



Universidade de Aveiro
2022

**João Fernando Reis
Ramos Rocha e Silva**

**Meta-análise da ecologia trófica da
megafauna marinha**

**Meta-analysis of marine megafauna trophic
ecology**



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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha Aplicada, realizada sob a orientação científica da Doutora Mónica Cordeiro de Almeida Silva, investigadora do Departamento de Oceanografia e Pescas da Universidade dos Açores e coorientação da Professora Doutora Etelvina Figueira, Professora Auxiliar com Agregação do Departamento de Biologia e CESAM da Universidade de Aveiro.

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agradecimentos

Começo por agradecer à Dra. Mónica Silva por nunca ter desistido de mim na orientação que me deu na elaboração desta dissertação. Por estar sempre disposta a esclarecer qualquer dúvida que tivesse, com uma paciência e compreensão inexaurível comigo. Agradeço profundamente todo o conhecimento e experiência de vida que me transmitiu.

Obrigado também à Prof^a Etelvina Figueira, que, não obstante a distância geográfica, nunca deixou de me dar o seu apoio na coorientação deste trabalho. As suas palavras, a sua ponderação e a sua sabedoria foram sempre motivo de grande aprendizagem e crescimento.

Um especial bem-haja ao Dr. Sergi Pérez-Jorge, investigador do IMAR (Instituto do Mar) – Universidade dos Açores, que não coube na elencagem dos orientadores apenas por questões formais. Ainda assim, representou uma enorme ajuda na coorientação do estágio e da elaboração da tese. Sem ele, teria sido tudo mais difícil e moroso. Soube incentivar-me a avançar e sempre que eu tinha dúvidas ele estava disponível. As suas palavras ficarão também como base de aprendizagem e serão lembradas para a vida.

Quero agradecer também à minha família por me ter apoiado constantemente em todas as horas, por puxarem por mim para que não desistisse de avançar com um sorriso no rosto. Dedico este trabalho aos meus avós, tios, primos, mãe, pai e irmão, porque todos eles dão-me a vontade necessária para continuar a esforçar-me e fazer garantir que aquilo que desejo esteja ao meu alcance.

Por fim, não posso deixar de agradecer a todos os colegas e amigos que ganhei no Departamento de Oceanografia e Pescas, que, apesar de não terem estado envolvidos em nenhuma parte do estágio ou tese, fizeram-me sentir como se estivesse numa nova casa. Guardarei recordações de todos os bons momentos passados seja em ocasiões de festa ou convívios simples. Em parte, em termos profissionais e pessoais, sou uma melhor pessoa graças a eles.

palavras-chave

Megafauna marinha, Meta-análise, conteúdos estomacais, modelos ecossistémicos, dietas, biomassa em peso, Açores.

resumo

Este estudo pretende, com base em dados dietéticos, perceber a influência dos predadores de topo da megafauna marinha nas interações tróficas entre predador-presa e predador-predador dos ecossistemas pertencentes à área oceanogeográfica dos Açores e restantes áreas marinhas da Europa. Para isto, foi necessário categorizar as espécies em diferentes grupos funcionais de acordo com a respetiva taxonomia e adquirir conhecimento sobre a sua dieta e relações que estabelecem com outros níveis da cadeia trófica. A meta-análise de dados estomacais convertidos em percentagem de peso (%W) foi o mecanismo escolhido para interpretar e representar as interações tróficas de uma forma realista. Em particular, a meta-análise foi aplicada a uma base de dados desenvolvida pelo projeto SUMMER (*Sustainable management of mesopelagic resources*), em curso desde 2019, que compila amostras de 145 fontes diferentes e de conteúdos estomacais de 65 espécies e 10,719 indivíduos (13% de peixes mesopelágicos e 87% de predadores). Por outro lado, foi necessário expandir esta coleção de amostras a partir de uma pesquisa bibliográfica para verificação dos dados compilados e adição de novos relativos ao arquipélago dos Açores e resto do Nordeste Atlântico e Mar Mediterrâneo. Foram feitas estimativas da contribuição das diferentes presas para a dieta de cada espécie e/ou grupos de predadores para permitir comparar a composição da dieta dos diferentes consumidores. Os cálculos foram desempenhados com recurso a uma média ponderada da biomassa em peso dos itens de presa, baseada nas áreas de amostragem dos respetivos estudos e no número de amostras por estudo. Os resultados revelaram uma maior semelhança dietética entre atuns, golfinhos, tubarões de profundidade e baleias teutófagas. Os cefalópodes mesopelágicos predominaram na dieta de metade dos predadores (em 17 das 34 espécies analisadas) e tiveram uma maior contribuição para as dissemelhanças entre dietas dos diferentes grupos de predadores (nomeadamente, entre atuns e baleias teutófagas, correspondente a $\pm 24,4\%$ e entre baleias teutófagas e tubarões de profundidade, correspondente a $\pm 23,4\%$). Desta forma, os cefalópodes, especialmente que habitam na zona mesopelágica, desempenham um papel crucial nas interações entre a megafauna marinha e os restantes níveis tróficos pertencentes aos ecossistemas oceânicos da Europa e contribuem para completar e desenvolver novos modelos ecossistémicos. No entanto, estudos sobre conteúdos estomacais futuros, principalmente direcionados para a região dos Açores, que impliquem meta-análise com observações visuais ainda requerem mais procura literária para garantir a verificação dos dados existentes e a compilação de novos registos que completam os modelos de gestão e preservação dos recursos naturais.

keywords

Marine megafauna, Meta-analysis, stomach contents, ecosystem-based models, diets, biomass by weight, Azores

abstract

Based on dietary data, this study aims, to perceive the influence of the top predators of marine megafauna on the trophic interactions between predator-prey and predator-predator among the ecosystems belonging to the oceanographical area of the Azores and the remaining marine areas of Europe. For this, it was necessary to categorize the species in different functional groups according to their specific taxonomy and to acquire knowledge about their diet and the relationships established with other levels of the food chain. Meta-analysis of stomach data converted into weight percent (%W) was the chosen mechanism to interpret and represent trophic interactions in a realistic way. In particular, the meta-analysis was applied to a database developed by the SUMMER (Sustainable management of mesopelagic resources) project, ongoing since 2019, which compiles samples from 145 different sources and stomach contents of 65 species and 10,719 individuals (13% of mesopelagic fish and 87% of predators). On the other hand, it was necessary to expand this collection of samples with bibliographic research to verify the compiled data and add new records related to the Azores archipelago and the rest of the Northeast Atlantic and the Mediterranean Sea. Estimates of the contribution of different prey to the diet of each species and/or groups of predators were made to allow comparing the diet composition of different consumers. The calculations were performed using a weighted average of the prey bulk data and based on the sampling areas of the respective studies. The results revealed a greater dietary similarity between teutophagous, tunas, dolphins and deep-sea sharks. Mesopelagic cephalopods predominated in the diet of half of the predators (in 17 of the 34 species analyzed) and had a greater contribution to the dissimilarities between the diets of different groups of predators (namely, between toothed whales teutophagous and tunas, corresponding to $\pm 24.4\%$ and between whales teutophagous and deep-sea sharks, corresponding to $\pm 23.4\%$). In this way, cephalopods, especially those that inhabit the mesopelagic zone, play a crucial role in the interactions between marine megafauna and the remaining trophic levels belonging to the oceanic ecosystems of Europe and contribute to complete and develop new ecosystem models. However, studies on future stomach contents, mainly directed to the Azores region, which imply meta-analysis with visual observations still require more literary search to ensure the verification of existing data and the compilation of new records that complete the management and preservation models of natural resources.

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1. Introduction

The diversity and increasing global pressures upon marine resources are threatening the natural ecosystem sustainability. Climate change, pollution, biological invasions, overfishing and habitat destruction are examples of the most frequently reported anthropogenic stressors in recent decades (Corrales *et al.*, 2018; Duarte *et al.*, 2020; FAO, 2020; Verma, 2021). These changes have many consequences that can disturb both predator-prey (feeding behaviour) and predator-predator (competition for food) interactions that are naturally present in the ecosystems. The threats include an increase in disease transmission, species extinction, and organism physiology and phenology anomalies, which contribute to the decrease in biological diversity and biomass and can affect the structure and functioning of entire food chains (Sala, 2006). Therefore, it is fundamental to have a good understanding of the current predator-prey interactions that compose the marine food webs to know more about the structure of trophic chains (Moon *et al.*, 2010; Tamaddoni-Nezhad, A. *et al.*, 2013).

Mesopelagic fish are crucial for assuring the energy transfer along the ocean food chains, since they constitute the group of marine vertebrates with the highest biomass in the world, estimated on approximately 10 billion tons (Irigoien *et al.*, 2014) and making up 90% of the global biomass of the ichthyofauna (Mann, 1984; Roberts *et al.*, 2020). Mesopelagic communities play a critical role in pelagic food webs, linking primary consumers (e.g., zooplankton) and higher trophic levels, as they are preyed on by a massive quantity of marine predators. Zooplankton, amongst various other larger mesopelagic invertebrates, such as squids, are capable of making diel vertical migrations in big masses to feed in shallow waters during the night and returning to the deep depths during the day (Clavel-Henry *et al.*, 2020; Mann, 1984; Roberts *et al.*, 2020). These aggregations of consumers constitute the main source of prey to key exploited fish stocks (e.g. tuna, swordfish) and charismatic taxa (e.g. cetaceans, pelagic sharks) which makes them decisive contributors for energy transference along the food chains. These marine top predators rely on the diel vertical migration of mesopelagic prey to feed, making deep dives that require large amounts of metabolic energy (Giménez *et al.*, 2018; Olafsdottir *et al.*, 2016). Understanding how prey characteristics drive predators' foraging

strategies and shape energy fluxes is one of the current challenges to improve our knowledge of ecosystem functioning and further ecosystem-based management approaches (Southall *et al.*, 2019). Additionally, mesopelagics also play a major role in carbon transport and sequestration, contributing to the creation and maintenance of the biological pump and providing an important ecosystem service for climate balance (Davison *et al.*, 2013; Proud *et al.*, 2017; Roberts *et al.*, 2020; St. John *et al.*, 2016). Mesopelagic organisms are still a very underexplored component in the ocean, but, because of their massive biomass, encompass an increasing fishing resource of interest over the last decade (Roberts *et al.*, 2020; St. John *et al.*, 2016). It could increase the availability of food for human consumption as the global demographic population keeps rising. However, the harvest of these marine communities, despite bringing many potential benefits, needs to be correctly balanced and managed, so that a sudden increase beyond its pre-established limits doesn't threaten the survival of the food chain basis and, consequently, the collapse of the entire marine ecological network (St. John *et al.*, 2016). Their value for the planet to function, climate stability and protection of ecosystem services is greater than their potential for food sustaining (Roberts *et al.*, 2020).

Moving up the trophic chain, the marine megafauna composes a very fragile group of predators in the ecosystems. Climate change (e.g., increase in temperature and salinity) and anthropogenic direct (e.g., overfishing) and indirect factors (e.g., pollution) are major threats to the abundance of predators unveiling consequences for the populations of lower trophic level (Geers *et al.*, 2016; Heymans *et al.*, 2014; Hsieh *et al.*, 2006; Jackson *et al.*, 2001; Kaschner *et al.*, 2006; Rijnsdorp *et al.*, 2009; Stevenson & Scott, 1999). As a side consequence, there is a permanent reduction in prey biomass that causes shifts in the food chains (Geers *et al.*, 2016; Heymans *et al.*, 2014). The decrease of food resources reduces consumer populations, especially the most vulnerable that belong to the marine megafauna, which encompasses the highest trophic levels (Geers *et al.*, 2016). Therefore, both the abundance of this group of predators and their biomass consumption are important bioindicators of existing shifts in the environment and trophic chains, hence the relevance to study their diet composition (Frank *et al.*, 2005; Johnson, 2011; Puerta *et al.*, 2020; Scopel *et al.*, 2018; Shackell *et al.*, 2012; Weise &

Harvey, 2008). Small pelagics and mesopelagic fish constitute the main consumed groups, but their availability represents a challenge for the top predators that are forced to look up for alternatives and compete for scarce food (Hsieh *et al.*, 2006). These demands enhance the efforts and raise faster losses of energy by high-trophic-level species (Hsieh *et al.*, 2006).

There is still very poor knowledge on the trophic links in pelagic food webs, especially of those involving mesopelagic organisms. This is why it is fundamental to create and develop models that aim to fill the gaps on understanding the ecosystem functioning. Primarily, scientists need to comprehend trophic interactions, based on observations of diets of consumers, to take conservation actions and make consensual management decisions. Several techniques exist to study feeding habits: visual identification of stomach, gut and faecal contents provide quantitative and/or qualitative diet information (Neves *et al.*, 2012; Nielsen *et al.*, 2018; Ramos *et al.*, 2015); however, models that contain quantitative data are commonly build using molecular sequencing of stomach, gut or faecal contents. This is an emerging method performed by manipulation and observation of DNA sequences to dismitify prey ingested by consumers and the composition of food webs (Nielsen *et al.*, 2018; Traugott *et al.*, 2013); bulk stable isotope analyses are conducted by estimation of the ratio of stable isotopes of carbon (^{13}C) and sulphur (^{34}S) to validate primary producers and the ratio of stable isotopes of nitrogen (^{15}N) to verify the trophic level position of the consumer and quantify its diet (Boecklen *et al.*, 2011; Nielsen *et al.*, 2018; Ramos *et al.*, 2015; Traugott *et al.*, 2013). Stable isotopes have the advantage to enable the aggregation of diet data acquired over long time intervals, but are restricted to one diet content tracing or two in case of using nitrogen (^{15}N) and carbon (^{13}C) stable isotopes (Nielsen *et al.*, 2018); Compound-specific stable isotope analysis is a method that focuses on measuring isotope compounds present in individual biomolecules (e.g. amino acids; fatty acids) allowing a more specific look to a wide number of diet contents, but it's an approach that always require more partitioning efforts than tracers to identify the species' taxonomy (McMahon *et al.*, 2016; Nielsen *et al.*, 2018); the fatty acid composition (from tissue samplings and dedicated to quantify energy during dietary catabolism) and fatty acid profile present inside the stomachs work as a common diet tracers for identification of distinct prey

items (Nielsen *et al.*, 2018; Traugott *et al.*, 2013). Nevertheless, it is hard to identify the whole spectrum of the diets because commonly half of the fatty acids are present in lower amounts (Brett, Eisenlord, & Galloway, 2016; Nielsen *et al.*, 2018). Trace metal accumulation (e.g. concentration estimates of Hg, Se, As, Cu, Mn, Cr and Zn) approach comes as an alternative or a complement for methods with slower paced processings and can be used for long period studies, but it is a more appropriate technique to apply in situations in which the organisms are evolutionarily close, otherwise trace metal accumulations may differ among the studied species (Soto *et al.*, 2016). Meta-analysis is a mechanism commonly used to quantify trophic relationships by combining data from multiple studies. Nowadays, it is an analysis widely used by the methods previously described that, by quantification of diet contents, purports to understand more about ecological interactions between different trophic levels of the food chain and comprehend the dynamics and functioning of marine ecosystems (Cardinale *et al.*, 2012; Nielsen *et al.*, 2018).

Stomach content analysis is a classic methodology that aims to quantify the bulk contribution of distinct prey to predators' diets (Neves *et al.*, 2012). This approach is made either by molecular or visual observations and both provide a high taxonomic resolution, meaning that prey items can be discriminated into species or genera (Nielsen *et al.*, 2018). Contributions to diet can be quantified by their relative abundance by number, or dry or wet weight, volume or by their frequency of occurrence in the stomachs of several predators of the same species (Baker, Buckland & Sheaves, 2014; Livingston *et al.*, 2017). Individual metrics can also be included into composite indices, when samples are large (Nielsen *et al.*, 2018). It is noteworthy that visual methods are not the best option when analysing partially digested material because it is more difficult to identify the diet items, leaving them as unidentified or only known to a certain taxonomical group. Furthermore, processing and identifying stomach contents is extremely time-consuming and challenging, requiring strong expertise in taxonomic identification of a wide range of organisms. Molecular techniques, on the other hand, have been frequently used because of their capacity to accurately identify the taxonomy of the prey even if it is rarely present or softly or highly degraded (Nielsen *et al.*, 2018). Nevertheless, despite

some limitations and challenges, visual methods provide the only way to provide robust quantitative information on diet composition with high taxonomic resolution (Ahlbeck, Hansson & Hjerne, 2012; Amundsen & Sánchez-Hernández, 2019; Bowen & Iverson, 2012). Additionally, the use of this technique for decades produced abundant information on stomach content records for numerous marine *taxa* (Ahlbeck, Hansson & Hjerne, 2012; Amundsen & Sánchez-Hernández, 2019; Baker, Buckland & Sheaves, 2014; Bowen & Iverson, 2012; Gül & Demirel, 2022; Hohmann & Huckschlag, 2005; Livingston *et al.*, 2017; Lydersen, Weslawski & Oritsland, 1991; Neves *et al.*, 2012; Nielsen *et al.*, 2018).

SUMMER (Sustainable Management of Mesopelagic Resources) is an ongoing project that focuses on estimating mesopelagic fish biomass, quantifying the ecosystem services provided by the mesopelagic community (food, climate regulation or bioactive compounds), and creating a decision support tool for the potential exploitation of mesopelagic resources without threatening the services provided by this community (SUMMER, 2020). One of the components of the SUMMER project is to investigate the consequences of mesopelagic exploitation on the structure, function and stability of pelagic food webs, including the energy transfer across trophic levels and functional groups. To reach this goal, SUMMER compiled a large database to identify and quantify trophic links between mesopelagic organisms, their prey and predators (Silva *et al.*, 2021). The database includes information assembled from 191 published and non-published sources on stomach contents, stable isotopes, estimates of diet composition, trophic level, energy densities, major and trace elements and fatty acid trophic markers (FATM). In terms of stomach content data, 65 species and 10,719 individuals were accounted for: 13% being mesopelagic fish (mostly myctophids) and 87% being mesopelagic predators. These data were obtained from sampling sites all over the Mediterranean (the majority being from the western side), northeastern and central Atlantic Ocean.

Meta-analysis of stomach content data from the SUMMER database could substantially increase sample size and enable diet assessment for a broader range of predator species. Additionally, it can help reduce errors and uncertainty in diet estimation from single studies, associated with the short temporal and spatial sampling, and problems and errors in prey

identification (Crespin de Billy, Dolegec, & Chessel, 2000; Sagarese *et al.*, 2016). Combining data from multiple studies can provide a more complete repertory of the feeding habits of a predators (Sagarese *et al.*, 2016). This can be done by averaging the relative weight composition of each prey across different studies (Chagaris *et al.*, 2015; Geers *et al.*, 2016; Livingston *et al.*, 2017). However, simple averages can lead to overestimation of rarely consumed prey in the diet of a predator, thereby misrepresenting its diet composition (Masi *et al.*, 2014; Sagarese *et al.*, 2016). Moreover, simple averages cannot take into account other factors (e.g., sampling duration, location, methodology) that might influence or be of interest for diet estimation. Weighted averages enable accounting for differences in methodology and sample size by applying weights to individual observations based on these factors, thus providing more accurate estimates of diet composition (Chagaris *et al.*, 2015; Geers *et al.*, 2016).

1.1. Objectives

The main goals of this thesis were to 1) produce a more accurate representation of the trophic links of the marine megafauna of the Azores, by estimating the diet composition of each predator based on a meta-analysis of data assembled from the Azores, entire North Atlantic and Mediterranean regions, using weighted averages of the relative weight of prey items reported across different data sources; and 2) produce a more realistic representation of the structure of the Azores food web, by assessing diet similarity and differences between and within marine megafauna trophic groups.

1.2. Specific objectives

The specific objectives of this study were to: 1) complement the existing database of stomach contents of the SUMMER project, by conducting a literature search to assemble more diet data of megafauna species of interest, with special emphasis on the ecosystem of the Azores and surrounding regions; 2) classify the marine megafauna species into functional groups, based on their taxonomic classification and ecology (e.g., diet, habitat, vertical distribution); 3) compare the diet composition within and between marine megafauna

functional groups by the observation of several graphs and other types of software plot representations using the original and pre-transformed diet data; and 4) finally contribute to the knowledge of trophic interactions in the Azores ecosystem.

2. Materials and Methods

2.1. Area of focus

The SUMMER database provides a valuable tool to investigate trophic links in pelagic food webs, and more specifically, the role of mesopelagic organisms for megafauna predators, for regions where in-situ information is scarce. One of such regions is the Azores archipelago. The Azores is the most remote oceanic archipelago in the north Atlantic, distancing about 1,400 km from continental Europe and 2,000 km from north America. The nine islands of the archipelago and the numerous seamounts surrounding it sits right on the mid-Atlantic ridge at a triple (tectonic plate) junction. The Azores region is characterized by various ocean currents that form a unique and complex current system: in the north, the cold North Atlantic Current and, in the south, the warm Azores Current, which forms the southeastern branch of the Gulf stream (Caldeira & Reis, 2017; Santos *et al.*, 1995). The interaction between the complex topography and physical oceanography is believed to form favourable foraging conditions to attract megafauna predators. Indeed, the oceanic area of the Azores is marked by a high diversity and abundance of megafauna species, such as cetaceans, large pelagic fish, sharks, turtles, and seabirds (Afonso *et al.*, 2020). Many of these species are thought to rely on mesopelagic food resources (Neves V. *et al.*, 2012; Neves V.C. *et al.*, 2012; Paiva *et al.*, 2018), but studies on their feeding habits are scarce, and the importance of mesopelagic organisms to this group cannot be accurately determined.

2.2. Meta-analysis of diet composition

a) Data sources and collection

In a first phase, a literature search was conducted to list the most frequent and abundant megafauna predators present in the Azores ecosystem. Then, an effort was made to compile all quantitative diet data (where prey importance was expressed as weight) from all these predator species. The data search focused on studies from the Azores and surrounding Central Atlantic oceanic regions but extended to the central and Northeast Atlantic and the Mediterranean Sea. The research was done online, resorting to two scout platforms: Google search engine, Google Scholar. A wide variety of references was searched, including biological field reports, academic theses, and peer-reviewed articles.

The trophic database created by the project SUMMER (<https://summerh2020.eu/>) was used to extract the diet information from the megafauna that was listed during the previous search (Silva *et al.*, 2021). Afterwards, an exploratory analysis was done to verify if the existing data could be applied for analysis and preview if they were able to furnish results. During this process, it was found several gaps regarding the representation of predator and prey species along with geographic areas and fundamental variables to measure throughout the methods' performance. Most records were of deepwater or pelagic sharks of the class Elasmobranchii (*Galeus melastomus*, *Etmopterus pusillus*, *Etmopterus princeps* and *Prionace glauca*) and pelagic fishes of the class Actinopteri (mainly, *Thunnus thynnus*). Cetaceans were the only representatives of the class Mammalia and were less than 10% of the data. Reptiles (1% of the data) consisted of a single species, *Caretta caretta*. *Mesoplodon bidens* was the only species included in toothed whale mesopelagic predator accounting for just 3 individuals, which constituted an underrepresentation of this functional group. The other categories didn't show as many gaps in terms of the number of species and/or individuals, except for baleen whales, turtles, and mesopelagic fish, which had also one species each (*Balaenoptera acutorostrata*, *Caretta caretta* and *Makaira nigricans*, respectively), although were not included in the main analyses. Seabirds and reptiles were poorly represented, both in sample size and specific diversity and accounted for 11% of the data records. They were composed by *Bulweria bulwerii*, *Calonectris diomedea*, *Larus argentatus*, *Larus michachellis*, *Puffinus baroli*, *Sterna hirundo*. Only

C. diomedea, which represented 35% of seabirds, was used for analysis since it was the most common species occurring in Azores. When it comes to geographic aspects, the data set showed no samples from the Central Atlantic for toothed whales, teutophagous, turtles, tunas, deep water sharks and dolphins.

The percentage of weight (%W) was the most important variable of interest to express the diet bulk data and 43% of the whole data had no records (including blank or zero values) related to this measurement. Therefore, all these gaps reinforced the need to add more diet evidence related to the archipelago zone, which is the purpose of this investigation. This was made also by progressive web research related to top predators showing scarce information about stomach samplings from the Central Atlantic Ocean. Other European areas such as the coast of Portugal, North and Celtic seas were also relevant to permit assumptions and comparisons to be made between diets based on the region of the predators' occurrence. Furthermore, it was considered also important to filter sources from a recent time period once this could provide insights of new feeding habits and diet shifts. If new records weren't found, old studies were included anyway. Most of the samples collected were of strandings. The most recent source added to the database was from stranding samplings of *Dalatias licha* in the Mediterranean Sea in the year 2018 and the oldest was of *Grampus griseus* stranded in England in 1983. However, former samples of *Hyperoodon ampullatus* were found recorded in 1946 but they were extracted from a larger collection between 1946 to 2009 in the North Sea. The same type of websites as the initial bibliographic scout were used to compile the new information (Google scholar and Google search engine). "Ecosystem-based models"; "high trophic levels"; "diet metanalysis"; "weight percentage; biomass; stomach contents were some of the key words used to gather the studies of interest.

b) Data records

The SUMMER database contained 7,915 records from 10,719 individual stomachs in 107 different studies with 65 species which nearly 70% corresponded to megafauna categories. The

measures of prey items were represented as absolute and relative number of individuals (N and %N); relative and absolute frequency of occurrence (FO and %FO); relative and absolute weight biomass (W and %W). Altogether, 18 sources were related to samplings from the Azores with a total of 2,198 individuals (20% of the total individuals). To perform the analyses, 287 samples of stomachs of 9 top predator species derived from 11 sources were added to the SUMMER pre-established data set. Within the new information, only one case regarded the Azores containing 71 individuals of pelagic sharks. All the other regions (Mediterranean; Celtic Seas & North Atlantic; Canary & Bay of Biscay; Other areas) were important to make comparative assumptions between the diets of predators that occur in the Azorean sampling area and in the rest of Europe. Table 1 divides the number of studies, number of stomachs and years when samplings were handled corresponding to each of the regions included in the examination. All the values registered in the table corresponded to the ones already included in the database plus the others that were new and inserted during the literature search.

REGION	Nº STUDIES	Nº STOMACHS	YEARS
AZORES & CENTRAL ATLANTIC	19	7604	1986-2013
MEDITERRANEAN	22	4760	1986-2018
CELTIC SEAS & NORTH ATLANTIC	18	1199	1885-2011
CANARY ISLANDS, MADEIRA & BAY OF BISCAY	15	942	1980-2016
ALL OTHER REGIONS	8	374	2001-2006
TOTAL	82	14879	

Table 1: Summary of compiled diet data used in this study by regions and the number of studies, number of stomachs and years of sampling that included them.

Figure 1 shows the geographic distribution of the predator samples on a map which are represented by sampling points with specific colours. This representation was created by inserting the coordinates from the database to the platform QGIS Desktop – version 3.24.3.

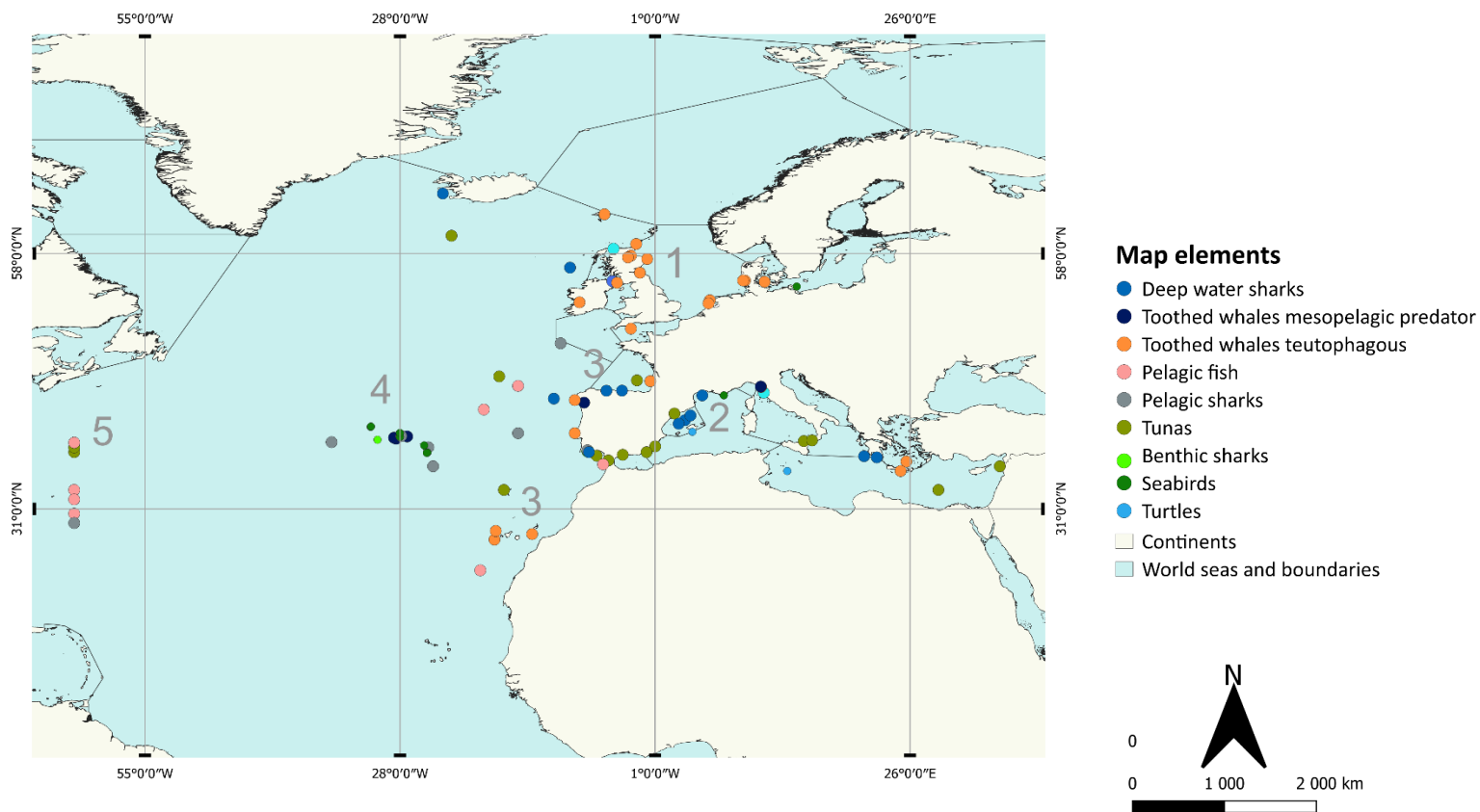


Figure 1: Map showing the sampling locations (coloured dots) of the different functional groups disposed in the legend that were added to SUMMER original matrix. The samples were grouped into regions 1: Celtic Seas & North Atlantic, including the regions of Faroe and Shetland Islands, the western channel of England, Scotland and the North Sea; 2: Mediterranean 3: Canary Islands, Madeira Islands & Bay of Biscay; 4: Azores & Central Atlantic; 5: All other regions (Mid-Atlantic Ocean). Maritime frontiers, which separate the different Atlantic Ocean and the seas, are represented by the black solid lines on the blue plane. The bigger dots represented the functional groups that were more important for the analysis: Deep water sharks; Toothed whale mesopelagic predator; Toothed whales teutophagous; Pelagic fish; Pelagic sharks and Tunas.

c) Diet estimation

One approach to integrate diet data from different studies is to simply average the weight (or %W) of each prey species across all studies (Hyslop, 1980). However, this method can lead to biased estimations of diet composition, especially when diet datasets are small. Simple averages tend to overestimate the contribution of large, but rarely consumed prey and underestimate the contribution of small frequent prey (Masi *et al.* 2014). Furthermore, in this study, given the lack of diet information for the Azores, diet data had to be integrated across distinct geographic regions, which likely differ in food web composition and structure. Therefore, to account for differences in the sample size and study locations between data sources, diet compositions were weighted by these factors, using the following formula, adapted from Sagarese *et al.* (2016):

$$w_{sj} = \frac{Reg_s}{Reg_{max}} + \frac{Loc_s}{Loc_{max}} + \frac{N_s}{N_{tot}}$$

where a weighting factor w for each data source s of each predator species j was calculated using a weighting value for region, by dividing the score of that sampling region (Reg_s) by the highest score assigned to factor region (Reg_{max}), a weighting value for study location (Loc_s/Loc_{max}), and for sample size, by dividing the number of individuals sampled in that data source (N_s) by the total number of individuals sampled for that predator species (N_{tot}). The factor region separated data sources into large marine biogeographic realms, following Costello *et al.* (2017). Regions, therefore, characterized data sources based on their dominant habitats, ecosystems, and biomes, rather than in terms of geographic distance (Costello *et al.* 2017). For region, data sources located in the Offshore & NW Atlantic, where the Azores is situated, received the highest score (Reg= 4), the Offshore S Atlantic (Reg= 3), NE Atlantic (Reg= 2), other regions in the North Atlantic (Reg = 1) (names of biogeographic regions follow Costello *et al.*, 2017) (see Figure 12 in the supplementary material). The factor location classifies the study sites of data sources in terms of their relative proximity to the Azores, where the highest score was assigned to studies conducted in the Azores archipelago and in the oceanic region around the Azores (Loc=3), followed by the area from Bay of Biscay to Canary Islands (Loc=2), Celtic and North Seas (Loc=1), and Loc=0 for all other locations.

Then, the percentage weight of each prey species i in the diet of a predator j ($\%W_{ij}$) was multiplied by the weighting factor of that data source (w_{sj}) (scaled to values from 0 to 1) to calculate the arithmetic weighted mean contribution of that prey ($\%WM_{ij}$):

$$\%WM_{ij} = \%W_{ij} \times \frac{w_{sj}}{\sum w_{sj}}$$

d) Defining predator and prey functional groups

The construction of functional groups for species was fundamental to simplify and synthesize the complexity of trophic interactions in the food chain (Table 2). The definition for each functional group was based on an Ecopath with Ecosim (EwE) ecosystem model developed for the Azores by Morato *et al.*, 2016. The groups were adapted according to the taxonomy of the including species, and previous knowledge about their habitat and feeding ecology. In this work, the group of toothed whales defined by Morato *et al.* (2016) was split into two new subcategories based on the preliminary analysis of the compiled diet information: mesopelagic predators, species that mostly feed on mesopelagic organisms, and teutophagous, which prefer to consume cephalopods. The baleen whales were not considered in this study due to the non-existence of stomach content data in weight related to them. Cases of prey contents contributing with less than 0.1% in weight and corresponding to inorganic/ completely undetected remains were also discarded from the analysis. Both FishBase (fishbase.org) and WORMS (worms.org) were the web platforms to correctly define the taxonomy of the predators and prey and, thus, helped to organize the species into their correct categories.

Table 2: Predator functional groups and respective species used in this study. The classification into functional groups was based on Morato *et al.* (2016).

Toothed whales mesopelagic predators	Toothed whales teutophagous	Dolphins	Deep-water sharks	Pelagic sharks	Benthic sharks	Tunas	Pelagic fish	Seabirds	Turtles
<i>Mesoplodon bidens</i>	<i>Globicephala melas</i>	<i>Delphinus delphis</i>	<i>Centroscymnus coelolepis</i>	<i>Galeorhinus galeus</i>	<i>Scyliorhinus canicula</i>	<i>Auxis rochei</i>	<i>Coryphaena hippurus</i>	<i>Calonectris diomedea</i>	<i>Caretta caretta</i>
<i>Ziphius cavirostris</i>	<i>Grampus griseus</i>	<i>Stenella coeruleoalba</i>	<i>Dalatias licha</i>	<i>Isurus oxyrinchus</i>		<i>Katsuwonus pelamis</i>	<i>Kajikia albida</i>		
<i>Mesoplodon europaeus</i>	<i>Hyperoodon ampullatus</i>	<i>Steno bredanensis</i>	<i>Deania calcea</i>	<i>Prionace glauca</i>		<i>Sarda sarda</i>	<i>Xiphias gladius</i>		
	<i>Kogia breviceps</i>	<i>Tursiops truncatus</i>	<i>Etmopterus princeps</i>			<i>Thunnus alalunga</i>	<i>Tetrapturus pfluegeri</i>		
	<i>Physeter macrocephalus</i>		<i>Etmopterus pusillus</i>			<i>Thunnus albacares</i>	<i>Makaira nigricans</i>		
	<i>Globicephala macrorhynchus</i>		<i>Etmopterus spinax</i>			<i>Thunnus obesus</i>			
			<i>Galeus melastomus</i>			<i>Thunnus thynnus</i>			

Individual prey species present in the stomach contents of predators were grouped into functional groups following the classification by Morato *et al.* (2016) (Table 3). For the analyses, some of the prey groups listed were excluded: anthropogenic debris, other benthos, other molluscs, other remains, seabirds, parasites. This was done because these groups were absent or uncommon in the predators' diet records and thus had a minor contribution to their diet.

Table 3: Functional groups for all identifiable prey species found in the predators' stomach records compiled in this study. The classification into functional groups was based on Morato *et al.* (2016).

Prey functional groups	
Amphipods	Non-mesopelagic decapods
Bathydemersal fish	Marine mammals
Bathypelagic fish	Pelagic fish
Benthic filter feeders	Rays and sharks
Benthic worms	Shallow water fish
Crustaceans	Tunas
Deepwater sharks	Turtles
Demersal fish	Unidentified fish
Mesopelagic cephalopods	Mesopelagic fish
Mesopelagic decapods	Non-mesopelagic cephalopods
Mesopelagic isopoda	Zooplankton

e) Data Analysis

Four types of data analyses were carried out a) Hierarchical cluster analysis; b) nMDS plot analysis; c) ANOSIM analysis and d) SIMPER analysis using PRIMER 6 (Version 6.1.13) + PERMANOVA (Version 1.0.3). Results were represented by graphs based on the original matrix of the database, discarding the values belonging to prey items previously mentioned as non-important for the research (amphipods, anthropogenic debris, other benthos, other molluscs, other remains, benthic filter feeders, seabirds, benthic worms, parasites, deep-water sharks, rays and sharks, turtles, mesopelagic isopoda). Afterwards, a transformation was applied to the new matrix so that the total could be equal to 100%.

I. Hierarchical cluster analysis

All the 11 predator groups were used in this analysis. The purpose of the hierarchical cluster analysis was to understand the similarities between predator species based on their diet by looking at the proximity of clusters to each other. A Bray-Curtis similarity index was used to quantify diet differences between species. This was only taken after applying a square root transformation, which shrinks the data of the original matrix showing lower values and obtaining a better suited for the creation of a Bray-Curtis resemblance mold. The index is calculated as:

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j}$$

Where i & j are the two species, S_i is the total number of prey counted on predator i stomach, S_j is the total number of preys counted on the predator j stomach, C_{ij} is the sum of only the lesser counts for the prey found in the stomachs of both predators.

II. nMDS plot analysis

A non-metric multidimensional scaling analysis (nMDS) was used to visualize diet similarity between predator species. Only predator functional groups with more than one species ($n=7$) were plotted.

III. ANOSIM analysis

A one-way permutation analysis of similarity (ANOSIM) was used to test the null hypothesis of no difference between predator functional groups. It is a classical approach applied to a resemblance matrix calculated among samples, like the Bray-Curtis, using a factor that describes them in a pre-established group structure (in this case, the predator functional groups) (Somerfield *et al.*, 2021). The ANOSIM R statistics compares the mean of ranked dissimilarities between groups (\bar{r}_B) to the mean of ranked dissimilarities within groups (\bar{r}_W):

$$R = \frac{(\bar{r}_B - \bar{r}_W)}{M/2}$$

Where $M = n(n - 1)/2$ and n is the total number of samples considered in the investigation. The R value ranges between 0 and 1, and the higher the R value (approximated to 1), the more dissimilar the diets are among the predator groups (Somerfield *et al.*, 2021).

The ANOSIM was done using the Bray-Curtis similarity coefficient with 9999 permutations (random samples from a large number) to obtain higher robustness. A significance value- $p < 0.05$ was used to reject the null hypothesis.

IV. SIMPER Analysis

A similarity percentage analysis (SIMPER) was conducted to assess the contribution of different prey functional groups to the dissimilarities within diets between predator groups. The analysis was done only for pairs of predators for which the ANOSIM table (Table 4) demonstrated statistically significant differences in diets (for which the value $P < 0.05$).

f) Analysis of the diet composition of predator functional groups

Finally, the diet composition of predator species by functional group was plotted to examine within-group consistency. The contributions of each prey to the diet of a predator were not extracted from the SIMPER analysis but used the weighted mean contribution of all prey to the diet of a given predator species (%WM_{ij}).

3. Results

3.1. Hierarchical cluster analysis

The hierarchical cluster analysis showed considerable diet differences between predator species of the same functional group. There was no functional group for which all species formed a single cluster. Overall, pelagic fish was the functional group with the lowest similarities between species, and they were all included in distinct clusters. The low similarity in diet was also observed within toothed whales mesopelagic predators, with *M. bidens*, *M. europeus* and *Z. cavirostris* fitting in very different clusters. Nevertheless, some species of the same functional group showed strong diet similarities and clustered together. That was the case of *G. griseus* and *G. melas*, and *K. sima*, *P. macrocephalus* and *H. ampullatus*, within the Toothed whales teutophagous group, and *T. thynnus*, *T. alalunga* and *A. rochei* within the Tunas group.

On the other hand, the only cluster with 100% diet similarity between its members was composed of four species from distinct functional groups: *S. frontalis* (Dolphin), *T. pfluegeri* (Pelagic fish), *M. europaeus* (Toothed whale mesopelagic predator) and *G. macrorhynchus* (Toothed whale teutophagous). These species also belong to different taxonomic ranks and differ markedly in ecology.

II. Hierarchical cluster graph

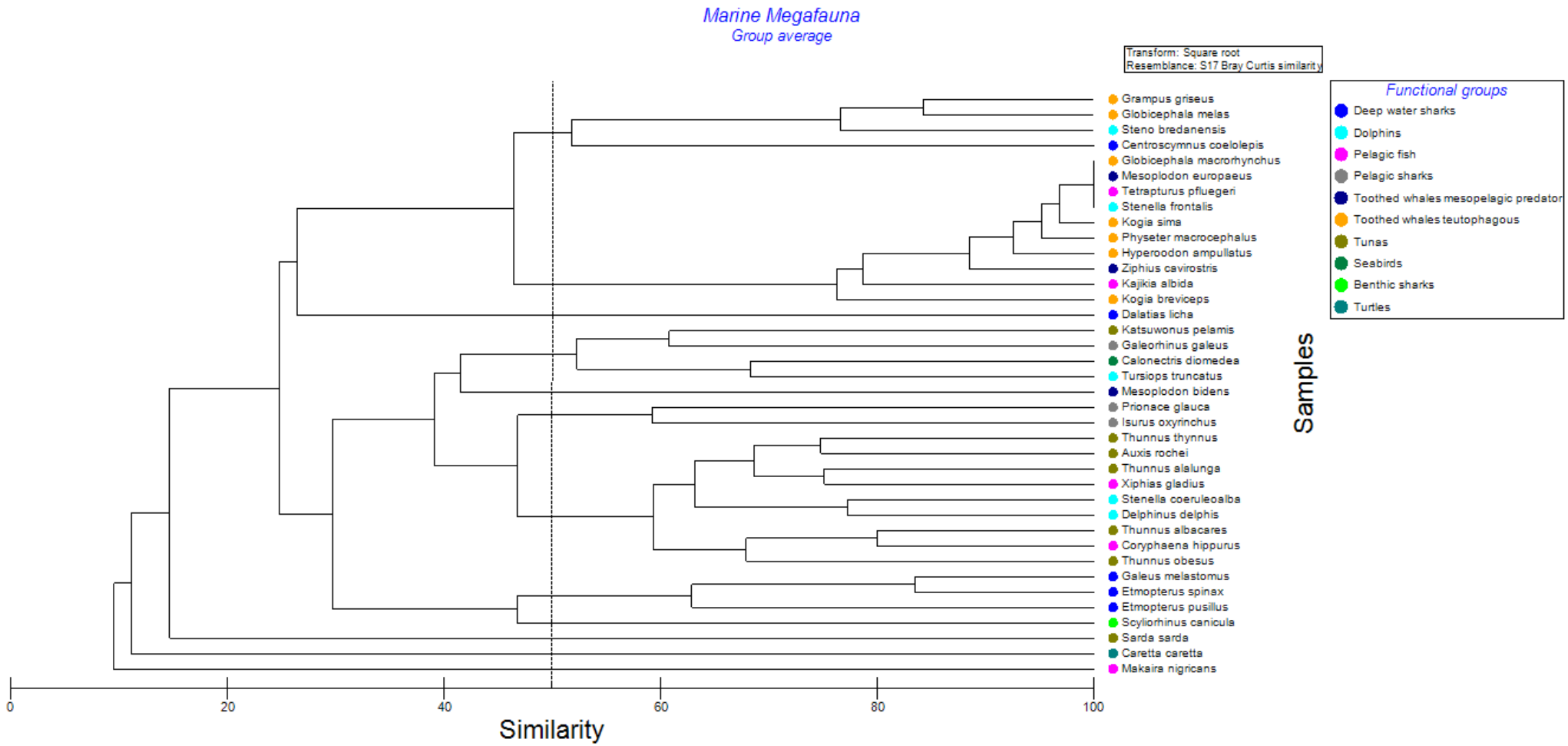


Figure 2: Hierarchical clustering dendrogram showing diet similarity by predator species. Functional groups are shown in different colours. The dendrogram was based on the Bray-Curtis similarity between predator diets calculated from square-root transformed %W of each prey functional group.

3.2. nMDS analysis

The non-metrical multidimensional MDS scaling analysis resulted in an ordination of species in two dimensions (Figure 3). Stress values quantify the existing difference between distance in the small scale dimension and the total space of the multidimensional plot graph. Values <0.05 represent an excellent plot fit and it's the interval in which there's the highest confidence, meaning that graph interpretations can be done with no risk. Figure 3 shows an overall stress value of 0.12, which represents a fair fit, although some scaling distances are misleading.

The nMDS distinguished four types of similarity groups and all of them are involved by the contour dashed line, implicating species sharing at least 20% of their diet preferences. If species are within the dashed blue contour, they share at least 40% of their prey preferences. In the black solid contour, species' diets are at least 60% similar to each other. Finally, species included in solid red contour share at least 80% of their diets. *M. nigricans* showed a residual representation since it had a diet less than 20% similar to the others and was discarded for this analysis.

The graph shows a heterogeneous distribution, i.e. species belonging to distinct functional groups can have a higher diet similarity than species of the same functional group. For example, *G. galeus*, a pelagic shark, had 60% diet similarity with *K. pelamis*, which is a tuna. Toothed whales - teutophagous formed two separate, and relatively distant, groups of species with strong diet similarity (80%). One group comprising four species - *K. sima*, *P. macrocephalus*, *H. ampulatus*, *G. macrocephalus* – formed a very tight cluster, which means that their diets were very similar, and a second group consisting of *G. melas* and *G. griseus*. There was one match of dots, meaning 100% of diet similarity, formed by species belonging to different functional groups: *G. macrocephalus*, a toothed whale teutophagous, *M. europeus*, a toothed whale mesopelagic predator, *T. pfluegeri*, a pelagic fish and *S. canicula*, a pelagic shark.

The pelagic sharks were associated with distinct circles, but *P. glauca* and *I. oxyrinchus* had 40% diet similarity. *S. canicula* and *G. galeus* were not associated in the same group and were isolated from all other predators, indicating they have very distinct diet preferences.

Except for *K. pelamis* and *S. sarda*, tunas showed relatively similar diets, with *A. rochei* showing a similarity of 60% with *T. thynnus* and *T. alalunga*, and *T. albacares* and *T. obesus* also displaying a similarity percent of 60%.

As for deep water sharks, only 3 species shared a similarity percent of 60 % with each other: *E. spinax*, *G. melastomus* and *E. pusillus*. The diets of the first two, *E. spinax* and *G. melastomus*, shared 80% of similarity.

III. nMDS plot graph

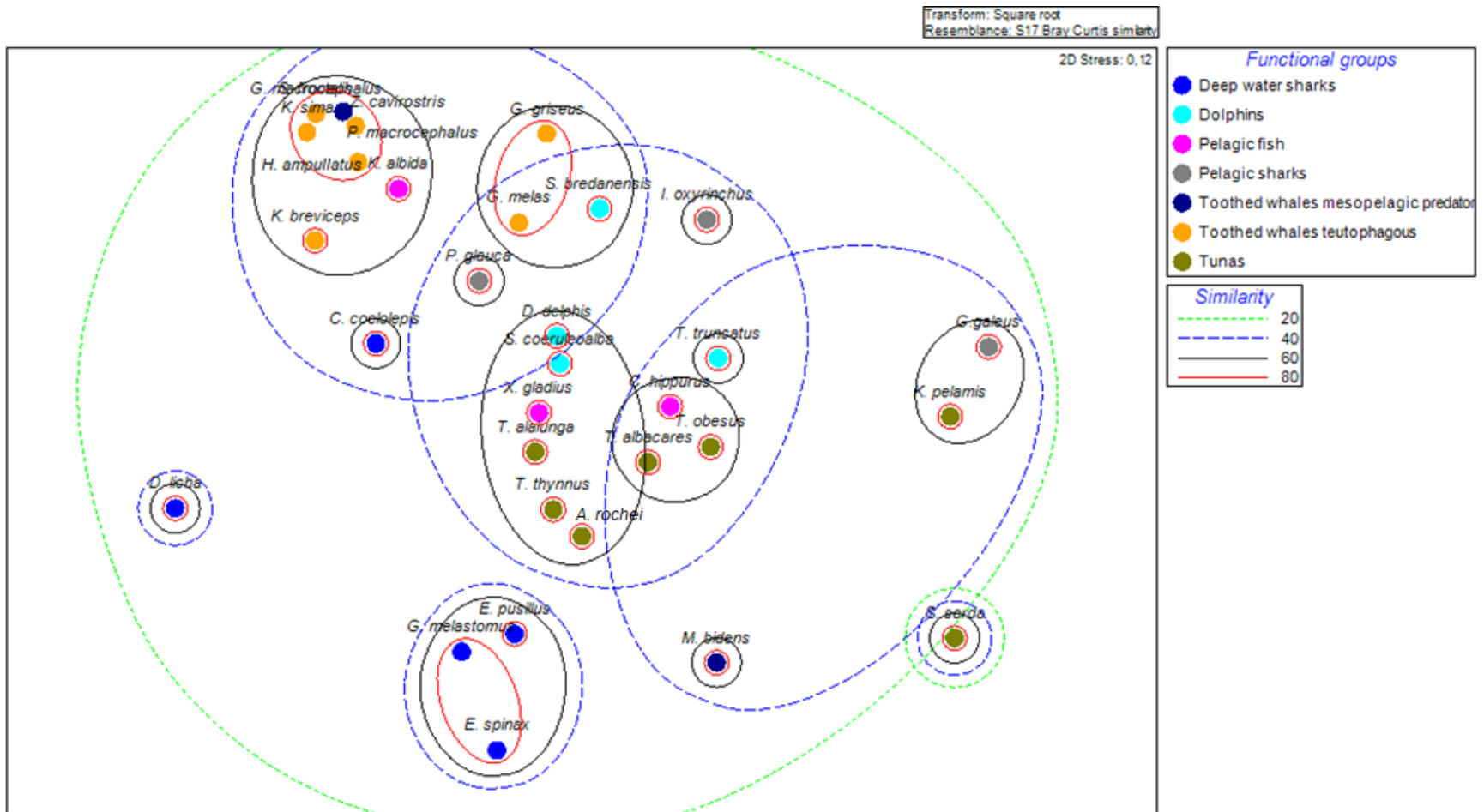


Figure 3: Ordination resulting from the analysis of a non-metric multidimensional scaling (nMDS) of predator species. The circles indicate the groups formed from the pool. Each contour of the circle has a specific colour that represents the percentage of similarity the different species have with each other: green dashed line = 20% of similarity; blue dashed line = 40%; black solid line = 60%; red solid line = 80%. Different predator species are represented by the coloured dots according to the functional groups they belong to.

3.3. One-way ANOSIM analysis

The results of the ANOSIM analysis showed various R statistical values for different pairs of functional groups to demonstrate quantifiably how distinct or similar they were from each other (Table 4). A total of 9 pairs of functional groups presented significant R statistic values ($P < 0.05$): deep water sharks and dolphins; deep water sharks and pelagic sharks; deep water sharks and toothed whales teutophagous; deep water sharks and tunas; toothed whales teutophagous and tunas; pelagic fish and tunas; dolphins and toothed whales teutophagous; pelagic sharks and toothed whales teutophagous and toothed whales – mesopelagic predator and tunas. The quantitative significance confirms the existence of differences between these functional groups causing the null hypothesis (there is no difference between groups) to be rejected.

The less dissimilar pair of functional groups was the toothed whales teutophagous and dolphins ($R = 0.292$). On the other hand, tunas and toothed whales – teutophagous showed the greatest differences in diet ($R = 0.852$). Intermediate values were registered for deep water sharks and toothed whales teutophagous ($R = 0.744$); pelagic sharks and toothed whales teutophagous ($R = 0.69$); deep water sharks and pelagic sharks ($R = 0.641$); toothed whales – mesopelagic predator and tunas ($R = 0.565$); deep water sharks and dolphins ($R = 0.54$); deep water sharks and Tunas ($R = 0.347$); pelagic fish and tunas ($R = 0.298$).

Table 4: Pairwise ANOSIM R statistics values for predator functional groups. Statistically significant values (value $P < 0.05$) are presented in bold.

PREDATOR GROUPS	Deep water sharks	Dolphins	Pelagic sharks	Toothed whales – mesopelagic predator	Toothed whales teutophagous	Pelagic fish	Tunas
DEEP WATER SHARKS	-	0.54	0.641	0.338	0.744	0.22	0.347
DOLPHINS	-	-	0.292	0.179	0.292	-0.022	0.217
PELAGIC SHARKS	-	-	-	0.056	0.69	0.097	0.349
TOOTHED WHALES – MESOPELAGIC PREDATOR	-	-	-	-	0.091	0.005	0.565
TOOTHED WHALES TEUTOPHAGOUS	-	-	-	-	-	0.253	0.852
PELAGIC FISH	-	-	-	-	-	-	0.298

3.4. SIMPER analysis

The SIMPER results (Figure 4) showed that the mesopelagic cephalopods constituted the prey group contributing more to diet dissimilarities between predators, with an average contribution of 20.2% across all predator pairs. On the other hand, the prey group contributing less to dissimilarities was crustaceans, with an average contribution of 2.2%.

The pair for which mesopelagic cephalopods contributed the most was toothed whales teutophagous and tunas, with a value of 24.4%. Secondly, for deep water sharks and tunas, the same prey category accounted for 23.4%, which approaches the value of 23.3% registered for deep water sharks and toothed whales teutophagous. However, there were three exceptional pairs that didn't have mesopelagic cephalopods as the primary contributor to diet dissimilarities. For dolphins and toothed whales teutophagous, non-mesopelagic cephalopods constituted the category contributing the most with a percentage of 25.8%, which is the highest number above all the results in the graph. Also, for deep water sharks and tunas, pelagic fish was the main prey contributor accounting for 19.2%. Mesopelagic cephalopods showed a very low value of 9.3%. Finally, for tunas and pelagic fish, pelagic fish showed the highest contribution with 20.6% and mesopelagic cephalopods contributed 19.5%.

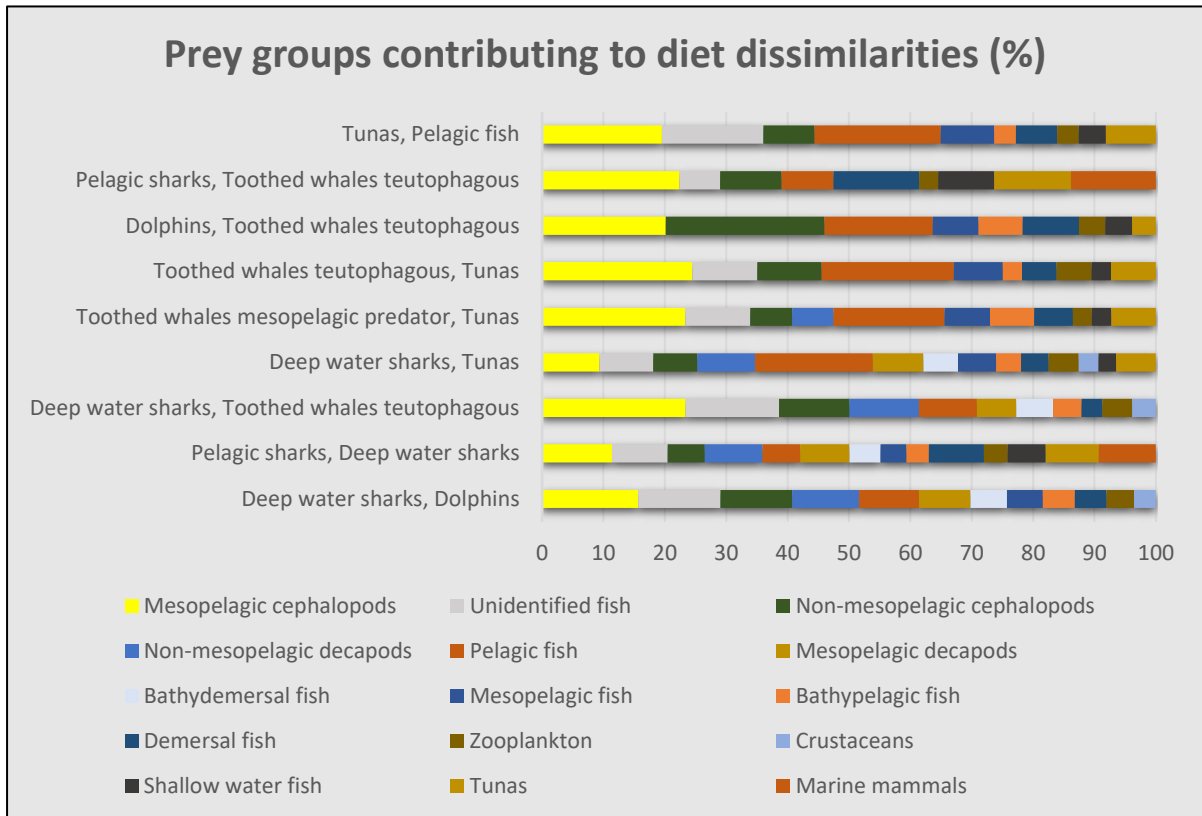


Figure 4: Cumulative contribution of prey functional groups to dissimilarities in diet among pairs of predators. Only predator groups with statistically significant differences in diet (as given by the ANOSIM R statistics in table 4) are shown.

3.5. Diet composition of the predator functional groups

Diet composition (Figures 5-11) showed great variances within predator functional groups, but mesopelagic cephalopods continued to be representative in terms of percentage showing a major preference for this prey type by most of the megafauna.

There was a huge variation in the diet between deepwater shark species (Figure 5). Unidentified fish represented the most important prey for *E. pusillus* (41%) and *E. spinax* (29%). On the other hand, non-mesopelagic decapods contributed the most for the diet of *G. melastomus* (35%). For these three species of deepwater sharks (*E. pusillus*, *E. spinax* and *G. melastomus*) the diet patterns were more or less the same because they all included the same type of preys on their diet. This was not registered for the remaining species: *C. coelolepis* was the only species that relied mainly on mesopelagic cephalopods (56%), while *D. licha* was the single one preferring small deepwater sharks (47%).

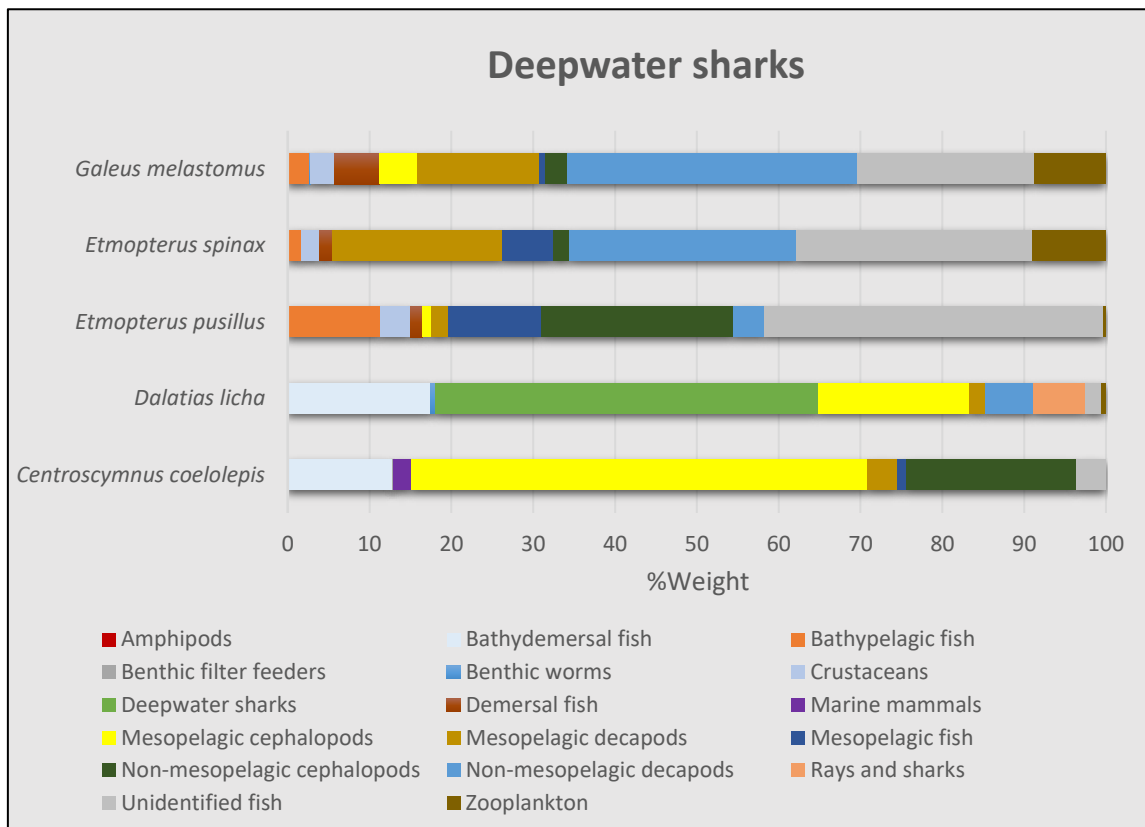


Figure 5: Percentage weight contribution of prey functional groups to the diet of deepwater shark species.

In general, the highest contribution to the diet of dolphin species (Figure 6) was from mesopelagic cephalopods (52% for *Delphinus delphis*; 56% for *Stenella coerulealba*; 100% for *Stenella frontalis*). The exception were *Steno bredanensis* and *Tursiops truncatus* which mostly preyed on non-mesopelagic cephalopods (Pelagic fish were also important prey of most Dolphin species, with a contribution ranging from 9% (*S. bredanensis*) to 17% (*D. delphis*)).

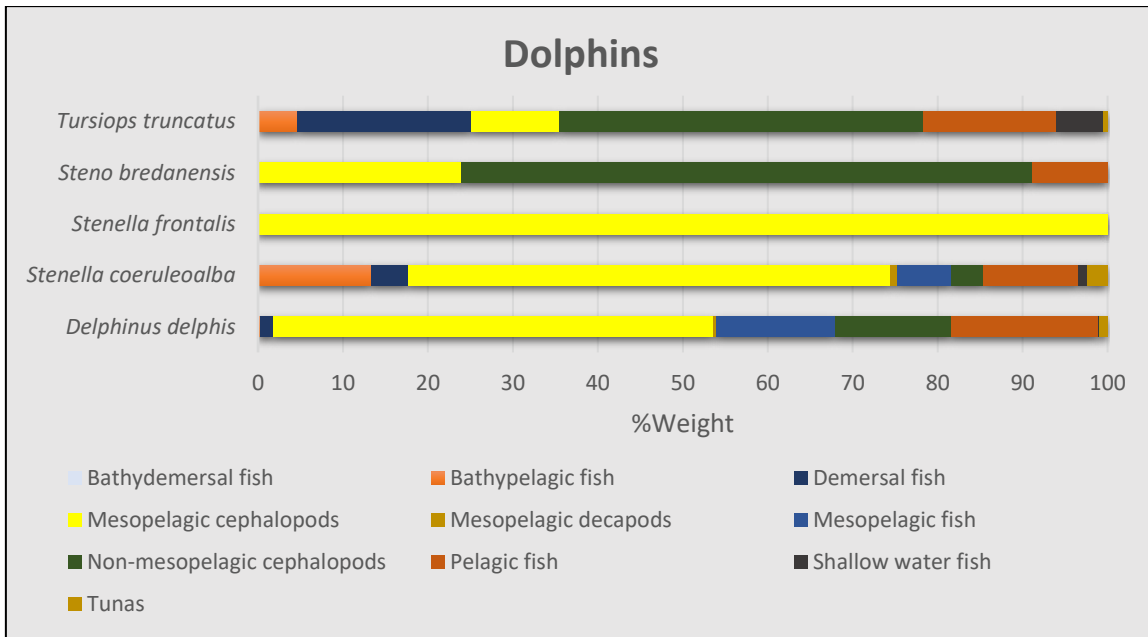


Figure 6: Percentage weight contribution of prey functional groups to the diet of Dolphin species.

Figure 7 showed the diets for pelagic fish species. *M. nigricans* is a pelagic fish that had 100% of the diet represented by unidentified fish and, therefore, depictions about the feeding behaviour of this species couldn't be handled. In parallel, mesopelagic cephalopods dominated the diet of the remaining three Pelagic fish studied, accounting for 100% of the diet of *T. pfluegeri*, 83% of *Kajikia albida*, and 37% of *Xiphias gladius*. Non-mesopelagic cephalopods were also important prey items for *X. gladius* (18%) and *K. albida* (17%). *Coryphaena hippurus* showed a more diverse diet, composed of unidentified fish (39%), pelagic fish (19%), mesopelagic cephalopods (14%) and shallow water fish (9%).

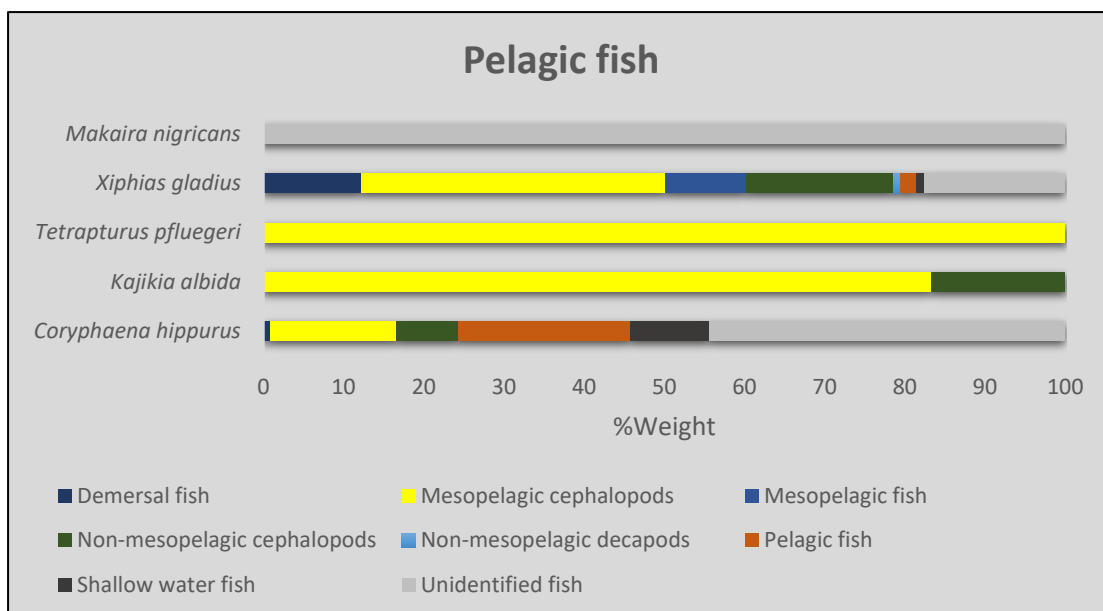


Figure 7: Weight contribution (%) of prey functional groups to the diet of Pelagic fish species.

Pelagic shark species showed marked differences in diet. *G. galeus* was the only species that didn't prey on marine mammals presenting the highest weight contribution for demersal fish (55%), followed by tunas (18%) and shallow water fish (17%) (Figure 8). The diet of the other two species was mainly based on mesopelagic cephalopods (62% for *P. glauca*, 29% for *I. oxyrinchus*) and marine mammals (27% for *P. glauca*, 20% for *I. oxyrinchus*), with Turtles being also an important item for *I. oxyrinchus* (23%).

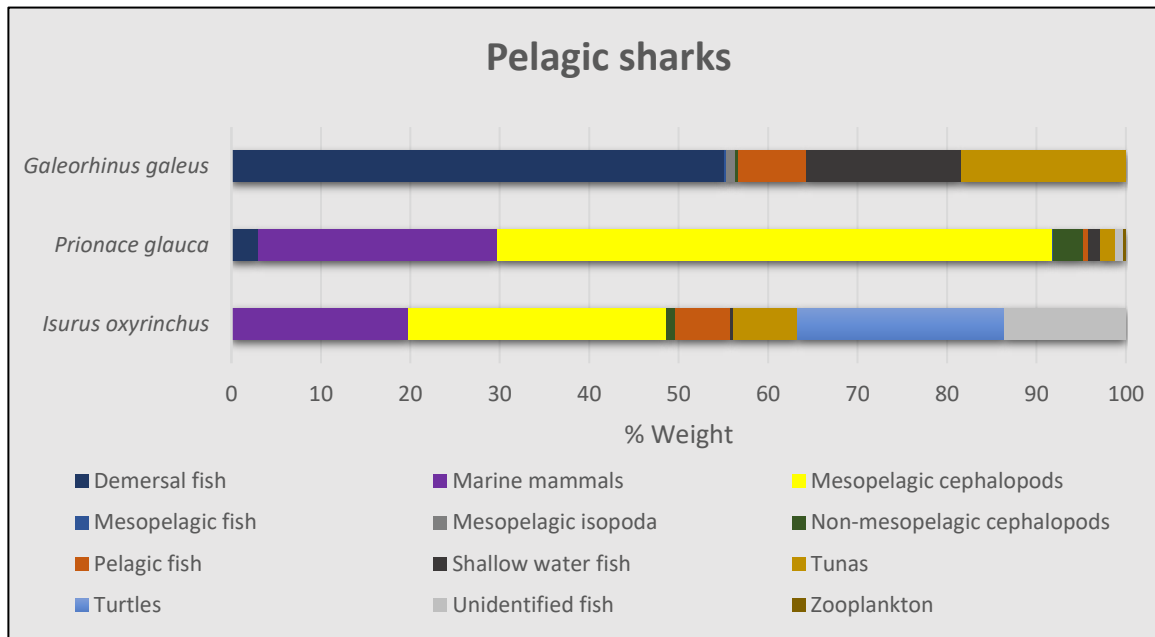


Figure 8: Weight contribution (%) of prey functional groups to the diet of pelagic shark species.

Toothed whales - mesopelagic predators included two species for which mesopelagic cephalopods were almost the single contributor to diet (*Z. cavirostris* with 97% and *M. europaeus* with 100%) (Figure 9). In sharp contrast, *M. bidens* did not consume mesopelagic cephalopods and had a wide range of prey items, including non-mesopelagic decapods (33%), bathypelagic fish (32%), pelagic fish (13%), demersal fish (12%), and non-mesopelagic cephalopods (8%).

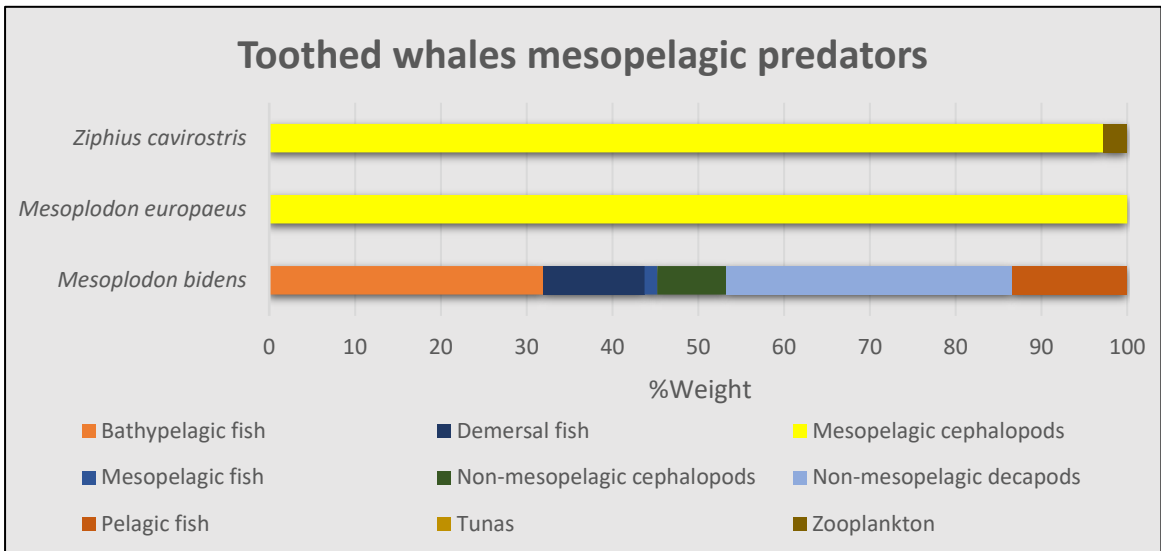


Figure 9: Weight contribution (%) of prey functional groups to the diet of Toothed whales - mesopelagic predator species.

In agreement with their classification, the diet of most Toothed whales – teutophagous was dominated by mesopelagic cephalopods, which accounted for 86% of the diet of all species (Figure 10). Nonetheless, mesopelagic cephalopods contributed less to the diet of *G. melas* and *G. griseus*, for which non-mesopelagic cephalopods were more important (57% and 54%, respectively).

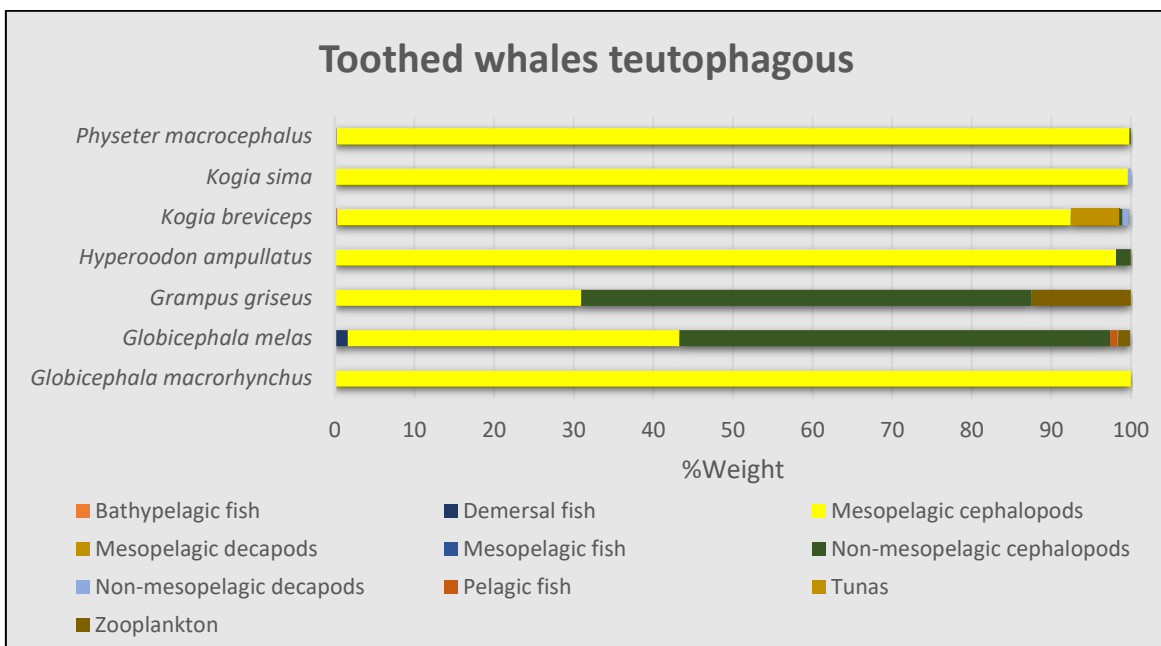


Figure 10: Weight contribution (%) of prey functional groups to the diet of Toothed whale - teutophagous species

Tunas showed a varied diet and greater diet variability between species (Figure 11). The diet of *Sarda sarda* was dominated by pelagic fish (97%). Small tuna species were important prey items for the larger tunas, *Katsuwonus pelamis* (50%) and *Thunnus obesus* (30%). In addition to pelagic fish, *T. alalunga*, *T. thynnus* and *Auxis rochei* also consumed mesopelagic cephalopods, non-mesopelagic cephalopods, demersal, mesopelagic, and unidentified fish. Unidentified fish made up most of the diet of *Thunnus albacares*, while zooplankton was also an important prey for *A. rochei*.

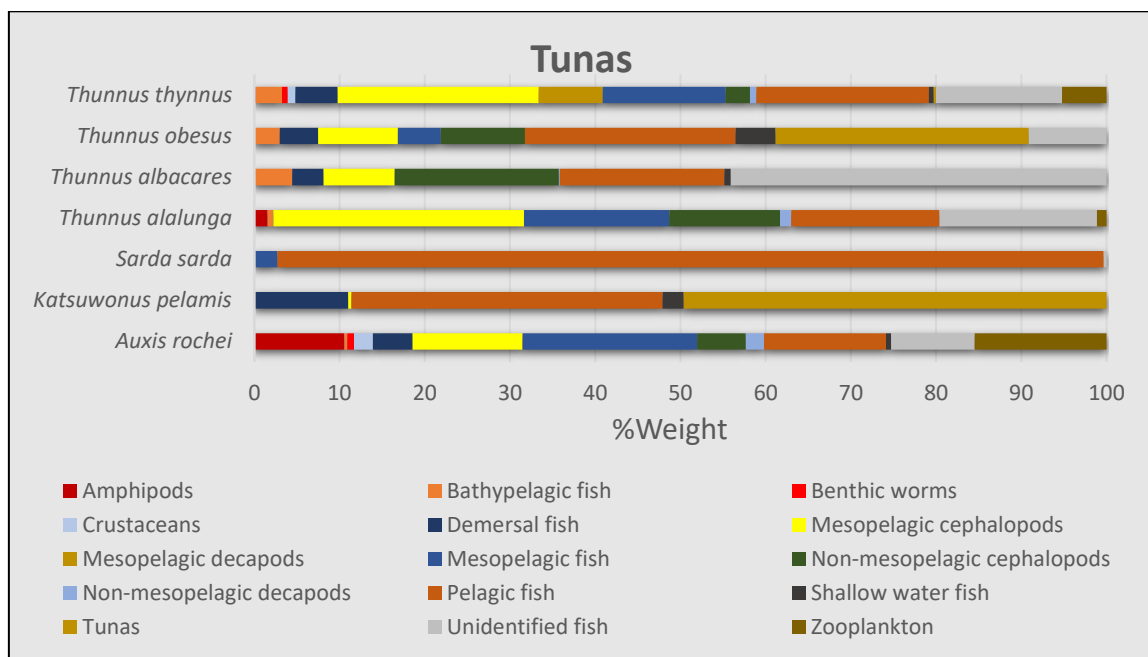


Figure 11: Weight contribution (%) of prey functional groups to the diet of tuna species.

4. Discussion

4.1. General diet considerations

In this study, I have complemented the existing database on predator's diet (Silva et al., 2021) with the objective to have a better understanding of the trophic ecology in European waters. The addition of new stomach content records from predators of the Azores contributed to improve the comprehension of ecological interactions between predators in that biogeographic area. Besides compiling more data, for the first time, I did a meta-analysis of all existing data to describe the diet of the megafauna predators occurring in the region of the Azores and surrounding areas of the Central Atlantic.

The composition of the diet data collected (represented in Figures 5, 6, 7, 8, 9, 10 and 11) showed that most of the megafauna predators (5 of a total of 7, being dolphins, pelagic fish, toothed whales mesopelagic predators and toothed whales teutophagous) preferred to feed on the mesopelagic zone and cephalopods constituted the main dietary item for most of them, with a weight contribution superior to 50%. This confirms the importance of the mesopelagic communities for predator species inhabiting the Azores region and shows the key role of mesopelagic organisms on the local food web. Deepwater sharks and tunas, though, showed different results. Sharks that inhabit deeper depths of the ocean seemed to feed more on fish that weren't completely identified, which doesn't clarify their main source of food. Considering only the detectable prey items, most deepwater sharks preferred non-mesopelagic invertebrates to feed. *G. melastomus* and *E. spinax* had non-mesopelagic decapods as their highest prey preference, while *E. pusillus* had non-mesopelagic cephalopods. *D. licha*, on the other hand preyed more on species of the same functional group because the data included contents taken from Navarro *et al.*, 2014 that showed small deep sharks were vital for the diet of this species. As the same article states, this indicates that *D. licha*, although also choosing other prey categories like bathydemersal fish and mesopelagic cephalopods to consume, can indeed be a major predator of sharks. *C. coelolepis* preyed substantially more on mesopelagic cephalopods which can give an insight that this is a species that chooses to remain on the mesopelagic oceanic zone to feed. Tunas, represented in figure 11, despite also frequently having cephalopod contents on their stomachs, showed to be pelagic and mesopredators as they had a high preference for pelagic and mesopelagic fish belonging to different categories, including unidentified for *T. albacares*. Inclusively, *K. pelamis* and *T. obesus* preferred to feed on species of the same functional group and other fish meaning both tunas presented a marked epipelagic diet (Romero *et al.*, 2021). The fact that the main prey preference is directed to cephalopods could be explained by what has been stated in other diet investigations, such as Kaschner *et al.*, 2006, that cephalopods have more hard parts (e.g.: beaks) making it harder to digest in quick rates and increasing their chances of accumulation in the stomachs.

4.2. Diet similarities between and within megafauna functional groups

Hierarchical cluster and nMDS graphs were chosen to interpretate the similarity percentages and to make assumptions about the proximity and distance between diets of each functional group and species. For the two representation plots, toothed whales teutophagous, tunas and deep water sharks were the predator functional groups closer to each other, which in turn explains that they feed in similar prey species and depth layers. As demonstrated in the sources from which the data was taken, both toothed whales teutophagous and deep water sharks tend to make vertical dives (diving down to 1000 m) to forage in the same depth interval where tunas inhabit and frequently hunt for prey (500 – 1000m) (Foskolos *et al.*, 2020; Karakulak, Salman, & Oray, 2009; Xavier *et al.*, 2012). The rest of the predator categories were observed to be more distant from each other, meaning that their diets were more dissimilar. This was mainly due to differences on the diet composition of predator species within the same functional group, as demonstrated by the nMDS (Figure 3). For example, in the pelagic shark functional group, *G. galeus* had demersal fish as the most important prey, whereas the stomachs of *I. oxyrinchus* and *P. glauca* showed to have mesopelagic cephalopods as a more predominant prey type. Also, there could be lack of data for some species. Nevertheless, only one reference was used to extract the prey contribution to the diet of *G. galeus* which doesn't prove if this pelagic shark is actually a true predator of demersal fish.

For the dissimilarity analysis (Figure 4), tunas and toothed whales teutophagous were the predator groups with the most dissimilarities (Table 4). Mesopelagic cephalopod contribution to diet dissimilarities showed to be the highest for this match of consumers, verifying the significance of mesopelagic prey to distinguish diets of pelagic predators and whales. Toothed whales teutophagous seemed to have the greatest dissimilarities from the rest of the groups being included also in the pair with the second and third highest dissimilarities (respectively, toothed whales teutophagous and deep water sharks; toothed whales teutophagous and pelagic sharks). This must be due to the way these whales and the sharks choose to feed by making multiple vertical dives that enables them to catch a greater diversity of prey (Foskolos *et al.*, 2020; Xavier *et al.*, 2012). The fact there were many samples for the shark predators could also explain the greater diversity of prey and there could be ontogenetic shifts in their diets proved by

the existence of different sized sampled individuals corresponding to distinct life stages that require various metabolic requirements and types of resources (Foskolos *et al.*, 2020; Valls *et al.*, 2011; Xavier *et al.*, 2012). Pelagic fish was the main contributor for dissimilarities between two functional groups belonging to the pelagic zone: the pelagic fish and tunas, which explains that the type of pelagic species these predators consume during their vertical dives is important to dictate their corresponding diets (Olafsdottir *et al.*, 2016). Also, a diverse menu of preys in the pelagic area for tunas and pelagic fish could explain the dissimilarities among them.

When it comes to unidentified fish, results related to prey contribution to diet dissimilarities (Figure 4) showed high percentages, except for dolphins and toothed whales teutophagous that always had identifiable prey in their stomachs. Though, differences between observed feeding habits of predator groups based on unidentified prey contents can never be assumed. It only demonstrates there was a great number of cases of low taxonomy resolution during the diet examinations. A great number of stomach content items that were ingested or degraded could explain the difficulty to define the taxonomy of the sample items, as stated by Nielsen *et al.* (2018).

4.3. Caveats and problems of the investigation

Acknowledging and evaluating the issues linked to the creation of groups for predators and preys were the most important aspects so that the objectives of analyses could be undertaken. Also, for there to be an easier distinction of prey functional groups within the several predators' diets, instead of creating a long and exhaustive list for the corresponding species, it was inevitable to group the various preys into categories. To define the functional groups, initially, the predators' taxonomy, general specific biological behaviours and feeding preferences were taken into account. The classification of predator and prey functional groups was created using a pre-established ecosystem-based model (EBM) by Morato *et al.*, 2016 containing species of deep-sea and open-sea ecosystems of the Azores databased (a database which was not published) on other sources, such as Bernal *et al.*, 2015, that identify mesopelagic species and information was extracted from Fishbase. Morato *et al.*, 2016 presents a collection of records that retain just a fraction of the Northeast Atlantic ecosystem condition where the samples were extracted. Models focusing on the Azores still miss a clear interpretation of high-

trophic-level and predator-prey interactions, which gives more value of importance to the research undertaken in the present thesis. The present research adds new diet information from stomach contents of megafauna predators recorded in recent articles and contributing with new data to one of the most complete datasets of top predators' stomach contents present in the European waters (Atlantic Ocean, the North and Mediterranean Sea) (Silva *et al.*, 2021).

Nevertheless, in the database, there was still a lack of predators and prey data from the Azores. A larger sample collection, especially in this area, would be necessary for a more robust analyses and with that increase diet similarities or dissimilarities between and within functional groups to distinguish to provide a clearer distinction. During the definition of the functional groups from SUMMER, only species that commonly occurred in the Azores region were accounted for, leaving seabirds, benthic sharks and turtles with just one species for analysis (respectively, *C. diomedea*, *G. galeus*, *C. caretta*). The hierarchical cluster analysis (Figure 2) purposed to give an initial general notion of the several diet proximities between functional groups. Due to the lack of content, the diets of the three types of predators were only applied for the hierarchical analysis. They looked very distant to each other, but, in contrast with the other two isolated groups (*G. galeus*, *C. caretta*), the cory's shearwater *C. diomedea* showed to have a diet close to the common bottlenose dolphin, *T. truncatus* (between 60 to 80% similarity). This hypothesizes that seabirds like *C. diomedea* occur in the same spot as the dolphin's food hunt takes place (as referred by Clua & Grosvalet in a study conducted in the Azores). However, this can only be conclusive when there is presence of the common bottlenose dolphin since, as shown in Figure 2, *T. truncatus* stays in a distant cluster from the rest of the dolphins meaning that its diet is unique among the species of dolphins. nMDS plot (Figure 3), on the other hand, although it also purposed to give a broad view of the data, showed the similarities between species. An insertion of seabirds, benthic sharks and turtles in this graph model, would result in positioning of their corresponding species in the residual area, where the diets are less than 20% similar with each other.

There is a constant debate in a vast number of papers regarding the best diet metric to describe and estimate species relationships in trophic studies. Prey weight (or biomass) was the most appropriate option as this information is necessary to be used in models that contain mass balances such as Ecopath (with Ecosim). The processing of data

converted in weight that is tabulated for the distinct prey items during sample collections is affected by factors like different digestion (Baker *et al.*, 2014) and ingestion rates between prey species (Baker *et al.*, 2014; Rindorf & Lewy, 2004). These aspects can lead to a big divergence between composition in bulk and composition in frequency of occurrence (%FO) (Baker *et al.*, 2014). A greater contribution by bulk composition can be explained, for example: (1) because of a more consumption of prey biomass; (2) because of a more slowly digestion of the prey item; (3) because the prey was consumed very recently; (4) or because the prey is clearer identified in a larger digestion spectrum over the other (Baker *et al.*, 2014). In order to increase the data records with information on prey weight, attempts have been made to convert prey frequency of occurrence (%FO) to relative weight, but this only allows approximated values and is solely used when there are no other estimates (Stobberup *et al.*, 2009).

The adaptation of the equation models from Sagarese *et al.* (2016) allowed to create an accurate arithmetic average that facilitates the observation of the biomass data extracted from a significant number of different studies and previously disposed in weight percentage. After the literature collection, bulk values in weight corresponding to different areas of investigation and many sample sizes, which were already aggregated in the database, needed to be taken into account to build a complex average. Simple averages, on the other hand, were not adequate for this data disposal because the sample sizes were frequently large and variable. Moreover, simple means are only based in number of diet observations and contributions rather than the prey compositions and areas of samplings ignoring the interdependence and variance of the accounting prey present in stomachs (Sagarese *et al.*, 2016).

This allowed the performance of the meta-analysis and transform the original raw data so that it could fit for graphic examinations. Moreover, the arithmetic mean was also based on the sampling region of the predator stomachs, which gives a quantitative perspective of the consumers' feeding habits taking into account the ecosystem they inhabit. This was the reason to weight the diet contributions per sampling regions instead of using a simple arithmetic mean. The formula for the weighted average also included locations and regions to distinguish the spots of the samples from the general dominant habitat where the predators were found. The locations were needed to build the weighted mean based on the geographic proximity from the Azores archipelago to the

sites where the samplings took place. The regions were even more important for the estimations since it accounted for the more specific spots where the samplings occurred giving significance to the dominant ecosystems of the different predators. The database used had records from a great variety of years of sampling (as shown in Table 1) providing simple visions of the diet habits over long time periods. Fluctuations in prey abundance in cycle systems are part of population dynamics in the environment and this can lead to changes in predator dietary selection as the years pass (Dale, Adams, & Bowyer, 1994; Pyke, 1984; Spencer, Newsome, & Dickman, 2017). Because of how the data disposes by corresponding the years of sampling to their respective predator stomach records, issues of consumers' ecological shifting over time don't need to be considered.

5. Future perspectives

The data provided in this thesis provides the basic information to support future ecosystem-based models or to update models that already exist (Morato et al., 2016) directed to the Azores archipelago biogeographic area. This approach could also be implemented in the scope of other dietary studies based on isotopic or molecular data.

The results of this thesis contribute to increasing the knowledge of the trophic relationships among megafauna predators in the Atlantic Ocean. Meta-analysis comes up as a helping tool for collecting data from a great number of different sources and for organizing the data composition that is disposed in various forms. It can also be implemented in research that aims to identify and understand the connections of species belonging to different functional groups based on any biological compounds and not just on their sampled stomach contents.

However, more research is needed on the ecological behaviours and interactions between predator and prey, mainly in regions with poor data such as the Central Atlantic Ocean, with the aim of increasing the accuracy and development of ecosystem-based management approaches. The final goal is to give a support tool to make prior decisions and act in favour for the conservation of species of apex predators that are not only so sensitive to the environmental shifts but are also crucial elements for the ecosystem stabilization and, thus, its protection.

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7. Supplementary material

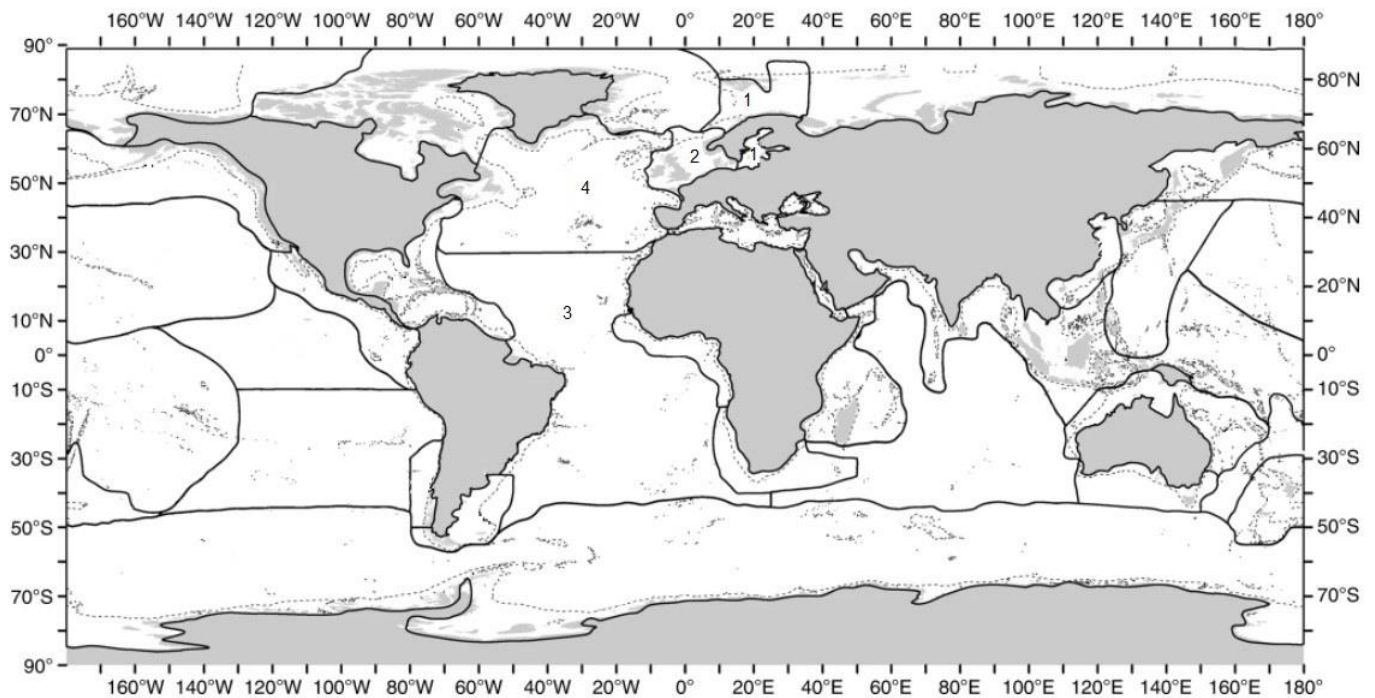


Figure 12: Biogeographic realms (denoted by solid lines) adapted from Costello *et al.*, 2017 and used as the basis for creating region factors to estimate the weighted arithmetic average: Offshore & NW Atlantic region (4); Offshore S Atlantic (3); NE Atlantic (2); North Atlantic (1). The dashed line shows the 1000 meters of depth contour.

(1) Direct link to the database created during the investigation:

https://uapt33090-my.sharepoint.com/:x:/g/personal/joaoreisramos_ua_pt/Ea4PtekWD0tCnCpRRpWV_GEBHcxxI1Nz_wgdoRW5wT0odg