

UNIVERSIDADE DE LISBOA  
FACULDADE DE CIÊNCIAS



**Ciências  
ULisboa**

**Effects of spatial and temporal scale on the assessment of biodiversity and  
ecosystems health, under the scope of the Marine Strategy Framework Directive**

*“Documento Definitivo”*

**Doutoramento em Ciências do Mar**

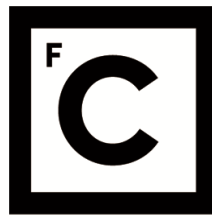
Maria Inês Sousa Machado

Tese orientada por:

Professor Doutor José Lino Oliveira Costa

Professor Doutor Henrique Nogueira Cabral

Documento especialmente elaborado para a obtenção do grau de doutor



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Documento especialmente elaborado para a obtenção do grau de doutor

Esta dissertação de Doutoramento foi financiada pela Fundação para a Ciência e Tecnologia através da Bolsa de Doutoramento com a referência: PD/BD/135065/2017



This PhD dissertation was supported by FCT –  
Fundação para a Ciência e Tecnologia, I.P, with a  
PhD scholarship attributed under the PhD  
Programme Earthsystems with the reference:  
PD/BD/135065/2017



# Acknowledgements

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*First and foremost, I would like to thank my family. Without knowing (or at least without being aware...), they hold their breath and dove into these studying years with me, bearing all its consequences, trusting me and always seeing the finish line as the main target! My grandmothers for the inspiration, my mother for the continuous support and my father for the pragmatic views that, apparently, never fall short. My uncles also for the moral strength, fierce debates and (many) moral meals.*

*My family/ friends that were also pure strength behind me, never accepting my tiredness as defeat and pointing out the light at the end of the tunnel whenever I failed to see it. Isa, for the fierce support and continuous energetic reminders, Formi for the perspective and strength, Miguel for being present and redundant throughout the entire process. A big thank you to them for putting up with my lame conversations and complaining!! They were always there and when my perspective failed, they all reminded me that the path is made forward and that everything was going to be all right. Guiga, for partnering in surf activities, that were especially important stress releases.*

*FCUL colleagues support was also important to my adaptation and to them I also leave a big thank you, for the support, cooperation, and teaching; specially Célia, Miguel, and Valter, and in a later stage Tiago, without forgetting my fellow PhD students; laughing with all of them made this period a lot easier and bearable! Célia for supporting my lame moments, for the academic insight and stimulating my scientific independence: Dona Dolores outfits will never be forgotten!!*

*To my supervisors, I would like to thank the guidance, the continuous relevant corrections, and their support in supplying training whenever necessary. And it was! Their availability, patience and effort made this journey possible with no major bumps – and yet, with the complete learning experience. To them I would like to express my gratitude: Henrique made sure I was welcomed into FCUL and accompanied me during the entire scientific journey, and Lino for continuing Henrique support in FCUL, and for being always available and helpful, even in these “crazy” pandemic times. To them, I also owe the possibility of participating in project PADDLE that contributed to expanding my knowledge and cooperation ability! I truly hope I was up to the challenge!*

*I am also grateful to Diana, Alberto and Marta for R tutorials, solutions, suggestions, and exchange of ideas: learning R from scratch was easier with their help and whoever learned knows... that this is a great debt!*

*I would like to thank IPMA for providing me with the possibility of collaboration in this dissertation work through Dr<sup>e</sup>. Ivone Figueiredo group. Within this group, I am especially thankful to Dr<sup>a</sup>. Teresa Moura for her scientific support, correction, and insightful comments through the entire period of the collaboration and for making sure all deadlines were achieved.*

*Last but not the least, I would also like to thank all the Earthsystems PhD programme colleagues, for the scientific and social interdisciplinarity! The international environment and interdisciplinarity contributed to keeping a lighter and healthy perspective of the PhD path, and of course, the official Friday motivational beers were also very helpful!!*

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# *Abstract*

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The environmental assessment and monitoring of marine systems aim is to reveal the condition of ecosystem components, assess the effects of anthropogenic pressures and evaluate the effects of management measures. In Europe, the Marine Strategy Framework Directive aims to assess and manage the quality of the European seas to achieve Good Environmental Status. This directive uses indicators to analyse the environmental status and anthropogenic pressures on the European seas. Even though the Directive provides an unparalleled comprehensive set of information, several issues are still undermining the correct assessment, masking the effects of anthropogenic activities and natural variability (e.g., climate change.). To overcome such difficulties the present dissertation thesis has addressed issues such as: 1) the implementation of the directive and the congruency of the scales used by the Member States (MSs) to report biodiversity indicators; 2) the use of distinctive spatial scales to assess the time series of biodiversity indicators, focusing on indicators addressing the status of sensitive fish species with no commercial value; 3) disaggregation of the spatial and temporal scales used in food webs assessment focusing on fish community indicators to identify the scales that detect prevailing patterns; and 4) food webs reporting in the NE Atlantic and the capability of food webs indicators detecting the effects of natural and anthropogenic drivers. Results showed that MSs used a wide range of criteria, metrics, and scales, which were selected independently and led to wide incongruencies in reporting. The evaluation of scales for biodiversity and food web indicators highlighted that spatial scales need to be downsized to detect species and community patterns. Downscaling allowed showing local and regional areas where indicators presented significant variability and were below the threshold, revealing that the spatial scales currently used are masking the effects of existing drivers. To validate these results, spatial scales were disaggregated for the assessment of the Portuguese continental waters, showing that smaller sized scales based on depth, sector and 1000km<sup>2</sup> units reveal estimates below the established threshold for fish communities in the South coast and on intermediary depths of the Southwest of the continental platform. On the contrary, temporal scales explained extremely low variance. Lastly, this study showed that anthropogenic drivers significantly influencing food web trends for fish elements were fishing and climate anomalies in the southern Bay of Biscay and Iberian coast, while eutrophication and chemical contamination had effects on trends in the Celtic seas and the North Sea. Results allowed to establish a direct relation between anthropogenic effects and food web patterns. Nonetheless, these were constrained since food webs data is still limited at relevant scales.

**Keywords:** Good environmental status, ecosystem-based assessment, detection of anthropogenic pressures, spatial boundaries, temporal scales.

## *Resumo*

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A avaliação ambiental e a monitorização do ambiente marinho têm como objetivo esclarecer a condição dos diferentes componentes do ecossistema, identificar os impactos das atividades antropogénicas ou avaliar os efeitos das medidas de gestão implementadas. Na União Europeia, a Diretiva Quadro Estratégia Marinha tem como objetivo avaliar e gerir a qualidade das áreas marinhas europeias. Esta Diretiva reuniu um leque de informação abrangente e inexistente até à data, no entanto, alguns aspetos poderão mascarar os efeitos das atividades antropogénicas ou da variabilidade natural existente (e.g., alterações climáticas). Para ultrapassar estes obstáculos a presente dissertação abordou os seguintes aspetos: 1) o nível de implementação e as escalas utilizadas pelos Estados Membros para reportar indicadores de biodiversidade; 2) a utilização de diferentes escalas espaciais e temporais na avaliação de indicadores de biodiversidade, focando na avaliação de espécies de peixes sensíveis; 3) a desagregação dos efeitos das escalas espaciais, temporais e das componentes ambientais – profundidade e temperatura – na variabilidade dos indicadores de cadeias tróficas; e 4) a implementação de indicadores de cadeias tróficas no Atlântico NE e a sua capacidade de deteção de efeitos das pressões antropogénicas e variabilidade natural, para detetar alterações no funcionamento do ecossistema. Os resultados demonstraram uma baixa cooperação entre Estados Membros e a utilização de um elevado número de indicadores, métricas e escalas que desencadearam várias incongruências na implementação. A avaliação das escalas utilizadas para estimar indicadores de biodiversidade e cadeias tróficas revelou que as escalas espaciais deveriam ser mais detalhadas para detetarem padrões locais e regionais ao nível das espécies e comunidades avaliadas. Ao aplicar as escalas identificadas à avaliação na plataforma continental portuguesa foi possível demonstrar que a utilização de escalas espaciais menores, definidas através da profundidade, sector ou unidades igualmente distribuídas de 1000km<sup>2</sup>, permite identificar indicadores abaixo dos limites estabelecidos em zonas da costa Sul e de zonas de profundidade intermédia no Sudoeste da plataforma continental portuguesa. Ao contrário das escalas espaciais, as escalas temporais explicaram uma variabilidade residual. Por último, este trabalho demonstrou que a implementação de indicadores de cadeias tróficas no Atlântico Nordeste é bastante incongruente no que diz respeito aos indicadores e elementos dos ecossistemas abordados. As pressões antropogénicas que influenciaram significativamente estes indicadores foram a pesca e alterações climáticas na Baía da Biscaia e plataforma Ibérica, e a eutrofização e a contaminação química no Mar Celta e no Mar do Norte. Estes resultados permitiram estabelecer uma relação entre as pressões e os padrões obtidos pela avaliação de cadeias tróficas, no entanto, é de salientar que apresentam vários constrangimentos, pois a avaliação de cadeias tróficas não foi feita para escalas relevantes.

**Palavras-chave:** Bom Estado Ambiental, avaliação com base no ecossistemas, detecção de pressões antropogénicas, escalas espaciais, escalas temporais.

## *Resumo alargado*

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A avaliação ambiental e a monitorização dos sistemas marinhos têm como objetivo esclarecer a condição dos diferentes componentes do ecossistema, identificar os impactos das atividades antropogénicas ou avaliar os efeitos das medidas de gestão implementadas. Nesse sentido, este processo requer a utilização de indicadores ambientais que consistam em métricas replicáveis e quantitativas que possam ser sistematicamente aplicadas no tempo e no espaço. Na União Europeia, a Diretiva Quadro Estratégia Marinha (DQEM) tem como objetivo avaliar e gerir a qualidade das áreas marinhas europeias para que estas atinjam o Bom Estado Ambiental (BEA) até 2020 (atualmente atualizado para 2024). Esta diretiva levou à implementação de um processo adaptativo de avaliação que utiliza onze descritores de estado ou de pressão para avaliar o estado dos ecossistemas e as pressões humanas exercidas no meio marinho, através da utilização de indicadores ambientais. Apesar da Diretiva ter reunido um leque de informação muito abrangente e inexistente até então, alguns aspetos têm vindo a impedir a realização de uma avaliação correta e poderão estar a mascarar os efeitos reais das atividades antropogénicas ou da variabilidade natural existente (e.g. alterações climáticas). Com o objetivo de contribuir para ultrapassar estes obstáculos, a presente dissertação de doutoramento examinou os diferentes descritores de biodiversidade (D1 - Biodiversidade, D2 - Espécies não-indígenas, D3 - Peixes e moluscos explorados para fins comerciais, D4 - Cadeias tróficas, D6 - Integridade dos fundos), abordando os seguintes temas: 1) identificar o nível de implementação e as escalas temporais utilizadas pelos Estados Membros para reportar indicadores de biodiversidade no primeiro ciclo de implementação da Diretiva; 2) analisar a utilização de diferentes escalas espaciais e temporais na avaliação de indicadores de biodiversidade, focando na estimativa de espécies de peixes sensíveis sem valor comercial, D1C2 - A abundância da população da espécie não é negativamente afetada pelas pressões antropogénicas; 3) desagregar os diferentes componentes dos indicadores de cadeias tróficas utilizados para avaliar o estado das comunidades de peixes, para compreender de que forma as escalas espaciais e temporais e as componentes ambientais – profundidade e temperatura – explicam os padrões de variabilidade destes indicadores (D4C2 - O equilíbrio da abundância total entre os grupos tróficos não é afetado negativamente pelas pressões antropogénicas. e D4C3 - A distribuição dos indivíduos por tamanho em todo o grupo trófico não é negativamente afetada por pressões antropogénicas); e 4) avaliar a implementação de indicadores de cadeias tróficas no Atlântico Nordeste durante o 2º ciclo da Diretiva e a sua capacidade de deteção de efeitos das pressões antropogénicas e da variabilidade natural, para detetar alterações na estrutura e funcionamento do ecossistema. Os resultados demonstraram que durante o primeiro ciclo da Diretiva, os descritores de biodiversidade foram implementados de uma forma incongruente pelos diferentes Estados-Membros,

no que diz respeito ao número e tipologia de métricas utilizadas, às espécies e habitats avaliados e em relação às escalas espaciais e temporais utilizadas. A cooperação entre os Estados-Membros foi insuficiente e a utilização de um elevado número de indicadores, métricas e escalas desencadearam várias incongruências na implementação. A implementação variou de acordo com o Estado-Membro e com os elementos taxonómicos avaliados. No que diz respeito à amplitude temporal utilizada, esta dependeu dos diferentes grupos e subunidade marinha analisada.

No que diz respeito à avaliação das escalas utilizadas para implementar indicadores de biodiversidade e de cadeias tróficas, foram utilizadas diferentes metodologias. Para testar as escalas de indicadores de biodiversidade foi utilizada a metodologia de *Breakpoint Analysis*, enquanto que, para testar os indicadores de cadeias tróficas, foram aplicados modelos gerais lineares (*Generalized Linear Models* (GLMs)). Nesta última metodologia, para além das escalas espaciais e temporais, foram também incluídas variáveis ambientais, tais como a temperatura e a profundidade. No entanto, os dois estudos revelaram que as escalas espaciais deveriam ser mais detalhadas para detetarem padrões significativos ao nível das espécies e comunidades avaliadas. No que diz respeito aos indicadores de biodiversidade, a redução das escalas espaciais permitiu identificar padrões locais e regionais de variabilidade com valores significativamente inferiores aos limites estabelecidos para a espécie *Microchirus variegatus* na costa Sul de Portugal. Embora não significativos, foi também possível identificar padrões populacionais distintos ao nível local da costa Sul de Portugal para as espécies *Pagellus erythrinus* e *Serranus hepatus* e ao nível do sector de Aveiro para *Argentina shpyraena*. A análise revelou ainda que os valores médios calculados para escalas abrangentes não permitem detetar estes padrões para esta espécie.

No que diz respeito aos indicadores de cadeias tróficas, foi elaborado um estudo no sentido de avaliar os efeitos da diminuição das escalas espaciais e temporais na deteção de impactes antropogénicos. Nesse sentido, foram testados quatro indicadores comumente utilizados para responder aos critérios estabelecidos pela DQEM - o Nível Trófico Marinho (MTL), o Nível Trófico Marinho com limites ( $MTL_{TL3,25}$  e  $MTL_{TL>4}$ ), a Proporção de Peixes Grandes (LFI) e a Abundância Média por Guilda Trófica (MATG). Os indicadores foram estimados a partir dos dados obtidos pelas campanhas de avaliação de stocks demersais, considerando seis escalas espaciais e quatro temporais e a adequabilidade das escalas foi comparada através de modelos aditivos generalizados. A diminuição das escalas espaciais permitiu detetar diminuições significativas dos indicadores de cadeias tróficas em relação aos limites estabelecidos e possibilitou compreender que as escalas apropriadas variam em função dos indicadores utilizados e de acordo com a sub-região em estudo: a Baía da Biscaia e plataforma continental Ibérica e o Mar Celta. O MTL apresentou diferenças significativas nas duas regiões de estudo quando os

modelos aplicados incluíam escalas locais e anuais. Já o cálculo do  $MTL_{TL>3.25}$  apresentou diferenças significativas quando o modelo utilizado incluiu escalas espaciais reduzidas, mas apresentou-se estável quando as escalas temporais dos modelos foram variadas. No Mar Celta, tanto o  $MTL_{TL>4}$  como o LFI foram explicados por modelos que utilizavam escalas locais e anuais. Por outro lado, na Baía da Biscaia, tanto o  $MTL_{TL>4}$  como o LFI foram adequadamente explicados por modelos que incluíam escalas geográficas ao nível da região e temporais ao nível do ano. A variabilidade do indicador MATG não foi afetada pelas escalas testadas. As análises permitiram verificar que as escalas temporais têm um papel menos preponderante do que as escalas espaciais na explicação da variabilidade existente. Ao aplicar as escalas identificadas pelos modelos à plataforma continental portuguesa foi possível validar a avaliação realizada pelo Estado Português. Esta estimativa demonstrou que escalas espaciais menores, definidas através da profundidade, sector ou unidades espaciais de 1000km<sup>2</sup> permitem identificar determinados indicadores de cadeias tróficas abaixo dos limites estabelecidos em zonas da costa Sul e de zonas de profundidade intermédia no Sudoeste da plataforma continental portuguesa. Ao contrário das escalas espaciais, as escalas temporais explicaram uma variabilidade residual para a plataforma continental portuguesa.

Por último, os trabalhos desenvolvidos no âmbito do quarto estudo, demonstraram que a implementação de indicadores de cadeias tróficas no Atlântico Nordeste é bastante incongruente no que diz respeito aos indicadores e elementos dos ecossistemas abordados. Nesta região, os indicadores de cadeias tróficas têm vindo a ser implementados principalmente pelo Reino Unido, Portugal e Espanha, ao contrário do que acontece com Irlanda e França. As pressões antropogénicas que influenciaram significativamente estes indicadores foram a pesca e alterações climáticas na Baía da Biscaia e plataforma continental Ibérica, e a eutrofização e a contaminação química no Mar Celta e no Mar do Norte. Estes resultados permitiram estabelecer uma relação entre as pressões e os padrões obtidos pela avaliação de cadeias tróficas, no entanto, é de salientar que esta relação apresenta vários constrangimentos: a avaliação de cadeias tróficas é incongruente em termo de critérios utilizados pelos Estados-Membros e a recolha de dados a escalas relevantes é atualmente muito limitada. Deste modo, a avaliação de cadeias tróficas não foi feita utilizando escalas ecologicamente relevantes, isto é, tendo em conta as escalas relevantes para avaliar as cadeias tróficas e as pressões antropogénicas existentes na área de estudo.

Os resultados obtidos neste trabalho contribuem para a harmonização e a calibração da implementação da DQEM, por parte dos Estados-Membros, no sentido de melhorar o nosso conhecimento sobre estado ambiental da biodiversidade e das cadeias tróficas no Atlântico Nordeste, e contribuir para a saúde dos ecossistemas marinhos.



## *List of research articles*

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This dissertation comprehends six chapters that correspond to an introduction, four scientific outputs, each corresponding to a peer-reviewed paper (chapters 2 to 5), and a conclusion chapter, summarizing major findings. Three scientific outputs were accepted and published in a peer-reviewed journal and one is under revision. These are the following:

### **Chapter 2**

Machado, I., Costa, J. L., Leal, M. C., Pasquaud, S., Cabral, H. 2019. Assessment level and time scales of biodiversity indicators in the scope of the Marine Strategy Framework Directive – A case study for the NE Atlantic. *Ecological Indicators*. 105: 242-253.

doi: <https://doi.org/10.1016/j.ecolind.2019.05.067>

### **Chapter 3**

Machado, I., Moura, T., Figueiredo, I., Chaves, C., Costa, J. L., Cabral, H. 2020. Effects of scale on the assessment of fish biodiversity in the marine strategy framework directive context. *Ecological Indicators*. 117: 106546.

doi: <https://doi.org/10.1016/j.ecolind.2020.106546>

### **Chapter 4**

Machado, I., Teixeira, C. M., Costa, J. L., Cabral H. 2020. Identifying assessment scales for food web criteria in the NE Atlantic: implications for the Marine Strategy Framework Directive, *ICES Journal of Marine Science*, fsaa217.

doi: <https://doi.org/10.1093/icesjms/fsaa217>.

### **Chapter 5**

Machado, I., Costa, J. L., Cabral, H. 2021. Response of food webs indicators to human pressures in the scope of the Marine Strategy Framework Directive for the NE Atlantic. *Frontiers in Marine Science*. 8:699566.

doi: <https://doi.org/10.3389/fmars.2021.699566>

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## *List of abbreviation*

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BBIC – Bay of Biscay and Iberian Coast

BD – Birds Directive

BPA – Breakpoint Analysis

CFP – Common Fisheries Policy

CS – Celtic Seas

EBA – Ecosystem based assessment

EBM – Ecosystem based management

EC – European Commission

EU – European Union

GES – Good Environmental Status

GNS – Greater North Sea

GAM – Generalized Additive Models

HD – Habitats Directive

HELCOM – The Convention on the Protection of the Marine Environment in the Baltic Sea Area of 1992 – the Helsinki Convention

ICES – International Council of the Exploitation of the Sea

LFI – Large Fish Indicator

MATG – Mean Abundance across Trophic Guild

MS – Member State

MSFD – Marine Strategy Framework Directive

MTL – Mean Trophic Level

$MTI_{TL>3.25}$  – Mean Trophic Index with TL higher than 3.25

$MTI_{TL>4}$  – Mean Trophic Index with TL higher than 4

OSPAR – The Convention for the Protection of the Marine Environment in the North-East Atlantic of 1992– the OSPAR Convention

The Bucharest Convention – The Convention for the Protection of the Black Sea of 1992

UN – United Nations

UNEP-MAP – The Convention for the Protection of Marine Environment and the Coastal Region of the Mediterranean of 1995 – the Barcelona Convention

WFD – Water Framework Directive

# 1 *General Introduction*

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**The world's oceans are facing unprecedented challenges due to the large-scale development of human activities that directly or indirectly have increased the risk of overexploitation and loss of vital habitats and biodiversity. Anthropogenic impacts are moving at a faster pace than management and protection measures and may rapidly push many ocean regions past critical tipping points of sustainability (Rouillard et al. 2018).** In the case of marine ecosystems, human-mediated degradations include pollution, overexploitation of marine resources (e.g., Pauly et al. 1998; Coll et al. 2016), habitat fragmentation or destruction, and more recently introduction of invasive species, climate change, and acidification (e.g., Link et al. 2015; Frazão Santos et al. 2020). Humans can have profound effects on marine ecosystems' health and many of these effects can act cumulatively (Halpern et al. 2008), and synergistically (Cabral et al. 2019). Furthermore, multiple stressors occurring in marine ecosystems have the potential to act in concert with climate change and cause even further degradation to the oceans' ecology and associated economies (Levin et al. 2010; Korpinen and Andersen 2016). Given the vast array of threats endangering marine ecosystems, simple management solutions have fallen short to achieve ecosystems sustainability.

Human related threats require a management response that moves beyond disjointed efforts and integrates ecosystem-based management (EBM) to increase adaptive capacity. EBM is an integrated approach to management that considers the entire ecosystem with the goal of providing the full suite of ecosystem goods and services that humans want or need (Levin et al. 2010). Adaptation practices aim to achieve objectives such as increasing resilience, flexibility, and climate-resistant populations, all to ensure the long-term delivery of ecosystem goods and services. Management actions employing adaptation commonly target the reduction of the human-related risk to the ecosystem. However, the specific actions needed to achieve effective adaptation are uncertain, and when a new management approach is employed, an examination is needed to verify how well the system is responding and to confirm if new approaches are needed. Effectively doing so requires the development of monitoring programmes and management plans. These are commonly implemented through robust indicators - quantitative measurements that provide an insight into the state of natural and socio-economic systems (Levin et al. 2010; Rombouts et al. 2013).

Due to the aforementioned concerns on the marine ecosystems' health and sustainability, in the last three decades, several worldwide and European agreements and policies have been implemented to ensure that the marine systems are in good environmental status and that resources are exploited sustainably through the implementation of an EBM. The United Nations sustainable development goals, the Regional Sea Conventions (RSC) – e.g., the Convention for the Protection of the Marine Environment in the North-East Atlantic (OSPAR), the Convention on the Protection of the Marine Environment in the Baltic Sea Area (HELCOM), etc. – and European policies such as the Habitats Directive (HD), the Birds Directive

(BD), the Water Framework Directive (WFD), the Marine Strategy Framework Directive (MSFD), and the EU 2020 Biodiversity Strategy have been developed to stimulate the health of marine ecosystems (United Nations 2015; Rouillard et al. 2018). All these initiatives have as a starting point, an initial assessment and the identification of information gaps concerning marine systems. As a result, in the last decades, there has been an attempt to tackle existing gaps. These actions led to an improvement of the areas covered by marine surveys, marine data has been made more accessible through freely accessible data portals (e.g., EMODnet, Copernicus, etc.), and the number of comprehensive monitoring programmes has increased. Importantly, this knowledge brought into light relevant aspects for key species and ecosystem dynamics, revealing several problems occurring in environmental assessments. These can arise from lack of representability in sampling, unaccounted natural environmental variability, unclear relation between anthropogenic pressure and environmental status, and the selection of appropriate spatial and temporal scales to be addressed in environmental assessments (e.g., Adams et al. 2017; Preciado et al. 2019).

### 1.1 Environmental Indicators

Indicators are widely used for assessing ecosystem status, the impacts of human activities, the effectiveness of management measures, and communication of complex anthropogenic impacts to a non-specialist audience (Shin et al. 2010). Bering this in mind, in this work, ecological indicators are defined as measures of the state of an ecosystem, i.e., the properties of a phenomenon, body, or a substance to which a magnitude can be assigned (Heink & Kowarik, 2010), consisting in quantitative measurements that stand for key attributes of interest. To quantify changes in indicators, appropriate metrics are developed to detect and measure a change in the indicator state, from a temporal baseline (Bedford et al. 2020; Rombouts et al. 2019). An indicator state is commonly compared to a baseline or a threshold to evaluate change, providing information on the status and trends of the studied components and attributes, usually those which address human and environmental impacts (Adams et al. 2017). When a suite of indicators is assembled, it can be used to assess ecosystem status, identify drivers of change, and develop management actions (Heim et al. 2021; Rice and Rochet 2005; Shin et al. 2010), supporting an Ecosystem-based Approach (EBA).

Environmental status indicators can range in complexity, from those that summarize responses from single-species indicators, through single-value indicators of diversity, to data ordinations, integrated indicators, and emergent properties of ecosystem models (Rice 2003). To provide reliable information on the effects of human and environmental pressures on communities, primary requirements are that ecological indicators are sensitive to the magnitude and direction of response to underlying attribute/pressure, have a basis in theory, be specific, be responsive at an appropriate time scale, and be cost-effective to monitor or to update (Rice 2003; Tam et al. 2017). In practice, the response of the indicator should be sensitive and specific to the pressures (Fulton et al. 2005; Rice and Rochet 2005;



Rombouts et al. 2013) but also distinguish environmental variability patterns (Henriques et al. 2008). Indicators can communicate incorrect information when the information content of the indicator has not been established rigorously. They can contribute to two types of errors: the indicator could fail to inform about events that have occurred in the real world or can provide false alarms about events that did not happen (Rice 2003). To tackle these issues, several authors have developed methods or frameworks to select the appropriate indicators for ecosystem assessment (e.g., Otto et al. 2018; Rice and Rochet 2005; Rombouts et al. 2013; Tam et al. 2017), however, marine ecosystems differ in the availability of data sets, monitoring capacity, human uses, and governance system, as well as in their ecological properties. All these factors may affect the utility of a specific indicator, making it obvious that no single suite of indicators is universally the best. In fact, ecosystems are considered so complex and unpredictable that suites of indicators are needed to give an adequate picture of their state (Rice and Rochet 2005).

Values of ecological indicators are commonly determined by metrics such as single species biomass indices (i.e., exploited species, endangered species), diversity indices, or by the relative numbers or biomass of species and/or body size categories in the community, among others (Rice 2003). An example of the latter type of indicators are food webs assessment metrics. Food webs consist of the networks formed by the trophic interactions between species in ecological communities and reflect many aspects of ecosystem dynamics. Depending on the indicators used, different structural and dynamic properties of food webs can emerge from the data. Well studied indicators, that can detect emergent properties, contribute to the assessment of cumulative pressures, integrate dynamic responses to existing pressures, detect direct and indirect consequences and provide a basis to manage the marine environment through an EBA (ICES 2015; Link et al. 2015). Nevertheless, ecosystem indicators can pose added challenges to interpretation because they supply a univariate summary of complex, multivariate, ecological phenomena. Good indicators should reduce the possibility of distortion of messages. To succeed, they must summarise a large body of technical information into a small number of values that can be interpreted unambiguously, providing simplicity and comparability. However, this also means that multiple and potentially different changes in community structure or function may result in the same overall change in an indicator value. This is called the challenge of multiple meanings. Recognizing this challenge, any indicator would ideally be used and interpreted in conjunction with a suite of component indicators that help to decompose the properties of the ecosystem accounting for changes in the indicator (Adams et al. 2017). Unfortunately, most community indicators are often used or reported in isolation (e.g., ICES 2014a), without explanatory decomposition. Furthermore, it is relevant to consider if the environmental status or trend estimated for a wide region is representative of trends across these regions as a whole, or whether it can be driven by local phenomena, occurring heterogeneously in space. Indicator trends may differ spatially across the study area, and whether different regions contribute differently to overall trend is unclear, since overall trends may not be representative of all areas (Adams et al. 2017). Furthermore, given constraints due to sampling resources, time series are usually based on data from surveys that are conducted once each year – discrete

annual surveys (e.g., trawl surveys). Even though the structure of the community in any defined time window may be influenced by seasonal changes in movement, migration, and phenology. There are temporal differences in indicator trends, such that clear trends may be confounded by phenological shifts. In general, indicators are unlikely to be immune to all these challenges of multiple meanings and sampling uncertainty due to scales, which questions their value for assessing status and trends.

## 1.2 Scales of assessment

A central question in ecology is how the scale of observation influences the description of existing patterns; each individual and species exist in the environment in a determinate range of scales and responds to variability differently (Levin 1992). Therefore, the distribution of species can depend on processes occurring at multiple spatial and temporal scales (OSPAR Commission 2012; Bradter et al. 2013). To address such phenomena, it is necessary to find ways to quantify patterns of variability in space and time, to understand how patterns change with scale, and to understand their causes and consequences. Once patterns are detected and described, it is possible to discover their determinants and the mechanisms that generate, maintain or disrupt those patterns. With an understanding of such mechanisms, one has a predictive capacity that is impossible with correlations alone. As a result, no assessment makes sense if the appropriate range of scales, that considers the ecological patterns of the organisms or processes, is not considered (Levin 1992).

The definition of proper scales for environmental assessment is linked to the purpose of the assessment, the specificities of the area under surveillance (habitat), the corresponding species or population(s) targeted (Walmsley et al. 2017) and prevailing anthropogenic pressures (OSPAR Commission 2012; Prins et al. 2014). When marine populations are well studied, the spatial and temporal scales addressed by sampling in environmental assessments are easier to be defined. However, aspects such as immigration and emigration by advection and migrations may be important components of change concerning spatial scales. For large, long-lived taxa, spatial scales which integrate over migration ranges may be appropriate, but these scales may span through different habitats and communities (OSPAR Commission 2012). Highly mobile and widespread populations, that cover large marine territories, require states coordination to survey across borders (time-consuming and expensive) or to incorporate climate variability effects, with variable effects. However, if assessment areas are too large, there is a risk that assessment of GES reflects the most frequent population pattern and fails to take into account significant but localized impacts that could result in a shrinking of the population's range or fragmentation of it. As a result, separate populations of a species that exist within a particular region or subregion should be assessed individually and integrated posteriorly in an increasing degree of complexity (Walmsley et al. 2017). Aggregation methods have been shown to work for several descriptors at the European scale for this purpose (Borja et al. 2019). Concerning temporal scales, assessments should incorporate the necessary time scale to assess growth, mortality and feeding components and should be annual to integrate over seasonal variability. More

frequent assessments, for example, those that could be undertaken monthly, may be required for specific taxonomic groups, however, they are operationally difficult to undertake and maintain, and their interpretation becomes complicated by seasonal dynamics (OSPAR Commission 2012). When the assessment targets populations for which knowledge is scarce, e.g., deep-sea species, other challenges may arise to achieve an assessment that considers the population patterns, spatial coverage, temporal variability, and lead to an efficient and representative assessment (OSPAR Commission 2012; Walmsley et al. 2017).

In general, indicators are commonly used to assess the environmental status and its evolution through time through comparable metrics. However, the spatial scale at which most indicators reflect temporal changes is basically unknown, a feature that is important both to identify the relevant scale for management actions and evaluations and to organize the spatial network of monitoring programmes for assessing environmental status over larger spatial scales (Östman et al. 2017). Even the dynamics of species and ecosystems and the development of the theoretic basis necessary to manage them would be greatly enhanced if scale challenges were properly addressed (Levin 1992). Nevertheless, only a few studies have examined how the spatial and temporal scale of an assessment influences indicator behaviour. According to Heim et al. (2012), if existing patterns are not assessed at an appropriate scale, the assessment may induce in error, since local trends may be masked, overestimated (propagation of local results) or underestimated (local divergence) (Figure 1.1).

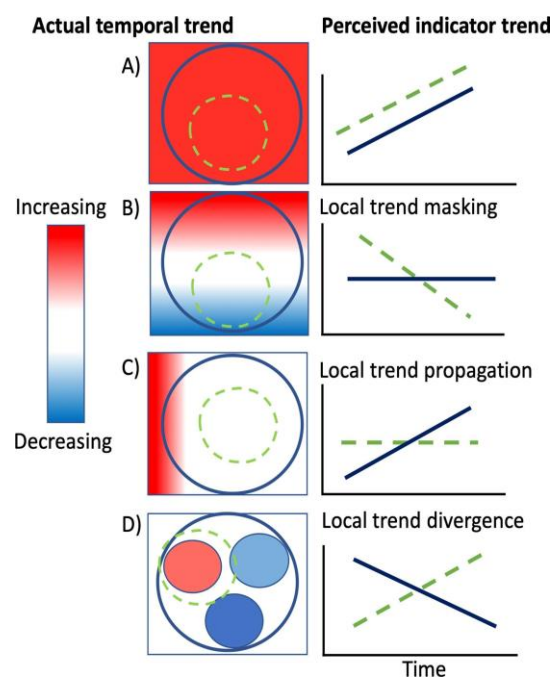


Figure 1-1 - Scenarios on how spatial extent and boundaries influence indicator trends. Solid blue circles (left panels) represent a large region over which one might summarize data and generate an indicator (shown as solid blue trendline, right panels). Green hatched circle (left panels) represents a smaller region over which one might summarize data and generate an indicator (green hatched trendline, right panels). Scenarios include (A) consistent trends perceived at all spatial scales; (B) when no trend is perceived at a large extent, but strong local trend(s) exist within the spatial domain; (C) when a trend is perceived at a large extent but driven by a highly localized trend; (D) when a trend is present at large extent, but a different trend is perceived at a local scale within this region (Heim et al. 2021).

### 1.2.1 Testing scales of assessment & ecosystem indicators – A review

The importance of scales has been pointed out conceptually for decades (Levin 1992). However, only in recent years, it was possible to have relevant data sets and robust methodologies to evaluate the importance of scales in the determination of environmental patterns or of patterns resulting from anthropogenic effects.

In recent years, several studies sought to tackle these issues. Research approaches differed among indicators studied, statistical methods employed, scales addressed, and case studies selected, reinforcing the idea that there are many scales options that researchers need to address before implementing most ecological indicators. A wider number of studies addressed concerns on the definition of spatial/geographical scales, while two studies tackled the effects of temporal scales selected. Nevertheless, all studies showed that the temporal and spatial boundaries used in environmental status assessment depended on the species or communities under study and that some scales were more appropriated to detect relevant patterns than others, depending on the subject under study. The most relevant studies and results obtained on the spatial and temporal scales of biodiversity indicators are shown in Table 1.1, below.

Engelhard et al. (2015), showed that the community indicator Large Fish Indicator (LFI) exhibited an uneven distribution in the North Sea, partly reflected by the distribution patterns of the two predominant species in the 'large fish component' of the LFI: cod and saithe (Greenstreet et al. 2011). Saithe have a generally northerly, deeper-water distribution (Homrum et al. 2013), and cod have shown major range shifts within the North Sea since the 1970s, to a distribution that is currently further north, east, and deeper than it has been throughout the 20<sup>th</sup> century (Dulvy et al. 2008; Engelhard et al. 2015). This study highlighted that only by using smaller sized assessment boundaries it was possible to detect existing community patterns and their response to fishing pressure. The shift in the cod distribution has been attributed to both climate change and fishing pressure (Dulvy et al. 2008), and the latter is in line with the significant relationships between LFI and trawling distribution reported. For the Celtic Sea, fine-scale spatial correlations between fishing effort and the LFI had been reported, but no link with temporal change in the LFI (Shephard et al. 2011).

Adams et al. (2017), reinforced results obtained by Engelhard et al. (2015) showing that, in the North Sea, indicator trends for LFI were spatially and species dependent, revealing that the assessment should be decomposed considering the species with higher biomass across ICES (International Council for the Exploration of the Sea) rectangles, to improve the identification of management needs. This study did not encounter significant differences when yearly data was partitioned and analysed by seasonal scales.

In the Baltic Sea, Östman et al. (2017), showed low spatial synchrony in the assessment of coastal fish community's status in the Baltic Sea. According to the authors, coastal fish indicators should be assessed on a local geographical scale and overall environmental status should be assessed from a wider network of

monitoring sites rather than a few but intensively studied monitoring sites. The authors argue that it is difficult to predict when and where hydroclimate variables may be important and therefore, these variables should be accounted for in the general status assessment over larger areas, most importantly temperature, which is easily measured or accessible. This applies to both geographical comparisons of coastal fish communities and temporal development of indicators in relation to changes in human activities/management (Östman et al. 2017).

In a distinct perspective, Preciado et al. (2019) crossed pressure and status datasets at small-scale spatial resolution and showed that, by using a detailed scale, it was possible to find a negative relationship between fishing effort and Mean Trophic Level (MTL) of benthic and demersal communities. As a result, these authors argue that finer spatial resolution scales should be used in the assessment of food webs indicators in the Bay of Biscay. In addition, since results showed a significant and decreasing trend in MTL with increasing fishing pressure, the authors claim that MTL can be a good indicator to monitor changes in food web structure, with a direct response to a manageable pressure such as fishing (Figure 1.2). This is particularly interesting because, under the European legislation (Commission Decision EU/2017/848), food web indicators (Descriptor 4 – Food webs) are considered as “surveillance” indicators, to monitor changes in the food web, rather than respond tightly to a manageable pressure (ICES 2014b). Nevertheless, Preciado et al. (2017) claim that MTL can achieve both goals since it responded to changes in food web structure but also showed a clear and negative relationship with a manageable pressure (bottom trawling effort). The relationships between indicators and pressures are also expected to be scale dependent. For example, the strength of predator-prey relationships or the negative impacts of bottom trawling on food webs, can weaken or strengthen depending on the spatial scale considered (Engelhard et al. 2015; Levin 1992; Preciado et al. 2019).

Heim et al. (2021) addressed scale patterns of ecosystem indicators on the US coast and found that the spatial scale of assessment selected can influence indicators results and that its degree of influence varies across indicators employed. The authors suggested that temperature indicators were the least sensitive to scale and that the sensitivity of marine community indicators increased along with trophic levels (i.e., phytoplankton, zooplankton, fish, and invertebrates). These results are largely consistent with theory and field-specific research (Levin 1992). More precisely, climatic variation and global warming operated across relatively broad spatial scales. Chlorophyll was similarly insensitive to scale and most zooplankton indicators were quite coherent across spatial subunits. Lastly, mid-trophic level indicators (fish and invertebrates) displayed the lowest spatial coherence and highest variation in trends, consistent with Östman et al. (2017) that found strong spatial coherence in physical pressures, but far less for indicators of the coastal fish community in the Baltic Sea.

Table 1-1 - Review of studies addressing the spatial and temporal scales of assessment of biodiversity and ecosystem indicators for ocean health and value (NES - Northeast United States Continental Shelf large marine ecosystem, TL - Trophic Level, LFI - Fish Indicator, ICES – International Council for the Exploration of the Sea, MTL - Mean Trophic Level).

Reference	Region of study	Scale	Elements of study	Indicators (metrics)	Scales of assessment			Results
					Wider	Medium	Smaller	
(Engelhard et al. 2015)	North Sea,	Spatial	Fish	LFI		ICES rectangles		LFI is significantly different over the ICES rectangles.
(Pranovi et al. 2016)	Mediterranean Sea	Spatial	Fish	Landing biomass	Mediterranean Sea basin	-	Eight ecoregions	Best explanative scales at 8 sea basins in the Mediterranean.
(Adams et al. 2017)	North Sea	Spatial	Fish	LFI		ICES rectangles		Significant differences for linear regression of ICES rectangles
	North Sea,	Temporal		LFI	Year	-		North Sea,
		Spatial	Fish	Perch abundance (numbers)	100 km	>100<400 km	1000 km	Spatial synchrony across all stations
		Spatial	Fish	Large Perch abundance (numbers)	100 km	>100<400 km	1000 km	No spatial synchrony f between 100-1000 km
(Östman et al. 2017)	Baltic Sea	Spatial	Fish	Mean Trophic Level	100 km	>100<400 km	1000 km	No spatial synchrony between 100-1000 km
		Spatial	Fish	Non-perch Piscivores	100 km	>100<400 km	1000 km	Spatial synchrony for stations <150 km
		Spatial	Fish	Diversity (Shannon diversity index)	100 km	>100<400 km	1000 km	No spatial synchrony between 100-1000 km
		Spatial	Fish	Large Piscivores; abundance (trophic level $\geq 4$ and size $\geq 30$ cm)	100 km	>100<400 km	1000 km	Spatial synchrony for stations <400 km
(Preciado et al. 2019)	Cantabrian Sea (Bay of Biscay)	Spatial	Food webs	MTL	Spanish part of the subregion (Bay of Biscay)			Small spatial resolution is crucial to detect the effects of spatially heterogeneous pressures - fishing.
(Bedford et al. 2020)	North Sea	Temporal	Plankton communities	5 plankton lifeform indices: Diatoms/Dinoflagellates, Phytoplankton/Non-carnivorous zooplankton, Pelagic/Tycho pelagic diatoms, Small/Large copepods, and	Long-term (1958–2017)	Medium-term (1990–2017)	Short-term (2004–2017)	Generally, reveals greater change when including data from further back in time.

			Holoplankton/Meroplankton.					
(Heim et al. 2021)	North Sea	Temporal	Plankton communities	Local contribution to beta diversity	Long-term (1958–2017)	Medium-term (1990–2017)	Short-term (2004–2017)	No relevant differences; but provides relevant ecological information.
	NES	Spatial	Physical environment	Bottom temp, Surface temp, Stratification, Summer SST, Marine heatwave days	250000 km <sup>2</sup>	125000–40 km <sup>2</sup>	20 km <sup>2</sup>	High spatial coherence (0.27 – 0.81)
	NES	Spatial	Lower TL (chlorophyll)	Chlorophyll-a, Chlorophyll-a (sat)	250000 km <sup>2</sup>	125000–40 km <sup>2</sup>	20 km <sup>2</sup>	0.71 - 0.76 spatial coherence
	NES	Spatial	Lower TL (Zooplankton)	Zooplankton (vol), <i>Centropages typicus</i> , <i>Pseudocalanus</i> spp., <i>Temora longicornis</i> , <i>Calanus finmarchicus</i> , Small/large copepod	250000 km <sup>2</sup>	125000–40 km <sup>2</sup>	20 km <sup>2</sup>	0.51 - 0.71 spatial coherence
	NES	Spatial	Mean TL (group)	Benthivore, Benthos, Piscivore, Planktivore, Sea scallop, American Lobster (biomass.tow <sup>-1</sup> )	250000 km <sup>2</sup>	125000–40 km <sup>2</sup>	20 km <sup>2</sup>	0.18 - 0.54 spatial coherence
	NES	Spatial	Mean TL (species)	Silver Hake, Spiny Dogfish (biomass.tow <sup>-1</sup> )	250000 km <sup>2</sup>	125000–40 km <sup>2</sup>	20 km <sup>2</sup>	0.2 - 0.3 spatial coherence

In general, all authors revealed that finer spatial scales of assessment were necessary to identify relevant patterns for indicators and that temporal scales provided more stable results, even though that could depend on the taxonomic group under study (Engelhard et al. 2015). The importance of spatial scales is also related to the complexity of ecosystem elements under study: climatic variables patterns are detected at wider scales, while community or food webs indicators, that involve a complex network of species and/or trophic levels, require smaller dimensional scales to identify patterns of variability.

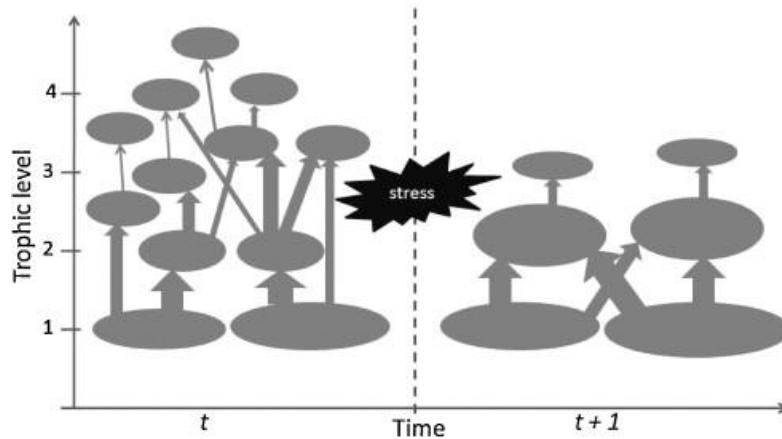


Figure 1-2 - Schematic diagram showing trophic network connectivity before (left) and after (right) a perturbation or stress. The depletion of higher-trophic level species simplifies the ecosystem structure while strengthening the connectivity and increasing the abundance of less-predated lower-trophic level species. Biomass (low-sized species) is represented by circle size. Flows from one trophic level to another are represented by arrows, while predation strength is represented by arrow size. (Rombouts et al. 2013, modified from Villanueva 2004).

### 1.3 The Marine Strategy Framework Directive

Recognizing an increasing pressure on natural marine resources and the often too high demand for marine ecological services, the European Community (EC) considered necessary to reduce the impact on marine waters regardless of where their effects occur. As a result, in 2008, the European Union (EU) implemented the Marine Strategy Framework Directive (MSFD), to evaluate the environmental status of the European Seas and established the first attempt to implement an EBA to management (Directive 2008/56/EC). The MSFD requires that the European Member States (MSs) that share a marine region or subregion cooperate when developing their marine strategies (European Commission, 2008). The initial aim was to ensure that the EU marine waters were in a Good Environmental Status (GES) by 2020, that marine goods and services were exploited in a sustainable way and that marine resources were preserved for future generations to use (European Commission, 2008). Because the Directive follows an adaptive management approach, the Marine Strategies must be kept up-to-date and reviewed every six years. To achieve this purpose the Directive defined 11 qualitative descriptors (see list in Table 1-2) requiring that MSs develop national marine strategies, that although being specific to their waters must also reflect the overall perspective of the marine region and subregions across European seas. The marine regions (and



subregions) were decided based on hydrological and biogeographic features and existing authorities appointed for coordination, established under the Regional Seas Conventions, e.g., the OSPAR for the North-East Atlantic, the Helsinki Convention (HELCOM) for the Baltic Sea, the Barcelona Convention for the Mediterranean Sea (UNEP-MAP) and the Bucharest Convention for the Black Sea. The North-East Atlantic region was further divided into subregions (Macaronesia, Bay of Biscay and Iberian Coast, Celtic Seas, Greater North Sea in the Atlantic) (Figure 1.3).

Table 1-2 – Qualitative descriptors for good environmental status identified in Annex I to Directive 2008/56/EC developed for the monitoring and assessment of predominant pressures and impacts and the current environmental status of marine waters (Commission Decision, 2017a).

<b>Descriptor</b>	<b>Description</b>	<b>Type of assessment</b>
<b>Descriptor 1: Biodiversity</b>	Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climate conditions.	Status
<b>Descriptor 2: Non-indigenous species.</b>	Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem.	Pressure
<b>Descriptor 3: Commercial species of fish and shellfish</b>	Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock.	Pressure
<b>Descriptor 4: Food webs</b>	All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.	Status
<b>Descriptor 5: Human-induced eutrophication</b>	Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters.	Pressure
<b>Descriptor 6: Sea-floor integrity</b>	Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected.	Status
<b>Descriptor 7: Hydrographical conditions</b>	Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.	Pressure
<b>Descriptor 8: Contaminants</b>	Concentrations of contaminants are at levels not giving rise to pollution effects.	Pressure
<b>Descriptor 9: Contamination of fish and seafoods</b>	Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.	Pressure
<b>Descriptor 10: Marine litter</b>	Properties and quantities of marine litter do not cause harm to the coastal and marine environment.	Pressure
<b>Descriptor 11: Energy and noise</b>	Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine	Pressure

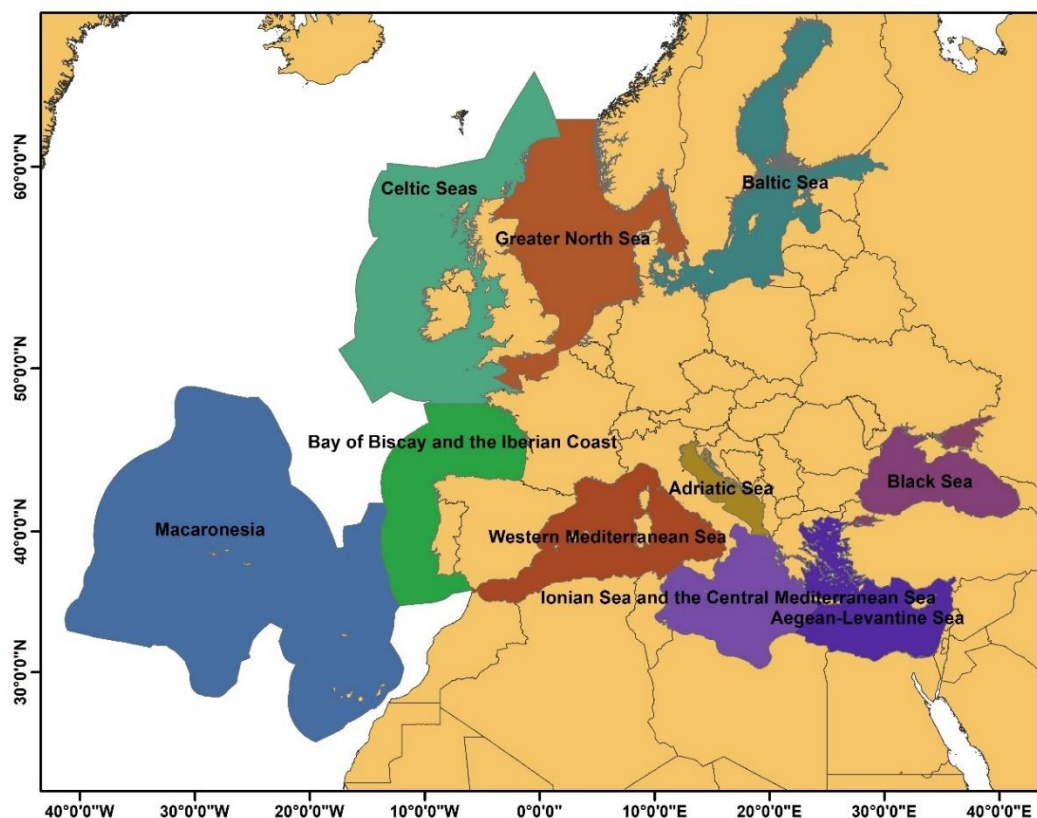


Figure 1-3 - Marine subregions listed in Article 4 of the Marine Strategy Framework Directive, together with other surrounding seas of Europe (EEA, 2021; European Commission, 2008).

The 11 descriptors defined in the Directive, address both ecosystem status and anthropogenic pressures, implementing an EBA to management (Borja et al. 2011). The development of indicators is an integral component of forming marine strategies under the European MSFD. A suite of indicators for biodiversity has been identified and developed for formal assessment under the MSFD, reflecting change in bulk, functional, and compositional aspects of community structure and ultimately, habitat state (Bedford et al. 2020). Indicators can address sensitive species and habitats, communities, commercially exploited species, and food webs, etc. However, the scale of assessment and aggregation of biodiversity related indicators (under descriptors D1 – Biodiversity, D2 – Non-indigenous species, D3 – Commercial Fish and Shellfish, D4 – Food webs, D6 – Sea floor integrity), that encompass many distinctive features and methods that need to be combined in a meaningful way, has not been developed yet (Figure 1-4).

The first reporting cycle occurred from 2010 to 2018 and revealed, for the first time, an overall perspective of the European seas state, but it also highlighted assessment inconsistencies and knowledge gaps that needed to be tackled (e.g., Palialexis et al. 2014; Elliott 2014; Elliott et al. 2017; Cavallo et al. 2016). Several EU projects (e.g., DEVOTES, EcArpha, PERSEUS) and authors have looked into the MSFD incongruencies (Teixeira et al. 2014; Preciado et al. 2019; Berg et al. 2015; Teixeira et al. 2016) and results pointed out inconsistent such as reporting of metrics, species and habitats, but also unbalanced reporting

across descriptors, discrepancies of temporal and spatial scales and lack of MSs cooperation within subregions (Palialexis et al. 2014). The most reported elements, habitats and criteria concerned aspects already targeted by environmental assessments mandated by previously implemented EU Directives such as the WFD, HD, BD and the Common Fisheries Policy (CFP) (Palialexis et al. 2014; Teixeira et al. 2016). Some countries reported a large number of assessment areas at various scales. For example, the reporting area ranged from 2 km<sup>2</sup> to 591 000 km<sup>2</sup>. This allowed very detailed reporting but caused difficulties in producing regionally coherent assessment maps when aligning with the reporting from other countries (Barrio and Holdsworth, 2016).

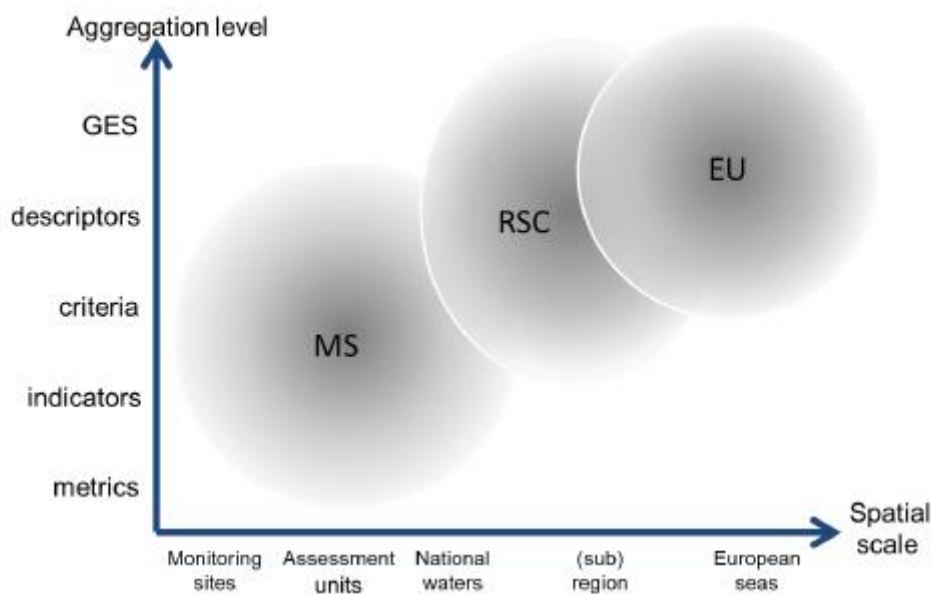


Figure 1-4 - Differences in information needs and associated spatial scales and aggregation levels (MS – Member State, RSC – Regional Sea Convention, EU – European Union) (Prins et al. 2014).

To address these concerns, in 2017, the Directive has undergone revisions to improve the consistency of the implementation. One legal diploma updated the criteria, the elements and the geographical area of assessment (European Commission, 2017a) and a second diploma updated the ecosystem elements and pressures addressed by each descriptor (European Commission, 2017b). In the revised Decision, specific assessment scales were laid down for each descriptor criteria element. According to these diplomas, MSs determinations of GES need to be consistent across the marine region or subregion, but the relevant assessment scales formally operate at three different geographic levels: the Marine Region, the Subregion and Subdivisions (Barrio and Holdsworth, 2016). The first two are defined within the Directive (Art. 4), while it is up to the MSs to apply any subdivisions, whether formally recognised or not. Each country is responsible to decide if the assessment considers subdivisions or spatial subunits and on what ecological aspects are these based upon (e.g., latitudinal, or longitudinal gradient, depth strata, key ecological dynamics) (European Commission, 2012). Assessments of the status of the marine environment has to

consider local or regional characteristics (e.g., specificities of the area being assessed, what ecosystem components and what pressures are important in this area), which may work out differently for the various descriptors or criteria and indicators within a descriptor due to differences in the spatial distribution of ecosystem components and human activities (Figure 1-4). The combination of spatial assessment scales with time scales for assessments is also another issue that needs further development (Prins et al. 2014; Borja et al. 2016, 2019). Therefore, one of the questions that needs to be addressed is what is the appropriate scale to assess each descriptor including its criteria, taxonomic elements and indicators?

Assessments of the elements can be undertaken at different geographical scales, using proper scales for each element (e.g., assessment at the regional or subregional scale, or suitable subdivisions of these). Even though generic scales for assessment are given in the revised Directive, MSs may wish to assess in their waters and later aggregate the results to regional or subregional scale together with other MS. However, MSs should agree at (sub)regional level using a ‘nested approach’ as far as possible. A combination of the element to be assessed and the appropriate scale for its assessment allows for the identification of the specific areas to be used for assessment within each region and subregion; the ‘nested assessment areas’ being used or developed by HELCOM and OSPAR can provide schemes for integrated assessments of a region or subregion, however other scales can be proposed, e.g., ICES units, Habitat Directive units. Nevertheless, the effects of uncertainty in data for assessment results and the risks of misclassification should be considered when more specific aggregation methods are developed (Barnard and Strong, 2014; Prins et al. 2014; Walmsley et al. 2017).

#### 1.4 Objectives and Thesis outline

Having into consideration the above-mentioned information concerning scales for environmental assessment of biodiversity and ecosystems health, the present thesis aims to study the congruency of biodiversity and ecosystem indicators in the framework of the MSFD and their corresponding scales in the North-Eastern Atlantic. To further elaborate on this topic and to improve complementarity, the appropriate scales of assessment of two main groups of indicators were tested - biodiversity (D1) and food webs (D4) - by using metrics that are specified in the Directive for this purpose. In this context, the following major research questions were addressed:

- Are MSs implementing MSFD congruently, in what concerns 1) the biodiversity indicators used (D1, D2, D4, and D6) and 2) the temporal scale range employed in the assessment of environmental status?
- Can assessment scales, currently being used in the assessment of biodiversity (D1) and food webs (D4), detect species or community patterns, and therefore distinguish environmental variability from anthropogenic patterns?

- Are indicators used to report food webs, in the MSFD context, responding to anthropogenic pressures at relevant spatial scales of assessment?

The study area of this work varied from the sea basin level to locally defined scales, ranging from the North Sea to the Iberian Peninsula continental platform, to tackle existing problems concerning biodiversity and ecosystem indicators assessment. Understanding the adaptive perspective of the directive, this work has initially focused on problems identified on biodiversity descriptors (D1 - Biodiversity, D2 – Non-indigenous species, D3 – Commercial Fish and Shellfish, D4 – Food webs, D6 – Sea floor integrity), including all ecosystem elements targeted by the directive. In a later stage, and to deepen this research topic, this dissertation focused on remaining inconsistencies, recognized by the EC, focusing on descriptors D1 – Biodiversity and D4 – Food webs assessment, at national and subregion levels. Indicators selected for this study are currently being used or considered as indicators of the state of biodiversity and food webs in the North Sea (Greenstreet et al. 2011; Probst and Stelzenmüller 2015), Celtic Sea (Shephard et al. 2011), North East Atlantic (Modica et al. 2014; Preciado et al. 2019; Arroyo et al. 2019) and Mediterranean (Pranovi et al. 2016), and in the framework of European directives (MSFD) (Ministério do Mar 2020; Ministerio para la Transición Ecológica (MITECO) 2019; Government of the UK 2019). Therefore, it is a timely issue to assess their performance and the potential for improving interpretations of it. For this purpose, distinct studies were developed.

The present dissertation is structured in six chapters, that correspond to four scientific publications. Hence, chapters two to five include four scientific outputs which are already published or under review (in a peer-reviewed journal) and include the main research chapters where research results are presented and discussed in detail. Chapter one and six correspond, respectively to the general introduction and major conclusions derived by the globality of the work developed. To answer the research questions described above, the work followed the outline described in Figure 1-5 below.

To address the first main research question, Chapter 2 - *Identifying assessment scales for food web criteria in the NE Atlantic: implications for the Marine Strategy Framework Directive*, presents a review of the first MSFD implementation cycle to understand the main strengths and knowledge gaps concerning the biodiversity and ecosystem indicators employed in the directive and the coherence of their corresponding scales. This review allowed to identify several aspects that required further research to improve MSFD assessment, pointing out knowledge gaps for specific descriptors, and the need for further cooperation and for the identification of common metrics and ecologically relevant scales at a transboundary level (subregion). Having this information into consideration the work focused next on answering the second research question: are the assessment scales of biodiversity indicators detecting existing patterns and effects of anthropogenic pressures? Bearing this aim in mind, Chapter 3 - *Effects of scale on the assessment of fish biodiversity in the marine strategy framework directive context*, and Chapter 4 - *Identifying assessment scales for food web criteria in the NE Atlantic: implications for the Marine Strategy Framework Directive*, depicted spatial and temporal scales of assessment of biodiversity and food webs indicators to answer the following question: are species and community patterns being pertinently addressed and detected by the scales used

in the assessment? Subsequently, Chapter 5 - *Response of food webs indicators to human pressures in the scope of the Marine Strategy Framework Directive for the NE Atlantic*, continues to look into food webs descriptor, which is one of the least developed descriptors within the MSFD. This chapter focuses on the third research question and it aims to understand if the environmental status of food webs is coherently reported and is detecting and/or responding to anthropogenic pressures within the MSFD framework.

# Chapter 1: General Introduction

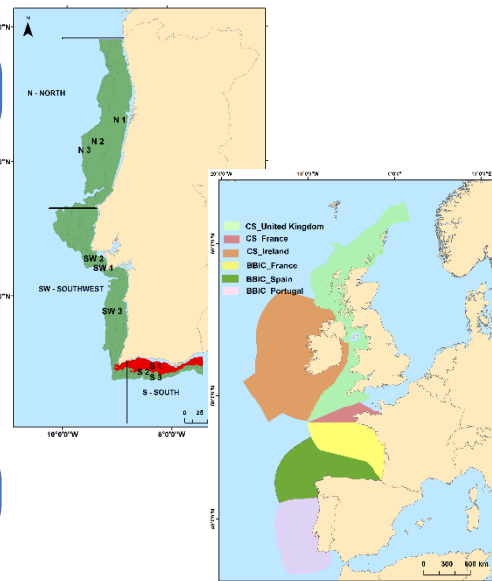
**1st Research Question: Is biodiversity assessment congruent, under the context of the MSFD?**

**Chapter 2: MSFD reporting and assessment scales for biodiversity descriptors (D1, D2, D3, D4 and D6)**



**2nd Research Question: Are the established scales adequate to evaluate environmental and anthropogenic patterns?**

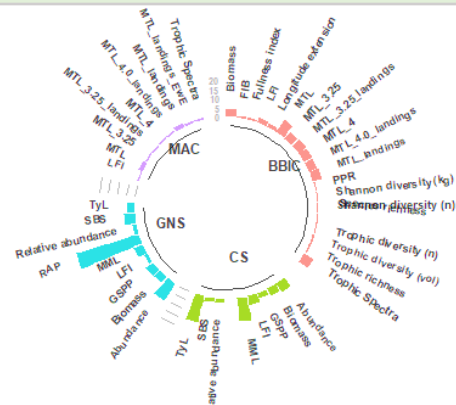
**Chapter 3: Effects of scale on the assessment of fish biodiversity in the MSFD context of D1**



**Chapter 4: Identifying assessment scales of food web indicators - D4**

**3rd Research Question: Are food-web indicators responding to anthropogenic pressures?**

**Chapter 5: Effects of pressures on food webs indicators (D4)**



# Chapter 6: Conclusion

Timeline and research flow

Figure 1-5 - Conceptual design and outline of the PhD dissertation thesis, showing the sequential workflow implemented throughout the PhD timeline, to answer the research questions posed in the objectives and in the thesis workplan.

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## *2 Assessment level and time scales of biodiversity indicators in the scope of the Marine Strategy Framework Directive: A case study for the NE Atlantic*

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**European Union Member States have made an unprecedented effort to implement the Marine Strategy Framework Directive (MSFD).** However, the coherent assessment of Europe's marine waters and Good Environmental Status by 2020 has not yet been achieved. This work analysed the implementation level and time scales used to report biodiversity criteria in the 1st MSFD cycle for two Biogeographic Regions: the Celtic Seas and the Bay of Biscay and Iberian Platform. Results were compared across Biogeographic Regions, Marine Sub-units, Criteria, and Biological Groups to assess congruency and integration possibilities. Reporting level was significantly different among Marine Sub-units within the same Biogeographic Region and country. France and Spain showed the highest implementation level and focused on criteria included in Biodiversity (1) and Food webs (4) Descriptors, while Ireland mostly reported Commercial Fish and Shellfish (3). Fish, Rocky and Biogenic Reefs and Sedimentary Habitat were the most reported Groups. Heterogeneous data was recorded for temporal scales used to report Groups and Criteria among Marine Sub-units within the same region, showing that each MS is working individually. Average temporal range used for reporting was wider in French Bay of Biscay, particularly for Marine turtles' Group, Food-web Criteria (Descriptor 4), and Ecosystem structure criteria (1.7). Ireland presented consistently shorter temporal data sets focusing on Commercial Fish and Shellfish descriptor (3) and Fish Group.

To enable the direct comparisons and integration of MSFD results, the use of historical and opportunistic data should be discouraged, the reporting timeframe of existing monitoring datasets (beginning/end) should be synchronized at regional level, and targeted habitats/species should be decided and prioritized regionally. This work pinpoints MSFD gaps and provides inputs for an improved 2nd implementation cycle, particularly in what concerns temporal scales used for reporting.

### 2.1 Introduction

Achieving healthy and sustainable oceans is a major concern for worldwide governments, policy-makers, researchers and Non- Governmental Organizations (NGO) as a consequence of the growing use of ocean space and increasing resources depletion. Anthropogenic impacts are increasing, and the marine environment has reached an unprecedented degradation status (e.g., fisheries collapse, pollution, and ocean

acidification.) (Borja et al. 2008; European Commission, 2008; UNCLOS, 1982; US Congress, 2002; Valdés et al. 2017). Worldwide organizations, such as the United Nations (UN), have expressed their concern by adopting ocean-related indicators, such as the Sustainable Development Goal (SDG) –GOAL 14: LIFE BELOW WATER – implemented by the UN global indicator framework (United Nations, 2016). In the past three decades, inter-governmental regional sea conventions (RSCs) (e.g., Oslo and Paris Conventions (OSPAR), Helsinki Convention (HELCOM), Barcelona Convention (UNEP-MAP) and Bucharest Convention (Black Sea Commission)) have played a crucial role in the achievement of ocean sustainability goals. RSCs implemented the first management guidelines and monitoring programs to assess environmental status, proposing common and comparable assessment methods, and defining common targets among geographic regions (Black Sea Commission, 1992; Helsinki Commission, 2010; OSPAR Commission, 1992; UNEP - MAP, 2002; Valdés, 2017). Yet, this task is far from easy because assessment methods must also incorporate a large diversity of ecosystems metrics, and concomitant thresholds, that often vary geographically (Borja et al. 2016a; Cavallo et al. 2016). In addition, RSCs are not legally binding and there are no sanctions if MSs do not comply with proposed measures (Cavallo et al. 2018). To address these challenges, in 2000 the European Commission (EC) has approved the Water Framework Directive (WFD) that provides the framework for the assessment and protection of inland surface waters, transitional waters, coastal waters, and groundwaters (European Commission, 2000). Subsequently, the Marine Strategy Framework Directive (MSFD) was approved in 2008 to establish the protection of the marine environment including Member State's Exclusive Economic Zone (EEZ) (European Commission, 2008). The MSFD aims to promote marine conservation and sustainability through an Ecosystem Based Approach (EBA) to achieve the sustainable use of marine goods and services while enabling ecosystems functions and their recovery whenever anthropogenic impacts occur (Borja et al. 2016a; Long et al. 2015). To attain this purpose, Member States (MS) must assess the environmental status to determine whether they are in Good Environmental Status (GES) as defined by the environmental targets established at national level. The assessment is made based on 11 qualitative descriptors: 1 – Biodiversity, 2 – Non-indigenous species, 3 – Commercial fish and shellfish, 4 – Food webs, 5 – Human-induced eutrophication, 6 – Seafloor integrity, 7 – Hydrographical conditions, 8 – Contaminants, 9 – Contaminants in fish and seafood, 10 – Litter, and 11 - Energy and noise (European Commission, 2008). These descriptors assess the environmental status from two perspectives: the state of the marine environment, and human pressures and impacts (European Commission, 2017). The descriptors represent the first EC attempt to implement an ecosystem-based approach to manage the marine environment as a coupled social-ecological system. Biodiversity related descriptors are among the most challenging ones as they include diverse biological or ecological elements (e.g., species, habitats, food webs and ecosystems, etc.) that need to be assessed and integrated (Borja et al. 2016a; Heiskanen et al. 2016) to reflect the global status of an ecosystem. However, the integration of biodiversity's vast set of information poses numerous problems, due to the numerous



elements, metrics and units, and to the introduction of novel features such as large-scale environmental assessments (Borja et al. 2008, 2014, 2016a).

Several problems have been identified during the first phase of the MSFD implementation, such as incongruent methodological approaches (Berg et al. 2015; Hummel et al. 2015; Palialexis et al. 2014), redundancy in reporting (Berg et al. 2015), heterogeneous establishment of thresholds within the same region (Cavallo et al. 2016; Hummel et al. 2015; Palialexis et al. 2014; Teixeira et al. 2014), different prioritization of taxa among countries (Cavallo et al. 2016; Peterlin et al. 2015), inconsistent temporal and spatial compartmentation of data (Bergström et al. 2016; Hummel et al. 2015; Patrício et al. 2016), and the lack of large-scale assessment of fluctuations (e.g. climate change) (Bellás, 2014; Cardoso et al. 2010; Santos et al. 2012). The definition of scales is of major importance to assess GES, as the detection of effects may be overlooked if the correct spatial area and/or temporal scope is not outlined. To define the spatial scale to assess GES, it is highly important to know factors, such as the geographical distribution of species/functional group and/or the extension of the habitat/ ecosystem. The temporal scale should have into consideration species' biological traits, such as life cycle, growth rate, reproductive cycle, and variability patterns, to ensure that intra- and inter-specific variability is correctly addressed (Teixeira et al. 2014). Scales are critical to understand if any deviations from GES are a consequence of natural variability or of human pressures; they can affect the establishment of reference values and thresholds, and have implications on the detection of environmental recovering trajectories (Bergström et al. 2016; Hummel et al. 2015; Rossberg et al. 2017). Incongruent scales can lead to even wider inconsistencies, such as unbalanced GES classification and the development of inefficient actions and monitoring plans (Berg et al. 2015; Borja et al. 2014; Cavallo et al. 2016). The geographical scales used in the 1st implementation cycle have been assessed by the EC (Prins et al. 2014), and the spatial scales that need to be assessed in the 2nd implementation cycle have been defined (European Commission, 2017). However, the temporal spectrum of reporting data is still largely overlooked, even though it is a recognized source of variability in environmental monitoring data that increases uncertainty and decreases the confidence levels of the assessments (Carstensen and Lindegarth, 2016).

The present work aims to assess the implementation level and the corresponding temporal data series used to assess environmental status during the 1st MSFD implementation cycle. Particular focus is given to biodiversity descriptors (i.e., #1 – biodiversity; #2 non-indigenous species; #3 – commercial fish and shellfish; #4 – food webs; #6 – seafloor integrity) implemented in two Biogeographic Regions of the North Eastern Atlantic (Berg et al. 2015; Hummel et al. 2015; Teixeira et al. 2014). This study hypothesizes that reporting level and temporal scales used by MSs in the initial assessment are appropriate, congruent, and comparable, and allow the isolation of natural variability from anthropogenic effects as recommended by the EC. To test this hypothesis, the frequency of reporting and time scales of biodiversity criteria were analysed for the first time and compared across Biogeographic Regions, MSs, Marine Sub-units, Criteria

and Biological elements (taxonomic groups and habitats). Ultimately, this assessment aimed to identify specific urgent actions and to provide novel inputs on how temporal scales can be improved in the 2nd implementation cycle.

## 2.2 Methods

### 2.2.1 Study area

The study area is located in the North Eastern Atlantic Sea and focus two Biogeographic Regions (the Celtic Seas, and the Bay of Biscay and Iberian Platform), five MSs (UK, Ireland, France, Spain and Portugal), and six Marine Sub-units (UK\_Celtic Seas, IR\_Celtic Seas, FR\_Celtic Seas, FR\_Bay of Biscay and Iberian Platform, SP\_Bay of Biscay and Iberian Platform and PT\_Bay of Biscay and Iberian Platform) (see Figure 2.1). The lowest spatial unit used to assess implementation levels and temporal scales was the Marine Sub-unit, which is a sub-division of the MSs territory according to its corresponding Regional Sea/Biogeographic Region (Peterlin et al. 2015).

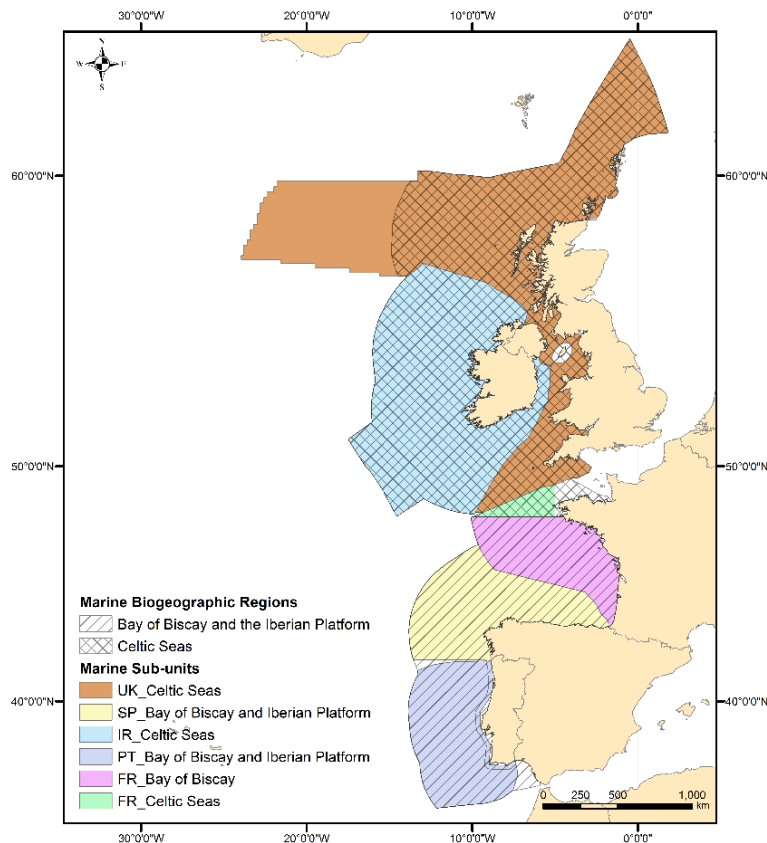


Figure 2-1 - Study area: Biogeographic regions and Marine Sub-units under study (EIONET Central Data Repository, 2018).

### 2.2.2 Data collection

MSs reporting level and the corresponding temporal range of biodiversity indicators was assessed by surveying Initial Assessment Reports, GES assessment reports, the European Environment Information

and Observation Network (EIONET) factsheets, Task-Group reports and peer reviewed papers (Alemany et al. 2012; Berg et al. 2015; Borja et al. 2011; Cardoso et al. 2010; Cavallo et al. 2018, 2017; DEFRA, 2012; EIONET Central Data Repository, 2018; Hummel et al. 2015; Irish Government, 2013; MAGRAMA et al. 2012; MAMAOT, 2012; Ministère de L'Écologie, du Développement Durable et de L'Énergie, 2012a, 2012b; Norton and Hynes, 2018; Olenin et al. 2010; Piet et al. 2010; Preciado et al. 2012; Punzón et al. 2012; Rice et al. 2010; Stuart Rogers et al. 2010; Sampedro et al. 2012; Teixeira et al. 2016; Velasco et al. 2012). In general, data were collected by crossing and comparing information from the MSs Initial Assessment Reports and reporting factsheets (EIONET Central Data Repository, 2018). Data from Portugal was limited to the information in the national report because this MS did not fill in the corresponding EIONET factsheet. The following biodiversity descriptors were selected: 1 - Biodiversity, 2 – Non-indigenous species (NIS), 3 – Commercial Fish and Shellfish, 4 – Food webs, and 6 – Seabed Integrity. Although not commonly considered a biodiversity descriptor, Descriptor 3 – Commercial Fish and Shellfish was also analysed as it reflects the environmental status of fish and crustacean biodiversity (Berg et al. 2015) and includes a consistent data series due to the long-term implementation of the Common Fisheries Policy (CFP) in Europe (European Commission, 2015). To assess reporting level, the number of indicators used to report was accounted and to assess time scale, the temporal range of each indicator was identified. Both variables were assessed for each Biogeographic Region, MS, Marine Sub-unit, Descriptor, Criteria, and for all reported biodiversity components. The assembled database included the following items: Biogeographic Region, MS, Marine Sub-unit, Descriptor,

Criteria, Indicator, Group, Specific taxa, Initial year of reporting/ Start year (initial year for which data are available) and Time range (number of years for which data are available, e.g., time series from 1992 to 2011 has a Time range of 19 years). Biodiversity components were merged into larger functional groups (hereafter designated by Groups) since MSs used different terminology across the same components (Peterlin et al. 2015). This standardization aimed to enable direct comparisons across defined Groups: Benthic habitats, Benthic species, Fish, Food-web, Marine mammals, Marine turtles, Plankton, Pelagic habitats, Rock & Biogenic Reef, Seabirds and Sedimentary habitat. A total number of 5 descriptors, 17 criteria, 35 indicators, and 11 functional groups/habitats were addressed (see Table 2.1). The data set was standardized to criteria level since not all MS reported up to the indicator level. To tackle differences in reporting level due to the unbalanced number of Indicators per Criteria (see Table 2.1), the average reporting level was calculated by summing all reported data in that particular Criteria, and by dividing it by the number of Indicators included in that Criteria – generating an average reporting level (e.g., Criteria 3.1 includes Indicators 3.1.1, 3.1.2, therefore all data reported in 3.1 was summarized and divided by the number of existing Indicators, “2”).

Table 2-1 - List of Descriptors, Criteria, Indicators and Groups assed for the case study region.

Descriptor	Criteria	Indicator	Group/ Habitat	
1 - Biodiversity	1.1 Species distribution	1.1.1	Fish; Marine mammals; Marine turtles; Pelagic habitats; Seabirds; Sedimentary habitat	
		1.1.2	Fish; Marine mammals; Marine turtles; Pelagic habitats; Seabirds	
		1.1.3	Benthic habitats	
	1.2 Population size	1.2.1	Fish; Marine mammals; Marine turtles; Pelagic habitats; Seabirds	
	1.3 Population condition	1.3.1	Fish; Marine mammals; Marine turtles; Pelagic habitats; Seabirds	
		1.3.2	Fish; Marine mammals	
	1.4 Habitat distribution	1.4.1	Benthic habitats; Fish; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
		1.4.2	Benthic habitats; Fish; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
		1.4.3	Sedimentary habitat	
	1.5 Habitat extent	1.5.1	Benthic habitats; Fish; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
		1.5.2	Benthic habitats; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
		1.5.3	Benthic habitats	
	1.6 Habitat condition	1.6.1	Benthic habitats; Fish; Marine mammals; Marine turtles; Pelagic habitats; Rock & Biogenic Reef; Seabirds; Sedimentary habitat	
		1.6.2	Benthic habitats; Fish; Marine mammals; Marine turtles; Pelagic habitats Rock & Biogenic Reef; Seabirds; Sedimentary habitat	
1.6.3		Benthic habitats; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat		
1.7 Ecosystem structure	1.7.1	Fish; Pelagic habitats; Seabirds		
2 – Non-Indigenous Species (NIS)	2.1 Abundance of NIS	2.11	Benthic species; Rock & Biogenic Reef; Pelagic habitats	
	2.2 Environmental impact of NIS	2.2.1	Benthic species; Rock & Biogenic Reef	
		2.2.2	Benthic species; Rock & Biogenic Reef	
3 - Commercial fish and shellfish	3.1 Fishing activity pressure	3.1.1	Fish; Marine mammals; Marine turtles	
		3.1.2	Fish; Marine mammals; Marine turtles	
	3.2 Reproductive capacity	3.2.1	Fish	
		3.2.2	Fish	
	3.3 Population age and size distribution	3.3.1	Fish	
		3.3.2	Fish	
		3.3.3	Fish	
3.3.4		Fish		
4 - Food webs	4.1 Biomass of key species/ trophic groups	4.1.1	Benthic habitats; Fish; Marine mammals; Marine turtles; Seabirds	
		4.1.3	Fish	
	4.2 Proportion of species at the top of food webs	4.2.1	Benthic habitats; Fish; Food-web; Seabirds	
	4.3 Abundance of key trophic groups/ species	4.3.1	Benthic habitats; Benthic species; Fish; Food-web; Marine mammals; Marine turtles; Plankton, Pelagic habitats; Rock & Biogenic Reef; Seabirds; Sedimentary habitat	
6 - Seafloor Integrity	6.1 Substrate physical damage	6.1.1	Benthic habitats; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
		6.1.2	Benthic habitats; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
	6.2 Condition of benthic community	6.2.1	Benthic habitats; Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
		6.2.2	Pelagic habitats; Rock & Biogenic Reef; Sedimentary habitat	
<b>SUM</b>	<b>5</b>	<b>17</b>	<b>35</b>	<b>124</b>

### 2.2.3 Statistical analysis

To test differences in MSFD reporting level, the number of criteria used to report each Biogeographic Region, Marine Sub-unit, Criteria, and Group as well as their interaction were tested using Generalized Linear Models (GLMs). The Poisson distribution was chosen since it is typically used for count data (Warton et al. 2016; Zeileis et al. 2008).

In this analysis, a nested design was prepared considering that each Marine Sub-unit was grouped within their corresponding Biogeographic Region. Descriptor showed strong collinearity with Criteria, and therefore it was not considered in the analysis. All factors were considered fixed (Underwood, 1997). To further disentangle variability patterns, a non-parametric multivariate analysis for categorical variables - Multiple Correspondence Analysis (MCA) - was used to understand how different variables explained the heterogeneity of reporting level. Similarly, the variables selected to perform the MCA analysis of reporting level were Biogeographic Region, Marine Sub-unit, Descriptor, Criteria, and Group.

On a second stage, the Time range used to report each biodiversity indicator was compared using a GLM with Gamma distribution, following data normality and homoscedasticity assessment procedures.

This analysis considered the following factors: Biogeographic Region, Marine Sub-unit (nested within Biogeographic Region), Criteria and Group. All factors were considered fixed. Similarly to the previous analysis, Descriptor exhibited a strong collinearity with Criteria, and therefore it was not considered in the analysis. Heterogeneity patterns among reporting Time range were analysed using Factor Analysis of Mixed Data (FAMD). This method is a mixture of two popular methods: Principal Component Analysis (PCA) which allows the ordination of quantitative datasets, and MCA which is suitable for exploring qualitative variables (Pagès, 2004). FAMD provides the classical results of a factorial analysis, such as ordination diagrams that assess the patterns of observations and allows to evaluate its relationships with the considered variables. The variables selected for the Time range FAMD analysis were: Biogeographic Region, Marine Sub-unit, Criteria, Group, Initial reporting year, and Time range. Cluster Analysis was applied to both non-parametric analyses using a probability of 0.05, creating an automatic similarity threshold to understand if reporting level and time scales show any patterns across the study area. All the analysis were performed using R software (R Core Team, 2014).

## 2.3 Results

### 2.3.1 Reporting variability

Reporting level was highly heterogeneous among Biogeographic Regions, MSs, Marine Sub-units, Descriptors, Criteria, and Groups. The Bay of Biscay and the Iberian Platform was the most reported region (n=1553), France was the country with the highest reporting level (n=1555), and French Bay of Biscay was the most reported Sub-unit (n=716). The most reported descriptor was 1 – Biodiversity

(n=2013), which notably contrasted with Descriptor 2 – NIS (n=139; Figure S1, Annex 1). Criteria and Group implementation was highly heterogeneous across MSs (Figure 2.2A and 2.2B, respectively). France, Portugal and UK highly reported Criteria 1.6 Habitat Condition, while Ireland focused on Criteria 3.3 Population age and distribution. Spain mostly reported Criteria 1.1 Species distribution (Figure 2.2A). The number of Groups reported per MS was the highest in the UK (n=9) and lowest in Ireland (n=6); this latter MS reported a large number of Criteria but concerning the same Group, i.e. Fish within the descriptor 3 – Commercial fish and shellfish. Fish was the most frequently reported Group for most MS except for France and UK that reported more often Rock & Biogenic Reef and Marine Mammals (Figure 2.2B).

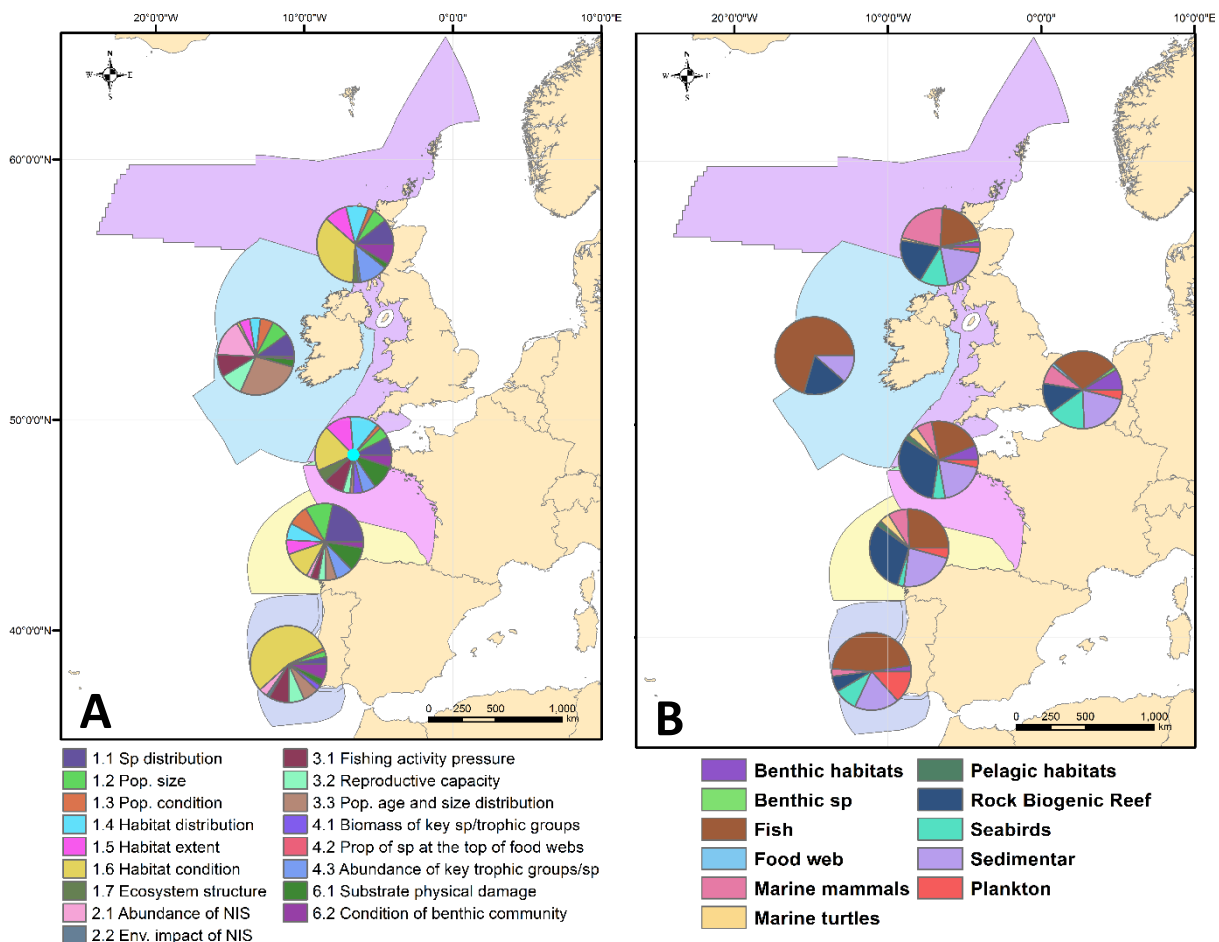


Figure 2-2 - A: Frequency distribution (%) of reporting per each Criteria and Member State; B: Frequency distribution (%) of reporting per Groups and Member State.

Marine Sub-units, within each Biogeographic Region, had a low contribution to explain reporting variability (2.62% of deviance explained;  $p=0.000$ ). Nevertheless, post-hoc tests confirmed that the Bay of Biscay and Iberian Platform reporting level was significantly higher in French Bay of Biscay in comparison to Spanish Bay of Biscay (with 3.3 and 1.6 average number of criteria, respectively), but similar to the Portuguese Sub-unit (with 2.0 average reporting number). In the Celtic Seas, the three Sub-units were significantly different but presented a smaller amplitude of variation (ranging from 2.6 in Ireland to 1.3 in

the UK unit). The most important explanatory variables were Group, the interaction between Marine Sub-unit and Criteria and Criteria. Group explained 23.3% of the existing variance and Rock & Biogenic Reefs and Fish Groups were significantly more reported than the overall Groups (with 5.6 and 4.9 average number of criteria) (Table 2.2). Benthic habitats, Pelagic Habitats, Food webs and Benthic species were the least reported Group (with 0.7, 0.3, 0.2 and 0.1 average number of criteria).

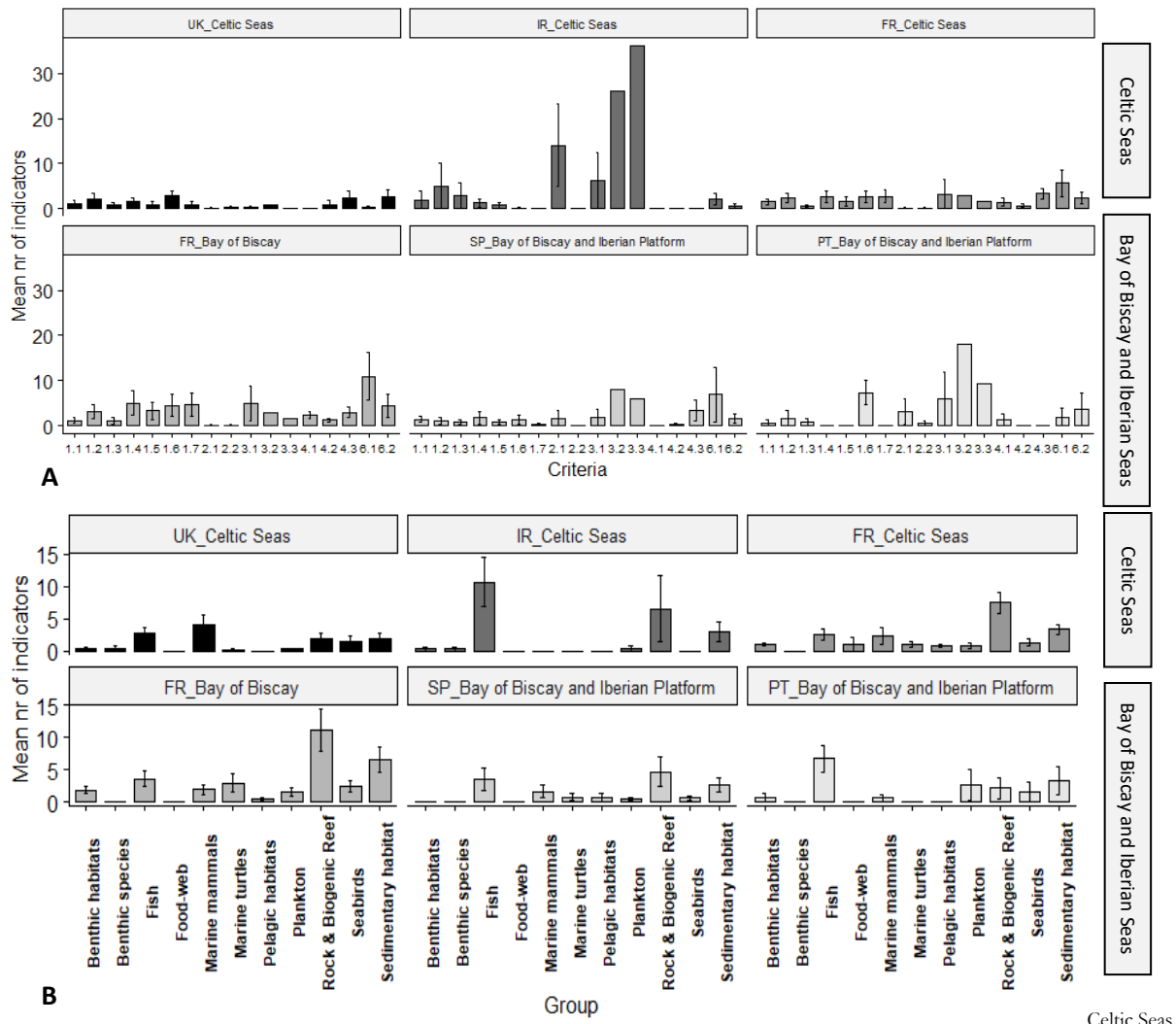


Figure 2-3 - Average number of indicators (lines above bars represent  $\pm$ SE) reported per Criteria, Marine Sub-unit and Biogeographic Region obtained by post-hoc tests (A) and average number of indicators (lines above bars represent  $\pm$ SE) reported per Group, Marine Sub-unit and Biogeographic Region obtained by post-hoc tests(B).

The interaction between Marine Sub-units (nested in Biogeographic Region) and Criteria explained 19.55% of variance ( $p=0.000$ ; Table 2.2). Post-hoc tests showed that Ireland implementation of 3.3 Population age and distribution and 3.2 Reproductive capacity of the stock were highest than the overall interactions (36.0 and 26.0 mean reporting number per Criteria/Sub-unit, respectively). Portugal also exhibited high implementation rates of the same descriptors (with 9.3 and 18.0 mean reporting number). By the contrary, French Bay of Biscay and Celtic Seas Sub-units highest reporting rates were 6.1 Substrate physical damage (mean of 11.0 and 5.8 mean reporting number per Criteria/Sub-unit, respectively). Spain focused on 3.2

Reproductive capacity of the stock and 6.1 Substrate physical damage (8.0 and 7.0 mean reporting number per Criteria/Sub-unit, respectively) and the UK addressed 4.3 Abundance of key trophic groups and 1 Habitat Condition (with 4.4 and 3.1 mean reporting number of Criteria/Sub-unit) (Figures 2.4 and 2.3A). Criteria explained 12.9% of the existing variance and Criteria 3.1 and 3.3 were significantly more reported than the overall Criteria (with 9.8 and 9.1 average reporting number per Criteria). Although in a lesser extent, Marine Sub-unit and Group interaction was also significant and explained as much as 10.8% of the variance ( $p=0.000$ ; Table 2.2). Post hoc tests showed highest implementation rates for Rock & Biogenic Reefs and Sedimentary habitats by the French Bay of Biscay (7.4 and 3.3 mean reporting number per Group/Sub-unit) and Fish by Ireland and Portugal Sub-units (10.6 and 6.7 mean criteria Group/Sub-unit). High reporting rates were also registered for French Celtic Seas and Rock & Biogenic Habitats (7.4 mean reporting number) (Figure 2.3). The analysis of Criteria and Group interaction was redundant, since the MSFD conceptual design implies that specific descriptors are focused on particular groups (e.g. Descriptor 3 focus on Fish group, 1.6 Criteria focusing on Benthic habitats, etc., see Table 2.1).

Table 2-2 - Cluster outputs obtained by the MCA analysis on reporting level (similarity level was established with a probability of 0.05).

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
6 – Seabed Integrity	1 - Biodiversity	4 – Food webs	2 - NIS	3 Commercial fish and shellfish
6.1 Substrate physical damage	1.6 Habitat Condition	4.3 Abundance of key trophic groups	2.1 Abundance of NIS	Fish
6.2 Condition of benthic community	1.5 Habitat extent	4.1 Biomass of key species/ trophic group	IR_Celtic Seas	3.3 Population size/age distribution
Rock & Biogenic Reef	1.4 Habitat distribution	Marine mammals	Rock & Biogenic Reef	3.1 Fishing activity pressure
Sedimentary habitat	1.1 Species distribution	4.2 Proportion of species at the top of food webs	2.2 Environmental impact of NIS	3.2 Stock reproductive capacity
Benthic habitats	1.2 Population size	Seabirds	Benthic species	IR_Celtic Seas
Bay of Biscay and Iberian Coast	1.7 Ecosystem structure	Marine Turtles	Celtic Seas	Celtic Seas
FR_Bay of Biscay and Iberian Coast	1.3 Population condition	FR_Celtic Seas	4.1 Biomass of key species/ trophic group	PT_ Bay of Biscay and Iberian Coast
SP_ Bay of Biscay and Iberian Coast	Plankton	Pelagic habitats	1.3 Population condition	2.2 Environmental impact of NIS
PT_ Bay of Biscay and Iberian Coast	Sedimentary habitats	SP_ Bay of Biscay and Iberian Coast	1.7 Ecosystem structure	Marine Turtles
Marine Turtles	UK_Celtic Seas	UK_Celtic Seas	3.2 Stock reproductive capacity	Pelagic habitats

MCA analysis explained 16% of the heterogeneity among reporting levels. A similarity threshold was automatically defined by a Cluster Analysis with a statistical probability of 0.05, creating five clusters with a similarity threshold of 3%. Clusters' similarity was based on Descriptor/Criteria, Group and, in a less extent, Marine Sub-unit. Descriptor 6 - Seabed Integrity and its corresponding criteria were associated with all benthic habitats and all Bay of Biscay Sub-units (Cluster 1). Cluster 2 included all Descriptor 1 criteria, Plankton Group and UK Sub-unit, while Cluster 3 included Descriptor 4 criteria and was associated to Marine mammals, Seabirds, Marine turtles and French Celtic Seas (Cluster 3). Descriptor 2 - NIS reporting and corresponding Criteria are associated with the Irish Sub-unit and Rock and Biogenic Reefs (Cluster



4). The Irish Sub-unit was partitioned in a second cluster (Cluster 5) together with Fish group and Criteria from Descriptor 3 - Commercial fish and shellfish and the Portuguese Sub-unit (see Table 2.3 and Figure S2; Annex 1).

### 2.3.2 Time range variability

Temporal scales varied largely across all factors under study: Biogeographic Region, MS, Marine Sub-unit, Descriptor, Criteria, and Group (Figure 2.4). Average Time range was wider for Bay of Biscay and Iberian Peninsula and for France and Spain. As for Marine Sub-units, French Bay of Biscay reported the widest ranges (average range of 28.4 years). Descriptor 4 – Food webs and 2 – Non-Indigenous species presented the largest time scales (with an average range of 25.3 and 20.8 years, respectively) and were represented by Criteria 4.1 Productivity of key species/trophic groups, 4.3 Abundance of key trophic groups/species, and 2.1 Abundance of NIS. Marine Turtles presented the widest time scales followed by the Benthic Species Seabirds group (51.5, 21.9 and 21.6 average temporal range) (Figure 2.4).

GLMs showed that all tested factors influenced mean Time range of reporting (Table 2.4). The GLM model that most influenced mean Time range was the interaction of Marine Sub-units (nested in Biogeographic Region) and Group (15.88% of deviance explained;  $p=0.000$ ). The interaction between nested Marine Sub-unit and Group showed that the widest Time ranges were used to report Marine Turtles by French Celtic

Seas and French Bay of Biscay (with 102.0 and 58.3 years in average); Plankton and Food webs in Spain (20.5 and 20.0 average years); Seabirds in the UK (27.0 average years) and Fish in Portugal (with 16.5 average years) (Figure 2.5A). Criteria explained 14.58% of the variance and, when in interaction with Marine Sub-unit (nested within Biogeographic Region), explained as much as 12.01% (Table 2.4; Figure 2.5B). Marine Subunit/ Criteria interaction showed that 3.1 Fishing activity pressure and 4.1 Biomass of key species/ trophic group were highest for French Celtic Seas (with 29.9 and 28.9 years in average) followed by 4.3 Abundance of key trophic groups/specie and 4.1 Biomass of key species/ trophic group for French Bay of Biscay (with 26.6 and 26.1 years in average). Criteria 4.1 Biomass of key species/ trophic group was widest in the Portuguese Subunit and 1.3 Population condition in Spain (21.30 and 20.8 average years). On the other hand, mean Time range for Descriptor 2 – NIS was very low for all Sub-units except for the UK, Ireland and Spain that presented historical data on NIS (Figure 2.5B). Marine Sub-unit nested within Biogeographic Region explained 13.32% of deviance ( $p=0.000$ ); French Bay of Biscay and Spanish Bay of Biscay presented the widest time-scales (average Time range of 18.6 and 16.0 years) and were significantly higher than the corresponding Portuguese unit.

French Celtic Seas and Ireland presented the lowest average reporting time (average Time range of 10.5 and 7.8 years). The first two dimension of the FAMC explained 16% of the variability. Geographically

related variables were the most relevant for Dimension 1 (e.g. Marine sub-unit and Biogeographic Region), while Dimension 2 was mostly explained by Criteria and Group (Figure 2.6B).

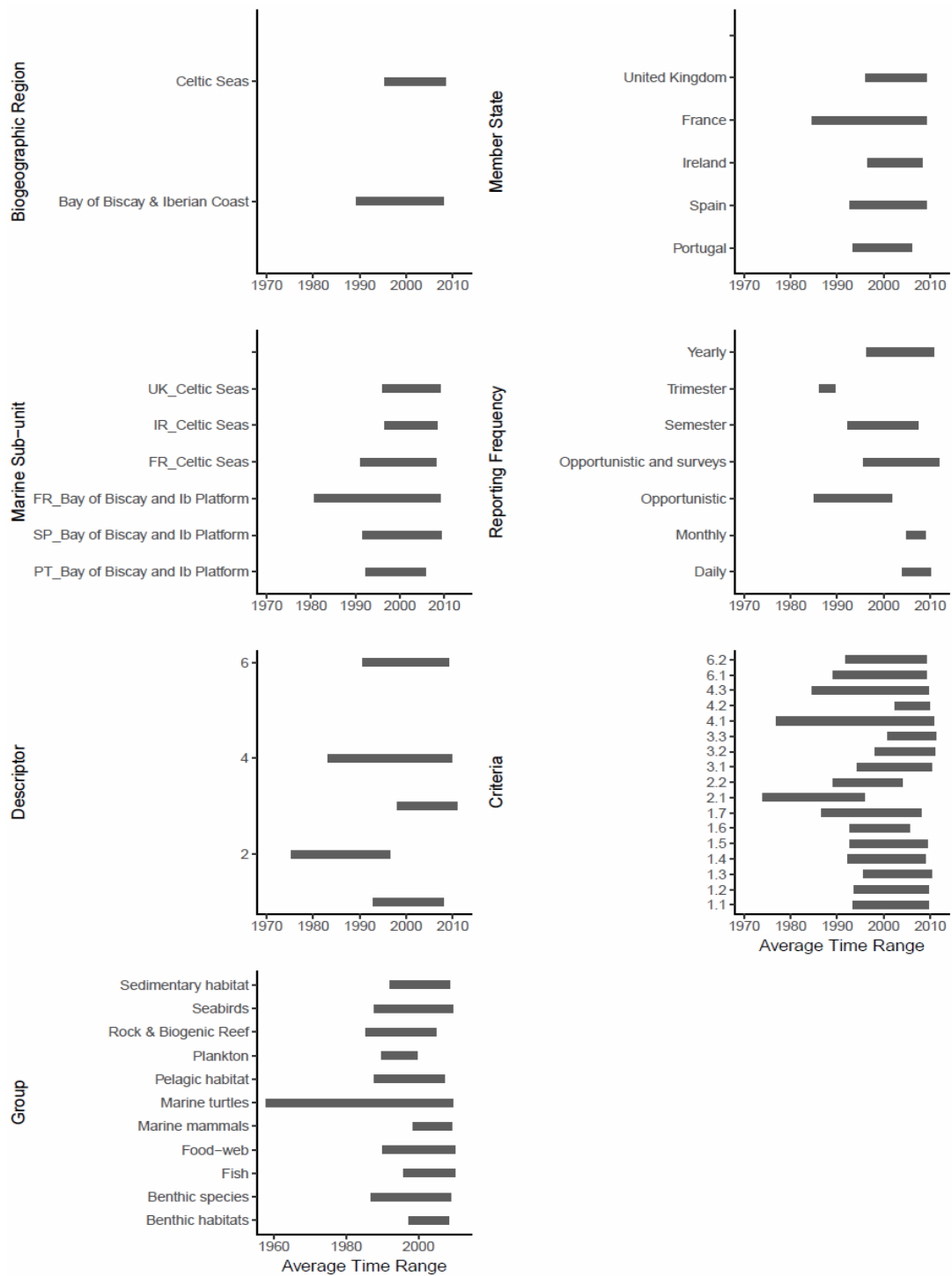
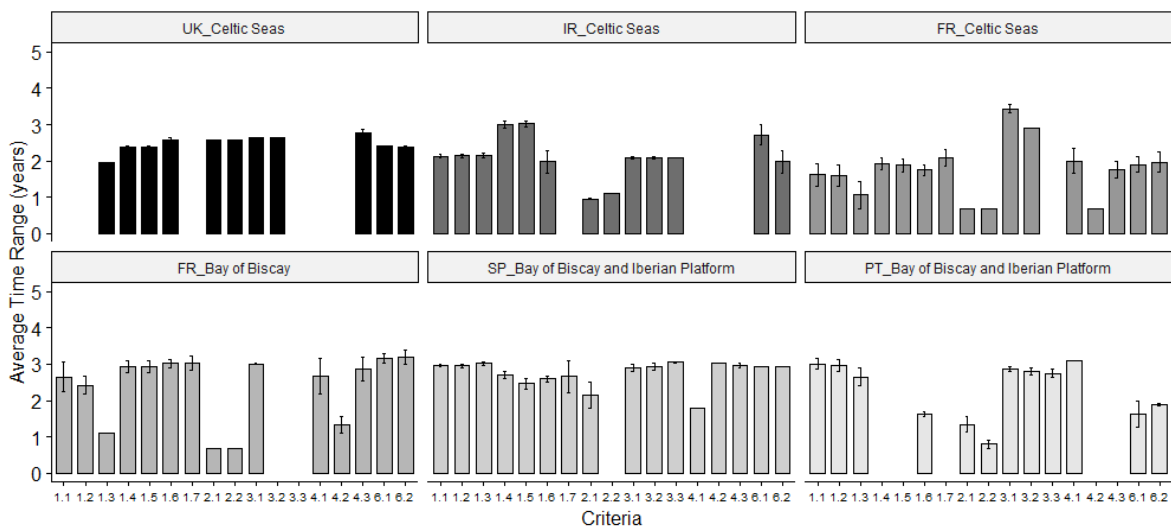


Figure 2-4 - Average initial and final reporting date per Member State, Marine Sub-unit, Biogeographic Region, Descriptor, Criteria and Group (see Table 2.1 for codes).

Although to a less extent, Time range, and Initial year of reporting also contributed to explain variability (Figure 2.6A). Using a statistical probability of 0.05, a similarity threshold was automatically defined by a

Cluster Analysis that resulted in four clusters. Clusters showed that time scales were highly related with Criteria, Marine sub-unit and Group (Figures 2.6B; S3 and Table S1 in Annex 1), suggesting that Time range used to report followed distinct patterns in accordance with these variables. Portuguese and Spanish Bay of Biscay and Iberian Platform data exhibited a similar intermediary Time range and Initial year of reporting. Both Subunits were included in the same cluster with Criteria 2.2 Environmental impacts of NIS and 1.6 Habitat Condition and Groups such as Benthic species, Rock & Biogenic Reef and Fish (Cluster 1). The categories most influenced by high Initial year of reporting and low Time range were Irish Celtic Seas, associated with Criteria 3.3 Population age and size distribution, 3.1 Fishing activity pressure, and Groups as Fish (Cluster 2). Although to a lesser extent, the third Cluster was also associated with a short Time range, including Rock & Biogenic Reef, Sedimentary habitat, and Criteria such as 1.4 Habitat distribution, 1.5 Habitat extent and 6.2 Condition of benthic community together with Celtic Seas units (French and UK Celtic Seas; Cluster 3). A fourth cluster included Marine Turtles, Seabirds and Food-web data together with 1.7 Ecosystem structure, 4.1 Biomass of key species/ trophic group, and 4.3 Abundance of key species. This group was associated with the French Bay of Biscay region, high Time range and low Initial year of reporting (Cluster 4) (Figure 2.6B).



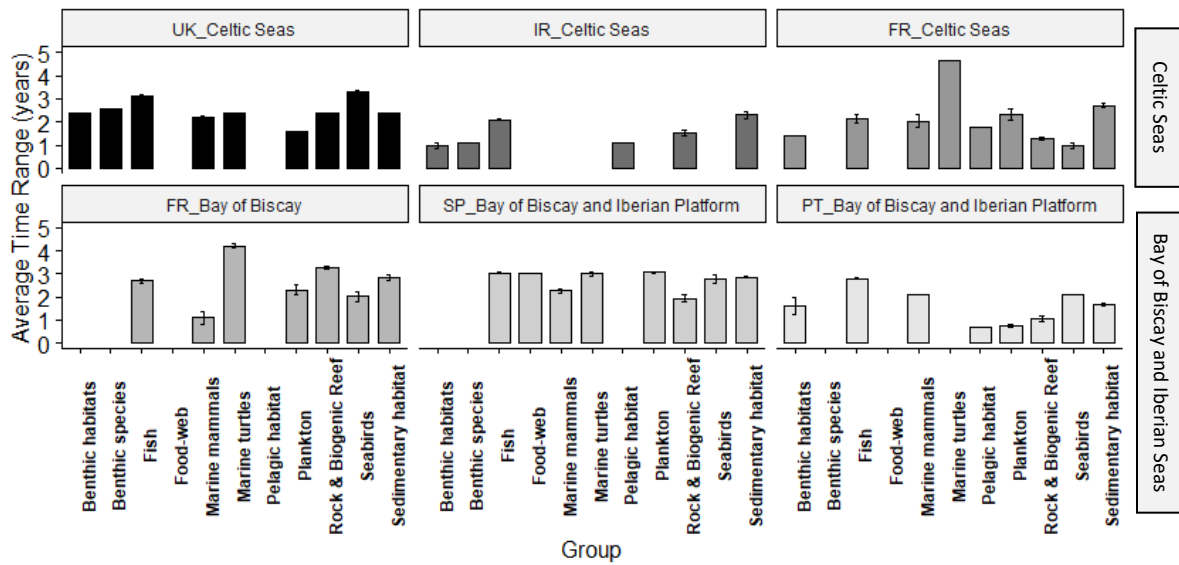


Figure 2-5 - Average time-range (lines on bars represent  $\pm$ SE) resulting from the interaction between groups of Biogeographic Region and Group (A) and groups of Biogeographic Region and Criteria (B); letters indicate significant differences obtained by post-hoc tests.

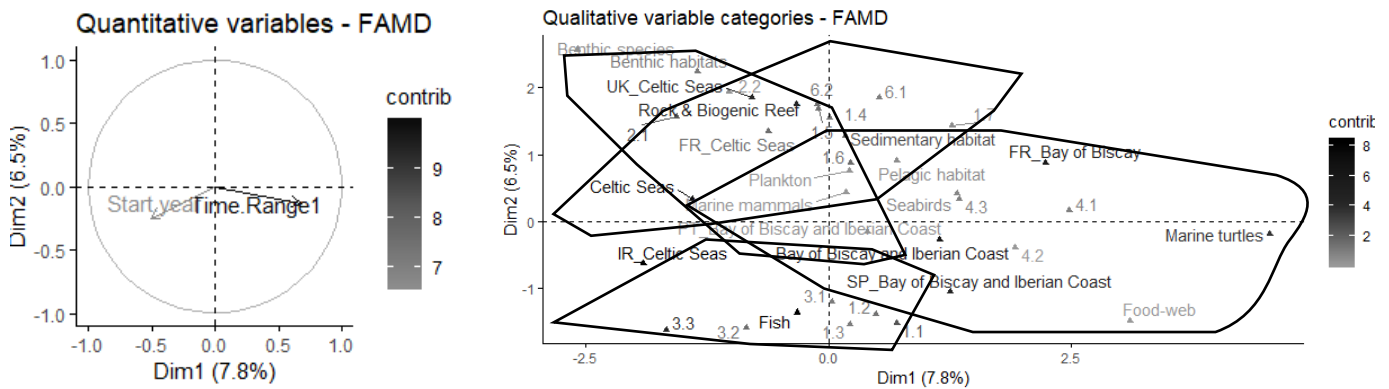


Figure 2-6 - FAMD results showing the contribution of A) - quantitative variables: Time range and Start year/Initial year of reporting and B) qualitative variables: Biogeographic Region, Member State, Marine Sub-unit, Criteria and Group, numbers indicate Clusters.

## 2.4 Discussion

MSFD implementation involved a great effort from MSs in searching, compiling, analysing, and reporting marine environment data. This unprecedented effort allowed MSs to achieve a high-level overview of existing information while concomitantly exposing existing problems. Analyses of the 1st implementation cycle, such as Hummel et al. (2015) and Palialexis et al. (2014), provided a holistic view of the MSFD implementation. These authors highlighted how differently each MS reported. However, their scope was limited to the evaluation of the implementation of Descriptor 1 (Hummel et al. 2015) or Descriptors 1, 2, 4 and 6 (Palialexis et al. 2014). Consequently, and due to the overarching aim of the MSFD, several critical aspects are yet to be addressed. By looking into the specific case study of two Biogeographic Regions, the present work showed how MSFD reporting level and, for the first time, temporal scales varied among sub-units and MSs within the same regional seas and RSC – i.e., OSPAR (OSPAR Commission, 1992).

Overall, different MSs sharing the same region reported differently, which highlights the need for further synchronization across neighbouring MS and Marine Sub-units. French Sub-units presented higher implementation rates and Ireland showed a different strategy from the additional Celtic Seas Marine Sub-units. Ireland reported a large number of indicators but focused on Fish Group and Criteria from Descriptor 3 – Commercial Fish & Shellfish and Descriptor 2 – NIS and Benthic species, thereby unbalancing MSFD implementation in the Celtic Sea region. This strategy is likely a result of a larger investment made by Ireland in obtaining data on commercial stocks. Due to their economic value and consequent commercial exploitation, fish populations have received a greater attention by the EU. The implementation of the CFP has obliged MSs to perform annual stock assessment and created a wide monitoring network (European Commission, 2015). Nevertheless, within each Biogeographic Region, countries should agree on what fish species need to be reported in order to guarantee congruency and Fish Criteria is not over/under-estimate in the same region. On the other hand, Irish results can also evidence different reporting strategies and/or commitment by different institutions; thereby, affecting reporting level and quality of information across descriptors.

The distinctive implementation and lack of coordination between national institutions was already pointed out by Cavallo et al. (2017). However, this question can only be addressed by a dedicated study, focused on implementation differences driven by reporting institution. Criteria and Groups from Descriptor 4 – Food webs and Descriptor 2 – NIS were significantly underreported when compared to criteria from Descriptors 1 - Biodiversity and 3 – Commercial Fish and Shellfish. Although related to the MSFD design, these outputs can also be associated with methodological weaknesses and the lack of fully operational indicators and data concerning these Descriptors (ICES, 2015; Lehtinen et al. 2016; Palialexis et al. 2014; Rombouts et al. 2013). Descriptor 4 – Food webs requires the establishment of relations between trophic levels and relies on long term datasets for implementation, but data gaps and incongruences within and across MSs are hindering its efficient application. Nonetheless, the development of new metrics, methodologies, and modelling tools are expected to increase Descriptor 4 -Food webs assessment and reporting (Borja et al. 2016b; Heiskanen et al. 2016). Descriptor 2 – NIS was also significantly underreported, which is driven by NIS impacts on ecosystems being a relatively recent problem when compared to other, well studied, human impacts (e.g. fisheries, etc.) (Heiskanen et al. 2016). Information from previously implemented directives (e.g. WFD, Birds and Habitats Directives and Common Fisheries Policy (CFP)) was often used in the MSFD reporting. This resulted in high reporting level of Descriptors 1 - Biodiversity and 3 – Commercial Fish and Shellfish and functional groups such as Fish, Birds, and Rocky & Biogenic Reefs. The use of data from previous Directives is highly recommended in order to concentrate efforts, avoid duplication of work, and stick to the very ambitious time frame of the MSFD. Inclusively, high level assessments of the 1st implementation cycle have concluded that MSs should go even farther in what regards using data from earlier policies (Bigagli, 2017; Palialexis et al. 2014). However, data from previous Directives should be carefully selected and standardized as it was partially collected

during national monitoring programmes that sometimes failed to focus in regional features, do not consider an inter-sectorial approach and therefore do not support an integrative EBA.

Temporal scales' variability was mostly explained by different Groups across each Marine Sub-unit (Figure 2.6). Bay of Biscay presented superior implementation rate and reporting time scales, suggesting that data sets in the region are consistent and lengthier. French and Spanish Bay of Biscay used a wide temporal window to assess Marine Turtles and Food webs criteria (e.g. 4.1 and 4.3), employing historic stranding data to report biological groups such as Marine Turtles and Marine mammals'. These data sets reached as far as 100 years' time span and were not reported congruently across MSs. Depending on the MS, these data sets were used to assess tendencies and/or to establish historical references, but their use should be restricted to the establishment of baselines/references, since stranding data can lack representability and statistical credibility (Peltier et al. 2014). On the contrary, Groups from Descriptor 2 - NIS were reported using the lowest Time range. Both descriptors were strongly influenced by the use of historic monitoring networks (Descriptor 4- Food webs) and sporadic research studies (Descriptor 2 - NIS) (e.g. historical marine turtles stranding networks, recent NIS monitoring programs) (Heiskanen et al. 2016), that increase incoherence. These datasets also have shortcomings, such as distinct methodologies and/or sampling designs and are not directly comparable between Marine Sub-units from the same regional sea or even from the same MS (Palialexis et al. 2014). Overall, for contemporary comparison between territories, the use of historical and opportunistic data for assessment should be discouraged. For this purpose, data should be obtained through integrated and harmonised tools and indicators should be measured in the same timeframe. Other Marine Sub-units, such as the UK and Ireland, have founded their reporting in consistent medium- (the UK) to short-term (Ireland) monitoring programmes (Patrício et al. 2016), but lack synchronized initial/ end time of reporting. As a result, data sets and their corresponding temporal scales are not comparable and require further harmonization. Although included in distinct Biogeographic Regions, Portugal and the UK used a similar mean Time range but focused on different ecosystem features and Criteria type. Portugal mostly reported Fish Group, while the UK Celtic Seas focused on Seabirds and 4.3 Abundance of key trophic groups criteria, somewhat similarly to the Irish Celtic Seas but different to French Celtic Seas. Irish data consistently exhibited a low Time range of reporting, and high (recent) Initial report date (i.e. 1997) (Figure 2.6). Descriptors 1 - Biodiversity and 3 - Commercial Fish and Shellfish exhibited a similar mean Time range, reflecting the use of data from previously implemented Directives. However the average initial/end time for each Descriptor was not synchronized across Marine Sub-units and, therefore, GES was assessed with data from distinct temporal windows at the sub-regional and regional level. Nevertheless, the initial reporting date occurred in the previous three decades, matching the results pertinently obtained by Patrício et al. (2016) when assessing MSFD monitoring programmes.

The present study suggests that, for the 1st MSFD cycle, any integration method to combine data across Marine Sub-units, Criteria, and Group should be hindered since temporal scales varied significantly, and

the associated uncertainty was not estimated. Furthermore, any management decision that is based on MSFD outputs should be made carefully. To improve MSFD congruency and to increase coordination mechanisms for Marine Sub-units of the same region, several measures need to be put in place. Marine sub-units should agree on the scales to be used in regional monitoring programs, while considering the specificities of the regional environment. More focus should be given to Descriptors 2 – NIS, 4 – Food webs and 6 – Seafloor Integrity monitoring programs to increase reporting confidence of these descriptors. Further work should be developed concerning Descriptors 1 – Biodiversity and 3 -Commercially exploited Fish and Shellfish, since the time length is similar across Criteria/Groups, but it is not synchronized; the initial and final date of reporting should be decided at a regional level. Establishing an initial time point for reporting would improve commonalities among data sets and enable comparison and integration at a regional level. Congruency should be promoted in the selection and prioritization of targeted habitats and species that have been properly characterized by baseline studies at a regional level, following the recommendation of the Commission Decision 2017/848/ EU (European Commission, 2017). This task has already been partially initiated for Descriptors 1 and 6 (Palialexis et al. 2018) but should be extended to all Biodiversity Descriptors. Lastly, reporting of opportunistic and historical data sets by MSs should be monitored and restricted to the establishment of reference values – more focus should be given to monitoring programmes that address regional seas or Biogeographic Region as a whole. This could be achieved by the development of guidelines at Biogeographic level, that address scales, targets, maximum uncertainty levels, and establishes thresholds, etc., together with a deeper calibration analysis to select inter-MS data to be considered in the regional assessment. Intercalibration, such as the one made for WFD, and uncertainty estimations can provide some perspectives on how to achieve further congruency. However, even though the MSDF and WFD overlap in some extent, the MSFD poses additional challenges, by targeting a much wider number of functional elements and a very wide-ranging criteria/indicators. To accomplish such measures and therefore improve regional coordination, the EC should develop policy instruments and focus in harmonizing methods, scales, and thresholds. Furthermore, Regional implementation could be enhanced by creating scientific institutions in each Biogeographic Region, that focus in the intercalibration and establishment of the regional goals previously suggested. Although very helpful, RSC consider highly diverse regions, their recommendations are not legally binding, and MS have some freedom to implement the Directive (Cavallo et al. 2018). Consequently, MS are interpreting and implementing MSFD differently, in accordance with national data and without a regional/sub-regional perspective.

This political de-synchronization in MSFD implementation contrasts with EBA concept, since the assessment of ecosystems status and anthropogenic pressures should be detached from political borders and should rely in cooperation within RSC and Biogeographic Region. Even though factors behind reporting differences have been widely identified (e.g. MSs politics, policies, funding and even the distinct

usage of maritime space (Cavallo et al. 2017)), the present work discloses the factors behind temporal patterns and highlights aspects that need to be addressed to improve MSFD reporting: 1) unbalanced reporting – each Marine sub-unit is working separately; 2) temporal incongruence due to the use of historical and opportunistic data, and 3) unsynchronized time spans even if using similar data from identical monitoring programs established by previous directives (e.g. CFP, Habitats Directive, etc.). In conclusion, reporting level and time scales used to report biodiversity descriptors in the NE Atlantic Ocean indicate low cooperation and integration of MSs data within Biogeographic Regions, not only in terms of implementation but also in terms of monitoring networks and selection of datasets to assess GES. If no changes are made to the action plan, the main purposes of the MSFD – conservation and sustainability through and integrative ecosystem approach - is very likely compromised.

## 2.5 Acknowledgements

This work is financed by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UID/MAR/04292/2019, granted to MARE - Marine and Environmental Sciences Centre.

The authors have received funding support from the FCT, through PhD and Post-Doc fellowships (PD/BD/135065/2017, SFRH/BPD/115298/ 2016 and SFRH/BPD/89480/2012).



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### 3 *Effects of scale on the assessment of fish biodiversity in the marine strategy framework directive context*

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**The marine strategy framework directive is currently undergoing its second implementation cycle while work is ongoing to promote further harmonization, especially regarding the European Commission standards on datasets, metrics, and thresholds.** Even though spatial scales for Biodiversity descriptor criteria concerning fish species group are set at the Subregion level, the scales of the assessment have never been confirmed across the multiple metrics, taxons and regions addressed by the Directive. In this work, Descriptor 1- Biodiversity and Criterion 2 - Population abundance of the species (D1C2) was evaluated and compared for six non-commercial fish species, at five geographical scales in the Portuguese Continental Platform, to understand if scales used in the assessment affect species group status and hinder the establishment of adequate monitoring or management measures. For comparability purposes, the methods used were identical to the MSFD; a Breakpoint analysis combined with a Trend analysis of the last five years. Results showed that assessments at Portuguese continental Economic Exclusive Zone level mask local population patterns that were visible when smaller size scales were used, and that each species had different scale requirements. *Argentyna sphyraena* had a low biomass index in the Northern region of Aveiro, and Algarve region presented distinct patterns for species analysed in the area. Downsizing scales revealed that *Microchirus variegatus* was not in good status – i.e., below threshold - in the Southern Coastal area, requiring further attention to understand how pressures are impacting populations locally. Although the overall status of the species was maintained, when species assessment was integrated, smaller sized assessment scales are required to understand how populations respond to pressures locally and therefore how monitoring and management of status and pressures should be implemented. Results highlight the need to consider species biology and population dynamics to define precise scales of assessment and to identify areas of risk at locally relevant scales.

**Keywords:** Marine Strategy Framework Directive, Descriptor 1 - Biodiversity, Environmental Status, Breakpoint and Trend Analysis, spatial scales, non-commercial fish species.

#### 3.1 Introduction

The marine environment has been exposed to a growing number of anthropogenic pressures with recognized impacts in different ecosystem components. The degradation of these systems as led to an increase of monitoring efforts and environmental assessments to evaluate the status of marine systems (Borja et al. 2008; Korpinen and Andersen, 2016). In Europe, the Marine Strategy Framework Directive (MSFD) was implemented to halt further degradation of all regional seas (European Commission, 2008). The MSFD tries to achieve good environmental status (GES), by monitoring and assessing 11 qualitative



descriptors, for which different assessment criteria have been proposed. Biodiversity criteria have been developed to assess the environmental status of biological diversity in marine waters and are included in Descriptor 1 – Biodiversity (D1 - Biodiversity). This descriptor aims to ensure that ‘there is no further loss of diversity, deteriorated attributes of biological diversity are restored, and the use of the marine environment is sustainable’. The assessment of biodiversity status is required at three main ecological levels: species groups, habitats and ecosystems, including food webs (European Commission, 2017a, 2017b). However, issues such as the precision of metrics, inconsistencies in methodological approaches and data scarcity have hindered the establishment of targets and thresholds, which are necessary for the correct implementation of this Descriptor in the initial assessments (Hummel et al. 2015; Palialexis et al. 2014). Given the issues raised, the European Commission (EC) requested the Regional Sea Conventions (RSCs), MSFD Task Groups and Integration Organizations (e.g., International Council for the Exploration of the Sea (ICES), Joint Research Centre (JRC), Oslo/ Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR), etc.) to advance research and standardization on such topics using the best available knowledge. As a result, MSFD regulation was updated prior to the 2<sup>nd</sup> cycle of implementation, to detail criteria and methodological standards (European Commission, 2017a). The Commission Decision (EU) 2017/848 defines the species groups and habitat types as the units for GES for each criterion assessed and the Commission Decision (EU) 2017/845 updates the ecosystem elements and the anthropogenic pressures and impacts that must be assessed (European Commission, 2017a, 2017b). Species-level aspects of biodiversity are considered for each ecosystem component (i.e., birds, mammals, reptiles, fish and cephalopods) and their ‘species groups’. Each species group is assessed using a set of representative species, and each species is assessed using defined criteria. Afterwards criteria results are combined for each species, and species are aggregated into species groups to assess the overall status of each group (Walmsley et al. 2017). Currently, the biodiversity descriptor includes the following criteria for fish: D1C1, the mortality rate per species from incidental by-catch is below the levels which threaten the species; D1C2, the population abundance of the species is not adversely affected due to anthropogenic pressures; D1C3, the population demographic characteristics; D1C4, the species distributional range is in line with prevailing physiographic, geographic and climatic conditions; and D1C5, the habitat for the species has the necessary extent and condition to support the different stages in the life history of the species (European Commission, 2018). Although several developments have been made to improve the coherency within those criteria across geographically relevant scales, assessing the status of non-commercial fish and cephalopods is still a challenge as the objectives established are very ambitious, especially when considering the datasets currently available (Palialexis et al. 2019). Due to the overarching framework of the directive, inconsistencies can arise due to the species selected, methodological mismatches, scales differences and/or targets established within the same region (Borja et al. 2019; Greenstreet et al. 2012; Hummel et al. 2015; Machado et al. 2019; Raicevich et al. 2017; Trenkel et al. 2015).

Following the Commission Decision (EU) 2017/848, each Member State (MS) should establish a list of representative fish species for each group and the appropriate ecologically relevant scales of assessment per species and group. In the Northeast Atlantic, the assessment areas vary among fish groups: subdivision of region or subregion (Bay of Biscay and Iberian waters) for coastal fish, subregion for pelagic and demersal fish and region (Northeast Atlantic) in the case of deep-sea fish (European Commission, 2017a; Walmsley et al. 2017). The dataset used for the assessments is decided at MS level; either by using a MSFD monitoring scheme or by making use of the data collected under the EU Data Collection Framework (DCF) implementing the Common Fisheries Policy (CFP) or other national or international projects (Palialexis et al. 2019). For wide-ranging mobile species, assessment areas may need to be as large, or larger than, subregions, to reflect the distribution of some species (e.g., cetaceans, fish, etc.). For commercially exploited species (assessed under Descriptor 3) the recommendation is to be assessed according to the stock assessment areas used under the CFP, established by appropriate scientific bodies (as, for example, the International Council for the Exploration of the Sea, ICES) (ICES, 2017a). ICES recommends that the assessment area should be defined according to hydrological, oceanographic and biogeographic criteria and as a result it uses sub-areas and divisions of sub-areas which form the basis for catch statistics and population monitoring (Prins et al. 2014). In the case of fish stocks occurring in Portuguese mainland waters, fish assessment can be conducted for subdivision 9a (Portuguese continental waters and Spanish Gulf of Cádiz), subdivisions 8c and 9a combined (including also the north of Spain up to the Basque country) or to a broader scale (e.g. Northeast Atlantic) (ICES, 2017b). However, if assessment areas are too large, there is a risk that assessment of GES reflects the most frequent population pattern and fails to take into account significant but localized impacts that could result in a shrinking of the population's range or fragmentation of it. As a result, separate populations of a species that exist within a particular region or subregion, should be assessed individually and integrated posteriorly in an increasing degree of complexity (Walmsley et al. 2017). Aggregation methods have been shown to work for several descriptors at the European scale, but for D1 assessment is still a challenge (Borja et al. 2019). There is a multiplicity of assessment scales that vary in accordance with indicators, species and groups of species selected across regional seas, especially for species that are not under Habitats Directive and have no-commercial interest (Walmsley et al. 2017). OSPAR recommends that a "case by case" approach is taken to define relevant assessment areas, addressing ecological challenges depending on species. If possible, the defined area should be compatible (or nested) within the sub-regions (OSPAR Commission, 2012; Borja et al. 2019).

Given the relatively broad assessment areas under the MSFD, it can be hypothesized if, to assess Biodiversity criteria, the use of wide range assessment scales can mask localized anthropogenic pressures that could otherwise be untangled if assessment units were downsized. For this purpose, the use of smaller sized units of assessment may contribute to the identification of potential localized effects of pressures on fish biodiversity.

In this work the D1C2 criterion was estimated to investigate how species status varies with the dimension of the assessment units. The aim was to evaluate if ecologically relevant scales, from wider to smaller assessment areas, have effects on Criterion D1C2's detection of localized pressures and therefore on D1C2 assessment results. With this purpose, this criterion was estimated for five pre-defined spatial ranges and for six ecologically relevant species with low or no commercial interest - coastal and demersal fish species. Since there are not yet any agreed threshold levels to define when a species' abundance meets GES, a trend-based approach was carried out (ICES, 2017b; OSPAR Commission, 2012); it combined a breakpoint analysis, to calculate both reference and assessment estimates, and a trend-based method, to evaluate the trend of the series in the last five years, to understand if species status varies in accordance with the scales used in the assessment. Outputs are expected to identify scales that show locally relevant results for each species and therefore improve the identification of anthropogenic impacts, increasing reporting coherence and promoting advancements on the selection of the scales used, to better accommodate MSFD objectives.

## 3.2 Methodology

### 3.2.1 Study area

The study area encompasses the Bay of Biscay and Iberian Waters sub-region, focusing on the Portuguese continental shelf and upper slope, from Caminha to Vila Real de Santo António. The area surveyed extends from latitude 41°20'N to 36°30'N, covering depth ranges from 20 m to 500 m (Figure 3.1a) (adapted from ICES 2017a).

### 3.2.2 Data set & analysis

The dataset was collected by the Instituto Português do Mar e da Atmosfera, I.P. (IPMA, IP), from 1982 to 2017, during the Autumn Groundfish Survey performed yearly under the EU data collection framework (PNAB/DCF), coordinated by the International Bottom Trawl Survey Working Group (IBTS) from ICES (Figure 3.1a). The current sampling design consists of a mixture of fixed and random tows, distributed over 12 sectors subdivided into three depth strata (ICES, 2017a) (Figure 3.1b). Despite changes in the survey design, since 1982, alterations have been made focusing on maintaining the data comparability among different series and covering a comparable spatial area. In each haul, the catch composition, number and weight were recorded for all species of fish, cephalopods, crustaceans and other invertebrates. More details on the Autumn demersal surveys' design, alterations and sampling are described in ICES (2017a).

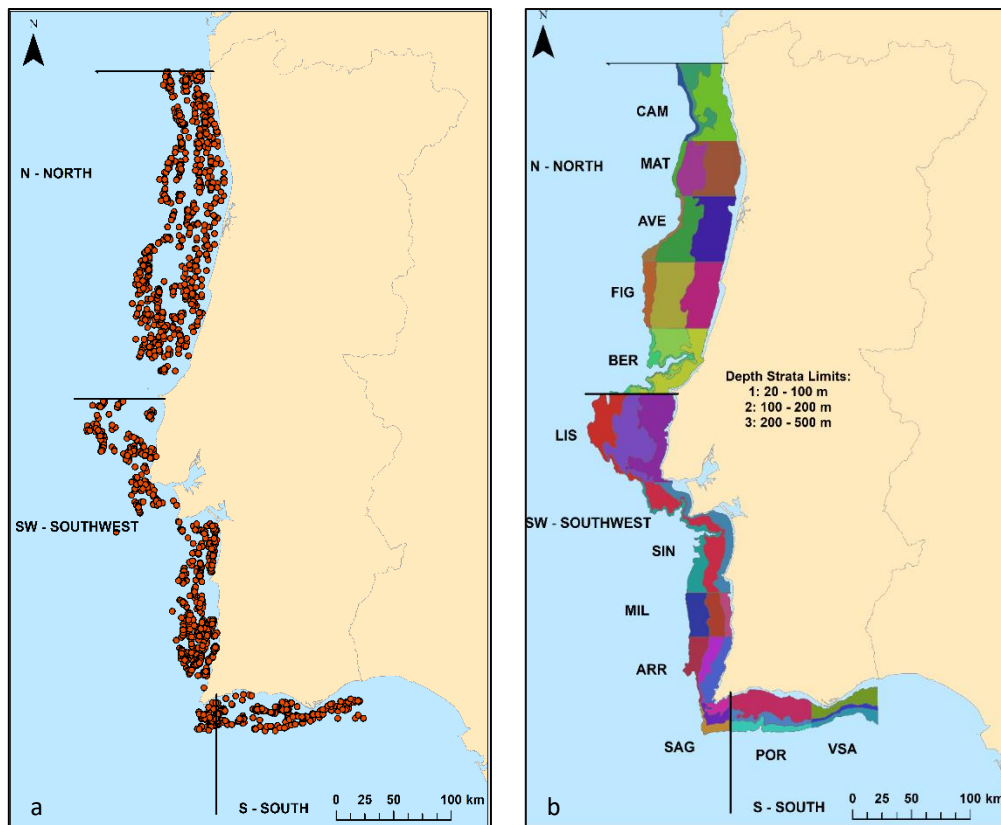


Figure 3-1 - a) Study area showing the location of each trawl held in the Portuguese survey – from 1982 to 2017 (source: IPMA); b) Study area showing the delimitation of each Sector and depth strata tested (ICES 2017a).

The methodologies followed the ones adopted to assess fish and cephalopods under Descriptor 1 and Criteria 2 - D1C2 on the 2nd evaluation cycle of the MSFD in the Portuguese continental waters (IPMA, 2020). As commercially relevant species are addressed in Descriptor 3, six coastal and demersal ecologically relevant species with low or no commercial value were selected (Table 3.1). An exploratory analysis was performed to characterize the distribution of each species along the Portuguese waters, to identify the global spatial area to further depict in the analyses. The scales selected are presented in Table 3.2 (see also Figure S1 to S6 in Annex 2). The geographical sectors, defined in the survey design were grouped into North, Southwest and South, following the criteria adopted in the two national MSFD assessment reports (2012 and 2020 (IPMA, 2020; MAMAOT, 2012)). Sector, Depth and the combination of both were also analysed, following the stratification used by the demersal groundfish survey. A biomass index ( $\text{kg}\cdot\text{h}^{-1}$ ) was determined as the mean biomass per year in each spatial unit considered (Table 3.2 and Figure 3.1b). For each species, the biomass index was analysed from a wider scale - (Portuguese continental EEZ) to the smallest spatial scale (Sector\*Depth strata) possible. For each spatial unit, the estimated biomass index was only considered when a minimum of three tows were carried out in each year. For the Sector\*Depth strata scale, a minimum of two tows were considered, due to the design of the survey, that establishes a minimum of two tows in each Sector\*Depth strata unit. In addition, spatial units with time series including less than 20% of the years, i.e. fewer than 8 years, were discarded. It should be remarked that these criteria were

applied for each species and spatial scale separately which resulted in distinct datasets and independent analysis for each spatial scale.

Table 3-1 - Species selected for analysis by species group and corresponding evaluation area.

Species Group	Scientific name	Common name	Areas evaluated	Depth evaluated
Coastal fish	<i>Callionymus lyra</i> (LYY)	Common dragonet	N, SW	20-190
	<i>Pagellus erythrinus</i> (PAC)	Common pandora	SW, S	20-140
	<i>Serranus hepatus</i> (SRJ)	Brown comber	S	20-160
Demersal fish	<i>Argentina sphyraena</i> (ARY)	Argentine	N, SW	50-250
	<i>Lepidotrigla</i> spp. (I-LEP)	Gurnards	SW, S	25-260
	<i>Microchirus variegatus</i> (MKG)	Thickback sole	N, SW, S	50-400

Table 3-2 - Spatial scales and corresponding spatial units selected for analysis. See the notation shown in Figure 3.1b for detailed information on spatial units' codes (Zone, Zone\*Depth strata, Sector and Sector\*Depth strata).

Scope of the analysis	Spatial Scales	Spatial units
Wider scale	Portuguese continental EEZ	Portuguese continental coast and upper slope
	Country demarcations: Zone	N- North; SW- Southwest; S- South
	Depth strata	1-Coastal (20-100m), 2-Medium (100 – 200m), 3-Deep (200-500m)
	Zone*Depth strata	N1, N2, N3, SW1, SW2, SW3, S1, S2, S3
	Sector	CAM, MAT, AVE, FIG, BER, LIS, SIN, MIL, ARR, SAG, POR, VSA
	Sector*Depth strata	CAM1, CAM2, CAM3, MAT1, MAT2, MAT3, AVE1, AVE2, AVE3, FIG1, FIG2, FIG3, BER1, BER2, BER3, LIS1, LIS2, LIS3, SIN1, SIN2, SIN3, MIL1, MIL2, MIL3, ARR1, ARR2, ARR3, SAG1, SAG2, SAG3, POR1, POR2, POR3, VSA1, VSA2, VSA3
Smaller scale		

Each time series was smoothed by a LOESS-smoother with a span of 0.3 to decrease interannual variability resulting from sampling errors (Probst and Stelzenmüller, 2015). The assessment of species status for each spatial unit followed the method proposed by Probst and Stelzenmüller (2015), to assess if the species status achieved the threshold established for the area. This is a two-stage method that includes a breakpoint analysis (BPA) combined with a trend analysis (TA). This method used the 'breakpoints'- function of the 'strucchange' – package (Zeileis et al. 2001) for R-statistical software (R Core Team, 2019), that identifies points in time (i.e. break points) in which a time series shifts from one stable period/ state into another (Bai and Perron, 1998; Zeileis et al. 2001). The algorithm implemented in the 'breakpoints' function fits the optimal number of linear, zero-slope regressions to the time series by minimizing the residual sum of squares (Bai and Perron, 2003). Although BPA provides reference values based on observed stable periods, this technique is unresponsive to changes occurring in the last 1 to 5 years of the time-series. Therefore,

this method combined the trend analysis (TA) of the last 5 years by applying a linear regression to assess whether the slope was significant and whether it was indicating a positive or negative trend. The combination of both methods has been regarded as adequate to assess the short- (1–5 years) and long-term (more than 5 years) status of the indicator time series. Summarizing, after obtaining the results for BPA, TA was used to confirm if the trend was increasing or declining, and to establish the approach required for management (Probst and Stelzenmüller, 2015) (Figure 3.2).

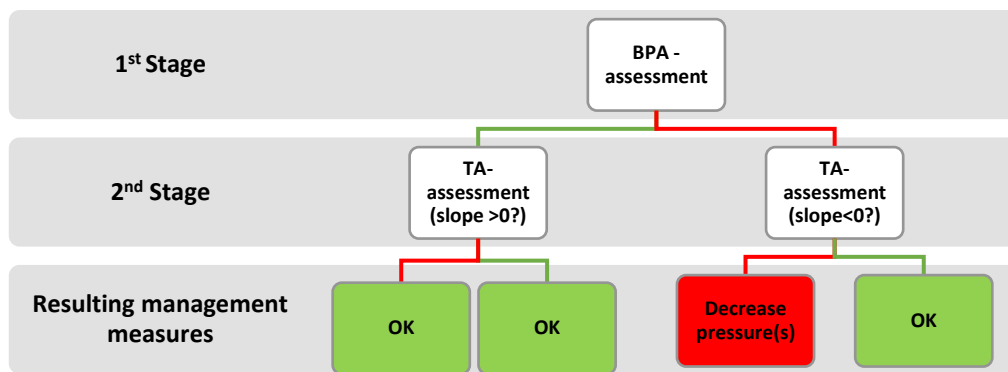


Figure 3-2 - Scheme showing the two-stage analysis applied to fish biomass index time-series: Breakpoint analysis (BPA) and Trend Analysis (TA). Green lines indicate Good status and red lines indicates Not Good status (adapted after Probst and Stelzenmüller 2015 and [IPMA, 2020](#)).

### 3.3 Results

#### ***Callionymus lyra* Linnaeus, 1758 - Common dragonet**

The assessment of *C. lyra* biomass index was based on data collected in the N region, in depths ranging between 20 m and 190 m. Data available was adequate to run the analyses for all the spatial scales established. The species was considered in a good status in all spatial units and spatial scales (Table 3.3 and Table S1, Annex 2). However, BPA results showed a significant decrease between the reference and the assessment periods for the Portuguese continental Economic Exclusive Zone (EEZ) assessment, and in several units, such as the Coastal and Medium depths, AVE, AVE2, BER1, CAM1 and FIG2 (see acronyms in Figure 3.1 and Table 3.2). The TA also provided evidence of decreasing trends, but none was significant. Considering the continental coast and upper slope, analysis revealed an historical peak of biomass in 1993 and an increase after 2013. When dividing into smaller sized areas, depth strata 1 and 2 presented similar time series with minimal differences in the biomass index. When analysing scales at the Sector level, AVE, CAM and FIG showed a similar time series. MAT had a similar peak, that occurred earlier, in 1991, and an additional peak in 2016. BER sector presented irregular time series with several peaks, such as 1986, 2001, 2008 and 2016, but no defined BPs. Sector\*Depth scales revealed similar patterns, especially for AVE2, FIG1, FIG2, and MAT1.

### ***Pagellus erythrinus* (Linnaeus, 1758) - (Common pandora)**

The assessment of *P. erythrinus* biomass index was based on biomass data collected in the SW and S regions, in depths ranging from 20 m to 140 m. Data available was adequate to run the analyses for all the spatial scales defined (Table 3.3 and Table S2, Annex 2). The comparison between the reference and the assessment averages and the TA showed that all scales were in line with existing thresholds. However, BPA showed a significant decrease in VSA1 time series and TA revealed a significant decrease for Sector MIL and SAG. The biomass index exhibited an increasing trend since 2005 – presenting a single BP in 2012. The historical maximum was recorded in 2017. Downsizing spatial scales showed that the biomass index in the S region and depth strata 2 presented typically three BPs in opposition to SW and depth strata 1 that presented a single BP in 2012, similarly to the Portuguese continental EEZ assessment. The 2012 BP was common to all Zones and depth strata units. All Sectors presented biomass peaks after 2011/2012, except for VSA, that presented a peak in 1986 and SIN and POR that presented a peak earlier, in 2011. POR and SIN also presented a biomass index peak in 1989. The majority of Sector\*depth strata units revealed very irregular time series due to data scarcity.

### ***Serranus hepatus* (Linnaeus, 1758) - (Brown comber)**

The assessment of *S. hepatus* biomass index was based on biomass data collected in S region, in depths ranging between 20 m and 170 m. Sector\*Depth strata units revealed very irregular time series due to data scarcity and was not analysed for this species. Analysis made at smaller size scales revealed no differences in species status, for BPA and TA, in relation to the Portuguese continental EEZ results (Table 3.3 and Table S3, Annex 2). The biomass increased from 1988 to 2005 – when the historical maximum was registered. After a steep decrease until 2009, biomass presented a growing trend between 2013 and 2017. BPs were registered in 1988, 1993, 2006. The analysis using depth scales showed four BPs for the depth strata 1 and depth strata 2. BPs in depth strata 2 had one to two years of temporal gap in relation to depth strata 1, but the overall trend was similar. Sector analysis showed that POR had similar BPs, biomass peaks and trends to the ones obtained in the Portuguese continental EEZ assessment, while VSA exhibited distinct BPs and trend.

### ***Argentina sphyraena* Linnaeus, 1758 - (Argentine)**

The assessment of *A. sphyraena* biomass index was based on data collected in the N and SW regions, in depths between 50 m and 250 m. Data available was adequate to run the analyses for all the spatial scales defined. Of all spatial scales and units analysed, BPA showed significantly lower averages in the assessment period in the Portuguese EEZ area and in spatial levels such as N, depth strata 2, N2, SW2, ARR, AVE, MIL, SAG, ARR2, BER2, CAM1, LIS2, MAT2, SAG2, and SIN2. TA revealed a decreasing trend for AVE2 (Table 3.3 and Table S4, Annex 2). Although included in distinct scale levels, Aveiro assessment units - AVE and AVE2 – failed to achieve BPA and/ or TA thresholds. The Portuguese continental EEZ

assessment revealed three biomass peaks: 1990, 1995 and 2006, followed by a decreasing trend. When downsizing scales, 1990 and 2006 peaks were especially relevant in the N region, while the 1995 peak was evident in SW. Depth strata 2 was similar to the Portuguese continental EEZ time series, while depth strata 1 showed a biomass index peak in 1989. Depth strata 3 regions had an irregular biomass index. N1 time series revealed a single biomass index peak in 1989, and N2 exhibited a biomass time series peaking in 1985, 1990 and 2006. The SW1 area exhibited a biomass peak in 1991 and SW2 area showed the highest biomass value in 1982 and 1995. Biomass was higher in N2 and SW2. At Sector level, all units had, at least, one biomass peak synchronized with the Portuguese continental EEZ time series; the most frequent peak was 1990 for CAM, MAT, AVE, BER, LIS and AR. AVE, MAT, LIS, ARR and SAG had an additional peak in 1985. The higher annual mean values were found for BER, MIL, SIN and SAG. Sector and Depth strata analysis showed that the biomass index in AVE1, BER2, and SIN2 was similar to the Portuguese continental EEZ assessment.

### ***Microchirus variegatus* (Donovan, 1808) - Thickback sole**

The assessment of *M. variegatus* biomass index was based on data collected in the entire Portuguese EEZ, in depths ranging from 50 m to 400 m. Data available was adequate to run the analyses for all the spatial scales defined. BPA showed a significantly lower biomass value in the assessment period for S, S1, S3, MIL, POR, SAG, MIL3, POR1, SIN1, SIN3, and VSA3 units. TA had a significant decreasing trend for S, N3, S1, BER, VSA and AVE2, CAM2, LIS2, MAT1, and SAG2 (Table 3.3 and Table S5, Annex 2). The S and S1 areas had a significant lower biomass in relation to the reference period, and a significant TA decline, failing to achieve established thresholds (Figure 3.3 to Figure 3.7). The Portuguese EEZ analysis showed a biomass index peak in 1993, and two BPs. Similar trends were found for the N (peak in 1993) and S regions (peaks in 1992 and 2005), but the biomass index was higher in the N than in the S. The SW region showed lower biomass and an irregular time-series. Depth strata 1 biomass index peaked in 1992, while depth strata 2 peaked in 1993 and 2011. Depth strata 3 biomass index peaked in 1992, 1995 and 2015. N1, N2 and N3 areas exhibited similar patterns to Portuguese continental EEZ time series, but higher biomass peaks. The SW area was distinct for each depth strata. S1 and S2 regions were similar to the S region patterns but presented three BPs. S3 had distinct BPs and the lowest biomass values. Regarding Sectors, CAM, MAT and FIG, had a similar time series to the overall N region. SW Sectors, e.g. LIS, ARR, and SIN, were highly irregular. The biomass index in FIG1, FIG2, SIN2 and ARR2 presented a similar time series to the Portuguese continental EEZ. Units such as CAM2, MAT3, and FIG3 from the N region, LIS2, LIS3, MIL2, MIL3, SIN1, and SIN2 from the SW region, and POR2, VSA1, VSA3 from the S region had a common peak between 2010 and 2015.

### ***Lepidotrigla* spp. - Gurnards**



The assessment of *Lepidotrigla* spp. biomass index was based on data collected in the SW and S regions, in depths between 25 m and 260 m. The gurnard species refers to the spiny gurnard *Lepidotrigla dieuzeidei* Blanc & Hureau, 1973 and the large-scaled gurnard *Lepidotrigla cavillone* (Lacepède, 1801) and were combined into the genus level due to possible misidentification in the past. Data available was adequate to run the analyses for all the spatial scales defined. The BPA comparison between the reference and assessment showed a significant biomass index decrease for ARR2 time series. The TA revealed significant decreases for units such as S, depth strata 1, S1, POR, SAG, VSA and SAG2 (Table 3.3 and Table S6, Annex 2). The Portuguese continental EEZ time-series presented two periods with high biomass index values, between 1986 and 1991 and between 2011 and 2017. The SW region presented a singular peak in 2017, while the S region was similar to the Portuguese continental EEZ time series. Depth strata 1 was identical to the latter and presented the highest biomass values in 2013. Depth strata 2 presented three peaks in 1987, 1992 and 2008 and depth strata 3 presented a peak in 1985. The three SW and S depth strata units were very distinct, but the units with highest biomass were SW1 and S1. As for Sector, SW regions such as ARR, MIL and SIN shared a common BP in 2012, but MIL, SIN and VSA had additional BPs. The three S sectors units were distinct concerning biomass index peaks and BPs (Table S6, Annex 2). Highest biomass values were found in MIL, SAG, and VSA. Sector\*Depth strata scales presented highly variable time series, but MIL1 registered the highest biomass index value in 2013.

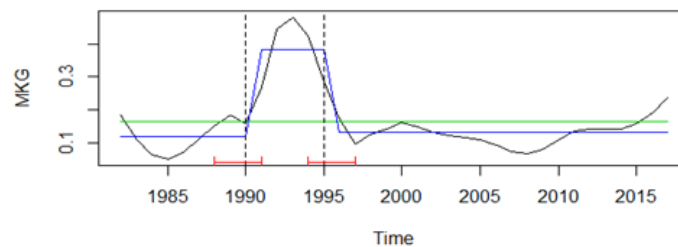


Figure 3 3 - Average smoothed biomass time series (kg.h<sup>-1</sup>), breakpoints and error for *Microchirus variegatus* in the Portuguese continental waters, using the demersal Autumn surveys (1982-2017) data sets (adapted from IPMA, 2020). Global average (green line), breakpoints (dashed line), average for each stable period (blue line) and error (red lines).

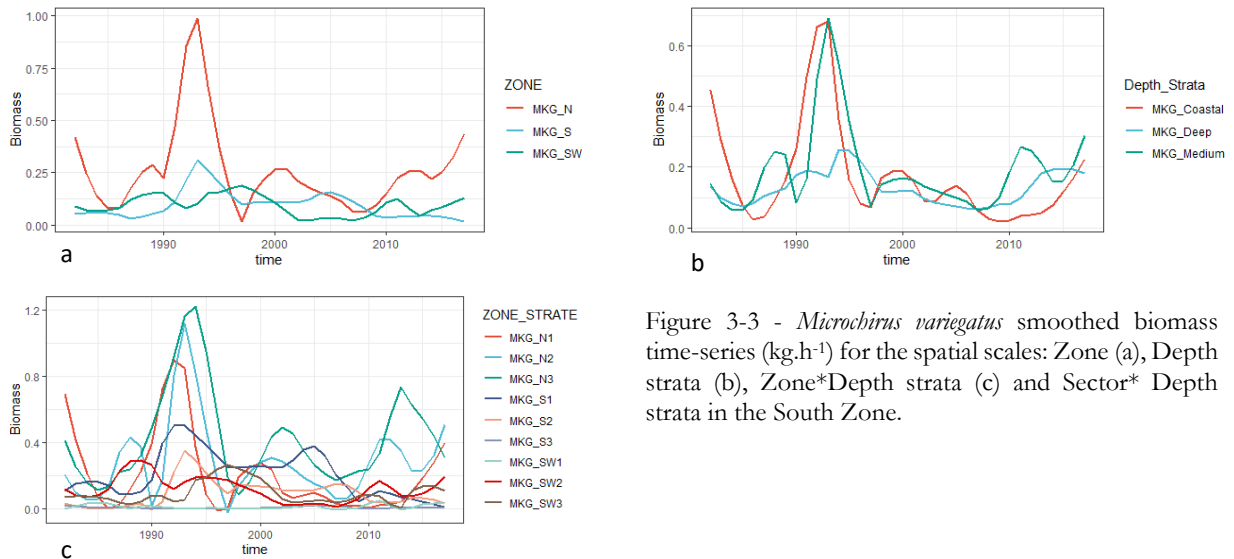


Figure 3-3 - *Microchirus variegatus* smoothed biomass time-series ( $\text{kg}\cdot\text{h}^{-1}$ ) for the spatial scales: Zone (a), Depth strata (b), Zone\*Depth strata (c) and Sector\* Depth strata in the South Zone.

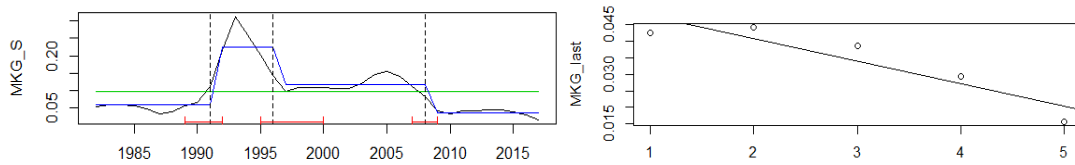


Figure 3-4 - BPA for the South (Zone spatial scale) for *Microchirus variegatus* time series (1982 to 2017): a) average smoothed biomass ( $\text{kg}\cdot\text{h}^{-1}$ ), global average (green line), breakpoints (dashed line), average for each stable period (blue line) and error (red lines), b) TA of the last five years of the time series: year = >2013.

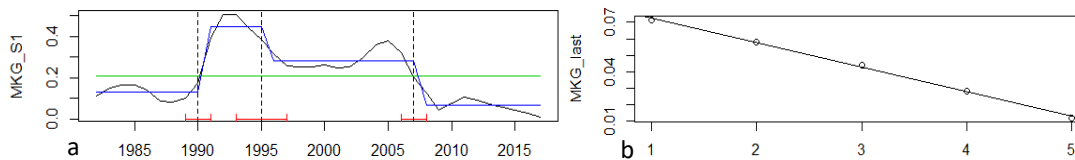


Figure 3-5 - BPA for S1 (Zone\*Depth strata spatial scale) for *Microchirus variegatus* time series (1982 to 2017): a) Average smoothed biomass ( $\text{kg}\cdot\text{h}^{-1}$ ), average (green line), breakpoints (dashed line), average for each stable period (blue line) and error (red lines), b) TA of the last five years of the time series: year = >2013.

Table 3-3 - BPA, TA and final assessment status for the coastal (*Callionymus lyra*, *Pagellus erythrinus*, *Serranus hepatus*) and demersal (*Argentina sphyraena*, *Microchirus variegatus*, *Lepidotrigla* spp.) fish species under analysis. Colors represent assessment outcome in relation to the threshold established in the methodology, red- “not-good”, green- “good”, grey – “area not assessed”, white – “area without the minimum number of trawls”. No BPs indicates that no Breakpoints were detected in the time series and NaN (Not Available) indicates that TA slope was 0.

Functional Group	Coastal									Demersal								
	<i>Callionymus lyra</i>			<i>Pagellus erythrinus</i>			<i>Serranus hepatus</i>			<i>Argentina sphyraena</i>			<i>Microchirus variegatus</i>			<i>Lepidotrigla</i> spp.		
Spatial Unit	BPA	TA	Stat	BPA	TA	Stat	BPA	TA	Stat	BPA	TA	Stat	BPA	TA	Stat	BPA	TA	Stat
Portuguese continental EEZ	Red	Green								Red	Green							
N	Red	Green								Red	Green							
SW				Green									Green					
S							Green						Red			Red		
1 – 0-100 m	Red	Green																Red
2 – 101-200 m	Red	Green								Red	Green							
3 – 201- 500 m																		
N1	Red	Green								Red	Green							
N2										Red	Green							
N3													Red					
SW1				Green						Red	Green							
SW2										Red	Green							
SW3																		
S1				Green			Green						Red			Red		
S2													Red					
S3													Red					Grey
CAM																		
MAT																		
AVE	Red	Green								Red	Green							
FIG																		
BER	No BPs													Red				
LIS																		
SIN																		
MIL							Red			Red	Green		Red					
ARR										Red	Green							
SAG													Red					
POR													Red					
VSA														Red				Red
CAM1	Red	Green								Red	Green							
CAM2																		
CAM3																		
MAT1																		
MAT2										Red	Green							
MAT3																		
AVE1																		
AVE2	Red	NaN									Red			Red				
AVE3																		
FIG1																		
FIG2	Red	Green																
FIG3																		
BER1	Red	NaN																
BER2										Red	Green							
BER3																		
LIS1																		
LIS2										Red	Green							
LIS3																		
SIN1																		
SIN2										Red	Green							
SIN3																		
MIL1																		
MIL2																		
MIL3																		
ARR1																		
ARR2										Red	Green					Red		
ARR3																		
SAG1																		
SAG2										Red	Green			Red				
SAG3																		
POR1																		
POR2																		
POR3																		
VSA1																		
VSA2																		
VSA3																		

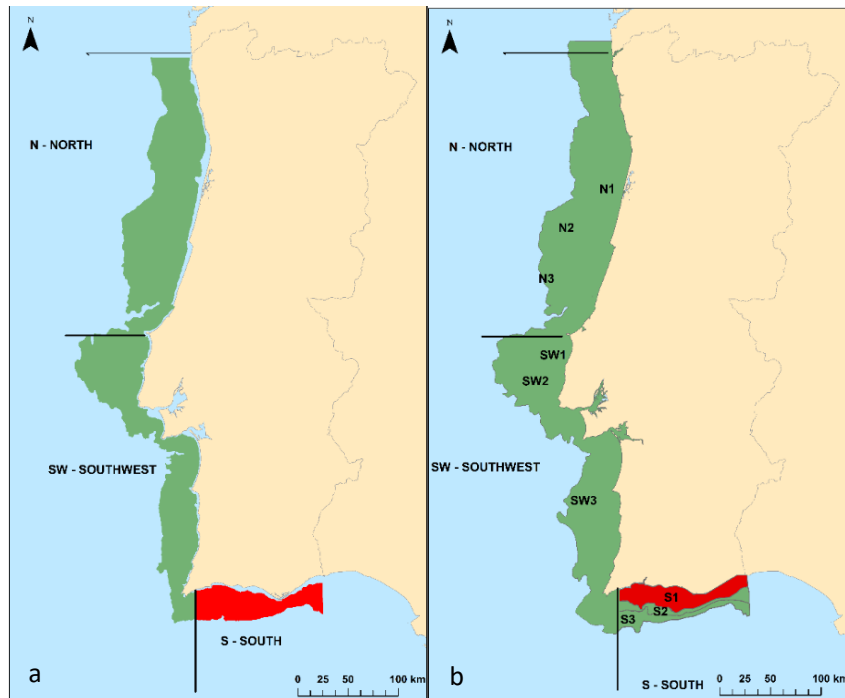


Figure 3-6 - D1C2 status for a) *Microchirus variegatus* assessment at Zone level and b) a) *Microchirus variegatus* assessment at Zone/Depth strata level.

### 3.4 Discussion

Assessing the status of the marine environment requires the establishment of common criteria, metrics, and units at ecologically relevant scales, to achieve a consistent assessment across the territory and the implementation of adequate monitoring and mitigation measures (Borja et al. 2011; OSPAR Commission, 2012; Raicevich et al. 2017; Borja et al. 2019; Palialexis et al. 2019). This study aimed to explore whether assessments at broader scales may indeed hinder the detection of impacts on species status at smaller scales, potentially deriving from local anthropogenic pressures.

In the case of commercially exploited live marine resources, traditional assessments are conducted at population or stock levels, which, in most of the cases, coincide with large distribution areas of the species that may occupy waters under different jurisdictions (ICES, 2017a; Froese et al. 2018; Borja et al. 2019). Nevertheless, and in the scope of MSFD, the identification of fractions of populations in poor status due to anthropogenic pressures, can help to prevent species populations from deteriorating in the long term. However, to reduce the scale of the assessments to smaller areas, it is important to have a good knowledge on species biology and distribution and an adequate monitoring program, to assure the quality of the assessments.

The three coastal species were found to be in good status regardless of the level of spatial disaggregation considered. However, *C. lyra*, *P. erythrinus* and *S. hepatus*, revealed distinct ecology and distribution, and, as a result, their BPA and TA assessments were differently affected by the scales used. *P. erythrinus* is a frequent

species in the SW and S regions whereas *S. hepatus* is abundant in the S region and, consequently, assessments were limited to those areas. For both species, time series exhibited similar patterns independently of the spatial scales used, with exception of VSA1 strata for *P. erythrinus*, and the VSA sector for *S. hepatus*. Although biomass was not significantly lower than the threshold established, BPA for these two species revealed distinct BPs and biomass peaks in this sector of the S region. Differences among spatial areas (within the same scale or not) were not unexpected given different community structure (and trophic web structure) and species ontogenetic distribution (e.g. larger specimens may distribute in other areas and/or depths) (Sousa et al. 2005). However, for these two species, distinct biomass index trends in the VSA1 and VSA units may also result from higher environmental influence from the Mediterranean (Ambar, 1983) or from higher fishing pressure. Studies in the Mediterranean, where *P. erythrinus* is a commercially important fish for shelf fisheries, have shown that fishing pressure may decrease *P. erythrinus* abundance and alter its trophic ecology (Fanelli et al. 2010). Data at a national scale indicates that this species is landed, in low numbers, by the demersal fish trawl fleet but is frequently discarded by the crustacean fishing fleet (Fernandes et al. 2007), which is known to exert an intense activity in the S area (Gonçalves et al. 2016). In Algarve (S zone), *P. erythrinus* is commonly caught by coastal multi-gear and multi-species fishing fleets (Coelho et al. 2010), especially trammel nets (Gonçalves et al. 2016), being commonly discarded due to damage or poor condition together with *S. hepatus* (Gonçalves et al. 2007).

On the other hand, the BPA for *C. lyra* time series on the Portuguese continental EEZ, reflected the characteristics from the depth strata 1, although is more abundant in the North. This species is one of the ten most discarded species, occurring in an average of 25% of the trawl fishing fleet landings, being also caught by the trammel net fleet and the crustacean and demersal trawl fleets (Fernandes et al. 2007; Gonçalves et al. 2007). Despite being frequent in discards, results indicate that the species is in a good status and that potential local pressures (as, for example, fishing) is not affecting negatively its abundance.

Contrary to coastal species, which showed no significant differences in status when scales were downsized, results for shelf species suggested that, depending on species, assessments should be partitioned. *M. variegatus*, species status was below threshold for specific scales, *A. sphyraena* showed decreasing patterns for a specific region, and *Lepidotrigla* spp. was not affected when scales were downsized.

The regional assessment for *M. variegatus*, revealed that biomass was below the ecological threshold for S zone. Disaggregating the scale showed that the poor status was specifically attributable to the coastal zone - S1. At the Portuguese continental EEZ level, this species had, from 2004 to 2005, a frequency of occurrence ~ 7-8% both in landings and discards by the demersal fish trawl fishery (Fernandes et al. 2007). It is also caught in the coastal trammel net fleet in Algarve (S zone) as well as the commercially valuable *Microchirus azevia* and *Microchirus ocellatus*, which are considered a frequent landing and by-catch species (Gonçalves et al. 2016; Stergiou et al. 2006). Mapping trammel nets fishing 'hot spots' showed that fishing effort is particularly high in Vila Real de Santo António coast (VSA1 scale), where *Pagellus* and *Microchirus*

spp. are caught (Gonçalves et al. 2016). The region includes recognized fishing banks used by all fishing fleets due to the: 1) species richness of the areas; 2) proximity to important fishing harbours and 3) high productivity of sandy, muddy and rocky substrate areas (Gonçalves et al. 2016, 2007; Monteiro et al. 2012). Given that these species are simultaneously exploited by small-scale gears nearshore and industrial gears (e.g., trawls, longlines) at depths greater than 50–100 m, their habitats are extensively subjected to fishing (Gonçalves et al. 2016; Stergiou et al. 2006). The significant decrease in the biomass index found for S coastal areas, highlighted the need to maintain and to adjust monitoring effort, at relevant and comparable scales, to establish a direct link between identified fishing pressures and *M. variegatus* biomass index decrease.

The assessment for *A. sphyraena* showed no differences in assessment status for smaller sized scales, however, it is worth pointing out a significant decrease in biomass index that may evidence pressures and/or population sensitivity at regional/ local level. In AVE sector, BPA revealed a significant decrease of biomass index, and, when downsizing scales, AVE2 showed a significantly decreasing TA for the last five years of the time series. Several reasons can drive population changes, such as environmental factors, anthropogenic forces or species sensitivity. *A. sphyraena* is a bathy-demersal species common in intermediary depths, that although with no recognized commercial value is caught as bycatch of mixed trawl fisheries (Serrat et al. 2018). The productivity of argentine stocks is often low, which makes them particularly vulnerable to overfishing (FAO, 2011). In Portugal, *A. sphyraena* is considered one of the most frequently discarded species by the Portuguese fishing fleets (Fernandes et al. 2007, 2017). From 2004 to 2007 and in 2011, discards were considered high (volume of 15 to 59 tonnes and a frequency of occurrence of more than 30% in discards) but afterwards they declined to lower values (Fernandes et al. 2007, 2017). The most recent advice from ICES, including the catches of the closely related *A. silus* and the mixed catches of *A. silus* and *A. sphyraena*, considers that fishing effort is currently below established reference thresholds (ICES, 2019). However, there is a general lack of information for this species in the Iberian region (9a area) and assessments may be biased by the relative composition of the two species (ICES, 2019).

In the case of *Lepidotrigla* spp., assessments concluded that the species is in good status in all units analysed. However, the species had a decreasing TA in the last 5 years in Southern units (S, S1, SAG, POR and VSA) (Table 3.3 and Table S6, Annex 2), suggesting that these species are being impacted and therefore should be further investigated in this region, focusing on where it is more abundant and looking into discards data - due to its small dimensions and economic value. In Portugal, *Lepidotrigla* spp., and particularly *Lepidotrigla cavillone*, are one of the ten most frequently discarded species of the demersal fish fleet – with a percentual value of 38.5% in 2004 and 2005 (Fernandes et al. 2007). More recent discard studies (from 2004 to 2011) (Fernandes et al. 2017), have not registered the presence of this species and it cannot be dismissed that, similarly to other coastal species, the decrease in abundance shown here, is a

consequence of a high fishing effort in the area. For example, in the Mediterranean, high relative biomass of gurnards was found in continental shelf areas with low trawling pressure and vice-versa (Colloca et al. 2020).

Spatial scales adopted showed species-specific aspects that should be addressed in future assessments; identified patterns were related with ecology and depth for *M. variegatus* – zone and depth strata, and to a minor extent, with latitude for *A. sphyraena* – sector. For most species, the spatial scale units located in the south showed distinct patterns in biomass indices when compared to the other areas in the Portuguese continental coast and upper slope. These differences were masked in a broader assessment, which may be related to a lower representativity of the area and/ or to the existence of specific and localized pressures. Walmsley et al. (2017) alerted for the use of global datasets to perform global assessments, which provide average outputs, illustrating the trend of the most frequent pattern or the most replicated unit for each species. For Portugal mainland, Sousa et al. (2005) and Moura et al. (submitted) studied the demersal assemblages and concluded that Northern, Southwestern and South regions have ecologically distinct communities at coastal and intermediate depths, varying seasonally. These areas have marked divisions in physical discontinuities of the coastline, as Nazaré Canyon and Sagres Cape, suggesting that country level assessments should consider such subdivision to address ecological variability. As a result, in Portugal, assessments should consider each ecological region separately and be carried out at the population level, however, for most of the species, population structure is still unknown.

Changes in the abundance and particularly in the species status can also be attributed to other factors, as, for example, management regulations in place (e.g., fisheries regulations as Total Allowed Catches (TACs) or recovery plans for other species, marine protected areas) or climate change. For example, in a climate change perspective, the coastal *S. hepatus* and *P. erythrinus* and the demersal *Lepidotrigla* spp. are expected to expand further north due to the increasing temperature trends that may benefit the movement of sub-tropical species into northern latitudes (Gamito et al. 2016; Punzón et al. 2016; Leitão et al. 2018). On the other hand, *A. sphyraena* is better adapted to the colder and more productive waters and will likely suffer a contraction of the southern limit of its distribution area – migrating north and/or to deeper areas (Serrat et al. 2018).

When looking into the global MSFD context, Portugal is included in the Bay of Biscay and the Iberian Coast sub-region, together with Spain and France. The criterion D1C2 was differently assessed by the three Member States in terms of scales used: in the Spanish Bay of Biscay, the assessment metrics used considered the time series trend of the global biomass for selected demersal species. This MS looked to the area as a whole and did not depict any ecologically relevant scale (Ministerio para la Transición Ecológica (MITECO), 2019a, 2019b). In the French Bay of Biscay, D1C2 was assessed by combining the same methodology used in the Portuguese assessment: the breakpoint analysis, and a method to identify species sensitivity based on life history traits (OSPAR, 2017). The geographic scale of assessment was the

whole area surveyed by the French demersal surveys, including the French Bay of Biscay and Celtic Seas areas (Brind'Amour et al. 2018). *A. shpyraena*, was the only species assessed by the three countries.

According to the MSFD, subregions should be the basis for defining assessment areas for biodiversity components, however, the use of partitioned scales, further integrated to larger areas, can enable the assessment of local impacts (OSPAR Commission, 2012; Prins et al. 2014). The assessment areas for mobile species should be based on species or population distribution, even though this approach needs to consider the practicalities of using and integrating multiple scales (OSPAR Commission, 2012). The global classification of species with distributional range beyond the national waters, requires the integration of assessments since species status would have a unique classification, facilitating the decision to apply measures to particular pressures at global population level (Borja et al. 2014; Walmsley et al. 2017).

The selection of an appropriate aggregation and integration method or tools, for each stage of the assessment, is an important part of building a robust assessment since it can have a significant effect on the assessment outcome (e.g. Ojaveer and Eero, 2011; Probst and Lynam, 2016). Integration methods for Descriptor 1 (Fish) are difficult to implement due to the multiplicity of scales, species, functional groups, and the lack of corresponding thresholds, especially for species that are not covered by the Habitats Directive or commercially exploited (Ojaveer and Eero, 2011; Walmsley et al. 2017). The challenges associated with different aggregation and integration methods, in each step of the assessment, has been widely demonstrated and described in literature, such as One Out All Out (OOAO) and its derivatives, averaging approach, defined threshold rules, hierarchical, weighted, multimetric indices etc. (Ojaveer and Eero, 2011; Barnard, S. and Strong, J., 2014; Borja et al. 2014; Probst and Lynam, 2016; Borja et al. 2019). However, depending on the method used, local pressures identified through spatial disaggregation, will not be considered after the integration procedure, even though the identification of localized impacts can provide relevant information for monitoring plans development and to inform decision making on risk assessment procedures. The lack of traceability of assessments that require management or mitigations measures, is one of the drawbacks of integration methods and tools (Borja et al. 2019). To overcome this challenge, Barnard, S. and Strong, J. (2014) suggested that, for high mobility species, the aggregation of assessment units should include the contribution of each unit to the overall assessment, such as weighting by surface area or by sampling effort of the unit assessed. While Borja et al. (2019), demonstrated that the use of NEAT tool, can include weighting procedures, and addresses traceability of different assessment status, to ensure that management measures are adequately driven. Independently of the method used, the common consensus is that spatial assessments units must be carefully defined prior to the assessment and consider population distribution and patterns (e.g. OSPAR Commission, 2012; Probst et al. 2013; Borja et al. 2014; Barnard, S. and Strong, J., 2014).

It is worth mentioning that assessments can be constrained by the data available. For example, in this case, both the species and the dataset used were selected from research surveys carried out under the data



collection framework, designed to provide scientific information for the stock assessment of species with relevant commercial interest as hake *Merluccius merluccius* (Linnaeus, 1758) or horse mackerel *Trachurus trachurus* (Linnaeus, 1758). In addition, outputs of the present study have also shown that as spatial scales become smaller (e.g. sector/ depth) data quality may decrease, as the number of fishing hauls is lower, which can further bias the assessment. If smaller assessment scales are needed, it may imply more sampling effort and more resources for monitoring. Therefore, the sampling procedure adopted might not be the most adequate to collect data for the species assessed under MSFD Descriptor 1.

### 3.5 Final Remarks

When identifying scales of assessment, the EC guidance recommends a priority risk-based approach, focusing monitoring efforts on areas where pressures caused by human activities are highest and/or ecosystem components are most vulnerable (OSPAR Commission, 2012). The present study exemplifies the importance of such a strategy, showing evidence on species sensitivity and hotspots of anthropogenic pressure, where risks were identified, and monitoring efforts should be focused. For the species assessed, spatial scale did not affect the status assessment, except for *M. variegatus*, which showed different status depending on the scale of the assessment. In fact, although spatial scales tested had no effect on D1C2 ecological status when all assessment status are integrated, they disclosed important species-specific patterns, that wide range assessment failed to demonstrate. Ideally, assessment areas should be defined case-by-case, combining defined population structure with ecologically defined areas for the Portuguese continental EEZ (Sousa et al. 2005). Outputs should be confirmed by localised monitoring, particularly by assessing species vulnerable status and the frequency and intensity of pressures, together with their spatial extent, at relevant and compatible scales (OSPAR Commission, 2012). There is also the need to improve the current scientific knowledge for these species, which are not considered as priority since they have no commercial interest. Not understanding the biology, ecology and distribution of a species may lead to the establishment of poorly defined assessment areas and deficient status classification. More information on potential impacts are also needed.

### 3.6 Acknowledgements

Authors are indebted to the Instituto Português do Mar e Atmosfera, particularly the cruise leaders and all the collaborators involved in the collection of survey data, and to the Portuguese Biological Sampling Program from the EU Data Collection Framework (PNAB/DCF). This work was financed by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04292/2020, granted to MARE - Marine and Environmental Sciences Centre, and under the project UIDB/50019/2020, granted to IDL – Instituto Dom Luiz. The leading author has received funding support from the FCT through a PhD fellowship (PD/BD/135065/2017).

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# *4 Identifying assessment scales for food web criteria in the NE Atlantic: implications for the Marine Strategy Framework Directive*

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**The development and operationalization of food-web criteria (Descriptor 4: Food webs) in the Marine Strategy Framework Directive context faces several difficulties, namely the lack of data for relevant taxa, absence of fully operational indicators, spatially and temporally limited datasets, etc.** This work aims to improve methodologies used to assess food webs by identifying ecologically relevant scales in two biogeographic subregions: the Celtic Seas (CS) and the Bay of Biscay and Iberian Coast (BBIC). Four food web criteria, used to detect fishing pressure on food webs (Mean Trophic Level (MTL), Mean Trophic Index (with different trophic level cut-offs:  $MTI_{TL>3.25}$  and  $MTI_{TL>4}$ ), Large Fish Indicator (LFI) and Mean Abundance across Trophic Guild (MATG)), were assessed using groundfish survey data and tested using GAMs, for six spatial scales and four temporal scales. Results showed that MTL was highly variable, requiring yearly and locally defined assessment scales in both subregions.  $MTI_{TL>3.25}$  improved significantly when downsizing spatial scales, but temporal variability was homogeneous in both subregions. In the CS,  $MTI_{TL>4}$  and LFI were explained by locally defined scales (ICES and 1000 km<sup>2</sup> rectangles) and yearly data. While in BBIC,  $MTI_{TL>4}$  and LFI patterns were identified at regional/depth strata area and yearly scales. MATG variability was marginally explained by scales. Using the scales identified, MTL, MTI and LFI were assessed for the Portuguese continental waters, considering the methods established in the Portuguese Directive and criteria failed to achieve Good Environmental Status in areas of the Southwest and South of the Portuguese continental waters. Smaller sized scales enabled the detection of decreasing patterns at local level for MTL time series, at local level and 5 year temporal scales for  $MTI_{TL>3.25}$ , and at regional/depth strata level for  $MTI_{TL>4}$  and LFI yearly series, suggesting that scale selection may hinder the assessment of anthropogenic effects. Although downsizing scales revealed that GES classification was below threshold at local/regional level, differences in GES classification are expected to be limited when spatial assessments are aggregated. This study identified the spatial and temporal scales that can further explain food webs variability in the CS and BBIC subregions, contributing to improve the detection of high sensibility and/or anthropogenic pressures areas, identifying areas where surveillance is needed.

**Keywords:** Marine Strategy Framework Directive, ecosystem-based assessment, food-web criteria, demersal fish communities, temporal scales, spatial scales.



## 4.1 Introduction

Sustainable ecosystem-based management calls for a thorough understanding of cause and effect relationships between human pressures and ecosystem states (Rombouts et al. 2013; Large et al. 2015) for a multitude of pressures affecting marine ecosystems (Tam et al. 2017). In the European Union (EU), the Marine Strategy Framework Directive (MSFD) underpins an attempt to incorporate an Ecosystem Based Assessment (EBA) through the establishment of 11 descriptors that include environmental status and anthropogenic pressure indicators (Cavallo et al. 2016). The Directive obliges Member States (MSs) to achieve healthy and productive ecosystems or in other words “Good Environmental Status” (GES) of the marine environment for all descriptors (European Commission, 2008). The network of feeding interactions between co-existing species and populations (food webs) are an important aspect of all marine ecosystems and biodiversity. The functioning of food webs (the networks formed by the trophic interactions between species in ecological communities) reflects many aspects of ecosystem dynamics and biodiversity (Tam et al. 2017). In the MSFD framework, Descriptor 4 – Food webs (D4) has been established to assess the environmental status of trophic guilds structure, functioning and dynamics. It aims to ensure that ‘All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.’ The first implementation cycle ended in 2018 and has been an important milestone on marine environmental policies at EU level, as it highlighted existing strengths and knowledge gaps (Palialexis et al. 2014). Analysis of D4 implementation pointed out problematic metrics, scarcity of fully operational indicators, dissimilar methodologies and data scarcity/incongruences as factors that have been hindering its correct implementation (OSPAR Commission 2012; Rombouts et al. 2013; Palialexis et al. 2014b; Machado et al. 2019). In fact, only a few indicators have been fully operationalized, i.e., they are quantitatively defined, assessed in relation to a defined threshold and respond clearly to anthropogenic activities (Rombouts et al. 2013). Similarly, the Oslo and Paris Regional Sea Convention (OSPAR) assessment from 2017 pointed out issues such as the lack of proper data and the difficulties in establishing clear reference points as the main knowledge gaps for a complete geographical analysis of D4 (OSPAR n.d.). The European Commission (EC) revised the decision on the methodological standards to determine GES (2017/848/EU), depicting methodological standards, re-defining the ecosystem elements and identifying the scales of the assessment to support the implementation of the MSFD (European Commission 2017a; 2017b). The assessment of food-web descriptor includes criteria classified as primary - D4C1 Trophic guild species diversity and D4C2 Abundance across trophic guilds, and secondary - D4C3 Trophic guild size distribution and D4C4 Trophic guild productivity (European Commission, 2017b). The revised Commission Decision (2017/848/EU) provided details of the elements for assessment: a) should take into account a list of trophic guilds to be assessed, that should be established by MSs through regional or subregional cooperation, b) include a minimum of three trophic guilds, c) two of the three guilds shall be non-fish, d) at least one guild shall be

a primary producer, and e) the trophic guilds assessed should represent at least the top, middle and bottom of the food chain. There has been an attempt to develop fully operational indicators that can integrate trophic structure and functions, together with their interactions. But the lack of comparable data between taxonomic groups has made such integration difficult (Rombouts et al. 2013; Tam et al. 2017; Ministério do Mar, 2020). When trying to disentangle if ecosystem status is directly linked to pressures, difficulties arise, since the environment is exposed to existing multiple pressures, such as natural and anthropogenic variability, that, allied to the temporal and spatial variation, make the diagnosis very difficult. Ideally, criteria should link pressure to ecosystem state at the appropriate spatial and temporal resolution (Henriques et al. 2008; Shin et al. 2010; Probst and Stelzenmüller, 2015; Preciado et al. 2019). Tam et al. (2017) identified food-web indicators that succeed in capturing effects of anthropogenic pressures. Among these, integrated trophic indicators (MTL, MTLI, etc.) and guild level biomass (guilds biomass) provide relevant indications on future surveillance and management actions for fish communities (Shannon et al. 2014; ICES 2015). Length based indicators were also considered appropriate metrics, especially when effects of fisheries on predators are targeted, providing relevant complementary information (Tam et al. 2017). However, further optimization is required, especially targeting incongruencies such as the guilds assessed, the development of targets/thresholds and the use of appropriate scales, since relevant assessment scale must be used to capture food webs variability patterns and detect existing trends. In the NE Atlantic, the scale of the assessment defined by the EC for food webs, is the subregion, with areas ranging from 1.857.164 km<sup>2</sup> for the Macaronesia and 491.305 km<sup>2</sup> for the North Sea, and subdivisions may be used if necessary (European Commission 2017a). Other assessment areas can be informally defined by the MS, but these (and the subdivisions) should be nested within the region/subregions being reported. The NE Atlantic subregions enclose a wide amplitude of environmental and oceanographic features, that together with distinct anthropogenic pressures may require different assessment scales to detect existing patterns. The effects of using different spatial scales in assessments have been widely studied for coastal and benthic communities (Cole et al. 2001; Antony J Underwood and Chapman 2013; Östman et al. 2017), that are easy to manipulate, but that is not true for highly motile species, such as fish communities, due to their motile properties and wide geographical distribution. For high mobility species, MSFD guidelines and OSPAR assessment have suggested that using wide assessment areas may fail to identify significant but localized impacts that could result in effects on ecosystems (OSPAR Commission, 2012; Walmsley et al. 2017). Even though, for large, long-lived taxa, spatial scales which integrate over migration ranges may be appropriate, these scales may span fundamentally different habitats and communities for lower trophic levels (e.g. plankton or benthos), to the point that a synthesis at this scale become questionable (OSPAR Commission 2012). Ultimately, the appropriate spatial scale at which food webs should be assessed will be set by the anthropogenic pressure under study rather than by any ecological considerations. The availability and spatial extent of monitoring data for key taxa, are also likely to influence the scale at which assessments are made (Rogers et al. 2010; OSPAR Commission 2012). In the Bay of Biscay, Preciado et al. (2019)

detected a direct relation between fishing pressure and ecological indicators response at small spatial scales (i.e. local level). While in the North Sea, Adams et al. (2017) showed that, size-based community indicators vary across space, species, and season, identifying International Council for Exploration of the Seas (ICES) rectangle units as an appropriate assessment scale. Furthermore, assessment scales should be agreed upon by MSs sharing subregions and should be nested into wider areas, to enable further spatial integration (Walmsley et al. 2017) and enable a global GES assessment .

This work tested four food-web criteria used to implement D4 in the Celtic Seas (CS) and in the Bay of Biscay and Iberian Coast (BBIC) over distinct spatial and temporal scales. We hypothesized that food web criteria estimates and the detection of pressures on foods webs may be affected by scales used in the assessment (from wider to smaller assessment areas and longer to shorter temporal periods), and therefore can affect the development of management procedures and implementation measures. Mean Trophic Level (MTL), Mean Trophic Index (MTI), Large Fish Indicator (LFI) and Mean abundance across Trophic Guild (MATG) were assessed using six spatial and four temporal scales, using groundfish survey data (Moriarty et al. 2019) and generalized additive models Generalized Additive Models (GAMs), to identify the spatial and temporal scales that significantly describe indicator's variability. Using the assessment scales identified for the BBIC subregion, food-web criteria were analysed, and compared with the Portuguese continental region assessment, to understand if scales had any effect in the criteria status. The methodologies used were identical to the ones applied in the MSFD 2nd cycle report, and since there are not any agreed threshold levels defined for food webs criteria, a trend-based approach was carried out (OSPAR Commission 2012; ICES 2017b; Ministério do Mar 2020); and the time series of each criteria was assessed through the non-parametric Mann-Kendall test, for which the significance and trend defined GES status. Outputs are expected to provide relevant information to increase reporting coherence and promote discussion concerning the most relevant scales to be used in D4 criteria assessment.

## 4.2 Material & Methods

### 4.2.1 Study area & Data set

The study area comprehends two ecological subregions of the North-Eastern Atlantic Ocean: the continental shelf of the CS (off the west coast of United Kingdom, surrounding Ireland, the northwest coast of France) and the BBIC (the west coast of France, north of Spain and west coast of Portugal) (Figure 4.1), with the exception of the Gulf of Cadiz.

The data set used was extracted from the Groundfish Survey Monitoring and Assessment Data Products (Moriarty et al. 2017, 2019). This dataset is based on the Database of Trawl Surveys (DATRAS), which is maintained by the International Council for Exploration of the Seas (ICES) and includes data from yearly trawl surveys, that have as aim to assess demersal communities and to collect suitable data to perform stock assessment in the framework of the Common Fisheries Policy (CFP) (European Commission 2015).

DATRAS has an integrated quality check, however, data available can vary with MSs survey features, MSs data uploading procedures, etc. and integration issues can arise. The features of each national survey are described in Table S1 (Annex 3). To solve discrepancies, Moriarty et al. (2017, 2019) made an extensive quality check across all MSs data sets, compiling absent data and, using existing parameters (e.g. swept area, etc.) to standardize the estimation of number of abundance and biomass per area (i.e. ind.km<sup>-2</sup> and kg.km<sup>-2</sup>, etc.) for all MSs. The full processing methods are outlined in the supporting documentation (Greenstreet and Moriarty, 2017; Moriarty et al. 2017, 2019). Groundfish survey data have been used at national and international level to assess food web status in the context of MSFD and RSC (MAMAOT 2012; OSPAR 2017a; Tam et al. 2017). The data set used included all surveys during the 4th quarter of the year (Q4 – from September to December), from 2002 to 2014, and using otter trawl (OT) as sampling gear (Figure 4.1). The depth range analysed varied between 15 to 581 m of depth.

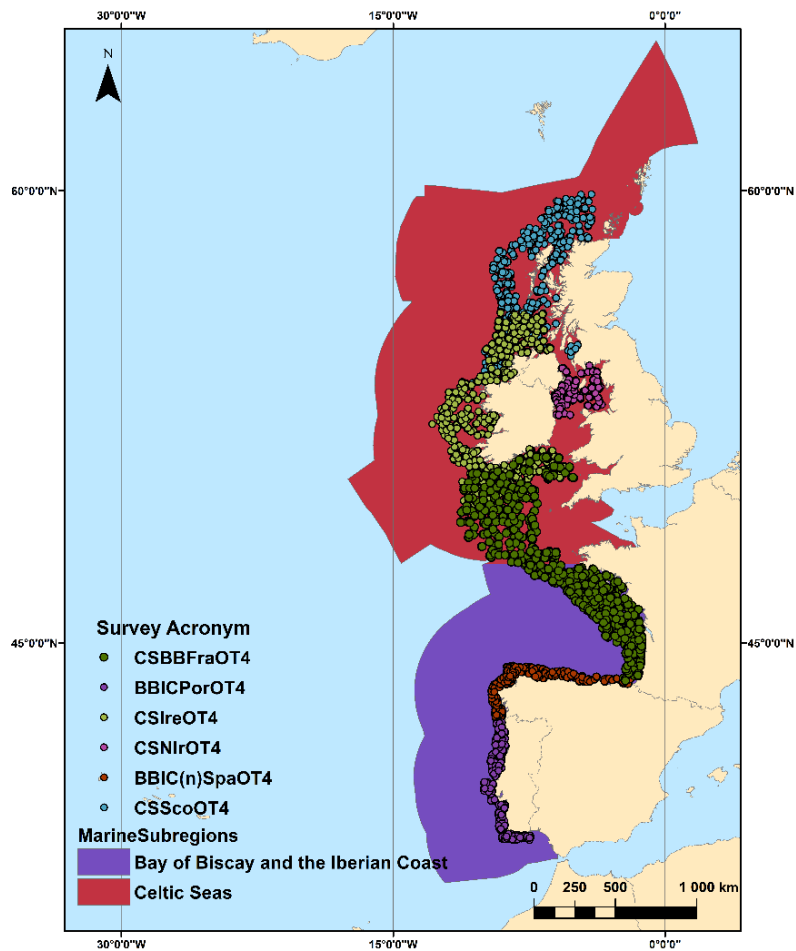


Figure 4-1 - Study area showing the MSFD delimitation for the Celtic Seas and Bay of Biscay and Iberian Coast and the location of each trawl held by MSs groundfish surveys, from 2002 to 2014 (Shapefile source: OSPAR, 2017; Moriarty, 2017). See supplementary Table S1 in Annex 3 for more details on the survey's acronyms and features.

#### 4.2.2 Food web criteria & scales analysed

Food-web indicators selected for this study were MTL, MTI, LFI and MATG. These are considered operational indicators for food webs assessment (Tam et al. 2017), they are complementary, and include

at least three trophic guilds (European Commission 2017a). According to ICES and MSFD guidance, MTL, MTI and MATG are adequate to report criteria D4C2 – Abundance across trophic guilds, while LFI reports D4C3 - Trophic guild size distribution (ICES 2015; Walmsley et al. 2017). They have been used by MSs to report D4 in both MSFD and OSPAR contexts (OSPAR 2017b; Ministério do Mar 2020). To calculate MTL, MTI and MATG, the trophic level (TL) and the trophic guild (TG) were assigned to each species. TL and TG were retrieved from online databases (e.g., Fishbase, Sealifebase (Froese and Pauly; Pauly and Christensen, 2000; Pauly and Watson, 2005; Beukhof et al. 2019; Palomares and Pauly, 2019)). TL values are worldwide averaged trophic level estimations and are attributed in accordance with each species position in the food chain, determined by the number of energy-transfer steps to that level (Froese and Pauly n.d., 2019). Due to the wide area under study, it was not possible to address regional particularities for each given region and therefore a fixed TL was used for each species (Reed et al. 2016). MTL was calculated as the average trophic position for each survey and includes all trophic guilds. MTI is calculated as the mean trophic position of species in relation to their relative biomass, following the formula below:

$$MTI = \frac{\sum_i (Y_{ik}) * (TL_i)}{\sum_i (Y_{ik})}, \quad (1)$$

where TL is the trophic level of species *i* and,  $Y_{ik}$  refers to the biomass of the species *i* in year *k* (1). Two TL cut-offs were applied to MTI to decrease the influence of pelagic species (i)  $MTI_{TL > 3.25}$  with a cut-off of all species with a TL < 3.25, including all consumer species and (ii)  $MTI_{TL > 4.0}$  with a cut-off of all species with a TL < 4.0 (Pauly and Watson, 2005; Shannon et al. 2014), addressing all predator species. MATG is the relative proportion of each guild's biomass in relation to the overall biomass (Auster and Link 2009). The guilds considered were planktivorous, benthivorous, piscivorous, omnivorous (Beukhof et al. 2019). To determine MATG values, the equation is as follows:

$$MATG = \frac{\sum BTG_j}{\sum B_{Total}}, \quad (2)$$

where BTG is the biomass of the trophic guild *j*, in year *k*. The Large Fish Index (LFI) was developed for the North Sea (Greenstreet et al. 2011), and uses the proportion of fish biomass density-at-length in relation to the overall biomass (3).

$$LFI = \frac{\sum_i B_{L > LLF}}{\sum_i B_{Total}}, \quad (3)$$

The length value, Large Length Fish (LLF) defining 'large fish' has been determined for the North Sea (LLF=40 cm) (Greenstreet et al. 2011), however demersal communities reflect differences in their composition and structure across environments, habitat conditions, and latitudinal gradient (Fisher et al. 2010). As a result, LLF values vary in the North Atlantic, and have been derived for the Celtic Sea (LLF=50 cm) (Shephard et al. 2011), the Bay of Biscay (LLF=35 cm) (Modica et al. 2014) and the Portuguese Iberian

Coast (LLF=30 cm) (MAMAOT 2012), using the methodology proposed by Greenstreet et al. (2011). All criteria were calculated excluding data from pelagic, pelagic-neritic and pelagic-oceanic species, to reduce the influence of environmental variability and the corresponding effects on pelagic communities' recruitment (Preciado et al. 2019b). In addition, since groundfish surveys do not target pelagic communities, data concerning these species are likely to be incomplete and underrepresented.

Considering the MSFD legislation, food webs (D4) assessment scales should be defined at the subregion level in the Northeast Atlantic (European Commission 2017a), therefore all criteria were assessed separately for the CS and BBIC subregions. Each criterion was calculated from wider to smaller sized spatial and temporal scales. The scales selected are presented in Table 4-1 and their spatial coverage is shown in Figures S1 to S4 in Annex 3. The spatial scales considered were Marine Subunit (MSU), Sector, Sector/Strata, ICES rectangles, and equally distributed 1000 km<sup>2</sup> squares and 100 km<sup>2</sup> squares. MSU are spatial areas defined in the MSFD framework that consider MSs subdivisions belonging to different subregions (e.g. France includes areas in the North Sea, the CS and the BBIC subregions). Sectors are geographical subdivisions defined by all MSs to support demersal survey design areas. The combination sector and depth were also analysed, following the stratification used by the demersal groundfish survey (ICES 2017a), however since MSs defined depth strata ranges differently, these were standardized according with the following depth ranges: 1-Coastal (20-100 m), 2-Medium (100 – 200 m), 3-Deep (200-500 m), 4-Slope (>500 m). ICES rectangles were used since they serve as basis for sampling stratification in some areas of the NE Atlantic Area (ranging between  $\approx$  7000 to 12000 km<sup>2</sup>). Artificial grid of 1000 km<sup>2</sup> and 100 km<sup>2</sup> rectangles were applied to the survey area and used as spatial assessment units. The temporal scales considered yearly datasets, and the aggregation of yearly data into five years datasets, three years data sets, and two years datasets. The temporal span was limited to the MSs with shortest time series, which was between 2002 to 2014. For each spatial and temporal unit, the estimated biomass index was only considered when a minimum of two tows were carried out. The analysis of temporal and spatial scales was made independently for each criterion and scale analysis.

Table 4-1 - Spatial and temporal scales and units used to assess food-web indicators in the CS and BBIC subregions (n: number of units tested per scale).

Scope of the analyse	Spatial scales	Spatial units		
		Celtic Seas	Bay of Biscay and Iberian Coast	
Wider scale	Marine Subunits (MSU)	MSs continental coast and upper slope: CS_Fra, CS_Ire, CS_Sco (n=3)	BBIC_Por, BBIC_Spa, BBIC_Fra (n=3)	
		Sector (Country level demarcations)	East Irish Sea, Irish Coast, ..., VIIb, windsock_lam (n= 22)	Cn, Cc, Cs, ..., SAG, POR, VSA (n=20)
	Smaller scale	Sector/Strata (Depth strata: 1-Coastal (20-100 m), 2-Medium (100 – 200 m), 3-Deep (200-500 m), 4-Slope (>500 m))	VIa1, VIa2, VIa3, VIIb1, ..., Cc2, Cn2, Cc4, Cs4 (n=35)	Gs1, Gs2, Gs3, Gs4, Gn1, Gn2, Gn3, Gn4, ..., VSA1, VSA2, VSA3 (n= 56)
		ICES Rectangles*	48E5, 48E4, 47E5, 47E4, ..., 26D9, 25E3, 25E2, 25E1, 25E0 (n=110) <sup>1</sup>	24E6, 24E5, 24E4, ..., 04E0, 03E1, 03E0, 02E2, 02E1 (n=65) <sup>1</sup>
		1000 km <sup>2</sup> Squares	298, 299, 301, 302, ..., 3440, 3441, 3442 (n=551)	3443, 3446, 3485, ..., 5824, 5826, 5827 (n= 298)
100 km <sup>2</sup> Squares	57182, 56899, 56898, ..., 25193, 25192, 25058 (n=1068)	25499, 25357, 25073, ..., 1044, 1043, 918 (n= 772)		
Temporal scales		Temporal units		
Wider scale	5 years	2002–2008, 2009–2014 (n=2)	2002–2008, 2009–2014 (n=2)	
	3 years	2005–2007, 2008–2010, 2011–2014 (n=3)	2005–2007, 2008–2010, 2011–2014 (n=3)	
	2 years	2002-2005, 2006-2007, 2008-2009, 2010-2011, 2013-2014 (n=5)	2002-2005, 2006-2007, 2008-2009, 2010-2011, 2013-2014 (n=5)	
Smaller scale	year	2002, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2013, 2014 (n=10)	2002, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2013, 2014 (n=10)	

<sup>1</sup> See further explanation on ICES rectangles nomenclature here: <https://www.ices.dk/data/maps/Pages/ICES-statistical-rectangles.aspx>.

#### 4.2.3 Model Selection

GAMs were employed to explore how each spatial and temporal scale contributed to explain ecological criteria and its corresponding residuals (Bradter et al. 2013). GAMs are powerful tools for exploring linear or non-linear response of variables to predictors without being constrained to an underlying parametric model of a specific form, which is particularly useful when ecological thresholds of non-linear responses are of interest (Wood 2006). Each scale was used as a model predictor and criteria estimates were the response variables. As a result, a model was built per spatial and per temporal scale for each ecosystem criteria. Environmental variables, such as depth and temperature were added identically to each model as explanatory variables. These variables are known to contribute widely to the existing variability of demersal communities, therefore including them in the model allowed to identify their contribution to the overall variability and to distinguish it from the variability obtained due to the scales tested (Pranovi et al. 2016; Preciado et al. 2019). Depth was available for each trawl surveyed, and the average temperature for Q4, was obtained using E.U. Copernicus Marine Service Information with a spatial resolution of 0.04 x 0.04 degrees (E.U. Copernicus Marine Service Information 2020). Spatial and temporal scales were parametric, and all environmental variables were continuous and were included as a smoothed variable in the model.

The Gamma distribution was used for all analyses since all response variables were continuous, had positive values and were slightly skewed, and the log identity link has been assumed (Zuur et al. 2009). The GAM models for all the food-web indices can be described as follows:

$$\text{MTL, MTI, LFI or MATG} \sim \beta_0 + f(\text{scale}) + s(\text{temp}) + s(\text{dep}) + \varepsilon$$

where MTL, MTI, LFI and MATG are the food web criteria;  $\beta_0$  is the intercept;  $f$  indicates the variables which were included as factors in the formula (i.e. each spatial and temporal scale);  $s$  is spline smoother and  $\varepsilon$  is the error term: scale represents the different spatial and temporal scales under test; temp is temperature; and dep is depth. To compare the performance of each spatial and temporal scale in predicting food web criteria, models with increasing complexity of scales were compared through the relative deviance explained by each model, and its corresponding Akaike's Information Criteria (AIC). ANOVA F-ratio test was also used to verify if smaller sized scales contributed significantly to explain deviance. The data set used in each GAM model was independent for all criteria. Afterwards, the most adequate spatial and temporal scale for each criterion were combined into a final GAM, to understand how each predictor influences ecosystem criteria. Additionally, p-values based on an ANOVA F-ratio test were used to evaluate the significance of each predictor tested. Prior to any analysis, the correlation between explanatory variables was tested for collinearity among all variables through pairwise correlation coefficient ( $r$ ) and Variance Inflation Factor (VIF). A mild negative collinearity was found between smaller sized spatial scales and temperature for some models (e.g.,  $r \approx -0.5$  and  $\text{VIF} < 4$ ). However, since models were not used to make predictions, which is the step where collinearity can have stronger effects (e.g., loss of predictive accuracy) and GAMs can perform relatively well in medium collinearity (Dormann et al. 2013), both predictors were considered in the models, to avoid losing relevant information. Nevertheless, results for the two variables were approached carefully. Data normality and homogeneity of variances were verified through Shapiro-Wilks test and Bartlett test, respectively. When data was not normal, transformations were applied (A J Underwood 1993; Zar 1999). All statistical analyses were performed using R software (R Core Team 2019), using the package "mgcv" to construct GAM models (Wood 2011).

#### 4.2.4 Effects of scales on the GES assessment – a case for the Portuguese continental shelf study

Using the scales identified in the previous section for the BBIC subregion, food web criteria were assessed and compared against MSFD results for the Portuguese continental shelf, to understand if spatial and temporal scales have effect on D4 assessment status. Portuguese authorities have assessed D4 – Food webs in the 1st and 2nd MSFD cycle, but the metrics and methods used differed. In the 1st report, MTL and LFI were implemented, while in the 2<sup>nd</sup>, MTL,  $\text{MTI}_{\text{TL}>3.25}$ ,  $\text{MTI}_{\text{TL}>4}$  and LFI were reported. The comparisons made in this work were limited to MTL,  $\text{MTI}_{\text{TL}>3.25}$ ,  $\text{MTI}_{\text{TL}>4}$  and LFI, since these were reported in the most recent assessment. In both reports, food-web criteria were assessed considering the continental platform subdivisions, that correspond to three spatial units: A – from Caminha to Peniche, B



– from Peniche to Lagos and C – from Lagos to Vila Real de Stº António; and yearly data sets, from 1989 to 2017 (MAMAOT 2012; Ministério do Mar 2020). To establish GES, a statistical trend analysis was applied to the time series of each assessment unit of MTL,  $MTI_{TL>3.25}$ ,  $MTI_{TL>4}$  and LFI. If the temporal trend was non-significant or if it significantly increased, the criteria was considered in GES. If the temporal trend exhibited a significant decrease, it was considered below GES. The statistical trend was investigated through the non-parametric Mann-Kendall test that was applied to each criteria and spatial unit of assessment. This test does not require datasets to be normally distributed and is frequently used to assess environmental and biological data to distinguish consistent trends from environmental variability. In the 2nd report all food web criteria assessed were in GES (see Table 4-1,; Annex 3) (Ministério do Mar 2020).

### 4.3 Results

#### 4.3.1 Identifying scales for food web criteria assessment in the North Atlantic subregions

##### 4.3.1.1 Celtic Seas (CS)

###### 4.3.1.1.1 *MTL*

GAM models comparison revealed that the best model to explain MTL included 100 km<sup>2</sup> spatial units as predictor, explaining 77.0% of the variance. The temporal model that best suited MTL included year as temporal scale and explained 33.2% of the variance (Table 4-2a). Although the GAM models showed that 100 km<sup>2</sup> spatial units per year were the most adequate scales, when downsizing the analysis in the final model, the number of spatial units that included two trawls per spatial and temporal unit was extremely low. As a result, the final GAM model, included 1000 km<sup>2</sup> units and year as scales, together with temperature and depth. The final model explained 61.6% of the variance and all predictor variables had a significant effect (Table 4-3). MTL increased widely from shallow areas to 100m of depth; it varied irregularly between 100 and 500 m of depth and decreased abruptly in deeper waters. MTL spatial distribution patterns varied irregularly. Year analysis showed that MTL peaked in 2002 and decreased abruptly after that until 2006. Afterword's MTL increased and two additional peaks were found, one in 2008 and a second in 2013 (Figure 4.2a).

###### 4.3.1.1.2 $MTI_{TL>3.25}$

AIC analysis revealed that the most suitable model to assess  $MTI_{TL>3.25}$ , included Sector/Strata spatial scale and explained 7.0% of the variance. Concerning temporal scales, AIC analysis showed that the best temporal model, included 3-year spatial units and explained 5.3% of existing variance (Table 4.2a). The final model included Sector/Strata, 3-year, temperature, and depth as variables, and explained 7.7% of existing deviance (Table 4-3). All variables were significant, except for temperature:  $MTI_{TL>3.25}$  was low in shallow depths and increased with depth until 80 m. After 100 m,  $MTI_{TL>3.25}$  decreased rapidly until 150 m, increasing irregularly until 500 m of depth.  $MTI_{TL>3.25}$  for spatial scales was very irregular.  $MTI_{TL>3.25}$  peaked in 2002, decreasing afterwards (Figure 4.2b).

#### 4.3.1.1.3 $MTI_{TL>4}$

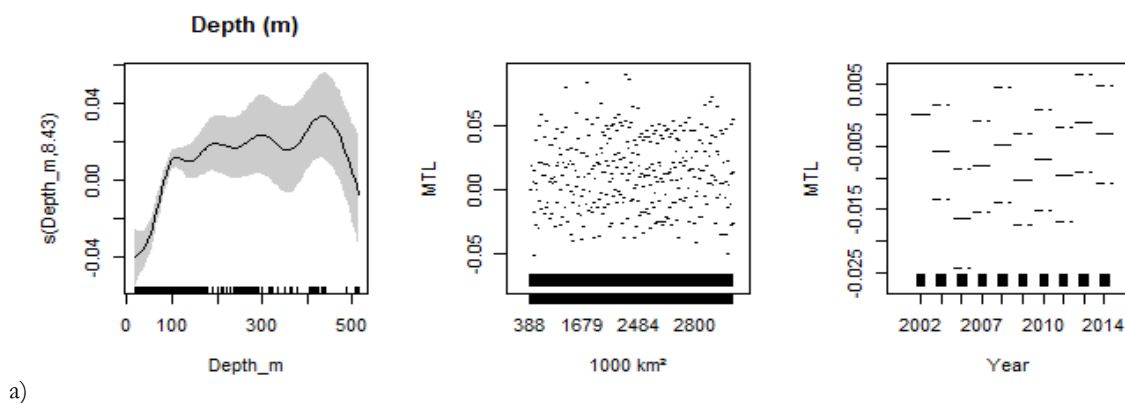
The spatial model presenting the lowest AIC values included ICES rectangles as a spatial scale and explained 20.6% of the variance. AIC analysis of temporal models revealed that the best performing model used year as temporal scale and explained 9.1% of the variance (Table 4.2a). In the CS region, the final GAM model for  $MTI_{TL>4}$  included ICES units, year, temperature, and depth as predictor variables and explained 22.5% of deviance (Table 4-3). All variables had a significant effect.  $MTI_{TL>4}$  decreased significantly with temperature. In relation to depth,  $MTI_{TL>4}$ , increased steadily until 300m of depth, and stabilized. ICES units at higher latitude had lower  $MTI_{TL>4}$  patterns.  $MTI_{TL>4}$  was highest in 2002, decreasing throughout the time series until 2008 and gradually increasing until the end of the time series (Figure 4.2c).

#### 4.3.1.1.4 LFI

The spatial scale model showing the lowest AIC results included 1000 km<sup>2</sup> as an assessment scale and explained 47.4% of the variance. The model using year as temporal scale presented the lowest AIC and explained 5.7% of the variance (Table 4.2a). The final model used 1000 km<sup>2</sup> and year as spatial and temporal scales, in addition to the overall predictors, and explained as much as 45.5% of the variance (Table 4-3). All variables had a significant effect except temperature. LFI increased significantly with depth. The 1000 km<sup>2</sup> analysis revealed that squares exhibited lower LFI values. Year analysis revealed that LFI was higher in 2006 and 2011, and lower in 2002 and 2010 (Figure 4.2d).

#### 4.3.1.1.5 MATG

For MATG, spatial scales models explained low values of deviance. The AIC comparison showed that including spatial scales in the model did not improve the model adequacy, however ANOVA test showed that including MSU spatial scale significantly explained deviance. Temporal scales did not contribute to decrease AIC and did not explain existing deviance (Table 4.2a). As a result, the final model for MATG included MSU, temperature and depth. The model explained 0.2% of the variance, but only depth had a significant effect on MATG (Table 4-3). MATG decreased significantly with depth (Figure 4.2e).



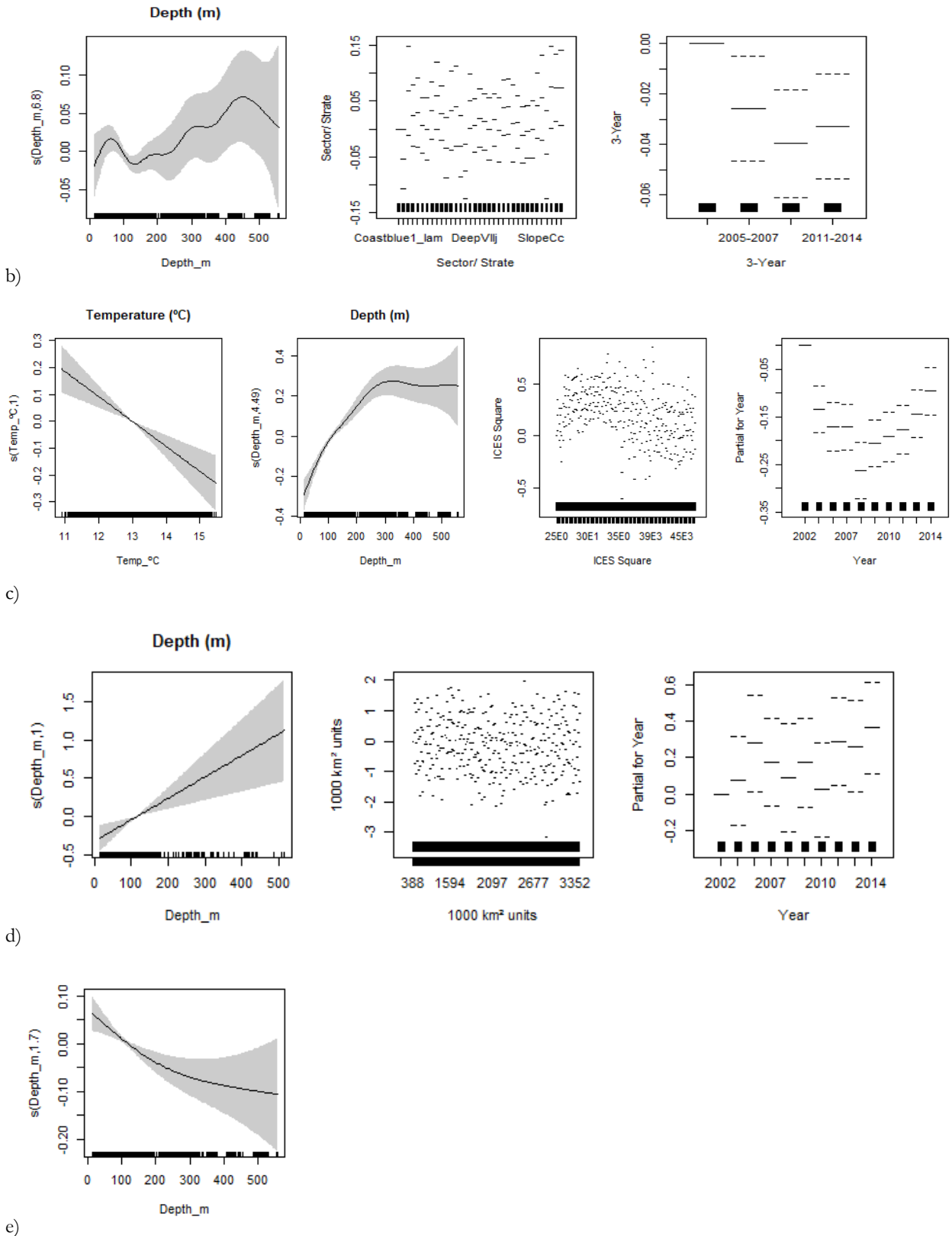


Figure 4-2 - Results of the GAM performed using spatial scale, temporal scale, temperature and depth as explanatory variables of changes observed for each food web criteria in the CS subregion: a) Results of the GAM performed using spatial scale (1000 km<sup>2</sup>) and temporal scale (year) as explanatory scales of changes observed in MTL; b) results of the GAM performed using spatial scale (Sector/Strata) and temporal scale (3-year) as explanatory scales of changes observed in MTI<sub>TL>3.25</sub>; c) results of the GAM performed using spatial scale (ICES rectangles) and temporal scale (year) as explanatory scales of changes observed in MTI<sub>TL>4</sub>; d) Results of the GAM performed using spatial scale (1000 km<sup>2</sup>) and temporal

scale (year) as explanatory scales of changes observed in LFI; e) Results of the GAM performed using spatial scale (MSU) as explanatory scales of changes observed in MATG. Only significant variables for each criterion are shown.

#### 4.3.1.2 Bay of Biscay and Iberian Coast (BBIC)

##### 4.3.1.2.1 *MTL*

AIC analysis revealed that the best model to explain MTL included 100 km<sup>2</sup> as a spatial scale and explained 72.6% of deviance. The most adequate temporal model included year as temporal units and explained 13.4% of deviance (Table 4.2b). Although GAM models showed that 100 km<sup>2</sup> spatial units were the most adequate, when downsizing the analysis in the final model, the number of trawls per spatial and temporal unit was lower than two for most of the 100 km<sup>2</sup> units. Therefore, the final GAM model for MTL in BBIC included 1000 km<sup>2</sup>, year, depth, and temperature as variables, and explained 60.8% of the variance. All variables had a significant effect (Table 4-3). MTL increased irregularly with temperature. As for depth, MTL presented low values at shallow depths, increasing steeply until 100 m of depth, where is stabilized. Spatial scale had a strong effect and the spatial units at the North of Spain and South of Portugal presented higher estimates. MTL lowest value was registered in 2006, increasing afterword's until 2009 – 2010, and decreasing until the end of the time series (Figure 4.3a).

##### 4.3.1.2.2 *MTI<sub>TL>3,25</sub>*

AIC analysis revealed that the most suitable model used 100 km<sup>2</sup> as spatial units, explaining 39.2% of the variance. The most suitable temporal model included 5-year dataset as a predictor, explaining 3.4% of the variance (Table 4.2b). The final model for *MTI<sub>TL>3,25</sub>* included 1000 km<sup>2</sup>, 5-year, depth, and temperature as predictors, and explained 29.8% of deviance. Significant effects were found for 1000 km<sup>2</sup> spatial units, temperature and depth (Table 4-3). *MTI<sub>TL>3,25</sub>* increased with temperature until 15°C, and then stabilized. At temperatures higher than 18°C *MTI<sub>TL>3,25</sub>* decreased. *MTI<sub>TL>3,25</sub>* increased non-linearly with depth: increasing until 100 m, stabilizing between 100 and 300 m, and increasing again between 300 m and 600 m. The 1000 km<sup>2</sup> unit's variability was higher in the upper Northern units and in South of the Portuguese peninsula. No patterns were found for 5-year (Figure 4.3b).

##### 4.3.1.2.3 *MTI<sub>TL>4</sub>*

The model presenting the lowest AIC values included Sector/Strata as a spatial scale and explained 22.0% of the variance (Table 4.2b). AIC analysis revealed that the best performing model included year as temporal scale and explained 9.6% of the variance (Table 4.2b). As a result, the final *MTI<sub>TL>4</sub>* model included Sector/Strata, year, temperature and depth as independent variables, explaining 23.9% of the variance - all variables had a significant effect (Table 4-3). *MTI<sub>TL>4</sub>* increased with temperature until 17° C, decreasing steeply until the maximum temperature registered. As for depth, *MTI<sub>TL>4</sub>* increased until 200 m, stabilizing between 200 and 400 m of depth. Spatial units revealed highly variable patterns, *MTI<sub>TL>4</sub>* was especially variable in the coastal units (20- 100 m), while in medium and deeper sector/strata unit's

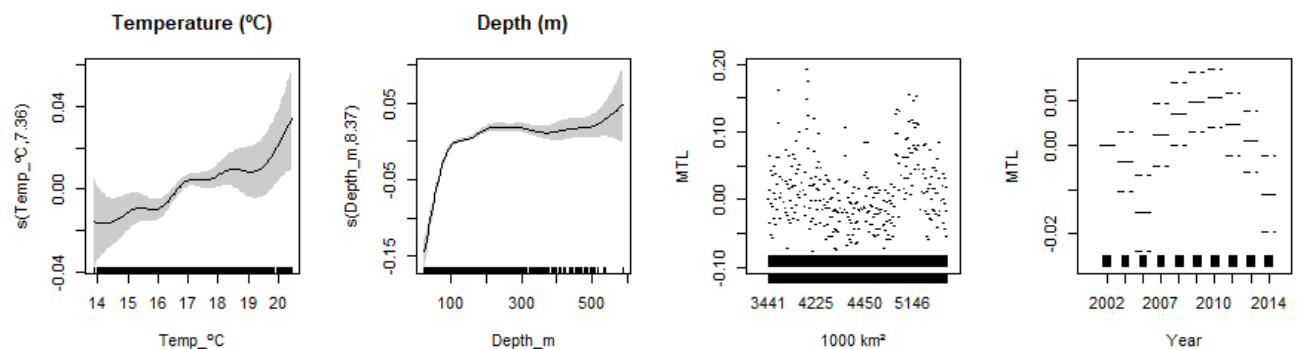
variability was lower. Year analysis revealed that  $MTI_{TL>4}$  decreased markedly in 2006, increased in 2009 and increased again until 2013 (Figure 4.3c).

#### 4.3.1.2.4 LFI

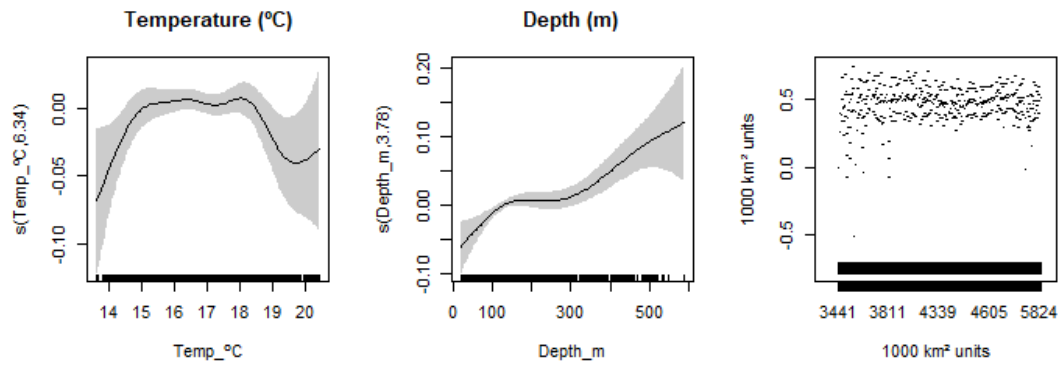
The spatial scale model showing lower AIC results included Sector/Strata units as assessment scale, explaining 28.4% of the variance. The most appropriate temporal model used yearly data and explained 25.8% of the variance (Table 4.2b). The final LFI model included Sector/Strata and year as scales and explained 30.9% of the variance. Spatial and temporal scales, and depth significantly influenced LFI estimates (Table 4-3). LFI increased abruptly with depth until 100 m. It decreased steeply between 100 and 300 m and increased again abruptly until 500m of depth. Sector/Strata units revealed high variability and but no clear pattern. Year analysis showed that LFI peaked in 2002 and 2006, and that its lowest value was registered in 2008 (Figure 4.3d).

#### 4.3.1.2.5 MATG

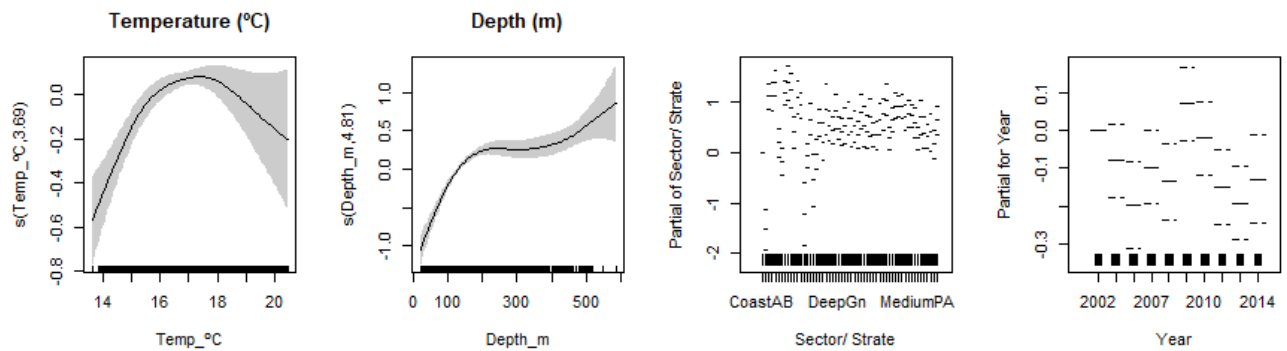
For MATG, spatial scales models explained low values of deviance. The AIC comparison showed that using MSU as assessment units improved model adequacy, explaining 0.4% of the variance. Temporal scales did not contribute to decrease models AIC (Table 4.2b). The final GAM model for MATG included MSU, temperature, and depth as independent variables, and explained 0.4% of the variance. All variables were significant (Table 4-3). MATG decreased linearly with temperature and decreased with depth, however, it showed an irregular pattern: increasing until 100 m, stabilizing until 400 m; and in deeper waters, from 400 m onwards, MATG exhibited an increasing trend. MATG was lowest in the French subunit, and highest in the Portuguese subunit (Figure 4.3e).



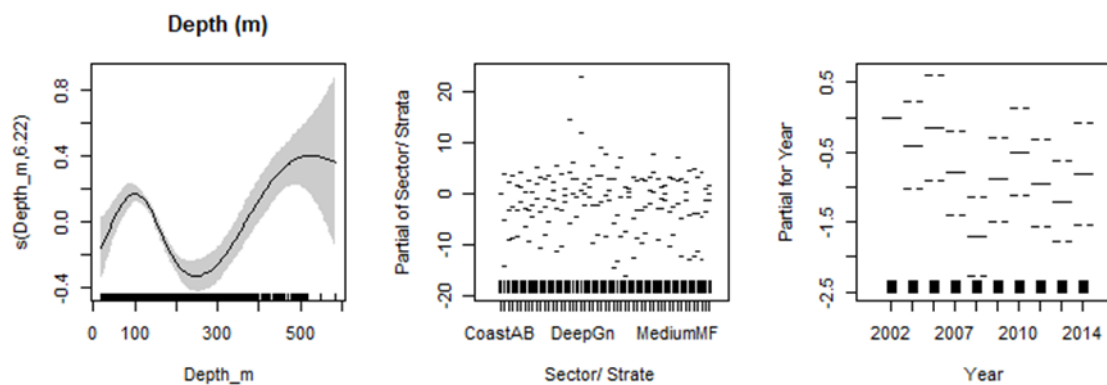
a)



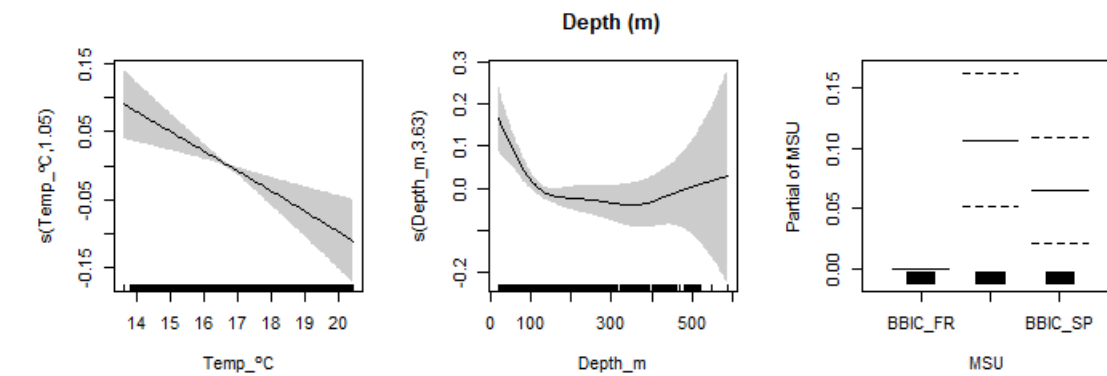
b)



c)



d)



e)

Figure 4-3 - Results of the GAM performed using spatial scale, temporal scale, temperature and depth as explanatory variables of changes observed for food web criteria in the BBIC subregion: a) Results of the GAM performed using spatial scale (1000 km<sup>2</sup>) and temporal scale (year) as explanatory scales of changes observed in MTL; b) results of the GAM

performed using spatial scale (1000 km<sup>2</sup>) and temporal scale (5-year) as explanatory scales of changes observed in MTI<sub>TL>3,25</sub>; c) results of the GAM performed using spatial scale (Sector/Strata) and temporal scale (year) as explanatory scales of changes observed in MTI<sub>TL>4</sub>; d) Results of the GAM performed using spatial scale (Sector/Strata) and temporal scale (year) as explanatory scales of changes observed in LFI; e) Results of the GAM performed using spatial scale (MSU) as explanatory scales of changes observed in MATG. Only significant variables are shown.

Table 4-2 - Model selection parameters for the food-web criteria - Scales models for the subregion a) Celtic Seas and b) Bay of Biscay and Iberian Coast.

<b>a) Celtic Seas</b>															
Food-web criteria	MTL			MIT <sub>TL&gt;3,25</sub>			MTI <sub>TL&gt;4</sub>			LFI			MATG		
Spatial scales model	AIC	R2	Explained deviance	AIC	R2	Explained deviance	AIC	R2	Explained deviance	AIC	R2	Explained deviance	AIC	R2	Explained deviance
Criteria ~ s(Temp) + s(depth)	-1946.90	0.29	29.6%	913.25	0.07	4.8%	3459.85	0.11	7.0%	-1508.30	0.04	3.9%	<b>2096.94</b>	<b>0.00</b>	<b>0.2%</b>
Criteria ~ MSU + s(temp) + s(depth)	-1962.71	0.29	30.3%	914.97	0.07	4.8%	3461.45	0.11	7.1%	-1522.98	0.05	4.3%	<b>2099.34</b>	<b>0.00</b>	<b>0.2%</b>
Criteria ~ Sector + s(temp) + s(depth)	-2008.54	0.34	36.3%	907.56	0.09	6.3%	3296.29	0.19	13.8%	-1803.87	0.13	12.4%	2125.09	0.00	0.3%
Criteria ~ Sec_Str + s(temp) + s(depth)	-2029.08	0.37	39.7%	<b>899.94</b>	<b>0.10</b>	<b>7.0%</b>	3297.73	0.20	14.6%	-1718.33	0.17	16.2%	2145.16	0.00	0.3%
Criteria ~ ICES + s(temp) + s(depth)	-2080.64	0.44	49.9%	918.25	0.14	15.1%	<b>3252.98</b>	<b>0.24</b>	<b>20.6%</b>	-2025.43	0.30	27.3%	2282.87	-0.01	0.4%
Criteria ~ 1000Km <sup>2</sup> + s(temp) + s(depth)	-2099.11	0.49	59.1%	1170.67	0.22	25.5%	3414.40	0.33	35.2%	<b>-2158.16</b>	<b>0.44</b>	<b>47.4%</b>	2935.49	-0.04	0.8%
Criteria ~ 100Km <sup>2</sup> + s(temp) + s(depth)	<b>-2113.72</b>	<b>0.57</b>	<b>77.0%</b>	1322.65	0.25	33.2%	3452.31	0.37	44.4%	-2054.05	0.45	53.5%	3350.51	-0.06	1.0%
<b>Temporal scales model</b>															
Criteria ~ s(temp) + s(depth)	-1946.90	0.29	29.6%	3916.83	0.07	4.8%	3459.85	0.11	7.0%	-1508.30	0.04	3.9%	<b>2096.94</b>	<b>0.00</b>	<b>0.2%</b>
Criteria ~ 5_year + s(temp) + s(depth)	-1947.98	0.30	30.6%	3918.28	0.07	4.9%	3461.84	0.11	7.0%	-1515.20	0.05	4.2%	2098.93	0.00	0.2%
Criteria ~ 3_year + s(temp) + s(depth)	-1960.74	0.31	31.7%	<b>3870.67</b>	<b>0.08</b>	<b>5.3%</b>	<b>3417.98</b>	<b>0.13</b>	<b>8.9%</b>	-1543.27	0.06	5.3%	2102.42	0.00	0.2%
Criteria ~ 2_year + s(temp) + s(depth)	-1977.70	0.32	32.4%	3887.99	0.08	5.3%	3436.59	0.12	8.1%	-1531.16	0.06	4.9%	2104.69	0.00	0.2%
Criteria ~ year + s(temp) + s(depth)	<b>-1982.58</b>	<b>0.33</b>	<b>33.2%</b>	3876.17	0.08	5.4%	<b>3423.70</b>	<b>0.13</b>	<b>9.1%</b>	<b>-1545.85</b>	<b>0.06</b>	<b>5.7%</b>	2113.25	0.00	0.2%
<b>b) Bay of Biscay and Iberian Coast</b>															
Food-web criteria	MTL			MIT <sub>TL&gt;3,25</sub>			MTI <sub>TL&gt;4</sub>			LFI			MATG		
Spatial scale model	AIC	R2	Explained deviance	AIC	R2	Explained deviance	AIC	R2	Explained deviance	AIC	R2	Explained deviance	AIC	R2	Explained deviance
Criteria ~ s(temp) + s(depth)	-2392.29	0.10	10.2%	-282.80	0.05	3.3%	612.55	0.12	8.4%	-593.44	0.17	12.8%	2154.52	0.00	0.2%
Criteria ~ MSU + s(temp) + s(depth)	-3171.02	0.35	35.5%	-282.00	0.05	3.5%	531.17	0.15	10.8%	-596.14	0.17	13.1%	<b>2145.93</b>	<b>0.01</b>	<b>0.4%</b>
Criteria ~ Sector+ s(temp) + s(depth)	-3589.64	0.46	46.6%	-356.70	0.12	8.4%	409.93	0.22	16.5%	-872.56	0.27	22.7%	2163.07	0.01	0.5%
Criteria ~ Sec_Str+ s(temp) + s(depth)	-3954.33	0.51	52.7%	-370.85	0.19	14.8%	<b>160.40</b>	<b>0.31</b>	<b>26.0%</b>	<b>-1001.41</b>	<b>0.33</b>	<b>28.4%</b>	2214.81	0.00	0.7%
Criteria ~ ICES + s(temp) + s(depth)	-3718.86	0.46	48.5%	-412.32	0.22	17.0%	215.22	0.30	26.6%	-925.59	0.31	26.8%	2235.60	0.00	0.7%
Criteria ~ 1000Km <sup>2</sup> + s(temp)+s(depth)	-4020.90	0.55	59.0%	-461.65	0.34	31.7%	168.19	0.38	38.0%	-876.85	0.37	36.1%	2594.31	-0.01	1.0%
Criteria ~100Km <sup>2</sup> + s(temp) + s(depth)	<b>-4073.02</b>	<b>0.62</b>	<b>72.6%</b>	<b>-528.34</b>	<b>0.38</b>	<b>39.2%</b>	230.56	0.45	48.5%	-830.81	0.42	47.6%	3092.53	-0.04	1.6%
<b>Temporal scale model</b>															
Criteria ~ s(temp) + s(depth)	-2392.29	0.10	10.2%	-282.80	0.05	3.3%	4085.84	0.12	8.4%	-593.44	0.17	12.8%	<b>2154.52</b>	<b>0.00</b>	<b>0.2%</b>
Criteria ~ 5_year + s(temp) + s(depth)	-2406.00	0.10	10.8%	-282.87	0.05	3.4%	4079.97	0.12	8.7%	-591.58	0.17	12.8%	2156.47	0.00	0.2%
Criteria ~ 3_year + s(temp) + s(depth)	-2426.24	0.11	11.6%	-278.02	0.05	3.4%	4081.30	0.12	8.8%	-619.68	0.18	13.9%	2159.29	0.00	0.3%
Criteria ~ 2_year + s(temp) + s(depth)	-2421.44	0.11	11.6%	-277.05	0.05	3.4%	4086.68	0.12	8.7%	-629.37	0.19	14.3%	2161.78	0.00	0.2%
Criteria ~ year + s(temp) + s(depth)	<b>-2463.92</b>	<b>0.12</b>	<b>13.4%</b>	<b>-269.22</b>	<b>0.05</b>	<b>3.5%</b>	<b>4069.96</b>	<b>0.13</b>	<b>9.6%</b>	<b>-693.27</b>	<b>0.31</b>	<b>25.8%</b>	2170.36	0.00	0.3%



Table 4-3 - Results of the final Generalized Additive Models (GAMs) performed for the four trophic criteria (MTL,  $MTL_{TL>3.25}$ ,  $MTL_{TL>4}$ , LFI, MATG) using the scales identified in the assessment for the CS and BBIC subregions. Degrees of freedom (df), relative importance ( $\Delta$  Deviance) and statistical significance of the explanatory variables of each GAM model are shown.

Celtic Seas	df/ edf	F	p-value	Bay of Biscay and Iberian Sea	df/ edf	F	p-value
<b>MTL (Deviance explained: 61.6%)</b>				<b>MTL (Deviance explained: 60.8%)</b>			
1000 km <sup>2</sup>	153	3.159	<0.001	1000 km <sup>2</sup>	129	14.29	<0.001
Year	9	4.782	<0.001	Year	9	7.89	<0.001
s(Temp_°C)	1.000	0.426	0.514	s(Temp_°C)	7.249	2.987	0.002
s(Depth_m)	8.438	8.990	<0.001	s(Depth_m)	7.937	29.647	<0.001
<b>MTI<sub>TL&gt;3.25</sub> (Deviance explained: 7.65%)</b>				<b>MTI<sub>TL&gt;3.25</sub> (Deviance explained: 29.8%)</b>			
Sec_Str	34	4.815	<0.001	1000 km <sup>2</sup>	246	5.573	<0.001
3_Year	3	4.926	0.002	5_year	1	3.674	0.055
s(Temp_°C)	1.002	2.760	0.601	s(Temp_°C)	6.336	2.323	0.018
s(Depth_m)	6.804	2.995	2.494	s(Depth_m)	3.785	7.240	<0.001
<b>MTI<sub>TL&gt;4</sub> (Deviance explained: 22.5%)</b>				<b>MTI<sub>TL&gt;4</sub> (Deviance explained: 23.9%)</b>			
ICES	110	8.074	<0.001	Sec_Str	52	14.356	<0.001
Year	9	12.198	<0.001	Year	9	5.722	<0.001
s(Temp_°C)	1.001	19.81	<0.001	s(Temp_°C)	3.849	13.85	<0.001
s(Depth_m)	4.488	19.98	<0.001	s(Depth_m)	5.167	24.02	<0.001
<b>LFI (Deviance explained: 45.5%)</b>				<b>LFI (Deviance explained: 30.9%)</b>			
1000 km <sup>2</sup>	145	3.541	<0.001	Sec_Str	52	12.16	<0.001
Year	9	2.366	0.013	Year	9	17.19	<0.001
s(Temp_°C)	1.370	0.485	0.678	s(Temp_°C)	3.114	1.735	0.141
s(Depth_m)	1.000	11.383	0.001	s(Depth_m)	6.244	28.525	<0.001
<b>MATG (Deviance explained: 0.17% %)</b>				<b>MATG (Deviance explained: 0.37%)</b>			
MSU	2	1.288	0.276	MSU	2	7.96	<0.001
s(Temp_°C)	1.590	0.411	0.636	s(Temp_°C)	1.047	12.681	<0.001
s(Depth_m)	1.701	8.152	<0.001	s(Depth_m)	3.633	4.562	0.001

#### 4.3.2 Effects of scales on the MSFD implementation - Portuguese continental waters case study

$MTL$ ,  $MTI_{TL>3.25}$ ,  $MTI_{TL>4}$  and LFI were estimated using the scales identified in the previous section, for the BBIC subregion. Since the most appropriate temporal scale for  $MTI_{TL>3.25}$  was wider (i.e., 5-year instead of an annual time series), the Mann-Kendall test was not applicable. As an alternative, a t-test comparison was made between criteria estimates for the first five years of the time series - considered the reference period - and the last five years of the time series - considered the assessment period. If the t-test was significant and the criteria average decreased, the criteria was considered below GES. If results were non-significant or significant and the criteria average increased, the criterion was considered in GES.

Assessment for D4 criteria showed that food webs were not in good status in all areas of the Portuguese continental waters.  $MTL$  estimates, using 1000 km<sup>2</sup> and year scales, demonstrated that for most units the time series were stable or had a significantly increasing trend. However, in the South region of Algarve – at intermediate and deep waters off Vila Real de S<sup>o</sup> Antonio – the time series for the spatial unit 5285 was significantly decreasing (Figure 4.4a, Table S3, Annex 3).  $MTI_{TL>3.25}$  was calculated using 1000 km<sup>2</sup> and 5-year scales and although most spatial units presented non-significant or significantly increasing values, the t-test revealed a significant decrease of  $MTI_{TL>3.25}$  in the 5685 spatial unit. This unit is located on the Southwest coast of Portugal, offshore V. N. Milfontes (Figure 4.4b, Table S4, Annex 3).  $MTI_{TL>4}$  was estimated using Sector/Strata and year scales and the Mann-Kendall analysis revealed that all Sector/Strata

time series were stable or increasing, except for ARR2 area, where the time series exhibited a significant decrease. ARR2 is located at intermediary depths off Arrifana, on the SW of Portugal (Figure 4.5a, Table S5, Annex 3). LFI was estimated for Sector/Strata and year scales. Results for the Mann-Kendell test showed that, in the South region, the time series for POR1 and VIG3 were significantly decreasing. These units are located in the coastal waters of Portimão, between 20 and 100 m, and offshore Vila Real de S<sup>o</sup> Antonio, between 200 and 500 m of depth (Figure 4.5b, Table S6, Annex 3).

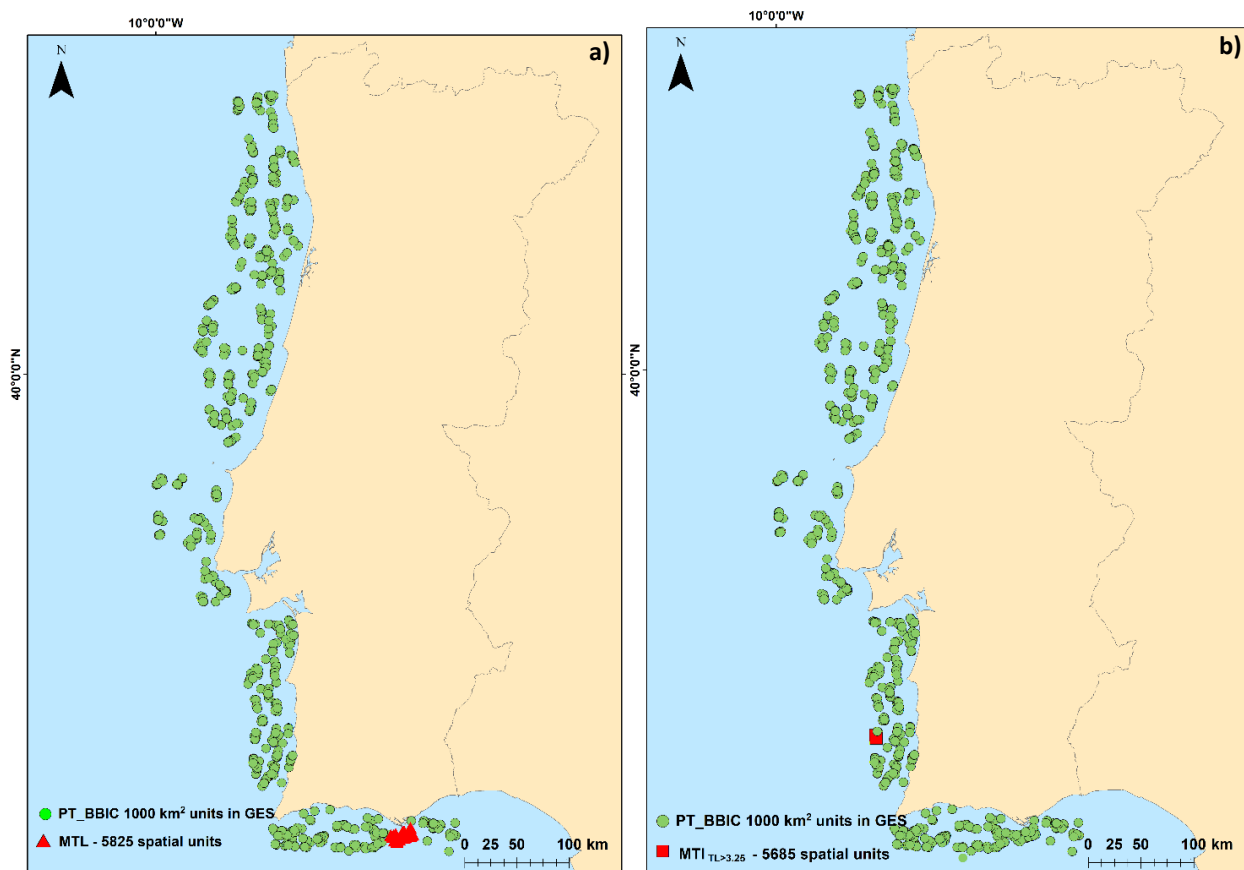


Figure 4-4 - GES assessment status for 1000 km<sup>2</sup> spatial units (information shown per haul): a) MTL calculated using 1000 km<sup>2</sup> spatial units and year as temporal units; and b) MTL<sub>TL>3.25</sub> calculated using 1000 km<sup>2</sup> spatial units and 5-year as temporal units. Green – Spatial units in GES; red – Spatial unit below GES.

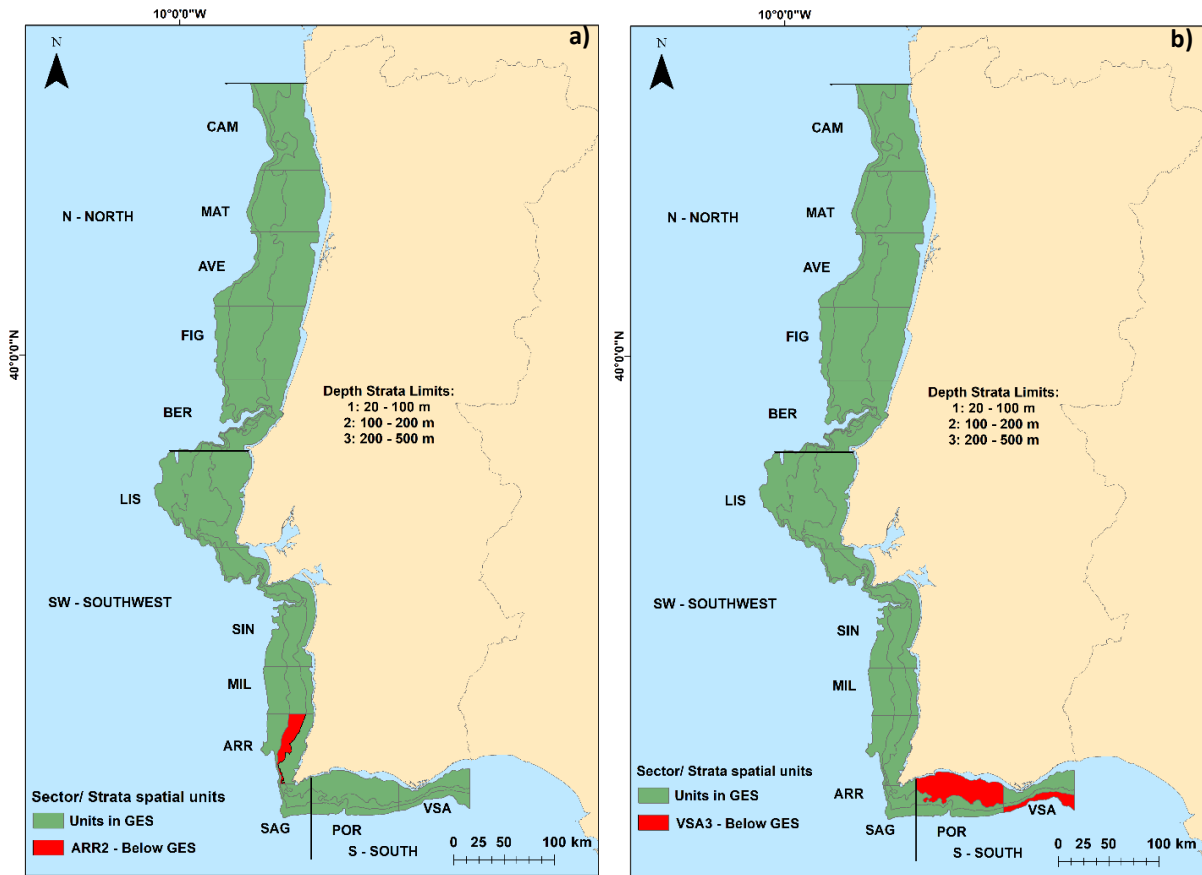


Figure 4-5 - GES assessment status for Sector/Strata spatial units: a)  $MTT_{TL>4}$  calculated using Sector/Strata and year as spatial and temporal scales; and b) LFI calculated using Sector/Strata and year as spatial and temporal scales. Green – Spatial units in GES; red – Spatial unit below GES.

#### 4.4 Discussion

Indicators are determinant to evaluate environmental status, to define management objectives and to establish measures that maintain healthy marine ecosystems (European Commission, 2008). Few studies have addressed scale effects on marine communities indices and are mostly focused on coastal ecosystems, specific taxa, and a scarce number of dimensions (e.g. one or two scales), in an attempt to model the relation of spatial scales with human pressures, environmental variables, and their impacts on indicators (Pranovi et al. 2016). By addressing a widespread number of scales across two geographical areas of the NE Atlantic, the present study isolated the effects of each scale and identified the scales that most adequately explained significant patterns of food web criteria in the CS and the BBIC.

This study revealed that spatial scales had wider effects than temporal scales in explaining all food-web criteria, for the two subregions. In fact, downsizing spatial scales of models allowed to identify significant community patterns for all criteria studied. In stable marine environments, studies contrasting spatial variability and temporal variability showed that spatial variability, arising from habitat heterogeneity, is likely greater than temporal variability, resulting from temporal fluctuations due to temperature, nutrients and pollution, well buffered in the marine environment (Barnard and Strong 2014). In the Baltic Sea,

interannual variation has been considered residual when compared to spatial variation, explained by habitat heterogeneity and natural local/regional environmental patterns, such as temperature, depth, etc. (Bergström et al. 2016). Such results suggest that depicting spatial areas of inference may improve results further than increasing resampling the same locations (Bergström et al. 2016; Östman et al. 2017). However, differences found in the present work can also be a consequence of a higher number of spatial scales being tested when compared to temporal.

The most appropriate scales identified for each criterion differed between CS and BBIC, except for MTL, that required similar sized spatial scales in both subregions, 100 km<sup>2</sup> units and year. Although downsizing the assessment to 100 km<sup>2</sup> spatial units could significantly improve variance explained, it had implications on the quality of the assessment, since the spatial units that have the minimum number of samples required for the analysis was low. As a result, the immediately upper spatial scale was used – 1000 km<sup>2</sup> square units. Similarly, in the Bay of Biscay the assessment of MTL revealed that small scale resolution was crucial to investigate heterogeneous pressures, such as fisheries impacts on benthic and demersal communities (Arroyo et al. 2019; Preciado et al. 2019b). This criterion includes all trophic levels, what can contribute to its extensive variability, and likely explains that small sized scales were required to explain existing deviance. Furthermore, although TL values are available in online databases (e.g. [www.fishbase.org](http://www.fishbase.org)), these are worldwide averages based on data from different ecosystems and may not reflect the characteristics of a given region. Mean TL values, averaged over time and area, may conceal high TL variability associated with food web dynamics (S Greenstreet 1997), environmental variation or human pressures (Chassot et al. 2008; Pinnegar et al. 2002; Vinagre et al. 2012), and ontogenetic changes. Nevertheless, when calculated with  $MTI_{TL>3.25}$  and  $MTI_{TL>4}$ , this metric can provide relevant information for the trophic guilds it represents - consumers species. These three criteria provide a ratio between TL limits for consumers (MTL), secondary consumers ( $MTI_{TL>3.25}$ ) and predators ( $MTI_{TL>4}$ ) (Shannon et al. 2014a), allowing to identify temporal trends across three trophic guilds. This indicator is associated with the detection of fishing pressure on secondary consumers and top predators which are targeted by fisheries, creating an effect known as “fishing down the food web” (Daniel Pauly and Watson 2005).

The most appropriate scales to assess  $MTI_{TL>3.25}$  were Sector/Strata and 3-year, in the CS, and 1000 km<sup>2</sup> and 5-year in BBIC. In the CS, downsizing spatial scales revealed a significant pattern based on region and depth strata together with a 3-year temporal scale. In the BBIC subregion, the most suitable assessment scale for  $MTI_{TL>3.25}$  were 1000 km<sup>2</sup> and 5-year showing that spatial variability was higher, when compared with the CS, while temporal variability was stable, i.e. yearly time series could be combined into five years data sets.

$MTI_{TL>4}$  assessment required ICES rectangular units and year in the CS, whilst in BBIC, the most suitable spatial scales were Sector/Strata and year. The most adequate scale in the CS, ICES rectangles, is used for gridding survey data to make simplified analysis and visualization (ICES, 2019), and amalgamate latitudinal

and longitudinal divided areas in rectangles; but the area of rectangles varies across latitude. In the CS subregion, their dimension varies between 12000 km<sup>2</sup>, in the Northernmost units, and 7000 km<sup>2</sup>, in the Southernmost units. Still, in this subregion, these units tend to be smaller than the Sector/Strata units, which cover wide areas of the Celtic waters. ICES statistical rectangles have been identified as the appropriate scale of assessment in the North Sea, to assess length-based community indicators. In this region, significant differences were found between LFI results for ICES rectangles (Engelhard et al. 2015; Adams et al. 2017).  $MTI_{TL>4}$  was higher at lower latitude rectangles (from the Northwest of France (25E0 unit) to the Southeast and Southwest of Ireland (35E0)) in opposition to the Northern units, what may result from local community trends (e.g., environmental factors, lower recruitment, etc.) or from higher fishing effort in the Northern areas. In the BBIC subregion, Sector/Strata and year explained higher variability, showing that spatial variability occurs at regionally and bathymetrically defined areas, while temporal scales vary annually. There was lower variability for  $MTI_{TL>4}$  criteria in this subregion, revealing that spatial scales used in the assessment can be wider when compared with MTL and  $MTI_{TL>3.25}$ . The decrease of mean trophic level, in heavily fished ecosystems, has been registered by Guénette and Gascuel (2012), using the total landings in the CS and BBIC, from 1950 to 2008. These authors showed that trophic level declined from 3.75 to 3.52, at a rate of 0.03 TL per decade, and at a steeper rate of 0.08 TL/decade between 1950 and 1970, concluding that a pervasive overexploitation has been occurring over the last 30 years.

To assess LFI criteria, the most adequate scales were 1000 km<sup>2</sup> units and year in the CS, whilst in BBIC, scales were identical to  $MTI_{TL>4}$ : Sector/Strata and year. In the CS subregion, LFI was adequately explained by finer assessment scales to capture spatial heterogeneity. Small-scale spatial heterogeneity in the CS LFI was observed previously, as LFI values showed positive spatial autocorrelation up to about 40 km, indicating regions of similar fish community size structure that remained stable. In the North Sea, LFI assessment at ICES rectangles level also showed markedly differing trends in LFI, probably driven by regional differences in habitat and benthic community (Adams et al. 2017), but these are averaged out at a larger scale. For the BBIC subregion, outputs provided an important implication for management since, assessing communities for higher guilds - predators (i.e.,  $MTI_{TL>4}$  and LFI), revealed consistency regarding the scales identified: sector/strata and year.

MATG was largely unexplained by scales in both subregions, showing that, for both regions, the most adequate spatial scale was MSU and that temporal scales had no significant effects. The rates of deviance explained were low (between 0.1% and 0.3%), and both spatial and temporal scales had a minor role in explaining deviance. Therefore, when evaluating anthropogenic impacts, MATG assessment should consider other sources of variability, that can have a greater role in explaining MATG heterogeneity and analysis should consider each guild separately (Pranovi *et al.* 2019). Still, further limitations can arise: groundfish surveys are designed with the purpose of sampling commercially exploited fish and shellfish,

and do not cover all guilds considered relevant in food web assessment. Therefore, several trophic guilds may be underrepresented (i.e. herbivorous, benthivorous). As a result, further research and development should be made considering MATG and monitoring programs should include all considered guilds (ICES 2015; Walmsley et al. 2017).

These results strongly support the idea that spatial scales have to be defined differently for each subregion and through the cooperation of MSs (ICES 2015; Walmsley et al. 2017). Furthermore, they also highlight the need to consider the population (or a sub-set of the population) targeted by the indicator used, since scales also vary in accordance. Overall, spatial variability patterns were disclosed when spatial assessment scales were downsized. Scales related with regional and depth physical features - Sector/Strata -, or with latitudinal and/or equally defined spatial scales, such as ICES rectangles or 1000 km<sup>2</sup> spatial units, significantly improved criteria estimation and detected significant differences at community level. As for temporal scales, even though its effects were significant in most final models, when compared to spatial scales, they had lower influence. Such outputs can be related with the size of the time series available (i.e., 14 years) (Blanchard et al. 2010) or with the lack of seasonal variability in the analysis, which is known to enclose higher ecological variability (Adams et al. 2017). MTL<sub>TL>3,25</sub> assessment showed that temporal scales could be merged, however, food webs assessments are recommended to consider annual averages (i.e. yearly time series), that enclose growth, mortality and feeding fluxes between food web components and integrate seasonal variability at the lowest trophic levels. In addition, the use of annual averages allows to address temporal trends to establish the status of communities over time (Blanchard et al. 2010; OSPAR Commission 2012).

Depth had a relevant role in explaining criteria variability, whilst temperature was less relevant. Food web patterns varied non-linearly with depth, that showed high influence in most criteria. Food web criteria were lower at shallow depths (from 20 m to 100-300 m of depth, depending on model), stabilized at intermediate depths – i.e. 200 - 300 m, and increased irregularly in deeper areas. The only exception was for LFI, in the CS, that exhibited a steep decreasing trend. In the North Sea, community trends showed the strongest decline in shallow waters, where high fishing effort occurs, while in the deep area this relationship was not observed (Piet and Jennings 2005). Similar patterns were found in the BBIC for trophic indicators, pointing out a different relation with depth in the upper continental slope of this region. However, an increasing trend of fishing effort in deeper waters may lead to a more acute decrease of food web indicators in deeper areas (Preciado et al. 2019b). For MTL and MTL, Heymans et al. (2014) found that ecosystem traits (i.e., latitude, ocean basins, depth) influence trophic level of the catch, thus suggesting the need to account for these confounding traits when evaluating fishing indicators and using them as ecosystem indicators. These drivers interact with fishing, making the impacts of various pressures difficult to disentangle and the setting of targets and thresholds even more problematic (Arroyo et al. 2019).

Temperature, on the other hand, exhibited irregular patterns per criteria and subregion.  $MTI_{TL>4}$  decreased with temperature in the CS. All other criteria were not affected. In BBIC, MTL increased with temperature.  $MTI_{TL>3.25}$  and  $MTI_{TL>4}$  increased with temperature until 18°C and decreased abruptly until 20°C. In the present study, the environmental stability of the Northeast Atlantic, especially in the CS subregion, appeared to be wide and therefore temperature reflected such aspects on the spatial areas surveyed (Barnard and Strong 2014). Studies in the CS revealed that fishing had a stronger effect than the temperature in size-based metrics patterns such as maximum length and time series trends (Blanchard et al. 2005). However, the time series used in the present study may be short to detect differences due to temperature, as historical time series are required to identify such changes. Temperature influence has been registered for the Portuguese coast and Mediterranean, where it had effects on fisheries landings for thermal affinity fish groups along the Portuguese coast (C. M. Teixeira et al. 2014) and at FAO spatial level in the Mediterranean (Pranovi et al. 2016). Therefore, it is important to recognize that long-term environmental changes could be impacting overall indicator values, because temperature can affect body size (e.g. Angilletta, 2004) and climate change can alter the depth distribution of species (Dulvy et al. 2008), altering community patterns. Results obtained in this study support that such effects are more likely to occur in the BBIC subregion.

The main findings of the present work suggest that long-term monitoring in reference areas is crucial for obtaining a historical baseline (Pauly, 1995; Pinnegar and Engelhard, 2008), however, the assessment scales of highly motile marine species would generally gain in adequacy by downsizing the size of spatial assessment units instead of increasing its frequency in time. Such outputs also emphasize the importance of assigning area-specific levels for assessments, that can after be aggregated, rather than relying on averaged values for wide areas, that can mask local results and have several implications for management (Walmsley et al. 2017). The need for further investigation concerning adequate criteria, metrics and methods together with assessment scales, has been widely acknowledged (MAMAOT, 2012; ICES, 2015; Walmsley et al. 2017). This work used GAM models to ascertain relevant scales for food web criteria estimation while addressing the role of additional environmental variables. Criteria varied mostly with depth, and scales, thus implying that these effects need to be accounted, to disentangle confounding variables, when building models to understand effects of anthropogenic pressures, e.g. fishing pressure (Shin et al. 2010; Heymans et al. 2014). These outputs provide important insights on factors influencing food-web criteria assessment, contributing to decrease scales mismatch in the detection of community patterns and/or anthropogenic effects (e.g. fishing impacts) when using groundfish datasets. The spatial scale at which these specific community indicators reflect changes was previously unknown, and this is an indispensable feature to identify relevant units of assessment, management actions and to organize the spatial network of monitoring programs that can address the environmental status over larger spatial scales (Östman et al. 2017). Spatial management of anthropogenic threats to populations of marine guilds can

only be effective where model predictions correctly identify key habitats, distribution patterns and threat hotspots (Maxwell et al. 2015).

Ideally, future studies should include additional factors, such as taxa/species contribution, to enhance criteria knowledge in the regions of study (Adams et al. 2017). It is worth mentioning that assessments were limited by the data available. The data sets used were retrieved in the framework of the CFP, under the data collection framework surveys, designed to provide scientific information for stock assessment of species with relevant commercial interest and are not designed for the specific assessment of food web criteria. As a result, assessments may also be limited by aspects such as differences between vessels and sampling gears used by each MS (Meadhbh Moriarty et al. 2020), and by the availability and spatial extent of data for key taxa. Outputs of the present study have also shown that as spatial scales become smaller (e.g. 100km<sup>2</sup>) data quality decreases, as the number of fishing hauls is lower, what can further bias the assessment.

#### 4.4.1 Scales effects on MSFD assessment for the Portuguese continental waters

The scales identified in the present work revealed distinct food webs patterns at local, regional and depth strata levels for the Portuguese continental waters. To some extent, these outputs are in agreement with studies made on the Portuguese coast, that showed assemblages were associated with depth patterns and with latitude (Sousa, Azevedo, and Gomes 2005; Moura et al. 2020). However, results suggested that further disaggregation of scales may be required, especially for criteria enclosing a wider range of trophic levels (i.e. MTL and  $MTI_{TL>3,25}$ ). Estimating food web criteria, considering the assessment scales identified in this work, revealed that GES was not achieved in specific units for MTL,  $MTI_{TL>3,25}$ ,  $MTI_{TL>4}$  and LFI criteria. MTL and  $MTI_{TL>3,25}$  analysis revealed that specific spatial assessment units of 1000km<sup>2</sup> squares, within the South and Southwest of the Portuguese economic exclusive zone, were below GES. More precisely, MTL was below GES at intermediate depths off Vila Real de Stº Antonio and  $MTI_{TL>3,25}$  off V.N. Milfontes; while  $MTI_{TL>4}$  and LFI exhibited units below the threshold, considering Sector/Strata units, in the Southwest and South coast: more precisely,  $MTI_{TL>4}$  was not in GES at intermediate depths off Arrifana (ARR2), and LFI registered a significant decrease in the coast of Portimão (POR1) and offshore V. R. de Stº Antonio (VIG3). Decreasing trends identified in the present work may result from specific communities' sensitivity, environmental variability, and anthropogenic pressures such as fishing and nutrient and organic enrichment, which are considered the main pressures exerted in food webs in the BBIC subregion (ICES 2019). By selectively extracting species, fishing can alter the structure of food webs, species richness, and predator-prey relation, etc. (Piet and Jennings 2005; ICES 2019; Preciado et al. 2019b). When studying MTL landings for Portugal mainland waters, Baeta et al. (2009) showed a decrease at a rate of about 0.005 per year, from 1970 to 2006, highlighting fishing pressure effects on the average trophic level of the catch. Eigaard et al. (2017) showed that, between 2010 and 2012, in the Portuguese Iberian region, the footprint of bottom trawling per unit landings was one of the largest in European



waters. In fact, the S area is heavily targeted by the Portuguese demersal fish and the crustacean fishing fleet, what can have impacts at the community level (Moura et al. 2020). Analysis of VMS data revealed that the main pressure exerted by the crustacean fishing fleet occurs in the S and SW Portuguese margins, on muddy and muddy-sand bottoms, between 100 m and 700 m water depths. Furthermore, a decrease in landings per unit of effort has also been registered for demersal fish in the SW and S areas (Bueno-Pardo et al. 2017). Despite such effort, it is important to recognize that the Portuguese coast is characterized by variable environmental drivers and is particularly affected by upwelling regimes (Moura et al. 2020; Sousa, Azevedo, and Gomes 2005), that can strongly affect community composition. In an attempt to control such effects, pelagic species were removed from the analysis, enabling the detection of fishing effects at higher trophic levels and on larger and long lived species (Shannon et al. 2014).

Downsizing the current spatial scale of assessment for the Portuguese continental waters enabled the detection of decreasing trends for food webs criteria, providing relevant information for management. The current MSFD assessment report established that food webs in the Portuguese continental waters were in GES, calculating the weighted average for three zones of the Portuguese continental waters: North, Southwest and South, using a time series between 1989 to 2017 (Ministério do Mar 2020). Even though the assessment of MTL, MTI and LFI considered, to some extent, spatial disaggregation, the results of the present study, using Sector/Strata as assessment scales and survey data from 2002 to 2014, revealed that significant food web patterns existing on the Southwest and South areas may be overlooked if smaller assessment units are not used. Recent studies, in the Portuguese continental waters, have shown that downsizing assessment scales for the fish group within Descriptor 1 (Biodiversity), revealed a significant biomass index decrease in the S area for ecologically sensitive species (i.e. *Microchirus variegatus*) (I. Machado et al. 2020). Criteria estimations that result from averaging wide spatial units may fail to reflect regional or locally defined food-web patterns related to communities specificities or anthropogenic pressures (OSPAR Commission 2012; Walmsley et al. 2017), that should be taken into consideration when designing monitoring/surveillance programs to inform management plans and conservation measures. After the assessment, several integration methodologies can be used to aggregate small sized spatial scales and trophic guilds into a final assessment classification for food webs at the subregion level (Barnard and Strong 2014; Walmsley et al. 2017).

#### 4.5 Final Remarks

Spatial scales revealed wider effect for all criteria and subregions, when compared with temporal scales. Outputs highlight that spatial scales may need to be downsized if relevant community patterns are to be identified for each subregion. Each subregion had different scale requirements, reflecting local and/or regional patterns. MTL models showed that using 1000 km<sup>2</sup> scales detected significantly different community patterns in both subregions. As for  $MTI_{TL>4}$  and LFI, these were significantly explained by ICES rectangles and 1000km<sup>2</sup> squares in the CS and by Sector/Strata in BBIC subregion, where scales

related with regional and depth strata patterns. MATG was marginally explained by spatial and temporal scales. Considering environmental variables, depth had a significant role in explaining criteria variability, while temperature had a low influence. Overall, food webs criteria assessment would benefit from downsizing the assessment scale, especially for criteria including higher variability, e.g. MTL, but there is also the need to improve the current scientific knowledge for lower trophic guilds, which are not considered as a priority since they have no commercial interest, especially at spatially relevant scales. The assessment of food web criteria for the Portuguese continental waters, using the spatial and temporal scales considered in the present study, showed that food webs present a decreasing trend in locally defined areas within the S and SW, for MTL and  $MTI_{TL3,25}$ , and in regionally defined areas of the SW and S, for  $MTI_{TL4}$  and LFI. Community patterns found here may result from natural variability, or from anthropogenic pressures, which are especially high in the SW and S of the Portuguese waters, but they pinpoint the need to detail food webs assessment in these regions for surveillance purposes. More information on potential impacts are also needed and these should be addressed at similar scales in future assessments, to match pressure-status effects.

#### 4.6 Data availability

The datasets analysed in this study are publicly available and can be found here: <https://data.marine.gov.scot/dataset>.

#### 4.7 Funding

This work was financed by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04292/2020, granted to MARE - Marine and Environmental Sciences Centre, and under the project UIDB/50019/2020, granted to IDL – Instituto Dom Luiz. The leading author has received funding support from the FCT through a PhD fellowship (PD/BD/135065/2017).

#### 4.8 Acknowledgments

The authors would like to acknowledge Dr. Meadbhaead Moriarty for kindly supplying the required data that ensured quality standards for all ecological analysis made here and therefore increased data consistency.

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## *5 Response of food webs indicators to human pressures in the scope of the Marine Strategy Framework Directive for the NE Atlantic*

Inês Machado, José L. Costa and Henrique Cabral

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**Understanding food webs environmental condition is a challenging task since evaluations are limited by data on key ecosystem elements, the availability of indicators that can incorporate all relevant guilds and the difficulty in establishing cause-effect relations between pressures and health status, as multiple overlapping pressures can affect taxonomic elements differently.** The present work aims to systematize the main aspects identified in the food webs assessment, under the Marine Strategy Framework Directive context, revealing existing gaps and pointing out future research. For this purpose, MSFD reports were surveyed for Descriptor 4 - Food webs information on criteria reported and assessment trends to understand reporting patterns in the NE Atlantic. A multivariate analysis was applied to fish ecosystem elements parallelly with anthropogenic pressures data to understand if pressures were detected by the assessment. Results revealed that reporting strategies varied between the EU Member States. High reporting effort was exhibited by the United Kingdom in opposition to Ireland or France. Reporting of other groups other than Fish and Plankton was limited, mainly due to data availability. Marine mammal and fish criteria presented increasing or stable trends while criteria for marine birds showed a decrease. The analysis applied to fish assessment reinforced that reporting strategies and trends differed between countries, although some similarities were found within the Bay of Biscay and Iberian coast and the Celtic Seas. The fish average trend was variable in Spain and was stable or increased in Portugal and the United Kingdom. Anthropogenic drivers significantly influencing food web trends for fish elements were fishing, and climate anomalies in the southern Bay of Biscay and Iberian coast, while eutrophication and chemical contamination had effects on trends in the Celtic seas and the North Sea. Results allowed to establish a relation between anthropogenic effects and food web patterns, however, these were limited since food webs data is still limited at relevant scales. This is crucial to advise the development of monitoring programmes and ecosystem-based management, as implemented by the directive. This study reinforced the necessity to further Member States harmonization and calibration to improve our understanding of the environmental status of food webs.

**Keywords:** ecosystem-based assessment, trophic webs, anthropogenic pressures, good environmental status, marine monitoring.

## 5.1 Introduction

Healthy marine systems depend on monitoring plans and management measures that consider competing societal interests such as the sustainability and productivity of the systems, human well-being, and the development of human activities (European Commission, 2008, 2020c; Korpinen et al. 2021). Several policies and legal instruments have been developed for that purpose and, in that scope, the Marine Strategy Framework Directive (MSFD), published in 2008 and revised in 2017, aims to implement an Ecosystem Based Approach (EBA) to the assessment and development of management measures in the European marine systems (European Commission, 2017b, 2017a). The directive is structured in eleven ambitious descriptors that are in different stages of development. Among the descriptors that target biodiversity, Descriptor 4 – Food webs (D4) aims to assess the status of the food chains, i.e., the network of predator-prey interactions between coexisting species and populations. This descriptor represents one of the most complex and unknown aspects of marine ecosystems, since the identification of simple indicators able to assess the status of the system with dynamic species interactions and the identification of underlying responses to pressures is challenging (Shephard et al. 2015; Otto et al. 2018). The assessment of the food-web descriptor includes criteria classified as primary - D4C1 Trophic guild species diversity and D4C2 Abundance across trophic, and secondary - D4C3 Trophic guild size distribution and D4C4 Trophic guild productivity (European Commission, 2017a). Nonetheless, the indicators and the methodology adopted may be different between Member States (MSs), which, are conditioned by existing data from national monitoring programs. Legislative updates have improved reporting consistency, considering the criteria selected and the spatial scale of the assessment (European Commission, 2017b, 2017a). However, in the 2018 assessment, more than 60% of the coastal food webs were considered ‘not assessed’, while the shelf ecosystems were either ‘not assessed’ or ‘unknown’ for 90% of the cases (European Commission, 2020a). The reasons behind low reporting and/or misreporting were the lack of appropriate metrics, the inexistence of appropriate datasets (that need to address an extensive number of ecosystem components), and the lack of knowledge on direct cause-effect relationships in Europe’s seas (European Commission, 2020a).

There has been an attempt to develop fully operational indicators that can integrate trophic structure and functions, together with their interactions. But the lack of comparable data between taxonomic groups has made such integration difficult (Rombouts et al., 2013; Tam et al., 2017; Machado et al., 2019; Ministério do Mar, 2020). According to Tam et al. (2017), food web indices should be sensitive to the magnitude and direction of response to underlying attribute/pressure, have a basis in theory, be specific, be responsive at an appropriate time scale, and be cost-effective to monitor or to update (Shin et al. 2010; Rombouts et al. 2013; Otto et al. 2018). The choice of a specific set of food web indicators can imply that some aspects of marine food webs are valued more than others. Therefore, a well-balanced selection process for indicators is required to encompass all currently known properties of marine food webs (Tam et al. 2017). As a result,

indicators considering ecosystem components such as fish and Phyto/zooplankton elements have been further implemented due to long-term stock assessment programs, implemented by the European Commission (EC) through the Common Fisheries Policy (CFP) and the Continuous Plankton Recorder monitoring programme in the Celtic Seas (CS) and Greater North Sea (GNS) (European Commission, 2020a; Machado et al. 2021). Even though these indices enable the evaluation of trophic guilds within ecosystem elements, they do not address the connectivity amidst ecosystem elements, hindering the assessment. As a result, a set of descriptors is commonly a recommended practice (Tam et al. 2017) and further emphasis on spatial and temporal resolutions should be further added (Machado et al. 2021). When trying to disentangle if ecosystem status is directly linked to pressures, further difficulties arise, since the environment is exposed to existing multiple pressures, and food web indicators lack the establishment of a clear and direct relation with anthropogenic pressures (pressure-status relationship) (Henriques et al. 2008; Shin et al. 2010; Crise et al. 2015; Probst and Stelzenmüller, 2015; Preciado et al. 2019). As a consequence, some authors have considered them as surveillance indicators, due to their limited interpretation of direct effects (ICES, 2015). On the other hand, results of food web surveillance can provide signals and indications on what multiple or combinations of pressure may be behind alterations. As a result, relevant indicators are those identifying emergent properties of food webs, which can address cumulative impacts, integrated dynamics, and responses to pressures, detect indirect and unintended consequences (Lynam et al. 2017; Tam et al. 2017). These are also often used in the context of evaluating trade-offs in management and mitigate impacts on food webs. In the last evaluation, indicators employed were considered short to show emergent proprieties that reflect the myriad of overlapping human pressures on food webs. Indices used incorporate a section of the system (fish elements) and mostly detect pressures driven by fisheries. In many instances, food webs assessments were incomplete, associated with high uncertainty, or are simply impossible due to a lack of suitable data. To overcome these obstacles, modelling approaches have been considered promising (Coll et al. 2016), but they also lack the appropriate data sets, what hinders their implementation. Solutions such as the use of long-term data series and cross-regional cooperation have been pointed out to further facilitate improved and consistent assessments (European Commission, 2020a).

This study aim is to systematize D4 - Food web assessment methods and environmental status, reported by MS under the MSFD framework, to identify existing inconsistencies and knowledge gaps. The environmental status and trends estimated by MSs were further used to disentangle if cause-effect relations, exerted by anthropogenic pressures, are being detected at the sea basin level. To achieve this purpose, the methodological criteria adopted to assess food webs in the second MSFD reporting cycle and their resulting assessment trends were surveyed, analysed and statistically compared across MSs to characterize reporting strategy and congruency across the North Eastern Atlantic MSs. In a second phase, a multivariate analysis was applied to food web criteria status, considering fish ecosystem elements trends, to understand if these are significantly influenced by spatially overlapping anthropogenic pressures occurring in the sea

basin. Information of human activities was used as a proxy of anthropogenic pressures to obtain information about potential exposure of food webs to anthropogenic pressure. It was hypothesized that fish food web indicators are detecting effects of anthropogenic pressure and therefore, they are contributing towards the assessment of anthropogenic effects on food webs functioning and structure.

## 5.2 Material & Methods

A systematic literature review was applied to MSFD reports submitted in the Central Data Repository (CDR) of the European Environment Information and Observation Network (EIONRT) Portal and the Marine Information System for Europe (WISE Marine) database (EEA, 2020, 2021) to survey results on the assessment of food webs criteria obtained within the scope of Descriptor 4 - Food webs. The search included all reports submitted until 2020, concerning the 2nd assessment cycle (2012-2018) in the Northeast Atlantic basin. The survey included the subregions Macaronesia (MAC), Bay of Biscay and Iberian coast (BBIC), Celtic Seas (CS) and the United Kingdom (UK) part of the GNS (Greater North Sea), as defined in Article 4(2) of Directive 2008/56/EC (European Commission, 2008) (Figure 5.1). The list of ecosystem elements (targets) and human-driven pressures considered are defined in the MSFD, for Descriptor 4 - Food webs, in the Commission Decision 2017/845 and 2017/848 (European Commission, 2017b, 2017a).

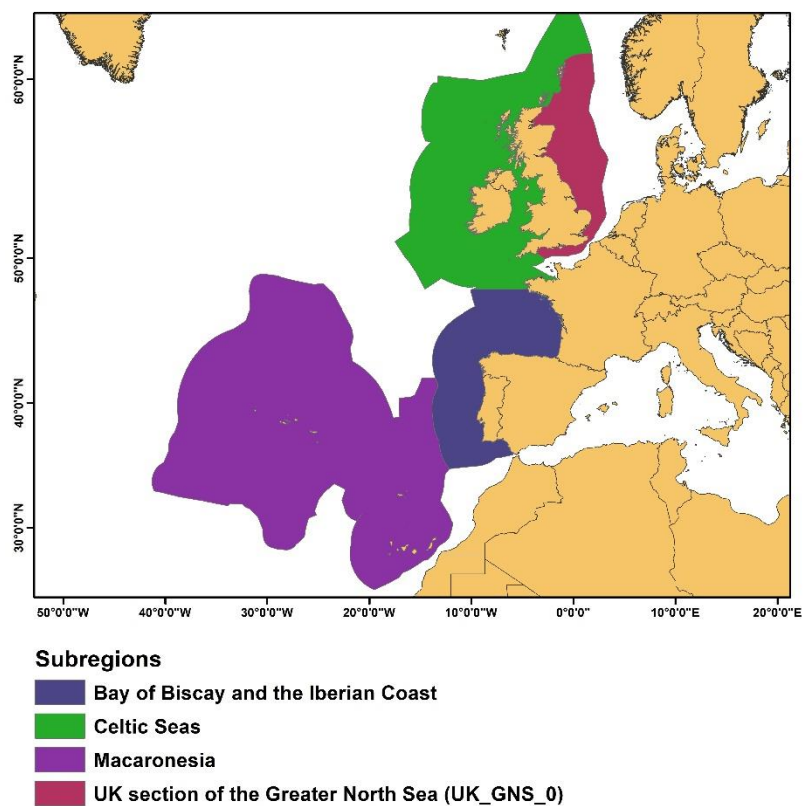


Figure 5-1 – North-Eastern Atlantic subregions included in the analyse (UK – United Kingdom).

### 5.2.1 Reporting data

The MSFD national reports included in the present work were from Ireland (IR), UK, France (FR), Spain (SP), and Portugal (PT), including the reports from the Autonomous Regions of the latter two countries. The existing information on food-web criteria assessed by each MS was retrieved from all reports. This survey collected the following data from each report: MSFD subregion, MS, food web criteria, ecosystem elements (target), human pressures, indicator, spatial scale, temporal scale, resulting trends: significance (i.e., significant, non-significant), direction (i.e., increase, decrease) and status of the assessment (i.e., GES, below GES). All these aspects were listed and counted, to understand MSs reporting patterns for this descriptor. If results were only available graphically, an image processing method was used to extract results from mean and standard error (e.g., Image J, software GraphClick, etc.). For comprehensive and in-depth analysis, spatial stratification groups were also devised for each MS (e.g., divisions and sub-divisions), whenever this information was available. The assessments were grouped based on the spatial unit of analysis (MSFD subregions, MSs divisions, and sub-divisions) available in the reports and regional estimations were obtained for each geographical scale of assessment. This allowed exploring the local pooled effects by classifying divisions and sub-divisions within MSFD sub-regions, whenever possible. Statistical maps were plotted to show the spatial distribution for the pooled results.

Since the only ecosystem element common to all MSs was Fish, a subset of the first database was built only including fish elements. This new dataset included food web criteria targeting Fish elements that were reported more than once, by more than one MS, to enable statistical significance and comparison across assessments. The food webs criteria fulfilling these thresholds (considering the Fish group) are identified in Table 5.1. Geographically referenced data on relevant anthropogenic pressures occurring in the marine environment was retrieved for each spatial unit of assessment. The pressures considered were fisheries, climate anomalies, noise, and input of nutrients, as these are the source of the most prevailing effects exerted by human activities in the European seas (Halpern et al. 2008; Crise et al, 2015; Korpinen et al. 2021). The data collected, its source and the temporal range are showed in Table 5.2.

Table 5-1 - Food web criteria selected by each MS concerning fish taxonomic elements.

<b>Food web criteria using fish ecosystem elements</b>	<b>MS</b>
Mean Trophic Level (MTL) surveys (and thresholds)	SP, PT
Mean Trophic Level (MTL) landings (and thresholds)	SP, PT
Large Fish Indicators (LFI)	UK, PT
Typical Length (TyL)	UK, IR
Mean maximum length (MML)	UK, IR

Table 5-2 - Anthropogenic data retrieved for the study: type of pressure, data, source and temporal scale of the dataset.

<b>Pressure</b>	<b>Type of data</b>	<b>Source of Data</b>	<b>Temporal range</b>
<b>Fisheries</b>	VMS data fisheries	EMODNET ("EMODnet Human Activities," n.d.)	2010-2018

	VMS bottom trawl fisheries	AIS derived high-resolution fishing effort layer for European trawlers of more than 15 meters long (Vespe et al. 2016)	2014-2015
	Number of hour fishing: (VMS data 2012-2016)	Daily Fishing Effort at 10th Degree Resolution by MMSI, version 2.0 (Kroodsmas et al. 2018)	(2012-2018)
	ICES landings (ICES database)	Official Nominal Catches. Catches in FAO area 27 by country, species, area, and year as provided by the national authorities (ICES, 2021a)	2006-2018
<b>Nutrients</b>	Chemical nutrients	Chemical on biota ( <i>Mytilus</i> spp.): Zinc, Cadmium, Copper, Fluor, Lead (ICES, 2021b)	2000-2016
	No3, Po4, Chlorophyll	Copernicus – Marine Data: (mean data) (E.U. Copernicus Marine Service Information, 2021a)	2010-2018
	Discharge Points	EMODnet (“Home   Emodnet Chemistry,” n.d.)	2010-2018
	Beach litter	EMODnet (“Home   Emodnet Chemistry,” n.d.)	2010-2018
	Seabed litter	EMODnet (“Home   Emodnet Chemistry,” n.d.)	2010-2018
<b>Climate Change</b>	SST & Sea surface Level	Calculated as a climate anomaly (mean data from 2010-2018, subtracted to data from the mean 2000-2010) (E.U. Copernicus Marine Service Information, 2021b)	2000-2018
	Climate anomaly	Air temperature changes (NCAR community, 2012)	2000-2018
	Ph, Si	Calculated as ph/ si anomaly (mean data from 2012-2018, subtracted to data from the mean 2000-2012) (E.U. Copernicus Marine Service Information, 2021a)	2000-2018
<b>Noise</b>	Nr of ports	EMODNet (“EMODnet Human Activities,” n.d.)	2018
	MRE & Offshore wind installations (nr of installations)	EMODNet (“EMODnet Human Activities,” n.d.)	2018
	Marine Traffic (VMS): All traffic	EMODNet (“EMODnet Human Activities,” n.d.)	2010-2018

### 5.2.2 Data Analysis

To compare the food webs assessment made to answer MSFD requirements for each subregion, MS, ecosystem element (target) and criterion, the number of reported indicators and the corresponding assessment trend were estimated for each of these parameters. Assessment trends for each indicator were transformed into dummy variables: -1 – Decreasing, 0 – Stable, and 1 – Increasing. A generalized linear model (GLM), using negative binomial distribution, was applied to the assessment trend, to understand if trend patterns were related to the categorical factors under study: subregion, MS, ecosystem element and criteria/indicator. Prior to the analysis, data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Cochran test) (Zar, 1999).

Since criteria addressing fish elements were common to all MSs, these criteria were selected for subsequent analysis. Multivariate analysis was used to find similarities between food web assessment trends across MSs units and to understand if anthropogenic pressures significantly explained variations observed. To delineate groups with distinct reporting strategy a hierarchical agglomerative clustering using arithmetic averages (CLUSTER) was used based on the Bray-Curtis similarity measure (Clarke, 1993) after the fourth-root transformation of reporting trends, according to Field et al. (1982). Afterwards, a principal coordinates analysis (PCO) was performed on anthropogenic pressures normalized data using the euclidean distance to determine if there is a spatial pattern of pressure variables that were associated with MSs food webs assessment (pressures data used: fisheries mean, ph, silica, seabed litter, beach litter, sea



surface temperature anomaly, sea surface level, chlorophyll, phosphates (po4), nitrates (no3), port number, distance to port, bottom trawl (mean), marine traffic, number of wind turbines, the average number of wind turbines, CPUE, zinc, lead, cadmium, copper and fluor across reported units (see data description in Table 5.2)) (Clarke K. R., 2001). A permutational multivariate analysis of variance (PERMANOVA) was used to test the hypothesis for significant differences among the cluster groups (defined by the Bray-Curtis measure on fourth root transformed data) (Anderson et al. 2008). The similarity percentages (SIMPER) routine was applied to identify which indicators contributed the most to the within-cluster similarity and the between-cluster dissimilarity (Clarke, 1993). Finally, the BEST (BIOENV) procedure was used to find the subset of pressure variables that significantly explained the clusters of food webs assessment trends determined in the cluster analysis, using the Spearman correlation (Clarke, 1993).

All statistical analyses were developed in R software environment (R Core Team, 2019) and multivariate analyses were performed in PRIMER 6.0 software (Clarke and Gorley, 2006).

### 5.3 Results

In the studied area, a total of 258 food web criteria were reported. The number of criteria implemented per subregion, MS, ecosystem element and criteria/indicator type is shown in Supplementary Table 1. Food web criteria were more reported in the CS subregion (115), followed by GNS (75) and BBIC (65) subregions. The MS with the highest number of reported criteria was the UK (159), followed by PT (38) and SP (35). Ireland and France showed a very low effort for D4 implementation with 22 and 12 criteria, respectively. The most reported ecosystem elements were Fish (121), followed by Plankton (100) and Marine Mammals (25) (Figure 5.2A). Ecosystem elements targeted varied between MSs: the UK assessment included a wider number of ecosystem elements (Fish, Plankton, Marine Mammals, Marine birds), while other MSs only reported Fish (PT, FR, SP) or Fish and Plankton (IR). Criteria reported varied between subregions and MS (Figure 5.2B and C). The most reported Criteria was the relative abundance of pairs (RAP), followed by maximum mean length (MML) and typical length (TyL) - all reported by the UK. Several criteria were only reported once across all studied MSs: Shannon diversity (kg), Shannon diversity (n), Species richness, Trophic diversity, Trophic diversity (vol), Trophic richness.

General linear models showed that factors influencing food webs average assessment trend were MSs, ecosystem elements and criteria, explaining 34.1% of existing deviance ( $p < 0.05$ ; Table 5.3). MSs and ecosystem elements explained 3.7% and 3.8% of existing deviance, respectively. MSs average trends significantly decreased in FR (-0.46; SE=0.16), while other MSs presented stable trends (Figure 5.3A). Significantly increasing trends were found for Marine mammal (0.16; SE=0.17), while a decrease was found for Marine birds (-0.42; SE=0.19); however, these two ecosystem groups were only reported by the UK (Figure 5.3B). As for criteria, results showed that this factor the highest percent of existing deviance with 21.22%; Fishing in Balance (FiB), Primary Production Required (PPR) and Fullness index (FI) presented significantly higher average trends (with 1; SE=0.00), while Trophic Diversity (n), Species richness (n),

Trophic richness and Relative abundance presented the lowest significant trend (with -1; SE=0.00) (Figure 5.3C).

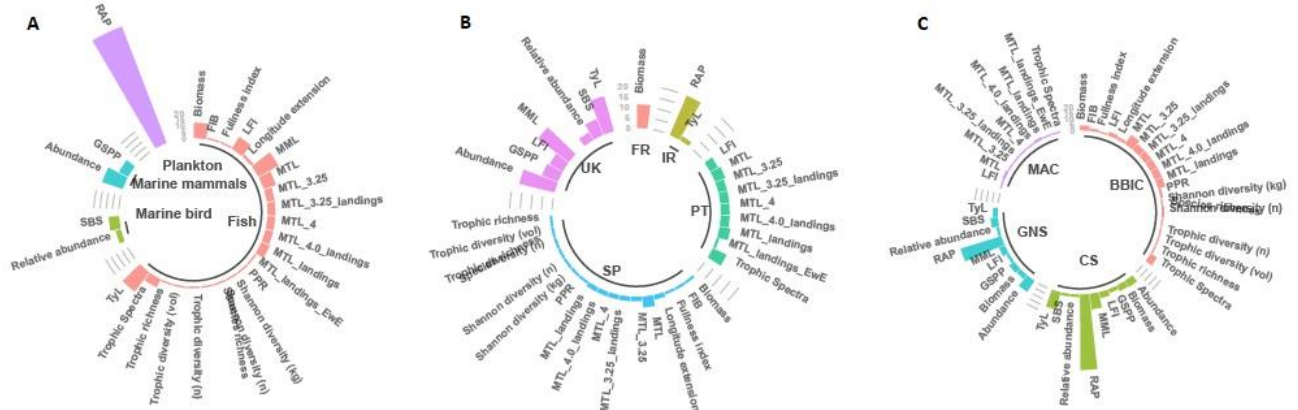


Figure 5-2 - Number of reported criteria per ecosystem element (A), Member state (B) and subregion (C).

When looking at food webs assessment targeting exclusively fish ecosystem elements, of the 115 criteria reported, 95 respected the established thresholds (were implemented more than once and were common to, at least, two MSs). These were reported mostly by BBIC (42), followed by the CS (33). As for MSs, UK was the country with the highest reporting number (42), followed by PT (30), while IR exhibited the lowest (2). The most reported criteria were TyL, MML, and Large Fish Indicator (LFI)/ Mean Trophic Level (MTL) (with 19, 18, and 11 criteria, respectively). There was a division between subregions: MSs reporting for the GNS and CS (UK and IR) subregion used TyL, MML and LFI fish criteria, while BBIC and MAC (SP and PT) used MTL, MTL with thresholds (using survey and landing data) and LFI (only in PT assessment) (Figure 5.4A).

Table 5-3 – Generalized linear models (GLMs) with gamma distribution applied to the average trend data for Descriptor 4 – Food webs, per subregion, Member State, criteria, ecosystem element and their corresponding interaction.

	Residual	Deviance	% explained	p-value
Subregion	0,14	22,24	0,64	0,534
Member State	0,83	21,56	3,69	<b>0,014</b>
Ecosystem element (Target)	0,85	21,53	3,81	<b>0,005</b>
Criteria	4,75	17,64	21,22	<b>&lt;0,001</b>
Subregion: Criteria	0,49	21,90	2,20	0,942
Subregion: Target	0,00	22,39	0,00	-
Criteria: MS	0,58	21,81	2,57	0,187
Target: MS	0,00	22,39	0,00	-
<b>Total explained</b>			<b>34,14</b>	

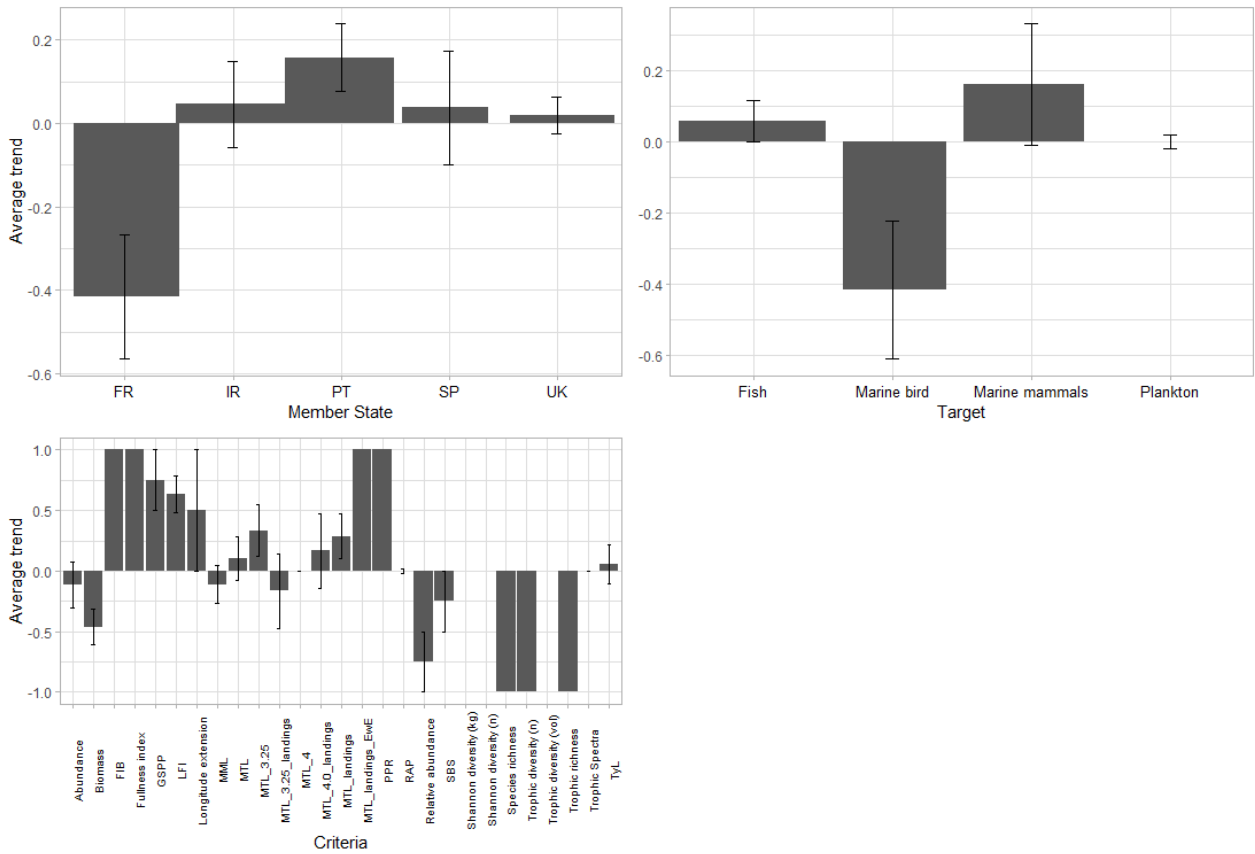


Figure 5-3- Average assessment trend for food web criteria per MS (A), ecosystem element (B) and criteria (C) resulting from the GLM analysis applied to Descriptor 4 – Food webs data in the NE Atlantic.

The average trend for fish criteria varied between 0 (SE=0.00) in MAC and 0.2 (SE=0.77) in GNS subregions. The MS with the highest average trend was IR, and PT, with 0.5 (SE=0.71) and 0.2 (SE=0.53), while the lowest trends were reported by SP and UK with a 0.1 average trend (SE=0.62 and SE=0.72, respectively). When analysing criteria, results showed that the highest trends were obtained for LFI and MTL\_3.25 (0.64; SE=0.50 and 0.43; SE=0.53, respectively), while the lowest trends were exhibited by MTL\_3.25\_landings and MML (-0.29; SE=0.76 and -0.11; SE=0.68, respectively) that exhibited decreasing patterns (Figure 5.4B).

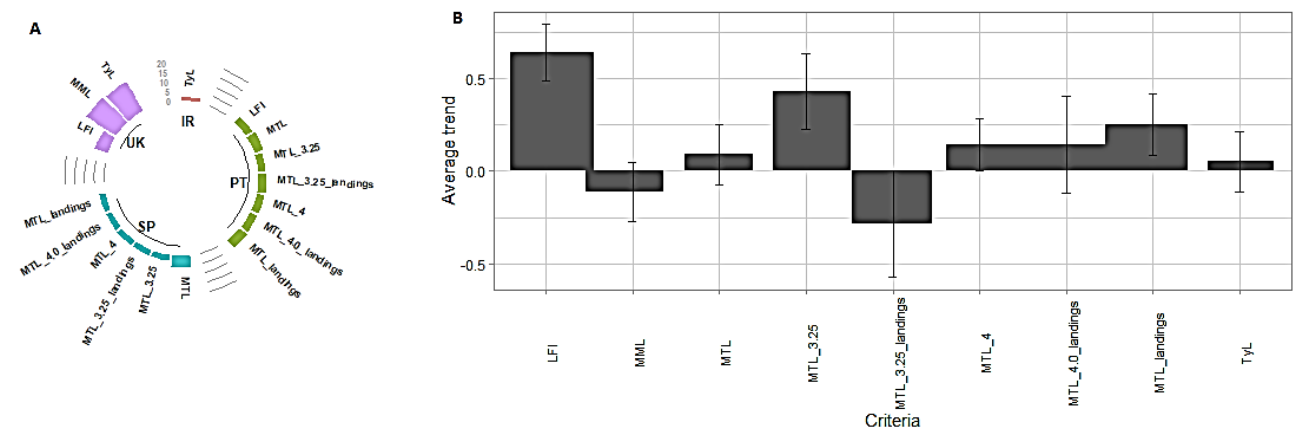


Figure 5-4 - Number of fish criteria reported per Member State (A) and average assessment trend for fish food web indicators (B).

In regards to the multivariate assessment, the cluster analysis revealed three groups (d, e and g), three separate units (b, c and f) and one outlier (a) at the distance level of 54.9% (Figure 5.5, left). When these clusters were superimposed with the spatial areas of assessment of each MS, a pattern could be observed (Figure 5.5, right). The first group included eight UK assessment units (cluster d) and presented an average similarity of ~86,99% within units. Two units also reported by the UK were separated from this cluster (cluster b and c, with 50 to 55% of similarity to cluster d). The second cluster included all PT units (including the Azores) and exhibited 93,24% of within-group similarity (cluster g). This cluster presented an average similarity of 61,69% to a single SP assessment unit (Cluster f). The third cluster included two SP assessment units (Cluster e) and presented 100% of within similarity (average trends were identical). The cluster plot showed that PT units had similarities to SP and UK reporting units (with 61.69%, and 15.11% of similarity). The similarity between SP and PT was due to MTL reporting, and the similarity between PT and UK was due to LFI reporting, while the SP units presented no similarity (0%) with the UK ones (Figure 5.5 left). In what concerns the outliers, a single SP assessment unit presented a dissimilarity of 97.5% from the overall units. Results showed that assessment for SP units was more heterogeneous, since it was separated in two cluster groups and one outlier, while PT and UK were more homogeneous (Figure 5.5 left and right).

The PCO analysis for pressure variables showed a relevant pattern for SST, SSL, cadmium, mean fishing, Marine Traffic and number of ports across Axis 1, which explained 28.1% of the variation. High values for these variables explained most of the PT and SP average trend results (clusters e, f, and g). Axis 2 explained 23% of the variation and was associated with a high amount of chemical nutrients (Lead, Copper, Zinc) and a high number of offshore wind turbines. This axis explained the UK units from the GNS and CS (cluster d and b) and SP (North Bay of Biscay units). Axis 3 explained 13% of variability, including variables such as no3, po4, port distance, and explained UK trend estimates (including clusters c and d). Axis 4 and 5, explained 10.1 and 9.1 21% of the variation, respectively. These had a high influence of CPUE and zinc, respectively. These two axes explained average trend patterns for partial units from the PT cluster (Zone B) and SP (North Bay of Biscay units) (Figure 5.6).

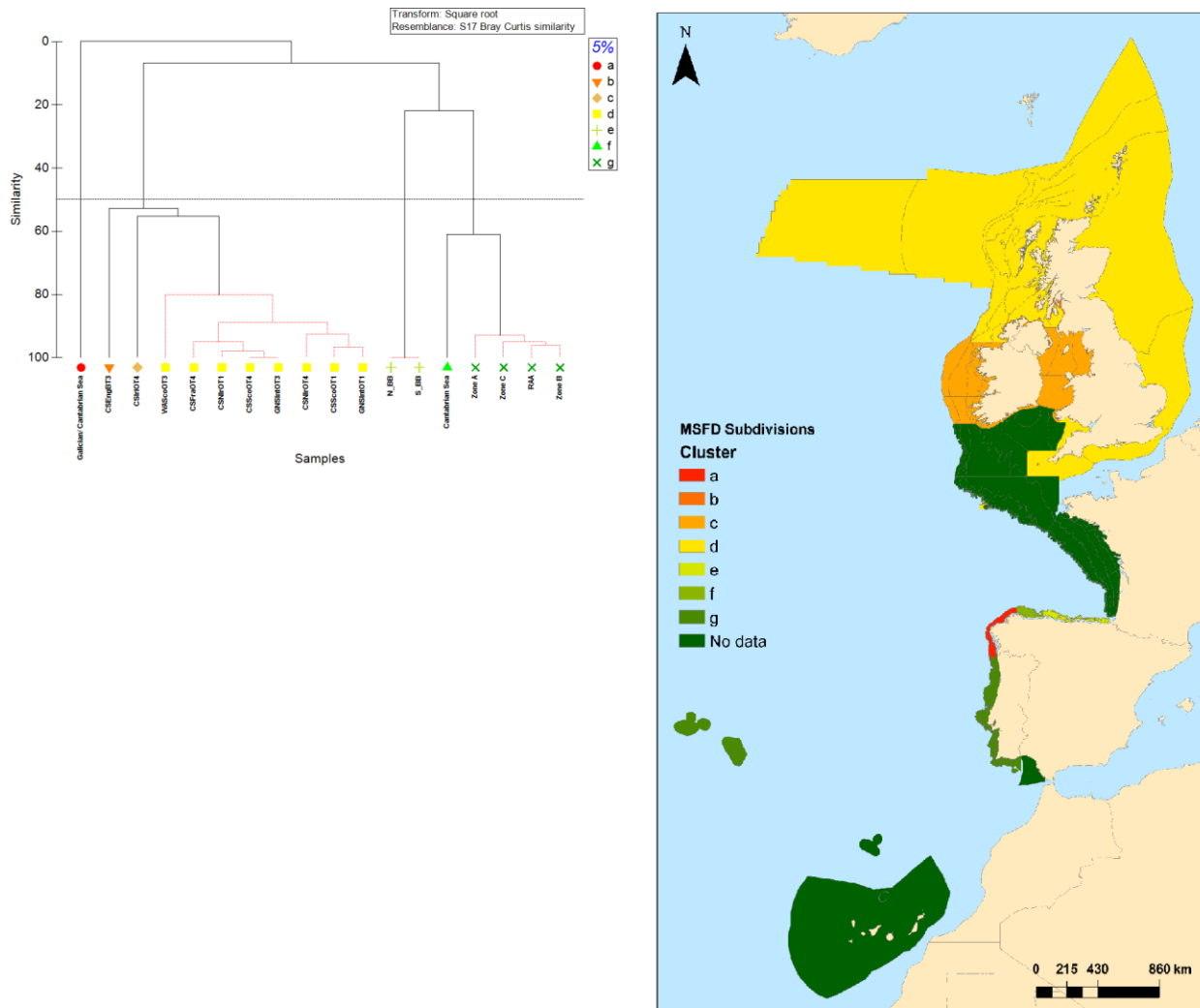


Figure 5-5 - Dendrogram resulting from cluster analysis of Food webs transformed average trend, reported by Member States (A) and spatial distribution of the cluster assemblages (B). Five assemblages and three outliers were identified at the 57% level of similarity.

PERMANOVA analysis showed significant differences between clusters ( $F=86,26$ ;  $P(\text{perm}) < 0,001$ ) and the pair-wise analysis revealed significant differences among UK and SP units (d, e) and UK and PT units (d-g). Cluster e showed significantly higher trends ( $e = 1.00$ ;  $SE=0.22$  average trend), while cluster a had significantly lower average trends ( $a=-1.00$ ;  $SE=0.30$  average trend), evidencing heterogeneity in the SP reporting.

The SIMPER analysis showed that the within-group similarity ranged from 85.61% to 100% (groups d and e, respectively) and that the criteria that mostly contributed to this were MML, and TyL in group d, and MTL in group e. The between-groups dissimilarity varied between 39.31 and 100%, and the main discriminating species are listed in Supplementary Table 2.

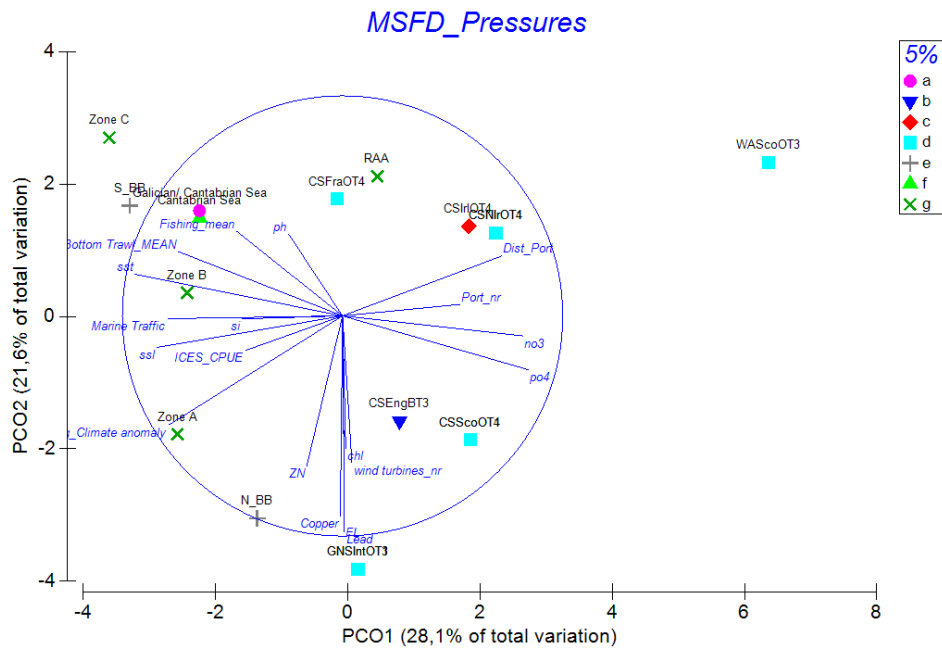


Figure 5-6 - Principal coordinates analysis (PCO) for the anthropogenic pressures' variables identified in Table 5.2; Clusters as obtained in the cluster analysis represented in Figure 5.5A.

The results of the BEST analysis, using anthropogenic data, revealed that the combination of SST, and mean bottom trawl provided the best match to explain the average reported trends. The correlation values were high ( $r=0.592$ ) and their influence was statistically significant ( $p<0.01$ ). When added to the combination, po4, Marine Traffic and Zinc also presented a significant correlation with the average reported trends ( $r=0.589$ ).

Anthropogenic pressures identified above have been analysed using descriptive (graphical) and statistical (correlation; spearman test) analysis to determine if they correlate with food web trends summaries for fish. The analyses showed that, in BBIC, an increase in SST and CPUE were associated with decreasing trends for food webs. While in the CS and GNS, high marine traffic and the number of offshore installations corresponded to food webs decreasing trends. However, none of these variables was significant.

#### 5.4 Discussion

The new MSFD report was published in 2020, pointing out broad progresses in relation to the 1st MSFD report, published in 2012. Improvements were largely driven by the recently published legal documents that defined aspects such as criteria, ecosystem components, anthropogenic pressures and spatial scales for reporting (European Commission, 2017b, 2017a), but were also motivated by MS effort, that revealed lessons learned from the 1st cycle and increased reporting coherence (European Commission, 2020a). However, particular descriptors are still not assessed accurately, are poorly coordinated or are underreported due to a lack of data and consensus on indicators or indices used. Food webs (D4)

assessment is complex by nature as it needs to measure energy flow across guilds through simple parameters, that require detecting changes on energy transfer (ICES, 2015).

This work assessed D4 implementation and assessment results across the NE Atlantic basin, including four subregions and six MSs, to disentangle if the current assessment encompassed the ecological aspects of food webs at subregion scale (European Commission, 2017a) and if trends detected emergent properties from single or cumulative anthropogenic pressures.

Results revealed that reporting strategies varied between MSs since each country supplied a distinct level of information. Relevant knowledge gaps were identified for IR and FR, which reported two or one indicators, while MSs such as UK, SP and PT showed an effort in reporting D4, employing fit-for-purpose methods (ICES, 2015; Tam et al. 2017). As a result, reporting differences were found between MSs, ecosystem elements and criteria selected, showing a lack of congruency in D4 implementation. The UK was the only MS addressing other ecological elements than Fish and Plankton (i.e., marine mammals, marine birds) using specific data sets and indicators. This was due to the adoption of long-term monitoring programs and data availability. Also, UK has closely followed OSPAR guidance in the implementation of indicators, which largely contributed to the choice of criteria (OSPAR, 2017; UK Marine Monitoring and Assessment Strategy and UK Monitoring and Assessment Reporting Group, 2019). That approach was not followed by other MSs in the CS, such as IR and FR.

In the BBIC subregion, SP and PT implemented food webs metrics indicated by ICES (2015) or Tam et al. (2017) and were coherent to some extent. On the other hand, the FR assessment reported raw information from stock assessment, not implementing food webs indicators and revealing lower reporting level and average trends. In the BBIC subregion, no higher-trophic (seabird and megafauna productivity) and lower-trophic ecosystem elements were addressed. Indicators assessing such groups are needed to reflect processes viewed from the opposite ends of the food web (e.g., PPR, zooplankton index, seabird productivity index, etc.). For example, PPR is an integrative indicator that represents the amount of primary productivity to sustain a fishery and enables the comparison of energy requirements across different fisheries (Pauly and Christensen, 1995; Chassot et al. 2010; Tam et al. 2017), while seabird productivity is an indicator of ecosystems health, through food availability (forage fish), accumulation of contaminants and environmental pollutants, and physiologic stress caused by environmental change (Mallory et al. 2010).

According to the Directive, EU Member States can monitor as many guilds as deemed appropriate (with a minimum of three), but at least two non-fish guilds should be addressed to ensure that not only fish are monitored (European Commission, 2017a). Even though indicators based on fish abundance and biomass can inform on the structural properties of food webs, they provide only partial information about its functioning, failing to consider complex trophic interactions and whole-system energy flow (Rombouts et

al. 2013). However, this procedure was only followed by the UK. The causes pointed out to explain this inequality are knowledge gaps in long term monitoring or the inexistence of minimum quality data that can support these assessments. Currently, the best data available comes from commercially exploited fish and shellfish stocks for which extensive monitoring programmes exist and from phyto- and zooplankton communities obtained through the Continuous Plankton Recorder; but even these are limited to certain areas of the North Atlantic Ocean (European Commission, 2020a). To overcome such issue, the EU has identified strategies such as the use of theoretical and empirical models to identify potential impacts and elucidate key properties that should be monitored and the necessity of harmonised monitoring programmes to generate proper assessments for trophic levels (and marine regions) (European Commission, 2020a).

When looking at the average trends established by the assessments these were stable or improved in the UK, SP, PT and IR, and registered a decrease in FR. The UK presented a significantly decreasing trend for Marine birds, that were considered below GES or at risk in CS and GNS. Decreasing patterns are of concern and direct management actions could be either top-down control rules aimed at relieving fishing pressure on lower-trophic species or bottom-up policies directed to improve water quality or habitat, which may also include improved management at land-sea interfaces (Mallory et al. 2010; Tam et al. 2017). On the other hand, marine mammals exhibited the highest average trend, evidencing increasing populations in the UK and indicating a recovery of mammal's populations. For the fish group, the average trend reported varied significantly between the type of metric employed, which resulted in high reporting heterogeneity.

When analysing food webs assessment approaches employed, the multivariate analysis applied to fish criteria revealed three similar food webs assessment groups including UK units, PT units, and SP units. Some units from IR and SP were considered dissimilar from the main groups. Within group similarities were based on MS and showed that UK, IR, PT and SP adopted distinctive reporting metrics and therefore resulting trends. Although UK and IR used similar indicators (MML and TyL), IR data sets did not enable a comprehensive assessment such as the UK, because the survey time-series from Irish waters is comparatively shorter (Machado et al. 2019); what has resulted in a dissimilar assessment for IR (European Commission, 2020a). To some extent, PT and SP used identical indicators (MTL and MTL with thresholds) increasing similarities. However, the PT assessment adopted indicators equally across all its spatial units, while the SP assessment employed indices heterogeneously across its territory. The SP assessment reported research findings obtained by EU funded projects (EcAlpha project), and peer-reviewed publications (Arroyo et al. 2019; Preciado et al. 2019). These studies included fit for purpose outputs concerning food webs assessment, but the metrics, and the temporal and spatial scales were dissimilar between them, what could explain part of the heterogeneity obtained in the SP assessments and the significant differences found between the SP clusters' average trends.



When looking at anthropogenic pressures and how they overlap with food webs assessment trends, results for UK units in the CS basin were influenced by high input of organic nutrients (no<sub>3</sub>, po<sub>4</sub>) and port distance, while GNS units were influenced by primary production (chl), chemical nutrients (Lead, Zinc, Cadmium) and the number of offshore installations. The high input of nutrients in the UK-CS units evidenced eutrophication and higher primary productivity, which can increase bottom-up effects (Cury and Roy, 1989). In the NE Atlantic, eutrophication has been recorded in the southern parts of the North Sea and along the North western coast of France. Nevertheless, nutrient inputs from point sources have significantly decreased; although inputs from diffuse sources, i.e. losses from agricultural activities, are still too high (European Commission, 2020b). In the GNS, chemical contamination has been decreasing due to regulations adopted, however, Cd levels are increasing in the Southern North Sea and need to be investigated. The assessment of chemical contaminants under the WFD showed that the worst scenarios can be found in the Baltic and the GNS, with 55% and 51% of the area assessed below GES (European Commission, 2020b). As for offshore installations (e.g., offshore renewable energies), long term impacts are still relatively unknown: offshore wind farms are a recently developed sector for which there are no long-term monitoring data. As a consequence, there is still a high level of uncertainty on the impacts of offshore wind parks on ecosystem structures and processes (Alexander et al. 2015). Noise affects especially marine mammals, but existing studies also show that habitat change, by adding artificial hard substrate in areas where mainly soft substrate occurs, can cause food webs shifts. Artificial reefs, such as offshore wind farms, are used by benthopelagic and benthic species as feeding grounds for prolonged periods (Mavraki et al. 2021).

In PT and SP units, anthropogenic pressures such as fishing (bottom trawl and mean fishing), seabed litter, SST, SSL, ph and marine traffic explained food webs trends. Similar results were found by Korpinen et al. (2021) for this region, identifying global warming (increasing SST), fisheries and shipping (underwater noise) as the major challenges that need to be addressed when considering cumulative anthropogenic effects. This region is characterized by narrow shelf areas (Korpinen et al. 2021), where trawling activities occur more intensely (Eigaard et al. 2017). Studies on the ratio of the trawling footprint over the landings showed that the highest ratios occurred in the Iberian Portuguese area, reflecting the higher level of exploitation when compared with some of the Atlantic management areas where fishing effort has been reduced (Eigaard et al. 2017). Regarding marine traffic, this activity is widely distributed in all EU marine regions and its intensity is highest along shipping corridors and near ports. Underwater noise from commercial shipping is considered one of the most pervasive noise sources. Underwater distribution and noise effects occurring in Europe are still unknown, however, impacts have been observed on all trophic levels, from invertebrates to fish, marine mammals, and diving seabirds (Dekeling et al. 2014; Barnett, 2020; Farcas et al. 2020). Climate anomalies (i.e., SST, SSL and ph) also explained food web average trends in the southern countries. Climate change effects on latitudes of species transition, such as the Iberian Peninsula, are expected to have wider degradation effects on food webs as the cold water species habitat

may be contracted, and warmer species habitat may expand (Serrat et al. 2018), changing food webs structure and resilience (Lynam et al. 2017). Even minor temperature changes can have significant effects on the onset of the spring phytoplankton bloom, the relative abundance of zooplankton, and the abundance and distribution of commercial fish species (Alexander et al. 2015).

The resulting matrix showed significantly high correlation values and as a result, it was possible to establish links between trends of abundance and distribution of fish elements and human pressures, such as fishing and climate anomalies and marine traffic in the southern region of the BBIC or the input of nutrients and/or chemicals in the UK waters. These findings indicate a relationship between food webs pressure and state and highlight the most relevant anthropogenic disturbances across the marine areas under study. Nevertheless, the methodological approach applied in this work, presented limitations since the assessment data set was limited by MSs reporting (only enabling the comparison of fish elements), it did not account for spatial and temporal variability and anthropogenic pressures were difficult to unequivocally distinguish from environmental variability. Notwithstanding, this work provides relevant insights on aspects that are hindering the detection of impacts and need to be considered in future assessments such as 1) the metrics/ indicators and ecosystem elements under assessment should be harmonized across the same subregion, 2) improvement of data mining and modelling for well monitored indicators (status and pressures) (Walmsley et al. 2017; Borja et al. 2019), 3) further development of monitoring networks, 4) improved spatial coverage and resolution of the assessment, since not all MSs are adopting similar scales (Machado et al. 2019, 2020), 5) methodologies and standards should account for the specificities of the region and detect region-specific sensitivity values (European Commission, 2020b).

Future work on food webs should use data that are often automatically recorded (e.g., automatic identification system (AIS) for shipping, vessel monitoring system (VMS) for fisheries), stored in permit databases (e.g., marine construction, dredging, dumping, fish catches), or observed from satellites (e.g., SST, chl, ph, oil spills, etc.). The use of these data sets could improve the assessment of single or cumulative effects on food webs, which in the past have been limited by data availability (Borja et al. 2019; Korpinen et al. 2021). However, more work is still needed in food web index implementation and development, to include non-linear responses and synergistic and antagonistic effects of pressures on ecosystem elements (Korpinen and Andersen, 2016; Taherzadeh et al. 2019).

In general, although the EU has surpassed previous assessments, food web evaluation is still lacking an appropriate ecological dimension. The present study emphasized the need for EU MSs to further improve their coordination and calibration at sea basin level, concerning ecosystems elements, criteria, indicators and the spatial and temporal scales used in food webs assessment. Only afterward's it will be possible to determine coordinated objectives and targets and having effective measures tackling the right pressures. The analysis proposed here allows, as a first step, to define the highest pressures by which managers can steer towards food web targets in the studied basins. Insights to this discussion are both timely and

relevant, especially as EU MSs are preparing their programme of measures to fulfil the aims of the MSFD, in which further inclusion is expected to be an important step towards fulfilling the commitments undertaken.

## 5.5 Conclusion

The present work highlights relevant aspects that need to be tackled in the assessment of food web in the context of the MSFD. The criteria and indicators selected need to be further calibrated, concerning the target element addressed and the metric employed, at subregion level. Although some advances have been made in this direction with the legal diploma 848/2017, the present work showed that food webs assessment is largely dependent on MS reporting. Only by using a harmonized set of indicators it will be possible to assess food webs status at an ecosystem level (at the subregion and sea basin level), to understand the effects of different human pressures, and to define effective management decisions. Importantly, the direct or indirect anthropogenic pressures were detected by trends assessed in the MSFD. The pressures of concern were fisheries, and climate change in the Iberian Peninsula, while eutrophication and chemical contamination affected CS and GNS surveyed regions. Overall, the MSFD assessment showed that human activities are not at an environmentally sustainable level and that pressures exert combined effects on food webs ecosystem components, i.e., fish. At the moment, the most urgent step would be to ensure a coherent assessment that foresees ecological relevant aspects of food webs and considers effects of relevant and on-going human pressures (Elliott et al. 2020), therefore promoting sea basin level calibration. A basis for this would be to continue to promote monitoring programmes and data rich platforms that can support assessments (Korpinen et al. 2021).

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## 6 *Final considerations and future perspectives*

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**Sustainable ocean management aims to meet the ‘needs of the present generation without compromising the ability of future generations to meet their own needs’** (Levine et al. 2014). To achieve this goal, environmental assessment and monitoring are essential. Environmental assessments are complicated since they attempt to comprehend the ecosystems complexity. In addition, multiple challenges affect the ability to measure ecosystem condition and report trends on a global scale (Borja, 2014; Borja et al. 2020; Garmendia et al. 2015; Halpern and Fujita, 2013). The vast ocean extent, diverse habitats, ecological complexity, and multiple ocean users with associated cumulative impacts make it extremely difficult to comprehensively measure and map ecosystem condition in the marine environment (Borja et al. 2014). Assessing the environmental status is commonly based on the use of ecological indicators that provide quantifiable metrics to understand if effects of anthropogenic pressure on environmental status are maintaining ecosystem services or, on the other, are leading to their degradation (Levin and Lubchenco, 2008). To achieve this objective, it is necessary to identify practical and reliable indicators to measure condition that can be used to track changes in the structure and functioning of ecosystems through time and space and allow to mitigate further environmental degradation (Smit et al. 2021; Tam et al. 2017).

Intending to contribute to the improvement of the environmental assessments of marine systems, the present dissertation aim was to evaluate the congruency of the MSFD implementation and to investigate if the scales used in environmental health assessments are detecting patterns of variability of the species or communities they aim to assess. Furthermore, this dissertation also aimed to understand if the assessment status responded to the effects of anthropogenic and/or natural drivers. To achieve this purpose the present PhD work plan focused on the application of biodiversity and ecosystem (or food webs) indicators, created or developed under the context of the MSFD.

In an initial stage, this thesis looked at MSFD reporting congruency in the North-eastern Atlantic, in terms of reporting level and temporal scales across geographic assessment scales (spatial reporting units). This work identified the factors responsible for incongruencies detected and paved the way into potential solutions for improvement. Outputs revealed that each MS was reporting independently with low cooperation at the region and sub-region level and that, frequently, most of the information used in the MSFD reporting was obtained from previously implemented directives (e.g., WFD, Birds and Habitats Directives and Common Fisheries Policy (CFP)) and not from dedicated programs conducted to answer the specific questions raised by MSFD. This resulted in a high reporting level of Descriptors 1 - Biodiversity and 3 - Commercial Fish and Shellfish and of functional groups such as Fish, Birds, and Rocky & Biogenic Reefs, and that criteria and Groups from Descriptor 4 - Food webs and Descriptor 2 - NIS were significantly underreported. As a result, temporal scales used to report in the NE Atlantic MSs,

varied significantly between different Groups across each Marine Sub-unit. Furthermore, in the first reporting cycle, the temporal ranges applied by MSs to assess biodiversity indicators were highly variable. French units and Spanish Bay of Biscay used a wide temporal window to assess Marine Turtles and Food webs criteria (e.g., 4.1 and 4.3), employing historic stranding data to report biological groups such as Marine Turtles and Marine mammals. On the contrary, Groups from Descriptor 2 - NIS were reported using the lowest temporal ranges. However, reporting variability was higher between groups across countries, which highlighted that differences between reporting strategies were nationally driven (Barrio and Holdsworth, 2016). Where resources and time-series length allow, assessing indicators over multiple temporal scales can provide different temporal scales of information to policy assessments; from detecting detailed changes within the most recent management cycle to providing broad-scale multi-decadal context to assessments. The selection of appropriate temporal scales is therefore a key example of the importance of co-production and dialogue between scientists and policymakers during the development of biodiversity indicators (Bedford et al. 2020) and should be further considered in the MSFD context.

In the following step, it was hypothesised if, assessing indicators over multiple relevant ecological scales can detect existing variability and patterns that would not be found over averages obtained for wider areas. The purpose was to identify the scales that reveal effects of anthropogenic or environmental stressors and therefore improve the accuracy of the ecosystem's assessment. To achieve this purpose the research work examined the responses of biodiversity and food webs indicators when using different scales of assessment in the North-Eastern Atlantic sea basins and the continental waters of Portugal. All the indicators and target elements selected for these studies have been used by MSs to report environmental status under the MSFD context. The methods (and metrics) employed to test the effects of scale varied per the type of indicators studied. In the first study, considering biodiversity (D1 – Biodiversity; C2 - Population abundance of the species), a Breakpoint Analysis (BPA) was applied to assess the temporal trend of biomass for three coastal and three demersal species, from wider to smaller sized scales. This study revealed that the three coastal species were found to be in good status regardless of the level of spatial disaggregation considered, although, some different patterns were detected at Vila Real the Sto António (VSA). For these species, distinct biomass index trends in the VSA1 and VSA units may result from higher environmental influence from the Mediterranean (Ambar, 1983) or higher fishing pressure. These species are landed in low numbers but are frequently discarded by the crustacean fishing fleet (Fernandes et al. 2007), which exerts an intense activity in the South area (Gonçalves et al. 2016). They are also commonly caught by coastal multi-gear and multi-species fishing fleets (Coelho et al. 2010), especially trammel nets (Gonçalves et al. 2016), being commonly discarded due to damage or poor condition (Gonçalves et al. 2007). Contrary to coastal species, results for demersal species suggested that, depending on species, assessments should be partitioned. *M. variegatus* species status was below the threshold for specific scales, and *A. sphyraena* showed decreasing patterns for a specific region. The regional assessment for *M. variegatus*, revealed that

biomass was below the ecological threshold for South zone. Disaggregating the scale showed that the poor status was specifically attributable to the South coastal zone - S1. The significant decrease in the biomass index found for South coastal areas, highlighted the need to maintain and to adjust monitoring effort, at relevant and comparable scales, to establish a direct link between identified fishing pressures and *M. variegatus* biomass index decrease. Spatial scales adopted showed species-specific aspects that should be addressed in future assessments; identified patterns were related with ecology and depth for *M. variegatus* – zone and depth strata and, to a minor extent, with latitude for *A. sphyraena* – sector.

When studying food web indicators (D4 – Food webs) a different approach was followed; Generalized Additive Models (GAMs) were built to disaggregate indicator components that explained food webs variability looking at scales and physical environment (i.e., temperature and depth). For this purpose, four food webs indicators were tested: MTL (Mean Trophic Level), MTI (Mean Trophic Index, with different TL thresholds), LFI (Large Fish Indicator) and MATG (Mean Abundance across Trophic Guild). Models for MTL showed that using 1000 km<sup>2</sup> scales detected significantly different community patterns.  $MTI_{TL>4}$  and LFI significant differences were explained when using ICES rectangles and 1000 km<sup>2</sup> as boundaries in the Celtic Seas (CS), while scales related with regional and depth strata patterns - Sector/Strata units- detected variability in BBIC (Bay of Biscay and Iberian Coast) subregion. On the contrary, MATG was only marginally explained by spatial and temporal scales. Outputs highlighted that the selection of spatial scales that identify relevant community patterns should be studied for each subregion, separately, since the subregions studied had different scale requirements, reflecting local and/or regional patterns. In general, food webs assessment patterns were detected at equally defined spatial scales, such as ICES rectangles or 1000 km<sup>2</sup> squares, in the CS, and at scales related with regional and depth features - Sector/Strata - in the BBIC. As for temporal scales, even though its effects were significant in most final indicators models when compared to spatial scales, they had lower influence. Depth had a relevant role in explaining criteria variability, whilst the temperature was less relevant. Food webs trends showed the strongest decline occurred in shallow waters, where high fishing effort occurs, while in the deep area trends stabilized or increased and the relationship with fishing was not observed (Piet and Jennings 2005). To validate model results, the spatial and temporal scales previously identified for BBIC were used to assess food web criteria for the Portuguese continental waters. The assessment showed that MTL and  $MTI_{TL>3,25}$  indicators were below the threshold in locally defined areas (1000 km<sup>2</sup> spatial units) within the South and Southwest zones. For  $MTI_{TL>4}$  and LFI indicators, areas defined by region and depth (Sector/ Strata) within the Southwest and South, were also inferior to threshold values.

It is also important to highlight how the methods used in the dissertation – BPA and GAMs – allowed to show the relevancy of components and still understand how scales can differently contribute to explain indicators assessment results. The solution offered by the decomposition-based method provided even more information for food webs indicators (i.e., MTL, MTL with thresholds, LFI, and MATG), supporting

more informed assessment and better management advice. Even though most food webs criteria assessments benefited from downsizing the assessment scale, this was especially true for criteria including higher variability, e.g., MTL. Scale requirements increased with the range of TLs included (Heim et al. 2021). There is also the need to improve the current scientific knowledge for lower trophic guilds, which are not considered as a priority since they have no commercial interest, especially at spatially relevant scales. Finally, the outputs showed it is more informative to include component parts of indicators (such as temperature, depth, etc.), to understand the main drivers behind the 'headline' indicator and changes in it. Introducing such component indicators complicates interpretation for managers, but the added insight also reduces the risk of a misguided assessment (Thompson et al. 2020). The greater complexity of interpreting a decomposition is unlikely to be as great as working with additional, unrelated indicators because the elements of the decomposition are conceptually unified under the headline indicator.

Followingly, this dissertation further investigated food webs assessment status established by the MSFD reporting by understanding the knowledge gaps that characterize this assessment and the operationalization of its indicators. This study aimed at understanding if, in the North-Eastern Atlantic, food webs assessment is responding to anthropogenic pressures or climatic variability and therefore can be seen as a status indicator or, as suggested by several authors, it should consist in a "surveillance" indicator (ICES, 2015; Tam et al. 2017). The present work highlighted relevant aspects that need to be tackled in the assessment of food web status. The criteria, target elements and indicators selected need to be further calibrated at the subregion level. Although some advances have been made in this direction with the legal diploma 848/2017, the present work showed that additional efforts need to be made as implementation was largely unbalanced across MSs. Only by using a harmonized set of indicators, it will be possible to understand ecosystem status across MSs and subregions and different human pressures to help management at all levels (from regional to national to international) and to make effective decisions. Notably, the direct or indirect anthropogenic pressures were linked with trends assessed in the MSFD. The pressures of concern were fisheries, and climate change in the BBIC, while eutrophication and chemical contamination affected CS and GNS surveyed regions. However, some effects were relatively weak. Overall, MSFD assessment showed that human activities are not at environmentally sustainable levels and their pressures exert combined effects on specific ecosystem components, i.e., marine birds and fish groups. At the moment, the most urgent step would be to ensure a coherent assessment that foresees ecological relevant aspects of food webs and considers effects of relevant and ongoing human pressures (Elliot et al. 2020), therefore promoting sea basin level calibration. A basis for this would be to continue promoting and enhance monitoring programmes and data-rich platforms that can support MSs calibrated assessments.

## 6.1 Does scale partition matter?

Overall, biodiversity and food web indicators analysed showed that increasing the resolution of spatial scales improved the detection of population and community patterns and highlighted areas where assessments differed from the wider area initially analysed, *i.e.*, patterns or trends that were not detected initially were disclosed with smaller sized scales. The scales that improved the detection of significant patterns and trends varied between subregions and between indicators under study, what evidenced the need to study variability *a priori*, before any environmental assessment status is performed, for a specific geographic region, ecosystem element or indicator. To validate the outputs obtained, the scales identified were applied to the Portuguese continental platform to analyse if scales resolution affected the outputs of the environmental assessment in this Member State. For the majority of the species analysed under the biodiversity criteria assessment, the spatial scale units located in the S (South) showed distinct patterns or significantly different trends (*i.e.*, *M. variegatus*) in biomass indices, when compared to the other areas in the Portuguese continental coast and upper slope. These differences were masked in a broader assessment, which may be related to a lower representativity of the area which is averaged out when a wider assessment area is considered (entire S zone) in the assessment. Differences may be explained by the existence of specific and localized pressures or natural variability. As for food webs, spatial variability patterns were disclosed when spatial assessment scales were downsized. Significant patterns were detected at intermediary depths on the Southwest (SW) continental slope and coastal S zones. In general, both studies showed that wider assessments are masking decreasing patterns occurring within these areas, at local (for MTL and MTL<sub>TL>3,25</sub>), sector/depth (for MTL<sub>TL>4</sub> and LFI) and zone/depth (for *M. variegatus* biomass) scales. Results reinforced the complexities of choosing a geographic region and season for the assessment of fish populations and community status (Adams et al. 2017). Species and community patterns detected may result from natural variability such as depth boundaries or climate patterns, or from anthropogenic stresses, especially fisheries that are known to exert high pressure in the SW and S of the Portuguese waters. The S area is heavily targeted by the Portuguese demersal fish and the crustacean fishing fleet, which can have impacts at the community level (Moura et al. 2020). Analysis of VMS data revealed that the main pressure exerted by the crustacean fishing fleet occurs in the S and SW Portuguese margins, on muddy and muddy-sand bottoms, between 100 m and 700 m water depths and a decrease in landings has also been registered for demersal fish in the SW and S areas (Bueno-Pardo et al. 2017). Furthermore, the results from this thesis also reinforced the idea that the most prevailing pressure in the BBIC subregion, in which the Portuguese continental waters are included, is intensive fishing pressure, which is likely due to the narrow continental platform (Eigaard et al. 2017). Despite all the effort exerted, it is also important to recognize that the Portuguese coast is characterized by variable environmental drivers and is particularly affected by upwelling regimes (Moura et al. 2020; Sousa et al. 2005) that can strongly affect community composition.

The spatial scales at which these biodiversity and community indicators reflect changes were previously unknown, and this is an indispensable feature to identify relevant units of assessment, management actions and to organize the spatial network of monitoring programs that can address the environmental status over larger spatial scales (Östman et al. 2017). Outputs highlight the need to assess pressures at relevant scales in the SW and S of the Portuguese continental waters to identify what are the drivers behind the patterns detected at finer resolution scales. Spatial management of anthropogenic threats to populations can only be effective where model predictions correctly identify key species and habitats, distribution patterns and threat hotspots (Maxwell et al., 2015).

This study serves as an important demonstration of how scale and boundaries can influence a multidisciplinary ecosystem assessment. Thus, the scale by which indicators are developed should be chosen carefully, and the consequences of different boundary systems should be explored to determine how they might influence indicator behaviour and uses in management. Although, the main message invoked (i.e., scale matters) probably is robust to all of these caveats, since the ‘problem of scale’ is a well-accepted issue across the biological sciences (Levin, 1992), this work goes further and shows which scales should be selected and why. Nevertheless, the degree to which choices related to scale influence an indicator and an ecosystem assessment are no doubt context-specific and may be less important in certain contexts than in others (e.g., a system with little or no warming) (Heim et al. 2021). As a result, the identification of scales for distinct marine systems requires the development of similar studies, in which local specificities may be included (e.g., species, season, etc.). Therefore, this work encourages those developing and applying environmental assessment through indicators to evaluate the importance of scale and indicators components in their applications. Furthermore, this dissertation highlights how the improvement of monitoring programmes, on which assessments are based, is of utmost importance to enhance the quality of existing assessments, improving the detection of patterns, and supporting ecosystem assessments that guarantee the sustainability of marine systems.

## 6.2 Assessment scales and the MSFD

According to the MSFD, subregions are the basis for defining assessment areas for biodiversity and ecosystem components, however, each MS can use spatial subdivision if considered pertinent. The use of partitioned scales, further integrated to larger areas, is recommended as it can enable the assessment of local impacts (OSPAR Commission, 2012; Prins et al. 2014). Even though the revised text of the MSFD indicates that the minimum required scales and boundaries of the assessment are the subregion, this dissertation thesis shows that MSs are using smaller reporting units for the same ecosystem elements and criteria using diverse scales and indicator metrics that lead to inconsistent assessments. Furthermore, this work shows that the selection of indicators used in the assessment should be preceded by specific research concerning the spatial and temporal scales used in the implementation. More precisely indicators should be studied considering the spatial distribution and time series variability of the species or communities



targeted, after the indicator's selection (Walmsley et al. 2017). This is a huge task, especially if considering all species and target elements that need to be evaluated per criteria. However, without doing so, there is a high probability of missing relevant impacts that are averaged out in wider assessment scales, limiting the comprehension of ecosystem status. OSPAR guidance for the implementation of the MSFD pointed out that the assessment areas for mobile species should be based on species or population distribution, even though this approach needs to consider the practicalities of using and integrating multiple scales (OSPAR Commission 2012). The global classification of species with distributional range beyond the national waters is especially problematic and requires the integration of assessments across neighbour MSs, since species status would have a unique classification, facilitating the decision to apply measures to particular pressures at the global population level (Borja 2014; Walmsley et al. 2017). Nevertheless, this is not yet in practice, though the current MSFD assessment for biodiversity and food webs indicators considered, to some extent, spatial disaggregation, to identify the appropriate distribution of species, it did not deepen scales of study to understand if spatial coherency occurred at small size relevant scales.

The current MSFD assessment report established that biodiversity and food webs in the Portuguese continental waters were in GES, by calculating the weighted average for three zones of the Portuguese continental waters: North, Southwest and South (Ministério do Mar, 2020). But results obtained by this thesis revealed that significant food webs and biodiversity patterns existing within the SW and S areas may be overlooked if smaller assessment units (i.e., local, sector and depth), are not used. When downsizing the current spatial scale of assessment for the Portuguese continental waters it was possible to detect decreasing trends for biodiversity and food webs criteria, providing relevant information for management. These studies showed that the average resulting from wider assessments are masking decreasing trends and relevant patterns occurring within the SW and S areas, at local, sectorial and depth-based scales. As a result, the environmental status should be assessed using smaller sized scales and only afterward be integrated using the methodology selected for this purpose. It is true that, depending on the representativity of the areas and the method selected for integration, detected trends and patterns may be concealed (Barnard and Strong, 2014) after integration; however awareness is raised, and depending on the relevancy of pressures and the sensitivity of the species or populations studied, they can point out areas of concern to managers.

Lastly, when addressing food webs assessment, this dissertation reinforced that several inconsistencies still need to be tackled in the following MSFD cycle (European Commission, 2020). Nevertheless, it also revealed that criteria being developed and operationalized by ICES and OSPAR for the MSFD (e.g., MTL, LFI, TyL, etc.) are, to some extent, responding to anthropogenic and environmental stressors. Such outputs emphasize that the development of Descriptor 4 is supplying relevant information on how ecosystem status (through food webs assessment) is responding to existing drivers. However, to improve

indicators response, further work should be done concerning the scales of assessment, to ensure that relevant effects are not being dismissed.

### 6.3 References

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ment.



# Annex 1

Supplementary Material of the manuscript “Assessment level and time scales of biodiversity indicators in the scope of the Marine Strategy Framework Directive - a case study for the North-eastern Atlantic”

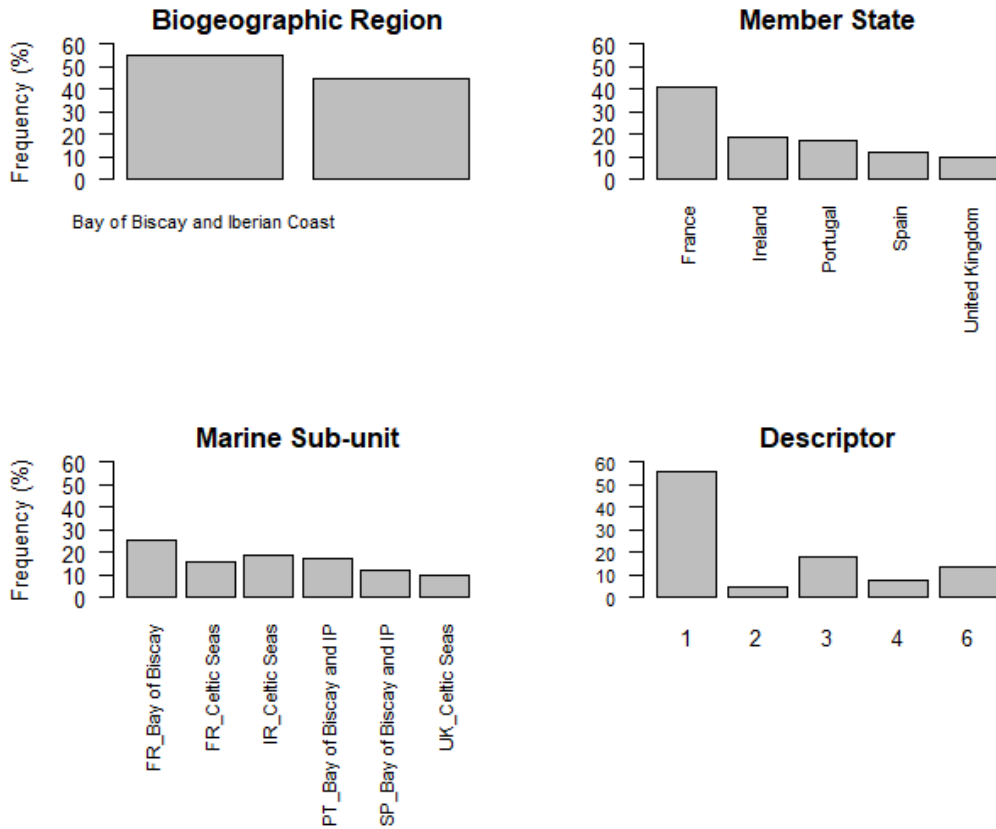


Figure S1 – Frequency distribution (%) of reporting per Biogeographic Region, Member State, Marine Sub-unit and Descriptor under analysis.

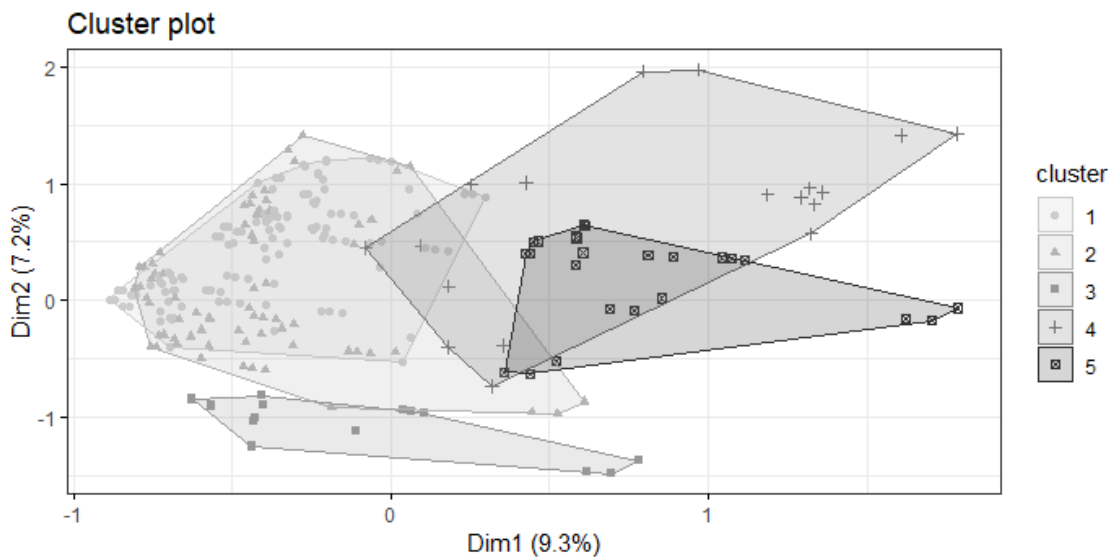


Figure S2– MCA and cluster Analysis applied to reporting data analyse – data is grouped by cluster analysis output with a similarity level of 3%.

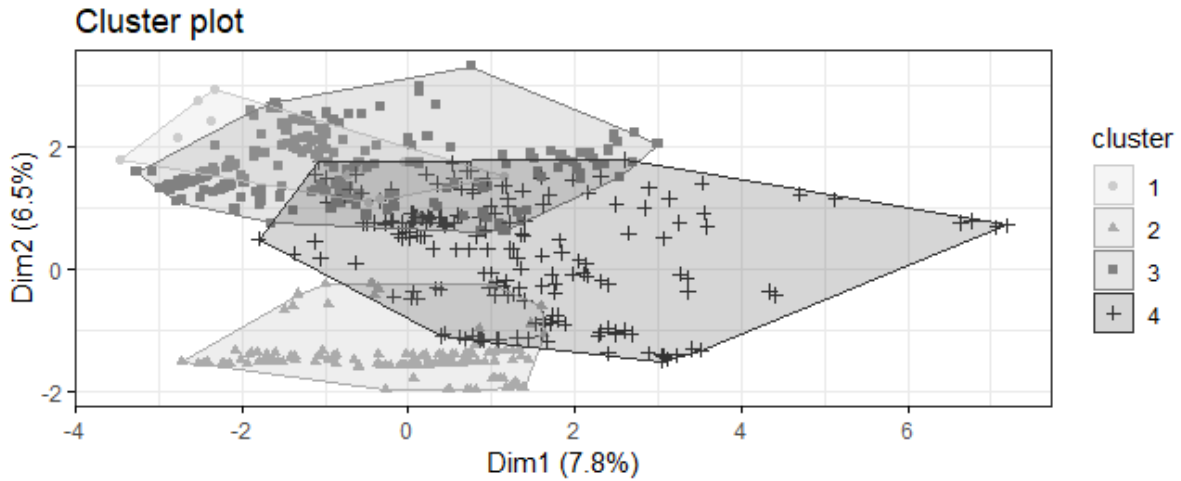


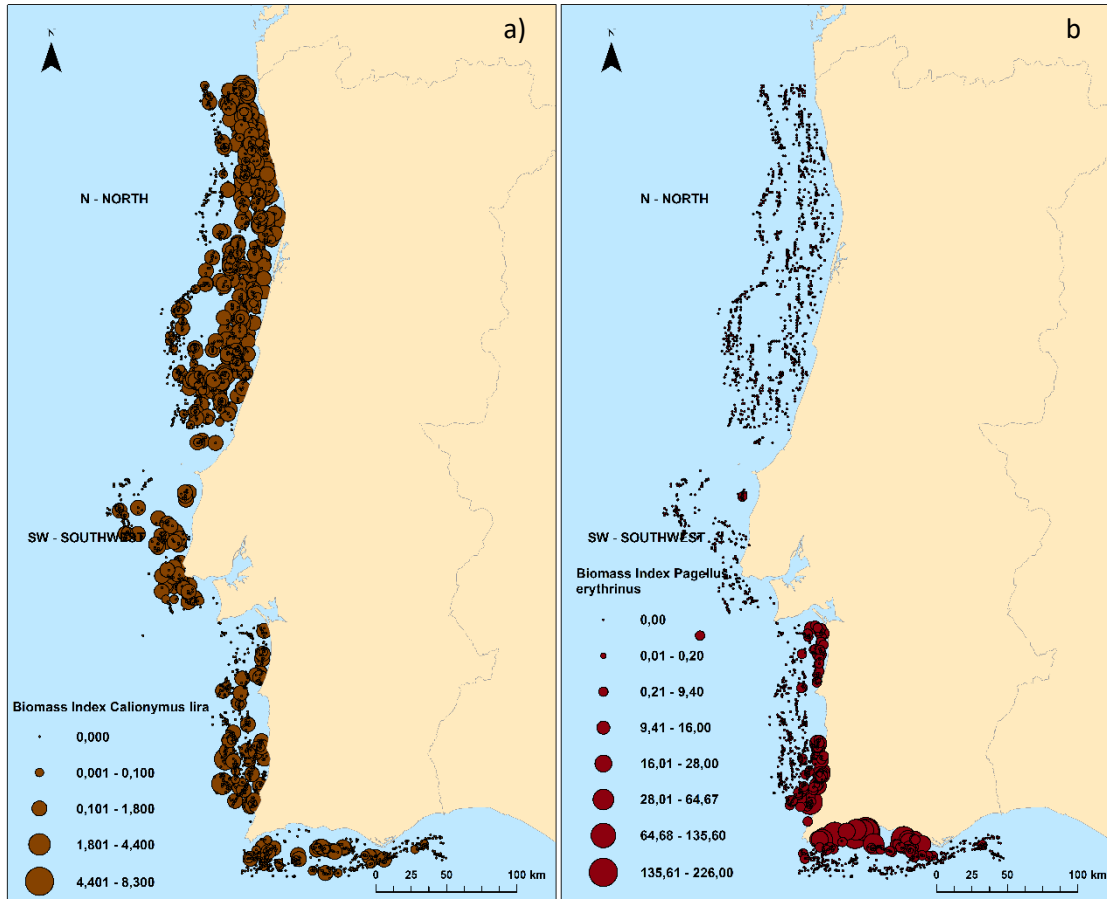
Figure S3– FAMD results showing the contribution of factors under analyse – data is grouped by cluster analysis output with a similarity level of 1.5%.

Table S1- Cluster analysis applied to FAMC results on reporting time range (similarity level was established with a probability of 0.05).

Cluster 1	Cluster 2	Cluster 3	Cluster 4
2.2 Environmental impacts of NIS	Fish	Rock & Biogenic Reef	1.6 Habitat Condition
Benthic species	3.3 Population size/age distribution	Sedimentary habitat	Marine mammals
Rock & Biogenic Reef	IR_Celtic Seas	1.4 Habitat distribution	4.3 Abundance of key trophic groups
PT_Bay of Biscay and Iberian Coast	3.1 Fishing activity pressure	1.5 Habitat extent	Seabirds
1.6 Habitat Condition	3.2 Stock reproductive capacity	6.2 Condition of benthic community	Bay of Biscay and Iberian Coast
SP_Bay of Biscay and Iberian Coast	1.1 Species distribution	2.1 Abundance of NIS	Plankton
Fish	1.2 Population size	6.1 Substrate physical damage	Marine turtles
-	1.3 Population condition	Celtic Seas	4.1 Biomass of key species/ trophic group

## Annex 2

Supplementary Material of the manuscript “Effects of scale on the assessment of fish biodiversity in the Marine Strategy Framework Directive context”



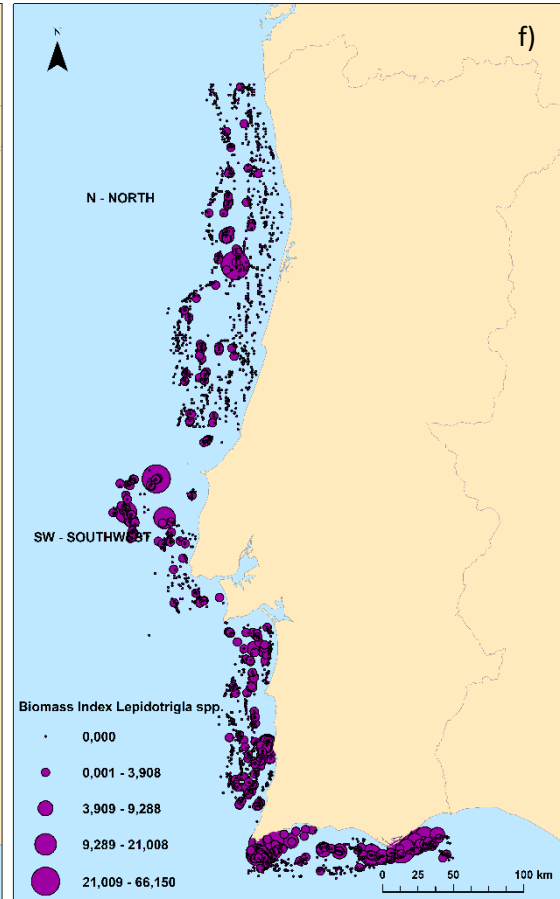
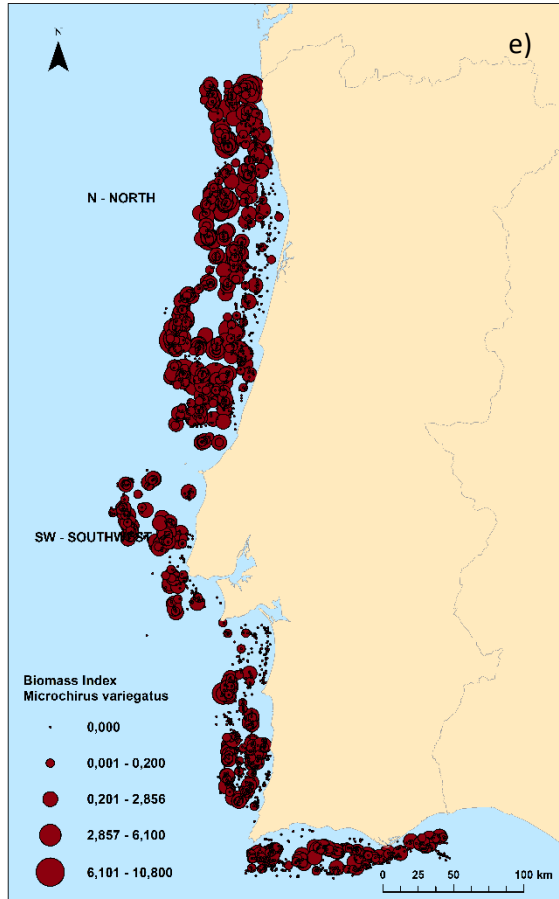
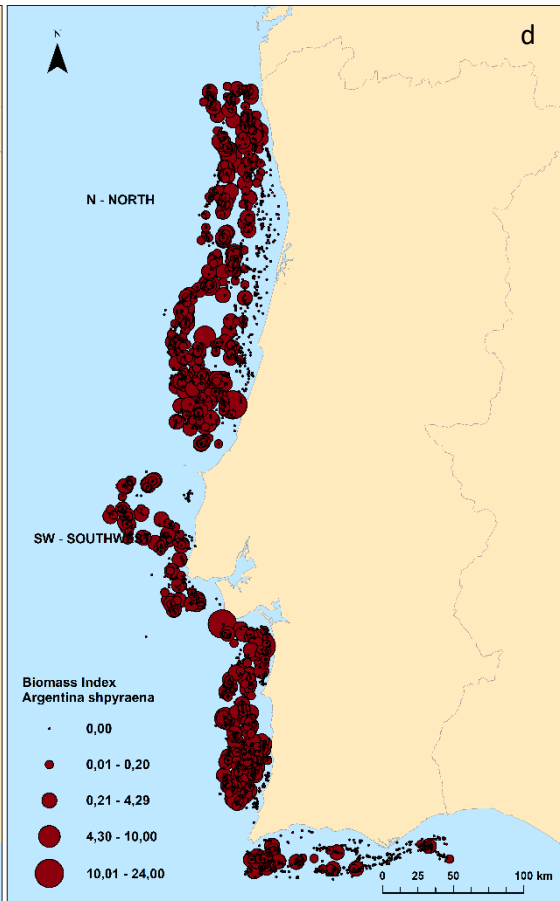
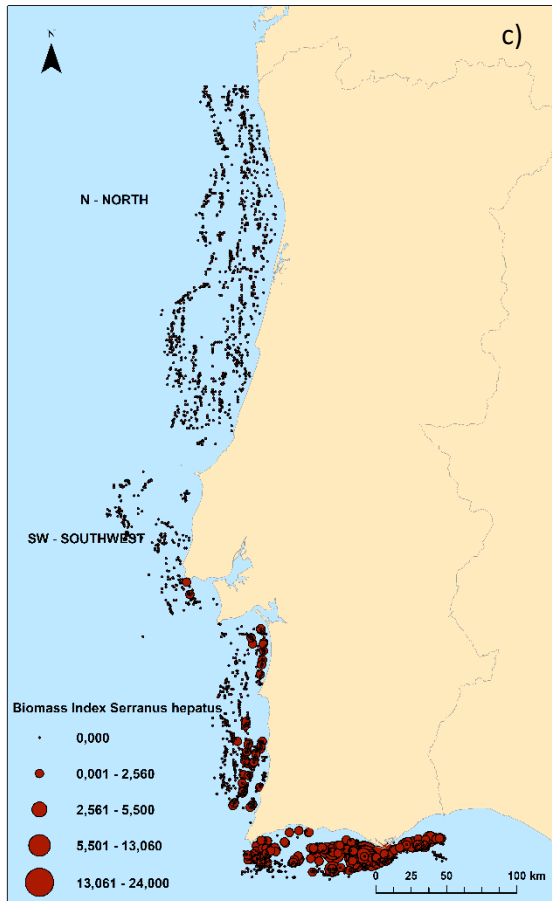


Figure S3 – Geographical distribution of a) *Callionymus lyra*, b) *Pagellus erythrinus*, c) *Serranus hepatus*, d) *Argentina shpyraena*, e) *Microchirus variegatus*, f) *Lepidotrigla* spp. for each trawl held in the Portuguese survey– from 1982 to 2017.

Table S1 - Results for *Callionymus lyra*: BPA + TA analysis

<i>Callionymus lyra</i>	Spatial Unit	Ref. Per	Assess. Per	Ref.	Assess.	p-value	>2013 slope	Trend
Portuguese continental EEZ	Global (N)	1982-1989	1994-2017	0.188	0.084	0.000	0.000	Increasing
Zone	N	1982-1989	1994-2017	0.188	0.084	0.000	0.000	Increasing
Depth strata	1 - Coastal	1982-1989	1994-2017	0.305	0.146	0.000	0.000	Increasing
	2 - Medium	1982-1989	1994-2017	0.026	0.010	0.002	0.009	Increasing
Zone*Depth strata	N1	1982-1989	1994-2017	0.303	0.146	0.001	0.000	Increasing
	N2	1982-1989	1994-2017	0.026	0.010	0.002	0.009	Increasing
Sector	AVE	1982-1990	1995-2017	0.013	0.006	0.032	0.119	Increasing
	BER	No BPs					0.005	Increasing
	CAM	1982-1989	1994-2017	0.048	0.037	0.256	0.000	Increasing
	FIG	1982-1990	1995-2017	0.003	0.007	0.082	0.056	Increasing
	MAT	1982-1988	2012-2017	0.005	0.021	0.128	0.040	Increasing
Sector * Depth strata	AVE1	1982-1986	2012-2017	0.117	0.175	0.190	0.860	Decreasing
	AVE2	1982-1989	1994-2017	0.048	0.001	0.018	NaN	Stable
	BER1	1982-1988	2000-2017	0.090	0.000	0.022	NaN	Stable
	BER2	1982-2012	2012-2017	0.012	0.066	0.204	0.013	Increasing
	CAM1	1982-1993	1993-2017	0.942	0.295	0.000	0.000	Increasing
	CAM2	1982-1986	1991-2017	0.020	0.011	0.378	0.404	Increasing
	FIG1	1982-1990	2003-2017	0.060	0.060	0.995	0.055	Increasing
	FIG2	1982-1989	1994-2017	0.022	0.001	0.020	0.040	Increasing
	MAT1	1982-1988	2012-2017	0.152	0.075	0.179	0.018	Increasing
	MAT2	1982-1987	2012-2017	0.039	0.021	0.158	0.018	Increasing

Table S2 - Results for *Pagellus erythrinus*: BPA + TA analysis.

<i>Pagellus erythrinus</i>	Spatial Unit	Ref. Per	Assess. Per	Ref	Assess	p-value	>2013 slope	Trend
Portuguese continental EEZ	Global	1982-2012	2012-2017	0.598	3.702	0.009	0.010	Increasing
Zone	SW	1982-2012	2012-2017	1.041	4.645	0.081	0.029	Increasing
	S	1982-1989	2012-2017	4.815	12.172	0.004	0.002	Increasing
Depth strata	1 - Coastal	1982-2012	2012-2017	2.879	14.111	0.014	0.018	Increasing
	2 - Medium	1982-1986	2011-2017	0.051	0.407	0.000	0.064	Increasing
Zone* Depth strata	SW1	1982-2012	2012-2017	1.715	7.412	0.048	0.031	Increasing
	SW2	1982-1986	2012-2017	0.051	0.328	0.000	0.002	Increasing
	S1	1982-1989	2012-2017	7.319	17.937	0.018	0.004	Increasing
	S2	1982-2011	2011-2017	0.016	0.348	0.046	0.102	Increasing
	ARR	1982-2012	2012-2017	1.430	8.627	0.222	0.042	Increasing
Sector	MIL	1982-2000	2012-2017	0.864	7.729	0.194	0.042	Decreasing
	POR	1982-1989	2012-2017	10.078	19.559	0.027	0.026	Increasing
	SAG	1982-2012	2012-2017	0.278	13.278	0.020	0.006	Decreasing
	SIN	1982-1986	2011-2017	0.377	1.179	0.029	0.273	Decreasing
	VSA	1982-1986	1986-2017	1.432	0.054	0.101	0.571	Increasing
Sector * Depth strata	ARR2	1982-1986	1986-2017	0.000	0.299	0.215	0.047	Increasing
	POR1	1982-1990	2011-2012	8.791	28.542	0.016	0.391	Increasing
	SIN1	1982-2008	2008-2017	0.912	2.904	0.005	0.869	Decreasing
	SIN2	1982-1994	2006-2017	0.009	0.218	0.005	0.406	Decreasing
	VSA1	1982-1986	1986-2017	2.875	0.097	0.012	0.182	Increasing

Table S3 - Results for *Serranus hepatus*: BPA + TA analysis.

<i>Serranus hepatus</i>	Spatial Unit	Ref. Per	Assess. Per	Ref	Assess	p-value	>2013 slope	Trend
Portuguese continental EEZ	Global (S)	1982-1988	2006-2017	0.257	0.742	0.000	0.518	Decreasing
Depth strata	1 - Coastal	1982-1986	2006-2017	0.246	1.436	0.000	0.864	Decreasing
	2 - Medium	1982-1987	2009-2017	0.026	0.046	0.002	0.992	Decreasing
Sector	POR	1982-1987	2006-2017	0.213	0.325	0.163	0.009	Increasing
	VSA	1982-1992	2006-2017	1.022	1.851	0.041	0.452	Decreasing

Table S4 - Results for *Argentina sphyraena*: BPA + TA analysis.

<i>Argentina sphyraena</i>	Spatial Unit	Ref. Per	Assess. Per	Ref	Assess	p-value	>2013 slope	Trend
Portuguese continental EEZ	Global	1982-1996	2008-2017	0.238	0.096	0.000	0.003	Increasing
Zone	N	1982-1988	2008-2017	0.154	0.078	0.001	0.000	Increasing
	SW	1982-1986	2012-2017	0.386	0.208	0.107	0.006	Increasing
Depth strata	1 - Coastal	1982-1986	2011-2017	0.006	0.063	0.018	0.064	Increasing
	2 - Medium	1982-1986	2008-2017	0.488	0.137	0.000	0.001	Increasing
	3 - Deep	1982-2001	2009-2017	0.028	0.040	0.286	0.054	Increasing
Zone*Depth strata	N1	1982-1986	1990-2017	-0.009	0.015	0.259	0.029	Increasing
	N2	1982-1992	2008-2017	0.312	0.137	0.001	0.000	Increasing
	N3	1982-1999	2012-2017	0.003	0.003	0.998	0.061	Decreasing
	SW1	1982-2012	2012-2017	0.025	0.154	0.038	0.171	Increasing
	SW2	1982-1986	1997-2017	0.650	0.116	0.016	0.007	Increasing
	SW3	1982-2002	2009-2017	0.052	0.083	0.248	0.005	Increasing
	Sector	ARR	1982-1995	1995-2017	0.208	0.049	0.000	0.005
AVE		1982-1991	1991-2017	0.106	0.032	0.004	0.097	Decreasing
BER		1982-1986	2007-2017	0.122	0.117	0.069	0.043	Increasing
CAM		1982-1991	2010-2017	0.090	0.083	0.705	0.043	Increasing
FIG		1982-1986	2010-2017	0.284	0.064	0.062	0.057	Increasing
LIS		1982-1993	2012-2017	0.144	0.184	0.600	0.057	Increasing
MAT		1982-1989	1993-2017	0.248	0.069	0.085	0.100	Increasing
MIL		1982-1986	2012-2017	0.847	0.133	0.025	0.092	Decreasing
SIN		1982-1986	1997-2017	0.697	0.155	0.775	0.014	Increasing
SAG		1982-1989	1994-2017	0.141	-0.017	0.001	0.003	Increasing
Sector * Depth strata	ARR2	1982-1995	1995-2017	0.329	0.070	0.000	0.017	Increasing
	AVE1	1982-2001	2011-2017	0.001	0.009	0.618	0.753	Decreasing
	AVE2	1982-1990	2008-2017	0.370	0.348	0.847	0.002	Decreasing
	BER2	1982-1992	2008-2017	0.380	0.087	0.000	0.068	Increasing
	CAM1	1982-1987	2002-2017	0.070	0.034	0.001	0.143	Increasing
	CAM2	1982-1986	2010-2017	0.080	0.032	0.188	0.000	Increasing
	FIG1	1982-2012	2012-2017	0.008	0.032	0.188	0.052	Increasing
	FIG2	1982-2003	2008-2017	0.084	0.098	0.764	0.994	Decreasing
	LIS1	1982-1995	2004-2017	0.008	0.130	0.010	0.107	Increasing
	LIS2	1982-1992	1992-2017	0.287	0.077	0.001	0.002	Increasing
	MAT1	1982-1990	2011-2017	0.053	0.122	0.206	0.710	Decreasing
	MAT2	1982-1986	1993-2017	1.813	0.118	0.021	0.093	Increasing
	MIL2	1982-1986	1986-2017	2.316	0.045	0.080	0.015	Increasing
	SAG2	1982-1989	1994-2017	0.192	0.014	0.025	0.001	Increasing
	SIN1	1982-2010	2010-2017	0.000	0.029	0.017	0.068	Increasing
	SIN2	1982-1986	1997-2017	0.899	0.201	0.043	0.012	Increasing

Table S5 - Results for *Microchirus variegatus*: BPA + TA analysis

<i>Microchirus variegatus</i>	Spatial Unit	Ref. Per	Assess. Per	Ref	Assess	p-value	>2013 slope	Trend
Portuguese continental EEZ	Global	1982-1991	1996-2017	0.114	0.122	0.649	0.002	Increasing
Zone	N	1982-1990	1995-2017	0.212	0.199	0.769	0.077	Increasing
	SW	1982-1986	2008-2017	0.075	0.084	0.448	0.000	Increasing
	S	1982-1991	2008-2017	0.057	0.036	0.014	0.027	Decreasing
Depth strata	1 - Coastal	1982-1989	1994-2017	0.160	0.104	0.315	0.001	Increasing
	2 - Medium	1982-1991	2009-2017	0.138	0.217	0.018	0.270	Increasing
	3 - Deep	1982-1989	2011-2017	0.102	0.180	0.000	0.995	Decreasing
Zone* Depth strata	N1	1982-1989	1994-2017	0.221	0.112	0.285	0.001	Increasing
	N2	1982-1991	1996-2017	0.189	0.235	0.407	0.322	Increasing
	N3	1982-1990	2011-2017	0.261	0.508	0.005	0.000	Decreasing
	SW1	1982-1987	2009-2017	0.023	0.023	0.927	0.024	Increasing
	SW2	1982-1986	2009-2017	0.094	0.119	0.203	0.043	Increasing
	SW3	1982-1993	2000-2017	0.060	0.078	0.159	0.197	Increasing
	S1	1982-1990	2007-2017	0.131	0.067	0.001	0.000	Decreasing
	S2	1982-1991	2009-2017	0.015	0.054	0.001	0.593	Decreasing
S3	1982-1986	2008-2017	0.013	0.005	0.049	0.749	Increasing	
Sector	ARR	1982-1988	1999-2017	-0.002	0.032	0.000	0.229	Increasing
	AVE	1982-1986	2013-2017	0.170	0.208	0.638	0.005	Increasing
	BER	1982-1986	1998-2017	0.193	0.114	0.163	0.000	Decreasing
	CAM	1982-1989	1994-2017	0.335	0.339	0.973	0.346	Increasing

	FIG	1982-1991	1996-2017	0.123	0.100	0.430	0.078	Decreasing
	LIS	1982-1996	2008-2017	0.207	0.160	0.251	0.005	Increasing
	MAT	1982-1989	1994-2017	0.147	0.317	0.020	0.055	Increasing
	MIL	1982-1994	1999-2017	0.097	0.062	0.003	0.232	Decreasing
	POR	1982-2000	2007-2017	0.133	0.044	0.000	0.966	Increasing
	SAG	1982-1991	2008-2017	0.001	0.028	0.045	0.857	Increasing
	SIN	1982-1995	2010-2017	0.050	0.092	0.007	0.541	Decreasing
	VSA	1982-1986	2000-2017	0.009	0.045	0.000	0.000	Decreasing
Sector * Depth strata	ARR2	1982-1992	1998-2017	0.040	0.053	0.696	0.036	Increasing
	ARR3	1982-1992	2011-2017	0.005	0.018	0.291	0.011	Decreasing
	CAM2	1982-2009	2009-2017	0.205	0.654	0.007	0.045	Decreasing
	LIS2	1982-1994	2009-2017	0.420	0.290	0.269	0.046	Increasing
	POR1	1982-1990	2003-2017	0.152	0.038	0.002	0.232	Decreasing
	SIN2	1982-1993	2010-2017	0.072	0.074	0.937	0.068	Decreasing
	VSA2	1982-1996	2010-2017	0.006	0.017	0.703	0.413	Decreasing
	VSA3	1982-1989	1989-2017	0.022	0.001	0.034	0.059	Decreasing

Table S6 - Results for *Lepidotrigla* spp.: BPA + TA analysis

<i>Lepidotrigla</i> spp.	Spatial Unit	Ref. Per	Assess. Per	Ref	Assess	p-value	>2013 slope	Trend
Portuguese continental EEZ	Global	1982-1986	2011-2017	0.239	1.568	0.000	0.388	Decreasing
Zone	SW	1982-2012	2012-2017	0.165	0.783	0.016	0.009	Increasing
	S	1982-1986	2011-2017	0.667	2.510	0.000	0.014	Decreasing
Depth strata	1 - Coastal	1982-1986	2011- 2017	0.770	3.554	0.000	0.000	Decreasing
	2 - Medium	1982-1986	2010 - 2017	0.080	0.182	0.026	0.000	Increasing
	3 - Deep	1982-1986	1986 - 2017	0.022	0.028	0.320	0.034	Increasing
Zone* Depth strata	SW1	1982-2011	2011 - 2017	0.169	1.754	0.000	0.079	Increasing
	SW2	1982-1986	2012- 2017	0.102	0.214	0.132	0.315	Increasing
	SW3	1982-1986	2010 - 2017	0.071	0.032	0.264	0.101	Increasing
	S1	1982-1986	2011 - 2017	1.734	4.714	0.008	0.026	Decreasing
	S2	1982-2003	2010 - 2017	0.141	0.214	0.131	0.193	Increasing
	S3	-	-	-	-	-	-	-
Sector	ARR	1982-2012	2012 - 2017	0.062	0.180	0.194	0.045	Increasing
	MIL	1982-1989	2012 - 2017	0.051	2.306	0.005	0.019	Increasing
	POR	1982-1987	1987 - 2017	0.022	0.265	0.000	0.020	Decreasing
	SAG	1982-1990	2004 -2017	3.015	3.145	0.868	0.012	Decreasing
	SIN	1982-1996	2012 - 2017	0.170	0.255	0.099	0.089	Increasing
	VSA	1982-1986	2011 - 2007	0.145	5.674	0.000	0.045	Decreasing
Sector * Depth strata	ARR2	1982-1985	1989 - 2017	0.050	0.035	0.040	0.169	Decreasing
	SAG2	1982-1987	2008 - 2012	0.660	1.227	0.176	0.029	Decreasing
	MIL2	1982-1989	2001 - 2017	0.114	0.031	0.252	-	Stable
	POR1	1982-1991	2000 - 2017	0.392	0.349	0.815	0.539	Decreasing
	POR2	1982-1985	2004-2017	0.000	0.010	0.069	0.055	Increasing
	SIN2	No BPs					0.145	Increasing
	SIN1	1982-2011	2011 - 2017	0.002	0.042	0.490	0.215	Increasing

## Annex 3

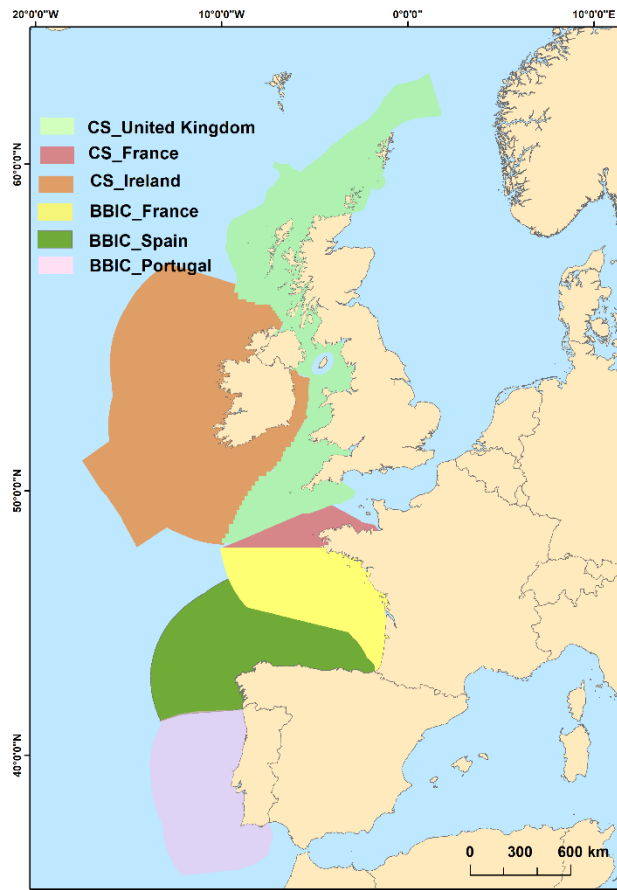
### Supplementary Material of the manuscript “Identifying assessment scales for food webs criteria in the NE Atlantic: implications for the MSFD”

**Table S1** – Groundfish surveys, subregion in which they operate, and period over which they were undertaken (Source: Greenstreet and Moriarty, 2017; OSPAR, 2017).

Marine Subregion	Survey Acronym <sup>1</sup>	Survey period	Collection methods
	BBIC(n)SpaOT4	1990–2017	Data collected by Instituto Español de Oceanografía (IEO) during the Spanish Survey (SP-IBTS)
<b>Bay of Biscay and Iberian Coast (BBIC)</b>	BBICPorOT4	2002–2014	Data collected by Instituto Português do Mar e da Atmosfera (IPMA) during the Portuguese Survey (PT-IBTS)
	CSBBFraOT42	1997–2017	Data collected by Institut français de recherche pour l'exploitation de la mer (Ifremer) during the French Celtic Sea/Bay of Biscay Groundfish Survey (EVHOE)
	CSIreOT4	2003–2015	Data collected by the Marine Institute (MI) during the Irish Atlantic/Celtic Sea Groundfish Survey (IE-IGFS)
	CSNIrOT4	1992–2015	Data collected by Agri-Food and Biosciences Institute (AFBI) during the Northern Irish Sea Groundfish Survey (NIGFS)
<b>Celtic Seas (CS)</b>	CSScoOT4	1995–2015	Data collected by Marine Scotland Science (MSS) during the Scottish West Coast Groundfish Survey (SWC-IBTS)
	CSBBFraOT42	1997–2017	Data collected by Institut français de recherche pour l'exploitation de la mer (Ifremer) during the French Celtic Sea/Bay of Biscay Groundfish Survey (EVHOE)

<sup>1</sup> Survey acronym convention: first two to four capitalised letters indicate the European Union Marine Strategy Framework Directive (MSFD) sub-region (BBIC: Bay of Biscay and Iberian Coast and CS: Celtic Seas). Next capitalised and lower-case letters signify the country/region involved (Spa: Spain; Por: Portugal; Fra: France, Ire: Republic of Ireland, Sco: Scotland, NI: Northern Ireland). Next two capitalised letters indicate the type of survey (OT: otter trawl). Final number indicates the season in which the survey is primarily undertaken (1: January to March; 2: April to June; 3: July to September; 4: October to December). <sup>2</sup>This is a single survey that operates across both the Celtic Seas and the Bay of Biscay and Iberian Coast sub-regions, from the southern coast of the Republic of Ireland and down the western Atlantic coast of France. For assessment purposes this single survey was split into its two sub-regional components.





**Figure S1** - Study area showing the delimitation of each Marine subunit (MSU) (EIONET Central Data Repository, 2018).



**Figure S2** - Study area showing the delimitation of each sector (grey lines) and depth strata (colour) tested (ICES, 2017).

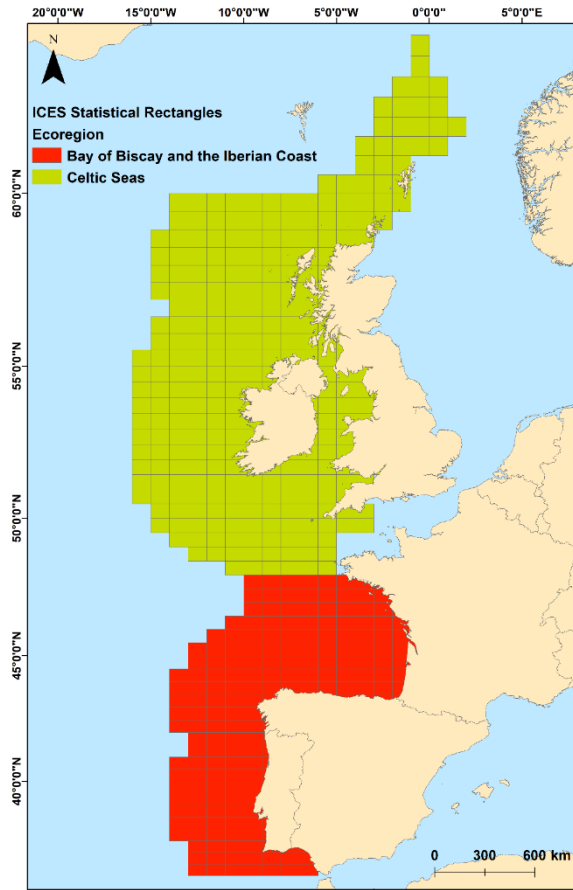
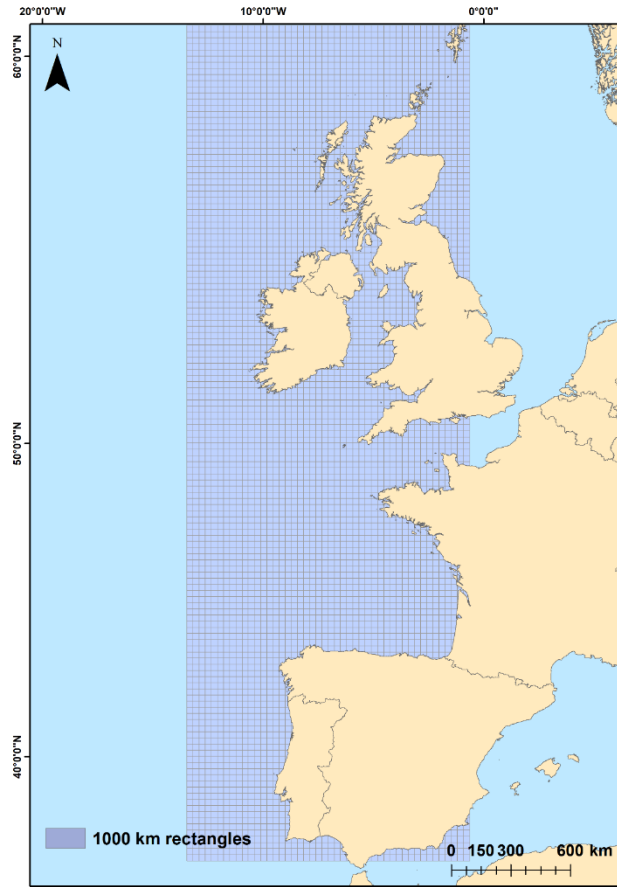


Figure S3 - Study area showing the delimitation of each ICES rectangle in the CS and BBIC subregions (ICES, 2017).



**Figure S4** - Study area showing the delimitation of each 1000 km<sup>2</sup> rectangle tested.

**Table S2** - Criteria assessment for Descriptor 4 (food webs) in the 2nd MSFD implementation cycle of the Portuguese continental waters (adapted from Ministério do Mar, 2020); Green – assessment status in GES.

Criteria	Area	MK Test (p-value)	Statistical Trend	GES
<b>MTL D4C2</b>	A	0.161	Stable	Green
	B	0.649	Stable	
	C	0.277	Stable	
<b>MTI<sub>TL&gt;3.25</sub> D4C2</b>	A	0.008	Increasing	Green
	B	0.441	Stable	
	C	0.353	Stable	
<b>MTI<sub>TL&gt;4</sub> D4C2</b>	A	0.707	Stable	Green
	B	0.244	Stable	
	C	0.333	Stable	
<b>LFI D4C1 (D4C3)</b>	A	0.002	Increasing	Green
	B	0.567	Stable	
	C	0.707	Stable	

Table S3 – Mann-Kendall test applied to MTL time series (p-value and trend) and GES assessment status per 1000 km<sup>2</sup> and year in the Portuguese continental waters. Green – Spatial units in GES. Red – Spatial unit below GES.

Spatial units	MK Test	Trend	GES
4831	0.368	Positive	Green
4876	-	-	Green
4920	-	-	Green
4921	1.000	Negative	Green
4965	0.386	Negative	Green
4966	0.734	Positive	Green

Table S4 - MTI<sub>TL>3.25</sub> estimates obtained per 1000 km<sup>2</sup> and 5-year scales. t-test results compare the means of the two temporal units and for the Portuguese continental waters. Green – Spatial units in GES. Red – Spatial unit below GES.

Spatial units 1000km <sup>2</sup>	Temporal units		t-test p-value	Status GES
	2002-2008	2009-2014		
4830	4.173	4.187	0.789	Green
4831	4.248	4.309	0.348	
4875	4.134	4.205	0.193	
4876	4.154	4.249	0.382	
4920	3.799	3.710	0.776	












































5010	0.721	Positive	Green	4921	4.004	4.272	0.209	Green
5011	0.711	Positive	Green	4922	3.818	4.121	0.268	Green
5055	-	-	Green	4965	3.925	4.154	0.346	Green
5056	0.754	Positive	Green	4966	3.838	4.009	0.457	Green
5101	-	-	Green	5010	4.129	4.000	0.350	Green
5144	0.348	Negative	Green	5011	3.587	3.815	0.106	Green
5145	-	-	Green	5012	-	-	-	Green
5146	0.807	Positive	Green	5055	4.042	4.118	0.703	Green
5190	0.251	Positive	Green	5056	3.648	4.018	0.012	Green
5191	-	-	Green	5099	4.188	4.147	0.378	Green
5235	0.175	Positive	Green	5100	-	-	-	Green
5280	0.308	Positive	Green	5101	4.022	4.165	0.587	Green
5323	0.902	Positive	Green	5144	4.170	4.215	0.249	Green
5369	0.386	Positive	Green	5145	3.573	4.052	0.532	Green
5414	0.108	Positive	Green	5146	3.842	4.060	0.515	Green
5459	1.000	stable	Green	5189	4.239	4.355	0.426	Green
5504	-	-	Green	5190	4.054	4.290	0.102	Green
5551	0.548	Negative	Green	5191	3.670	4.239	0.004	Green
5552	0.308	Negative	Green	5234	4.331	2.920	0.417	Green
5595	-	-	Green	5235	4.103	4.264	0.127	Green
5641	0.211	Negative	Green	5236	3.763	3.957	0.717	Green
5686	0.764	Negative	Green	5279	-	-	-	Green
5731	0.175	Negative	Green	5280	3.798	3.787	0.976	Green
5777	-	-	Green	5323	3.611	3.818	0.353	Green
5781	-	-	Green	5369	3.517	3.947	0.039	Green
5822	0.592	Positive	Green	5413	-	-	-	Green
5823	0.721	Negative	Green	5414	3.618	4.051	0.036	Green
5824	1.000	Negative	Green	5459	3.840	4.142	0.102	Green
5825	0.035	Negative	Red	5504	3.765	4.163	0.017	Green
5826	0.175	Positive	Green	5505	-	-	-	Green
				5507	-	-	-	Green
				5550	1.336	1.853	0.701	Green
				5551	3.158	3.006	0.742	Green
				5552	3.306	3.195	0.746	Green
				5595	4.134	3.438	0.416	Green
				5596	2.269	3.659	0.024	Green
				5641	3.220	3.173	0.869	Green
				5685	3.188	2.147	0.032	Red
				5686	3.620	3.425	0.464	Green
				5730	1.889	2.305	0.435	Green
				5731	3.394	3.594	0.337	Green
				5732	3.306	3.451	0.483	Green
				5777	-	-	-	Green
				5781	3.591	3.504	0.820	Green
				5821	3.204	3.503	0.408	Green
				5822	3.345	3.727	0.158	Green
				5823	4.057	3.798	0.125	Green
				5824	3.534	3.605	0.749	Green
				5825	3.951	3.827	0.463	Green
				5826	3.943	3.989	0.622	Green

Table S5 - MK test results (p-value and trend) and GES assessment status for  $MTI_{TL>4}$  calculated using Sector/Strata and year as spatial and temporal scales. Green – Spatial units in GES. Red – Spatial unit below GES.

Sector/ Strata	MK Test	Trend	GES
CAM1	0.211	Positive	Green
MAT1	0.902	Positive	Green
AVE1	0.721	Negative	Green
FIG1	0.107	Positive	Green
BER1	0.050	Positive	Green
LIS1	0.260	Negative	Green
SIN1	0.387	Positive	Green
ARR1	-	-	Green
SAG1	-	-	Green
POR1	0.211	Negative	Green
VIG1	-	-	Green

Table S6 - MK test results (p-value and trend) and GES assessment status for LFI calculated using Sector/Strata and year as spatial and temporal scales. Green – Spatial units in GES. Red – Spatial unit below GES.

Sector/ Strata	MK Test	Trend	GES
CAM1	0.032	Positive	Green
MAT1	0.999	Positive	Green
AVE1	0.371	Positive	Green
FIG1	0.592	Positive	Green
BER1	0.107	Positive	Green
LIS1	0.060	Positive	Green
SIN1	0.009	Positive	Green
ARR1	-	-	Green
SAG1	-	-	Green
POR1	0.002	Negative	Red
VIG1	-	-	Green

CAM2	0.537	Positive		CAM2	0.536	Positive	
MAT2	0.592	Negative		MAT2	1	Negative	
AVE2	0.266	Negative		AVE2	0.266	Negative	
FIG2	0.371	Positive		FIG2	1	Negative	
BER2	0.754	Negative		BER2	-	-	
LIS2	0.592	Positive		LIS2	0.371	Negative	
SIN2	0.592	Positive		SIN2	0.032	Positive	
MIL2	0.858	Positive		MIL2	0.371	Negative	
ARR2	0.007	Negative		ARR2	0.127	Negative	
SAG2	1	Positive		SAG2	0.368	Negative	
POR2	0.764	Positive		POR2	0.548	Negative	
VIG2	0.049	Positive		VIG2	0.548	Negative	
CAM3				CAM3	-	-	
MAT3	0.260	Negative		MAT3	0.707	Positive	
AVE3	-	-		AVE3	1	Stable	
FIG3	0.452	Negative		FIG3	1	Stable	
BER3	-	-		BER3	-	-	
LIS3	0.221	Negative		LIS3	0.221	Positive	
SIN3	0.371	Negative		SIN3	0.211	Positive	
MIL3	0.592	Negative		MIL3	0.474	Negative	
ARR3	0.707	Negative		ARR3	1	Positive	
SAG3	1	Positive		SAG3	1	Negative	
POR3	0.754	Positive		POR3	0.917	Negative	
VIG3	1	Negative		VIG3	0.049	Negative	

## Annex 4

**Supplementary Material of the manuscript** “Response of food webs indicators to human pressures in the scope of the Marine Strategy Framework Directive for the NE Atlantic”

**Supplementary Table 1.** Food-web criteria reported per subregion, EU Member State, ecosystem element and for each spatial unit.

Subregion	Member State	Ecosystem target/ element	Criteria type	n
GNS	FR	Fish	Biomass	4
		Fish	Large Fish Indicator (LFI)	2
	UK	Fish	Mean Maximum Length (MML)	5
		Fish	Typical Length (TyL)	4
		Marine bird	Relative abundance	2
		Marine bird	Seabird Breeding Success (SBS)	6
		Marine mammals	Abundance	11
		Marine mammals	Grey Seal Pup Production (GSPP)	5
		Plankton	Relative Abundance of Pairs (RAP)	36
		Fish	LFI	5
CS	UK	Fish	MML	13
		Fish	TyL	13
	Marine bird	Relative abundance	2	
	Marine bird	SBS	2	
	Marine mammals	Abundance	6	
	Marine mammals	GSPP	3	
	Plankton	RAP	44	
	Fish	TyL	2	
	IR	Plankton	RAP	20
	FR	FR	Fish	Biomass
Fish			Biomass	3
Fish			Biomass	1
Fish			Fishing in-Balance (FIB)	1
Fish			Fullness index	1
Fish			Longitude extension	2
Fish			Mean Trophic Level (MTL)	5
Fish			MTL_3.25	2
Fish			MTL_3.25_landings	2
Fish			MTL_4	2
SP		Fish	MTL_4.0_landings	2
		Fish	MTL_landings	2
		Fish	Primary Production Required (PPR)	1
		Fish	Shannon diversity (kg)	1
		Fish	Shannon diversity (n)	1
		Fish	Species richness	1
		Fish	Trophic diversity (vol)	1
		Fish	Trophic richness	1
		Fish	LFI	3
		Fish	MTL	3
PT	Fish	MTL_3.25	3	
	Fish	MTL_3.25_landings	3	
	Fish	MTL_4	3	
	Fish	MTL_4.0_landings	3	
	Fish	MTL_landings	3	
	Fish	Trophic Spectra	6	
	Fish	LFI	1	
	Fish	MTL	2	
	MAC	Fish	MTL_3.25	1
		Fish	MTL_3.25_landings	1
Fish		MTL_4	1	
Fish		MTL_4.0_landings	1	

Fish	MTL_landings	2
Fish	MTL_landings_EwE	1
Fish	Trophic Spectra	1

**Supplementary Table 2.** Three criteria that most contributed to the between-clusters dissimilarity.

Groups	Av. Dissimilarity (%)	Species		
f vs b	100.00	MML	MTL_3.25	MTL
f vs d	99,05	MTL_3.25	LFI	MML
b vs d	47,55	LFI	TyL	MML
d vs c	45,86	LFI	MML	MML
d & a	100	LFI	MML	TyL
d & e	97.39	LFI	MML	TyL
f & g	39.31	MTL_3.25	LFI	MTL
b & g	100.00	MML	MTL_4.0_landings	MTL_landings
c & g	100.00	TyL	MTL_4.0_landings	MTL_landings
a & g	100.00	MTL_4.0_landings	MTL_landings	LFI
d & g	83,70	MTL_4.0_landings	MTL_landings	MTL_3.25
e & g	76,53	MTL_4.0_landings	MTL_landings	LFI