from frequently caught species (*Etmopterus* spp., *G. melastomus*, and *S. ringens*) randomly chosen from each selected haul. Blood was collected as quickly as possible from dead sharks via the caudal peduncle using a 1 ml heparinized syringe, followed by sampling from alive sharks. Dead sharks were prioritized over live ones, as blood flow decreases over time *post-mortem*, making collection increasingly difficult. Because each fishing trip takes *ca*. three full days, plasma was obtained by centrifugation of blood samples on board within 2h post-collection, stored at -20°C and kept frozen until laboratory analysis. The alive sharks were released after blood collection. At the laboratory, the concentrations of metabolites (i.e., glucose, lactate and urea) and electrolytes (i.e., phosphorus, chloride, magnesium, and calcium) were measured by colorimetric assays using Spinreact® kits (Girona, Spain) with a Multi-Mode Microplate Reader BioTek Synergy™ 4 (BioTek® Instruments, Winooski, VT, USA). Sodium and potassium concentrations were determined using a flame photometer (BWB-XP PerformancePlus, BWB Technologies, UK).

3.2.6 Statistical analysis

3.2.6.1 At-vessel mortality

A binomial Generalized Linear Model (GLM) was used to evaluate the influence of several predictors on the likelihood of shark mortality (dead or alive). To ensure predictors were suitable for the GLM, an initial *Logit* model was run using the package *lessR* (Gerbing, 2021), which allowed for identifying potential collinearity among predictors. The initial predictor variables included codend weight, codend mesh size, total length, fishing velocity, fishing effort, fishing depth, species, and temperature differences between surface and bottom waters (hereafter simply 'temperature differences'). If in this first step, a collinearity is identified by the Tolerance coefficient - as was the case with the variable 'depth' found to exhibit high collinearity (Tolerance = 0.17) - than this variable is excluded from further GLM analyses.

The *glmulti* package (Calcagno and Mazancourt, 2010) was used to identify the best predictor variables (excluding 'depth') by fitting a series of binomial GLMs with AVM as the binary response variable. The *logit* link function ensured response probabilities remained within the 0–1 range, appropriate for binary outcomes. Model selection relied on the Akaike Information Criterion (AIC), where a lower AIC (lower than 2 units) indicates a better model balance between fit and complexity. Only the main effects of each variable were considered, as the data were collected randomly and did not provide sufficient differences between some predictor combinations. This approach helped prevent potential overfitting issues from interaction terms that lacked adequate contrast. The *glmulti* package identified the importance of terms to be used in the final GLM, using a diagnostic plot to highlight predictors with an importance higher than 50%, hence suggesting exclusion of predictors that fell below that threshold. Finally, a GLM was conducted with the selected predictors, followed by a check of the model's suitability, which was once more conducted by the *Logit* model in the *lessR* package. The following diagnostics were conducted with the *Logit*: (1) the absence of multivariate outliers, (2) linear relationships between continuous predictors and the logit-transformed response, and (3) no collinearity among predictors.

3.2.6.2 Capture and handling stress

To test the usefulness of using dead animals' values as reference values, stress responses concentrations (i.e., metabolites and electrolytes concentrations) in plasma were compared between dead and alive specimens of *E. pusillus* and *G. melastomus* using the t-Test or the equivalent non-parametric Mann-Whitney test. The latter was applied whenever the assumptions of normality and homoscedasticity of each evaluated response were not met using the Shapiro-Wilk test and the Bartlet test respectively (at a $p < 0.05$). Due to the low number of specimens for the species *E. spinax* and *S. ringens* (< 5 observations per dead or alive specimens) only mean (SD) and median (IQR) values on the plasma related stress responses concentrations were reported.

All statistical analyses were done with the open-source R environment (R Development Core Team, 2024).

3.3 Results

3.3.1 At-vessel condition

A total of 1,559 specimens belonging to 18 deep-sea species of sharks (15 species) and skates (3 species) were evaluated. Collectively they were mostly dead (n=1258) or in a poor condition (n=224), with very low numbers of specimens in good condition (n=70) and even lower in excellent conditions (7). All species presented a much higher number of deceased specimens (mean = 84) or specimens in poor condition (mean =15), than in excellent (mean = 2) and/or good conditions (mean = 6; Table 3.2). Species that presented the worst condition were *Oxynotus paradoxus* and *Neoraja iberica* with all specimens dead (however with low number of evaluated specimens), followed by *Deania calceus* and *D. profundorum* (Table 3.2). Species that presented comparatively better at-vessel condition (i.e., higher rates in good and excellent conditions) were the *Centrophorus* spp. with no deceased specimen. *Mitsukurina owstoni* also did not present immediate AVM but only one specimen was sampled (Table 3.2).

Table 3.2 Percentages (%) of the at-vessel condition of deep-sea elasmobranchs caught by a crustacean trawler at the southern region of Portugal.

		At-vessel condition (%)			
Species	**n**	**Excellent**	**Good**	**Poor**	**Dead**
Centrophorus granulosus	10	0	30	70	0
Centrophorus squamosus	5	20	20	60	0
Centroscymnus coelolepis	4	0	0	50	50
Centroselachus crepidater	17	0	6	47	47
Chlamydoselachus anguineus	3	0	33	0	67
Dalatias licha	13	0	8	31	62
Deania calceus	46	0	0	4	96
Deania profundorum	208	0	1	11	88
Dipturus nidarosiensis	19	0	0	16	84
Dipturus oxyrinchus	37	0	3	22	76
Etmopterus pusillus	131	1	2	12	85
Etmopterus spinax	303	0	6	8	86
Galeus atlanticus	64	0	3	14	83
Galeus melastomus	494	1	7	19	74
Mitsukurina owstoni	1	0	0	100	0
Neoraja iberica	2	0	0	0	100
Oxynotus paradoxus	4	0	0	0	100
Scymnodon ringens	198	1	1	12	86
All	**1559**	**0.4**	**4**	**14**	**81**

3.3.1.1 At-vessel mortality

Of a total of 1,126 specimens, the majority belonged to *G. melastomus*, followed by *E. spinax*, *S. ringens*, and *E. pusillus* (Table 2). At-vessel mortality presented a much greater proportion of dead (81%) than alive (19%) specimens (Table 3.3), where the species *Galeus melastomus* presented the lowest proportion of dead specimens in relation to alive specimens in comparison with the other sharks (Table 3.2).

Dead specimens were more numerous in hauls using a codend mesh size of 55 mm (846; 75%) than 70 mm (71; 6%). Additionally, codend weight and temperatures (surface, bottom and differences among the two) showed higher mean values in deceased specimens compared to alive ones, while the sharks' total length, fishing effort, and depth presented greater values in alive specimens (Table 3.3).

Table 3.3 Total number of deep-sea sharks' specimens sampled by at-vessel mortality (AVM) categories (Alive and Dead). Mean and standard deviation (±SD) of the total length, fishing depth, fishing effort, fishing velocity, weight of the net codend, temperature differences between the surface and bottom waters, and bottom and surface temperatures by the at-vessel mortality categories.

	AVM categories	
	Alive	**Dead**
AVM predictors	(n=209)	(n=917)
Total length (cm)	42.4 ± 16.0	32.2 ± 12.6
Depth (m)	640.9 ± 229.0	617.4 ± 196.3
Fishing effort (h)	5.3 ± 1.3	4.8 ± 1.1
Velocity (nm/h)	2.5 ± 0.39	2.5 ± 0.3
Codend Weight (kg)	191.7 ± 85.7	211.2 ± 116.3
Temp. differences (°C)	4.1 ± 1.7	4.3 ± 1.9
Bottom Temp. (°C)	13.0 ± 0.6	13.2 ± 0.6
Sea surface Temp. (°C)	17.1 ± 1.5	17.5 ± 1.5

The best GLM model included predictors such as total length (TL), codend mesh size and weight, species, fishing effort, and temperature differences (Table 3.4), achieving an overall prediction accuracy of 83.5%, with 97.4% of mortality predicted and 22.5% of survivorship predicted. The results indicate that several predictors significantly affect the AVM categories in different ways (Table 3.4). Species differences played a strong role: *Etmopterus spinax* and *Galeus melastomus* have significantly lower probabilities of mortality compared to the species (*S. ringens*), while *E. pusillus* shows significantly higher probabilities of mortality relative to *S. ringens* (Table 3.4). Additionally, a 70 mm codend mesh size corresponds to a significantly lower mortality rate compared to 55 mm mesh. Larger TL and increased fishing effort also correlate with lower mortality rates (Table 3.4). In contrast, increasing codend weight and temperature differences are associated with higher mortality likelihood (Table 3.4).

Table 3.4 Results from a generalized linear model (GLM). Predictors included the categorical variables "species" (*Etmopterus pusillus, E. spinax, and Galeus melastomus*), and "codend mesh size" (70 mm), and the coefficients for the categorical variables are "*Scymnodon ringens*" and "55 mm" respectively. Continuous and numerical variables included total length, fishing effort, codend weight and temperature differences among surface and bottom waters.

GLM coefficients presented are the estimate, standard error, odds ratio with its confident interval (C.I. 95% lower and upper limits) and the significance of each the predictor (p-value, p < 0.05*; p< 0.001***).

Predictor	Estimate	Std. Error	Odds Ratio	C.I. (95%) lower	C.I. (95%) upper	p-value	
(Intercept)	-6.215	0.908	0.002	-1.778	1.782	<0.001	***
E. pusillus	-2.201	0.476	0.111	-0.822	1.044	<0.001	***
E. spinax	2.712	0.485	15.042	14.091	15.993	<0.001	***
G. melastomus	2.889	0.453	17.947	17.059	18.835	<0.001	***
Codend mesh size (70 mm)	0.843	0.361	2.324	1.616	3.032	0.019	*
Total length (cm)	0.063	0.008	1.065	1.049	1.081	<0.001	***
Fishing effort (h)	0.332	0.097	1.394	1.204	1.584	<0.001	***
Codend Weight (kg)	-0.005	0.001	0.995	0.993	0.997	<0.001	***
Temp. differences (°C)	-0.158	0.065	0.854	0.727	0.981	0.016	*

3.3.2 Capture and handling stress

Alive *E. pusillus* and *G. melastomus* were larger, generally caught at greater depths, and presented greater duration of capture and handling procedures when compared with dead specimens (Table 3.5). For *E. spinax* the same was observed but that comparison was made considering only one dead specimen. Alive *S. ringens* presented larger size and were mainly caught at shallower depths. In this species, however, the capture and handling times between dead and alive specimens were similar (Table 3.5).

Table 3.5 Mean, minimum and maximum values of total length (TL, cm), depth of fishing (m) and capture and handling time (h) for sharks' species (*Etmopterus pusillus*; *E. spinax*; *Galeus melastomus* and *Scymnodon ringens*) and at-vessel mortality (AVM) categories (alive and dead) with the respective number of specimens.

Species	AVM	n	TL (cm)	Depth (m)	Capture and handling time (h)
E. pusillus	Alive	6	38.8 ± 3.6	647.4 ± 98.0	5.8 ± 0.7
	Dead	15	32.9 ± 6.3	483.7 ± 51.8	4.7 ± 0.6
E. spinax	Alive	14	29.3 ± 5.8	542.8 ± 99.4	4.9 ± 0.9
	Dead	1	33 ± 0.0	723.6 ± 0.0	5.8 ± 0.0
G. melastomus	Alive	13	48.7 ± 11.9	572.4 ± 67.7	5.4 ± 0.8
	Dead	8	36.9 ± 11.6	527.0 ± 69.1	4.4 ± 0.8
S. ringens	Alive	5	54.6 ± 9.7	603.7 ± 99.9	6.2 ± 0.3
	Dead	3	40.8 ± 6.3	702 ± 0.0	6.2 ± 0.0

Plasma analyses revealed varying concentrations of metabolites and electrolytes for the different species (Appendix 3.1). Glucose and urea presented significantly higher concentrations for alive specimens of the species *E. pusillus* and *G. melastomus* and higher concentrations for alive *S. ringens* in comparison with dead specimens. However, urea presented higher values for deceased *S. ringens* in relation to alive specimens (Figure 3.2; Appendix 3.2). Lactate concentrations were not significantly different but were also higher for alive specimens of *G. melastomus* and *S. ringens* (Figure 3.2). For *E. pusillus* higher concentrations were found for deceased specimens in comparison with alive ones (Figure 3.2). Alive specimens of *E. spinax* presented the highest maximum values of all metabolites in relation to the dead specimen (Figure 3.2).

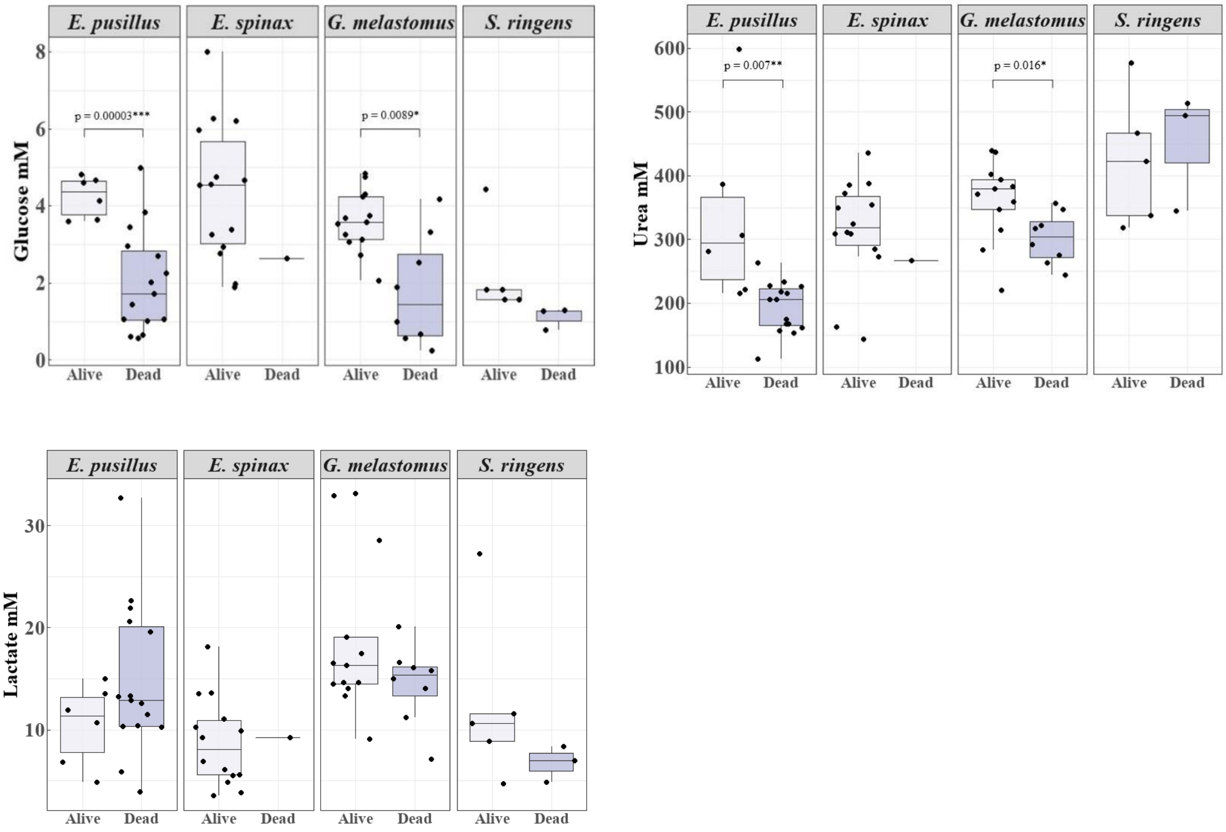

Figure 3.2 Boxplot representation of the concentrations of the plasma metabolites (glucose, lactate and urea) in mM for alive and deceased specimens of the sharks *Etmopterus pusillus, E. spinax, Galeus melastomus and Scymnodon ringens*. The boxplots show the median (horizontal lines) with 50% (boxes) and 95% intervals (vertical lines). Significant differences in glucose and urea concentrations between deceased and alive specimens of *E. pusillus and G. melastomus* are presented through the p-value of the t-Test (glucose) and Mann-Whitney test (urea).

In relation to the electrolytes, significant differences were found for potassium and magnesium (Figure 3.3; Appendix 3.2). Potassium concentrations were significantly higher for dead specimens of *E. pusillus* and *G. melastomus* (Figure 3.3; Appendix 3.2) and higher for dead specimens of *S. ringens* (Figure 3.3). Magnesium concentrations were significantly higher for *G. melastomus* deceased specimens but higher for *E. pusillus* and *S. ringens* alive specimens (Figure 3.3; Appendix 3.2). Calcium was higher for alive specimens of *E. pusillus* and *S. ringens* but for *G. melastomus* it was higher for dead specimens (Figure 3.3). Chloride concentrations were higher for alive specimens of *E. pusillus* and *G. melastomus* whereas *S. ringens* deceased specimens presented higher concentrations (Figure 3.3). Sodium concentrations were higher in deceased *G. melastomus* and *S. ringens* but higher for alive *E. pusillus* (Figure 3.3). Phosphorus concentrations were higher in deceased *E. pusillus* and *G. melastomus* and a bit higher in alive *S. ringens.* The only dead specimen of *E. spinax* presented the highest value of sodium (449.7 mM), in comparison with alive specimens of the same species and all the other specimens of the other species (Figure 3.3; Appendix 3.1). Chloride, potassium and sodium were all greater for this dead *E. spinax* specimen when compared to the alive specimens, whilst calcium, and magnesium were greater for alive specimens in comparison with the dead specimen (Figure 3.3).

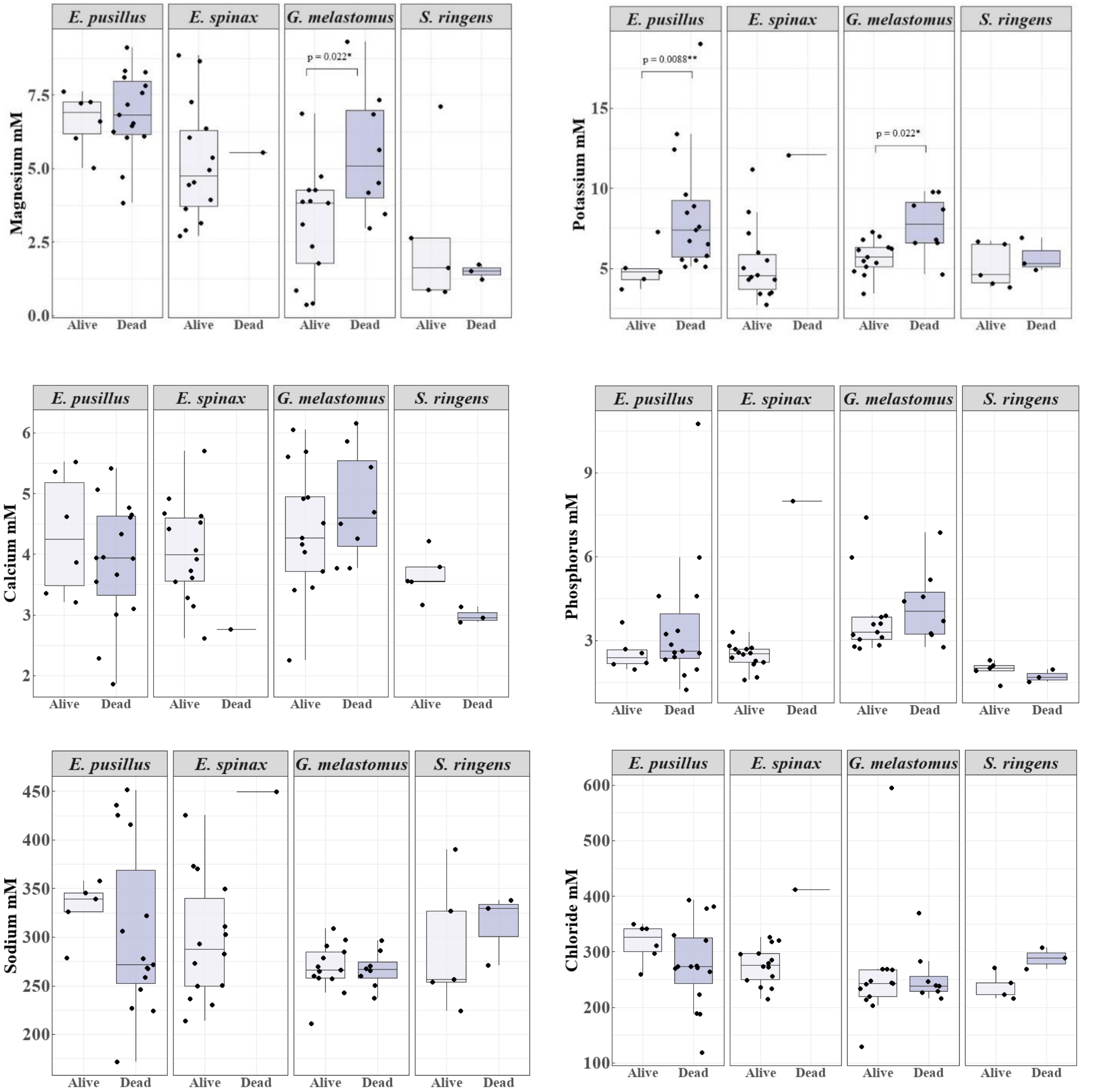

Figure 3.3 Boxplot representation of the concentrations of the plasma electrolytes (calcium, chloride, magnesium, phosphorus, potassium, and sodium) in mM for alive and deceased specimens of the sharks *Etmopterus pusillus, E. spinax, Galeus melastomus and Scymnodon ringens*. The boxplots show the median (horizontal lines) with 50% (boxes) and 95% intervals (vertical lines). Significant differences in potassium concentrations between deceased and alive specimens of *E. pusillus and G. melastomus* are presented through the p-value of the Mann-Whitney. For magnesium, significant differences between deceased and alive specimens of *G. melastomus* are presented by the p-value of the t-Test.

3.4 Discussion

3.4.1 At-vessel condition

This study presents data on the at-vessel condition of DSE caught in bottom trawling operations, where most specimens were either dead upon retrieval (80.7%; n=1,258) or in poor condition (14.4%; n=224). Only a few were in excellent (0.4%; n=7) or good condition (4.5%; n=70). Specimens classified as in poor condition are unlikely to survive when released back into the sea (e.g., Morgan and Burgess, 2007; Skomal, 2007; Skomal and Mandelman, 2012; Brooks et al., 2015; Talwar et al., 2017). Consequently, the PRM (including dead specimens or in poor condition) of the studied DSE species is expected to be extremely high, averaging 95% across all of the 18 DSE species sampled. This includes exceptionally high estimated PRM for the most numerous species like *G. melastomus* (93%), *E. spinax* (94%), *E. pusillus* (97%), *S. ringens* (98%) and *D. profundorum* (99%). Additionally, PRM is thought to be critically high for species considered endangered in Europe by the International Union for Conservation of Nature (IUCN) (Nieto et al., 2015), including *Centroscymnus coelolepis* and *Deania calceus* (100%), as well as *Dalatias licha* (93%). However, some species of conservation concern showed a higher proportion of specimens in relatively better condition compared to other DSE species. These include the critically endangered (Nieto et al., 2015) *Centrophorus granulosus* (30% in good condition), the endangered *Centrophorus squamosus* (20% in excellent and 20% in good condition), and the least-concern but rare *Chlamydoselachus anguineus* (33% in good condition).

Most at-vessel condition studies on sharks and skates focus on demersal species caught by longline and gillnets presenting varying mortality estimates (0-100%) but generally lower than for bottom trawling (Revill, 2012; Dapp et al., 2016; Ellis et al., 2016; Talwar et al., 2017). Studies specifically on bottom trawling are scarcer (Dapp et al., 2015) and mainly involve resilient demersal species like *Scyliorhinus canicula*, which showed relatively low mortality rates between 2–53% (Kaiser and Spencer, 1995; Revill et al., 2005; Rodríguez-Cabello et al., 2005; Barragán-Méndez et al., 2019). Only recently has research assessed the condition of a few DSE species in bottom trawling, as in a study of 12 demersal elasmobranch species in the Asinara Gulf, where specimens were classified into ‘active,’ ‘inactive,’ or ‘dead’ conditions (Scacco et al., 2023). Scacco et al. (2023) reported high rates of inactive or dead specimens for *Dipturus oxyrinchus* (85%), *E. spinax* (88%), and *G. melastomus* (91%), aligning with our findings. Though our estimates of dead or poor-condition specimens were still slightly higher (98%, 94%, and 93%, respectively).

Using several technical, environmental and biological predictors, the GLM achieved a high prediction of 83.5% of sharks' AVM (dead and alive), which was specifically linked to several factors which included specimen size (TL), codend weight and mesh size, temperature differences, species, and fishing effort. Mortality rates were inversely related to shark size, with smaller specimens more likely to die in bottom trawling procedures than larger ones. Similarly, Ellis et al. (2008) reported higher mortality in skates under 50 cm in the North Sea trawling fishery, and Talwar et al. (2017) observed higher mortality in smaller *Squalus cubensis* in the Exuma Sound longline fishery. This trend is also consistent with findings for other elasmobranchs (Rodríguez-Cabello and Sánchez, 2017; Scacco et al., 2023) and bony fishes (Suuronen et al., 1996; Wileman et al., 1999; Ingolfsson et al., 2002). Larger specimens generally exhibit greater swimming endurance and are less prone to injury. In contrast, smaller specimens are more vulnerable to physical damage and higher mortality rates when caught in trawling nets, primarily due to the increased volume and composition of the catch in the codend. Higher catch volumes intensify contact and abrasion among organisms, raising the risk of injury for smaller species (Broadhurst et al., 2006). This agrees with the present study findings where heavier codend presented significantly higher probability of mortality (although with a small effect), than lighter codend. However, caution is needed when interpreting these results, as codend weight was estimated visually by the skipper, introducing the potential for under- or overestimation of the weight. Future studies could use a codend weigher, a tool that measures the codend's weight as it is brought on board (Caslake, 2009). This would enhance the accuracy of codend weight measurements and contribute to a better understanding of how codend weight affects mortality rates in sharks.

Another important technical predictor on the sharks' mortality was codend mesh size, where hauls using smaller mesh sizes (55 mm) targeting prawns and shrimp had significantly higher mortality rates than those with larger mesh sizes (70 mm) targeting Norway lobster. Smaller mesh sizes increase retention, causing the net to clog more quickly and capturing a wider range of specimen sizes (Murawski, 1996; Broadhurst, 2000). This congestion may cause mortality by compression and asphyxia. This would suggest that adjusting mesh sizes based on target species and fishing depth could help reduce mortality in non-target deep-sea shark populations. However, the relationship between codend mesh size and mortality is so far only supported in studies with bony fish. Studies with bony fish suggest that larger mesh sizes reduce injuries, and scale loss by allowing more fish to escape (Main and Sangster, 1991; Sangster et al., 1996; Lowry et al., 1996), although some research found no significant impact of mesh size on escapee survival (Suuronen et al., 1996; Wileman et al., 1999). In this study, depth—a key predictor of mortality—was excluded from the GLM due to collinearity with other factors, as smaller mesh sizes (55 mm) were predominantly used at greater average depths (623 m ± 286 m), while larger meshes (70 mm) were used at shallower depths (473 m ± 58 m). This does not rule out the potential impact of mesh size on shark mortality, but further research using varied mesh sizes at similar depths and conditions could provide clearer insights into its effects specifically on mortality rates in deep-sea sharks.

With larger temperature differences (between bottom and surface waters) there was an increase in mortality in the sharks analysed for this study. Similar results were reported in deep-sea sharks caught by longliners in the Cantabrian Sea (NE Atlantic; Rodríguez-Cabello and Sánchez, 2017) and in Bahamian waters where the sea surface temperature reached 30°C (Brooks et al., 2015; Talwar et al., 2017). Deep-sea species inhabit cold waters, and when brought to warmer surface temperature may increase the stress and mortality (Davis, 2002; Morgan and Burgess, 2007; Braccini et al., 2012; Weltersbach and Strehlow, 2013; Prohaska et al., 2021). Most sharks are ectothermic which means that they regulate their body temperature with the surrounding water and have a relatively narrow range of temperature in which they can thrive and reproduce (Elliot, 1981). Water temperature was already suggested as a proxy to determine time/area closures when it reaches a certain threshold (Morgan and Burgess, 2007). This approach may become even more important as global water temperature rises due to climate change but will require an understanding of species-specific reactions to local conditions.

Fishing effort is generally positively related to mortality, as prolonged procedures elevate mortality rates (Díaz and Serafy, 2005; Morgan and Burgess, 2007; Morgan and Carlson, 2010). In static gear like longlines and gillnets, longer soak times increase mortality, particularly for species reliant on ram ventilation for oxygenation (Carlson et al., 2004; Dapp et al., 2015). However, in this study, higher fishing effort was related with a slightly increased odds of finding alive specimens than dead ones. Unlike static gear, trawling does not allow for pinpointing the exact moment of capture, so fishing effort in this study is likely overestimated, measured from the start of each haul rather than the exact entry time of each specimen in the net. Hauls in this study ranged from 2:54 to 8:36 h, meaning that specimens were exposed to at least 2:54 h of fishing activity. It is possible that the alive specimens from longer hauls were captured closer to the end of the haul, increasing their chance of survival. This could suggest that shorter hauls, potentially less than 2:54 h, may reduce shark mortality. However, longline studies have shown significant mortality in deep-sea sharks even with soak times under 3 h (Talwar et al., 2017; Rodríguez-Cabello and Sánchez, 2017). Additional controlled studies could help identify if there is an optimal haul duration that would minimize shark mortality.

Shark species exhibited markedly different mortality likelihoods, aligning with findings in other studies (Morgan and Carlson, 2010; Braccini and Waltrick, 2019; Scacco et al., 2023). Deep-sea squaliform sharks tend to have particularly vulnerable and conservative life histories which includes lower fecundity and smaller litter size, making them less resilient to the pressures exerted by fisheries (García et al., 2008; Simpfendorfer and Kyne, 2009; Neat et al., 2015). Indeed, the squaliform sharks (*Etmopterus* spp. and *S. ringens*) showed the highest mortality rates, contrasting with the carcharhiniform shark (*G. melastomus*). These differences may be partly attributed to the presence of an anal fin in *G. melastomus* and its absence in squaliform species. The anal fin is thought to enhance swimming stability (Ebert et al., 2021), which could provide *G. melastomus* with improved swimming abilities, potentially reducing its mortality in trawling situations. However, this adaptation does not prevent its capture by trawlers. Further research is required to confirm this hypothesis.

3.4.2 Capture and handling stress

In general, specimens appeared stressed by the conditions and procedures conducted during fishing activities, as indicated by plasma concentrations of secondary stress response indicators (e.g., metabolites and electrolytes). These concentrations were different when compared to baseline values for elasmobranchs from previous studies (Frick et al., 2010; Heard et al., 2014) and stress indicators for demersal sharks caught by bottom trawlers (e.g., Ruiz-Jarabo et al., 2022; Falco et al., 2023). In this study, significant differences in glucose, urea, magnesium, and potassium concentrations were observed between deceased and alive specimens of *E. pusillus* and *G. melastomus*.

Potassium and urea levels could act as mortality markers for *E. pusillus* and *G. melastomus*, with deceased specimens showing significantly higher potassium and lower urea levels. For *G. melastomus*, significantly higher magnesium levels were also observed in deceased individuals.

Although no statistical differences were noted for *S. ringens* and *E. spinax* due to low sample numbers, deceased specimens exhibited higher potassium levels. *Post-mortem* potassium and urea concentrations have previously been linked to mortality in *Galeocerdo cuvier* and *Prionace glauca*, alongside other markers like lactate, phosphorus, and calcium (Wosnick et al., 2017; Moyes et al., 2006). High potassium levels may lead to hyperkalemia, disrupting muscle cell membrane excitability, causing myocardial dysfunction in *S. acanthias* and muscle tetany in *Mustelus antarcticus* (Martini, 1974; Cliff and Thurman, 1984; Frick et al., 2010). Plasma potassium above 7 mM in this study consistently indicated poor at-vessel condition or death, supporting previous findings (Butcher et al., 2015), and suggesting cell disruption leakage. Similarly, low urea can signal osmotic imbalance, possibly increasing ion exchange across osmoregulatory tissues—a stress response linked to capture in *S. acanthias* (Piermarini and Evans, 2000; Mandelman and Skomal, 2009).

Stressed fish generally exhibit significant increases in glucose levels (Skomal and Bernal, 2010; Marshall et al. 2012; Skomal and Mandelman, 2012), which is attributed to the mobilization of glucose for energy production (e.g., Dobson and Hochachka, 1987; Girard and Milligan, 1992). However, in the present study, glucose was lower for dead specimens of *S. ringens* and significantly lower for dead specimens of *E. pusillus* and *G. melastomus* in comparison with alive ones. Talwar et al. (2017) also found that, lower blood glucose levels, resulted in a higher likelihood of mortality of deep-sea sharks in the Bahamas. Cliff and Thurman (1984) observed that moribund or dead *C. obscurus* specimens had lower glucose levels compared to those that were alive. This might imply that, an inability to further mobilize glycogen stores and glucose depletion may contribute to metabolic failure and ultimately, death.

Lactate has been proposed as a mortality marker (Moyes et al., 2006; Marshall et al., 2012; Wosnick et al., 2017), given that increased lactate levels indicate a strong physiological response, often due to anaerobic respiration triggered by high stress or intense activity in low-oxygen conditions (Dobson and Hochachka, 1987; Girard and Milligan, 1992; Skomal and Bernal, 2010). In this study, lactate levels did not significantly differ between deceased and alive specimens of *E. pusillus* and *G. melastomus*, though across all four species studied, lactate levels were high when compared to the unstressed 5 mM level reported for species like *Carcharhinus obscurus*, *C. plumbeus*, and *S. acanthias* (Cliff and Thurman, 1984; Spargo, 2001; Mandelman and Farrington, 2007). Pelagic sharks with lactate levels above 16 mM often show high mortality (Skomal and

Bernal, 2010). Notably, in this study, maximum lactate values for *G. melastomus* (33.12 mM) and *S. ringens* (27.27 mM) were among the highest recorded for elasmobranchs, comparable to species like *Alopias vulpinus* (27 mM; Heberer et al., 2010) and *Carcharhinus brachyurus* (42 mM; Dapp et al., 2016). Elevated lactate levels in *G. melastomus* and some other alive specimens from this study suggest potential PRM, warranting further investigation into species-specific lactate responses (Marshall et al., 2012).

Comparing interspecific responses, *S. ringens* displayed distinct concentrations of phosphorus, magnesium, and urea. This could indicate either a unique response to fishing procedures on the crustacean bottom trawler or naturally different baseline levels of these metabolites and electrolytes. Prohaska et al. (2021) found species-specific and ecological differences in stress responses among deep-sea sharks, with deeper-dwelling species showing distinct reactions compared to those at shallower depths. In this study, *S. ringens* was generally found at greater depths and tended to be larger than other studied species, particularly among live specimens. This size difference may relate to reduced fight intensity, potentially helping larger sharks better manage stress (Talwar et al., 2017).

The observed differences in plasma secondary stress responses between alive and deceased and the high dispersion within a single species suggest individual variability in coping mechanisms (Edwards, 1988) and that stress responses may be triggered at an individual level (Wosnick et al., 2017). Variability within parameters and species also likely reflect the differences each animal experiences while caught which are impossible to estimate in this study – i.e., actual time in codend, level of contact with the gear and other organisms, place within the organism's mass in the codend and opportunity to struggle or even access to oxygenated water. Studies on elasmobranchs indicate that plasma metabolites and electrolytes reach peak levels over different timeframes: lactate (~2 hours), urea (~6 hours), and sodium (~5 hours) (Cliff and Thurman, 1984; Frick et al., 2010, 2012; Chapman and Renshaw, 2009; Heard et al., 2014; Ruiz-Jarabo et al., 2022). This highlights the importance of further species-specific studies where animals are exposed to various stressors in different fisheries, with blood sampling over extended time intervals to fully characterize stress responses following events like capture or air exposure (Frick et al., 2009). However, samples collected immediately after capture may not fully reflect physiological impact. Conducting controlled experiments with deep-sea elasmobranchs poses challenges due to the need to replicate their natural environment (low temperature, high pressure, darkness) and minimize captivity-related stress. Thus, endpoint concentrations of metabolites are often assessed in deceased specimens (Moyes et al., 2006; Hight et al., 2007; Hutchinson et al., 2015; Wosnick et al., 2017). In this study, only potassium, magnesium, and urea showed potential as markers for mortality in deceased *E. pusillus* and *G. melastomus*, suggesting that further studies should include additional species, sample sizes, and varied fishing conditions.

3.4.3 Recommendations for decreasing impacts of bottom trawling on deep-sea elasmobranchs

In the European Union, DSE are protected by regulations such as Regulation 2024/257, which prohibits the landing of several shark species, effectively setting a zero TAC to prevent targeted fishing. However, despite this protection, DSE continues to be caught as bycatch in bottom trawling operations and are subsequently discarded, often dead or dying, and under stress, as observed in this study. This is particularly concerning, as discarded bycatch is rarely recorded in fisheries logbooks, limiting our understanding of DSE's population dynamics and distribution. The ongoing mortality of DSE, combined with the absence of accurate catch and discard records, underscores a significant yet unrecorded impact on these vulnerable populations. This raises concerns about the long-term sustainability of DSE species in EU waters, as the true extent of their decline remains largely unknown. Given the unknown limits to which these species can withstand fishing pressures, a precautionary approach is necessary by avoiding the capture of DSE in the first place.

Studies on gear modifications provide a valuable foundation for mitigating bycatch. Turtle excluder devices and the Nordmøre grid have proven effective in reducing bycatch and are widely applied in elasmobranch studies (e.g., Isaksen et al., 1992; Brewer et al., 1998, 2006; Chosid et al., 2012; Vasapollo et al., 2019). These are angled panels with spaced bars, that allows smaller target crustaceans through while excluding larger, unwanted species such as sea turtles and elasmobranchs without impacting greatly the target catch rates (Isaksen et al., 1992; Brewer et al., 1998, 2006; Chosid et al., 2012; Vasapollo et al., 2019). A modified version of the Nordmøre grid has been applied in Portuguese crustacean trawl fisheries, where it effectively reduced bony fish bycatch while maintaining the commercial catch of crustaceans (Fonseca et al., 2005). However, while effective for larger elasmobranchs, these devices still allow smaller DSE to pass through, meaning smaller-bodied DSE remain at risk. Therefore, further actions and gear modifications are encouraged to address the bycatch of smaller-bodied DSE species.

Adjusting the codend mesh size and weight in bottom trawling presents a viable approach to reducing bycatch and mortality of sharks. In Portugal, research on bottom trawler's codend mesh configuration suggests that increasing mesh size and switching from diamond to square mesh significantly enhances selectivity, allowing smaller specimens to escape (Campos et al., 2002; Campos and Fonseca, 2003; Campos et al., 2003a, b; Fonseca et al., 2007). Diamond mesh tends to close under tension, trapping smaller species, while square mesh stays open, facilitating the escape of non-target species without reducing the catch of commercially valuable fish (Campos et al., 2002; Campos and Fonseca, 2003; Campos et al., 2014). Codend weight also plays a significant role in bycatch mortality as previously discussed. Heavier codend increase mortality not only directly, through the physical injuries caused by increased contact and abrasion among organisms (Broadhurst et al., 2006), but also indirectly by prolonging sorting time onboard, thereby raising the risk of air exposure. Although air exposure was not measured in this study, the benefits of reducing fishing duration suggest that adjusting effort duration and codend weight could further reduce bycatch mortality. Shortening fishing hauls, when possible, could reduce both the codend's weight and handling time, minimizing air exposure and stress for non-target species.

Closures based on specific areas, seasons, and/or depths have proven effective in reducing bycatch, as they help to protect critical elasmobranchs' aggregations (Gupta et al., 2020). For instance, a depth-based ban on bottom trawling below 800 m was implemented in the Northeast Atlantic in 2017 (Regulation 2016/2336) to protect deep-sea species, including DSE. Yet, for further spatial-temporal closures to be optimized, especially in Portuguese waters, a comprehensive understanding of DSE pupping and breeding grounds, would facilitate the establishment of targeted closures, maximizing conservation benefits for DSE populations. This can be achieved using a spatial distribution modelling approach (Guisan and Thuiller 2005; Elith and Leathwick 2009). While DSE distribution patterns have been modelled for the Azores (Das et al., 2022), mainland Portugal still lacks crucial data on DSE distribution and habitat use. Integrating electronic monitoring programs, alongside onboard observers, can also support data collection on DSE bycatch, enhancing data on abundance and distributions, ensuring compliance and enabling adaptive management.

Implement onboard handling DSE protocols may aid in the mitigation of their mortality rates. Studies on elasmobranch PRM demonstrate that careful handling increases survival likelihood, even for species prone to stress-related mortality (Musyl et al., 2011; Poisson et al., 2014; Gupta et al., 2020). Despite the high AVM rates in this study, improved handling techniques could be beneficial to minimize physical trauma, especially for conservation concern species like *C. coelolepis*, *Centrophorus squamosus*, *C. granulosus*, and *D. calceus*. Training fishers in these techniques, informed by the high mortality rates observed for DSE in bottom trawling, would be an actionable step toward reducing bycatch impacts.

3.5 Conclusion

The vast majority (95%) of the DSE caught in trawling operations conducted for this study were either dead upon retrieval or are unlikely to survive after being discarded. At-vessel mortality rates of deep-sea sharks were species-specific and influenced by some factors, including the small size of specimens, large temperature differences between bottom and surface waters, and the use of 55 mm codend mesh (targeting prawns and shrimps) instead of 70 mm (targeting Norway lobster), and heavier weight in the net codend. Metabolites and electrolytes levels suggest that the studied sharks are stressed, with potassium, urea, and magnesium identified as potential mortality indicators for certain species, while high lactate levels in *G. melastomus* pointed to elevated PRM. Species-specific and individual stress responses, as indicated by varying metabolite and electrolyte levels, suggest that further research is needed to clarify the stress pathways in different species and improve bycatch survival rates. To address these issues, immediate actions should focus on a precautionary approach, preventing DSE bycatch where possible. Complementary measures should focus on mitigating the impacts of bottom trawling activities on these vulnerable species. Translating research findings into actionable conservation strategies requires a coordinated, multi-stakeholder effort involving researchers, the fishing industry, and regulatory bodies. This collaboration is essential for developing effective management strategies that safeguard DSE populations while promoting sustainable practices in crustacean bottom trawl fisheries.

Conflict of Interest - The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3.7 Appendices

Appendix 3. 1 Median and interquartile (IQR) and mean and standard deviation (±SD) of the concentrations in mM of the secondary stress responses of alive and deceased deep-sea sharks, subjected to crustacean bottom trawl fisheries.

	Etmopterus pusillus		***Etmopterus spinax***	
	Alive (6)	Dead (15)	Alive (14)	Dead (1)

Metabolites and electrolytes (mM)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	
Glucose	4.36 (0.89)	4.24 (0.53)	1.71 (1.79)	2.02 (1.33)	4.55 (2.65)	4.37 (1.79)	2.64
Lactate	11.33 (5.36)	10.48 (3.92)	12.87 (9.72)	14.78 (7.44)	8.06 (5.32)	8.73 (4.31)	9.22
Urea	294.13 (129.69)	335.24 (143.08)	205.49 (57.91)	192.95 (39.82)	318.01 (76.78)	314.62 (82.43)	266.6
Phosphorus	2.38 (0.49)	2.54 (0.61)	2.62 (1.60)	3.52 (2.54)	2.54 (0.47)	2.44 (0.45)	7.98
Magnesium	6.92 (1.08)	6.63 (0.97)	6.83 (1.80)	6.88 (1.42)	4.75 (2.58)	5.20 (2.01)	5.55
Chloride	326.36 (41.25)	316.8 (34.81)	273.68 (82.31)	276.63 (77.10)	276.49 (46.22)	275.47 (34.39)	411.7
Calcium	4.25 (1.70)	4.32 (1.00)	3.94 (1.30)	3.88 (1.00)	3.99 (1.04)	4.06 (0.82)	2.77
Potassium	4.80 (0.70)	5.02 (1.37)*	7.4 (3.55)	8.47 (3.85)	4.55 (2.18)	5.29 (2.32)	12.1
Sodium	339.20 (19.2)	329.44 (30.80)*	272 (116.65)	304.65 (87.06)	287.75 (90.08)	297.30 (62.53)	449.7

Metabolites and electrolytes (mM)	***Galeus melastomus***				***Scymnodon ringens***			
	Alive (13)		Dead (8)		Alive (5)		Dead (3)	
	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)
Glucose	3.58 (1.13)	3.61 (0.80)	1.45 (2.10)	1.80 (1.44)	1.81 (0.24)	2.24 (1.24)	1.27 (0.26)	1.11 (0.29)
Lactate	16.28 (4.58)	18.78 (7.71)	15.39 (2.84)	14.49 (3.89)	10.61 (2.70)	12.62 (8.60)	7.00 (1.76)	7.76 (1.77)
Urea	379.21 (46.84)	362.47 (60.57)	304.51 (56.03)	302.21 (40.08)	422.49 (129.25)	424.15 (104.70)	494.82 (84.49)	451.15 (92.57)
Phosphorus	3.31 (0.79)	3.79 (1.37)	4.05 (1.48)	4.24 (1.33)	2.019 (0.17)	1.94 (0.34)	1.69 (0.23)	1.72 (0.23)
Magnesium	3.83 (2.49)	3.12 (1.90)	5.08 (2.98)	5.53 (2.17)	1.62 (1.75)	2.61 (2.62)	1.52 (0.25)	1.50 (0.25)
Chloride	243.30 (48)	259.71 (107.17)	238.61 (26.91)	256.14 (50.00)	223.43 (21.00)	235.58 (22.17)	288.30 (19.69)	288.43 (19.69)
Calcium	4.27 (1.22)	4.39 (1.07)	4.60 (1.40)	4.81 (0.92)	3.56 (0.24)	3.65 (0.39)	2.95 (0.13)	2.99 (0.13)

Potassium	5.70	5.70	7.75	7.73	4.60	5.14	5.30	5.70
	(1.20)	(1.08)	(2.53)	(1.86)	(2.40)	(1.37)	(1.00)	(1.06)
Sodium	265.90	268.38	266.75	266.76	256.60	290.28	329.90	312.90
	(26.60)	(25.14)	(16.58)	(18.73)	(72.90)	(67.43)	(33.30)	(36.41)

*only five values

Appendix 3. 2 *Etmopterus pusillus* and *Galeus melastomus* paired test among alive and dead specimens using stress secondary responses concentrations that arises from a parametric distribution (t Test which are the ones showing the degrees of freedom df) and non-parametric distribution (Mann-Whitney test, without df). The test statistic is presented, the degrees of freedom and the p-value where p<0.05*, p< 0.005**, p< 0.0005***

Species	**Stress responses**	**Statistic**	**df**	**p-value**
E. pusillus	Glucose	5.448	18.95	0.00003***
	Lactate	-1.719	17.113	0.1037
	Urea	80		0.007216**
	Phosphorus	34		0.4135
	Magnesium	39		0.6768
	Chloride	1.645	18.472	0.1169
	Calcium	0.926	9.2815	0.3779
	Potassium	7		0.0088**
	Sodium	0.94	17.735	0.3598
G. melastomus	Glucose	3.2655	9.6893	0.00885**
	Lactate	64		0.4137
	Urea	85		0.01592*
	Phosphorus	38		0.3363
	Magnesium	-2.5875	13.415	0.022*
	Chloride	51		0.9711
	Calcium	-0.959	16.706	0.3513
	Potassium	20		0.0224*
	Sodium	54		0.916

Chapter 4: A GLIMPSE INTO THE TROPHIC ECOLOGY OF DEEP-WATER SHARKS IN AN IMPORTANT CRUSTACEAN FISHING GROUND

Graça Aranha, Sofia; Teodósio, Alexandra; Baptista, Vânia; Erzini, Karim, & Dias, Ester. 2023. A glimpse into the trophic ecology of deep-water sharks in an important crustacean fishing ground. *Journal of Fish Biology,* 102(3), 655-668. https://onlinelibrary.wiley.com/doi/10.1111/jfb.15306

Abstract

Deep-water sharks are among the most vulnerable deep-water taxa because of their extremely conservative life history strategies (i.e., late maturation, slow growth, and reproductive rates). Yet, little is known about their biology and ecology. Thus, this study aimed at investigating the trophic ecology of five deep-water shark species, the birdbeak dogfish (*Deania calcea)*, the arrowhead (*D. profundorum)*, the smooth lanternshark (*Etmopterus pusillus*), the blackmouth catshark (*Galeus melastomus*) and the knifetooth dogfish *Scymnodon ringens* sampled onboard a crustacean bottom-trawler off the southwest coast of Portugal. We combined carbon and nitrogen stable isotopes with RNA and DNA (RD) ratios to investigate the main groups of prey assimilated by these species and their nutritional condition, respectively. Stable isotopes revealed overall small interspecific variability in the contribution of different taxonomic groups to sharks' tissues, as well as in the origin of their prey. *Scymnodon ringens* presented higher δ^{15}N and δ^{13}C values than the other species, suggesting reliance on bathyal cephalopods, crustaceans, and teleosts; the remaining species likely assimilated bathy-mesopelagic prey. The RD ratios indicated that most of the specimens had an overall adequate nutritional condition and had recently eaten. This information, combined with the fact that stable isotopes indicate that sharks assimilated prey from the local or nearby food webs (including commercially important shrimps), suggests a potential overlap between this fishing area and their foraging grounds, which requires further attention.

Keywords: bottom trawl, diet, ecophysiology, Northeast Atlantic, RNA/DNA, stable isotopes.

4.1 Introduction

Deep-sea chondrichthyans (i.e., sharks, skates, rays, and chimaeras living below 200 m depth) are among the deep-water taxa that are most vulnerable to extinction due to their highly conservative life histories (i.e., slow growing, late to mature, and low reproductive rates; Cortés, 1999), and thus slow population recovery rates (Simpfendorfer and Kyne, 2009). According to the European Red List of Marine Fishes, approximately 30% of the deep-sea shark species with zero total allowable catch, are threatened, facing an elevated risk of extinction, while approximately 5% are classified as "data deficient" (IUCN; Nieto et al., 2015; Regulation EC n° 2021/91). Despite the existing regulations, they still compose a large portion of the bycatch in European fisheries, especially the deep-water crustacean bottom trawl fishery (Borges et al., 2001). However, little is known about their biology, ecology, and population status (Kyne and Simpfendorfer, 2007; Cotton and Grubbs, 2015). This is primarily due to the inherent difficulties in studying species in inaccessible habitats, which require expensive equipment and rigorous logistical protocols (Brooks et al., 2015). The scarcity of basic biological and ecological information compromises the development of proper management and conservation strategies for deep-sea sharks (Kyne and Simpfendorfer, 2010), creating uncertainty regarding the potential effects of their removal on the structure and functioning of deep-water ecosystems.

Deep-sea sharks have multiple feeding habits, varying with species (e.g., Cortés, 1999), size (e.g., Xavier et al., 2012; Besnard et al., 2022), space (e.g., Mauchline and Gordon, 1983; Ebert et al., 1992; Preciado et al., 2009; Pethybridge et al., 2011), season (e.g., Anastasopoulou et al., 2013), and resource availability (e.g., Dunn et al., 2013). In general, deep-sea sharks are meso- to top-predators (Cortés, 1999; Churchill et al., 2015), which implies they can have top-down interactions in the marine food webs. Thus, the removal of these predators due to overfishing, for instance, has the potential to affect the overall structure of marine food webs due to changes in prey composition or availability or by impacting communities lower down the food web (Brooks and Dodson, 1965; Heithaus et al., 2008; Shipley et al., 2017). Therefore, understanding the role played by sharks in the deep-water food webs is necessary to improve predictions of ecosystem responses to ongoing perturbations (e.g., Stergiou and Karpouzi, 2002). The most widely used approaches to study sharks' diet and estimate their trophic position are the analyses of stomach contents and stable isotopes (Hussey et al., 2012). Stomach content analysis can provide a precise assessment of sharks' diet (Hyslop, 1980). However, this method is time-consuming, highly invasive, and require a large number of samples, while only providing a snapshot of the recently consumed prey (Simpfendorfer et al., 2001; Baker et al., 2014). Moreover, given the opportunistic feeding behaviour of many sharks (e.g., Jones and Geen, 1977; Olaso and Rodríguez-Marín, 1995), stomach content data are usually insufficient to adequately characterize trophic position, except in rare instances where regular and long-term stomach content data sets are available (Caut et al., 2013). On the other hand, carbon and nitrogen stable isotopes provide time-integrated information about the prey assimilated, rather than recently ingested, by a given consumer (Peterson and Fry, 1987). Carbon isotope ratios ($\delta^{13}C$: $^{13}C/^{12}C$) are frequently used to distinguish among different autotrophs at the base of the food web because they differ among primary producers with respect to C source and fixation (Smith and Epstein, 1971; Cloern et al., 2002; Dias et al., 2016). Carbon isotopes are also useful for identifying general patterns of inshore/benthic vs. offshore/pelagic feeding preferences (France, 1995; Lawson and Hobson, 2000; McMahon et al., 2013). Nitrogen isotope ratios ($\delta^{15}N$: $^{15}N/^{14}N$) are more commonly used to estimate trophic position in food webs (Vander-Zanden et al., 1997) as they generally exhibit high trophic fractionation between prey and consumers (Caut et al., 2009).

Monitoring the nutritional condition can provide additional information about prey consumption, indicating if a given specimen has been feeding or not for the previous days (Buckley et al., 1999). Among the most used condition indices at the organism level in marine ecology are the nucleic acid-derived indices, such as RNA/DNA values (RD). This ratio has been successfully applied as an indicator of growth (e.g., Bulow, 1987; Caldarone et al., 2003; Tavares et al., 2006), nutritional condition (e.g., Chícharo and Chícharo, 1995; A. Chícharo et al., 2003; Alves et al., 2020), productivity (e.g., Cruz et al., 2017), health status (e.g., Tavares et al., 2006), and as an indicator of natural or anthropogenic impacts in marine populations and communities (Chícharo and Chícharo, 2008; Müller et al., 2020). The RD values provide a short-term measure of nutritional condition (1-3 days Buckley et al., 1999), based on the fact that DNA concentrations within specimen cells remain relatively constant (Wallace, 1992) while RNA concentrations increase as protein synthesis increases (Buckley et al., 1999). The RD values vary with life stage, sex, size, disease state, and environmental conditions (e.g., Bulow, 1970; Buckley, 1980; Ferron and Leggett, 1994; Suthers et al., 1996). Thus, a recently well-fed, active-growing specimen should have a relatively high RD value compared to a starving specimen of the same species (Bulow, 1987; Robinson and Ware, 1988; Richard et al., 1991). This approach was used in different marine taxa such as fishes (e.g., Morais et al., 2017; Baptista et al., 2019), crustaceans (e.g., Gonçalves et al., 2021), bivalves (e.g., Chícharo et al., 2007), cephalopods (e.g., Sykes et al., 2004), marine turtles (Vieira et al., 2014), and in marine mammals (Alves et al., 2020). To the best of our knowledge, only Cruz-Ramírez et al. (2017) and Tavares et al. (2006) have used RD in chondrichthyans, recognizing that this technique could be essential to evaluate instantaneous growth and health status in shark species. Thus, obtaining information on elasmobranchs' nutritional traits such as resource uptake and use, may help identify critical life stages or areas for conservation and management.

Because fisheries are expanding to deeper waters worldwide (Cotton and Grubbs, 2015), and given the vulnerability of sharks to anthropogenic pressures (García et al., 2008; Simpfendorfer and Kyne, 2009), more information on the biology and ecology of deep-water sharks is urgently needed. Thus, the present study combined stable isotopes and nucleic acids to investigate the feeding ecology of free-ranging deep-water sharks coexisting in a crustacean bottom trawling fishing ground on the Southwest coast of Portugal. This study area was selected because it is one of Portugal's most important fishing grounds for crustacean bottom trawl fisheries, where high levels of bycatch of deep-water sharks have been reported (Borges et al., 2001). The goals of this study were to 1) identify the main groups of prey (teleost, crustaceans, and cephalopods) assimilated by deep-water shark species commonly found on the Southwest coast of Portugal (ICES, 2020) as well as their origin (bathyal or bathy-mesopelagic), 2) determine their trophic position, and 3) estimate their short-term nutritional condition. We hypothesized that sharks are tertiary/quaternary consumers (trophic position ≥ 4; Cortés, 1999; Barría et al., 2015), feeding on a variety of deep-water prey from the local or nearby marine food webs (Dunn et al., 2013), including commercially important shrimps (Santos and Borges, 2001).

4.2 Materials and methods

4.2.1 Field sampling

Sampling took place off the Southwest coast of Portugal (Figure 4.1) in February 2018 during a four-day commercial fishing trip onboard a crustacean bottom trawler targeting the giant red shrimp *Aristaeomorpha foliacea* (Risso 1827) and the scarlet shrimp *Aristaeopsis edwardsiana* (Johnson 1868) and operating at depths beyond the continental shelf (1,107-1,350 m). A total of six hauls were conducted with an average duration of 6 h. At the end of each haul, deep-sea sharks (hereafter, sharks) and their potential prey species, were immediately separated from the remaining catch. Sharks that were alive were immediately placed inside three containers of 80x40x30 cm filled with flowing seawater. Each specimen was identified (following Compagno et al., 2005), measured (total length -TL: from the tip of the snout to the tip of the caudal fin; ± 0.1 cm), weighed (± 0.1 g), and sexed (male- claspers present; female- claspers absent). Life stage was identified (adult or juvenile) for all species based on Coelho and Erzini (2005), Paiva et al., (2012) and Ebert et al. (2021), except for *Scymnodon ringens*, for which there is no available information to conduct such identification.

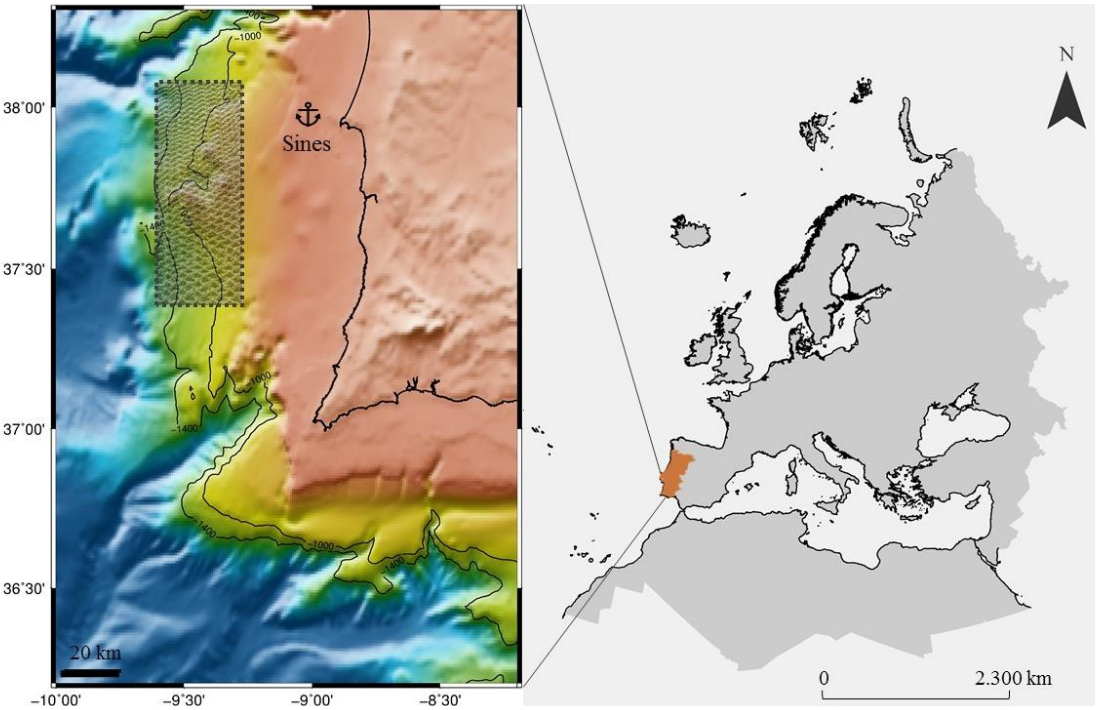

Figure 4.1 Study area off the southwest coast of Portugal (SW- Europe) showing the fishing port of Sines, and the isobaths (black lines) of the sampling area (1000-1400 m) (Created with Mirone software).

Muscle samples were collected following a modified procedure developed for teleosts (Henderson et al., 2016), which consisted of an incision in the base of the first dorsal fin on the left side of each specimen's body. A subsample was collected for stable isotope analysis and another for RNA/DNA (RD) analysis, which were stored frozen at -20°C and in RNA Riboreserve^{TM}, respectively, for subsequent analysis. The entire procedure lasted a maximum of two minutes for the live sharks, which were returned to the sea.

The main groups of sharks' potential prey were selected based on previous studies on stomach content analysis, including in nearby areas (Appendix 4.1). Potential prey included crustaceans (shrimps, prawns and squat lobsters, Scarlet lobsterette, and crabs), teleosts and cephalopods (squid and octopus) which were frozen prior to laboratory analysis. Additionally, zooplankton samples were collected to characterize the isotopic baseline of the local marine food web. For that, a plankton net (500 µm mesh size) was towed vertically from a maximum depth of 80 m during the night - considering diel vertical migration of zooplankton from deeper waters - and samples were immediately preserved in 70 % ethanol after collection.

4.2.2 Laboratory analysis

Shark muscle samples were dried at 60 °C for at least 48 h and ground to a fine powder with a mortar and pestle. Urea and lipids were removed following Carlisle et al. (2017) as both compounds are known to impair $\delta^{15}N$ and $\delta^{13}C$ values respectively.

Potential prey were thawed and identified to the lowest taxonomic level possible (Gibbs, 1984; Bauchot, 1986; Whitehead et al., 1987; Jereb et al., 2016; Froese and Pauly, 2019). Muscle was collected from the dorsal region of each fish. From crustaceans, muscle tissue was collected from the tail of shrimps and lobster and from the crabs' appendages. Cephalopod muscle samples were collected from the mantle (squids) or from the appendages (octopuses). All samples were dried at 60 °C for at least 48 h and ground to a fine powder with a mortar and pestle.
Zooplankton was sorted, and copepods were selected and dried (60 °C) for 24 h.

Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage IRMS via Conflo IV interface (MARINNOVA, University of Porto). The raw data were normalized by three-point calibration using the international reference materials IAEA-N-1 ($\delta^{15}N = +0.4$ ‰), IAEA-NO-3 ($\delta^{15}N = +4.7$ ‰), and IAEA-N-2 ($\delta^{15}N = +20.3$ ‰) for nitrogen isotopic composition, and two-point calibration using USGS-40 ($\delta^{13}C = -26.39$ ‰) and USGS-24 ($\delta^{13}C = -16.05$ ‰) for carbon isotopic composition. Stable isotope ratios are reported in δ notation, $\delta X = (R_{sample}/R_{standard} - 1) \times 10^3$, where X is the C or N stable isotope, and R is the ratio of heavy/light stable isotopes. The $\delta^{13}C$ and $\delta^{15}N$ are expressed in units per mil (‰) relative to Vienna Pee Dee Belemnite and air, respectively. The analytical error, the mean standard deviation (SD) of the replicate reference material, was 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$.

The nutritional condition was determined using RNA and DNA ratios from a microplate fluorescent assay (Caldarone et al., 2001). Samples were cleaned with distilled water, dried on a paper sheet, placed in a new vial, and frozen at -80 °C prior to lyophilization. Samples were freezedried under a pressure of -10 atm at -40 °C for about 36 h. Nucleic acids were chemically and mechanically extracted and determined following procedures described in Esteves et al. (2000).

In order to reduce the differences in RD values among protocols and allow for future comparisons among studies, we used the standardized RD (hereafter RD) values to evaluate the nutritional condition of sharks, which was based on the DNA and RNA standard's slope ratio of 3.73 and the reference slope ratio of 2.4, as described in Caldarone et al. (2006). Then, to ensure that the RNA digestion was complete, and that no DNA degradation occurred, "only-DNA" and "only-RNA" control samples were run in each plate, where the samples were previously analysed, and a RNAase digestion was applied to all the samples (except for the "only-RNA").

4.2.3 Data analysis

Inter-specific differences in the sharks' $\delta^{13}C$ values were tested using a non-parametric test Kruskal-Wallis followed by a pairwise multi-comparison Dunn's test; differences between the $\delta^{15}N$ values were tested using a one-way analysis of variance (ANOVA one-way) with a Tukey's HSD *post-hoc* test for paired contrasts.

When dealing with predators that feed on multiple species, a reduced set of prey species or consolidating prey species is necessary due to overlapping isotopic values (Phillips et al., 2005). In this case, prey were grouped according to their taxonomic group (teleosts, squids, octopus, crabs, lobsters, and shrimps) and habitat (bathyal and bathy-mesopelagic). Because Myctophids are considered important prey for some deep-sea sharks (Appendix 4.1), and since they were not collected during this study, estimates from the Mediterranean were used instead (8.4 ± 0.2 ‰ $\delta^{15}N$ and -20.6 ± 0.8 ‰ $\delta^{13}C$; Barría et al., 2015). Although stable isotope values may vary between geographic areas, zooplankton (Myctophid's main prey; Hulley, 1990) $\delta^{13}C$ and $\delta^{15}N$ estimates are similar between these two areas (McMahon et al., 2013). Therefore, small differences are expected between the stable isotope values of Myctophidae from the Mediterranean and the Southwest coast of Portugal. Groups of the most likely prey for each shark species were identified using $\delta^{13}C$ and $\delta^{15}N$ biplots where sharks' $\delta^{13}C$ and $\delta^{15}N$ values were compared to each potential prey's $\delta^{13}C$ and $\delta^{15}N$ values, after adjusting for trophic fractionation (Phillips et al., 2014). The relative contribution of the most likely prey to the diet of sharks was quantified using the Bayesian stable isotope mixing model MixSIAR v3.1.12 (Stock and Semmens, 2016). To run the models, the stable isotope values of sharks and their most likely prey groups were input as raw data, using noninformative priors because of the general lack of dietary information for the species in the study area. Model convergence was assessed via Gelman–Rubin and Geweke diagnostics (Geweke, 1991; Gelman et al., 2013). Posterior distributions obtained from the MixSIAR analyses are expressed as median and 95% credibility intervals. For the mixing model, the $\delta^{13}C$ and $\delta^{15}N$ values were adjusted to one trophic level using the trophic fractionation estimates from Hussey et al. (2010; 2.3 ± 0.2 ‰ $\delta^{15}N$, 0.9 ± 0.3 ‰ $\delta^{13}C$), which were obtained from controlled experiments with lemon sharks (*Negaprion brevirostris*, Poey 1868) and sand tiger sharks (*Carcharias taurus*, Rafinesque 1810). Because the resulting isospace was narrow when compared to ecosystems like estuaries or shallower areas of the ocean, we ran three models for each shark species: all prey groups, prey groups combined according to their position in the isospace (squids, lobsters, octopus+tel1, crabs+shrimps+tel2), and prey group selection based on the proximity of prey in relation to a given consumer. Models were compared using the function *compare_models* available in the package loo (Vehtari et al., 2017). The best model was the one presenting dLOO=0 and the resulting errors were lower or close to 1 (Stock and Semmens, 2016). The errors obtained for the species *Deania calceus* and *Deania profundorum* were greater than 1, indicating that sources were mixing or that there was a structure in the data that was not resolved with the available information (Stock and Semmens, 2016). For that reason, only a qualitative analysis was conducted for these species.

Copepods and teleosts (with C:N > 3.5) $\delta^{13}C$ values were corrected for lipid content according to the mass balance correction models of Smyntek et al. (2007, equation 5) and Hoffman and Sutton (2010, equation 6), respectively. Copepods were also corrected for ethanol preservation (0.4 ‰ $\delta^{13}C$, 0.6 ‰ $\delta^{15}N$, Feuchtmayr and Grey, 2003).

Shark trophic position (TP) was determined following the scaled framework proposed by Hussey et al. (2014b):

$$TP = \frac{log(\delta^{15}\,N_{lim} - \delta^{15}N_{copepod}) - \, log(\delta^{15}\,N_{lim} - \delta^{15}N_{sharks})}{k} + TP_{copepod}$$

where $\delta^{15}N_{lim} = -\, {}^{\beta_0}/_{\beta_1}$,the intercept β_0 and slope β_1 were 5.92 and -0.27, respectively (Hussey et al., 2014a). The $\delta^{15}N_{copepod}$ was 4.7 ‰ which is the direct measurement of the $\delta^{15}N$ values for the baseline organisms, in this case copepods, which are assumed to belong to the $TP_{copepod}$ 2; $\delta^{15}N_{sharks}$ is the direct measurement of the $\delta^{15}N$ values for each shark; k is the rate at which $\delta^{15}N_{TP}$ approaches $\delta^{15}N_{lim}$ per TP step, i.e., $k = - \log (\beta_0 - \delta^{15}N_{lim}/- \delta^{15}N_{lim})$ (Hussey *et al.*, 2014b).

The nucleic acid-derived indices were calculated in relation to dry weight (µg RNA mg^{-1} DW, µg DNA mg^{-1} DW, and RD). The RD was obtained by the ratio of the RNA mg^{-1} and the DNA mg^{-1} resulting in the RD value. Since the effect of size (TL) may influence these indices, a linear regression model was conducted and no significant relationships were found between those indices and size (Appendix 4.2; Suthers et al., 1996; M.A. Chícharo et al., 1998). A one-way ANOVA was conducted to test for differences in the RD between shark species.

Because there are no estimates of critical RD values for sharks (i.e., threshold values to determine if sharks are in a good or poor condition), the nutritional condition was evaluated using the percentile approach (Meyer et al., 2012; Alves et al., 2020). The percentile approach shows that if the RD mean values are closer to the 75th percentile, then, the specimens' samples from a certain species are in an adequate nutritional condition and have fed in the last 1-3 days (Meyer et al., 2012; Alves et al., 2020). On the other hand, if the RD mean values of the specimens' samples are closer to 10th percentile, specimens are considered to be in poor nutritional condition (Meyer et al., 2012; Alves et al., 2020).

To test for the differences (at $p < 0.05$) between the stable isotope values between sharks or in the RD values, various parametric and non-parametric analyses were used depending on whether normality and homoscedasticity hypotheses were verified. All statistical analyses were conducted with the open-source statistical language R (R Development Core Team, 2021).

4.3 Results

4.3.1 Stable isotopes analysis

A total of 34 sharks were collected, belonging to five species (Table 4.1). The mean $\delta^{13}C$ (Kruskal-Wallis: $Chi^2 = 16.5$, $F = 4$, $p = 0.002$) and $\delta^{15}N$ values (ANOVA: $F_{(3,24)} = 15.78$, $p = 8.98^{-07}$) were different between species. *Scymnodon ringens* was ^{15}N- enriched when compared to all the other species (pairwise tests, $p < 0.001$), and ^{13}C- enriched than *D. profundorum* (pairwise tests, $p = 0.009$) which was ^{15}N- depleted when compared to *D. calceus* (pairwise tests, $p = 0.02$) (Table 4.2).

Table 4.1 Shark species, and number of specimens (n), collected in February 2018 off the Southwest coast of Portugal. Mean (± SD), total length (TL) and weight of the specimens collected from each species by sex, male (M) or female (F), the life stage (adults [A], juveniles [J], or not available [n/a]). The overall condition of each specimen was determined as good (G), poor (P), or dead (D).

Species	**n**	**Sex (n)**	**Life stage (n)**	**TL (cm)**	**Weight (g)**	**Condition (n)**
Deania calceus	8	M (3)	A (3)	84.3 ± 3.1	2067 ± 152.8	P (1) D (2)
		F (5)	J (5)	70.3 ± 14.8	1514 ± 1112	P (3) D (2)
Deania profundorum	4	F (4)	J (4)	44.3 ± 6.1	287.4 ± 98.3	P (1) D (3)
Etmopterus pusillus	5	M (2)	A (2)	41.0 ± 0.0	315.0 ± 35.0	G (1) D (1)
		F (3)	A (2) J (1)	41.8 ± 4.1	343.3 ± 89.9	D (3)
Galeus melastomus	5	F (5)	A (5)	61.8 ± 5.6	678 ± 168.7	P (2) D (3)
Scymnodon ringens	12	M (4)	n/a	51.9 ± 2.2	957.5 ± 316.7	P (2) D (2)
		F (8)	n/a	59.5 ± 11.7	1382.5 ± 958.7	P (6) D (2)

Table 4.2 Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ values (‰) after correction for lipids and urea, trophic position (TP), RNA, DNA, and standardized RNA/DNA values (RD) values of each shark species collected in February 2018 off the Southwest coast of Portugal.

Species	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**TP**	**RNA/mg**	**DNA/mg**	**RD**
Deania calceus	10.8 ± 0.6	-19 ± 0.7	4.2 ± 0.2	2.2 ± 1.1	3.3 ± 0.7	0.4 ± 0.2
Deania profundorum	9.8 ± 0.1	-19.8 ± 0.2	3.8 ± 0.0	1.5 ± 0.3	4.1 ± 0.9	0.3 ± 0.1
Etmopterus pusillus	10.5 ± 0.4	-19.2 ± 0.2	3.9 ± 0.1	3.1 ± 0.6	4.8 ± 0.9	0.4 ± 0.1
Galeus melastomus	10.6 ± 0.2	-19.3 ± 0.1	4.1 ± 0.1	2.6 ± 0.6	2.8 ± 0.4	0.6 ± 0.2
Scymnodon ringens	11.8 ± 0.4	-18.5 ± 0.2	4.5 ± 0.2	2.0 ± 1.3	3.5 ± 0.8	0.4 ± 0.2

The teleosts, crustaceans, and cephalopods collected during this study (57 specimens) included a variety of species occupying different habitats (mesopelagic to bathyal), habits (pelagic and demersal) and with different migratory behaviours (i.e., migratory, non-migratory, diel vertical migrations - DVM) (Table 4.3). Overall, the $\delta^{13}C$ and $\delta^{15}N$ values of the sharks collected – after adjusting for trophic fractionation – were intermediate between $\delta^{13}C$ and $\delta^{15}N$ values of several prey groups, indicating reliance on different sources (Figure 4.2). The low $\delta^{13}C$ and $\delta^{15}N$ values of *E. pusillus*, *G. melastomus*, *D. calceus*, and *D. profundorum*, indicate they assimilated ^{13}C- and ^{15}N-depleted sources, such as shrimps, squids and lobsters (Figure 4.2). *Scymnodon ringens* assimilated more ^{13}C- and ^{15}N-enriched than the previous shark species indicating a higher contribution of bathyal prey, including teleosts (Tel1, Table 4.3) and octopus (Figure 4.2).

Table 4.3 Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ values (‰) of each species collected in February 2018 in the southwest coast of Portugal, grouped according to their taxonomic group, stable isotope values and/or habitat (group codes): Teleosts are divided into two major groups, Tel1 are bathyal and Tel2 are bathy-mesopelagic. Cop are copepods. For some species, information about their habitats and diet is not available (n/a), and others perform diel vertical migratory movements (DVM).

Groups	Species	n	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Diet	Habitat
Tel1	*Gadomus* sp.	4	12.6 ± 1.4	-18 ± 0.4[†]	Copepods, amphipods	Bathyal
	Aldrovandia phalacra	3	12.4 ± 0.2	-18.1 ± 0.2[†]	Copepods, amphipods	Bathyal
	Trachyrincus scabrus	2	13.7 ± 0.6	-17.5 ± 0.7[†]	Copepods, mysids, shrimps, cephalopods, fish, polychaets	Bathyal, non-migratory
	Nezumia sclerorhynchus	4	14.0 ± 0.2	-18.1 ± 0.5[†]	Copepods, amphipods, decapods, mysids and polychaets	Bathyal, non-migratory
	Cetonurus globiceps	1	12.5	-18.9	Small fishes, planktonic crustaceans	Bathyal
	Chaunax pictus	1	12.7	-18.9[†]	Shrimps and crabs	Bathyal
	Bathypterois dubius	3	12.8 ± 0.1	-18.5 ± 0.1[†]	Mysids and benthopelagic copepods	Bathyal
	Alepocephalus rostratus	3	11.4 ± 0.2	-19.2 ± 0.1	Euphausiids, decapods and mysids	Bathyal
	Anoplogaster cornuta	2	11.6 ± 1.1	-19.8 ± 0.8	Crustaceans, shrimps, fishes and cephalopods	Bathyal
	Serrivomer beanii	1	9.3	-19.3	Euphausiids, decapods, mysids, cephalopods and fishes	Bathyal, DVM
	Total	**24**	**12.5 ± 1.2**	**-18.5 ± 0.7**		
Tel2	*Chauliodus sloanii*	1	10.5	-19.6	Mid-water crustaceans and fishes, mainly mictophids	Bathy-meso pelagic, DVM
	Melanonus zugmayeri	3	10.7 ± 0.3	-18.6 ± 0.2	n/a	Bathy-meso pelagic
	Rouleina maderensis	1	11.4	-18.2[†]	n/a	Bathy-meso pelagic
	Hoplostethus mediterraneus	2	11.2 ± 0.6	-18.6 ± 0[†]	n/a	Bathy-mesopelagic
	Omosudis lowii	1	10.4	-20	Cephalopods and fishes	Bathy-meso pelagic

	Myctophidae‡	2	8.4 ± 0.2	-20.6 ± 0.8	Copepods, euphasiids	Mesopelagic, DVM
	Total	**10**	**10.6 ± 0.9**	**-19.1 ± 0.8**		
Lobster	*Nephropsis atlantica*	**2**	**8.9 ± 0.2**	**-18.5 ± 0.3**	n/a	Bathyal
Crab	*Geryon longipes*	**3**	**11.5 ± 0.3**	**-18.8 ± 0.2**	n/a	Bathyal
Shrimp	*Aristaeopsis edwardsiana*	1	10.9	-17.6	n/a	Bathy-Mesopelagic
	Aristaeomorpha foliacea	3	10.8 ± 1.3	-18.8 ± 0.8	Crustaceans and fishes	Mesopelagic
	Polycheles typhlops	4	10.8 ± 0.3	-18 ± 0.4	n/a	Bathy-Mesopelagic Bathyal
	Dichelopandalus bonnieri	1	11.3	-18.7	n/a	n/a
	Total	**9**	**10.9 ± 0.8**	**-18.3 ± 0.8**		
Octopus	Octopodidae	1	12.4	-18.3	n/a	Bathy-mesopelagic
	Opisthoteuthis sp.	2	11.9 ± 0.2	-19.1 ± 0.1	n/a	Bathyal
	Total	**3**	**12.2 ± 0.3**	**-18.7 ± 0.6**		
Squid	*Mastigoteuthis* sp.	2	11.5 ± 0.2	-20.6 ± 0.3	n/a	Bathyal
	Histioteuthis sp.	1	10.5	-20.1	n/a	Bathyal
	Total	**3**	**11 ± 0.7**	**-20.3 ± 0.4**		
COP	Copepods		**4.7 ± 0.3**	**-20.7 ± 0.1**	Phyto-zooplankton or organic matter	Epipelagic, Mesopelagic

†Species with $\delta^{13}C$ (‰) values corrected for lipid content.

‡Mean values extracted from Barría et al. (2015) based on two specimens from Mediterranean

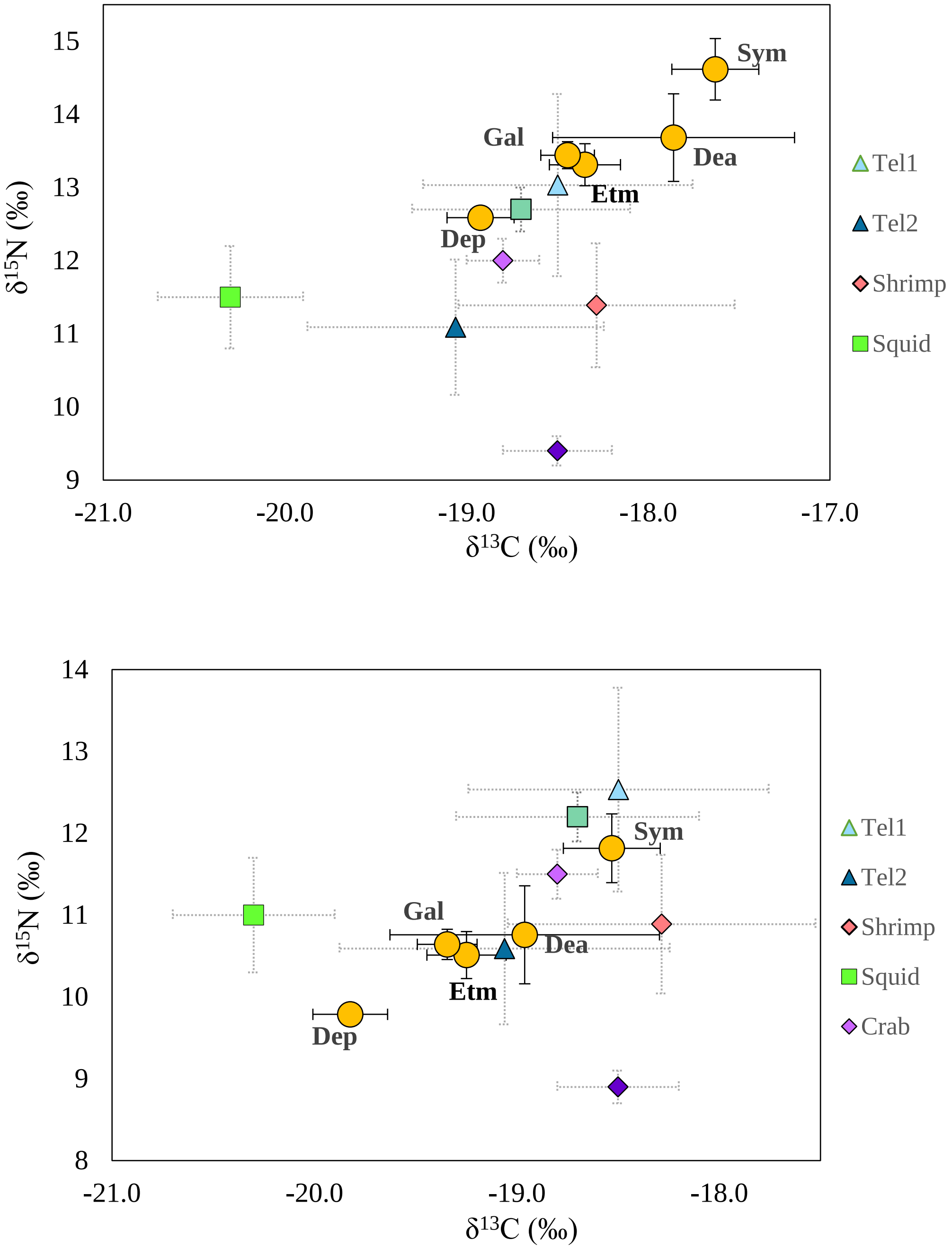

Figure 4.2 Mean (±SD) δ^{15}N and δ^{13}C values (‰) of sharks collected off the southwest coast of Portugal (SW-Europe) not adjusted for trophic fractionation (upper) and adjusted for trophic fractionation (lower; 2.3 ± 0.22 ‰ δ^{15}N, 0.9 ± 0.33‰ δ^{13}C; Hussey et al., 2010). Teleosts were grouped into bathyal (Tel1) and bathy-mesopelagic (Tel2) represented by the triangles; Crustaceans (Lobster, Crab and Shrimp) are represented by the diamond; and Cephalopods (Octopus and Squid) by squares. Sharks are represented by yellow circles: *Deania calceus* (Dea), *Deania profundorum* (Dep), *Etmopterus pusillus* (Etm), *Galeus melastomus* (Gal), and *Scymnodon ringens* (Sym).

The dual-stable isotope mixing model results indicate that overall, cephalopods and crustaceans were the main prey contributing to the tissues of the analysed sharks, followed by bathyal teleosts (Figure 4.3). Bathyal squids were the main prey assimilated by *G. melastomus* and *E. pusillus*, which also relied on mesopelagic and bathyal crustaceans (Figure 4.3). *Scymnodon ringens* assimilated mostly bathyal species of cephalopod, crustacean, and teleost groups (Figure 4.3).

Overall, sharks from this study, were classified as tertiary consumers (TP close to 4). Trophic position (TP) values varied between 3.8 for *D. profundorum* and 4.5 (± 0.2) for *S. ringens* (Table 4.2).

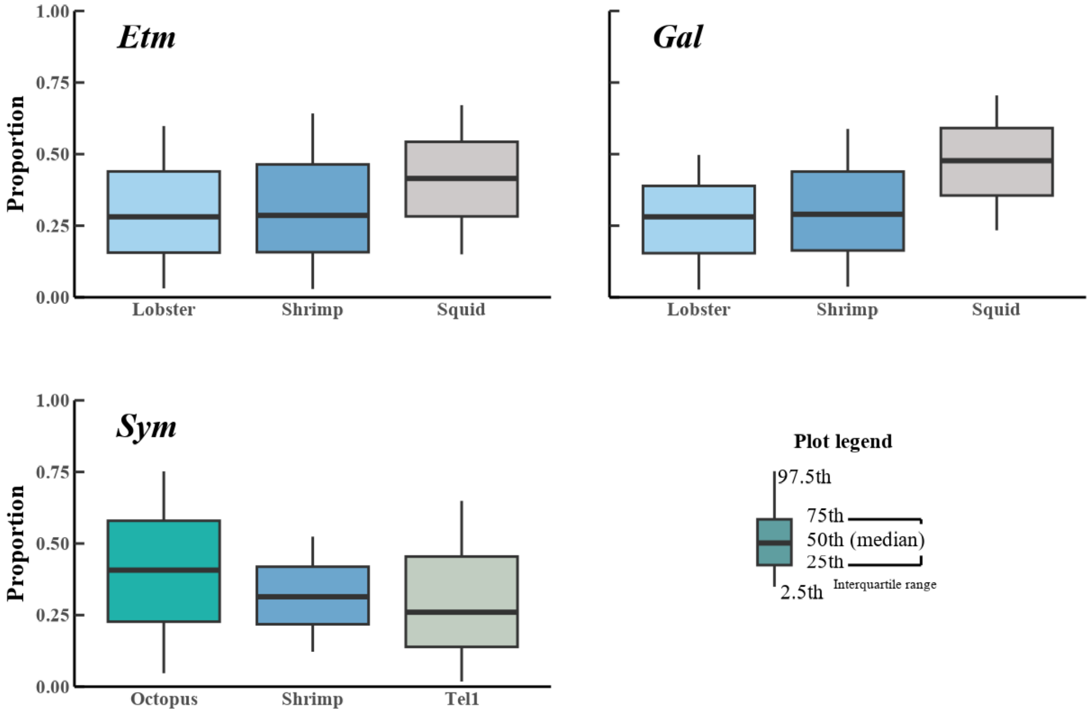

Figure 4.3 Relative contribution of each prey group based on the stable isotope mixing models of the sharks *Etmopterus pusillus* (Etm), *Galeus melastomus* (Gal), and *Scymnodon ringens* (Sym). The prey groups include: Tel1 – bathyal teleosts, Squid, Octopus, Shrimp, and Lobster. Boxplots depict median (horizontal line) with 50% (box) and 95% credible intervals (vertical line).

4.3.2 Nutritional condition

A non-significant positive relationship was found between the nucleic acid-derived indices and size (TL) for *D. profundorum*, *E., pusillus*, and *S. ringens.* Contrarily, a non-significant negative relationship was found for *D. calceus* and *G. melastomus* (Appendix 4.2). Likewise, the values of RD did not vary significantly between the shark species (ANOVA: $F_{(6, 30)} = 1.5$, p= 0.2).

Galeus melastomus presented the highest mean RD values among the species studied and the one with the lowest was *D. profundorum* (Table 4.2). The interquartile analysis showed that mean RD values, were generally close to the 75th percentile for all species except for *G. melastomus* where the mean RD value was close to the 10th percentile (Figure 4.4).

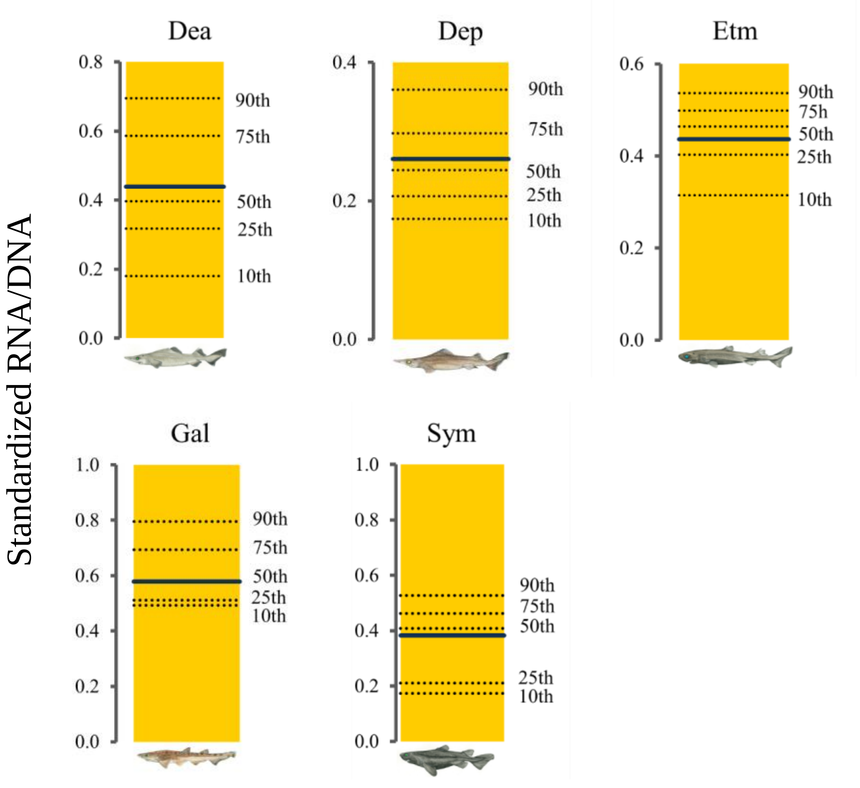

Figure 4.4 Percentile approach of the standardized RNA/DNA of the shark species with n> 3, collected off the southwestern coast of Portugal. Dotted lines are percentiles 10th,25th,50th,75th, and 90th, and the dark blue line are the RD mean values of the species *Deania calceus* (Dea), *Deania profundorum* (Dep), *Etmopterus pusillus* (Etm), *Galeus melastomus* (Gal), and *Scymnodon ringens* (Sym). This approach shows that when the mean is closer to the 75th and far from the 10th percentile, the species has a high number of specimens with an adequate nutritional condition.

4.4 Discussion

The present study combined, for the first time, stable isotopes, and nucleic acids to investigate the feeding ecology of free-ranging deep-sea sharks coexisting in a crustacean bottom trawling fishing ground on the Southwest coast of Portugal. The stable isotopes revealed that sharks are tertiary consumers and the mixing models showed low interspecific variability in the prey groups assimilated and their origin. The RD analysis suggests that overall, most sharks were in a good condition and had recently fed, most likely in the area where sampling occurred or nearby areas.

4.4.1 Stable isotopes analysis and trophic position

Bathyal cephalopods (pelagic and demersal) presented the highest relative contribution to the tissues of *E. pusillus*, *G. melastomus*, and *S. ringens*, followed by bathy-mesopelagic crustaceans, and teleosts, although with some variability between species. This pattern of prey assimilation does not mirror the relative importance of each prey group derived from stomach content analysis; in general, shrimps and teleosts are identified as the most frequent and abundant prey groups (e.g., Neiva et al., 2006; Xavier et al., 2012; Muñoz, 2015; Barría et al., 2018). Nonetheless, a previous study conducted in the Mediterranean showed that the relative importance of cephalopods in *G. melastomus* stomachs can be as high as the one found in this study (close to 50%) (Barría et al., 2018). Also, they compared the relative contribution of each group of prey derived from stomach contents and stable isotope analysis, which suggests that in some cases, stomach content analysis alone might underestimate the relative importance of cephalopods to sharks' tissues. Whether the results in our study reflect prey availability or isotopic routing (i.e., differential allocation of isotopically distinct dietary components to different tissues; Schwarcz, 1991) is unclear because we did not analyse the stomach contents of the sampled sharks. Moreover, stomach contents studies may fail to characterize the entire prey spectrum of a given consumer, due to the inherent limitations associated with such studies: i) they provide a snapshot of what the specimen ate in the last hours; thus a high number of stomachs, and a good temporal resolution, is necessary to capture the diversity of all their prey due to the imbalance between easy-to-digest and difficult-to-digest, ii) deep-sea sharks generally present empty stomachs (e.g., Preciado et al., 2009; Mauchline and Gordon, 1983) and may regurgitate food when brought to the surface (Bowman, 1986), iii) prey items from deep-water communities often are fragile and difficult to identify (Cailliet et al*.,* 1999; Drazen et al*.,* 2001; Robinson et al*.,* 2007). Nonetheless, we cannot exclude the possibility that we have not sampled all the possible prey groups. This seems to have been the case for *D. profundorum*, given their position in relation to that of the sampled prey, which suggests that ^{15}N- and ^{13}C- depleted prey may be missing in this dataset. Thus, future studies in this area should combine stable isotopes with traditional stomach content analysis or more recent metabarcoding approaches (e.g., Dunn et al., 2010; van Zinnicq Bergamnn et al., 2021) to identify the predator-prey links to improve the quantification of their importance.

The similarity in the stable isotope values of *E. pusillus* and *G. melastomus* suggests they likely share the same trophic niche. Although estimating trophic niche size and overlap was out of the scope of this study (low number of specimens per group; Jackson et al., 2011), the stable isotope mixing models indicate they assimilated mostly bathyal squids, followed by bathy-mesopelagic shrimps, and teleosts. *Scymnodon ringens*, on the other hand, showed higher $\delta^{15}N$ and $\delta^{13}C$ values than the remaining species indicating the assimilation of ^{13}C- and ^{15}N- enriched prey such as bathyal octopus and bathyal teleosts. The fact they rely on different groups of prey suggest some degree of resource partitioning between *S. ringens* and the other species. The reasons for that are unclear, because little is known about the biology and ecology of *S. ringens* (Finucci et al., 2021).

Groups of commercially important shrimps contributed to the diet of all the sharks studied, although in general, only with a small contribution (median < 31). Despite the low contributions, the consumption of commercially important shrimps might be among the reasons why those sharks are the most frequently caught in the studied area, as observed by an ongoing study [Graça Aranha et al., (unpublished results)].

It is possible that demersal predators, such as deep-sea sharks, exploit different depths gradients to help increase net energy gain, similarly to pelagic species (Watanabe et al., 1999; Schabetsberger et al., 2000). This was not seen in our study since we did not sample prey from habitats other than the bathyal, although some of the sampled prey are also from the mesopelagic. Furthermore, the group Tel2, which also contains teleosts that perform diel vertical migrations (DVM), was not included as potential prey of the studied sharks, due to high errors resulted from the models, but some authors have suggested that *E. pusillus* (Coelho and Erzini, 2007; Xavier et al., 2012) and *D. calceus* (Clark and King, 1989) might perform DVM following their prey.

The lack of a clear preference for any prey group (median contributions lower than 50%) suggests that these species may be generalists' predators. Although this study does not allow concluding about their feeding behaviour, a generalist/opportunistic behaviour was previously reported for *G. melastomus* in the Cantabrian and the Ionian Sea (Olaso et al., 2005; Anastasopoulou et al., 2013). Nonetheless, the narrow range in prey $\delta^{13}C$ and $\delta^{15}N$ values, which probably reflects the low number of available sources of productivity in the deep-sea environment, introduced some challenges to the quantitative analysis. The ranges of the stable isotopes of Tel2 overlapped with those from crabs and shrimps, which poses a limitation to the interpretation of mixing models (Phillips et al., 2014). Although the models obtained in this study were robust (low errors and median values close to mean values), we cannot reject the possibility that they could also be assimilating fish from the group Tel2. In fact, previous stomach content studies reported the consumption of mesopelagic fish such as Myctophidae by the species *D. calceus*, *D. profundorum*, *E. pusillus* and *G. melastomus*. Thus, it is possible they also assimilate mesopelagic fish along with bathyal and bathy-mesopelagic crustaceans, as previously reported on stomach content studies (Appendix 4.1).

The TP estimates obtained during this study, position the deep-water shark species analysed as tertiary consumers, with TP varying between 3.8 in *D. profundorum* and 4.7 in *S. ringens*. These estimated values agree with other studies that used stable isotopes (Chouvelon et al., 2012; Colaço et al., 2013) or stomach content analysis (Cortés, 1999). Furthermore, these TP values are close to the 4.5 obtained for deep-sea top-predators such as the *Hexanchus griseus* (Froese and Pauly, 2022) which also inhabits this same area and depths. This might indicate that these sharks are also top-predators in this food web. The high trophic levels suggests that they might not sustain direct or indirect exploitation (Pauly et al., 1998). While most deep-sea sharks are protected in European waters (EC council regulation 2021/91) with zero total allowable catch they are still frequently caught as bycatch in bottom-trawlers and longliners and most often discarded dead or in a poor condition (Rodríguez-Cabello and Sánchez, 2017) which calls for better fisheries management to avoid their catch in the first place.

However, caution is necessary when comparing TP between studies, since these estimates can vary with the input value for values used for trophic fractionation and also with the method applied to generate the TP estimates. The most used fractionation value is 3.4 ‰ (Post, 2002) which is usually assumed to be constant across trophic levels (e.g., Pethybridge et al., 2012; Colaço et al., 2013; Iitembu and Richoux, 2015). Nevertheless, experimental studies conducted under controlled situations, proved that there is a wide variation in $\Delta^{15}N$ ($\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{prey}$) values among species and taxa (Caut et al., 2009). Thus, we used the scaled $\delta^{15}N$ framework approach proposed by Hussey et al. (2014a) since it improves the ability to accurately measure absolute TP variation, extending the length of the food web in comparison to conventional constant fractionation frameworks.

Another critical aspect to consider when conducting diet reconstruction and estimation of TP through stable isotopes analysis is the use of proper trophic fractionation values. The trophic fractionation values are usually estimated by conducting controlled feeding experiments, where animals are fed a variety of prey types for an extended period until isotope values reach a plateau

(Hussey et al., 2010). However, there are a limited number of trophic fractionations estimates for this group of species because of the difficulties of keeping chondrichthyans in captivity for long periods (Shipley et al., 2017). Moreover, the existing estimates, based on controlled experiments, which do not include deep-sea species, are highly variable ($\Delta^{15}N$: 2.3-5.5‰; $\Delta^{13}C$: 0.9-3.5‰) varying according to the consumer species, prey species, prey tissue-type, consumer tissue-type, natural conspecific variation, and species-specific metabolic rates (e.g., Hussey et al., 2012; Kim and Koch., 2012; Malpica-Cruz et al., 2012; McClain et al., 2012). Because there are no trophic fractionation estimates derived from captivity studies with deep-sea elasmobranchs, we used those obtained from muscle tissues of two reef-associated shark species (*Negaprion brevirostris* and *Carcharias taurus*) fed with a fish diet for over two years (Hussey et al., 2010). In many fish species, there is a positive relationship between body size, trophic position (Romanuk et al*.,* 2011) and $\delta^{15}N$ values, where consumers feeding on prey at higher levels of the food-web would be larger animals (Hussey et al., 2012), thus, likely resulting in lower trophic fractionation values with increasing trophic position and body size (Hussey et al. 2014a). However, Churchill et al. (2015) found no relationship between average body size and mean $\delta^{15}N$ values in deep-water sharks from the Gulf of Mexico, suggesting this might be due to reduced resource pathways in the deep-sea habitat along with high levels of scavenging contributing to compressed food webs.

4.4.2 Nutritional condition

Based on the percentile RD analysis, we were able to conclude that the sharks analysed during this study were in an overall adequate nutritional condition (Meyer et al., 2012; Alves et al., 2020) and that their food likely came from the study area, or nearby areas, due to the short window of time provided by the RD analysis (Buckley et al., 1999). The species that presented the highest values of RD was *G. melastomus*, but it was also the only species with specimens in a poor nutritional condition. If *G. melastomus* was a selective feeder, the poor nutritional condition could mean that their preferred prey was absent from the study area. Since they are considered generalist feeders, other reasons could have been the motive for their poor nutritional condition. Because RD values decrease with death and since the specimens collected were either in poor condition or dead, another possible explanation could be related to their condition upon arrival at the boat. However, if this was true, the same should have been observed in other specimens arriving dead or in poor condition, which was not the case. Thus, we consider that the most likely explanation is related to the fact that the specimens collected from *G. melastomus* were all adult females, and that may have recently given birth, since these conditions are usually associated with low RD values as the energetic reserves of females are transmitted to the offspring (e.g., Chícharo et al., 2003; Pérez-Camacho et al., 2003; Garrido et al., 2007).

Even though RD values seemed to increase with shark' size for the species *D. profundorum, E. pusillus*, and *S. ringens* and decrease for the species *D. calceus* and *G. melastomus,* none of those relationships were significant (Appendix 4.2). Previous studies attempted to apply nucleic acid-derived indices to evaluate the nutritional condition of other elasmobranchs where an inverse relationship was found between RD values and size (Tavares et al., 2006), and age (Cruz-Ramírez et al., 2017). However, we cannot establish direct comparisons with the above studies because their RD values were not standardized as in this study. Another reason is that RD is an index of cellular protein synthesis capacity and might take days to weeks to change (Chícharo and Chícharo, 2008), thus it cannot be used as an instantaneous growth index as done by Tavares et al. (2006) and cannot be biased by stress related to capture events as stated by Cruz-Ramírez et al. (2017).

To the best of our knowledge this is the first attempt to evaluate the nutritional condition of deep-sea sharks using this approach. Nevertheless, because there are no estimates for the critical RD values for the studied species and due to the small number of specimens analysed, these conclusions should be interpreted cautiously. Further studies are necessary to confirm the usefulness of RD values as indicators of the nutritional condition in deep-sea sharks, which should include a higher number of specimens of different sex and maturation stages when compared to those used in this study.

4.5 Conclusion

This study was the first to combine dietary information and nutritional condition of deep-sea shark species in an important Portuguese deep-water crustacean fishing ground. Despite the small sample size of these especially inaccessible organisms to trophic studies, we were able to show that the sharks studied here are tertiary consumers, assimilating cephalopods, crustaceans, and teleosts with bathyal and mesopelagic origins from the local food webs or from nearby areas. The fact that they assimilated different groups of prey but showed no high relative contribution of any group to their tissues, suggests they could be generalists predators. The RD percentile approach indicated that most of the species were in an adequate nutritional condition and had recently eaten in the days before sampling. Thus, the fact they had recently eaten prey from the local or nearby food webs, which included groups of prey targeted by the crustacean bottom trawl fisheries, suggests there is some potential for overlap between their foraging grounds and the most important fishing areas for deep-water crustaceans in this country. Further studies are necessary to determine the complete prey array and their feeding behaviour and estimate the actual overlap between sharks' foraging grounds and fishing areas, including a more exhaustive monitoring programme, covering a greater number of species, and using complementary approaches that combine predator-prey relationships with shark habitat use.

Ethical approval - All the study was conducted in accordance with the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of animals and enforced by CCMAR. CCMAR staff are certified to house and conduct experiments with live animals, and their facilities are also certified in accordance with the three "R" policy, national and European legislation, and with guidelines defined by the ethical committee ORBEA CCMAR-CBMR.

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Authors' contributions - S.G.A., K.E., A.T. and E.D. conceived/designed the study. S.G.A. collected the raw data S.G.A. and E.D. performed the stable isotopes analyses and wrote the manuscript. S.G.A., V.B. and A.T. performed the nucleic acids analysis. K.E. provided funding. S.G.A and E.D. wrote the manuscript. All authors gave final approval for publication.

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4.7 Appendices

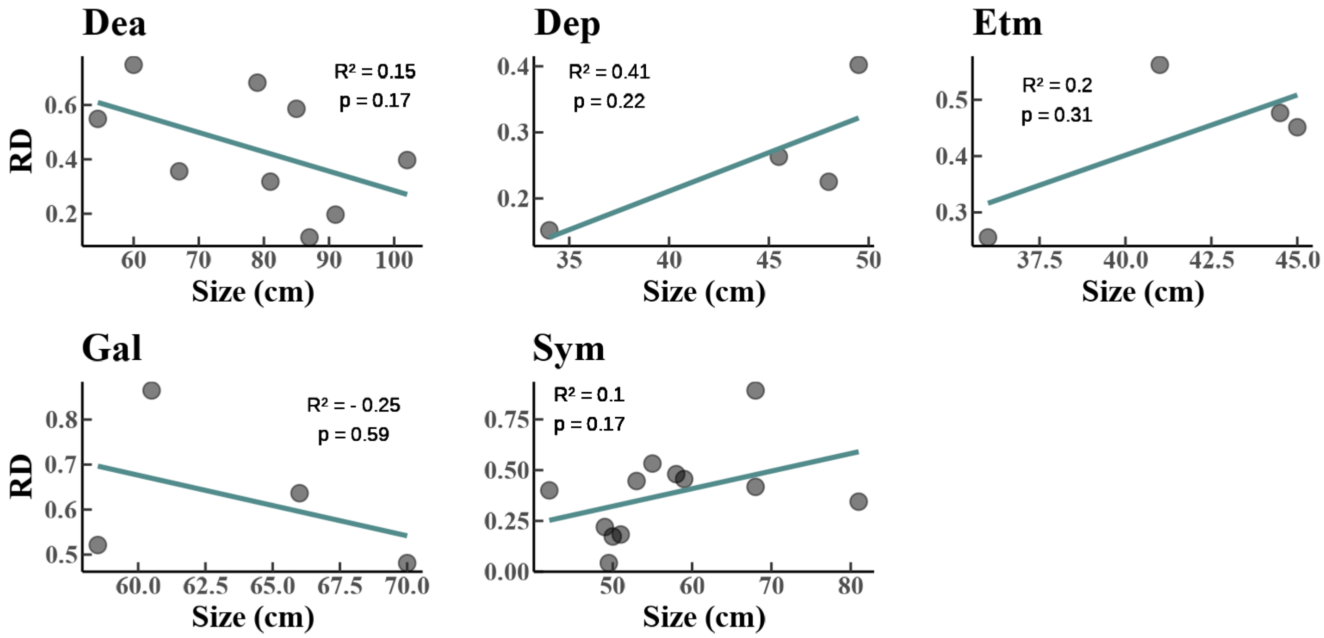
Appendix 4.1 Summary of dietary information from the literature combining studies on stomach content analysis and stable isotopes for the shark species evaluated in this study from the southwest coast of Portugal. The reported size range is a combination of total lengths from the cited studies per species from minimum to maximum, whilst the reported depth of occurrence is the minimum and maximum depth of occurrence reported for the species worldwide.

Taxa	Size range (cm)	Depth of occurrence (m)	Diet	Taxonomic groups	References
Squaliformes					
Centrophoridae					
Deania calceus	20 to 111	60 to 1504	Fishes, crustaceans and squids	*Gaidropsarus macrophthalmus* (eaten by larger fish), M*icromesistius poutassou* and *Phycis blennoides* (smaller fish), Myctophidae, *Helicolenus dactylopteru*s, Merluciids, Macrouridae, *Todarodes sagitattus*	Mauchline and Gordon (1983); Marshal and Merret (1977); Dunn et al. (2013); Preciado et al. (2009)
Deania profundorum		205 to 1800	Small benthic and midwater bony fishes, including myctophids, as well as squids and crustaceans	Myctophidae	Ebert et al. (2021)
Etmopteridae					

Etmopterus pusillus	12 to 50	274 to 1200	Mesopelagic and demersal prey. High contribution of teleosts when adults and almost entirely crustacean diet when juveniles	*Micromesistius poutassou*, Myctophidae, *Pasiphaea sivado*, Natantia, *Gadiculus argenteus,* Squids	Santos and Borges (2001) †; Xavier et al. (2012) †; Muñoz (2015) †
Somniosidae					
Scymnodon ringens	52.5	200 to 1600	fish bones and fragments of muscle tissue and few crustaceans' fragments		Mauchline and Gordon (1983); Ebert et al. (2021)
Carcharhiniformes					
Pentanchidae					
Galeus melastomus	20 to 79	200 to 2000	fishes, cephalopods and dendrobranchiatan and caridean shrimps	Myctophidae, *Robustosergia robusta, Histioteuthis meleagroteuthis*, Pasiphaeidae (*Pasiphaea multidentata, Pasiphaea tarda and Pasiphaea sivado*), Oplophoridae (*Acanthephyra*	Santos and Borges (2001) †; Olaso et al. (2005); Neves et al. (2007) †; Preciado et al. (2009); Anastasopoulou et al. (2013); Barría et al. (2018)

pelagica), epibenthic crustaceans (*Munida perarmata and Geryon longipes*)

[†] Diet information from the South of Portugal



Appendix 4.2 Linear regression of standardized RD values and size (total length in cm) of the species *Deania calceus* (Dea), *D. profundorum* (Dep), *Etmopterus pusillus* (Etm), *Galeus melastomus* (Gal) and *Scymnodon ringens* (Sym).

Chapter 5: TROPHIC ECOLOGY OF DEEP-SEA ELASMOBRANCHS AND NOTES ON THE OVERLAP WITH CRUSTACEAN BOTTOM TRAWL FISHERIES

Graça Aranha, Sofia; Teodósio, Alexandra; Marsili, Tiago; Pires Da Rocha, Pedro; Baptista, Vânia; Cruz, Joana; Figueiredo, Ivone & Dias, Ester. Trophic ecology of deep-sea elasmobranchs and notes on the overlap with crustacean bottom trawl Fisheries. ***In Preparation***

Abstract

Deep-sea elasmobranchs (DSE) are critical components of deep-sea ecosystems, acting as meso-and top-predators influencing community structure and creating stability in food webs. Understanding their trophic ecology is crucial for assessing their ecological roles and the potential impacts of their removal on marine ecosystems due to anthropogenic impacts such as fishing. Hence, the present study brings information on the trophic ecology of sympatric DSE in the South and Southwest coasts of Portugal. Carbon and nitrogen stable isotopes were used to determine DSE trophic position, trophic niche widths, and trophic niche overlap between sympatric species. Nucleic acid-derived indices (i.e., RNA and DNA ratios) were used to investigate their short-term nutritional condition and to evaluate the potential overlap between DSE foraging areas and fishing grounds. The 13 DSE species studied (e.g. *Deania* spp., *Etmopterus* spp., *Galeus* spp., *Dipturus* spp.) are mesopredators in the local food webs, although *Centrophorus* spp. and *Scymnodon ringens* can act as top-predators. Wider niches and higher trophic niche overlap were generally observed for the South coast suggesting that DSE assimilated a higher diversity of prey in this region, when compared to the Southwest, and that resources' sharing was also higher. Furthermore, DSE also overlap their foraging grounds with crustacean bottom trawlers in these areas given their sRD values suggest recent feeding events. Nonetheless, their good nutritional condition suggests that the available resources and conditions are likely sufficient to meet DSE's energetic requirements. Ontogenetic and sexual, differences were also spotted in regards of trophic niche width. These differences across areas, maturity stages and sex, indicate a trophic plasticity for some DSE species, suggesting they can adjust their trophic behaviour according to the environmental variability and disturbance levels, and maintain a good nutritional status. Further studies evaluating resources abundance, and more in-depth dietary data of DSE are necessary to indicate the state of the resources in the regions and DSE dietary preferences. However, the complexity and variety of the DSE behaviours in relation to their trophic ecology highlights the need for species-specific studies integrating spatial, seasonal, biological, and ecological factors through standardized and multidisciplinary approaches.

Keywords: stable isotopes, nucleic acids, RNA:DNA, trophic position, nutritional condition, Iberian Peninsula, Portugal

5.1 Introduction

Deep-sea elasmobranchs (DSE), sharks and skates predominantly inhabiting habitats below 400 m (ICES, 2020; O'Hea et al., 2020), are critical components of deep-sea ecosystems acting as meso- and top-predators (e.g. Cortés, 1999; Graça Aranha et al., 2023) influencing community structure and creating stability in food webs (e.g., Heithaus et al., 2008; Ruppert et al., 2013; Barley et al., 2017). For instance, sharks can regulate prey populations through top-down control, connect benthic and pelagic energy pathways, and link spatially segregated food webs (Heithaus et al., 2008; Baum and Worm, 2009; Ferretti et al., 2010; Bird et al., 2018; Graça Aranha et al., 2023). However, DSE species are particularly vulnerable to extinction due to their conservative life histories, characterized by slow growth, late maturity, and low reproductive rates (García et al., 2008; Simpfendorfer and Kyne, 2009; Villagra et al., 2022). This vulnerability is exacerbated by their significant presence as bycatch and high mortality in European fisheries, despite the existing regulations [Borges et al., 2001; Graça Aranha et al., (unpublished results)]. Elasmobranchs display a variety of ecological strategies and feeding behaviours and their ecological roles vary between species and regions (e.g., Cortés, 1999; Wetherbee and Cortés, 2004; Shiffman et al., 2019); however, for deep-sea species, these are largely unknown. The lack of comprehensive biological and ecological information on DSE, primarily due to the logistical challenges of studying their habitats, hampers the development of effective management and conservation strategies (Simpfendorfer and Kyne, 2009; Brooks et al., 2015). Understanding DSE trophic ecology is crucial for assessing their ecological roles and the potential impacts of their removal on marine ecosystems (Heithaus et al., 2008; Ferretti et al., 2010). Traditionally, stomach content analysis has been the primary method in studies of trophic ecology in aquatic organisms, including elasmobranchs (e.g., Cortés, 1999; Barría et al., 2018). Stomach content data provides high taxonomic resolution; however, it has several limitations, including the need for large sample sizes, the relative importance of different prey items may be biased by differences in digestion rates, difficulties in identifying partially digested prey, and large percentage of empty stomachs (e.g., Hyslop, 1980; Benson et al., 2001; Hammerschlag and Sulikowski, 2011; Albo-Puigserver et al., 2015). Additionally, since techniques for sampling stomachs' contents can be harmful or lethal, gathering data for species that are rare, endangered, or challenging to capture like DSE, may be logistically impractical or ethically questionable (Hammerschlag and Sulikowski, 2011). Conversely, biochemical markers like stable isotopes ratios of carbon ($\delta^{13}C$: $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) can be used to characterize trophic structure, niche breadth, and energy flow over extended periods, offering quantitative approaches (Layman et al., 2011; Shipley et al., 2017). This method provides time-integrated data on assimilated prey, requires relatively smaller sample sizes in comparison with stomach content analysis, and can be non-lethal, making it suitable for studying vulnerable species (Peterson and Fry, 1987). Carbon isotopes ratios are useful in identifying the origin of the organic matter sources supporting food webs, due to differences on C source and fixation between producers (e.g., Smith and Epstein, 1971; Fry and Sherr, 1989; Cloern et al., 2002; Layman et al., 2011; Dias et al., 2023), while nitrogen isotope ratios are more commonly used to estimate trophic position in food webs (Vander-Zanden et al., 1997) due to predictable enrichment between prey and predators (Cabana and Rasmussen, 1994; Post, 2002; Caut et al., 2009). Also, both $\delta^{13}C$ and $\delta^{15}N$ have been associated with foraging depths in benthopelagic communities (France, 1995; Lawson and Hobson, 2000; Trueman et al., 2014). Consequently, the combination of carbon and nitrogen isotopes provides information on the trophic ecology of consumers, essential for evaluating the ecological role of a species or population in a given ecosystem (Newsome et al., 2010).

In addition to stable isotopes analysis, nucleic acid-derived indices such as standardized RNA:DNA ratios (sRD), offer valuable insights into the nutritional condition of marine organisms. These indices have been applied as indicators of growth (e.g., Bulow, 1987; Caldarone et al., 2003; Tavares et al., 2006), nutritional condition (e.g., Chícharo and Chícharo, 1995; Chícharo et al., 2003; Alves et al., 2020), productivity (e.g., Cruz et al., 2017), health status (e.g., Tavares et al., 2006), and anthropogenic impacts (Chícharo and Chícharo, 2008; Müller et al., 2020) across various marine taxa, including teleosts, crustaceans, and marine mammals. Additionally, the RD ratio provides a short-term measure of nutritional condition, reflecting recent feeding activity (Buckley et al., 1999), which could help in identifying foraging areas of slow moving DSE (Graça Aranha et al., 2023). The effectiveness of sRD as indicators of nutritional condition stems from the fact that cellular DNA levels remain relatively constant, regardless of any changes. In contrast, RNA levels increase with the cells demand for protein synthesis and growth (Chícharo and Chícharo, 2008). Therefore, sRD reflect the cell's protein synthesis capacity, which fluctuates in response to environmental factors like temperature and food availability. Poor nutritional status leads to reduced protein synthesis and slow growth, resulting in low sRD (Ikeda et al., 2007; Buckley et al., 2008). Consequently, individuals with a good nutritional condition tend to have higher sRD than those experiencing dietary limitations exhibiting lower RNA levels and, in turn, a reduced sRD (Frommel and Clemmesen, 2009). Nucleic acids' ratios application to elasmobranchs is relatively new and limited to three studies. Cruz-Ramírez et al. (2017) and Tavares et al. (2006) demonstrated its potential to assess growth and health status in shark species through non-standardized RD values. More recently, Graça Aranha et al. (2023) applied this method to investigate the condition of deep-sea sharks caught in crustacean bottom trawling fisheries in the Southwest coast of Portugal suggesting an overlap of sharks' feeding grounds with trawlers' activities. The integration of both stable isotopes with nucleic acid-derived indices can enhance our understanding of the trophic ecology and feeding grounds of DSE. Such combined approaches can help to identify critical life stages and/or areas for conservation and management amidst increasing fishing pressure, ultimately contributing to more effective strategies for preserving these vulnerable marine species (Newsome et al., 2010).

Hence, the present study aimed at investigating some aspects of the trophic ecology of DSE in the southern coast of Portugal, including their trophic position, trophic niche widths, and dietary overlap considering potential differences between areas (South vs Southwest), maturity stage (i.e., mature vs immature) and sex (males vs females). For that, carbon and nitrogen stable isotopes were used. The potential for overlap between DSE' foraging areas and crustacean bottom trawlers fishing grounds was evaluated using sRD ratios, on the basis that they can reflect recent feeding activity (Buckley et al., 1999). Because the species targeted in this study are frequently found in the southern coast of Portugal [e.g., *Galeus* spp., *Deania* spp., *Etmopterus* spp., *Scymnodon ringens;* Graça Aranha et al. (unpublished results)], we hypothesized that this area includes feeding grounds for DSE, thus indicating potential for overlap with crustacean bottom trawl fishing areas.

5.2 Materials and methods

5.2.1 Ethics statement

This study was conducted following the Guidelines of the European Union Council (2010/63/UE) and Portuguese legislation "The protection of Animals Used for Scientific Purposes" (DL 113/2013). All the procedures were approved by CCMAR Animal Welfare Committee (ORBEA CCMAR - Organization Responsible for Animal Welfare of CCMAR) and the *Direção-Geral de Alimentação e Veterinária* (DGAV) of the Portuguese Government. All animal protocols were performed under Group-C licenses from the DGAV, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, Portugal.

5.2.2 Surveys

Fishing surveys were conducted in a commercial trawler and in a research vessel from June 2020 to May 2022. Ten trips were conducted opportunistically in a commercial bottom trawler targeting crustaceans. This vessel contained two bottom trawl nets with a codend diamond mesh size of 55 mm and 70 mm for targeting shrimps and prawns, and Norway lobster (*Nephrops norvegicus*) respectively. In the South area (Figure 5.1) the vessel hauled in velocities of 1.5-3.7 knots, hauls lasted from 2.7-6.3 h, were conducted at depths of 257-810 m, and bottom temperature varied from 11.5 to 14.2°C. In the Southwest area (Figure 5.1) the vessel hauled in velocities of 2.4-2.8 knots, hauls lasted from 2.9 to 8.6 h, were conducted at depths of 403-1244 m, and the bottom temperature varied from 11.9 to 13.2°C .

Two trips were conducted onboard the R/V Mário Ruivo from the Portuguese Institute for the Sea and Atmosphere (IPMA, Portuguese acronym) for the biological sampling campaigns "*Crustáceos*" and "*Demersais*" in the scope of the EU Data Framework Collection. This vessel contains a bottom trawl net with a codend mesh size of 20 mm for targeting prawns, shrimps and Norway lobster. In the South area (Figure 5.1), the vessel hauled at velocities of 2.3 to 3.4 knots, hauls lasted from 0.3 to 1.4 h at depths of 243 to 776 m, and the bottom temperature varied from 12.6 to 13.4°C. In the Southwest (Figure 5.1), the vessel hauled at velocities of 2.7 to 3.3 knots, hauls lasted from 0.5 to 0.6 h at depths of 221 to 423 m, and the bottom temperature varied from 11.9 to 12.0°C.

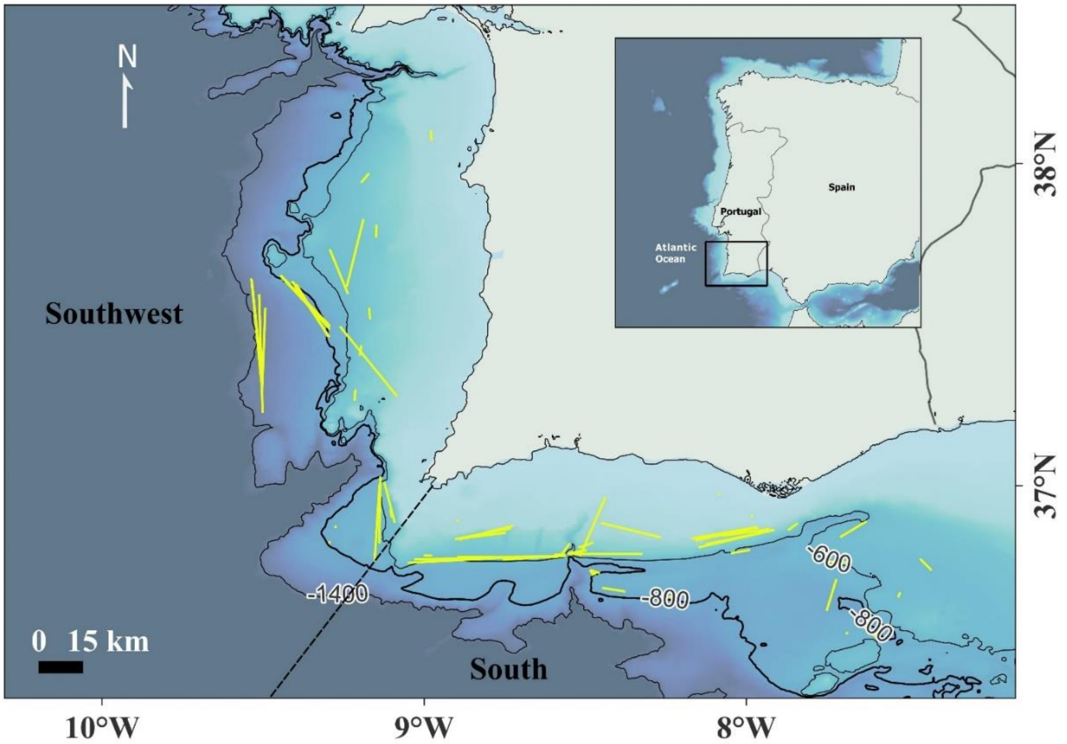


Figure 5.1 Map presenting the study area in the South and Southwest of Portugal. The yellow lines are the path in which the hauls were conducted by a commercial crustacean bottom trawler and a research vessel.

For both the commercial and the RV, sampling depth (m) and bottom temperature (°C) were recorded using a DST-CTD mini logger device from Star-Oddi®. Coordinates marking the start and end of each haul were obtained using a GPS system linked to a laptop onboard, with data automatically input into Olrac DDL ® software provided by OLSPS Marine. The haul duration, defined as the time elapsed (in hours) from when the net first made contact with the seabed to when it began to be retrieved, was also logged into the software. Using the recorded haul coordinates and duration, the Olrac DDL ® software automatically computed the average vessel speed in knots.

Following each haul, DSE were immediately separated on the sorting table and placed inside cooled water tanks prior to identification. Once the sorting ended, elasmobranchs were then identified following Williams (2014), counted, measured (total length in cm -TL, from the tip of the snout to the tip of the caudal fin), weighed (kg), and sexed (males with claspers and females without claspers). Maturity stage was assessed macroscopically using the criteria provided by Stehmann (2002), with immatures classified as males and females in stages I and II, and matures as males in stages III and IV, and females in stages III-VII. As female gonad maturity can only be assessed by internal inspection, and no live females were sacrificed for this purpose, the maturity stage of live females was inferred from available literature based on the specimen TL (Coelho and Erzini, 2007 [*Etmopterus* spp.]; Ebert et al., 2021).

Muscle samples were collected using a modified technique adapted from Henderson et al. (2016) for teleosts, consisting in an incision made with a scalpel near the base of the first dorsal fin for sharks and at the dorso, close to the vertebrae, for skates. The tissue sampled was then placed into two Eppendorf vials: one vial for R/D stored with RNA Riboreserve™ and the other for Stable Isotope Analysis (SIA). Both types of samples were immediately frozen onboard. After tissue removal, wound treatment was performed to alive specimens, by applying a small amount of Fish Bandage™ into the wound. Fish Bandage™ is a non-toxic, non-allergenic cellulose-based powder that, when mixed with water, forms a transparent, viscous gel. This gel can be used to treat skin ulcers and close open wounds in fish to minimize risk of infection. The entire process took a maximum of two minutes, after which the alive DSE were returned to the water. However, because *post-mortem* RD tends to decrease, dead specimens were sampled first.

5.2.3 Laboratory analysis

To prepare elasmobranchs' muscle tissue for SIA, a combination of the approaches from Logan and Lutcavage (2010), Churchill et al. (2015a), and Carlisle et al. (2017) was followed. First, for the removal of urea, frozen muscle was homogenized and cleaned approximately 3x consecutively with 5ml of ultra-pure water (MiliQ™). Then, the sample was placed in ultra-pure water inside an Ambar glass vial for an ultrasound bath for 15 min in ice cold water. After this, the supernatant was removed by centrifugation. After repeating this procedure, the sample was placed in the oven at 60°C for around 48h, and ground to powder prior to lipids' removal. A mixture of chloroform and methanol (2:1) was placed inside each vial to *ca*. 5x the volume of the sample following Bligh and Dyer (1959). Samples were placed in a vortex for 2 minutes, left undisturbed for another 30 minutes and centrifuged for 10 minutes. After this procedure the supernatant was removed and the entire process was repeated until the supernatant was clear, indicating complete lipid removal. To remove residual reagent from the sample, the previous procedure was done, but this time with ultra-pure water three times. Finally, samples were placed in the oven at 60°C for a maximum 36 h to once again dry completely and ground to a fine homogeneous powder prior to analysis.

Teleosts were identified (Albuquerque, 1956), and measured for total length (TL, cm). Muscle tissue was collected from the dorsal region (often on the left side), avoiding scales and skin. All the samples were dried at 60°C for at least 48 h and grounded to a fine powder prior to stable isotopes analysis.

Stable isotopes ratios were measured using a Thermo Scientific Delta V Advantage IRMS via a Conflo IV interface (Marinnova, University of Porto). Stable isotope ratios are reported in δ notation, $\delta X = (R_{sample}/R_{standard} - 1) \times 10^3$, where X is the C or N stable isotope, R is the ratio of heavy:light stable isotopes. The $\delta^{13}C$ and $\delta^{15}N$ are expressed in units per mill (‰) relative to Vienna Pee Dee Belemnite and air, respectively.

The raw data were normalized by three-point calibration using the international reference materials IAEA-N-1 ($\delta^{15}N$ = +0.4‰), IAEA-NO-3 ($\delta^{15}N$ = +4.7‰) and IAEA-N-2 ($\delta^{15}N$ = +20.3‰) for nitrogen isotopic composition, and two-point calibration using USGS-40 ($\delta^{13}C$ = -26.39‰) and USGS-24 ($\delta^{13}C$ = -16.05‰) for carbon isotopic composition. The analytical error, the mean standard deviation (SD) of the replicate reference material, was 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$.

To determine elasmobranch's nutritional condition, RNA and DNA were extracted from a microplate fluorescent assay, as outlined by Caldarone et al. (2001). Nucleic acids were extracted both chemically and mechanically, following the procedures described in Esteves et al. (2000) and Graça Aranha et al. (2023).

To minimize discrepancies in RD values across different protocols and to facilitate future comparisons between studies, we employed standardized RD values to assess the nutritional condition of elasmobranchs (Caldarone et al., 2006). These standardized values were derived using the slope ratio of 3.53 for the RNA and DNA standards, with a reference slope ratio of 2.4, as described by Caldarone et al. (2006). To confirm the completeness of RNA digestion and to ensure that no DNA degradation occurred, control samples labelled as 'only-DNA' and 'only-RNA' were included on each plate, where samples were previously analysed, and RNAase digestion was applied to all samples except the 'only-RNA' controls.

5.2.4 Data analysis

Deep-sea elasmobranchs' trophic position (TP) was determined following the scaled framework proposed by Hussey et al. (2014b):

$$TP = \frac{log(\delta^{15}N_{lim} - \delta^{15}N_{myct}) - log(\delta^{15}N_{lim} - \delta^{15}N_{specim})}{k} + TP_{myct}$$

where TP is trophic position, $\delta^{15}N_{lim} = {}^{\beta 0}/_{\beta 1}$ and the intercept β0 and slope β1 were 5.92 and 0.27, respectively (Hussey et al., 2014a). The $\delta^{15}N_{myct}$ was 8.9‰ in the South and 9.4‰ in the Southwest, which is the direct measurement of the $\delta^{15}N$ values for the baseline organisms, in this case myctophids (*Myctophum punctatum* and *Lampadena* sp.) and other teleosts (*Polymetme corythaeola* and *Argyropelecus hemigymnus*), which are known to perform diel vertical migration movements hence connecting the pelagic and benthic food webs (e.g., Torres et al., 1979; Childress et al., 1980; Eduardo et al., 2021). These organisms are assumed to be secondary consumers and thus present a TP of 3 (Olivar et al., 2018). The $\delta^{15}N_{specim}$ is the $\delta^{15}N$ values directly measured for each analysed consumer, and k is the rate at which $\delta^{15}N_{TP}$ approaches $\delta^{15}N_{lim}$ at each TP, *i.e.*, k = - log (β_0 - $\delta^{15}N_{lim}/\delta^{15}N_{lim}$; Hussey et al., 2014a).

To estimate the trophic niche width for each elasmobranch species, the stable isotope values were used to calculate the standard ellipse area (SEA) using the SIBER (Stable Isotope Bayesian Ellipses in R) package (Jackson et al., 2011). The SEA is a bivariate measure of the distribution of individuals in the trophic space (Jackson et al., 2011). To account for the small sample size, the corrected standard ellipse areas (SEA_C; considering 40% of central data points) and the corresponding 95% Bayesian ellipse areas (SEA_B) were estimated for each species per area (Jackson et al., 2011). When overlap was observed, the extent of overlap (%) was calculated using the SEAc, which represents the overlap between the core dietary niches of any pair of species (Jackson et al., 2011).

The correction for the decadal decrease in atmospheric $\delta^{13}C$ (i.e., Suess effect), was not applied to DSE's $\delta^{13}C$ values prior to the statistical analyses. The decrease is ca. -0.02‰ yr-1 in the Atlantic Ocean (Gruber et al., 1999; Körtzinger et al., 2003) which would result in a maximum difference between the measured and corrected values of 0.04‰ in $\delta^{13}C$. This difference is not ecologically meaningful.

All values are reported as mean and standard deviation (±SD) even for data not following a normal distribution, to facilitate comparisons between studies. All the analyses were conducted using the open-source statistical software R (R Development Core Team, 2024).

Nucleic acid-derived indices were calculated based on dry weight (expressed as µg RNA mg−1 DW, µg DNA mg−1 DW, and RD). The RD index was determined by dividing RNA concentration (mg−1) by DNA concentration (mg−1), yielding the RD value. To assess whether these indices were influenced by size (total length, TL), a linear regression analysis was performed, which revealed no significant relations between the indices and size ($p > 0.05$), except for the species *Galeus atlanticus* ($p = 0.001$) in which the residual index of RNA mg-1 (i.e., resRNA) was used to account for the allometric effect of size (Suthers et al., 1996; M.A. Chícharo et al., 1998).

Since no critical RD thresholds are established for elasmobranchs—values that would indicate whether they are in good or poor nutritional condition—the assessment was carried out using the percentile approach (Meyer et al., 2012). According to this method, if the mean RD values for a species or group are near the 75th percentile, the individuals are in good nutritional condition, indicating they have likely fed within the past days or weeks, hence feeding in the study area or nearby areas. Conversely, if the mean RD values are closer to the 10th percentile, the individuals are deemed to be in poor nutritional condition (Meyer et al., 2012).

5.3 Results

5.3.1 Trophic ecology

A total of 301 specimens belonging to 12 DSE species were studied in the South and 190 specimens belonging to nine DSE’ species were studied in the Southwest (Appendix 5.1). Overall, sharks and skates occupy a similar position in the analysed food webs presenting slightly higher values for the South in comparison with the Southwest. Deep-sea elasmobranch’s TP varied between 3.1 and 5.0 in the South, and between 3.3 and 4.2 in the Southwest (Appendix 5.1). The highest TP average value was presented by *Centrophorus granulosus*, whereas the smallest TP average value was presented by *G. atlanticus* (Appendix 5.1). The difference between the TP values for each species in the same area was small (SD < 0.7; Appendix 5.1). However, when comparing between areas, the *S. ringens* in the South was one trophic level above the *S. ringens* from the Southwest presenting an average TP of 4.5 and 3.7 respectively.

Trophic niches were wider in the South than in the Southwest for all the evaluated DSE, except for *S. ringens* which presented an obvious opposite pattern (Table 5.1). The shark *Deania profundorum* and the skates *Dipturus* spp. presented the widest trophic niches among areas (Table 5.1). In the South, the species with the widest and smallest core trophic niche were *Dipturus oxyrinchus* and *S. ringens*, respectively, whereas in the Southwest *Dipturus nidarosiensis* showed the widest trophic niche width as opposed to *G. atlanticus* (Table 5.1).

Estimates from the SEAc for both areas and species were generally the same as the modes generated from the SEA$_B$ and fell within lower and minimum 95% credible intervals, suggesting that SEAc provides a reliable approximation of the isotopic niche width (Table 5.1).

Table 5.1 Community niche metrics for deep-sea elasmobranchs in the South (S) and Southwest (SW) coasts of Portugal. Standard ellipse area corrected for small sample sizes (SEAc; ‰), and Bayesian standard ellipse area (SEAB; ‰2) presenting the modes and upper and lower 95% credible intervals inside parenthesis.

	SEAc (‰)		**SEA$_B$ (‰^{2})**			
Species	**S**	**SW**	**S**		**SW**	
Galeus atlanticus	2.0	0.4	2.1	(1.5 - 2.8)	0.4	(0.3 - 0.5)
Galeus melastomus	2.0	0.9	2.0	(1.5 - 2.5)	0.9	(0.6 - 1.2)
Deania calceus		0.6			0.5	(0.3 - 0.9)
Deania profundorum	2.4	1.7	2.4	(1.6 - 3.6)	1.4	(0.8 - 2.8)
Etmopterus pusillus	1.7	0.5	1.7	(1.3 - 2.3)	0.5	(0.2 - 1.1)
Etmopterus spinax	1.4	1.6	1.4	(1.0 - 1.9)	1.6	(1.1 - 2.2)
Scymnodon ringens	0.3	0.9	0.3	(0.2 - 0.5)	0.9	(0.6 - 1.2)
Dipturus nidarosiensis		2.1			1.8	(1.1 - 3.5)
Dipturus oxyrinchus	3.0		3.0	(1.7 - 4.9)		
Neoraja iberica	0.8		0.8	(0.4 - 1.2)		

Patterns in trophic niche width among maturity stages and sexes varied according to species and study area. In the South immature specimens of *E. pusillus* showed a wider niche width than mature specimens (Figure 5.2). In the Southwest, mature *D. calceus* showed a wider trophic niche width (Figure 5.2), while the opposite was found for *D. nidarosiensis* with wider trophic niche width for immature specimens than mature (Figure 5.2).

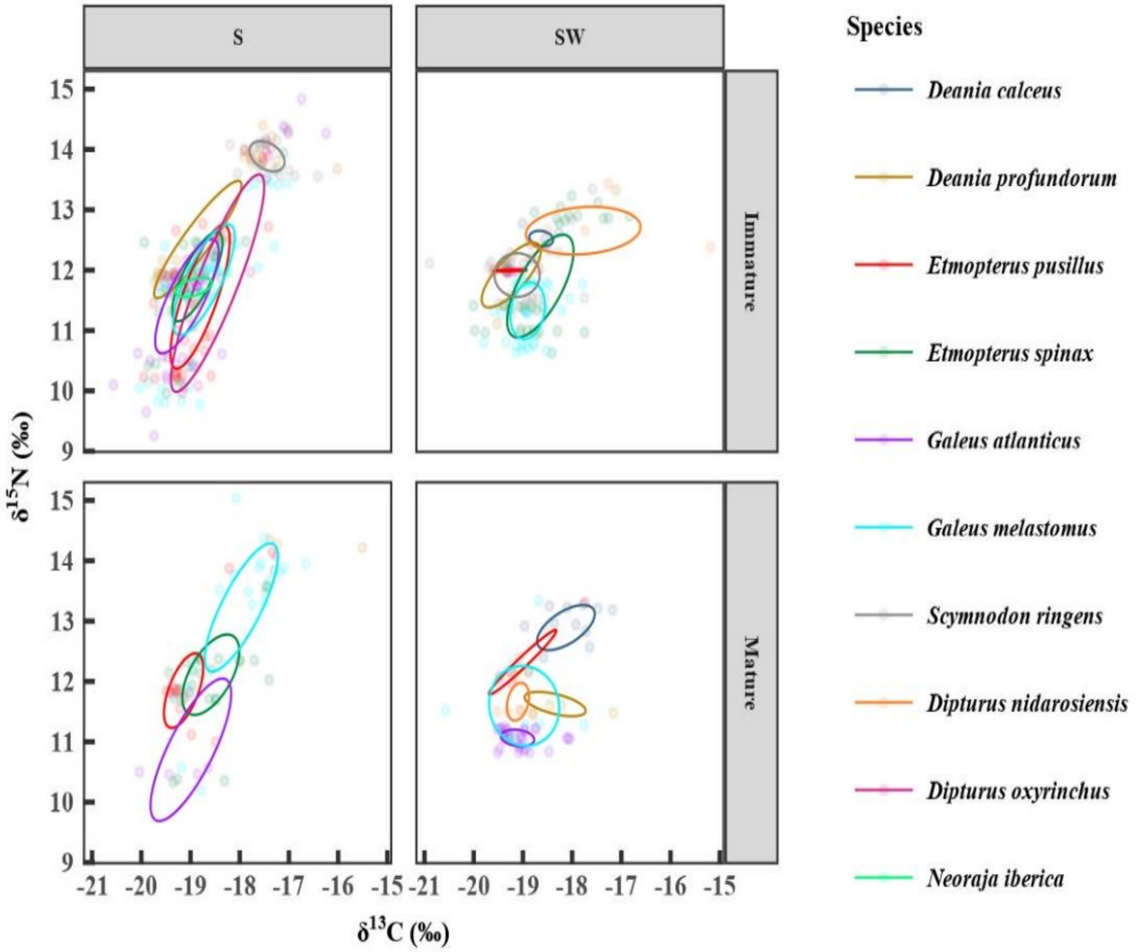


Figure 5.2 Standard ellipse area comprising 40% of the data of each deep-sea elasmobranch species off the South (S) and Southwest (SW) coasts of Portugal separated by species and maturity stage.

The trophic niche width differed between males and females, and patterns varied between the species. In the South, females of *E. pusillus* and *G. melastomus* presented wider trophic niches than males, while the opposite was observed for *E. spinax,* and *D. oxyrinchus* (Figure 5.3). In the Southwest females of *D. profundorum* presented wider trophic niches than males, while the opposite pattern was observed for *D. calceus* (Figure 5.3).

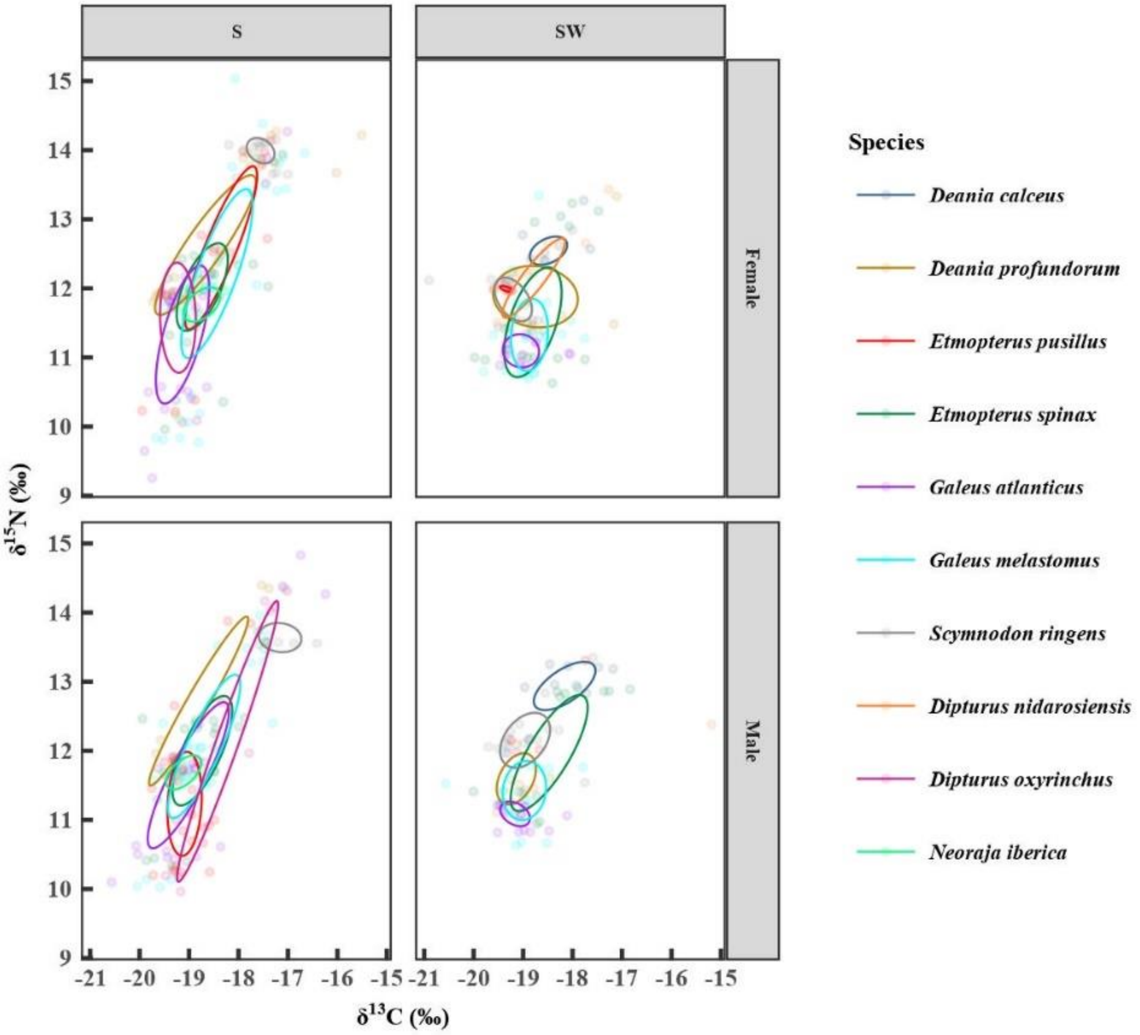


Figure 5.3 Standard ellipse area comprising 40% of the data of each deep-sea elasmobranch species off the South (S) and Southwest (SW) coasts of Portugal separated by species and sex.

The overlap between shark species, skate species, and between sharks and skates was overall high in the South (Table 5.2). Some exceptions included the *S. ringens*, showing small to no overlap with the remaining species analysed (0 to 3%; Table 5.2). The highest values of core trophic niche overlap were observed between *G. atlanticus* and *E. pusillus* (71%) and between *Etmopterus* spp. (69%) (Table 5.2).

In the Southwest, niche overlap was variable according to species. The trophic niche of *D. profundorum* overlapped with that of all species, although the proportion of overlap was low with *G. atlanticus* and with the congener *D. calceus* (Table 5.2). *Galeus atlanticus* was the species showing the lowest values of core trophic niche overlap with other species and the highest values were recorded with the congener *G. melastomus* (39%; Table 5.2). The highest values of core trophic niche overlap were observed between *D. profundorum* and *E. spinax* and *D. nidarosiensis* (58%; Table 5.2).

Table 5.2 Overlap between the core trophic niche (%) of pairs of elasmobranch species collected off the South and Southwest coastal areas of Portugal.

South

Species	***Galeus melastomus***	***Deania profundorum***	***Etmopterus pusillus***	***Etmopterus spinax***	***Scymnodon ringens***	***Dipturus oxyrinchus***	***Neoraja iberica***
Galeus atlanticus	53	19	71	63	0	54	39
Galeus melastomus		31	61	61	0	65	37
Deania profundorum			21	23	3	29	10
Etmopterus pusillus				69	0	58	46
Etmopterus spinax					0	46	56
Scymnodon ringens						0	0
Dipturus oxyrinchus							27

Southwest

Species	***Galeus melastomus***	***Deania calceus***	***Deania profundorum***	***Etmopterus pusillus***	***Etmopterus spinax***	***Scymnodon ringens***	***Dipturus nidarosiensis***
Galeus atlanticus	39	0	4	0	18	0	0
Galeus melastomus		0	28	0	45	15	14
Deania calceus			1	11	4	1	6
Deania profundorum				16	58	40	58
Etmopterus pusillus					3	38	19
Etmopterus spinax						20	42
Scymnodon ringens							37

5.3.2 Nutritional condition

The condition of 524 specimens from 11 DSE species were evaluated using sRD. In the South, data from 326 specimens belonging to ten species were analysed whilst in the Southwest, data from 198 specimens from nine species were accessed (Appendix 5.2). Differences between the sRD means and the 10th and 75th percentiles (Figure 5.4) indicate that the analysed species are in a good nutritional condition since all values are closer to the 75th percentile (bar with smaller blue area; Figure 5.4).

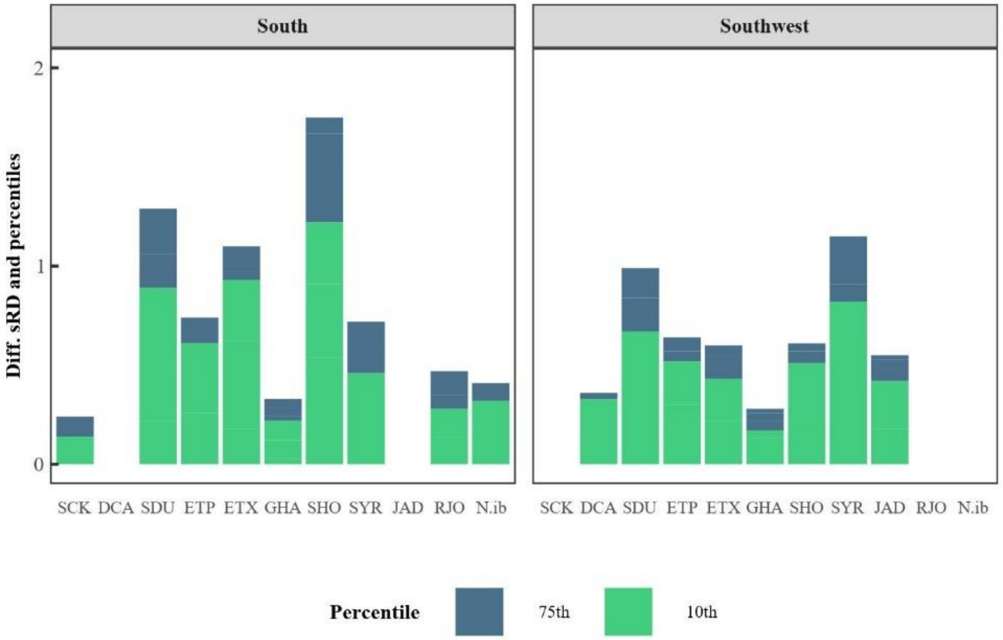


Figure 5.4 Difference between the mean sRD values and the 10th (green - lower part of each bar) and 75th percentiles (blue- upper part of each bar) in the South and Southwest Portugal per deep-sea elasmobranch species. The larger the area between the mean and the percentile, the greater is the difference, hence better (closer to 75th - blue) or worst (closer to 10th - green) nutritional condition. SCK = *Dalatias licha*, DCA = *Deania calceus,* SDU = *Deania profundorum*, ETP = *Etmopterus pusillus*, ETX = *Etmopterus spinax*, GHA = *Galeus atlanticus*, SHO = *Galeus melastomus*, SYR = *Scymnodon ringens*, JAD = *Dipturus nidarosiensis*, RJO = *Dipturus oxyrinchus*, N.ib = *Neoraja iberica*.

5.4 Discussion

This study evaluated some aspects of the trophic ecology and nutritional condition of 13 DSE species caught off the southern coast of Portugal. According to our analyses, the species evaluated are mostly mesopredators in the local food webs since their trophic level varied, overall, between 3 and 4; however, some trophic plasticity seemed to occur for *S. ringens* between the studied areas. The trophic niche width varied according to species and location, but they were overall wider in the South than in the Southwest. Intraspecific comparisons on the trophic niche width between maturity stages and sex, showed variations but no clear pattern. The trophic niche of most species overlapped at both areas, especially at the South, but a few species presented no overlap with the other co-occurring DSE suggesting trophic niche partitioning. The DSE evaluated for this study presented a good nutritional status, which indicates that, not only they are feeding in the study area or nearby areas, but also that their feeding strategies support their nutritional needs.

5.4.1 Trophic ecology

The DSE caught in the South presented, overall, higher TP values, wider trophic niches, and higher trophic niche overlap between species than those caught in the Southwest. Also, intraspecific differences in the stable isotopes according to the maturity stage and sex were more frequent in the South than in the Southwest. Factors influencing the observed patterns may be related with prey availability (diversity and/or abundance) and with specific foraging strategies.

The DSE analysed for this study can act as meso- or top-predators in the food webs. The TP estimates indicate that many of these species behave as mesopredators with trophic levels varying between 3 and 4, in line with other studies relying on stable isotopes (Chouvelon et al., 2012; Colaço et al., 2013; Albo-Puigserver et al., 2015; Barría et al., 2015; Graça Aranha et al., 2023) and stomach contents' analyses (Cortés, 1999; Stergiou and Karpouzi, 2002; Dunn et al., 2013; Albo-Puigserver et al., 2015; Barría et al., 2015). However, in the South species such as *Centrophorus* spp. and *S. ringens* showed TP values above 5, which indicates they can behave as top-predators in this food web, similarly to other known deep-sea top-predators like *Hexanchus griseus* (TP = 4.0 - 4.7; Froese and Pauly, 2024) and *Dalatias licha* (TP = 4.6 - 4.8; Navarro et al., 2014). In the case of *S. ringens*, while this species behaved as top-predator in the South, in the Southwest, their TP values (between 3.5 and 4.2) indicate they act as mesopredators, suggesting some degree of trophic plasticity in this species. These differences do not seem to be related with size since the mean TL for *S. ringens* specimens was close between both areas (South 35.5 ± 5.5 cm and Southwest 56.2 ± 18.3 cm). The present study does not allow to conclude on the reasons for this apparent flexibility in the feeding behaviour, but previous research showed that trophic plasticity can result from competition with co-occurring species (Creel and Creel, 1996, 1998) or anthropogenic pressures such as fisheries (Shiffman et al., 2019). Thus, when facing limitations to their preferred resources (e.g., crustaceans or teleosts), *S. ringens* and other DSE may be able to adapt by preying on species positioned higher in the food web.

Trophic niche widths were generally wider in the South in comparison with the Southwest. For instance, *G. melastomus* and *G. atlanticus* showed a core trophic niche 5 or 2 times wider in the South than in the Southwest, respectively. Wider trophic niches indicate that the species in the South used a higher diversity of prey than those in the Southwest. Likewise, trophic niche overlap between species was generally higher in the South than in the Southwest. For instance, while the overlap between *Galeus* spp. and *Etmopterus* spp. in the South was the highest observed between any pair of species (from 61 to 71%), in the Southwest, the overlap between *Galeus* spp. and *E. pusillus* was inexistent and the overlap between the congeners *E. pusillus* and *E. spinax* was minimal (3%). Indeed, *G. melastomus* and *E. spinax* have been reported to differ in the extent of niche overlap, with some studies reporting high overlap (Macpherson, 1981; Preciado et al., 2009; Valls et al., 2011) while others reported small overlap among them (e.g. Valls et al., 2017). Previous studies suggested that high overlap between species is related to low prey availability, while low overlap suggests that there are a variety of prey suitable for the species to partition resources (Shiffman et al., 2019). There is a generalized lack of information on the temporal variability of DSE potential prey for the studied areas. However, differences in prey availability may occur due to differences in oceanographic characteristics (e.g., upwelling events), habitat features (e.g., submarine canyons), and anthropogenic pressures (e.g., fishing activities).

Areas influenced by the upwelling of nutrient-rich cold waters, such as the Southwest of Portugal between spring and late summer (Relvas et al., 2007; Loureiro et al., 2008; de Oliveira Júnior et al., 2024), tend to present ^{15}N- and ^{13}C-depleted organisms and simplified food web (Sommer and Stibor, 2002; Stukel et al., 2018; Décima and Landry, 2020; García-Seoane et al., 2023). Upwelling events bring nitrate-rich waters from the deep waters to the surface (Sigman et al., 2009), where phytoplankton, benefiting from renewed nitrate availability, exhibits isotopic discrimination against the heavier nitrogen isotope (^{15}N). As a result, the phytoplankton that form the base of the food web in upwelling areas have, not only relatively low δ^{15}N values, but also low δ^{13}C values (Lopez-Lopez et al., 2017; García-Seoane et al., 2023). This effect continues up in the food web, with herbivorous and small carnivorous organisms reflecting these lighter isotopic values. As a result, upwelling-driven productivity promotes large populations of low to mid-trophic level organisms, concentrating energy at these levels and limiting the abundance of top-predators due to energy losses with each trophic transfer (Alcaraz and Calbet, 2007; Décima, 2022; García-Seoane et al., 2023). Since the South experiences less intense upwelling, a slower deposition of particulate organic matter and microbial degradation can lead to an enrichment of organisms from that food web (Mintenbeck et al., 2007).

Habitat features such as submarine canyons may also influence isotopic signatures and predator-prey relations. Submarine canyons enhance the connectivity between coastal and deeper offshore waters by channelling nutrient-rich currents and organic matter from the shelf to deeper areas, which facilitates the growth of a diverse range of marine organisms (Fernandez-Arcaya et al., 2017; Santora et al., 2018). These canyons not only create shelter areas for DSE from the intense bottom trawling activity within this region (e.g., Bueno-Pardo et al., 2017; Campos et al., 2021), but also create zones of organic enrichment and depositional areas, where particulate organic matter accumulates and undergoes distinct degradation processes (Martín et al., 2006; Zúñiga et al., 2009). Canyon systems also induce high turbulence and mixing due to interactions between topography and hydrography, which further enriches the food web and allows for a range of trophic interactions, supporting top-predators that feed on ^{15}N- and ^{13}C-enriched organic matter sources (Gardner, 1989; Dell'Anno et al., 2013). Due to the heterogeneity of habitats within submarine canyons, these features often support higher trophic diversity (Demopoulos et al., 2017). This likely explains the greater isotopic diversity observed in the South (i.e., wider trophic niches and greater TP) compared to the Southwest. In the South, three submarine canyons—Portimão, Lagos, and Faro—contribute to this complexity, whereas the sampled areas in the Southwest lack such features.

Another not mutually exclusive factor that may result in a decrease in prey availability is related to the fishing pressure. In the South, crustacean bottom trawling is more prevalent than in the Southwest (Bueno-Pardo et al., 2017; Campos et al., 2021). This fishery is expected to exert an increased pressure on mid-trophic level prey like crustaceans (with trophic levels of 2–3), which may originate differences in their availability between areas. To the best of our knowledge, there are no temporal estimates on the availability of this group of potential prey, but this may also contribute to explain the fact that some species were positioned higher in the food web in the South than in the Southwest. For instance, overfishing in the Mediterranean has been pointed out as the reason for a shift in diet of *D. licha* from its preferred teleosts (lower TP) to demersal sharks (higher TP; Navarro et al., 2014). Similarly, this study showed that in the South, *S. ringens* was one trophic level higher in the food web than in the Southwest. The variability in the availability of mid-trophic level prey between areas could also explain the overall wider trophic niche widths and higher trophic niche overlap between species found in the South when compared to the Southwest. This suggests that in the South, DSE have to prey on a different range of sources to meet their energy requirements and that the variety of prey suitable for this species to prey on is low to allow resources' partitioning. Furthermore, in the Southwest, the depths where *S. ringens* were sampled (below 800 m) have been under a bottom-trawling ban since 2017 (Regulation 2016/2336). It is possible that the current fishing ban likely alleviated fishing pressure on mid-trophic level crustaceans at those depths allowing those prey populations to gain some recovery, hence allowing *S. ringens* to act as a meso-predator in this area. Further evidence from 2018, based on stable isotopes analyses, showed that *S. ringens* specimens collected at depths below 800 m, were acting as a top-predator in the Southwest of Portugal (Graça Aranha et al., 2023). This likely reflects isotopic signatures from prey assimilated over a year prior to sampling at the start of the fishing ban in 2017, given the low isotopic turnover rates in shark muscle tissue (Logan and Lutcavage, 2010; Kim et al., 2011), thus still representing the prey assemblage influenced by the fishing effort targeting *Aristaeopsis edwardsiana* at the sampled depths. However, it is not possible to know at which depths these specimens were feeding prior to sampling. sRD ratios suggest they were feeding recently but it is unclear at which depths. Furthermore, although *S. ringens* is frequently caught as bycatch in European Union bottom trawling operations, there are only a couple of dietary studies that indicates that this species typically preys on cephalopods, teleosts, and commercially valuable crustaceans, which could represent ca. 30% of their diet (Mauchline and Gordon, 1983; Graça Aranha et al., 2023). Hence, due to the lack of data on the availability of potential prey, the role of fisheries in DSE foraging ecology remains speculative and requires further investigation.

Ontogenetic dietary shifts are well-documented in elasmobranchs (e.g., Lowe et al., 1996; Yamaguchi and Taniuchi, 2000; Farias et al., 2006). Several fishes exhibit a positive relationship between body size and TP (Romanuk et al., 2011), and thus foraging on prey with higher TP values is expected to be done by larger specimens while the opposite is expected for smaller specimens (Hussey et al., 2012). In the case of DSE, consumption of crustaceans generally decreases with size, whereas the opposite occurs regarding fish consumption (Wetherbee and Cortés, 2004; Xavier et al., 2012; Valls et al., 2017; Besnard et al., 2022; D'Iglio et al., 2021). This may be the case of the species *G. melastomus*, and *D. profundorum* where immature presented lower TP than mature, possibly feeding at lower TP prey such as crustaceans as supported by previous studies (Santos and Borges, 2001; Preciado et al., 2009; Dunn et al., 2013; D'Iglio et al., 2021). However, *D. nidarosiensis* in the Southwest exhibited the reverse pattern, with immatures displaying higher TPs than mature specimens—also observed in other DSE (Churchill et al., 2015b). One possible explanation would be that mature specimens could feed on larger prey but from lower TP than immature. This is observed for *Galeocerdo cuvier,* a known generalist top-predator, which exhibits lower TP with increasing size (Shiffman et al., 2012), because they are able to prey on large herbivorous sea turtles (Lowe et al., 1996) while juveniles present a large array of prey including teleosts. However, the limited dietary information for *D. nidarosiensis* cannot support such assumptions for ontogenetic shifts in diet due to the lack of immature stomach content data (Gordon and Duncan, 1989; Follesa et al., 2012). Hence, further studies would help address *D. nidarosiensis* dietary preferences at different life stages.

As shifts in diet occur as elasmobranchs grow, dietary differences may arise between males and females due to variations in reproductive requirements, morphology, and behaviour (Wearmouth and Sims, 2008). Sexual dimorphism in size and/or feeding structures is often associated with those mentioned dietary differences and may function as a strategy to minimize intraspecific competition between sexes (Wearmouth and Sims, 2008; 2010), which was already reported for different shark species (Matallanas, 1982; Yano and Tanaka, 1983; Hanchet, 1991; Simpfendorfer et al., 2001; Sousa et al., 2009; Barría et al., 2018). In this study, intraspecific sexual differences did not present a pattern, females would present wider (e.g., *E. pusillus* and *G. melastomus*) or smaller niches than males (e.g., *E. spinax* and *D. oxyrinchus*). For instance, *E. pusillus* females in the South exhibited wider niches than males, indicating they rely on prey from different TP and/or prey with different sources of organic matter. Previous investigations in the same area, relying on stomach content analyses, found no dietary differences between sexes (Xavier et al., 2012; Muñoz, 2015). The contrasting findings—where isotopic signatures in this study suggest distinct dietary preferences between females and males, while earlier studies detected no such differences—could be attributed to variations in the methods used to assess trophic ecology or potential temporal changes in sexual dietary behaviour. Sexual dietary behaviours might reflect differences in the proportions of the same prey consumed by each sex or a trophic plasticity due to prey availability in the studied areas. This plasticity could allow *E. pusillus* to partition resources between sexes as a strategy to adapt to changes in prey availability or habitat characteristics. However, further studies on prey structure and *E. pusillus* population and distribution would help clarify the apparent differences in the trophic behaviour.

Finally, other factors other than the cited upwelling cycles, canyons and fishing activities, may play a role in the differences observed in this study. For instance, spatial variations in the phytoplankton carbon composition ($\delta^{13}C$) are influenced by phytoplankton community and physiology (e.g., Popp et al., 1998, 1999; Maranon, 2009; Lara et al., 2010), sea-surface water temperature temperatures (e.g., Sackett et al., 1965; Lara et al., 2010), and concentration of dissolved CO_2 (e.g., Rau et al., 1997; Fischer et al., 1998). Furthermore, other anthropogenic influences could also affect stable isotopes signatures in DSE such as pollutants (e.g., Bezerra et al., 2021). Hence, further investigations would provide a better understanding of the influence of other factors in the prey availability and DSE trophic behaviour.

5.4.2 Nutritional condition and fisheries overlap

The sRD mean values were closer to the 75th percentile than to the 10th suggesting that the elasmobranchs analysed during this study were in an overall good nutritional condition (Meyer et al., 2012). This indicates recent feeding activity in the days or weeks prior to capture (Buckley, 1980; Clemmesen, 1987; 1989). Given that DSE are relatively slow swimmers, compared to their pelagic counterparts (Treberg et al., 2003; Condon et al., 2012; Pinte et al., 2020), it is likely that feeding occurred in or near the areas where they were captured. This finding suggests an overlap between DSE foraging grounds and crustacean bottom trawl fisheries, as hypothesized earlier by Graça Aranha et al. (2023), which concentrates their activities in the southern region of Portugal (Bueno-Pardo et al., 2017; Campos et al., 2021).

Deep-sea elasmobranchs are commonly caught as bycatch by crustacean bottom trawlers in Southern Portugal (Borges et al., 2001; Monteiro et al., 2001; Carbonell et al., 2003; Coelho et al., 2005; Costa et al., 2008), especially in the Southwest, where a greater biomass of DSE was observed at greater depths, summing up to 58% of the weight of the total catch [Graça Aranha et al., (unpublished results)]. This is concerning because, despite the ongoing protection measurements for most deep-sea sharks in the Northeast Atlantic ocean (Regulation n° 2023/194) and skates (Regulation n° 2024/257), they present a collectively very high at-vessel mortality rate [81%, Graça Aranha et al. (unpublished results)] and even if returned alive to the ocean, their chances of survival are minimal (Coelho and Erzini, 2007; Brooks et al., 2015; Rodríguez-Cabello and Sánchez, 2017; Talwar et al., 2017).

5.5 Conclusion

This study provided insights on the trophic ecology and nutritional condition of 13 DSE species off the South and Southwest coasts of Portugal. Deep-sea elasmobranchs function as mesopredators and, in some cases, as top-predators within the local food webs. Differences were observed between areas with DSE presenting higher TP, wider trophic niches, and greater interspecific niche overlap in the South compared to the Southwest. These variabilities suggest a trophic plasticity, likely reflecting regional differences in prey availability which could be attributed to variable oceanographic conditions, habitat features and fishing intensity. Further ontogenetic and sexual differences in trophic niches were detected among sympatric specimens without any clear pattern, suggesting shifts in diet among maturity stages and different dietary behaviours among females and males. Furthermore, DSE good nutritional condition indicates recent feeding activity in the studied areas or in nearby areas. Despite a potential for competition within and between species, and overlap with fisheries, the DSE seemed to maintain an adequate nutritional status. This further supports the observed trophic plasticity as a way to adapt to different environmental conditions and disturbance levels, as specifically highlighted for some species like *S. ringens*.

The heterogeneity observed in trophic ecology among DSE further underscores the complexity of their ecological roles and the need for species-specific studies. While this study contributes to filling knowledge gaps on DSE trophic ecology, it also highlighted the general lack of ecological and biological information about these species, which limits our capacity to draw further conclusions on their trophic strategies. In order to understand seasonal shifts in diet and movements and the full extent of overlap between DSE and fisheries, future studies should consider combining multiple approaches. This would include using data from DSE collected throughout the year (according to sex and maturity stage) and also of their potential prey, to understand strategies used according to temporal differences in prey availability considering the use of multiple ecological factors, and standardized methodologies.

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Conflict of Interest - The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5.7 Appendices

Appendix 5.1 Taxa (order and species) of deep-sea sharks and skates caught off South (S) and Southwest (SW) coasts of Portugal with their number of specimens (n), mean ± S.D. of the total length (TL), isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$ ‰), and trophic position (TP) by sex (Female-F and Male-M) and by maturity stage (Immature-Im. and Mature-Mt.). Values in bold are mean ± S.D. of all specimens from a species.

Taxa	**Sex/ Stage**	**n**		**Depth (m)**		**TL (cm)**		**$\delta^{13}C$ (‰)**		**$\delta^{15}N$ (‰)**		**TP**	
		S	**SW**	**S**	**SW**	**S**	**SW**	**S**	**SW**	**S**	**SW**	**S**	**SW**
Carcharhiniformes													
Galeus atlanticus	**All**	**46**	**33**	**557 ± 114**	**414 ± 68**	**22.6 ± 7.7**	**37.6 ± 7.6**	**-18.9 ± 0.9**	**-19.1 ± 0.4**	**11.6 ± 1.3**	**11.1 ± 0.3**	**3.6 ± 0.4**	**3.5 ± 0.1**
	F	22	15	571 ± 119	408 ± 36	21.8 ± 6.9	37.7 ± 10.2	-19.0 ± 0.7	-19.0 ± 0.4	11.4 ± 1.2	11.2 ± 0.3	3.6 ± 0.4	3.5 ± 0.1
	M	24	18	544 ± 111	419 ± 85	23.5 ± 8.4	37.6 ± 5.0	-18.8 ± 1.0	-19.1 ± 0.4	11.8 ± 1.3	11.1 ± 0.3	3.7 ± 0.5	3.5 ± 0.1
	Im.	41	3	568 ± 115	486 ± 0	20.6 ± 5.4	15.3 ± 3.2	-18.9 ± 0.9	-19.2 ± 0.1	11.7 ± 1.3	12.0 ± 0.0	3.7 ± 0.4	3.7 ± 0.0
	Mt.	5	30	462 ± 54	407 ± 66	39.0 ± 1.9	39.9 ± 2.7	-18.8 ± 1.0	-19.0 ± 0.4	11.2 ± 1.6	11.1 ± 0.1	3.5 ± 0.5	3.5 ± 0.0
Galeus melastomus	**All**	**67**	**39**	**552 ± 155**	**525 ± 255**	**35.0 ± 16.1**	**36.9 ± 14.4**	**-18.5 ± 0.8**	**-18.9 ± 0.5**	**12.1 ± 1.3**	**11.4 ± 0.6**	**3.8 ± 0.4**	**3.6 ± 0.2**
	F	37	24	559 ± 154	548 ± 281	37.5 ± 15.9	38.2 ± 14.9	-18.4 ± 0.8	-18.9 ± 0.4	12.2 ± 1.4	11.4 ± 0.6	3.9 ± 0.5	3.6 ± 0.2
	M	30	15	543 ± 158	489 ± 209	31.8 ± 16.0	34.8 ± 13.7	-18.7 ± 0.8	-19.0 ± 0.6	12.1 ± 1.1	11.4 ± 0.5	3.8 ± 0.4	3.6 ± 0.1
	Im.	48	32	527 ± 131	443 ± 175	27.4 ± 11.0	32.0 ± 10.6	-18.7 ± 0.7	-18.9 ± 0.4	11.8 ± 1.1	11.3 ± 0.5	3.7 ± 0.3	3.5 ± 0.1
	Mt.	19	7	614 ± 193	901 ± 227	54.1 ± 9.5	59.2 ± 4.4	-18.0 ± 0.8	-19.0 ± 0.8	13.1 ± 1.3	11.7 ± 0.8	4.2 ± 0.5	3.7 ± 0.3
Squaliformes													
Centrophorus granulosus	**M | Mt.**	**5**		**801 ± 0**		**89.1 ± 3.0**		**-17.3 ± 0.2**		**14.6 ± 0.2**		**4.8 ± 0.1**	
Centrophorus squamosus	**All**	**2**		**796 ± 8**		**118.0 ± 11.3**		**-17.4 ± 0.9**		**14.3 ± 0.1**		**4.7 ± 0.0**	
	F	2		796 ± 0		118.0 ± 11.3		-17.4 ± 0.9		14.3 ± 0.1		4.7 ± 0.0	
	Im.	1		790 ± 0		110.0 ± 0.0		-16.7 ± 0.0		14.2 ± 0.0		4.7 ± 0.0	
	Mt.	1		801 ± 0									
Dalatias licha	**All**	**6**		**535 ± 34**		**78.6 ± 50.3**		**-19.1 ± 1.1**		**11.3 ± 1.6**		**3.6 ± 0.5**	
	F	4		518 ± 9		95.6 ± 55.3		-19.5 ± 0.7		10.5 ± 0.9		3.3 ± 0.3	
	M	2		570 ± 42		44.5 ± 0.7		-18.2 ± 1.5		12.9 ± 1.8		4.1 ± 0.6	
	Im.	4		546 ± 37		46.1 ± 2.0		-18.7 ± 1.1		11.9 ± 1.7		3.7 ± 0.6	
	Mt.	2		513 ± 0		143.5 ± 2.1		-19.8 ± 0.9		10.1 ± 0.0		3.2 ± 0.0	
Deania calceus	**All**		**18**		**1237 ± 9**		**88.2 ± 6.2**		**-18.3 ± 0.6**		**12.8 ± 0.4**		**4.0 ± 0.1**
	F		8		1229 ± 9		90.9 ± 8.6		-18.4 ± 0.5		12.6 ± 0.3		3.9 ± 0.1

	M		10		1243 ± 2		86.0 ± 2.1		-18.2 ± 0.7		12.9 ± 0.4		4.0 ± 0.1
	Im.		5		1228 ± 9		86.8 ± 8.5		-18.6 ± 0.3		12.6 ± 0.2		3.9 ± 0.1
	Mt.		13		1240 ± 7		88.7 ± 5.5		-18.2 ± 0.6		12.9 ± 0.4		4.0 ± 0.1
Deania profundorum	**All**	**27**	**11**	**646 ± 132**	**942 ± 285**	**41.1 ± 11.9**	**54.9 ± 21.5**	**-18.5 ± 1.2**	**-18.7 ± 0.9**	**12.7 ± 1.2**	**11.9 ± 0.6**	**4.0 ± 0.5**	**3.7 ± 0.2**
	F	21	7	644 ± 134	1013 ± 282	40.6 ± 12.0	59.7 ± 21.2	-18.4 ± 1.2	-18.5 ± 1.0	12.7 ± 1.1	12.0 ± 0.6	4.0 ± 0.5	3.7 ± 0.2
	M	6	4	654 ± 137	819 ± 283	42.7 ± 12.7	46.5 ± 22.2	-18.8 ± 1.0	-19.1 ± 0.5	12.7 ± 1.3	11.6 ± 0.4	4.1 ± 0.5	3.6 ± 0.1
	Im.	25	6	633 ± 129	1057 ± 282	38.7 ± 8.3	38.5 ± 11.3	-18.7 ± 1.0	-19.0 ± 0.9	12.6 ± 1.1	12.0 ± 0.7	4.0 ± 0.4	3.8 ± 0.2
	Mt.	2	5	806 ± 6	805 ± 247	70.8 ± 12.4	74.6 ± 10.5	-16.4 ± 1.3	-18.4 ± 0.8	14.3 ± 0.1	11.7 ± 0.3	4.7 ± 0.0	3.6 ± 0.1
Etmopterus pusillus	**All**	**46**	**8**	**550 ± 105**	**954 ± 388**	**28.9 ± 8.3**	**35.0 ± 12.7**	**-18.8 ± 0.7**	**-19.1 ± 0.6**	**11.8 ± 1.2**	**12.2 ± 0.5**	**3.7 ± 0.4**	**3.8 ± 0.2**
	F	17	5	579 ± 130	940 ± 414	26.2 ± 8.8	33.2 ± 13.7	-18.4 ± 0.8	-19.4 ± 0.1	12.4 ± 1.3	12.0 ± 0.0	3.9 ± 0.5	3.7 ± 0.0
	M	29	3	533 ± 85	978 ± 423	30.5 ± 7.7	38.0 ± 13.0	-19.0 ± 0.5	-18.6 ± 0.8	11.4 ± 1.0	12.5 ± 0.7	3.6 ± 0.3	3.9 ± 0.2
	Im.	35	4	547 ± 113	675 ± 378	25.6 ± 6.0	24.8 ± 9.8	-18.8 ± 0.7	-19.2 ± 0.4	11.7 ± 1.3	12.0 ± 0.0	3.7 ± 0.4	3.7 ± 0.0
	Mt.	11	4	561 ± 79	1233 ± 11	39.5 ± 5.0	45.3 ± 0.5	-18.9 ± 0.6	-18.9 ± 0.8	12.1 ± 1.0	12.4 ± 0.6	3.8 ± 0.4	3.9 ± 0.2
Etmopterus spinax	**All**	**49**	**34**	**610 ± 116**	**421 ± 104**	**27.0 ± 6.8**	**24.5 ± 2.8**	**-18.7 ± 0.7**	**-18.6 ± 0.8**	**12.0 ± 0.9**	**11.8 ± 0.9**	**3.7 ± 0.3**	**3.7 ± 0.3**
	F	27	19	610 ± 117	432 ± 110	28.3 ± 7.2	25.1 ± 2.6	-18.6 ± 0.7	-18.7 ± 0.7	12.0 ± 0.9	11.6 ± 0.9	3.8 ± 0.3	3.6 ± 0.3
	M	22	15	610 ± 118	406 ± 97	25.3 ± 6.1	23.7 ± 2.9	-18.8 ± 0.7	-18.4 ± 0.9	12.0 ± 0.9	12.0 ± 0.9	3.7 ± 0.3	3.7 ± 0.3
	Im.	24	33	542 ± 68	411 ± 88	22.3 ± 6.6	24.2 ± 2.4	-18.9 ± 0.6	-18.6 ± 0.8	11.9 ± 0.9	11.8 ± 0.9	3.7 ± 0.3	3.7 ± 0.3
	Mt.	25	1	675 ± 116	742 ± 0	31.5 ± 2.7	32.5 ± 0.0	-18.5 ± 0.7	-19.3 ± 0.0	12.1 ± 0.9	11.1 ± 0.0	3.8 ± 0.3	3.5 ± 0.0
Scymnodon ringens	**All**	**19**	**33**	**796 ± 6**	**1076 ± 231**	**35.5 ± 5.5**	**56.2 ± 18.3**	**-17.4 ± 0.4**	**-19.0 ± 0.6**	**13.9 ± 0.3**	**12.0 ± 0.5**	**4.5 ± 0.1**	**3.7 ± 0.2**
	F	13	18	794 ± 5	1020 ± 250	34.9 ± 5.1	57.6 ± 22.2	-17.6 ± 0.3	-19.2 ± 0.6	13.9 ± 0.2	11.9 ± 0.4	4.6 ± 0.1	3.7 ± 0.1
	M	6	15	801 ± 0	1144 ± 194	36.8 ± 6.6	54.6 ± 12.8	-17.1 ± 0.5	-18.8 ± 0.6	13.7 ± 0.3	12.1 ± 0.5	4.5 ± 0.1	3.8 ± 0.2
	Im.	19	30	796 ± 6	1060 ± 237	35.5 ± 5.5	54.8 ± 18.5	-17.4 ± 0.4	-19.0 ± 0.6	13.9 ± 0.3	12.0 ± 0.5	4.5 ± 0.1	3.7 ± 0.1
	Mt.		3		1237 ± 11		71.0 ± 3.6		-19.0 ± 0.5		12.4 ± 0.4		3.9 ± 0.1
Rajiformes													
Dipturus nidarosiensis	**All**	**2**	**12**	**661 ± 183**	**1034 ±248**	**140.0 ± 5.7**	**135.9 ± 27.8**	**-18.2 ± 1.4**	**-18.4 ± 1.2**	**13.1 ± 1.7**	**12.1 ± 0.6**	**4.2 ± 0.7**	**3.8 ± 0.2**
	F	1	9	790 ± 0	1080 ± 241	144.0 ± 0.0	136.7 ± 30.6	-17.2 ± 0.0	-18.7 ± 0.7	14.3 ± 0.0	12.2 ± 0.7	4.7 ± 0.0	3.8 ± 0.2
	M	1	3	531 ± 0	910 ± 272	136.0 ± 0.0	133.3 ± 22.3	-19.2 ± 0.0	-17.7 ± 2.2	11.8 ± 0.0	11.8 ± 0.5	3.7 ± 0.0	3.7 ± 0.2
	Im.		5		1232 ± 10		107.3 ± 7.6		-17.5 ± 1.4		12.7 ± 0.5		4.0 ± 0.2
	Mt.	2	7	661 ± 183	894 ± 238	140.0 ± 5.7	156.3 ± 14.5	-18.2 ± 1.4	-19.1 ± 0.3	13.1 ± 1.7	11.7 ± 0.3	4.2 ± 0.7	3.6 ± 0.1
Dipturus oxyrinchus	**All**	**15**	**2**	**544 ± 125**	**1242 ± 0**	**48.7 ± 33.8**	**52.0 ± 2.8**	**-18.6 ± 0.9**	**-19.1 ± 0.3**	**11.9 ± 1.6**	**12.1 ± 0.2**	**3.7 ± 0.5**	**3.8 ± 0.0**
	F	8		525 ± 77		58.4 ± 43.5		-19.0 ± 0.7		11.6 ± 1.1		3.6 ± 0.3	
	M	7	2	565 ± 168	1242 ± 0	37.7 ± 13.8	52.0 ± 2.8	-18.2 ± 1.0	-19.1 ± 0.3	12.1 ± 2.0	12.1 ± 0.2	3.9 ± 0.7	3.8 ± 0.0
	Im.	12	2	547 ± 141	1242 ± 0	33.5 ± 13.1	52.0 ± 2.8	-18.5 ± 1.0	-19.1 ± 0.3	11.9 ± 1.8	12.1 ± 0.2	3.8 ± 0.6	3.8 ± 0.0

	Mt.	3	531 ± 0	109.7 ± 9.3	-19.3 ± 0.2	11.8 ± 0.0	3.7 ± 0.0
Neoraja iberica	**All**	**17**	**604 ± 158**	**18.4 ± 7.1**	**-18.8 ± 0.5**	**11.7 ± 0.6**	**3.7 ± 0.2**
	F	10	575 ± 146	19.1 ± 7.9	-18.8 ± 0.4	11.6 ± 0.6	3.6 ± 0.2
	M	7	644 ± 177	17.4 ± 6.4	-18.9 ± 0.7	11.9 ± 0.7	3.7 ± 0.2
	Im.	16	626 ± 132	17.6 ± 6.6	-18.9 ± 0.4	11.6 ± 0.4	3.6 ± 0.1
	Mt.	1	243 ± 0	30.5 ± 0.0	-17.5 ± 0.0	13.5 ± 0.0	4.2 ± 0.0

Appendix 5.2 Taxa (order and species) of deep-sea sharks and skates caught off South (S) and Southwest (SW) coasts of Portugal with their number of specimens (n), mean ± S.D. of the total length (TL in cm), RNA and DNA mg, and standardized nucleic acids ratios (sRD) by sex (Female-F and Male-M) and by maturity stage (Immature-Im. and Mature-Mt.). Values in bold are mean ± S.D. of all specimens from a species.

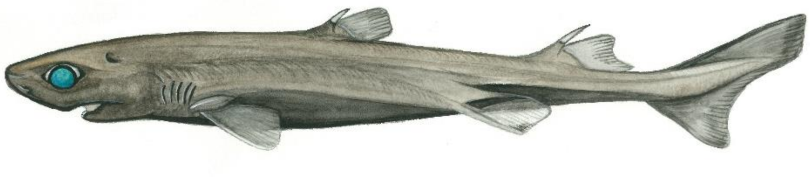
Taxa	**Stage/Sex**	**n**		**TL (cm)**		**RNA mg-1**		**DNA mg-1**		**sRD**	
		S	**SW**	**S**	**SW**	**S**	**SW**	**S**	**SW**	**S**	**SW**
Carcharhiniformes											
Galeus atlanticus[1]	**All**	**44**	**34**	**22.99 ± 7.86**	**37.44 ± 7.62**	**0.24 ± 0.15**	**0.23 ± 0.17**	**1.06 ± 0.61**	**1.71 ± 1.80**	**0.11 ± 0.07**	**0.11 ± 0.07**
	F	23	16	22.04 ± 6.91	37.31 ± 9.97	0.23 ± 0.14	0.23 ± 0.21	0.93 ± 0.37	1.74 ± 2.37	0.11 ± 0.07	0.10 ± 0.09
	M	21	18	24.02 ± 8.85	37.56 ± 4.98	0.25 ± 0.16	0.24 ± 0.14	1.20 ± 0.78	1.68 ± 1.15	0.11 ± 0.07	0.11 ± 0.06
	Im.	39	4	20.94 ± 5.62	19.25 ± 8.26	0.26 ± 0.16	0.57 ± 0.21	1.08 ± 0.62	5.40 ± 3.22	0.12 ± 0.07	0.24 ± 0.08
	Mt.	5	30	39.00 ± 1.87	39.87 ± 2.69	0.15 ± 0.06	0.19 ± 0.10	0.91 ± 0.58	1.22 ± 0.70	0.06 ± 0.02	0.09 ± 0.05
Galeus melastomus	**All**	**94**	**41**	**32.27 ± 14.95**	**35.14 ± 13.36**	**1.86 ± 1.84**	**1.06 ± 1.04**	**1.97 ± 2.52**	**1.17 ± 0.86**	**0.52 ± 0.37**	**0.47 ± 0.45**
	F	55	26	35.14 ± 14.46	33.97 ± 13.16	1.70 ± 1.73	1.11 ± 1.07	1.84 ± 2.65	1.09 ± 0.90	0.54 ± 0.41	0.57 ± 0.53
	M	39	15	28.23 ± 14.87	37.17 ± 13.93	2.08 ± 1.99	0.97 ± 1.03	2.15 ± 2.34	1.30 ± 0.82	0.49 ± 0.31	0.31 ± 0.17
	Im.	76	35	27.09 ± 10.67	31.49 ± 10.74	1.90 ± 1.93	1.09 ± 1.10	2.11 ± 2.72	1.17 ± 0.91	0.48 ± 0.29	0.50 ± 0.48
	Mt.	18	6	54.13 ± 9.73	56.45 ± 2.26	1.67 ± 1.39	0.92 ± 0.69	1.38 ± 1.19	1.20 ± 0.61	0.70 ± 0.58	0.30 ± 0.12
Squaliformes											
Dalatias licha	**All**	**8**		**70.13 ± 45.48**		**1.79 ± 1.26**		**2.39 ± 1.06**		**0.31 ± 0.13**	
	F	4		95.63 ± 55.30		2.11 ± 1.61		2.80 ± 1.28		0.30 ± 0.14	
	M	4		44.63 ± 5.94		1.48 ± 0.93		1.99 ± 0.73		0.31 ± 0.15	
	Im.	6		45.67 ± 4.90		1.26 ± 0.80		2.00 ± 0.85		0.28 ± 0.15	
	Mt.	2		143.50 ± 2.12		3.38 ± 1.11		3.56 ± 0.75		0.37 ± 0.09	
Deania calceus	**All**		**14**		**87.14 ± 6.53**		**2.01 ± 0.60**		**0.77 ± 0.45**		**1.15 ± 0.46**
	F		6		89.17 ± 9.45		1.84 ± 0.32		0.52 ± 0.18		1.47 ± 0.56
	M		8		85.63 ± 3.02		2.14 ± 0.74		0.95 ± 0.51		0.91 ± 0.11

	Im.		4		84.75 ± 8.26		1.90 ± 0.40		0.56 ± 0.18		1.38 ± 0.59
	Mt.		10		88.10 ± 5.93		2.06 ± 0.68		0.85 ± 0.51		1.05 ± 0.39
Deania profundorum	**All**	**27**	**12**	**40.59 ± 12.16**	**54.33 ± 20.61**	**1.35 ± 0.72**	**1.48 ± 1.00**	**1.44 ± 0.94**	**2.18 ± 3.30**	**0.59 ± 0.50**	**0.53 ± 0.33**
	F	22	8	40.08 ± 11.96	58.25 ± 20.08	1.44 ± 0.77	1.66 ± 0.89	1.45 ± 0.98	2.80 ± 3.96	0.64 ± 0.54	0.54 ± 0.34
	M	5	4	42.80 ± 14.22	46.50 ± 22.25	0.97 ± 0.23	1.11 ± 1.24	1.42 ± 0.87	0.95 ± 0.57	0.36 ± 0.15	0.52 ± 0.37
	Im.	25	7	38.17 ± 8.48	39.86 ± 10.93	1.41 ± 0.71	1.67 ± 1.17	1.31 ± 0.85	2.80 ± 4.33	0.63 ± 0.50	0.60 ± 0.36
	Mt.	2	5	70.75 ± 12.37	74.60 ± 10.46	0.60 ± 0.42	1.21 ± 0.73	3.05 ± 0.22	1.31 ± 0.54	0.08 ± 0.07	0.43 ± 0.29
Etmopterus pusillus	**All**	**33**	**16**	**28.09 ± 8.71**	**29.81 ± 9.50**	**1.55 ± 0.72**	**2.20 ± 1.88**	**1.25 ± 0.77**	**3.26 ± 3.85**	**0.60 ± 0.35**	**0.41 ± 0.29**
	F	12	8	23.08 ± 7.33	31.25 ± 11.31	1.69 ± 0.89	2.73 ± 1.87	1.60 ± 1.06	4.11 ± 3.82	0.50 ± 0.23	0.35 ± 0.19
	M	21	8	30.95 ± 8.25	28.38 ± 7.81	1.47 ± 0.61	1.67 ± 1.85	1.05 ± 0.45	2.41 ± 3.93	0.66 ± 0.40	0.48 ± 0.36
	Im.	26	13	24.92 ± 6.40	26.23 ± 6.22	1.61 ± 0.72	2.01 ± 1.86	1.34 ± 0.81	3.57 ± 4.19	0.57 ± 0.32	0.36 ± 0.25
	Mt.	7	3	39.79 ± 5.57	45.33 ± 0.58	1.31 ± 0.73	3.06 ± 2.08	0.92 ± 0.51	1.91 ± 1.46	0.72 ± 0.48	0.66 ± 0.35
Etmopterus spinax	**All**	**59**	**38**	**24.87 ± 7.45**	**24.88 ± 3.52**	**1.77 ± 1.97**	**1.08 ± 0.78**	**2.27 ± 3.00**	**1.29 ± 0.79**	**0.51 ± 0.37**	**0.43 ± 0.30**
	F	33	21	25.52 ± 8.24	25.88 ± 3.52	1.99 ± 2.38	1.06 ± 0.70	2.69 ± 3.52	1.25 ± 0.72	0.54 ± 0.40	0.48 ± 0.37
	M	26	17	24.04 ± 6.37	23.65 ± 3.19	1.50 ± 1.28	1.10 ± 0.90	1.73 ± 2.13	1.35 ± 0.88	0.48 ± 0.33	0.36 ± 0.18
	Im.	37	34	20.92 ± 6.50	24.06 ± 2.57	1.91 ± 2.37	1.07 ± 0.81	2.82 ± 3.59	1.24 ± 0.79	0.44 ± 0.29	0.44 ± 0.31
	Mt.	22	4	31.50 ± 2.71	31.88 ± 2.66	1.55 ± 1.01	1.19 ± 0.61	1.35 ± 1.18	1.76 ± 0.63	0.65 ± 0.46	0.31 ± 0.12
Scymnodon ringens	**All**	**21**	**28**	**35.90 ± 6.79**	**57.36 ± 19.97**	**1.90 ± 0.96**	**1.43 ± 0.72**	**1.73 ± 1.70**	**0.91 ± 0.53**	**0.69 ± 0.52**	**0.74 ± 0.49**
	F	16	17	36.37 ± 7.60	58.38 ± 22.77	1.96 ± 1.05	1.32 ± 0.79	1.96 ± 1.90	0.93 ± 0.58	0.69 ± 0.59	0.72 ± 0.58
	M	5	11	34.40 ± 3.21	55.77 ± 15.58	1.71 ± 0.67	1.60 ± 0.61	1.01 ± 0.24	0.89 ± 0.47	0.71 ± 0.23	0.75 ± 0.33
	Im.	21	24	35.90 ± 6.79	55.08 ± 20.72	1.90 ± 0.96	1.49 ± 0.75	1.73 ± 1.70	0.96 ± 0.56	0.69 ± 0.52	0.76 ± 0.52
	Mt.		4		71.00 ± 2.94		1.11 ± 0.55		0.65 ± 0.24		0.57 ± 0.19
Rajiformes											
Dipturus nidarosiensis	**All**	**3**	**11**	**147.67 ± 13.87**	**133.50 ± 27.85**	**2.38 ± 2.04**	**1.73 ± 0.97**	**2.78 ± 2.14**	**1.59 ± 0.83**	**0.34 ± 0.05**	**0.60 ± 0.43**
	F	2	8	153.50 ± 13.44	133.56 ± 31.07	3.44 ± 1.28	1.89 ± 0.97	4.00 ± 0.47	1.43 ± 0.81	0.33 ± 0.06	0.70 ± 0.44
	M	1	3	136.00 ± 0.00	133.33 ± 22.30	0.27 ± 0.00	1.32 ± 1.03	0.34 ± 0.00	2.03 ± 0.84	0.36 ± 0.00	0.32 ± 0.27
	Im.		5		107.30 ± 7.61		1.78 ± 0.36		0.87 ± 0.35		0.90 ± 0.35
	Mt.	3	6	147.67 ± 13.87	155.33 ± 15.68	2.38 ± 2.04	1.70 ± 1.34	2.78 ± 2.14	2.19 ± 0.56	0.34 ± 0.05	0.34 ± 0.31
Dipturus oxyrinchus	**All**	**18**	**4**	**46.85 ± 30.96**	**67.75 ± 31.69**	**2.37 ± 1.88**	**1.31 ± 0.91**	**2.36 ± 1.45**	**1.37 ± 0.76**	**0.41 ± 0.15**	**0.43 ± 0.26**
	F	9		57.24 ± 40.55		2.46 ± 2.59		2.39 ± 1.85		0.42 ± 0.18	
	M	9	4	36.46 ± 12.23	67.75 ± 31.69	2.28 ± 0.87	1.31 ± 0.91	2.33 ± 1.02	1.37 ± 0.76	0.41 ± 0.14	0.43 ± 0.26
	Im.	15	3	34.39 ± 11.70	53.67 ± 17.79	2.73 ± 1.84	1.38 ± 1.10	2.69 ± 1.34	1.00 ± 0.20	0.42 ± 0.16	0.50 ± 0.27
	Mt.	3	1	109.67 ± 9.29	110.00 ± 00	0.58 ± 0.57	1.10 ± 0.00	0.72 ± 0.61	2.48 ± 0.00	0.38 ± 0.18	0.21 ± 0.00

Neoraja iberica	**All**	**19**	**18.35 ± 6.72**	**1.52 ± 0.89**	**1.37 ± 0.85**	**0.58 ± 0.28**
	F	11	19.01 ± 7.46	1.57 ± 0.99	1.41 ± 1.03	0.64 ± 0.34
	M	8	17.44 ± 5.92	1.44 ± 0.79	1.32 ± 0.57	0.49 ± 0.12
	Im.	18	17.67 ± 6.21	1.55 ± 0.90	1.37 ± 0.87	0.60 ± 0.28
	Mt.	1	30.50 ± 0.00	0.91 ± 0.00	1.45 ± 0.00	0.30 ± 0.00

[1] residual RNA values were used to account for the allometric effect of size.

PART II - MONITORING AND MITIGATION OF IMPACTS IN DEEP-SEA ELASMOBRANCHS



Chapter 6: REMOTE MONITORING OF THE BYCATCH OF DEMERSAL ELASMOBRANCHS USING VIDEO IMAGERY: A CASE STUDY FROM A DEEP-WATER CRUSTACEAN TRAWLER

da Rocha, Pedro; Marsili, Tiago; Barkai, Amos; Figueiredo, Ivone; Dias, Ester; Modesto, Teresa; Relvas, Paulo; Teodósio, Alexandra & **Graça Aranha, Sofia**. Remote monitoring the bycatch of demersal elasmobranchs using video imagery: a case study from a deep-water crustacean trawler. ***Under revision at Marine Ecology Progress Series***

Abstract

Effective monitoring and reporting of fisheries are crucial for successful management and are typically done by at-sea observers and fishers, respectively. However, this system can produce biased information due to economic and social limitations. Electronic monitoring and reporting systems (EMR) are becoming more prevalent and seen as a solution to combat illegal, unreported, and unregulated fishing. The present study aimed to test the effectiveness of an integrated EMR in identifying demersal and deep-sea sharks and skates (hereafter chondrichthyans) bycatch in the Portuguese crustacean bottom trawl fishery. 42 h of footage were thoroughly examined and provided identification of 2182 specimens representing 11 taxa, the majority up to genus level and some, even at species level. Only 0.9% of the chondrichthyans were not able to be identified. Furthermore, the highest bycatch rates of chondrichthyans were the from the gender *Etmopterus* spp. and *Galeus* spp. The technology limitations are discussed and suggestions for improvement are made, to enhance future research proposals and improve the system's overall design. However, the successful implementation of the EMR in this study and other case studies worldwide, demonstrates its upscaling potential to the entire Portuguese fleet and contributes significantly to more sustainable fishing practices and better management of marine resources.

Keywords: Electronic monitoring, electronic reporting, fisheries logbook, fisheries management, sharks, skates.

6.1 Introduction

Ensuring the sustainable future of fisheries relies on the successful implementation of effective monitoring and reporting systems, an ongoing challenge faced globally (Boenish et al., 2020). Diverse monitoring schemes are employed worldwide, such as dockside and at-sea observation programs, fisheries surveys, interviews, collaborative sampling initiatives, smartphone reporting, and electronic monitoring and reporting (EMR) (Gilman et al., 2012; Mangi et al., 2015). In the European Union, how fisheries are reported depends on the size of the vessel: paper forms are used for vessels from 10 to 12 m in length and electronic reporting is used for vessels ≥ 12 m in length (Regulation 404/2011). However, lately legislative proposals emphasized compulsory camera use for boats exceeding 18 m in length (Press Releases PECH 31-05-2023; Regulation 1224/2009). Following the EU, recently in Portugal, the Directorate-General for Natural Resources, Safety and Maritime Services (DGRM - Portuguese acronym) developed an electronic reporting (ER) system (*Diário de Pesca Electrónico-DPE*) which is focused on the landed catch. However, this type of data absence reliability because is reported by fishers usually with no formal training to record data according to the prescribed data collection methods (Stobberup et al., 2021). Moreover, fishers can generally underestimate, or simply not record, the number or volume of captured and discarded organisms because of economic and regulatory concerns (Brown, 2001; Walsh et al., 2002, 2005; Gilman et al., 2020).

Human at-sea observers are so far the most widespread, accurate, and reliable source of fisheries monitoring (Mangi et al., 2015) being this method recommended by the Common Fisheries Policy (Regulation 1380/2013) and adopted by countries in the European Union (Portugal included). Nevertheless, this type of monitoring program presents its own set of challenges. Observers are not always available to record data because of basic physiological needs (sleep, nutrition) or may be coerced by fishers to not report certain situations. Also, the manual transfer of information from data collected onboard to digitalized paper sheets may result in long delays in the analytical process and can introduce room for error and inaccuracy of results (Saila, 1983; Alverson et al., 1994; Kennelly, 1995; Liggins et al., 1996). Hence, this system has been changing to electronic monitoring systems (EM), which are proving to be more reliable and efficient, providing means for speeding up the analysis (Lee Son et al., 2023). Further, those systems are already implemented in countries such as Australia, New Zealand, the Netherlands, and the United States (Borges, 2015; van Helmond et al., 2020).

The EM has been suggested as a solution to combat illegal, unreported, and unregulated fishing (IUU), as well as biased information (Barkai et al., 2010; van Helmond et al., 2020; Stobberup et al., 2021). It is also considered an important solution to meet authorities' requirements regarding the catching of endangered and protected species since these discards are not normally reported and information on these animals is scarce (Suuronen and Gilman, 2020; Pierre et al., 2022). Likewise, it is helpful for monitoring species with total allowable catch (TAC) subjected to landing obligations (Catchpole et al., 2017). Hence, EM could and should be used to monitor bycatch and discard rates of protected species as deep-sea sharks (Regulation 2021/91), or that have a TAC, such as the skates of the order Rajiformes (Regulation 2024/1015). This is particularly relevant in fisheries with high bycatch of sharks and skates such as the crustacean bottom trawling in the southern coast of Portugal [Borges et al., 2001; Monteiro et al., 2001; Coelho and Erzini, 2008; Graça Aranha et al., (unpublished results)]. The general lack of (or biased) data about the sharks and skates discarded by bottom trawling fisheries in Portugal emphasize the need for combining electronic monitoring systems with electronic reporting for data verification (Barkai and Meredith, 2007). This was the premise for the implementation of the project Electronic Monitoring and Reporting Technology for Fisheries in Portugal (EMREP). The goal of the EMREP was to integrate an existing and commonly used commercial fishing logbook technology (eLog) with footage from onboard cameras, creating an integrated electronic monitoring and reporting solution (iEMR) for the Portuguese crustacean bottom trawling fleet. This solution aimed to monitor and report bycatch and discards of chondrichthyans (i.e., sharks, skates and chimaeras). The Olrac® eLog technology developed by OLSPS® was selected for this purpose and it comprises a vessel-based eLog software application named Olrac Dynamic Data Logger® (Olrac® DDL) and a web-based fleet management application named Olrac Dynamic Data Manager® (Olrac® DDM). The Olrac® DDL is certified and used by commercial fisheries and government agencies in several countries (e.g., Australia, New Zealand, United Kingdom); however, in Portugal, this was the first trial of the software. For the present study, the Olrac® iEMR was used to remotely count and identify demersal and deep-sea chondrichthyans to the lowest taxonomic level possible.

6.2 Materials and methods

The EMR trials were conducted in a volunteer commercial crustacean bottom trawler (23 m total length, eight meters width). The vessel operates off the South and Southwest coasts of Portugal (7.7° - 9.6° W; 36.7° - 37.8° N), the most important crustacean fishing ground for Portuguese bottom trawlers (Borges et al., 2001).

6.2.1 Operational system

The operational system consisted of two cameras (Marine HD PoE®, model 0482-6030), with a frame rate of 25/30 fps, a pixel count of 1920x1080, and a network switch with 16-way power over Ethernet. The cameras were installed in the participating fishing trawler in areas allowing the best view of discards and fishing activities (e.g. net arrival, catch sorting) while guaranteeing the crew members' privacy. The cameras were positioned above the sorting table (Figure 6.1) and on the main deck, aiming to cover the net arrival and deployment area (Figure 6.2).

Figure 6.1: Camera installed at the sorting table (top) and images with zoom, from the integrated Electronic Monitoring and Reporting solution, the Olrac® iEMR (bottom).

Figure 6.2: Camera aiming at the main deck (top), with its daylight (bottom left), and nightlight (bottom right) view accessed from the images of the integrated Electronic Monitoring and Reporting solution, the Olrac® iEMR.

The cameras recorded the fishing activity in an external hard drive with 2 or 4 TB of space, which was manually replaced every fifteen days for future analysis. The recording system activates with motion detection in a designated frame area previously selected by the user. Records started every time a movement was detected in the main deck and/or sorting table, and the footage record would stop when no activity was detected. The version of the Electronic Reporting system, the Olrac Dynamic Data Logger (Olrac® DDL), was then integrated with an electronic monitoring system using footage from the at-sea cameras, creating the integrated electronic monitoring and reporting solution (Olrac® iEMR) (Figure 6.3).

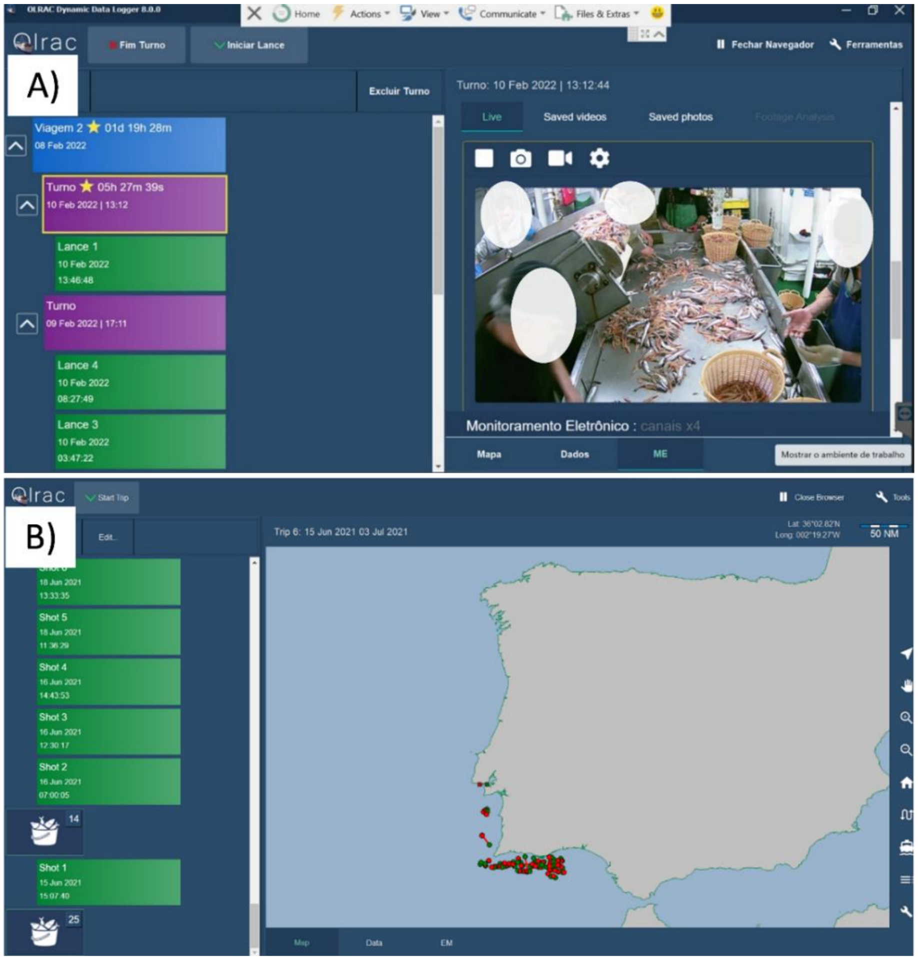


Figure 6.3: **A)** General view of the Portuguese version of the integrated Electronic Monitoring and Reporting solution, the Olrac® iEMR, showing the data input during operation for each haul (left side) and the general view from the at-sea cameras (right side), and **B)** The English version of the integrated Electronic Monitoring and Reporting solution the Olrac® iEMR showing the map area and the points of start (green dots) and end (red dots) of each haul conducted by a crustacean bottom trawler (dummy data).

The Olrac® iEMR holds essential information related to the target species and discards for compliance with the authorities, and holds an option to add information about each specimen studied (e.g. size), Vessel coordinates (automatically registered using a GPS), fishing depth (m), codend mesh size (mm), along with catch and bycatch information, were inserted into the Olrac® iEMR either by the skipper or by researchers when on board. Whenever researchers were not onboard, data such as target type, fishing depth, season, vessel coordinates, and start and end of each haul were collected using the information inputted by the skipper in the Olrac® iEMR. Following each haul, sorting took place until there was no more activity on the sorting table, hence, one haul is related to one sorting event. Sorting time was measured from the moment the ship's crew began separating the catch until the end of the sorting procedures.

6.2.2 Footage analysis

A total of 210 hours of footage from sorting events were analysed from the data collected in September 2021, and February, March, and April 2022. Approximately 20% of this footage, totaling 42 hours and 20 minutes and representing 27 sorting events, was randomly analysed. This approach follows recommendations from the European Fisheries Control Agency (EFCA, 2019) and common practices in Europe for demersal trawlers (van Helmond et al., 2020).

The sorting events conducted in September 2021 and the first week of February and March 2022, researchers were onboard the vessel monitoring the functioning of the system. The remaining footage, the last three weeks of February and in April 2022, where there were no researchers onboard.

The selected footage was thoroughly examined by a human video analyst, with expertise in the identification of chondrichthyans, using the open-source software Milestone Xprotect® Smart Client 2020 R3. Additionally, because all skates are subjected to TAC in European waters (except for *Raja undulata* in the subarea IX) they were identified up to order, i.e., Rajiformes. The images were reviewed at normal speed, with varying zooms and focus adjustments were made as necessary for the precise identification of each specimen. Key events, including the time when a chondrichthyan was spotted, the initiation and conclusion of catch sorting events, periods of camera signal loss, and chondrichthyan count by species and/or genus, were systematically recorded in an Excel spreadsheet. The dynamic nature of the analysis allowed for pauses, zooming in, or playing backward whenever specific details required closer scrutiny. After the footage analysis, data for each examined haul – such as fishing depth, season, and vessel coordinates – were verified using the Olrac® iEMR as previously mentioned.

6.2.3 Data analysis

To examine the effectiveness of the taxonomic identification the EM method, screenshots of chondrichthyans are provided (Appendix 6.1) in which is possible to identify sharks up to genus level and skates up to order, or up to genus, There was an attempt to conduct *in situ* observations from the same sorting procedures as the ones evaluated through the Olrac ® iEMR, where at-sea researchers passively evaluated the catch identifying the chondrichthyans from the same distance as the cameras. Unfortunately, the sorting events in which those procedures were conducted were the same in which there was signal loss from the cameras, hence the cross validation with *in situ* data was not conducted.

The number of chondrichthyans per minute of footage was calculated considering the number of chondrichthyans observed during the sorting of the catch. The calculation was done by dividing the total number of chondrichthyans observed by the minutes that each sorting event lasted for each month. The chondrichthyans per minute of footage calculation did not account for footage signal loss (where applicable) or the total footage time.

6.3 Results

A total of 42:20:46 hours of footage were analysed, of which 21:04:43 hours were from the catch sorting procedure. These corresponded to 27 different hauls (sorting events) conducted across four months, with an average fishing effort of 5.17 hours per haul. The average duration of each sorting procedure was 47 minutes.

Sorting events that took place in September 2021 and at the first week of February 2022 presented connection problems, representing a footage loss of 14% and 36%, respectively (Table 6.1). Results show the improvement of the EM system, resulting in no signal loss in the months following the adjustments. This improvement was achieved after changing some of the camera settings and fixing connection cables (Table 6.1).

Each minute of footage analysed from the sorting procedure presented at least one elasmobranch specimen for the months September, February and March and more than two for April 2022 (Table 6.1).

Table 6.1 The evaluated months with the amount of sorting events, total amount of footage analysed, duration of each sorting event, camera signal loss, total number of chondrichthyans specimens and number of chondrichthyans per minute of footage from an electronic monitoring trial in the S and SW coast of Portugal.

Months	**Sorting events**	**Average effort (h ± SE)**	**Footage Time analyzed (h:mm:ss)**	**Sortin g time (min)**	**Signal loss (h:mm:ss)**	**Chond. specimens (n)**	**Chond./ minute**
Sep. 2021	5	4.45 ± 0.31	05:53:47	94	00:48:00	141	1.5
Feb. 2022 (1st week)	2	4.03 ± 0.09	02:53:57	82	01:03:00	99	1.2
Feb. 2022 (2-4 weeks)	16	5.82 ± 0.51	26:51:16	853	0	1422	1.7
Mar. 2022	3	4.07 ± 0.22	05:08:50	192	0	482	2.5
Apr. 2022	1	5.93 ± 0	01:32:56	41	0	51	1.2

A total of 11 taxa representing 2,195 specimens were identified to the order (1), genus (4), or species (6) level by analysing the video footage. Notably, only 13 sharks were not able to be identified in the footage analysis, constituting a small percentage (0.9%) of the overall specimens observed (Table 6.2). Regarding the most captured taxa the genus *Galeus spp.* was the most representative, followed by *Etmopterus* spp. and *Deania* spp.

Table 6.2 Taxa of demersal and deep-sea chondrichthyans identified using the integrated electronic monitoring and reporting solution (iEMR) and the total number of specimens. The taxon skates refer to demersal species of Rajiformes that are not deep-sea.

Taxa	**Number of specimens**
Galeus melastomus/atlanticus	922
Etmopterus spinax/pusillus	870
Scymnodon ringens	97
Deania calceus/profundorum	199
Scyliorhinus canicula	72
Dalatias licha	4
Galeorhinus galeus	2
Chlamydoselachus anguineus	1
Dipturus spp.	6
Skates	6
Chimaera monstrosa	3
Unidentified deep-water shark	13
Total	2195

Due to taxonomic concerns, specimens that require manipulation for correct identification, or that did not have satisfactory details in the footage to allow identification to species-level, were grouped at the genus level. This was the case for species from the genus *Galeus*, *Etmopterus*, *Deania,* and *Dipturus*. These species differ by details that could not be identified in the footage (Table 6.3). For instance, *G. melastomus* and *G. atlanticus* are distinguished by the colour in the labial furrows. *D. calceus* and *D. profundorum* are differentiated by the subcaudal keel on the underside of the caudal peduncle. *E. spinax* and *E. pusillus* are identified by their slight differences in colour and denticles (Compagno, 1984). Additionally, skates comprise non-deep sea Rajiformes that were not identified to the species level using the footage observations.

The key taxonomic characteristics used to identify the species/genus in this study and outlines the identification limitations using the footage are exemplified in Table 6.3. Additionally, Appendix 6.1 shows footage examples from some of the chondrichthyans identified in the present study.

Table 6.3 Taxonomic key characteristics (Compagno, 1984) used to identify the chondrichthyans specimens using the footage in the present study and identification limitations.

Genus	Characteristics	Species	Characteristics	Footage species identification limitations
Galeus	Colour light grey or brown with dark barred, blotches and spots pattern; long and wedge-shaped snouts.	*Galeus melastomus*	White colour of the groove formed by the labial furrows; caudal upper edge with small denticles	Species identification requires manipulation
		Galeus atlanticus	Blackish colour of the groove formed by the labial furrows; caudal upper edge with greater denticles	Species identification requires manipulation
Deania	Colour dark grey or brown; Extremely long snout, large, grooved spines on dorsal, no anal fin.	*Deania calceus*	No subcaudal keel on caudal peduncle, extremely long, low, first dorsal fin.	Species identification requires manipulation
		Deania profundorum	Subcaudal keel on underside of caudal peduncle, first dorsal fin short and high.	Species identification requires manipulation
Etmopterus	Short to moderate snout; small sized body; Colour variable, from blackish to tan, often with prominent dark markings on underside of head and caudal peduncle.	*Etmopterus pusillus*	Truncate denticles	Species identification requires manipulation
		Etmopterus spinax	Denticles not in lines and with long slender cusps	Species identification requires manipulation
Dalatias	Snout broadly conical, rounded, and short; colour greyish to black or blackish brown, sometimes violet with black spots.	*Dalatias licha*	A moderate-sized, short and blunt snouted shark with two almost equal-sized spineless dorsal fins; only species that occurs in Portugal.	None
Scymnodon	Moderate long to short snout; dark colour; no anal fin; pectoral fins with broadly rounded free rear tips	*Scymnodon ringens*	Black coloration; small dorsal fin spines; no anal fin; short snout; geographical distribution (Portugal)	None
Galeorhinus	Snout moderately long and parabolic in dorso-ventral view; eyes horizontally oval and lateral; mouth broadly arched and long; second dorsal fin much smaller than first; an extremely	*Galeorhinus galeus*	The only member of the genus.	None

	long terminal caudal lobe about half the dorsal caudal margin.			
Chlamydoselachus	Eel-like shark with 6 gill slits; dark brown or grey in colour, sometimes paler below	*Chlamydoselachus anguineus*	Dorsal fin small and lobe-like originating over pelvic fin bases to behind anal fin origin; geographical distribution	None
Scyliorhinus	Colour pattern extremely variable, ranging from simple dark saddles, reticulating dark bars, or large dark spots on a light background to combinations of light and dark spots and saddles; second dorsal fin much smaller than first.	*Scyliorhinus canicula*	A slender, dark-spotted catshark; second dorsal fin much smaller than the first.	Can be mistaken with Scyliorhinus stellaris
Dipturus	Snout long and pointed; anterior disc margin concave	*Dipturus batis*	Black colour; belly with dark and white spots; absence of black mucus in the abdomen; distance between	Species identification requires manipulation
		Dipturus oxyrinchus	Black colour; belly with dark and white spots; absence of black mucus in the abdomen; rostrum 60% longer than head	Species identification requires manipulation
		Dipturus nidarosiensis	Uniformly dark colour in the ventral and dorsal sides; dark mucus in the abdomen.	Species identification requires manipulation
Chimaera	Long tapering bodies and large heads; elongate body; colour vary from black to pale blue to brownish grey; smooth skin; large eyes, rabbit appearance	*Chimaera monstrosa*	A reddish-brown, silver-grey body coloration; longitudinal stripes on dorsal side; large, conical snout; elongate body.	None

6.4 Discussion

Electronic monitoring and reporting technology for fisheries was tested for the first time in Portugal by developing an integrative electronic monitoring system (video cameras) with an electronic reporting system (eLog Olrac® DDL) resulting in the Olrac® iEMR. This solution was tested by identifying demersal and deep-sea chondrichthyans through video analysis.

The quality of the footage and camera settings of the Olrac® iEMR was satisfactory to identify and count chondrichthyans' specimens of the species *S. ringens*, *C. anguineus*, *D. licha*, and *G. galeus* and from the order Rajiformes. This could help decrease IUU fishing and support and enhance fisheries monitoring and management, by providing information on their abundance and distributions which is essential for effective conservation efforts (Ruiz-García et al., 2023). Understanding their abundance and distribution will contribute to increasing the scientific knowledge and help to map high-density bycatch areas. This is crucial for mitigating impacts on endangered species and species of conservation concern such as *C. anguineus, D. licha and G. galeus*. Thus, the use of EM for deep-sea chondrichthyans can support information for the implementation of measures such as fishing closures or serving as a bycatch avoidance tool by authorities and skippers.

In the case of specimens from the order Rajiformes, which have biannual quotas and are subject to landing obligations, EM could provide the necessary information on their discards. This would eliminate the need for fishermen to land them, as this group of species has small commercial value. Landing obligations were initiated in 2015 through the Common Fisheries Policy (Article 15 of Regulation 1380/2013) to reduce unwanted catches by European Union fishing vessels. This was achieved in the north Atlantic, by asking fishers to land all species subjected to TAC, with some exemptions. To facilitate the implementation of the landing obligations and avoid the risk for early closures of fisheries, TAC was increased above the scientific advised, in general by 36% (up to 60% for demersal species; Borges, 2020). However, fishers are not complying with the landing obligations (Savina, 2019; Borges, 2020). Hence, the discard issue in EU fisheries remains and has worsened due to a combination of TAC top-ups and the non-compliance of the landing obligations. This issue could be overcome with the Olrac ® iEMR which was effective in counting and identifying Rajiformes specimens. This system could equally help in identifying other TAC species. Thus, further trials should be conducted to test the effectiveness of this system in counting and identifying other species of interest.

The largest limitation of the EM in the present study was the impossibility to identify most specimens up to the species level, because of the remarkable morphological similarity between some congener species. The species of the genus *Galeus*, *Etmopterus*, *Deania* and *Dipturus* are difficult to identify without careful manipulation due to intricate details that separate the species. Even so, are still difficult to tell apart by less experienced at-sea observers. For instance, is reported that the identification of *G. melastomus* and *G. atlanticus* are commonly confused (Rey et al., 2006). For example, *Deania* spp. are mainly distinguished by the presence of a sub-caudal keel in the ventral side of the caudal peduncle of *D. profundorum* (Compagno,1984). This is concerning since those similar congener species are frequent in the bycatch of crustacean bottom trawlers [Oliver et al., 2015; Graça Aranha et al., (unpublished results)]. Some of these species are not protected, such as *Galeus spp., E. pusillus,* and *D. profundorum.* Others, like *E. spinax, E. princeps,* and *D. calceus*, are included in the EU list of deep-sea sharks, which means they have a zero Total Allowable Catch (TAC) (Regulation 2021/91). Additionally, other species of chondrichthyans that were not captured in this study can occur on the Portuguese coast and may present the same challenges. For instance, *Chimaera notafricana* and *Hydrolagus lusitanicus*, *Scyliorhinus canicula* and *Scyliorhinus stellaris*, *Centrophorus squamosus* and *Centrophorus granulosus*, *Etmopterus princeps* (Almeida and Biscoito, 2019).

Other concern relates with the adoption of EM tools by fishermen and agencies, and the time-consuming review of footages (van Helmond et al., 2020). The latter issue is being addressed in the longline fishery through the implementation of machine learning algorithms using artificial intelligence (AI) which automates and speed up monitoring procedures (Oliver et al., 2015; Awalludin et al., 2020; Kay and Merrifield, 2021; Mei et al., 2021). In bottom trawlers, the EMREP project (unpublished data) is testing the usefulness of AI in automatically identifying deep-sea elasmobranchs among the catch from onboard footage. However, the similarity between congener species, combined with the procedures conducted onboard the study vessel, poses a significant challenge. These procedures include fast bycatch discarding, crew overlapping or shading the images, and animals overlapping with each other. This challenge is particularly pronounced in muddy bottoms, where the catch is usually covered in mud.

Nevertheless, AI can be beneficial for some trawlers, due that each vessel operating in different settings (e.g. crew number, sorting procedures, different sorting tables layouts). Modifications to sorting procedures in trawl fisheries, such as using a sensor with cameras at the conveyor belt (Vilas et al., 2020) or placing conspecifics inside baskets or separately in the sorting table or discarding belt (van Helmond et al., 2020), could potentially facilitate the use of AI for the bottom trawling sector. However, it is believed that even with the highest footage quality, and the great advancements in the AI, identifying specimens up to species level is an issue that may remain. In this matter, in order to make use of the available EM tools to remotely monitor elasmobranchs bycatch, an effort could be made to set uniform regulatory measures for these congener species, which would require further bio-ecological studies to improve the existing knowledge about their populations and distributions.

Given that EM is still undergoing trials and requires refinement, especially in the context of bottom trawl fisheries, the continued deployment of trained at-sea observers is still necessary when applicable (e.g. deep-sea fisheries) for accurately identifying specimens and ensuring proper handling. Furthermore, at-sea observers have access to critical information on the sexual maturation, age and specimen life status, which still cannot be addressed by EM (Sylvia et al., 2016; Suuronen and Gilman, 2020) and are crucial to understand elasmobranchs species dynamics. On the other hand, the EM's ability to monitor elasmobranchs without an at-sea observer onboard may be beneficial. This can mitigate potential bias introduced by variations in skipper behavior when an observer is present (Borges et al., 2008). Therefore, for the time being, the combined use of EM and at-sea observers to control and monitor IUU catches and enforcement of regulatory measures in bottom trawlers is advised.

Despite these constraints, it is crucial to acknowledge EM as an innovative technology in Portuguese fisheries. The Olrac® iEMR has the potential to streamline the feedback process for fisheries data analysis, aiding fishery managers, improving data treatment for the scientific community, and guiding fishers toward more selective practices. This not only addresses existing knowledge gaps but also holds promise for advancing monitoring and reporting technology, both within Portugal and globally.

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Ethical standards - This study was conducted in accordance with the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of animals and enforced by CCMAR. CCMAR staff are certified to house and conduct experiments with live animals, and their facilities are also certified in accordance with the three “R” policy, national and European legislation, and with guidelines defined by the ethical committee ORBEA CCMAR-CBMR.

Declaration of Competing Interest - The authors declare that they have no known competing commercial interests or personal relationships that could have appeared to influence the work reported in this paper.

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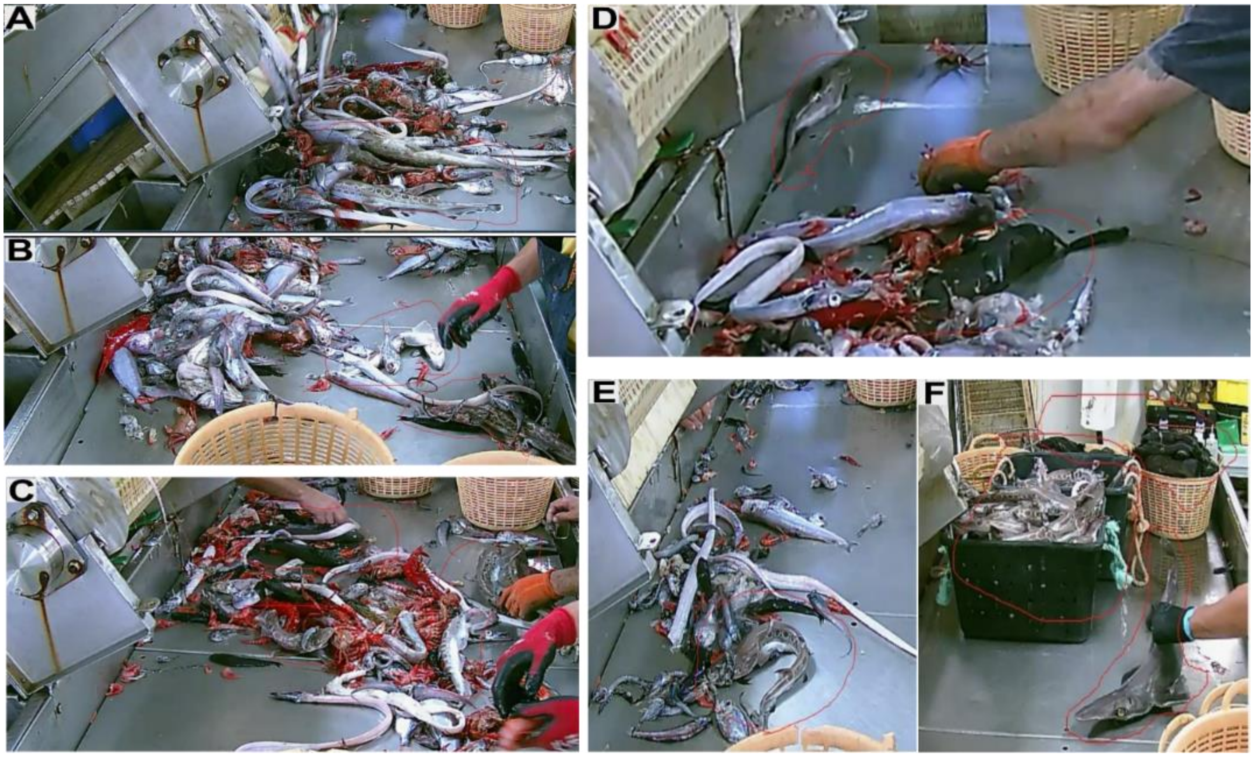
6.6 Appendices

Appendix 6.1 Examples of footage identification of elasmobranchs found in the present study. A) Catch overlapped, presence of *Galeus* spp. *Etmopterus* spp. and possibly *Scymnodon ringens*. B) Catch overlapped, presence of *Galeus* spp. C) Catch less overlapped, presence of *Galeus* spp. *Scymnodon ringens* and possibly *Etmopterus* spp. D) Image zoomed in on the species *Scymnodon ringens*. E) Image zoomed in on the species *Galeus* spp. F) Example of the catch sorted in buckets that could facilitate species identification using AI, one bucket with *Galeus* spp. and another with *Scymnodon ringens* mixed with *Etmopterus* spp. and a single *Deania* spp.



Chapter 7: HANDLING PROTOCOL FOR SHARKS AND SKATES FOR BOTTOM TRAWL FISHING VESSELS

Graça Aranha, Sofia; Teodósio, Alexandra; & Dias, Ester. 2024. Handling protocol for sharks and skates for bottom trawl fishing vessels: case of study on a crustacean trawler in southern Portugal within the scope of the Delasmop project "Deep-sea elasmobranchs of Portugal" [Portuguese online version]

7.1 Summary

This handling guide was developed within the scope of the Delasmop project, an acronym for "Deep-sea elasmobranchs of Portugal," which aims to provide information about the elasmobranchs (sharks and skates) that inhabit the deeper waters off the Portuguese coast. The project was carried out as part of a PhD thesis in Marine, Earth, and Environmental Sciences at the University of Algarve. It benefited from valuable partnerships with the Center for Marine Sciences (CCMAR/CIMAR LA), the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), the company OLSPS International Ltd. and OLSPS Marine, and was funded by the Save Our Seas Foundation (#SOSF501), the EEA Grants (#PT-INNOVATION-007), and the Foundation for Science and Technology (FCT).

Through scientific activities conducted aboard a commercial vessel over two years (2020 to 2022), information was collected on the conservation, ecology, and biology of deep-sea shark and skates species. This information was disseminated at scientific events, participatory meetings, in the media (e.g., the Biosfera TV show from RTP), on social media, in workshops (with children in schools and "Centro de Ciência Viva" from the North to the South of Portugal), and through the production of educational and informational materials (e.g., educational videos, infographics, reproductions of the studied species) aimed at various target audiences such as the scientific community, fisheries managers, associations, fishermen, ship owners, and the general public.

This best practices' guide is intended for crustacean trawling vessels that have significant interactions with these animals. In addition to recommending best practices for handling these animals on board, it addresses the main identification characteristics of deep-sea sharks and skates in Portugal. The drawings of these animals were all hand-drawn by the artist Luis Thiem and kindly

offered to the Delasmop project. They are accurate illustrations; however, they are not scientific illustrations, so there may be some differences between the drawing and the natural specimen.

This guide is structured as follows: an introduction to the theme and the problematic of bycatch of sharks and skates; suggestions for best practices for handling sharks and skates on board trawlers in Portugal (using the available literature and Delasmop project results as a case study); presentation of the species found in Portuguese waters; various appendices containing the main results of the Delasmop project; and a general discussion on strategies to mitigate the impacts of bottom trawling on elasmobranch mortality.

By summarizing the Delasmop project results and incorporating knowledge from specialized literature, the following pages of this protocol offer a comprehensive and practical approach to promote more conscious and effective handling of deep-sea elasmobranchs, aiming to mitigate the harmful impacts of bottom trawling on these vulnerable animals.

A booklet with information on handling sharks and skates on board trawlers was developed and printed for quick reference.

We hope this guide is useful to you. We are available for suggestions and to answer any questions.

Contact us via this Google form.

7.2 Background

Crustacean bottom trawling is an ancient and traditional fishery with significant importance in the Portuguese economy. Although it targets species such as shrimp, prawns, and Norway lobster, the low selectivity of this method results in considerable amounts of bycatch, meaning all other organisms that are not the fishing target and may be retained for sale or personal consumption, or discarded due to low economic interest or regulatory measures. On the Portuguese coast, specifically in the South and Southwest, crustacean trawling has on average, a 70% discard rate (Borges et al., 2001), and a significant proportion (up to 58%) consists of deep-sea elasmobranchs [Borges et al, 2001; Monteiro et al., 2001; Costa et al., 2008; Graça Aranha et al., (unpublished results)] (Figure 7.1).

Figure 7.1 Discards in crustacean trawlers account for about 70% of the total catch in southern Portugal, with deep-sea elasmobranchs comprising a significant portion of these discards (up to 58%).

Deep-sea elasmobranchs are sharks and skates that mostly live beyond 500 m of depth and have low resilience, as this group consists of species with long and slow reproductive cycles, long lifespans, and slow growth rates (Stevens et al., 2000). Moreover, despite being returned to the sea, most specimens are already dead or dying by the time they reach the vessel's deck (Wetherbee and Nichols, 2000; Coelho and Erzini, 2007; Rodríguez-Cabello and Sánchez, 2017; Graça Aranha et al., 2023). This makes them highly susceptible to overexploitation, which can negatively impact their populations and consequently disrupt the fragile deep-sea ecosystem. Because they are often discarded, they remain largely unknown to science and the general public, unlike the more iconic great white shark, whale shark, and manta ray, for example.

To mitigate the impacts of crustacean trawling on deep-sea elasmobranchs, some strategies can be adopted, such as developing guides and protocols for best practices on board (Gilman et al., 2008; Benoît et al, 2010), highlighting the importance and objective of this guide.

7.3 Elasmobranch’s handling[1]

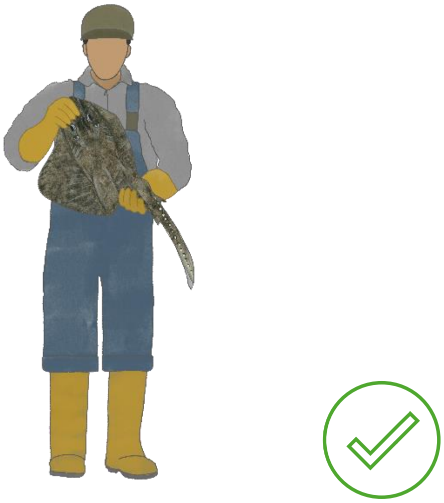
7.3.1 What to do?

- **Small sharks and skates (less than 1 m)**

Hold at the base of the tail and behind the pectoral fins.

Or

Hold by the dorsal fin, supporting the animal's body on your arm.



Hold the animal preferably by the snout and support the base of the tail. For species with a slippery snout, you can opt to hold by the spiracles. Keep the animal as upright as possible to avoid bending the tail and compromising the vertebrae.

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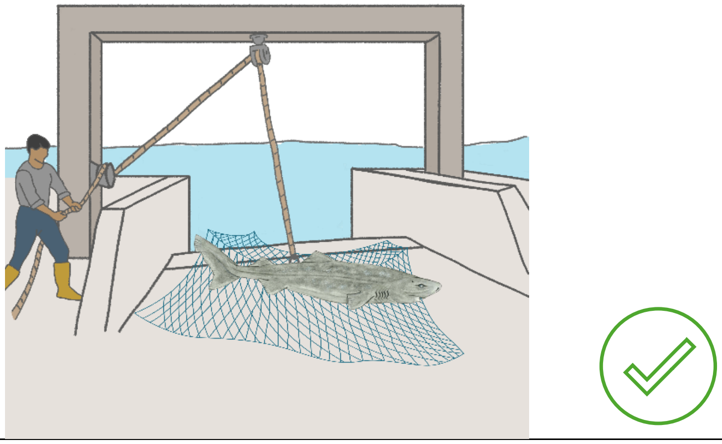
[1] Images inspired by the “Shark’s Trust Best Handling Guides” and Sandra Sharma “Shark and Ray Handling Practices” (AFMA)

- **Medium-sized sharks and skates (1-2 m)**

For these animals, it is ideal for two people to handle them simultaneously: one person should hold the tail, while the other holds the dorsal and pectoral fins (at the level of the gill slits). It may be necessary to place a fish in the mouth to prevent bites.
Alternatively, you can support the body from underneath and hold by the pectoral fin, as shown in the image.

Two people hold the skate by its wings, supporting their hands on the snout or around the wings as shown in the image.

- **Very large sharks (> 2 m)**



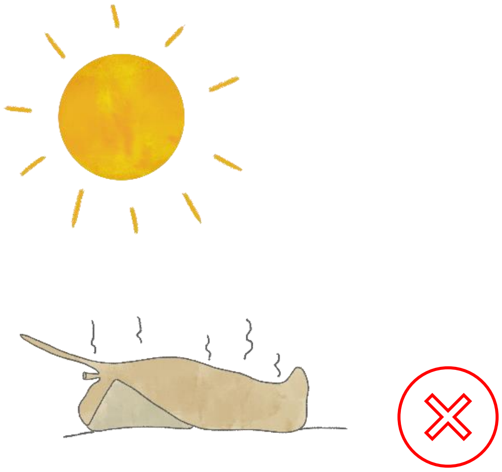
Before removing the animal from the fishing net, place a piece of unused net on the deck and position the animal on top of it. Stop the vessel and gently pull the net back into the water with the help of a crane.

7.3.2 What not to do?

Never hold sharks by the tail alone.

Never hold skates by the tail alone.

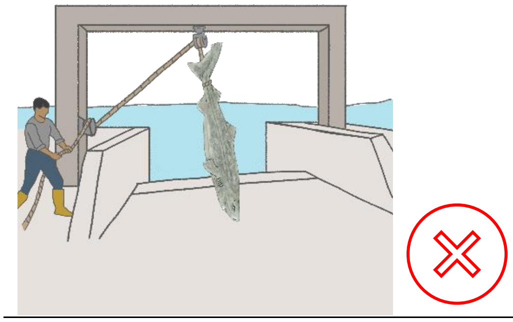
Never hold skates or sharks by the gill slits



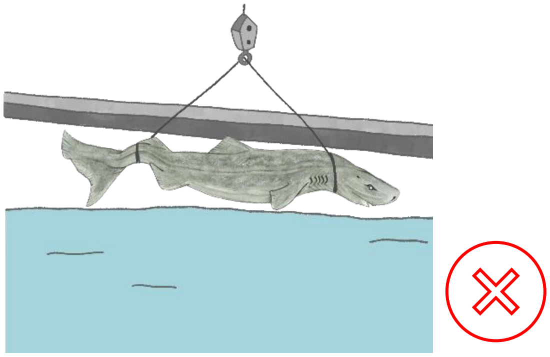
Never leave the animals exposed to the sun.

Do not move the animal using gaffs or other objects.

Do not move the animal using gaffs or other objects.



Do not lift very large sharks by the tail alone (or by tail and head together), as this can cause damage to their vertebrae.



Do not lift sharks with cables tied to their extremities, as this can cause damage to their vertebrae.

7.4 Delasmop Project

7.4.1 Field

Over two years, from June 2020 to May 2022, 10 trips were conducted on a commercial crustacean bottom trawling along the southern (coordinates ~37°-36°N and 9°-7.5°W) and southwestern (coordinates ~39°-37°N and 9°-11°W) coasts of Portugal (Figure 7.2).

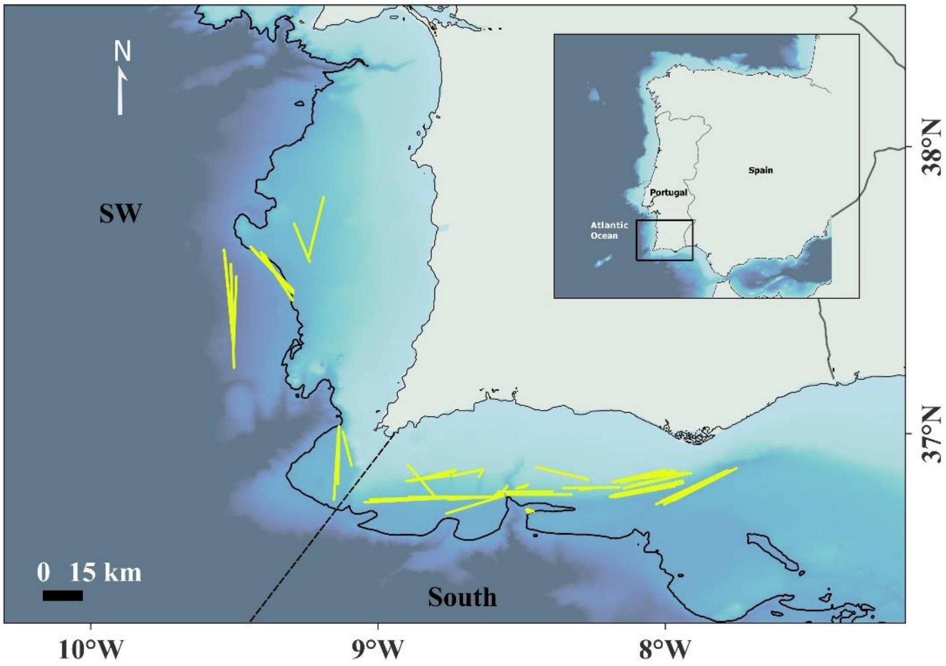


Figure 7.2 Map of the area sampled by a crustacean trawling vessel as part of the Delasmop project. The yellow lines are the tracks from the hauls conducted.

During the trips, researchers from the Delasmop project collected several types of information about the fishing activity and the animals caught, which were recorded in an Olrac DDL® fishing logbook adapted for scientific purposes (Figure 7.3).

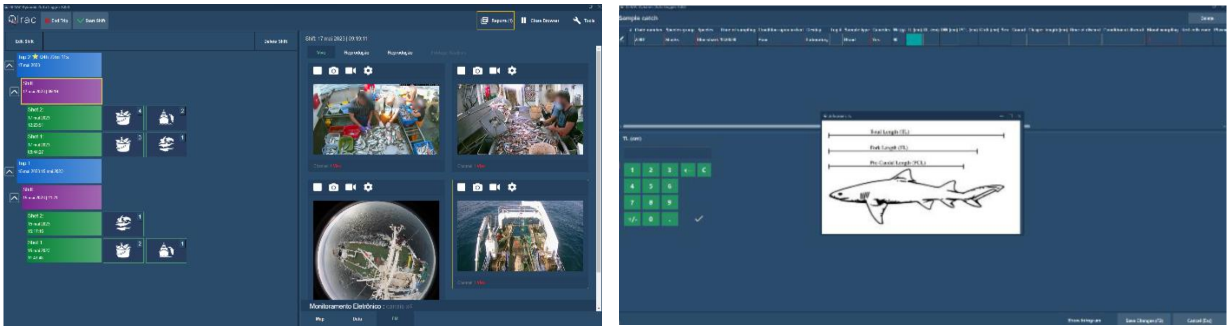


Figure 7.3 Olrac® DDL electronic monitoring software and fishing reports adapted for scientific use within the Delasmop project, for collecting information on sharks and skates in crustacean bottom trawl fishery.

Fishing Information

- Fishing effort (h; calculated from when the net touches the bottom until it starts to be hauled)
- Fishing speed (nm/h; automatically calculated by the Olrac DDL® software based on the start and end distance of the haul and the haul time)
- Fishing haul coordinates (automatically entered into the Olrac DDL® software via GPS)
- Net mesh size (55 or 70 mm)
- Target species (information provided by the skipper at the start of each haul)
- Net weight (Kg; estimate made by the skipper)
- Depth (m; obtained through the vessel's Scanmar® device or the Star Oddi® mini-CTD)
- Bottom and surface temperature (°C; measured using the Star Oddi® mini-CTD)
- Salinity (measured using the Star Oddi® mini-CTD)
- Air exposure (minutes; measured from the moment the net is out of water until the sorting of the catch begins)

Animal Information

- Identification of shark and skate species (Compagno et al., 2005; Ebert et al., 2021)
- Sex (male: presence of claspers on the pelvic fins; female: absence of claspers)
- Weight (Kg; measured with an onboard scale or for animals > 1m, the weight was estimated using available literature)
- Total length (cm; measured from the tip of the snout to the tip of the caudal fin)
- Muscle sample (for isotopic and nucleic acid analyses)
- Blood with plasma separation on board (for stress analyses)

7.4.2 Characterization of fishing and bycatch of elasmobranchs

Where are crustacean trawlers operating in Portugal?

Crustacean trawling activities are concentrated in the South of Portugal due to the extended continental shelf with more uniform depths, which provides access to deeper waters closer to the coast (Figueiredo, 1989). There is a higher fishing effort along the southern coast compared to the southwestern coast (Cascalho et al., 1984; Pestana, 1991; Borges et al., 2001; Pita et al., 2001; Bueno-Pardo et al., 2017). This was also detected during our study, where the fishing effort in the South was three times greater than in the Southwest. A total of 259 h, divided into 61 hauls (2-6 h each), were conducted in the South of Portugal at depths ranging from 96 to 810 m. In the Southwest, 92 h of fishing effort were conducted, divided into 16 hauls (3-9 h each), at depths between 403 and 1244 m.

Due to the different fishing depths in the southern and southwestern regions, the physicochemical characteristics of these regions are not comparable. Therefore, analyses were conducted separately for each coast.

The southern coast is characterized by a typically narrow continental shelf and, as it is located at the northern edge of the Eastern North Atlantic Upwelling Region, it is significantly affected by seasonal coastal upwelling phenomena (Relvas et al., 2007). Cold, nutrient-rich waters are brought to the surface between June and October, while warmer waters from offshore regions reach the shelf between November and May. The steep slope of the continental shelf, between 800-1500 m, limits bottom trawling activities to depths of up to 800 m.

The southwestern coast is influenced by the mixing of Atlantic Intermediate Water and Mediterranean Water flowing through the Strait of Gibraltar, which provides relatively warm (~13°C) and salty (~36.3) water at intermediate depths (~900 m; Tanhua et al., 2013; Aldama-Campino and DööS, 2020). In this region, the continental shelf slope is moderate, allowing easier access to depths beyond 800 m.

Who are the deep-sea sharks and skates of Portugal?

A total of 1559 specimens belonging to 18 species (15 sharks and 3 skates) were studied (Table 7.1). In the South, out of the 14 identified species, the most frequently caught species was the blackmouth catshark. In the Southwest, out of the 16 captured species, the most frequent was the knifetooth dogfish shark. All hauls conducted in the Southwest contained DSE, whereas in the south, only 71% of the hauls contained DSE.

Table 7.1 Numbers of sharks and skates caught by a crustacean trawler in the South and Southwest of Portugal.

Species	Common names	Areas	
		South	Southwest
Sharks			
Order			
Carcharhiniformes			
Galeus atlanticus	Atlantic sawtail catshark	47	17
Galeus melastomus	Blackmouth catshark	450	44
Hexanchiformes			
*Chlamydoselachus anguineus**	Frilled shark		3
Lamniformes			
Mitsukurina owstoni	Goblin shark		1
Squaliformes			
*Centrophorus granulosus**	Gulper shark	10	
*Centrophorus squamosus**	Leafscale gulper shark	3	2
*Centroscymnus coelolepis**	Portuguese dogfish	1	3
*Centroselachus crepidater**	Longnose velvet dogfish		17
*Dalatias licha**	Kitefin shark	9	4
*Deania calceus**	Birdbeak dogfish	1	45
Deania profundorum	Longsnout dogfish	167	41
Etmopterus pusillus	Smooth lanternshark	115	16
*Etmopterus spinax**	Velvet belly	264	39
*Oxynotus paradoxus **	Sailfin roughshark		4
*Scymnodon ringens **	Kinfetooth dogfish	101	97
Skates			
Order			
Rajiformes			
Dipturus nidarosiensis	Norwegian skate	4	15
Dipturus oxyrinchus	Longnosed skate	30	7
Neoraja iberica	Iberian pygmy skate	2	

* Deep-sea Sharks for which Retention is Prohibited for Vessels in the European Union (Regulation 2024/257)

Where do they concentrate?

To evaluate which areas and depths exhibited higher numbers of sharks and skates, the index of catch per unit effort (CPUE) was used. This index calculates the number of specimens and the weight of a species relative to the duration of the haul.

A higher CPUE by number (CPUE n) and CPUE by weight (CPUE kg) of specimens was identified between 500-600 m depth between Portimão and Sagres. Some species, such as the knifetooth dogfish and longsnout dogfish, showed high CPUE n in the Portimão Canyon at around 800 m depth. In the Portimão Canyon, high CPUE kg was also identified for species threatened with extinction, such as the Portuguese dogfish and leafscale gulper shark, and the Norwegian skate, which is near threatened (Figure 7.4).

Higher CPUE by number (CPUE n) was observed between 500-700 m. However, the highest values of CPUE by weight (CPUE kg) were observed beyond 1200 m, indicating the presence of larger animals at greater depths, as opposed to what was observed at shallower depths. Some species were exclusively found below 1200 m, such as the critically endangered birdbeak dogfish and Portuguese dogfish, and the rare goblin shark and frilled shark (Figure 7.4).

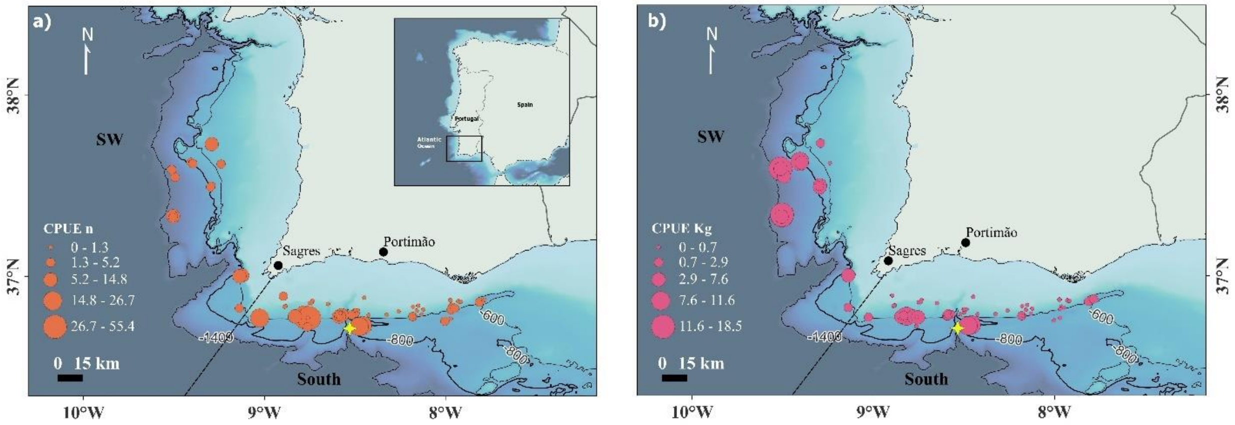


Figure 7.4 Maps of the study area showing the catch per unit effort (CPUE) values for the number of specimens (a) and weight in kg (b) of deep-sea sharks and skates.

Overlap with bottom trawling activities

To assess whether the feeding areas of deep-sea elasmobranchs overlap with bottom trawl fishing areas on the South coast of Portugal, the main prey were identified and evaluated to determine if they had been consumed in the days preceding the captures.

The diet determination was conducted through the analysis of stomach contents[2] from specimens of species that arrived dead on board (smooth lanternshark, blackmouth catshark, knifetooth dogfish, longnosed skate), and also through stable isotope analysis of nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) and carbon ($\delta^{13}C$: $^{13}C/^{12}C$) for frequently captured species (birdbeak dogfish, longsnout dogfish, velvet belly, blackmouth catshark, and knifetooth dogfish).

Nucleic acid ratios (RNA:DNA) were used to determine if the animals had been feeding in the days or weeks prior to capture, thus assessing their nutritional status and condition. Analysis of percentiles by species, given the lack of baseline values for such comparisons, indicated that the average values were closer to the 75th percentile. This was considered indicative of recent

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[2] Work conducted by Sofia Quaresma under the supervision of Dimitri Araújo and of Ester Dias from Ciimar, University of Porto, and Sofia Graça Aranha from CCMAR, University of Algarve.

feeding and good condition, as opposed to the 10th percentile, where the condition would be imminently weaker.

Thus, it was concluded that the deep-sea elasmobranch species evaluated during the Delasmop project feed on crustaceans, including commercially important species to varying extents (e.g., blackmouth catshark and knifetooth dogfish), along with other fish and cephalopod species (Graça Aranha et al., 2023). Moreover, the majority of species showed average RNA:DNA values closer to the 75th percentile, suggesting that the animals were likely using the area or nearby zones for feeding, indicating an overlap of their feeding areas with crustacean trawl fishing on the South coast of Portugal.

7.4.3 Impact of trawling on sharks and skates

Deep-sea elasmobranchs are a frequent bycatch in crustacean trawling. The weight of elasmobranch bycatch on the South coast varied between 0-47% of the total net weight, while on the Southwest coast, the values varied between 1-58%.

At-vessel mortality rates

Onboard mortality rates were assessed for the most abundant shark species. Whenever a shark was spotted during the fish sorting process, it was immediately placed in a cooled (temperature 13°C) and oxygenated tank, where body and spiracle movements were assessed, as well as the presence or absence of lesions on the body. Animals that were dead or in poor condition showed no (or few) body and spiracle movements and could have wounds on the body. Animals in good and excellent condition showed strong and vigorous body and spiracle movements and also few or no lesions on the body.

The number of dead and poor-condition specimens (95%, i.e. 1073) was higher than the number of specimens in good and excellent condition (5%; 53). The blackmouth catshark had the lowest number of dead specimens (76%), in comparison with knifetooth shark and velvet belly (86%) and the smooth lanternshark (85%).

To assess which parameters are responsible for higher shark mortality, logistic regression models were used. The models indicated that the knifetooth dogfish presented higher mortality than the other species, and that smaller specimens have a higher risk of mortality than larger specimens in general. Furthermore, specimens from hauls that presented greater temperature differences between the surface and bottom waters, that contained greater catch (i.e. heavier codend) and hauls that used the codend with a mesh size of 55 mm (for catching shrimp and prawns) compared to a mesh size of 70 mm (for catching Norway lobster), also caused higher mortality rates of specimens of the species in question.

Stress[3]

In order to assess the potential stress to which deep-sea sharks are subjected during bottom trawling activities, some physiological parameters were measured in their blood plasma and correlated with fishing procedures and environmental factors. The target species for this analysis were the smooth lanternshark, velvet belly, blackmouth catshark, and the knifetooth shark.

On board, blood samples were collected from the caudal vein of sharks that presented excellent, good or poor conditions, using a 1 ml heparinized syringe (Figure 7.5); the plasma was separated by centrifugation and frozen until laboratory analysis. The sharks were released after the procedure.

Figure 7.5 Researcher drawing blood from the caudal vein of a deep-sea shark on board a crustacean trawler.

In the laboratory, the concentrations (mmol/L) of metabolites (glucose, urea and lactate) and electrolytes (phosphorus, potassium, chloride, sodium, magnesium and calcium) in blood

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[3] This work was developed in collaboration with Aurélien Tambuté from La Rochelle University and CCMAR researchers namely Alexandra Alves, Teresa Modesto and Pedro Guerreiro"

plasma were determined using commercial kits (Spinreact®) related to the physiological response to stress.

An increase in the concentration of some of these physiological indicators was observed due to greater fishing depths, greater temperature differences and also higher fishing speed. As they live in an environment characterized by cold waters and higher pressures, an abrupt transition in temperature and rapid changes in pressure may result in higher stress and mortality rates. However, greater temperature differences are related to lower concentrations of glucose (for the velvet belly), potassium (for smooth lanternshark) and urea (blackmouth catshark), which may indicate that the physiological responses to the stress of capture had not yet been triggered or that they were already returning to baseline levels (Barkley et al., 2017; Talwar et al., 2017; Prohaska et al., 2021).

The fishing effort showed, for the most part, a negative correlation with the plasma levels of glucose, chloride, magnesium, potassium and sodium, i.e., longer hauls are associated with lower concentrations of these indicators. This could indicate a stabilization of the response after long hauls, or perhaps the specimens studied entered the net just before the end of each haul and these responses had not yet been triggered, which could be the case for some specimens studied, but is unlikely to happen to the majority. Therefore, the first hypothesis is most likely, but further studies are needed to assess the onset and stabilization times of these indicators.

The maximum chloride and magnesium values for the velvet-belly (349.8 and 7.61 mmol/L respectively) and the smooth lanternshark (325.8 and 8.85 mmol/L respectively) were very high when compared with those found in 46 articles on stress indicators in elasmobranch plasma. In relation to three articles on deep-sea sharks (Barkley et al., 2017; Talwar et al., 2017; Prohaska et al., 2021), the highest lactate levels were found for the blackmouth shark (33.11 mmol/L) and sodium for the velvet-belly (357.8 mmol/L) and smooth lanternshark (425.5 mmol/L).

The plasma lactate levels are related to an extreme physiological response, since this glucose metabolite is generated under greater energy demand due to anaerobic respiration caused by high stress or high physical activity. The lactate values, all above 5 mmol/L in the species analysed in this study, indicate that the sharks may be under stress. The literature indicates that, for pelagic sharks, values above 16 mmol/L are correlated with higher mortality rates (Skomal and Bernal, 2010), which may indicate that the blackmouth shark in this study may not survive the fishing procedures, even if they are in good or excellent physical condition.

Although there are numerous physiological indicators that identify whether a fish is under stress, our understanding of what the absolute levels of these indicators mean is rudimentary, especially in non-experimental contexts in wild sharks. As the baseline levels of these plasma indicators of stress for deep-sea sharks are not known, some caution is advised when interpreting the results obtained. High levels of an indicator may signal a fish under stress, but lower levels of an indicator do not necessarily mean the opposite.

More research is therefore needed to establish a more robust analysis of the relationship between physiological indicators of stress and fishing variables, which could provide the basis for establishing additional recommendations aimed at improving the welfare and effective management of discarded deep-sea sharks.

7.4.4 Conversations with the sectors

Meetings and workshops were held to present the project and its main objectives to fisheries managers and fishers, and to hear questions and concerns about elasmobranch bycatch. In addition, fishers were trained in the use of an electronic monitoring tool under the EMREP project.

What are the fishers' perceptions[4]?

To find out how fishers perceive deep-sea elasmobranchs, surveys were conducted in the main fishing ports in southern Portugal, where around nine crustacean trawling vessels were approached, totalling 34 fishermen aged between 25 and 76.

Several questions were raised, specifically about the frequency of fishing, identification, survival and handling of the species caught, as well as regulations involving these animals.

Among the main parameters that could affect the survival of elasmobranchs, fishers pointed out the weight of the net, the speed of the net ascent and the duration of the haul as the factors with the greatest impact.

In general, the vast majority of fishers demonstrated a limited understanding of the issues raised: 92% indicated incorrect handling techniques as the most frequently used, others were unable to identify (from the elasmobranchs' illustrations within the online guide) the species with which they have the most contact and 68% reported believing that the majority of animals returned to the sea survive. Furthermore, more than 85% stated that they were not aware of the regulations

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[4] This work was carried out within the scope of the Delasmop project, authored by Matilde Romão under the supervision of Ana Hilário from CESAM, University of Aveiro, co-supervised by Sofia Graça Aranha from CCMAR, University of Algarve

regarding deep-sea elasmobranchs. These responses, among others provided by the surveys, indicate a limited understanding and therefore there is a need to promote to fishers more awareness-raising and clarification actions on the subject.

7.4.5 Conclusion

The Delasmop project identified a negative impact of bottom trawling on the survival of deep-sea elasmobranchs. The high mortality rates and low growth and reproduction rates of these animals, combined with the insufficient knowledge of fishers who deal with these species daily, highlights the urgent need for improved management strategies and the need for greater communication between researchers, managers and fishers. This is essential to gain greater understanding of the issues that permeate both parties and to discuss ways to mitigate the current impact of fishing on local populations of deep-sea elasmobranchs.

7.5 Strategies to mitigate the impacts of bottom trawling on sharks and skates

7.5.1 Reducing bycatch

Prevention measures

Reducing the bycatch of deep-sea elasmobranchs is one of the main strategies for mitigating the impact of bottom trawling on these animals. One way to reduce catches is to avoid spatial (horizontal and vertical) and temporal overlap between fishing operations and areas where there is a known concentration of deep-sea elasmobranchs. To achieve this, it will be necessary to gain greater knowledge of habitat use and the times of year when the animals are most susceptible to capture.

- Recommendations for Portugal:

According to the information collected by the Delasmop project (section 7.4 "Delasmop Project"), in order to avoid bycatch of deep-sea elasmobranchs on the southern coast of Portugal, it is recommended to limit crustacean bottom trawling whenever possible to a depth of 500 m, especially in the areas between the cities of Portimão and Sagres and also in the Portimão canyon (close to 800 m). These were the areas and depths that presented the highest CPUE n and CPUE kg values. On the southwest coast, it is suggested to avoid crustacean bottom trawling activities beyond 800 m in accordance with the recommendations of Regulation (EU) 2016/2336, especially in areas below 1200 m, which is where the highest CPUE kg values of rare and endangered species were obtained.

It is also recommended that further studies be carried out to identify other sensitive habitats, such as nursery areas, and a temporal analysis of the interaction between fishing and the occurrence of these species.

Bycatch reduction mechanisms

The use of weights to stir the substrate at the mouth of the net to help increase the catch of target species may result in an increase in the bycatch of elasmobranchs, especially skates. Removing these weights has proven effective in reducing the catch of elasmobranchs. However, this removal may also result in a decrease in the catch of some target species (Kynoch et al., 2015).

The use of devices in trawl nets also helps to reduce bycatch of elasmobranchs and other unwanted species and has shown positive results in some countries. They consist of modifications made to the nets, where a grid (firm or flexible) is placed in front of the entrance to the codend, which allows the target species to pass through, diverting elasmobranchs out of the net through a lower or upper opening. These devices depend on the type of species to be excluded, i.e. whether they have more demersal habits (lower opening, Figure 7.6) or more pelagic habits (upper opening). These devices have shown a significant reduction in elasmobranch catches in some trials carried out in several countries (Brewer et al., 2006; Brčić et al., 2015; Kynoch et al., 2015; Willems et al., 2016; Wakefield et al., 2017). However, the effectiveness of such measures in reducing bycatch of deep-sea elasmobranchs, specifically in the European crustacean trawl fishery, requires further investigation.

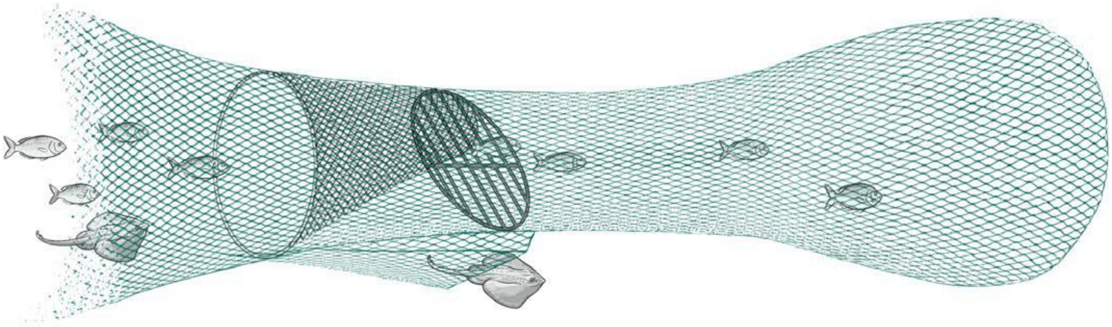

Figure 7.6 Codend with a device to exclude bycatch of demersal fish, such as skates. Illustration taken from Sacchi (2021).

- Recommendations

Given the proven effectiveness of the use of grids to exclude elasmobranchs in crustacean trawlers in other countries, and due to the lack of strategies to reduce elasmobranch bycatch in trawl fisheries in Portugal, it is suggested that trials be carried out to test the functionality of such mechanisms in reducing bycatch, as a way of mitigating the current impacts that crustacean trawling has on these animals.

Identification of discards

Deep-sea elasmobranchs are discarded by crustacean bottom trawlers, either due to existing regulations or because they have low commercial value. This results in a low number of records of the occurrence of these species (Walsh et al., 2002; Campana et al., 2016), which makes it difficult to obtain information on the status of populations and distribution patterns. The landing obligation (Article 15 of Regulation (EU) Nº. 1380/2013) applies to species with a total allowable catch (TAC), such as skates and rays in the Northeast Atlantic Ocean. This means that specimens with a TAC must be landed, even if they are not marketed, so that their numbers are counted against quotas, unlike when they are discarded. However, since 2019, when its implementation was achieved by European Union countries and fisheries (with some exceptions), there has been little adherence from the fishing industry (Savina, 2019; Borges, 2020). In this regard, efforts to identify what is discarded need to be made without fishers continuing to be hampered with the obligation to report discarded species and the obligation to land species with a TAC. Therefore, it is essential to intensify and diversify monitoring measures, in order to obtain data on discards in a more autonomous way. Monitoring by on-board observers is the most common method for collecting data in commercial fisheries. However, in the case of deep-sea fisheries, such as crustacean trawling, only 20% of the commercial fleet should be monitored (Regulation (EU) Nº. 2016/2336). In this regard, electronic monitoring using on-board cameras would be an added value, as it could be applied to the entire fleet, maintaining a high level of identification remotely [da Rocha et al., (unpublished results)].

- Recommendations for Portugal:

An electronic monitoring trial was carried out during the activities of the Delasmop project. Surveillance cameras on board a crustacean trawling vessel, integrated into electronic fishing logbook, made it possible to identify sharks and skates down to the genus level and many specimens down to the species level da Rocha et al., (unpublished results)].

It is understood that the use of electronic monitoring could help in the identification not only of elasmobranchs, but also of other species subject to regulations and expanded to other fisheries. However, its functionality would need to be tested and, in this regard, it is suggested that tests of this technology be carried out in different fisheries in order to identify discards of other species of conservation interest.

7.5.2 Reducing mortality

Reducing mortality depends on greater control of factors and variables involved in fishing activities. During fishing, a series of factors such as duration of air exposure, fish handling, net configuration, fishing duration and vessel speed, along with environmental variables such as water temperature and pressure changes, can contribute to at-vessel mortality [Jean, 1963; Olla et al., 1998; Davis et al., 2001; Davis and Olla, 2002; Graça Aranha et al., (unpublished results)]. Deep-sea elasmobranchs often arrive on board in poor condition, with little or no body movement, which suggests that their survival after discards is unlikely.

After discards, mortality can be attributed to internal injuries caused by barotrauma or crushing within the fishing net, in addition to stress resulting from fishing activities and handling on board. This stress can lead to an increase in the concentration of certain plasma markers, which could result in the death of the animals shortly after discards (see subsection “Stress” under section 7.4.3 “Impacts of trawling on sharks and skates”).

- Recommendations for Portugal:

Depth is strongly linked to the distribution of deep-sea elasmobranchs, so that high depths (> 400 m) in addition to presenting a higher probability of bycatch of deep-sea elasmobranchs [ICES, 2020; O’Hea et al., 2020; Graça Aranha et al., (unpublished results)] may be associated with higher mortality, since differences in pressure and temperatures (between the bottom and surface waters) can cause barotrauma and thermal shock, respectively.

- To mitigate such effects, it is suggested:
    - Reducing the fishing depth, maintaining < 800 m in accordance with the recommendations by law (Regulation (EU) 2016/2336), however, if possible, maintaining < 500 m. This will result in a decrease in the catch of deep-sea elasmobranchs, but also in a decrease in the probability of mortality due to barotrauma and thermal shock.

    - Restricting fishing to times of the year when surface water and air temperatures are lower or when there is little stratification in the different water layers is recommended, since rising temperatures have been associated with decreased condition in many taxa and decreased survival [Jean, 1963; Olla et al., 1998; Davis et al., 2001; Davis and Olla, 2002; Graça Aranha et al., (unpublished results)].
    - Decreasing the hauling speed of the net, to allow a more gradual transition between zones with different pressures and temperatures.
- If the animal arrives on board alive and has good physical condition, i.e. strong body and spiracles movements and does not present significant injuries, its return should be prioritized. To this end, better handling practices are essential to increase the chances of survival of these animals after their release (Campana et al., 2009; see section 7.3 "Elasmobranch's handling").
- To increase post-discard survival rates, the most effective measure would be to limit the air exposure time (Neilson et al., 1989; Richards et al., 1994; Parker et al., 2003; Stobutzki et al., 2002; Rodríguez-Cabello et al., 2005; Mandelman and Farrington, 2007; Rulifson, 2007), for which a reduction in sorting time is necessary.
    - One way to reduce sorting time would be to reduce haul duration. This would reduce the number of animals in the net per haul, as well as the weight of the net, reducing the possibility of death by crushing.

Keep the haul speed to the minimum possible, since greater speed was associated to increase stress levels in some shark species (see subsection "Stress" in section 7.4.3 "Impacts of trawling on sharks and skates").

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7.6 References

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PART III – FURTHER INSIGHTS INTO THE KNOWLEDGE OF POORLY KNOWN DEEP-SEA ELASMOBRANCHS

Chapter 8: NEW INSIGHTS ON THE ECOLOGY AND BIOLOGY OF THE RARE *OXYNOTUS PARADOXUS* FROM RECENT RECORDS

Graça Aranha, Sofia; Dias, Ester; Marsili, Tiago; Pires Da Rocha, Pedro; Teodósio, Alexandra; & Figueiredo, Ivone (2024). New insights on the ecology and biology of the rare *Oxynotus paradoxus* from recent records. *Cybium*, 48(3): 211-217. https://doi.org/10.26028/CYBIUM/2024-013

Abstract

Oxynotus paradoxus, documented in the Eastern Atlantic Ocean from Senegal to Scotland at depths ranging between 92 and 1044 m, has been a subject of limited scientific attention in Portugal. Despite its presence in various Portuguese occurrence checklists, only two scientific studies have reported *O. paradoxus*, one off the mainland and the other off the Azores islands. In this study, conducted during fishing campaigns, four *O. paradoxus* specimens were caught off the southwest coast of Portugal (SW Iberian Peninsula) at depths ranging between 742 and 1238 m. Notably, an adult female achieved a new maximum depth record for this species, measuring 650 mm in total length. By examining this adult female, we provide novel insights into the ecology and biology of *O. paradoxus*. Morphometric measurements are compared with previous studies, revealing some intra-specific variability. The observed low hepato-, gonadosomatic, and ecophysiological indices suggest that the adult female had refrained from feeding for some time before collection. This specimen-focused approach contributes significantly to the understanding of this poorly known species, especially considering its rarity. This study marks a noteworthy effort to enhance knowledge and emphasizes the importance of specimen-based investigations when targeting rare species.

Keywords: Bycatch, SW Iberian Peninsula, Gonadosomatic index, Hepatosomatic index, Morphometry, Ecophysiological index

8.1 Introduction

The Oxynotidae constitute a family of elasmobranchs within the order Squaliformes, exclusively represented by the genus *Oxynotus*. Species in this genus exhibit a compressed body, a triangular cross-section, two high-spined dorsal fins, very rough skin, a small thick-lipped mouth, large closely positioned nostrils, and a flat blunt snout (Ebert et al., 2021).

Out of the five globally occurring species of *Oxynotus*, only two inhabit European waters: the Angular Roughshark *Oxynotus centrina* (Linnaeus, 1758) and the less studied Sailfin Roughshark *Oxynotus paradoxus* (Frade, 1929). The latter is distributed along the continental slopes of the Northeast Atlantic, ranging from Scotland to Senegal, including the Azores and Canary Islands, but is absent in the Mediterranean.

Previous studies have reported *O. paradoxus* at depths varying between 92 and 877 m in the British Isles (e.g., Blacker, 1962; Norman, 1932; Tucker and Palmer, 1949; Rae and Lamont, 1960; Went, 1968; Quigley and Flannery, 1994), along the French Basque coast (e.g., Frade, 1932; Harambillet et al*.,* 1976), and in the Galicia Bank seamount (Bañón et al., 2016). Pajuelo et al. (2016) captured the species in NW Africa during experimental fishing trips at depths ranging between 803 and 1044 m. In SW Iberian waters, particularly in Portugal, *O. paradoxus* has been reported in areas surrounding the mainland and the islands of Azores and Madeira (Krefft and Tortonese, 1979; Santos et al., 1997; Carneiro et al., 2014; Biscoito et al., 2018). Despite its occurrence in Portuguese waters, the available scientific literature on *O. paradoxus* is scarce. Azevedo et al. (2003) described dermal denticles from two specimens caught off the Azores in 1993 and 1995 (one female caught at 600 m depth with a gill net and a male with no depth indication). Moura et al. (2015) presented the barcoding of one specimen of *O. paradoxus* caught off the coast of mainland Portugal during a bottom-trawl research survey conducted by the Portuguese Institute for the Sea and Atmosphere (IPMA), reaching a maximum depth of 750 m.

Oxynotus paradoxus specimens are born with a total length (TL) of 25 cm and can attain a maximum TL of 128 cm (O'Riordan, 1984). Males reach maturity at around 75 cm TL, but information on females' maturity is lacking (Ebert et al., 2021). According to IUCN criteria, O. paradoxus is globally classified as Vulnerable (Finucci et al., 2021), and in Europe, it is classified as Data Deficient (Soldo et al., 2015), signifying insufficient information for a direct or indirect assessment of its risk of extinction based on distribution and/or population status (IUCN, 2022).

In the present study, we describe a new depth record for *O. paradoxus* off the SW Iberian Peninsula slope, including morphometric measurements and new insights into the biology of this poorly understood species.

8.2 Materials and methods

Between June 2020 and May 2022, a total of 106 trawling hours were conducted by both a commercial crustacean bottom-trawler and the RV Mário Ruivo of the Institute for the Sea and Atmosphere (IPMA) along the southwest coast of Portugal. The fishing operations were carried out at depths ranging from 27 to 1244 m, within the coordinates of 37-39°N and 9-10°W.

During these operations, four specimens of *Oxynotus paradoxus* were collected by the commercial vessel while targeting the giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) and the scarlet shrimp *Aristaeopsis edwardsiana* (Johnson, 1868). The collection depths varied from 742 to 1238 m and took place during both summer and winter trips in 2021 (Figure 8.1).

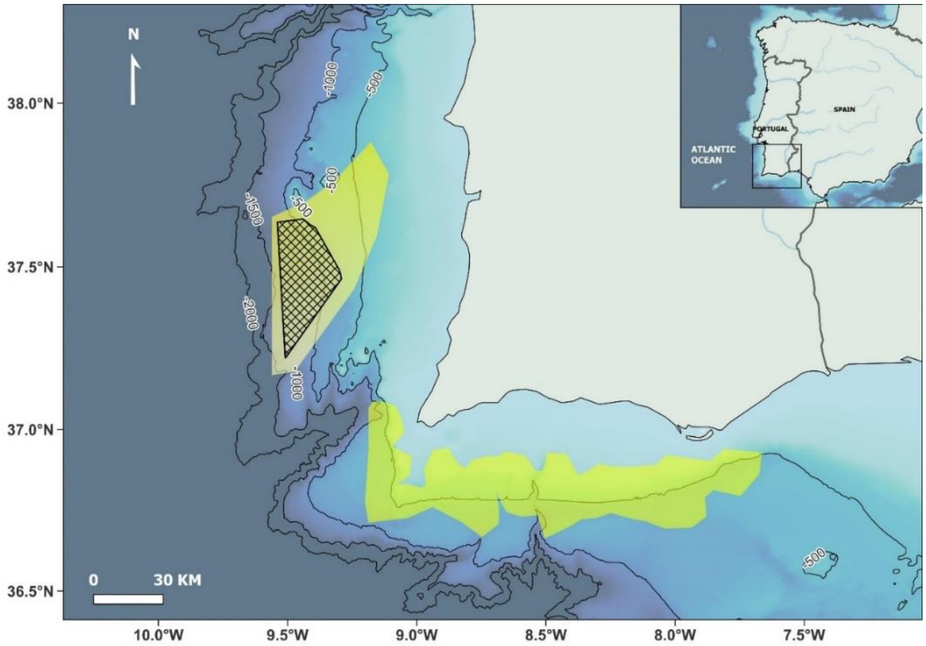


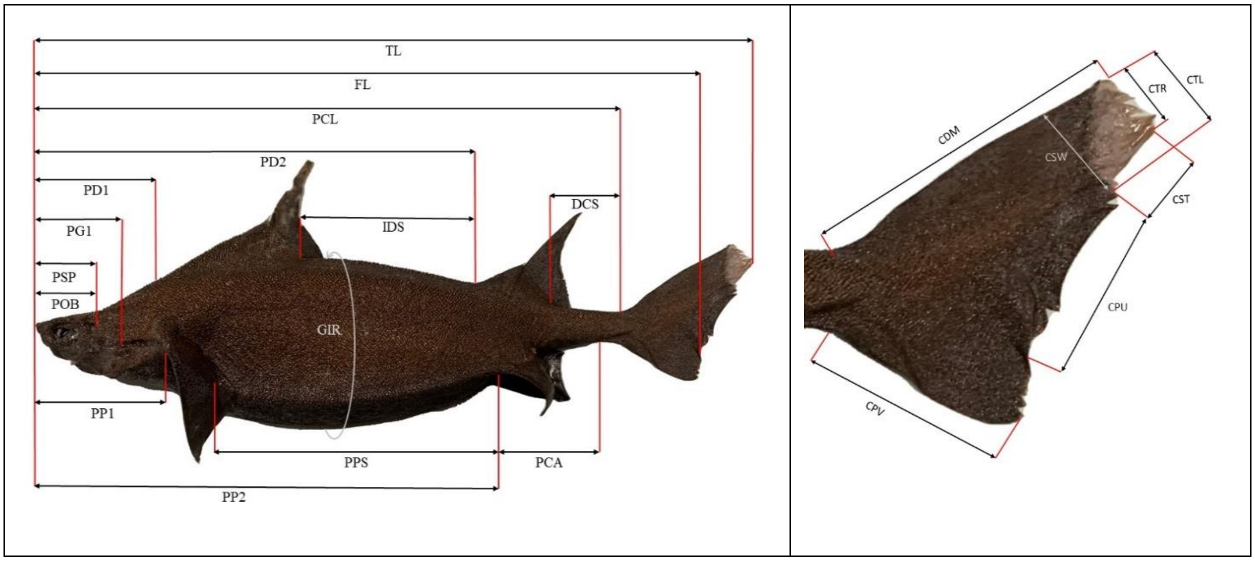
Figure 8.1 Study area where the yellow polygons represent the areas where fishing was conducted, and the black dashed polygon corresponds to the area where *Oxynotus paradoxus* specimens were caught.

The fishing depths (m) were recorded using a Star Oddi® mini-CTD. The GPS points, vessel velocity, and vessel track were automatically integrated into the Olrac Dynamic Data Logger (DDL)® software.

Upon collection, the specimens of *O. paradoxus* were frozen onboard and subsequently transported to the Fisheries Biodiversity and Conservation laboratory (CCMAR) at the University of Algarve. Each specimen was weighed uneviscerated (g), and its total length (TL) was measured in millimetres. Sex was macroscopically determined, and maturity stages were assigned following Stehmann's maturity scale (Stehmann, 2002). Recognition of adult females was based on enlarged oocytes and well-rounded ovaries.

For the ecophysiological index, the RNA/DNA standardized ratio (sRD) was determined in accordance with Graça Aranha et al. (2023) and references therein. The sRD index is widely employed as a nutritional condition index at the organism level in marine ecology (Chícharo and Chícharo, 2008).

The specimen caught at the greatest depth (Table 8.1, #4.093 Matos et al., 2024) underwent further investigation. After being thawed and frozen twice, external morphometric measurements were taken using a digital calliper (to the nearest 0.5 mm) or a metric tape (for measurements exceeding 120 mm). Given that the morphometry of *Oxynotus* species differs from most sharks due to the unusual shape of their dorsal fins and the absence of anal fins, graphic illustrations depicting the measurements conducted in this study were provided (Figure 8.2). These measurements were expressed both in millimetres and as a percentage of total length (% TL) and were compared with the average % TL obtained by Yano and Matsuura (2002) for a 555 mm TL male and a 508 mm TL female from Western Sahara and Ireland, respectively (Table 8.2).



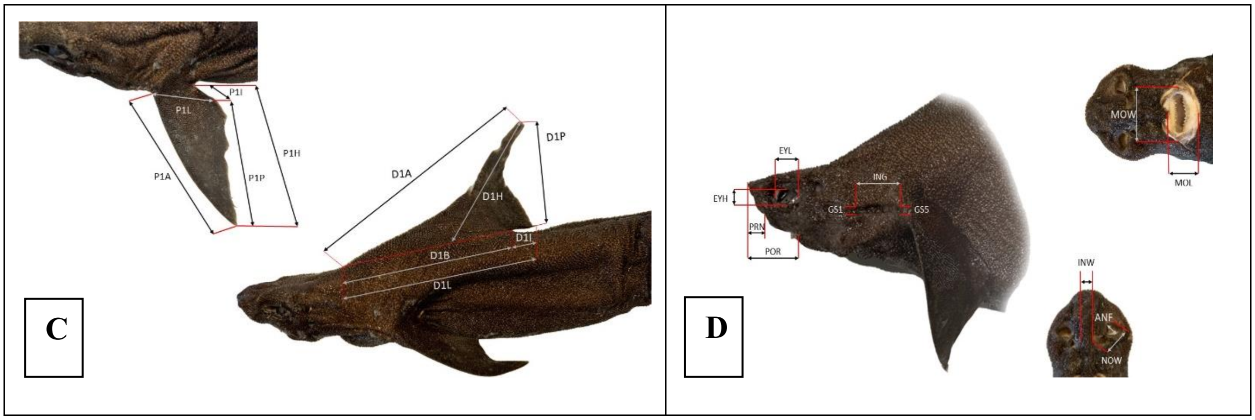


Figure 8.2 This figure is not scaled and shows external measurements of the *Oxynotus paradoxus* specimen #4.093 (see Table II for further details). **A**: Full body measurements before dissection and after the first thaw; **B**: Caudal fin measurements; **C**: Pectoral and first dorsal fin measurements after dissection and second thaw; **D**: Eye, gill slits, nostrils, and mouth measurements.

The liver and gonads were extracted from specimen #4.093. Subsequently, these organs were weighed to the nearest 0.01 g to calculate the hepatosomatic and gonadosomatic indices (Table 8.1).

The hepatosomatic index (HSI) was calculated as:

$$HSI(\%) = \frac{liver\ weight(g)}{Total\ body\ weight\ (g)} * 100$$

and the gonadosomatic index (GSI) as:

$$GSI(\%) = \frac{gonad\ weight(g)}{Total\ body\ weight\ (g)} * 100$$

8.3 Results

Among the collected *O. paradoxus* specimens, one was identified as a juvenile male (#8.086), while two were juvenile females (#8.085 and 8.087), and one was an adult female (#4.093; Table 8.1).

The adult female exhibited an HSI of 18.6% (liver weight = 374.12 g) and a GSI of 1.33% (gonads weight = 26.87 g). Notably, this adult female displayed the lowest sRD values among all the specimens captured (Table 8.1).

Table 8.1 General information on the *Oxynotus paradoxus* specimens collected off the SW Iberian Peninsula with the code for each specimen, total length (TL) in mm, weight in g, RNA/DNA standardized ratio (sRD), sex (female or male), maturity stage according to Stehmann (2002), mean haul depth in meters, season, latitude, and longitude of the start of the haul.

Code	TL (mm)	Weight (g)	sRD	Sex	Maturity stage	Mean depth (m)	Season	Latitude (ºN)	Longitude (ºW)
#4.093	650	2015.0	0.08	F	3 (mature)	1238	Winter	37.589884	-9.512294
#8.085	240	62.5	0.62	F	1 (immature)	765	Summer	37.630817	-9.405929
#8.086	285	154.0	0.41	M	1 (immature)	752	Summer	37.49418	-9.298284
#8.087	440	596.0	0.71	F	2 (maturing)	742	Summer	37.622667	-9.39556

Out of the 65 measurements conducted, only 36 were deemed comparable due to differences in the measurement protocols employed by different authors. The present study adhered to the methodology outlined by Ebert et al. (2021), while Yano and Matsuura (2002) followed Yano and Tanaka (1983).

Although the measurements conducted in the present study were generally similar to those presented by Yano and Matsuura (2002), notable differences were observed. Specifically, the inter-dorsal space (IDS) and spiracle length (SPL) were smaller in the present study, while the pre-orbital length (POB) and pelvic insert to caudal lower origin (PCA) were greater in the present study (Table 8.2).

Table 8.2 External morphometric measurements (mm) and percentage of total length (% TL) of a female *Oxynotus paradoxus* (code #4.093) from the SW Iberian Peninsula collected at 1238 m depth, and as a mean of the % TL for a female and male for Yano and Matsuura (2002). * Measurements not presented in Figure 8.2.

Measurements	Code	#4.093		Yano and Matsuura (2002)
		n = 1		n = 2
Sex		F		F-M
Range of TL (mm)		650		508–555
		mm	**% TL**	**% TL**
Pre-orbital length	POB	36	5.54	3.8
Pre-narial length	PRN	16	2.46	1.8
Pre-oral length	POR	38.5	5.92	5.8
Pre-spiracular length	PSP	71	10.92	9
Pre-first dorsal length	PD1	92	14.15	16.6
Pre-second dorsal length	PD2	344	52.92	56.4

Measurements	**Code**	**#4.093**		**Yano and Matsuura (2002)**
		n = 1		n = 2
Sex		F		F-M
Range of TL (mm)		650		508–555
		mm	**% TL**	**% TL**
Pre-pectoral length	PP1	115	17.69	19.6
Pre-pelvic length	PP2	350	53.85	
Inter-dorsal space	IDS	95	14.62	21.9
Dorsal caudal space	DCS	68	10.46	11.4
Pectoral-pelvic space	PPS	222	34.15	37.8
First dorsal anterior margin	D1A	205	31.54	
First dorsal height	D1H	108.5	16.69	16.2
First dorsal posterior margin	D1P	111	17.08	15
First dorsal base	D1B	145	22.31	18.9
Second dorsal anterior margin	D2A*	139	21.38	
Second dorsal height	D2H*	106	16.31	14.6
Second dorsal posterior margin	D2P*	103	15.85	14.4
Second dorsal base	D2B*	82	12.62	12.6
Pelvic anterior margin	P2A*	78.5	12.08	9.9
Pelvic height	P2H*	61	9.38	
Pelvic posterior margin	P2P*	44	6.77	
Pelvic base	P2B*	46	7.08	6.3
Pelvic inner margin	P2I*	35	5.38	
Dorsal caudal margin	CDM	124	19.08	22
Terminal caudal margin	CTR	31	4.77	
Sub-terminal caudal-fin margin	CST	29	4.46	
Upper post-ventral caudal margin	CPU	100	15.38	
Caudal preventral margin	CPV	87	13.38	
Head length	HDL*	121	18.62	
Mouth length	MOL	8	1.23	
Mouth width	MOW	31.5	4.85	5.4
First gill slit height	GS1	10	1.54	1.3
Fifth slit height	GS5	8	1.23	1.1
Inter-gill length	ING	28	4.31	
Spiracle length	SPL*	5	0.77	2.2
Nostril width	NOW	16.5	2.54	
Anterior nasal flap length	ANF	11.5	1.77	

Measurements	**Code**	**#4.093**		**Yano and Matsuura (2002)**
		n = 1		n = 2
Sex		F		F-M
Range of TL (mm)		650		508–555
		mm	**% TL**	**% TL**
Internarial width	INW	9	1.38	1.3
Eye spiracle space	ESL*	12.5	1.92	
Eye length	EYL	28.5	4.38	4.5
Eye height	EYH	9.5	1.46	1.8
Head width	HDW*	51	7.85	
Trunk width	TRW*	74.5	11.46	
Caudal subterminal width	CSW	37	5.69	
Caudal terminal lobe	CTL	39	6.00	
First dorsal inner margin	D1I	29	4.46	
First dorsal length	D1L	175	26.92	23.2
Second dorsal inner margin	D2I*	27.5	4.23	
Second dorsal length	D2L*	107	16.46	14.8
Pelvic length	P2L*	63	9.69	11.4
Fork length	FL	576	88.62	75.7
Precaudal length	PCL	515	79.23	78.6
Snout to vent	SVL*	358	55.08	
Pelvic insert to caudal lower origin	PCA	118	18.15	9.9
Interorbital space	IOS*	39	6.00	8.5
Caudal peduncle width	CPW*	14	2.15	
Lower labial furrow length	LLA	16	2.46	
Girth	GIR	350	53.85	
Pectoral anterior margin	P1A	131	20.15	15
Pectoral radial length	P1R*	50	7.69	7.2
Pectoral inner margin	P1I	24	3.69	
Pectoral posterior margin	P1P	91	14.00	9.9
Pectoral height	P1H	115	17.69	
Pectoral length	P1L	57	8.77	

8.4 Discussion

This study presents a noteworthy finding of an *O. paradoxus* specimen captured at 1238 m, marking the highest depth ever reported for this species. This observation supports the hypothesis that the species may occur at greater depths than previously documented (Azevedo et al., 2003; Soldo et al., 2015).

The limited information available on this species in EU waters could be attributed to various factors. Firstly, the rarity of the species, possibly due to its preference for deeper waters, may contribute to the scarcity of sightings. Additionally, areas deeper than 750 m are not covered by the research surveys carried out in Portuguese waters under the EU Data Framework Collection for Fisheries. Furthermore, the imposition of a total allowable catch of 0 for this species since 2010 by the EU (Regulation n° 2021/91) prohibits its landing. In support of the first hypothesis, it is noteworthy that in NW Africa, 66 specimens were caught at depths ranging between 803 and 1044 m (Pajuelo et al., 2016).

The capture of an adult female *O.s paradoxus* at 1238 m, a depth surpassing that of the three juveniles caught during the summer at shallower depths (742 to 765 m), has led us to hypothesize potential ontogenetic segregation by season, depth, or both. This phenomenon has been reported for other deep-sea sharks such as *Centroscymnus coelolepis, Centrophorus squamosus,* and *Deania calceus* (Moura et al., 2014). The relatively small area where the specimens were collected in this study, compared to the total surveyed area, raises the possibility of a sparse distribution, as also previously suggested by Viana and Lisher (2018) for an *Oxynotus* sp. in the western Indian Ocean.

The adult female, measuring 650 mm TL, displayed large well-rounded ovaries at maturity stage III, while the two females were juveniles at maturity stages I and II (Stehmann, 2002). Although no reproductive studies are available for *O. paradoxus*, Capapé et al. (1999) observed that the smallest adult females of the congener *O. centrina* in the Mediterranean and East Atlantic had a TL of 640 mm.

For adult females of *O. centrina*, the HSI range is 23.4-42.0% (Capapé et al., 1999), and an immature female of *Oxynotus* sp. caught in the western Indian Ocean presented a 36.6% HSI (Viana and Lisher, 2018). On the other hand, Megalofonou and Damalas (2004) found 18.8% HSI in an *O. centrina* gravid female in the Mediterranean Sea, supporting that HSI decreases sharply in breeding females as liver reserves are used for gonadal products (Capapé et al., 1999;

Megalofonou and Damalas, 2004). The adult female captured in this study had an HSI of 18.6%, closer to the value found by Megalofonou and Damalas (2004); however, since the GSI values were 1.33%, it appears that hepatic reserves were not used in gonadal products (Capapé et al., 1999). Since HSI depends on lipid deposition derived from food intake, the low HSI values suggest the use of hepatic reserves due to food scarcity (Craik, 1978). In addition, the sRD of the adult female was also very low compared to the values obtained for the other *O. paradoxus* in this study and also to those reported by Graça Aranha et al. (2023) for five deep-sea shark species sampled in the same area. This may indicate reduced nutritional condition, suggesting that this female did not feed in the short to medium term prior to collection (Buckley et al., 1999).

Given that deep-sea sharks in this area feed on commercially important crustaceans (Graça Aranha et al., 2023), and that adult females of *O. centrina* are also known to feed on crustaceans (Capapé, 1975; Compagno, 1984; Barrull and Mate, 1996), it is possible that *O. paradoxus* also prey on crustaceans. Since its spatial distribution overlaps with deep-water fisheries for crustaceans (e.g., Borges et al., 2001; Campos et al., 2021), some overlap between their foraging grounds and fishing areas may occur. Unfortunately, dietary information was not available in the present study due to the small number of specimens collected. Given the metabolic rates and feeding habits of deep-sea shark species (e.g., Graça Aranha et al., 2023), a large number of stomachs from specimens that died during fishing procedures should be considered for this purpose. This approach, coupled with stable isotope analysis, would enhance our understanding of *O. paradoxus'* preferred prey and potential interactions with fisheries resources.

Morphometric measurements in this study were generally similar to those presented by Yano and Matsuura (2002), with some differences noted in PCA, IDS, SPL, and POB measurements. These variations could be attributed to specimen preservation or divergences in measurement protocols, as highlighted by Viana and Lisher (2018), thus, a standardized approach to *Oxynotus* spp. measurements is recommended for future comparisons.

Despite the limitations imposed by the low number of specimens, our specimenized approach contributes to the advancement of knowledge on this rare species.

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Ethical standards - This study was conducted in accordance with the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of animals and enforced by CCMAR. CCMAR staff are certified to house and conduct experiments with live animals, and their facilities are also certified in accordance with the three “R” policy, national and European legislation, and with guidelines defined by the ethical committee ORBEA CCMAR-CBMR.

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Chapter 9: DATASET OF THE COMPLETE MITOGENOME OF THE SAILFIN ROUGHSHARK, *OXYNOTUS PARADOXUS* FRADE, 1929

Matos, Ana; Gomes-dos-Santos, André; **Graça Aranha, Sofia**; Dias, Ester; Verissimo, Ana; Teodósio, Alexandra; Figueiredo, Ivone; C. Castro, L. Filipe; & Froufe, Elsa. 2024. Dataset of the complete mitogenome of the deep-sea sailfin roughshark, *Oxynotus paradoxus* Frade, 1929. *Data in Brief*, 52, 109836. https://doi.org/10.1016/j.dib.2023.109836

Abstract

Chondrichthyans comprise a diverse group of vertebrate species with extraordinary ecological relevance. Yet, multiple members of this evolutionary lineage are associated with significant extinction risk. The sailfin roughshark *Oxynotus paradoxus* is a deep-water benthic shark currently listed as vulnerable due to population declines in parts of its range. Here we provide the first complete mitochondrial genome of *O. paradoxus*, comprising also the first record for the genus and family Oxynotidae. These data can facilitate future monitoring of the genetic diversity in this and related species. Genomic DNA was extracted from *O. paradoxus* collected in the eastern North Atlantic off western Portugal (37.59°N, 9.51°W) and sent for Illumina Paired-End (2x150bp) library construction and whole genome sequencing on a Novaseq6000 platform. Trimmomatic (version 0.38) was used to remove adapters and MitoZ (version 3.4) to assemble and annotate the mitogenome. This mitogenome with 17 100 bp has a total of 38 genes, 13 of which are protein-coding genes, 23 transfer RNA genes, and 2 ribosomal RNA genes. Eight transfer RNAs and 1 protein-coding gene (NADH dehydrogenase subunit 6, NAD6) are in the complementary strand. In the provided phylogenetic inference, with all available and verified Squalomorphii mitogenomes, the four orders are well separated, and as expected, *O. paradoxus* is placed in the Squaliformes order. This data reinforces the need for more genomic resources for the Oxynotidae family.

Keywords: Chondrichthyes; Shark; Oxynotidae; Mitochondrial; Phylogeny

Table 9.1 Specifications table

Subject	Biological Sciences
Specific subject area	Bioinformatics, Marine Biology, Phylogeny and Evolution
Data format	Raw, Analyzed
Type of data	Figures
Data collection	A specimen of *Oxynotus paradoxus* was collected at 37.59°N, 9.51°W in the eastern North Atlantic off western Portugal (Figure 9.1). The species was identified at morphological and genetic levels. Genomic DNA was sent to Macrogen (Seoul, South Korea) for Illumina Paired-End (2x150bp) library construction and whole genome sequencing on a Novaseq6000 platform. Adapters were removed using Trimmomatic (version 0.38) and the mitogenome was assembled and annotated with MitoZ (version 3.4). Genome coverage information was obtained by running BBMap (BBMap Guide - DOE Joint Genome Institute).
Data source location	One *O. paradoxus* specimen, collected at 37.59°N, 9.51°W, was deposited at Centre of Marine Sciences, Universidade do Algarve (Portugal) (CCMAR) (contact person: Sofia Graça Aranha, sgramos@ualg.pt) with voucher name 4.93.
Data accessibility	Repository name: GenBank Data identification number: Accession numbers OQ627801 and OQ645448, and BioProject accession number PRJNA1033629 Direct URL to data: https://www.ncbi.nlm.nih.gov/nuccore/OQ627801.1/ https://www.ncbi.nlm.nih.gov/nuccore/OQ645448 https://www.ncbi.nlm.nih.gov/sra/PRJNA1033629

9.1 Value of the data

- The Class Chondrichthyes includes two sister groups: Holocephalans (or chimaeras) and Elasmobranchs (sharks and rays), distributed throughout the marine, brackish and freshwater ecosystems. Despite the importance of this group, the available genomic resources are still scarce. These are essential for assessing species diversity and population structure and applying conservation policies.

- Among Elasmobranchs, the sharks comprise roughly 50% of the taxa in the group and include various orders, of which the Squaliformes is the second most diverse. The only family within the Squaliformes without mitochondrial genomes reported is the Oxynotidae.
- The data provided in this study comprises the first mitogenome of *O. paradoxus* which is also the first record for the genus *Oxynotus* and family Oxynotidae. These data can facilitate future monitoring of the genetic diversity in this and related species.
- Researchers, conservation managers, and policymakers can benefit from these data.
- This dataset can be reused by other researchers when studying phylogenetic relationships in sharks and their genetic structure.

9.2 Data description

Oxynotidae family comprises benthic deep-sea sharks with a wide distribution range and all belonging to the same genus, *Oxynotus*. Currently, this genus comprises five species, including *Oxynotus paradoxus* Frade, 1929 (sailfin rough shark) which is listed as vulnerable by the International Union for Conservation of Nature (IUCN). This article describes the mitochondrial genome of *O. paradoxus* (Figure 9.1). This mitogenome has 17 100 bp with a gene content of 38 genes: 13 protein-coding genes, 23 transfer RNA genes and 2 ribosomal RNA genes (Figure 9.2; Supplementary Material 1-3), as other elasmobranchs (Johri et al., 2020; Kim et al., 2021; Pearce et al., 2021). Of these 38 genes, 9 were in the complementary strand (8 transfer RNAs and 1 protein-coding gene (NADH dehydrogenase subunit 6, NAD6). The phylogenetic analysis here presented separates with high node support the four taxonomic orders of Squalomorphii. *O. paradoxus* is placed within the Squaliformes clade, as expected, and in a separate branch basal to the families Squalidae and Somniosidae (Figure 9.3).

Genome coverage information and graphical displays are available in Appendices 9.1 to 9.3. The mitogenome was deposited in GenBank with accession number OQ645448, and raw sequencing data was deposited in NCBI with BioSample accession SAMN38037946, BioProject number PRJNA1033629, and SRA study SRP469130.

9.3 Experimental design, materials and methods

A female specimen (identified by the absence of claspers on the pelvic fin) of *O. paradoxus* was collected at 37.59°N, 9.51°W in the eastern North Atlantic off western Portugal (Figure 9.1). The sex of the specimen does not influence the results of the study. The species was first identified at the morphological level. A further test was performed by amplifying the mitochondrial cytochrome oxidase subunit 1 (COI) gene. The sample was obtained in a dead state. Genomic DNA was extracted following a standard high-salt protocol (Sambrook et al., 1989), and the COI gene was amplified with LCOI and HCOI primers (Folmer et al., 1994). The PCR mixture (final volume of 25 µL) contained 2.5 µL of Invitrogen PCR buffer (Invitrogen, Waltham, MA, USA), 1.5 µL 50 mM $MgCl_2$ (Invitrogen, Waltham, MA, USA), 0.5 µL 10mM dNTPs, 0.5 µL of each primer (10 mM), 0.1 µL Invitrogen Taq DNA Polymerase (Invitrogen, Waltham, MA, USA) and 1 µL of genomic DNA. The PCR program consisted of an initial denaturation at 94°C for 3 minutes followed by 38 cycles at 94°C for 30 seconds, 45°C for 40 seconds, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. PCR product was sequenced in both directions by Macrogen (Madrid, Spain), using the same primers. The obtained sequence was submitted to GenBank under the accession number OQ627801.

Figure 9.1 Species reference image of *Oxynotus paradoxus* (photograph by Tiago Marsili).

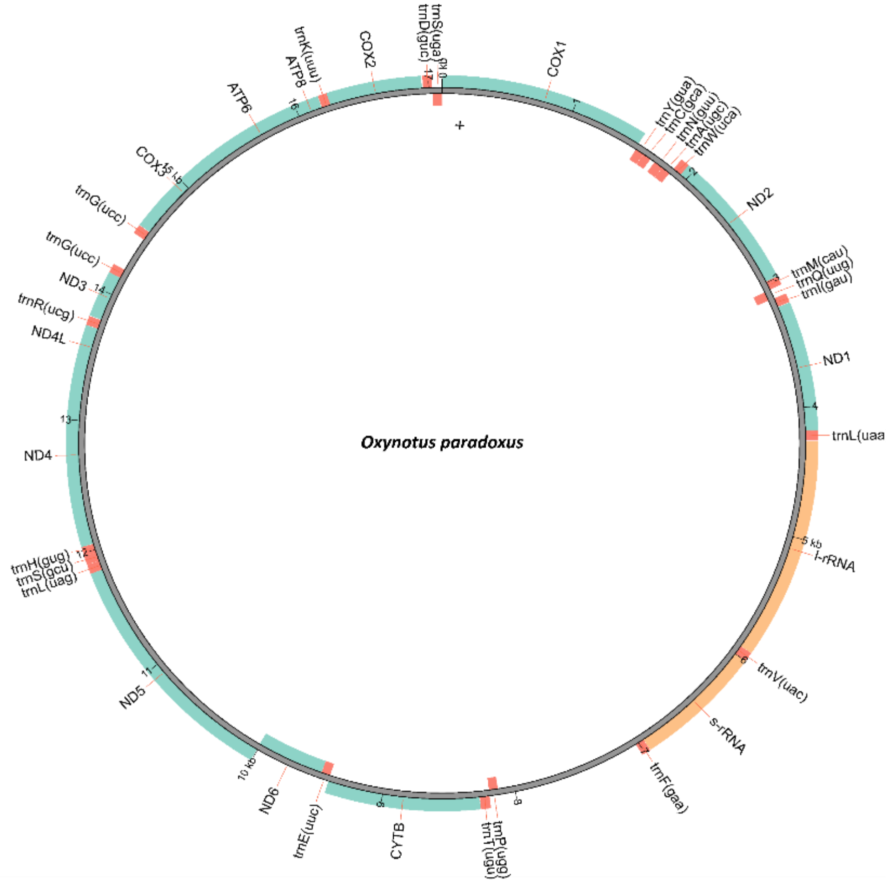

Figure 9.2 Mitogenome map of *Oxynotus paradoxus*.

CIIMAR ethical committee and CIIMAR Managing Animal Welfare Body (ORBEA), according to the European Union Directive 2010/63/EU, approved the present work. *O. paradoxus* specimen was deposited at Centre of Marine Sciences, Universidade do Algarve (CCMAR) (contact person: Sofia Graça Aranha, sgramos@ualg.pt) with voucher name 4.93.

The extracted genomic DNA was sent to Macrogen (Seoul, South Korea) for Illumina Paired-End (2x150bp) library construction and whole genome sequencing on a Novaseq6000 platform. Trimmomatic (version 0.38) was used to remove adapters in the raw PE sequencing reads, using parameters leading and trailing set to 5, minlen of 36, and a “sliding window” set to 4bp with the required quality of 15 (Bolger et al., 2014). The mitogenome was assembled and annotated with MitoZ (version 3.4) (Meng et al., 2019), using the clean PE reads and default parameters. Genome coverage information was obtained by running BBMap (BBMap Guide - DOE Joint Genome Institute) (splice-aware global aligner for DNA and RNA sequencing reads) and a graphical display produced using the genome browser Artemis (Carver et al., 2012)

(Appendices 9.1 and 9.2) and following the Generating Sequencing Depth and Coverage Map for Organelle Genomes protocol (Ni et al., 2023) (Appendix 9.3).

All available and verified Squalomorphii mitogenomes (n=43) were downloaded from GenBank (accession date: 08/02/2023). Furthermore, four mitogenomes representing the shark orders Heterodontiformes, Orectolobiformes, Lamniformes, and Carcharhiniformes were also downloaded from GenBank as outgroup taxa. The 13 protein-coding genes of the downloaded mitogenomes were aligned with MAFFT (version 7.505) (Katoh and Standley, 2013). The resulting alignment was trimmed and concatenated with trimAL (version 1.2) (Capella-Gutiérrez et al., 2009) and FasConCAT-G (version 1.05.1) (Kück and Longo, 2014), respectively, resulting in a final alignment of 22 866 bp. The identification of partition-scheme, their best-fit nucleotide substitution models and Maximum Likelihood phylogenetic inference were conducted on IQ-TREE (version 1.6.12) (Nguyen et al., 2015; Kalyaanamoorthy et al., 2017).

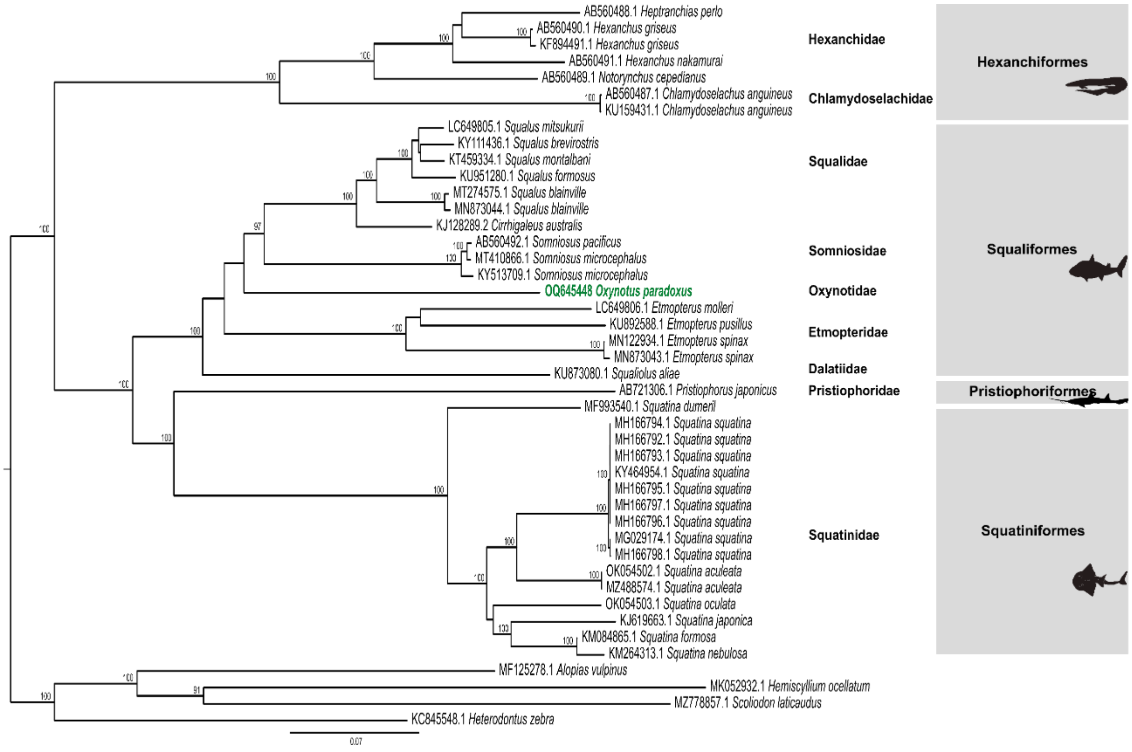

Figure 9.3 Maximum Likelihood phylogenetic inference obtained with the sequences of all protein-coding genes from the 43 verified and available Squalomorphii mitogenomes. Bootstraps above 90% are shown, above the nodes.

Ethics statement - The authors have read and followed the ethical requirements for publication in Data in Brief. The authors confirm the current work does not involve human subjects, animal experiments, or any data collected from social media platforms. CIIMAR ethical committee and

CIIMAR Managing Animal Welfare Body (ORBEA), according to the European Union Directive 2010/63/EU, approved the present work.

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Declaration of competing interests - The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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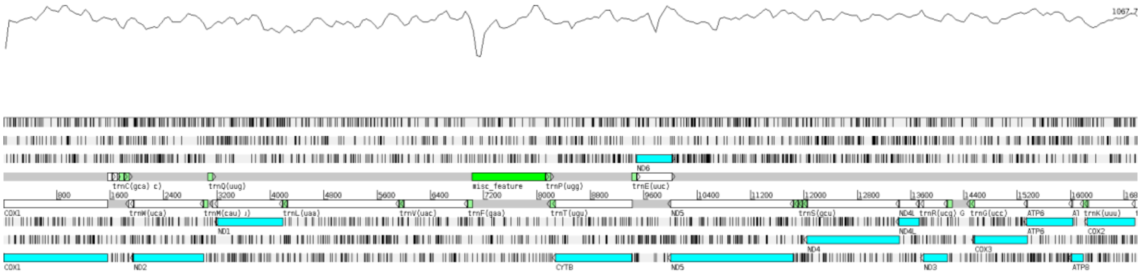
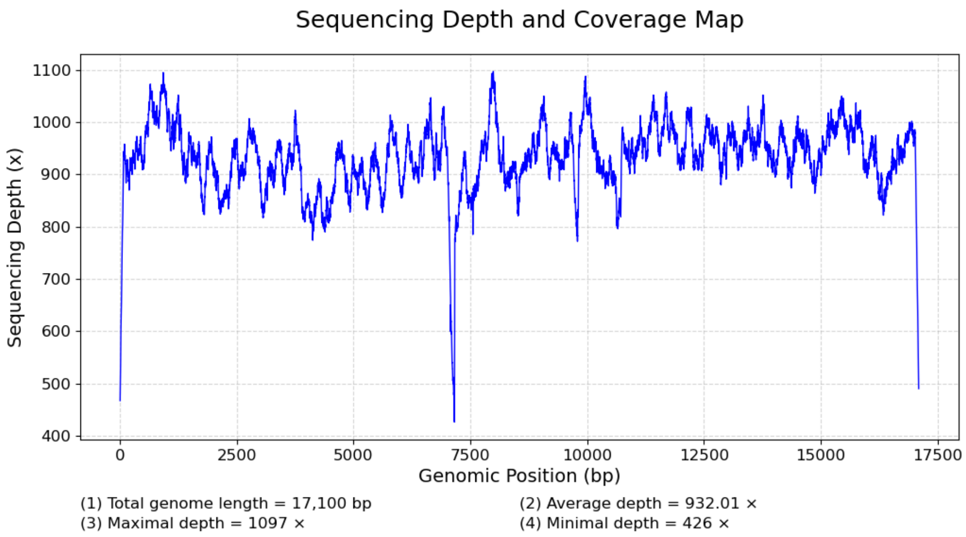
9.5 Appendices



Appendix 9.1 Read coverage depth map of the *Oxynotus paradoxus* mitochondrial genome.

Appendix 9.2 Genome coverage information of *Oxynotus paradoxus*.

Identification	Average fold	Length	Ref GC	Covered Percentage	Covered Bases	Plus Reads	Minus Reads	Read GC	Median Fold	Standard Deviation
Oxynotus paradoxus	935.3006	17100	0.3621	100.0000	17100	53659	53603	0.3618	940	66.12
Identification	Average fold	Length	Ref GC	Covered Percentage	Covered Bases	Plus Reads	Minus Reads	Read GC	Median Fold	Standard Deviation
Oxynotus paradoxus	935.3006	17100	0.3621	100.0000	17100	53659	53603	0.3618	940	66.12



(1) Total genome length = 17,100 bp
(2) Average depth = 932.01 ×
(3) Maximal depth = 1097 ×
(4) Minimal depth = 426 ×

Appendix 9.3 Sequencing depth and coverage map of *Oxynotus paradoxus.*

Chapter 10: THE COMPLETE MITOCHONDRIAL GENOME OF THE ENDEMIC IBERIAN PYGMY SKATE *NEORAJA IBERICA*, SÉRET, COSTA & BARO 2008 (ELASMOBRANCHII, RAJIDAE)

Gomes-dos-Santos, André; Machado, André; **Graça Aranha, Sofia**; Dias, Ester; Verissimo, Ana; C. Castro, L. Filipe, & Froufe, Elsa. 2021. The complete mitochondrial genome of the endemic Iberian pygmy skate *Neoraja iberica* Stehmann, Séret, Costa & Baro 2008 (Elasmobranchii, Rajidae). *Mitochondrial DNA B Resour*. 15;6(3):848-850. https://doi.org/10.1080/23802359.2021.1884030

Abstract

Skates, Chondrichthyes fishes from order Rajiformes, are the most species-rich group of all Batoidea. However, their phylogenetic relationships and systematics is still a highly discussed and controversial subject. The use of complete mitogenome has shown to be a promising tool to fill this gap of knowledge. Here, the complete mitogenome of the Iberian pygmy skate *Neoraja iberica* (Stehmann, Séret, Costa & Baro 2008) was sequenced and assembled. The mitogenome is 16,723 bp long and its gene content (i.e. 13 protein-coding genes, 22 transfer RNA, 2 ribosomal RNA genes) and arrangement are the expected for Batoidea. Phylogenetic reconstructions, including 89 Rajiformes and two outgroup Rhinopristiformes, recovered family Rajidae as monophyletic, further divided in the monophyletic tribe Rajini, sister to tribes Amblyrajini and Rostrorajini. The newly sequenced *N. iberica* mitogenome is the first representative of tribe Rostrorajini.

Keywords: Chondrichthyes, Elasmobranchii, Iberian Peninsula, Mitogenome, Phylogenetics

Within the Batoidea (skates, stingrays, sawfishes, electric rays and guitarfishes), the order Rajiformes (skates) is one of the most species rich (250 species so far described) despite its seemingly morphological stasis (Ebert and Compagno, 2008). Skates also tend to have very localized distributions, reflected in a high degree of endemism with several new species being described recently (Stevenson et al., 2004; Stehmann et al., 2008; Iglésias et al., 2010). Nevertheless, phylogenetic relationship inferences and systematics of the Batoidea remain controversial mostly due to low taxon sampling, unresolved/poorly supported topologies using either morphological and molecular data, and also incongruent morphological and molecular topologies (Douady et al., 2003; McEachran and Aschliman, 2004; Aschliman, 2011; Serra-Pereira et al., 2011; Aschliman et al., 2012; Rodríguez-Cabello et al., 2013; Gaitán-Espitia et al., 2016; Last et al., 2016b). Although the application of molecular approaches has been fundamental to understand the phylogenetic relationships and assert the systematics within Rajiformes, these are still under discussion with several interpretations available (Last et al., 2016a; 2016b). Consequently, from here forward we follow the nomenclature proposed by the most recent taxonomic/systematic review of Rajiformes (Last et al., 2016a).

Skates (Order Rajiformes) are among the most threatened groups of vertebrates and are particularly prone to overexploitation (Dulvy et al., 2014; Davidson et al., 2016). Thus, the inability to efficiently infer phylogenetic relationships and assert the systematics of the group is clearly a concern for fishing management and conservation planning. Most species have low fecundity, late sexual maturity and long generation times that hinder efficient population restocking and since skates' meat and gill rakers are valuable commercial resources, over-fishing has devastating effects in many populations (Serra-Pereira et al., 2011; Wannell et al., 2020). This is further aggravated by frequently bottom trawling bycatch of these organisms (Wannell et al., 2020). Complete mitogenomes have been successfully used for comparative studies and phylogenetic inferences in cartilaginous fishes (e.g., Inoue et al., 2010; Alam et al., 2014; Gaitán-Espitia et al., 2016; Gomes-dos-Santos et al., 2020). This is especially relevant in cartilaginous fish due to their slow mtDNA mutation rates that can hinder the efficient resolution of traditional partial mitochondrial markers (Martin, 1995; Gaitán-Espitia et al., 2016).

The Iberian pygmy skate *Neoraja iberica* (Stehmann et al., 2008) was recently characterized as an endemic species from the south coast of Portugal and Spain (Stehmann et al., 2008; Serra-Pereira et al., 2011). The very few studies published to date on this species relied on morphological analyses, with only a few partial COI mitochondrial sequences being available. Furthermore, no mitogenomes for *Neoraja* genus are currently available. Therefore, producing a complete mitogenome is a timely and valuable resource.

Liver and muscle tissue samples from a *N. iberica* specimen were collected and stored in 96% ethanol. The specimen was captured in June 2020 off the south coast of Portugal (between Lat: 36.777.215, Long: -8.817.514 and Lat: 36.779.223, Long: -8.612.814), onboard of a commercial crustacean bottom trawler and at depths of approximately 500 m. Morphological identification was performed onboard. The specimen is stored at the Interdisciplinary Centre of Marine and Environmental Research (specimen code Neoib001). Liver tissue was used for genomic DNA extraction and whole-genome sequencing of 150 bp paired-end (PE) reads were obtained using Hiseq X Ten machine at Novogene Europe.

Complete mitogenome assembly and annotation was obtained using MitoZ v2.3. (Meng et al., 2019). Annotation was further validated by comparison with mitogenomes from other members of the Family Rajidae available on NCBI, including representatives of the two rajid tribes: Amblyrajini and Rajini. For the phylogenetic analysis, all available mitogenomes from species from Family Rajidae and from two species from Order Rhinopristiformes (Accession numbers NC_023951.1 and NC_022821.1) were retrieved from GenBank (03/12/2020). The 13 protein-coding genes (PCG) were specimenly aligned using MAFFT v7.453 (Katoh and Standley, 2013) and afterwards concatenated using FASconCAT-G (https://github.com/PatrickKueck/FASconCAT-G) (final length: 11,435bp). The best partition-scheme best fitted evolutionary models and Maximum Likelihood (ML) phylogeny were obtained using IQ-TREE (v.1.6.12) (Nguyen et al., 2015; Kalyaanamoorthy et al., 2017). The newly sequenced complete mitogenome of *N. iberica* is available in GenBank under the accession number MW377218. The length of the mitogenome is 16,723 bp and the gene composition and arrangement is, as expected for Batoidea, the typical for vertebrate mtDNA: 13 PCGs, 22 transfer RNA, 2 ribosomal RNA genes, with a 14 tRNA, 2 rRNA all PCG (except NAD6) being present in the heavy strand (Satoh et al., 2016).

The resulting phylogenetic tree (Figure 10.1) recovered Family Rajidae as monophyletic, further divided in the monophyletic tribe Rajini, sister to tribes Amblyrajini and Rostrorajini. The newly sequenced *N. iberica* represents the first mitogenome sequenced Rostrorajini taxa. The present study highlights the importance of increasing the sampling and mitogenome sequencing of

Rostrorajini, as well as other skates, to clarify the phylogenetic relationships within the most species-rich group of Chondrichthyes.

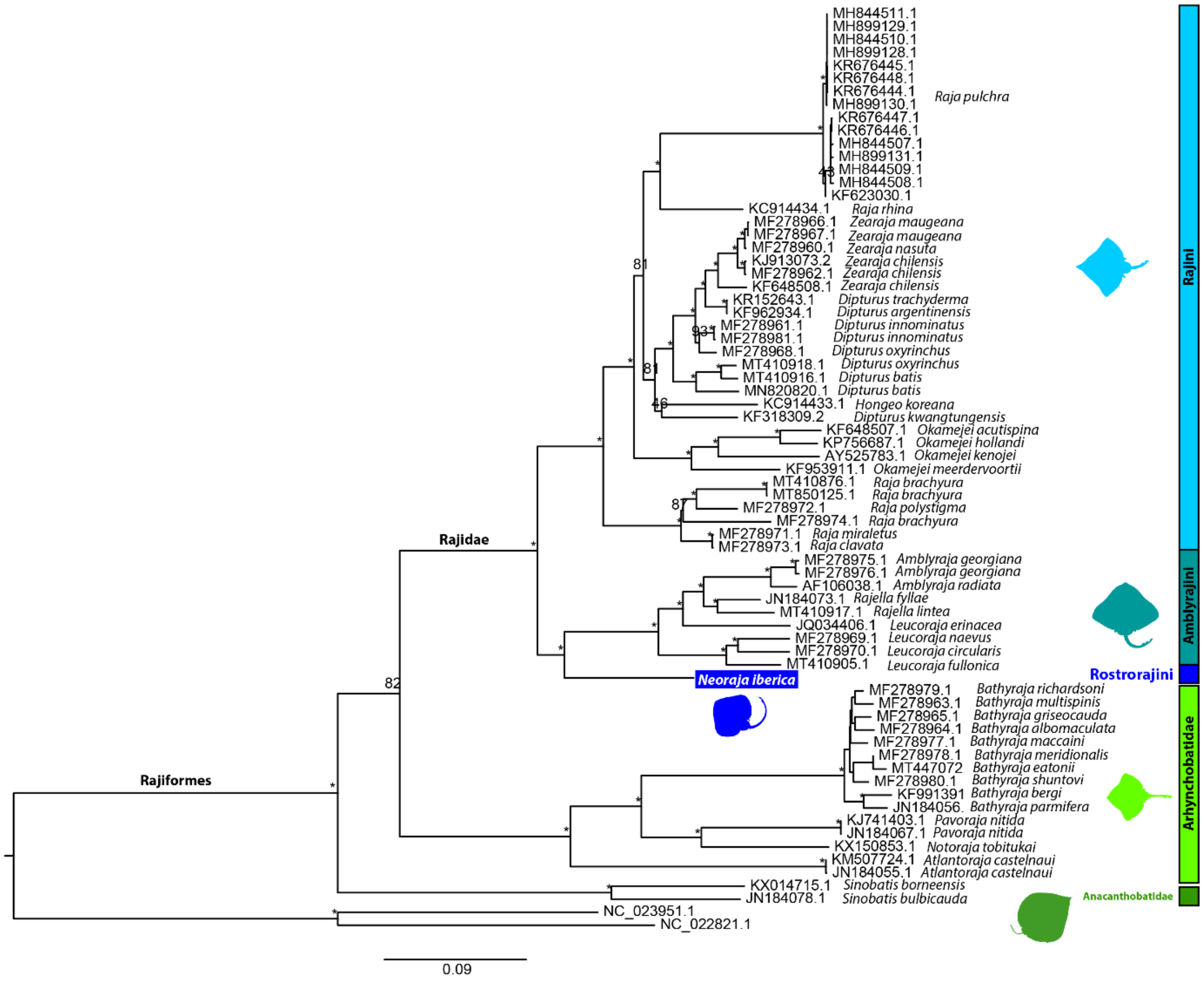


Figure 10.1 Maximum likelihood phylogenetic tree based on concatenated sequences of 13 protein-coding genes from 89 Rajiformes and two outgroup Rhinopristiformes mitogenomes. GenBank accession numbers are presented before species names. The * above the branches indicate both bootstrap support values above 95%.

Acknowledgements - This work was funded by the Project The Sea and the Shore, Architecture and Marine Biology: The Impact of Sea Life on the Built Environment (PTDC/ART-DAQ/29537/2017) from FCT/MCTES through national funds (PIDDAC) and co-financing from the European Regional Development Fund (FEDER) POCI-01-0145-FEDER-029537, in the aim of the new partnership agreement PT2020 through COMPETE 2020 – Competitiveness and Internationalization Operational Program (POCI) and by FCT - Foundation for Science and Technology UIDB/04423/2020, UIDP/04423/2020 which also supported A.G.S. (SFRH/BD/137935/2018), S.G.A (SFRH/BD/147493/2019) and A.V. (DL57/2016). Additional funding was provided by FEDER Funds through the Operational Competitiveness Factors Program COMPETE and by national funds through FCT within the scope of Project PTDC/ASP-PES/28053/2017.

Disclosure statement - The authors declare no conflict of interest.

Data availability statement - The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession number MW377218. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA694536, SRS8105111, and SAMN17526303, respectively

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Chapter 11: GENERAL CONCLUSIONS AND REMARKS

The results presented in this doctoral study addressed the main objective and specific goals proposed, which aimed to evaluate the impacts of bottom trawling activities in the condition and survival rates of DSE off the southern Portugal. It was seen that despite the good nutritional condition of DSE these are negatively impacted by crustacean bottom trawling because of their high bycatch rates, vulnerability to fishing, high post release mortality and stress rates. Mitigation strategies and monitoring tools adapted for DSE were provided. Further aspects of ecology, biology and genetics of two rare or poorly studied DSE are provided. Together these may not only contribute for decreasing the interactions and negative impacts that bottom trawlers have on DSE species, improve the management strategies to be adopted for these species and improve the scientific knowledge available for these species of conservation concern.

Insights into bycatch rates, mortality, and stress levels of DSE were derived from *in situ* catch data, where a pressing issue is highlighted, as these species are frequently caught in large numbers, face high mortality and seemingly high stress rates. Crustacean trawling, especially when conducted at non-permitted depths (i.e., below 800 m; Regulation 2016/136) targeting the deep-sea shrimp *Aristaeopsis edwardsiana*, was responsible for the highest bycatch biomass of DSE in comparison with permitted depths (< 800 m). In some hauls, sharks and skates, including vulnerable and data deficient species such as *Deania* spp., *Etmopterus pusillus*, and *Dipturus nidarosiensis*, account for up to 60% of the total catch biomass in the Southwest coast of Portugal. Furthermore, DSE are preferentially distributed in certain depths; some are commonly found at depths < 800 m, for instance *Galeus melastomus* and *Etmopterus spinax*, while others most commonly found at depths greater than 800 m like *Centroselachus crepidater* and *Deania calceus*. The majority of the frequently caught DSE species present a grouping behaviour, since they were consistently caught in numbers higher than three specimens per haul like *Galeus* spp., *Etmopterus* spp., *Scymnodon ringens* and *Deania* spp., while those less frequent species were generally caught in numbers lower than three specimens per haul like *Dipturus* spp., *Centrophorus* spp., and *Dalatias licha*.

Following capture, mortality rates among DSE are alarmingly high, with 95% of specimens found dead or in extremely poor condition by the time they are hauled aboard, effectively reducing their chances of post-release survival. Among the four studied deep-sea sharks (i.e., *E. pusillus, E. spinax, G. melastomus* and *S. ringens*), *S. ringens* have higher chances of mortality in this fishery. Likewise, smaller specimens and those caught in fisheries using smaller codend mesh sizes of 55 mm (targeting shrimps and prawns) instead of 70 mm (targeting the Norway lobster), and from hauls with more catch (i.e., heavier codend weight) are especially vulnerable. Furthermore, larger temperature differences between the surface and bottom waters also increase significantly the mortality rates. These sharks also seem to experience substantial physiological stress during capture as indicated by their plasma concentrations of potassium, urea, and magnesium and as compared with the available literature. Potassium, urea and magnesium were suggested as potential indicators of mortality for the species *E. pusillus* and *G. melastomus*. Additionally, high lactate levels in species such as *G. melastomus* suggest that even those few sharks that are released alive and in good conditions face a high risk of post-release mortality.

The DSE are in general tertiary consumers or so-called meso-predators, assimilating varied prey from the taxa crustaceans, teleosts and cephalopods from the study area or nearby regions. Stable isotope analyses showed that shrimps (which included commercially important species) represented *ca.* 30% of the diet of the species *E. pusillus, G. melastomus* and *S. ringens*, which may justify their high frequence in the bycatch of this fishery. This was further supported by the sRD ratios which reflected recent feeding activities, suggesting overlap with crustacean bottom trawling in the southern coasts of Portugal. A comparison of niche widths and overlaps between the two studied regions—South and Southwest—revealed some differences. In the South, DSE exhibited larger niche sizes and greater overlap between sympatric species. In contrast, in the Southwest, species presented smaller niches and reduced overlap. For instance, *G. melastomus* and *G. atlanticus* showed a core trophic niche 5 or 2 times wider in the South than in the Southwest, respectively. The overlap of *Galeus* spp. and *E. pusillus* in the South was the highest (71% and 61%) while in the Southwest their overlap was non-existent. Wider trophic niches and high overlap indicate that the species in the South share a wide variety of resources while those in the Southwest presenting smaller niches and low overlap, suggest reliance on a smaller variety of prey and resource partitioning. A contrasting pattern was observed for the species *S. ringens*, which in the South presented a smaller niche and no overlap with the sympatric species whilst in the Southwest presented a larger niche and overlap with the other DSE. In fact, *S. ringens* was the only evaluated DSE species that presented differing trophic roles acting both as meso- and top-predator within the same area but at different sampling periods and also between studied areas within the same sampling periods. Ontogenetic and sexual differences were spotted within specimens of the same species in the same studied area without clear patterns. Sometimes immature presented larger (e.g., *E. pusillus* and *D. nidarosiensis*) or smaller (e.g., *D. calceus*) niches than mature specimens. The same occurred for sexual differences, where females presented wider (e.g., *E. pusillus* and *G. melastomus*) and smaller niches (e.g., *E. spinax* and *D. oxyrinchus*) in comparison to males. These results suggests that some DSE have evolved a trophic plasticity under different environmental variability and disturbance levels (e.g., frequency and strength of upwelling events, habitat features, anthropogenic pressures), likely without impairment of their nutritional conditional as seen by their good nutritional status through the sRD ratios. Further studies evaluating resources abundance and more in-depth dietary data on the evaluated DSE are necessary to indicate their dietary preferences. However, the complexity and variety of the DSE behaviours in relation to their trophic ecology highlights the need for species-specific studies integrating spatial, seasonal, biological, and ecological factors through standardized and multidisciplinary approaches.

Measures to monitor DSE bycatch were proposed through the development of an integrated electronic monitoring and reporting system (iEMR), which allowed to monitor remotely the bycatch, identifying 2,195 specimens of 11 species of DSE (including chimaeras), through the analysis of video imagery collected onboard. The majority of specimens were successfully identified to genus or species level with only *ca*. 1% not identified due to camera's connection issues or physical barriers blocking the view (e.g., fishers body part, animals overlapping). The iEMR demonstrated a high level of effectiveness, with reliable species identification, but faced challenges in distinguishing species with subtle morphological differences, such as those congeners (e.g., *G. melastomus* and *G. atlanticus; D. calceus* and *D. profundorum*) which still requires manipulation of specimens to differentiate between species, and even so, are still increasingly challenging. This system proved successful in achieving the proposed goals and have potential to be escalated to other fisheries and species of interest, even to monitor TAC regulated species which are under landing obligations such as skates. However, due to the constraints in reaching species level at most times, it is believed that *in situ* monitoring by onboard observers is still required if the objective is to access species-specific data. An alternative would be to include congener species under the same regulations so that EM could be used to identify those species at least up to genus level, hence still demonstrating potential to support more sustainable fishing practices and contribute to better management of marine resources.

Mitigation of the impacts on DSE is provided through a handling protocol, which highlights important handling techniques for fishers to employ onboard, such as that to avoid injuries, limit air exposure, reduce mortality, and preventing additional stress by ensuring the DSE are either quickly discarded or kept in shaded, cooler areas. The importance of the handling protocol lies in its potential to increase the survival rates of these species, thereby contributing to the overall preservation of deep-sea ecosystems. By implementing better handling practices and reducing at-vessel and post-release mortality, fishers may aid in the mitigation of the long-term population declines of DSE caused by bycatch. The protocol also serves as an educational tool for fishers, with accurate illustrations of DSE from Portugal which help with the identification of the species caught, and by raising awareness of the critical role they play in reducing the impact of fishing on these vulnerable species and promoting more sustainable fishing practices.

Progress on the understanding of rare and poorly studied DSE, specifically *Oxynotus paradoxus* and *Neoraja iberica* were also provided. For *O. paradoxus*, a new maximum depth record of 1,238 m was reported, along with novel insights into its biological traits, including intraspecific morphometric variability and ecophysiological conditions. To the best of my knowledge, this study is the first to provide information on the maturity stage of a female *O. paradoxus* where an adult female specimen with a 65 cm TL presented a maturity stage III and was in a depth stratum (1,238 m) distinct of the three other *O. paradoxus* juveniles (~753 m) suggesting a potential for ontogenetic segregation. The relatively small area in which those specimens were caught in relation to the total area surveyed suggests a sparse distribution. Insights on feeding behaviour were provided from hepato-gonadosomatic and nucleic acid indices to which, because of the low estimates, suggest a deprecated nutritional condition for the adult female caught at the greatest depth. The differences found in morphometry between this study and others from the literature may result from intraspecific differences or from variability in the sample storage methods and protocols, hence standardized procedures between studies are encouraged. Additionally, the first complete mitochondrial genome for *O. paradoxus* was sequenced, marking a critical resource for monitoring genetic diversity and phylogenetic studies within the Oxynotidae family. For *N. iberica*, the complete mitochondrial genome was also sequenced, filling a key gap in the species' genetic data. This genomic information enhances understanding of the evolutionary history of *N. iberica* within the Rajidae family, supporting more accurate classification and phylogenetic positioning.

Finally, the outreach activities and other outputs mentioned in the "Outputs" section were designed to engage a diverse range of stakeholders, including fishers, managers, researchers, children, and the general public. These activities underscored the fact that DSE are not only highly unfamiliar to the general public but also to fishers, who encounter these species in their daily activities. Unlike iconic and widely recognized species such as the manta rays (*Manta spp.*) or the great white sharks (*Carcharodon carcharias*), DSE are rarely encountered by the general public because they inhabit remote, deep-sea environments and are typically never landed. The lack of familiarity, especially by the crustacean trawling fishers, extends to critical areas such as the regulations governing DSE and appropriate handling procedures, revealing a significant gap in knowledge. Addressing this gap is crucial, as it directly affects the sustainable management and conservation of DSE. Targeted interactions bridging the scientific community to fishers and other stakeholders, could ensure each one is equipped with the knowledge and tools necessary for the effective management and conservation of DSE. These interactions could include (but not only) tailored workshops, hands-on training, accessible informational materials such as the handling protocol, media campaigns, and public engagement (e.g., through educational activities), each designed to meet the specific needs and realities of each group of stakeholders. These approaches not only support the objectives of this study but also contribute to the broader goal of protecting vulnerable DSE.

This study highlighted that DSE from the southern coasts of Portugal; despite sharing the same habitats, resources and life-history traits, are seemingly different in their responses to fishing pressures, and this is true even for congener species. Hence, when studying DSE's and depending on the goals of the research, a species-specific approach is encouraged, and where possible, taking into consideration the maturity stages, sex and temporal variability. The vulnerability of DSE to crustacean bottom trawling activities, combined with their conservative life-history traits and the poor knowledge we have (and share) about those species is a matter of concern. However, the implications of the present findings for the conservation of the DSE populations and the food webs from these areas are unknown and require a deeper investigation. Hence a precautionary approach is suggested where the improvement/sharing in knowledge and avoidance of the bycatch of these species should be prioritized among other solutions thoroughly discussed in the chapters of this thesis. Briefly, EM tools would aid in enhancing our understanding of DSE population structures and dynamics, by remotely monitoring the bycatch of DSE. Adjustments in the current regulations, e.g. grouping congeners species into the same regulatory measures, and adding deep-sea species (e.g. *Mitsukurina owstoni)* in the deep-sea shark list (regulation 2024/157), would help regulate their bycatches more efficiently. Solutions such as bycatch excluder devices, gear modifications, and spatio-temporal closures are highly encouraged to decrease the bycatch of DSE. Additionally, improved handling and fishing practices would help to reduce the mortality of the small proportion of individuals (approximately 5%) that might survive the catch-and-discard process. Finally, outreach activities would help spread the knowledge acquired to the specific stakeholders. These practices should be actively proposed, discussed and adopted especially by fishers and managers who regularly deal with DSE. In combination, these approaches may establish a robust foundation for further research, conservation initiatives, and regulatory adjustments aimed at safeguarding DSE populations while promoting sustainable fishing practices for the benefit of all, the Earth, Oceans, marine resources and present and future generations.

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