SOFIA GRAÇA ARANHA CARVALHO RAMOS

DIET AND TROPHIC POSITION OF DEEP-SEA SHARKS IN THE SOUTHWEST COAST OF PORTUGAL

USING STABLE ISOTOPES ANALYSIS AND NUCLEIC ACIDS RATIOS (RNA/DNA)

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FACULDADE DE CIÊNCIAS E TECNOLOGIA

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Master in Marine and Coastal Sciences

Work performed under the supervision of:

Advisor: Karim Erzini (CCMAR, UAlg)

Co-advisor: Ester Dias (CIIMAR, Porto)

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“Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.”

"I declare to be the author of this work, which is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references. "

SOFIA GRAÇA ARANHA CARVALHO RAMOS

"We but mirror the world. All the tendencies present in the outer world are to be found in the world of our body. If we could change ourselves, the tendencies in the world would also change. As a man changes his own nature, so does the attitude of the world change towards him. This is the divine mystery supreme. A wonderful thing it is and the source of our happiness. We need not wait to see what others do."

- **MAHATMA GANDHI**

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ABSTRACT

The deep-sea sharks are vulnerable to exploitation, and their productivity is amongst the lowest observed to date. Sharks are, in general, predators and thus crucial to maintain the balance of their direct and indirect preys. Due to their fragility and role in the marine food web, this study aimed at assessing the nutritional condition, diet, and trophic position (TP) of deep-sea sharks at the southwest coast of Portugal using for the first time a combination of two non-lethal approaches with deep-sea elasmobranchs: RNA/DNA (R/D) ratios, and stable isotope analysis (SIA). Muscle samples were collected from deep-sea shark species: *Centrophorus squamosus*, *Centroselachus crepidater*, *Deania calcea*, *Deania profundorum*, *Etmopterus pusillus*, *Galeus atlanticus* and *Scymnodon ringens.* Their potential prey were also collected and included, teleosts, crustacean and cephalopods. Overall, sharks presented a good nutritional condition indicating they have been feeding and that their diet might be supported by the species from the study area. The species with higher R/D values (e.g. *G. atlanticus*) are eating more frequently than species with lower ratios (e.g. *D. profundorum*). Sharks were divided in three groups according to SIA. The first group was composed by *D. profundorum*, *E. pusillus*, and *G. atlanticus* presenting low $\delta^{13}C$ and $\delta^{15}N$ values, indicating they were feeding on preys with low $\delta^{13}C$ and $\delta^{15}N$ values such as crustaceans and diel vertical migratory teleosts (group T3), presenting a TP range of 4.1-5.1. Second group of consumers is composed by *S. ringens* and *C.squamosus* with ^{13}C and ^{15}N-enriched values, an indication they were feeding on preys with higher values of $\delta^{13}C$ and $\delta^{15}N$ such as migratory teleosts and cephalopods and presented a TP range of 5.6-6.1. The third group is composed by *D.calcea* and *C.crepidater* with a high isotopically variability suggesting intra-specific variation on the diet or a generalist behavior with the presence of a wider trophic niche and TP range of 5.1-6.3.

Keynotes: squaliformes; elasmobranchs; ecophysiology; eastern Atlantic Ocean; nutritional condition; trophic niche

RESUMO

Tubarões de profundidade são vulneráveis à exploração e sua produtividade está entre as mais baixas observadas até o momento. Os tubarões são, em geral, predadores e, portanto, são cruciais para manter o equilíbrio do ambiente em que habitam. Devido à essa fragilidade e importância, este estudo teve como objetivo avaliar o estado nutricional, a dieta e a posição trófica (TP) de tubarões de profundidade na costa sudoeste de Portugal utilizando pela primeira vez uma combinação de duas abordagens não letais com elasmobrânquios de profundidade: Rácios de RNA/DNA e análise de isótopos estáveis (SIA). Amostras de músculos foram coletadas para tubarões - *Centrophorus squamosus*, *Centroselachus crepidater*, *Deania calcea*, *Deania profundorum*, *Etmopterus pusillus*, *Galeus atlanticus e Scymnodon ringens* e também para potenciais presas: teleósteos (divididos pelos seus valores de $\delta^{13}C$ e $\delta^{15}N$ nos grupos T1 à T4), crustáceos e cefalópodes. Em geral, os tubarões apresentaram uma boa condição nutricional indicando que eles têm se alimentado e que sua dieta pode ser sustentada pelas presas da área de estudo. As espécies com maiores rácios de R/D (por exemplo, *G. atlanticus*) se alimentam mais frequentemente do que as espécies com menores rácios (por exemplo, *D. profundorum*). Os tubarões podem ser agrupados em três grandes grupos de consumidores de acordo com SIA. O primeiro é composto pelas espécies *D. profundorum*, *E. pusillus* e *G. atlanticus* com baixos valores de $\delta^{13}C$ e $\delta^{15}N$ indicando, portanto, uma dieta composta por presas com baixos valores de $\delta^{13}C$ e $\delta^{15}N$ como crustáceos e espécies de teleósteos que realizam migração vertical diária, apresentando também uma variação de TP de 4,1 a 5,1 . O segundo grupo de consumidores é composto por *S. ringens* e *C. squamosus* que possuem valores enriquecidos de ^{13}C e ^{15}N, um indicativo de que eles se alimentam de presas com valores maiores de $\delta^{13}C$ e $\delta^{15}N$ como algumas espécies migratórias de teleósteos e cefalópodes e possuam uma variação de 5,6 a 6,1 de TP. O terceiro grupo é composto por *D. calcea* e *C. crepidater* onde apresentaram alta variabilidade isotópica sugerindo variação intraespecífica na dieta e/ou comportamento generalista com a presença de um nicho trófico mais amplo, e uma variação de TP 5,1 a 6,3.

Palavras-chave: squaliformes; elasmobrânquios; ecofisiologia; oceano atlântico leste; condição nutricional; nicho trófico

Table of Contents

Index of Figures and Tables

SUMÁRIO

Os peixes de profundidades são considerados um recurso extremamente frágil, uma vez que não suportam altos níveis de exploração devido às características do seu ciclo de vida que incluem extrema longevidade, baixa taxa de crescimento, maturação tardia e baixa fecundidade. Os elasmobrânquios de profundidade são ainda menos resistentes à exploração e a sua produtividade está entre as mais baixas observadas até o momento, entre os organismos de profundidade. Embora o seu papel varie entre espécies e regiões, os tubarões são geralmente aceites como predadores nas cadeias alimentares marinhas, sendo de extrema importância para a manutenção do equilíbrio de todo o ecossistema através da ligações tróficas que estabelecem. Sendo assim, é crucial a avaliação das suas características ecofisiológicas e biológicas por meio de abordagens não-letais, a fim de compreender a sua vulnerabilidade e o seu papel dentro de ecossistemas específicos. Os ácidos nucleicos como os rácios de RNA/DNA (R/D) são ferramentas importantes porque fornecem uma medida de curto prazo das condições ecofisiológicas dos animais (por exemplo, 1-3 dias), embora nunca tenham sido aplicados em estudos com elasmobrânquios de águas profundas. A análise de isótopos estáveis (SIA) é uma ferramenta muito útil para estudar não só as interações tróficas em cadeias alimentares aquáticas, mas também inferir o uso do habitat e os padrões de movimento de indivíduos e populações. Ambas as metodologias podem ser usadas como uma abordagem não letal para o estudo de animais frágeis e únicos, como os tubarões. Como informações sobre organismos de profundidade são ainda muito escassas (especialmente no caso de tubarões), o objetivo principal do presente estudo foi contribuir para aumentar o conhecimento existente sobre o estado nutricional, a dieta e a posição trófica dos tubarões de profundidade da costa sudoeste de Portugal combinando pela primeira vez rácios de R/D e SIA em tubarões.

Para este fim, foi realizada uma campanha de amostragema em Fevereiro de 2018, durante três dias, a bordo de um barco de pesca de arrasto de fundo para captura comercial de crustáceos. As recolhas foram feitas entre 1.107 e 1.350 m de profundidade e amostras musculares das espécies de tubarões e suas potenciais presas foram recolhidas para análise. Os tubarões encontrados na área foram o lixa *Centrophorus squamosus* (n=2); sapata preta *Centroselachus crepidater* (n=2); sapata *Deania calcea* (n=9), sapa branca *D. profundorum* (n=4) xarinha preta *Etmopterus pusillus* (n=5); *Galeus atlanticus* (n=5) e o arreganhada *Scymnodon ringens* (n =12). As potenciais presas incluíram teleósteos divididos em grupos i.e. T1 com elevado $\delta^{15}N$ e composto por espécies não-migratórias, T2 com espécies migratórias, T3 com espécies com baixos valores de $\delta^{15}N$ que realizam migrações verticais diárias e T4 com valores médios de $\delta^{15}N$ incluindo, crustáceos (caranguejos, camarões e lagostas) e cefalópodes (lulas e polvos). Fêmeas representaram a maioria dos tubarões capturados (69%). Foram apanhados mais juvenis (36%) do que adultos (33%), embora para *S.ringens* (31%) não haja informação suficiente, para fazer esta classificação. No geral, os tubarões apresentaram uma boa condição nutricional dado a média dos rácios estandarditizados de R/D foi superior a 0. Isso indica que eles se alimentaram no últimos 1-3 dias, e também, que talvez a sua dieta seja sustentada pelas espécies da área de estudo. Espécies que apresentaram rácios mais elevados, como o *G.atlanticus* (0,63) indicam que

estão se alimentando com maior frequência do que as espécies com rácios menores como *D.profundorum* (0,26).

Os resultados do modelo de mistura de isótopos estáveis (95 % CI) indicam que os crustáceos foram os principais contribuintes (3-78%) para a biomassa de *D. calcea*, seguidos pelos teleósteos do grupo T2 (0-65%). Por outro lado, *D. profundorum* apresentou uma dieta de contribuições iguais dos dois grupos de teleósteos T2 e T3, crustáceos e cefalópodes, batidemersais e pelágicos. *Etmopterus pusillus* exibiu uma preferência de T3 (21-83%), o que reforça a ideia que *E. pusillus* também podem realizar migrações diárias verticais para se alimentar. O *Galeus atlanticus* apresentou maior contribuição do grupo de teleósteos T3 (11-69%), seguido pelos crustáceos (05-60%). A espécie *Scymnodon ringens* foi a única com uma preferência maior por um grupo específico, neste caso os crustáceos (46-89%) e, por apresentar um nicho trófico mais pequeno, pode indicar que poderá ser ser um predador mais seletivo. Algumas espécies, como *D. profundorum*, *E. pusillus* e *G. atlanticus*, apresentaram valores demasiado baixos de $\delta^{15}N$, quando comparados com os das presas potenciais capturadas. Isto poderá indicar que nem todas as presas relevantes para a dieta dessas espécies foram amostradas neste estudo,e que poderão incluir mictofídeos e eufasiáceos.

As fêmeas juvenis de *D. calcea* e *D. profundorum* mostraram a maior amplitude de nicho o que pode indicar que elas são predadores generalistas. Os indivíduos da espécie *D. calcea* parece estar a alimentar-se mais frequentemente do que *D.profundorum* devido ao fato de que *D.profundorum* apresentou menores rácios de R/D em comparação com *D.calcea* e outras espécies de tubarões.

De uma forma geral, as espécies de tubarões foram as que apresentaram a maior posição trófica (TP 4,8 a 6,3), seguidos pelos teleósteos (TP 4,0 a 5,0), cefalópodes (TP 3,8 a 4,4) e crustáceos (TP 3,4 a 4,1).

Apesar de este estudo apresentar algumas limitações, como em relação ao número de amostras e tecidos analisados, os resultados aqui obtidos são, em geral, satisfatórios para a interpretação dos principais objetivos inicialmente propostos. Novos estudos são necessários a fim de fornecer uma informação mais detalhada do estado nutricional, composição da dieta e maior resolução trófica dos tubarões de profundidade. Para isso, é aconselhável o uso de diferentes tecidos para a realização de SIA - uma vez que os tecidos assimilam a proteína ingerida em tempos diferentes '*turnover'* (dias, anos e até décadas) - juntamente com um maior período de coleta de dados para caracterizar a sazonalidade da dieta; o uso de diferentes fatores de enriquecimento trófico para diferentes tipos de organismos; uma boa escolha do organismo de base que tem que estar de acordo com a cadeia trófica em estudo; um número maior de indivíduos para avaliar mudanças da dieta ao longo do desenvolvimento e entre géneros; amostragem em diferentes limites batimétricos, pois acredita-se que algumas espécies agregam por tamanho e sexo em diferentes profundidades; finalmente, o uso de diferentes tipos de ferramentas para amostrar diferentes grupos de presas.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Fishing the deep-sea

Continental shelf break is located at approximately 200 m depth and function as the boundary between 'shallow' and 'deep sea', i.e. everything below 200 m depth is considered the deep-sea realm and it covers more of the Earth's surface than any other habitat (Gage and Tyler 1991). The bathyal zone extends from a depth of 1,000 to 4,000 m below the ocean surface. At this zone, physicochemical parameters such as temperature and salinity reach constant values. Because of its constant darkness it is also called the "midnight zone" and the only light coming from those depths, and below it, results from animal bioluminescence (NOAA 2017).

The deep-sea was believed to be a depauperated ecosystem due to its high pressures with an average of 400 atm, low temperatures with a mean of 4° C and little to no light penetration (Rowe 1983). However, today it is among the biomes with the highest biodiversity on Earth (Hessler and Sanders 1967; Grassle and Maciolek 1992; Snelgrove and Smith 2002). Still, little is known about these habitats due to its remoteness and difficulty in sampling at great depths (Snelgrove and Grassle 2001). Some commercially important species are known to inhabit deep-sea areas, e.g. the redfish (*Sebastess* spp.); the orange roughy (*Hoplostethus atlanticus*) (Norse *et al*. 2012) as well as some crustacean species such as the giant red shrimp (*Aristaeomorpha foliaceae*) and the scarlet shrimp (*Aristaeopsis edwardsiana*) (Figueiredo *et al*. 2001).

Despite the apparent higher levels of productivity over seamounts and similar features (Koslow *et al*. 2000) species in the deep-sea cannot support high levels of exploitation due to their life-history characteristics which includes extreme longevity, slow growth rate, late maturity, and low fecundity (Koslow *et al*. 2000; Morato *et al*. 2006; Norse *et al*. 2012). Thus, stock depletion is more rapid and recovery is consistently much slower than for species in shallow waters (Roberts 2002).

Commercial marine fishing has been occurring at increasing depths around the globe since 1970 which coincided with the collapse of shallow water stocks (Roberts 2002; Morato *et al*. 2006). The need to fish at higher depths stimulated the development of new and robust fishing gear; nonetheless, the access to deep-sea habitats it is still difficult because it requires expensive equipment and rigorous logistical protocols, and its success depends upon regional- or local-scale production processes. It seems likely that deep-sea fishing can only be profitable if pursued in the present mode of serial depletion (Roberts 2002). Thus, the need for site-specific

information and a precautionary approach is required as the footprint of fisheries expands (Norse *et al*. 2012; Brooks *et al*. 2015).

The fishing sustainability of some deep-sea species is related to (a) the ability of these species to also inhabit systems shallower than 200 m; (b) relatively high population resilience, and (c) the use of low-tech, non-trawl methods. Therefore, bottom trawling fisheries are not sustainable for any deep-sea species (Merrett and Haedrich 1997; Norse *et al*. 2012).

Bottom trawling fisheries pressure at the Portuguese continental waters is high (ICES Sub-area IXa). Over 100 trawlers using cod-ended mesh sizes, ranging from 55 to 70 mm, fish a large number of species (Campos *et al*. 2007). For the years 2012 to 2014 crustacean bottom trawlers landed 3.481 tons of catch and the most representative species were the rose shrimp (*Parapenaeus longirostris*) accounting for 44% of the total catch, followed by *Nephrops norvegicus* (12%), *Merluccius merluccius* (10%), and *Octopus vulgaris* (9%) (Bueno-Pardo *et al*. 2017). The seabed integrity indices for bottom-trawling fisheries in Portuguese waters are among the lowest of all Europe, and this is the result of a large footprint per unit of landing (ca. 17 km^2/t) and of a large total area (93.6%) where the trawling takes place (Eigaard *et al*. 2017) highlighting the intense pressure on deep-sea benthic habitats by bottom trawler fisheries.

Deep-sea sharks

Sharks are one of the most abundant and diverse groups of consumers in the ocean being found throughout the world's oceans – from coastal waters to the open ocean, from the surface to depths of 3,000 m (Priede *et al*. 2006) and presenting all the reproductive traits from vertebrates, from egg laying to placental viviparity (Cahmi 2008). They also have a variety of feeding habits, preying upon smaller sharks, marine mammals, teleosts, crustaceans, and zooplankton. Although their role in the marine food webs varies between species and regions, they are generally accepted as predators (Cortes 1999; Simpfendorfer and Dulvy 2017). As such, they are extremely important for the entire ecosystems balance, by regulating not only their direct main preys, but also second and third degree non-prey species through trophic linkages (Schindler *et al*. 2002).

Chondrichthyans (sharks, rays and chimeras) are among the most vulnerable taxa due to their extremely conservative K-selected life history strategy (i.e. slow growing, late to mature, small number of descendants) (Cortes 1999) and thus, are characterized by slow population turnover rates which makes them especially vulnerable to fisheries. Deep-sea chondrichthyans are overall less resilient to fisheries pressures than coastal and epipelagic species due to parameters such as high pressures and low temperatures which are known to slow down the metabolism of

the animal, directly affecting the growth rates (Vetter and Lynn 1997; Gordon 2001) and because of that, their productivity is amongst the lowest observed to date (Simpfendorfer and Kyne 2009). In these animals, overexploitation can occur even with low levels of fishing mortality and once they start to decline, it can take decades for populations to recover (Anderson 1990; Stevens *et al*. 2000; Pauly 1980; McCann and Shuter 1997; Gordon 1999),

Some of the world's elasmobranch (sharks and rays) populations are in rapid decline, and present a high potential risk of extinction in the future (García *et al*. 2008). However, almost half have insufficient data to support any form of assessment (Heupel and Simpfendorfer 2010). In Europe, according to the European Red List of Marine Fishes (IUCN, Nieto *et al*. 2015) the most threatened taxa is the Chondrichthyes comprising 40.4% of the endangered European marine species, while elasmobranchs represent 100% (n = 15) of 'critically endangered' (CR) species. The most relevant threat to marine fishes is the over-exploitation of both targeted and non-targeted species, more specifically, several deep-sea elasmobranch populations observed catastrophic declines in recent years, e.g. the picked dogfish, *Squalus acanthias* (Linnaeus, 1758) had its northeastern population depleted to about 5% of the original biomass (Hammond and Ellis 2004); the blue skate, *Dipturus batis* (Linnaeus, 1758) went locally extinct in the Mediterranean (Abdulla 2004). This is of great relevance taking into account that deep-sea chondrichthyans comprise approximately half (47.6%) of extant taxa (Cotton and Grubbs 2015). Thus, the lack of information raises concerns over the health of deep-sea chondrichthyan populations and creates uncertainty regarding potential effects of their removal on deep-sea ecosystem structure and function, since for tropical communities it was proved that the removal of sharks affected the condition and abundance of other fishes, across many trophic levels (Stevens 2000; Cox *et al*. 2002). For example, the seasonal presence of tiger sharks (*Galeocerdo cuvier*) in Shark Bay, Australia, was found to limit the habitat use and abundance of dolphins and dugongs in productive shallow areas (Heithaus and Dill 2002; Heithaus *et al*. 2006; Wirsing *et al*. 2007). More recently, Hammerschlag *et al.*, (2018) found that shark declines at unprotected coral reefs in western Australia can induce physical changes in some reef fishes (e.g. smaller fins and eyes) in comparison to areas where shark populations are healthy.

Similarly to other regions in the world, the distribution of elasmobranch species along Portuguese waters is poorly known (Albuquerque 1956; Figueiredo *et al*. 1996). At the south coast of Algarve (S- Portugal) sixty species of sharks were identified, including several deep-sea species such as the smooth lantern shark (*Etmopterus pusillus*), knifetooth dogfish (*Scymnodon ringens*), the birdbeak dogfish (*Deania calcea*), arrowhead dogfish (*D. profundorum*), leafscale gulper shark (*Centrophorus squamosus*) and the longnose velvet

dogfish (*Centroselachus crepidater*) which are commonly caught as bycatch of longline and crustacean trawlers fisheries (Borges *et al*. 2001; Coelho and Erzini 2008; Leitão *et al*. 2014). The great majority of deep-sea sharks including those from the families Scyliorhinidae and Etmopteridae, have little or no commercial value and therefore are frequently discarded (Monteiro *et al*. 2001; Coelho and Erzini 2008).

Decreases in the abundance of apex or meso-predators such as sharks may cause alterations in ecosystems through competitive release (i.e. without its predator, the immediate prey would increase its number and thus decrease the numbers of their direct preys), which would therefore, alter fish population dynamics (Stevens 2000). Thus, the understanding of the trophic ecology of sharks is of extreme importance to evaluate the possible consequences of their stock's reduction. Given the role of sharks in the marine ecosystems and due to their increasing vulnerability to overfishing and habitat degradation (Ferretti *et al*. 2010; Worm *et al*. 2013) they became the focus of several marine conservation studies which aim at understanding their ecology and behavior in order to develop effective management and conservation strategies (Simpfendorfer *et al*. 2011; Molina *et al*. 2012; Jordan *et al*. 2013; Kynoch *et al*. 2015).

Nutritional condition, diet and trophic position of sharks

Condition and growth of organisms can be estimated with biochemical techniques, and to date, the most widely-used index is the bulk ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) analysis (RNA/DNA). The RNA/DNA (R/D) is able to provide a short-term measure of ecophysiological conditions (i.e., past 1–3 days) (Buckley *et al*. 1999), based on the concept that DNA concentrations within individual cells remain fairly constant while RNA concentrations increase as protein synthesis increases and varies with age, life-stage, organism size, disease-state and with changing environmental conditions (Bulow 1970; Buckley 1980; Ferron and Leggett 1994; Suthers *et al*. 1996). Thus, a recently well-fed, metabolically active growing individual, should have a relatively high R/D compared to a starving, metabolically inactive individual (Bulow 1987; Robinson and Ware 1988; Richard *et al.* 1991).

For about fifty years, nucleic acid ratios have been used as a biochemical indicator of the physiological and nutritional state of aquatic organisms in natural environment (Holm-hansen *et al*. 1968). R/D is a macromolecular index frequently used as an indicator of protein synthesis and is considered a reliable indicator of instantaneous condition and growth (Rooker *et al*. 1997; Okumura *et al*. 2002; Islam and Tanaka 2005; Vidal *et al*. 2006). To date, a number of studies in ecology, toxicology, aquaculture and fisheries have used this approach to assess the condition of different organisms, mainly plankton (Sutcliffe 1965; Holm-Hansen *et al*. 1968; Dortch *et*

al. 1983; Berdalet and Dortch 1991; Gorokhova and Kyle 2002; Cruz *et al*. 2017), larval fish (Buckley 1984; Bulow 1987; Caldarone *et al*. 2003; Chícharo *et al*. 2003; Garcia *et al*. 2003; Caldarone 2005; Buckley *et al*. 2008), bivalves (Wright and Hetzel 1985; Chícharo and Chícharo 1995; Dahlhoff and Menge 1996; Chícharo *et al*. 2001), cephalopods (Clarke *et al*. 1990; Sykes *et al*. 2004; Vidal *et al*. 2006), crustaceans (Grémare and Vétion 1994; Lemos *et al*. 2002; Chícharo *et al*. 2007) and vertebrates like sea turtles (Roark *et al*. 2009; Vieira *et al*. 2014), juvenile and adult fishes (Bulow 1970; Thorpe *et al*. 1982; Carter *et al*. 1998; Buckley *et al*. 1999; Smith and Buckley 2003; Islam and Tanaka 2005; Mercaldo-Allen *et al*. 2006; Caldarone *et al.* 2006; Chícharo *et al.* 2007; Vinagre *et al.* 2008a).

Although the R/D approach has been widely used on many different marine organisms, including vertebrates, only one published study applied this tool on sharks. Tavares *et al*. (2006) analyzed the physiological condition of juveniles of smooth dogfish shark (*Mustelus canis*) at the northern coast of Venezuela using R/D ratios. No studies on deep-sea sharks were found.

The fact that samples from deep-sea species are not easy to obtain (Brooks *et al*. 2015), biological and ecological studies on deep-sea sharks, especially for the Portuguese coast are very scarce. Nonetheless, it is possible to find studies on deep-sea sharks' diet based on stomach content analysis (Costa 1998; Santos and Borges 2001; Coelho and Erzini 2007, 2008; Xavier *et al.* 2012; Coelho *et al*. 2015; Muñoz 2015; Gamito *et al.* 2016) which is the most commonly used method to analyze the diet and estimate trophic position of consumers in the aquatic food web. Perhaps, the most important study on sharks' diet and trophic position is that of Cortés (1999) where the author showed, based on stomach content analysis of more than 149 shark species, that sharks are top predators being predominantly tertiary consumers (trophic level TL > 4) consuming a wide range of secondary consumers, i.e. carnivorous preys with a trophic level of 3.

Stomach content analysis provides detailed information on the food sources consumed at a given time. However, it does not provide information on the food sources which are assimilated by the consumer and in most cases requires the death of the animal. Also, stomach content analysis can be biased due to the presence of unrecognized prey items, loss of prey by regurgitation induced by stress with handling, among others (Vander Zanden *et al*. 1997; Pinnegar and Polunin 1999; Pinnegar *et al*. 2001; Renones *et al.* 2002). Deep-sea teleosts and elasmobranchs often regurgitate food as they are brought to the surface (Bowman 1986) and prey items from deep-sea communities often are fragile and difficult to identify (Cailliet *et al*. 1999; Drazen *et al*. 2001; Robinson *et al.* 2007).

Over the past decades, stable isotope analysis (SIA) has emerged as a useful biochemical tool in ecological research to study trophic interactions in aquatic food webs and also to infer habitat use and movement patterns of individuals and populations (Hobson 1999; Hussey *et al.* 2012; Layman *et al*. 2012). The analysis of stable isotopes, in particular of nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) and carbon ($\delta^{13}C$: $^{13}C/^{12}C$), is a useful method to clarify the structure and dynamics of aquatic food webs. The $\delta^{15}N$ values are usually used to determine the trophic level of a certain consumer while the $\delta^{13}C$ values are useful indicators of the origin of the food sources assimilated by consumers (Polunin *et al*. 2001). This technique is based on the relationship between the isotopic composition of the organic matter (OM) in the ecosystem and the isotopic composition of the consumer's tissues that incorporate this OM into their structural components and energy reserves (Peterson and Fry 1987). Thus, the stable isotope ratio of a consumer reflects its diet, demonstrating an average trophic fractionation (i.e., the difference between the consumer and its diet) of + 0.4 for $\delta^{13}C$ and + 3.4 for $\delta^{15}N$ per trophic level (Post *et al*. 2004). The trophic fractionation may vary according with the consumer's nutritional status (Hobson *et al*., 1993) lipid content, quality of the diet, ontogeny, size, age, and with the type of tissue analyzed (Minagawa and Wada 1984; Zanden and Rasmussen 2001; Post 2002; Caut *et al*. 2008; Caut *et al.* 2010).

A series of improved quantitative approaches for analyzing stable isotopes over the past decade contributed to the improved understanding of food webs, for example, providing new insight into food-chain length (Post 2000), elucidating trophic relations in aquatic food-webs (Vander-Zanden *et al*. 1997; Post 2002) niche variation (Moore and Semmens 2008; Martínez Del Rio *et al*. 2009; Semmens *et al*. 2009; Votier *et al*. 2010) and human-driven shifts in community structure (Layman *et al*. 2007; Schmidt *et al*. 2007).

For large highly mobile animals inhabiting environments where they are difficult to observe, SIA is a useful tool and has been applied in shark ecology studies (Shiffman *et al*. 2012; Shipley *et al*. 2017), providing information on trophic structure (Estrada *et al.* 2003; Layman *et al*. 2007; Hussey *et al.* 2014, 2015; Churchill *et al*. 2015b), resource use (Matich *et al.* 2011, 2017; Heithaus 2013), diet ontogenetic shifts (Estrada *et al*. 2006; Madigan *et al*. 2015a), and movement patterns (Carlisle *et al*. 2012; Munroe *et al*. 2015).

Stable isotopes analysis (SIA) in elasmobranch may target different tissues including muscle, whole blood, red blood cells, cartilage, plasma, and liver (Hussey *et al*. 2012). The choice of a certain tissue will depend on the temporal resolution of interest (Pinnegar and Polunin 1999). For instance in sharks, the plasma, muscle, and cartilage will reflect feeding behaviors from the previous months, years, and decades, respectively (MacNeil *et al*. 2005; Caut *et al*. 2009; Kim

et al. 2012a). White muscle provide long-term dietary estimation due to its slower turnover rates in comparison to blood or liver, which exhibit high metabolic activity and thus, provides information of the diet for the previous months (Pinnegar and Polunin 1999; Estrada *et al*. 2006; l *et al*. 2015b). White muscle it is one of the most commonly sampled tissues because it can be non-lethal and can be sampled from multiple individuals of different size, sex or maturity to provide an integrated view of a species over ontogeny (Papastamatiou *et al.* 2010; Abrantes and Barnett 2011; Hussey *et al.* 2011, 2012).

SIA is also becoming increasingly applied in deep-sea systems (Polunin *et al*. 2001; Pethybridge *et al.* 2012; Churchill *et al.* 2015b; Shipley *et al.* 2017), because it allows to quantify ecologically significant community interactions, unique to deep-sea systems. For instance, trophic interactions among deep-sea sharks in the Gulf of Mexico allowed to understand that the stable isotopes varied as a matter of regions, ontogenic factors, time and gender (Churchill *et al*. 2015b); Pethybridge *et al*. (2012) correlated the biomagnification of total mercury levels (THg) in the deep-sea community of Southeastern Australia, with physical-chemical (bathome affinity) and community structure (presumably species composition and food chain length), and more recently Preciado *et al.* (2017) pointed out that the values of trophic position (TP) from SIA were significantly higher than the values from stomach content analysis for a seamount benthic community of deep-sea fishes on the northeast Atlantic.

In Portugal, SIA has been applied in ecological studies since the year 1984, in studies on food web dynamics involving teleosts (Vinagre *et al.* 2008b; França *et al.* 2011; Vinagre *et al.* 2011, 2012, 2015; Colaço *et al.* 2013; Farias *et al.* 2014; Rossi *et al*. 2015; Dias *et al.*, 2017). For deep-sea fauna, only a few studies were carried out off the eastern coast of Portugal along with the islands of Azores and Madeira (Correia *et al*. 2011; Colaço *et al*. 2013; Farias *et al*. 2014). Nothing was found in the literature on SIA studies of elasmobranchs or of deep-sea fauna off the southern coast of Portugal. This is relevant because it shows that there is a lack of data for SIA on deep-sea species for Portugal in general and especially for elasmobranchs.

In addition, SIA in elasmobranchs have never been associated to an indicator of nutritional and health state such as the RNA:DNA ratio. This is relevant because in order to study trophic position and dietary composition of marine organisms, it is important to assure that the sampled organisms have been feeding in the area and this can be assessed by condition and growth indicators, without the need of killing fragile and endangered animals such as sharks to conduct stomach content analysis, since only a very small portion of muscle is necessary to conduct the analysis.

Therefore, this study aims at assessing the ecophysiological condition, diet and trophic position of deep-sea sharks from the southern-west coast of Portugal combining non-lethal approaches. R/D analysis was performed to evaluate the ecophysiological condition while carbon ($\delta^{13}C$: $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$: $^{15}N/^{14}N$) stable isotope analysis (SIA) were used to access dietary and trophic position information. Specifically, the aims of this study were to:

- Assess the nutritional condition of sharks using R/D ratios;
- Identify the main preys and quantify its importance to sharks' biomass using SIA;
- Determine the trophic niche overlap between shark species;
- Determine the sharks' trophic position.

This was done with the intention to contribute to fill the extant gap and improve the knowledge on the deep-sea sharks from the southwest coast of Portugal.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 Data Collection

The Portuguese coast provides a great variety of marine and coastal habitats being located in a biogeographic transition zone between subtropical and temperate waters allowing an overlap of species limits between the northern and southern regions (Cardoso *et al.* 2019). This coast is also characterized by specific hydrographical features such as the presence of a relative warm and salty water at intermediate depths (~1000 m), resulting from the mixing between the Atlantic Intermediate Water and the Mediterranean Water (MW) flowing through the Strait of Gibraltar (Ambar 1982).

One field sampling of three days was conducted in February 2018 on board of a crustacean bottom trawler off the southwest coast of Portugal, which targets the giant red shrimp (*Aristaeomorpha foliacea*) and the scarlet shrimp (*Aristaeopsis edwardsiana*) leaving from the fisheries port of Sines, Portugal. Sampling took place between 38°07.12 N / 9°23.09 W and 37°51.19 N / 9° 33.058 W (Figure 2.1).

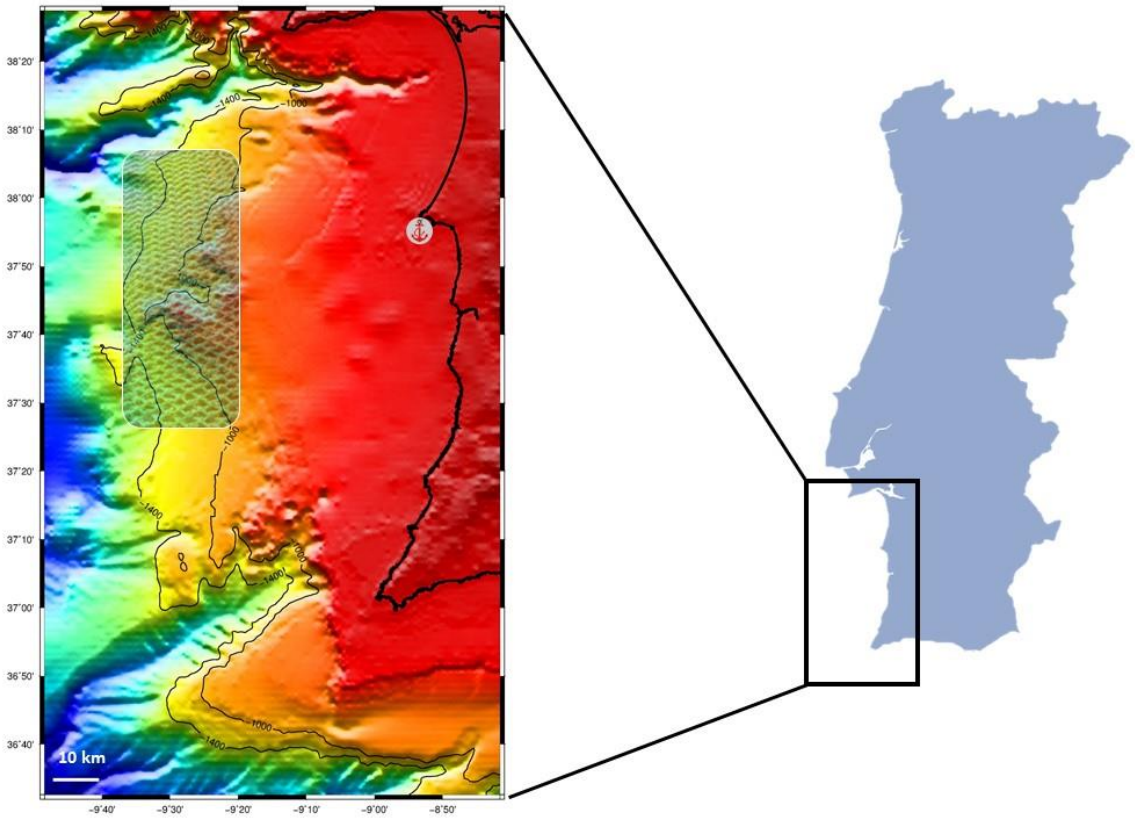

Figure 2.1: Study area at the southern-west coast of Portugal presenting the fishing port of Sines and the isobaths of sampling (Created with Mirone software).

Sharks and its potential prey were sampled using a 90 m- bottom trawl (with a meshsize of 70 mm in the codend) towed for 4 h at a velocity varying from 2.1 to 2.7 knots. Six hauls were

performed in these conditions at depths varying between 1107 and 1350 m although the data was collected from only five hauls, due to the absence of organisms of interest for this study from the haul # 4. The temperature sensor EcloThermocron™ series 415BC904000000F3 was placed inside a led lure capsule (Figure 2.2), which is used for the squid fisheries, and was attached to the mouth of the net on the inner upper side. This lure capsule is known to resist pressures around depths of 400 m and therefore the temperature was only recorded for the first two hauls (for each 5 minutes).

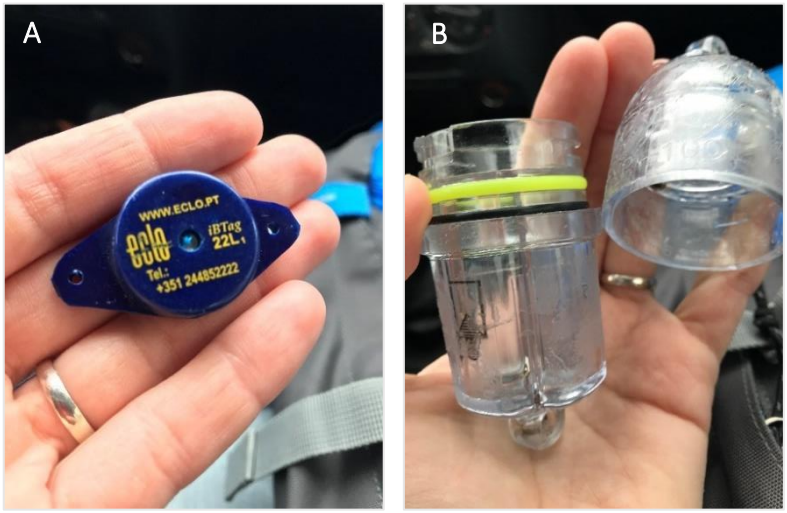

Figure 2.2: **A** EcloThermocron™ sensor; **B** led lure capsule

While the target species were being selected by the fishermen, the live sharks were selected among the by-catch species and placed inside two large containers filled with sea water (Figure 2.3). Based on a rapid external observation, sharks were analyzed following the order: 1) in good condition 2) in poor condition, and 3) dead sharks (that died during the tows). These three categories were chosen according to previous studies (Benoît *et al*. 2010; Braccini *et al*. 2012; Rodríguez-Cabello and Sánchez 2017) where:

- Good condition: strong movements and lively swimming;
- Poor condition: spiracles movement, floats in the container with water, no body movement;
- Dead: no response

Each individual was measured (total length -TL: from the tip of the snout to the tip of de caudal fin (cm) - in order to assess the life stage (adult or juvenile), weighted (g), sexed and identified onboard, or photographed for later identification whenever it was not possible to identify it immediately. Sharks were identified following the field book of Compagno *et al*. (2005). Their vulnerability was assessed through the European Union (EU) list of deep-water sharks (EU Regulation, Nº: 1182/2013) which have a zero total allowance catch (TAC) of some of the deep-

sea shark species, and also by the European Red List from the International Union of Conservation of Nature IUCN (EU 2013; Nieto *et al*. 2015).

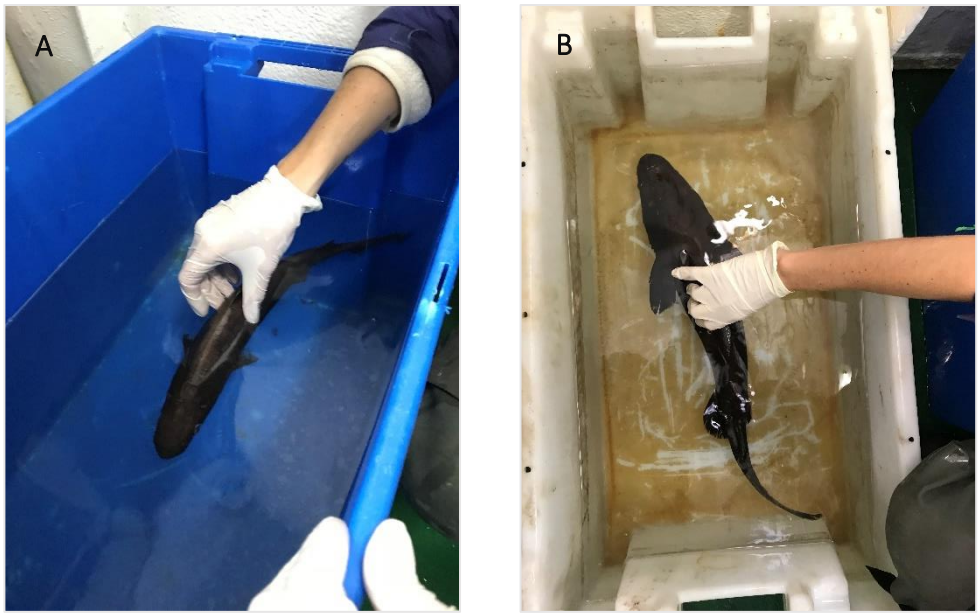

Figure 2.3: **A, B** Live sharks being handle inside the containers with sea water.

Muscle samples were collected following a modified procedure developed for teleosts by Henderson *et al*. (2016). With the help of a scalpel, a medical-grade biopsy punch (Kai Medical) of 4-mm-diameter was gently inserted at approximately 3 cm inside the muscle. First an incision was done with a scalpel next to the base of the first dorsal fin, often at the left side of the sharks body, then the biopsy punch was used to remove the tissue which was placed inside two Eppendorf's vials (Figure 2.4 A-C): one for R/D stored with RNA Riboreserve^{TM} and the other for SIA. Both type of samples were immediately frozen onboard. This procedure was conducted in order to avoid the collection of dermis since the collagen and fiber, which are the primary constituents of the dermis (Meyer and Seegers 2012), typically drive enriched ^{13}C values in relation with the diet (Kim and Koch 2012). After the removal of the tissue, the treatment of the wound was performed in two steps: first a small portion of powder from a grinded algae rich in iodine called 'cochayuyo' (*Chondracanthus chamissoi)* was placed on the top of the wound, and afterwards three drops of Betadine^{TM} were added (Figure 2.4 D). This procedure formed a viscous gel bandage to close the wound and reduce chance of infection. The entire procedure lasted 2 min maximum for the live sharks which were returned to water still alive.

Figure 2.4: Muscle collection procedure and bandage for the live sharks. **A** Incision on the skin with a scalpel; **B**, wound opened; **C** incision with the biopsy punch and **D** wound with bandage.

The sharks' potential preys were selected from the by-catch and frozen prior to laboratory analysis. Up to 5 specimens of each potential prey species were selected.

Zooplankton samples were collected in order to represent the baseline to calculate the trophic position of the organisms. To collect zooplankton, a vertical plankton net of 50 µm meshsize was towed vertically at night from a maximum depth of 80 m to the surface - due to zooplankton diel vertical migration, even the deep zooplanktonic organisms' approach more superficial waters in the absence of light. These samples were immediately fixed in ethanol 70%.

2.2 RNA/DNA ratios (R/D)

The preparation of the muscle samples prior to R/D and SIA were made at the "Fisheries, Biodiversity and Conservation" and "ECOREACH" laboratories both belonging to the University of Algarve under the responsibility of groups from the Centro de Ciências do Mar (CCMAR), Campus de Gambelas, Faro, Portugal.

The RNA and DNA were determined from muscle samples of sharks specimens and the protocol followed is a modification of methods by Caldarone *et al.* (2001) and Chícharo *et al.* (2007). The samples were defrozen and cleaned with distilled water, dried in a paper sheet and placed inside a new Eppendorf vial, which were kept frozen at - 80° C prior to lyophilization.

Samples were lyophilized at the "RX Diffraction" laboratory from University of Algarve under the responsibility of the "Centro de Investigação Marinha e Ambiental" (CIMA), under a pressure of -10 atm at - 40° C for about 36 h and afterwards they were again frozen at -80° C. Samples were weighted in order to keep the sample weight between 0.9-1.3 mg and the remainder of the sample was again placed inside the same Eppendorf flasks and kept frozen at -80° C for future analysis if needed.

After weighing, 600 µl of Sarcosina-tris (0.5%) was added to each sample which were sonicated for more or less 1 minute, for 3 pulses of 65 s intervals, placed on a vortex for 30 minutes and immediately after, centrifuged for 15 minutes at 12000 rpm at 0-4° C in a refrigerated centrifuge. In a black plate, 50 µl of each sample was placed with a duplicate, to each sample was added 120 µl of Tris Buffer, 30 µl of RNAse and 30 µl of Gel Red. For the first two columns of the plate, a calibration curve was made for RNA and DNA, and the RNAse was added to the DNA wells but not to the RNA wells. For the first round, fluorescence was read at 365 nm excitation and 590 nm emission. Then the plate was placed inside an incubator for 30 min under 37° C and again read for a second round with the same fluorescence excitation and emission. The results were generated with the help of the software Gen5^{TM} which provided the readings from both rounds. All R/D values were standardized (sRD) based on the assay specific ratio of the slopes of the standard curves (DNA slope/RNA slope), standardized to a reference slope ratio of 2.4, as described in Caldarone *et al.* (2006).

To detect statistical differences in the R/D values of the sharks a Shapiro-Wilk Test was performed to check the normality of the data in order to understand which type of analysis would have to be done. Since this data presented a normal distribution, a one-way analysis of variance was performed with the Levene's test (to check for the homogeneity of the variances) with the open source statistical language R (R Development Core Team 2007) at a 95% confidence.

2.3 Stable Isotopes Analysis (SIA)

For the stable isotopes analysis (SIA) muscle samples from sharks were dried at 60° C in an oven for at least 48 h, and ground to a fine homogeneous powder. Sharks' potential prey included teleosts, crustaceans, and cephalopods. Teleosts were identified according with Albuquerque (1956), measured (TL cm) and weighed (g). Muscle was collected from the dorsal region avoiding scales and skin. Crustaceans were identified according with Falciai and Minervini (1995), measured (cephalothorax length, cm), weighted (g). A piece of tail muscle was collected for shrimp and lobsters, while leg muscle was collected for crabs, in order to

avoid the chitin which is rich in carbon and may bias the results. Cephalopods were identified with the help of Roper *et al*. (2010) and Jereb *et al*. (2016), measured (mantle length, cm), and weighted (g). Muscle samples were collected from the mantle (ooegopsida, squids) or from the appendices (octopoda, octopuses). Zooplankton was processed as a whole following procedures from Cartes *et al*. (2007). The samples were sorted and copepods were separated and placed directly into the tin capsules. The tin capsules were also dried at 60° C in an oven for at least 48 h. Stable isotope ratios were measured using a continuous flow isotope mass spectrometry (CF-IRMS) (Preston and Owens 1983), on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyzer for online sample preparation by Dumas-combustion (Stable Isotopes and Instrumental Analysis Facility" (SIIAF) at the University of Lisboa - Portugal). Stable isotope ratios were reported in δ notation (Eq 1):

(Eq 1) $$\delta X = (R_{sample}/R_{standard} - 1) \times 10^3$$

where X is the C or N stable isotope, *R* is the ratio of heavy:light stable isotopes. Pee Dee Belemnite and air are standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The analytical error, the mean standard deviation of replicate reference material, was 0.08‰ for $\delta^{13}C$ and 0.25‰ for $\delta^{15}N$ values. The reference materials used were USGS-25, USGS-35, BCR-657 and IAEA-CH7 (Coleman and Meier-Augenstein 2014); the laboratory standard was Protein Standard OAS/Isotope Elemental Microanalysis, UK. Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of laboratory standard (Protein Standard OAS/Isotope), interspersed among samples in every batch analysis, was ≤ 0.1‰. The major mass signals of N and C were used to calculate total N and C abundances, using Protein Standard OAS (Elemental Microanalysis, UK, with 13.32%N, 46.5%C) as elemental composition reference material. A replicate of the samples was performed for the species with only one individual and the average between one round to the second was presented. This was done in order to guarantee the degree of credibility of the results obtained.

Lipids are depleted in ^{13}C when compared to protein and carbohydrates (DeNiro and Epstein 1977) and therefore, consumers $\delta\ ^{13}C$ values require correction. Because it was not possible to conduct lipid extraction on the samples collected during this study, muscle tissue data from prey species with $C:N_{bulk} > 3.5$ (Post *et al.* 2007) were corrected for lipid content (Eq 2) following the mass balance correction for fish muscle proposed by Hoffman and Sutton (2010-Eq. 6), which uses estimates of $C:N_{protein}$ and $\Delta\delta^{13}C_{lipid}$ that are similar to those from the muscle

tissue found for other fish (e.g., Sweeting *et al*. 2006) and taxonomic groups (e.g., shrimp and zooplankton; Smyntek *et al.* 2007) although this one is particular for deep-sea fishes.

(Eq 2) $$\delta^{13}C_{PTN} = \delta^{13}C_{bulk} + (-6.39\ ‰ \times (3.76 - C{:}N_{bulk}))/C{:}N_{bulk}$$

where $\delta^{13}C_{PTN}$ is the lipid free value after mathematical correction, and the $\delta^{13}C_{bulk}$ and $C{:}N_{bulk}$ the values before lipid correction.

All the sharks species presented at least one individual with a C:N > 3.5, therefore, they had their lipid corrected by the above equation (Eq 2), following the same procedure performed by Barría *et al*. (2015). However, mathematical corrections of lipids are not encouraged to correct lipids for elasmobranchs tissue (Shipley *et al.* 2017) unless a relationship between C:N values and the change in carbon isotope values is proven (Post *et al.* 2007). Therefore, sharks' $\delta^{13}C$ values were plotted against C:N, to see if there was a potential effect of lipids on its values (DeNiro and Epstein 1977). Also, $\delta^{13}C_{bulk}$ values were compared with those resulting from mathematical correction (Eq 2), using a t-test. Since this relation was not significant ($p > 0.05$), $\delta^{13}C_{bulk}$ values were used in the following analyses.

Since the zooplankton samples were fixed in ethanol, values were corrected according with Feuchtmayr and Grey (2003) ($\delta^{13}C$: -0.4 and $\delta^{15}N$: -0.6).

To test for possible differences in the $\delta^{13}C$ and $\delta^{15}N$ values between species, the one-way permutational multivariate analysis of variance (PERMANOVA) was performed using PRIMER v 6.1.11® (Clarke and Gorley 2006) with PERMANOVA+1.0.1. add-on package (Anderson 2008). PERMANOVA tests the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods (Anderson 2001). This means that the probabilities are generated by permutation and randomization of the data. The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. Whenever the number of the possible permutations was lower than 150, the Monte Carlo-p value (pMC) was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons, to determine which pairs of species were significantly isotopically different assuming a significance level of $p < 0.05$.

The $\delta^{13}C$ and $\delta^{15}N$ bi-plots were used to examine and choose the potential prey for sharks. To quantify the proportional contribution of each source to sharks' biomass, a dual-stable isotope mixing model that uses Bayesian inference to solve indeterminate linear mixing equations (i.e. for two stable isotope ratios and more than three diet sources) was used. For modeling purposes, prey were grouped according to a cluster routine combined with ecological information on

habitat use and feeding behavior. Indeterminate linear mixing equations produce a probability distribution that represents the likelihood of a given source to contribute to the consumer's diet (Parnell *et al*. 2010). The model Stable Isotope Analysis in R (SIAR) was used because it allows each of the sources and the trophic enrichment factor (TEF; or trophic fractionation) to be assigned a normal distribution (Parnell *et al.* 2010). SIAR produces the distribution of feasible solutions to the mixing problem and estimates credibility intervals (95% CI), which is analogous to the confidence intervals used in frequentist statistics. SIAR also includes a residual error term. In the SIAR mixing model, the $\delta^{13}C$ and $\delta^{15}N$ values were adjusted for one or two trophic levels using the TEF estimates from Hussey *et al.* (2010), which were obtained from controlled experiments in aquaria with lemon sharks (*Negaprion brevirostris*) and sand tiger sharks (*Carcharias taurus*): $\Delta^{15}N$ 2.3 ± 0.22 and $\Delta^{13}C$ 0.9 ± 0.33.

The trophic niche size of each shark species and overlap between species were estimated using SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson *et al*. 2011). SIBER fits bi-variate ellipses to stable isotope data using Bayesian inference to describe and compare isotopic niches. Several parameters were used to describe and compare the trophic niche of each species: SEAc (corrected standard ellipse area) and TA (total area). Trophic niche overlap was estimated after assigning the maximum likelihood fitted standard ellipses area estimates between two ellipses considering a probability of 95%. A Bayesian model was fitted to data to estimate the standard ellipse area for each species. As with SIAR, the model used 10^4 posterior draws from a Markov chain Monte Carlo simulation to estimate the SEA. The model output plots display measures of uncertainty and central tendency, including the mode, the 50th, and the 95th percentiles.

To determine the trophic position of each shark species, a common equation was used (Eq 3):

(Eq 3) $$TP = \lambda + \frac{(\delta^{15} N_{consumer} - \delta^{15} N_{base})}{\Delta_n}$$

where λ is the trophic position of the organisms used to estimate the $\delta\ ^{15}N_{base}$, in this case the copepods, which are assumed to belong to the trophic position 2; Δ_n is the TEF in ^{15}N per trophic level, which was 2.3 (Hussey *et al.*, 2010) for sharks, 3.2 for teleosts and 3.4 for invertebrates (crustacean and cephalopods) (VanderZanden and Rasmussen 2001); the $\delta\ ^{15}N_{consumer}$ is the direct measurement of the $\delta\ ^{15}N$ for the sharks and potential preys.

All other statistical analyses were performed either in 'R' (version 3.0, R Development Core Team, 2007), Primer, or Excel 2016 and the results are normally expressed as average ± SD (standard deviation) unless otherwise pointed out.

CHAPTER 3

RESULTS

3. RESULTS

3.1 Sharks and potential preys

A total of 96 individuals from 27 different taxa were sampled in this study (Figure 0.1 in the ANNEX B). All individuals were found at depths ranging between 1.107 and 1.350 m, after performing diurnal and nocturnal hauls. The temperature varied between 10.8 and 11.8 °C (diurnal measurements). Sharks (infraclass Selachii) accounted for 39 individuals belonging to 2 orders, 5 families, and 7 different species, followed by Teleostei (bone fishes) with 36 individuals from 9 orders, 12 families, and 18 species; Crustacea (crabs, shrimps, lobsters and zooplankton) with 18 individuals from 3 orders, 5 families, and 6 species; and also Cephalopoda (squids and octopuses). with 6 individuals from 2 orders, 4 families, and 4 species Zooplankton was pooled in two samples containing only copepods (Figure 3.1 and Table 0.1 of ANNEX A). Pictures from the species sampled are in the ANNEX B.

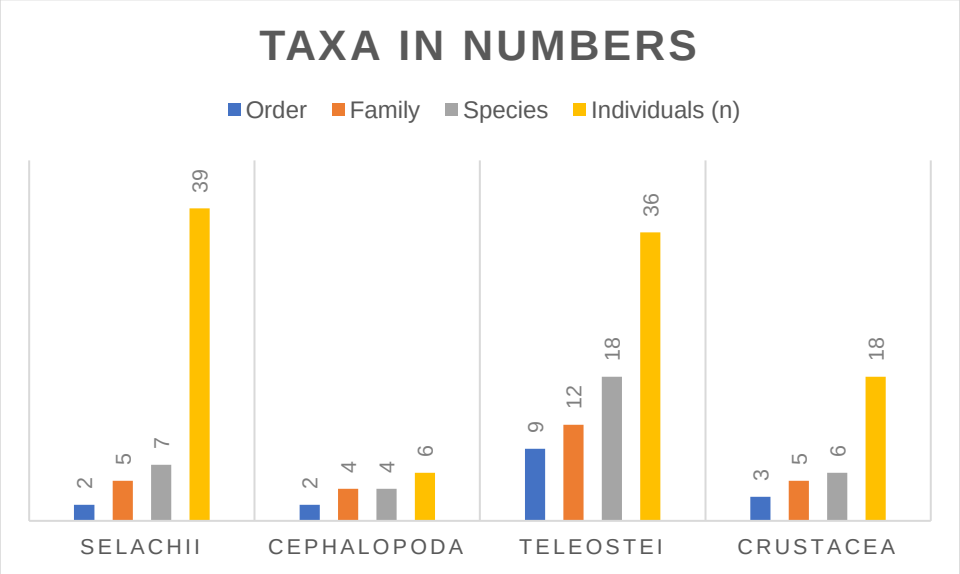

Figure 3.1: Taxa sampled in this study. The consumers (sharks) are Selachii and the sources include the taxa Cephalopoda, Teleostei and Crustacea with its respective orders (excluding zooplankton copepods), families, species and individuals (n).

Shark species found in the area were the leafscale gulper shark *Centrophorus squamosus* (Bonnaterre, 1788), the longnose velvet dogfish *Centroselachus crepidater* (Barbosa du Bocage & de Brito Capello, 1864), birdbeak dogfish *Deania calcea* (Lowe, 1839), arrowhead dogfish *D. profundorum* (Smith & Radcliffe, 1912), smooth lantern sharks *Etmopterus pusillus* (Lowe, 1839), Atlantic sawtail catshark *Galeus atlanticus* (Vaillant, 1888), and the knife tooth dogfish *Scymnodon ringens* (Barbosa du Bocage & de Brito Capello, 1864).

Only two specimens from *Centrophorus squamosus* were collected, during this study. Both were mature males, one in a very poor condition and the other was dead, with an average (±SD)

size of 101.5 ± 0.5 cm, and an average (±SD) weight of 7.7 ± 3.25 kg (Table 3.1). Also, two specimens from *C. crepidater* were collected, one was a juvenile male and other a pregnant female, which gave birth to five dead offspring's onboard. Both *C. crepidater* individuals were dead and had an average total length and weight (±SD) of 73 ±12 cm and 1.5 ± 0.5 kg respectively (Table 3.1). *Deania calcea* was the second most representative species collected during this study presenting three males (two adults and one juvenile) and six females (all juveniles). Five individuals were in a poor condition and four were dead. They presented an average total length and weight (±SD) of 78.5 ± 14.5 cm and 2.3 ± 1.8 kg, respectively (Table 3.1). *Deania profundorum* were all juvenile females, one was in a poor condition and the other three came onboard already dead. They presented an average (±SD) of 44.3 ± 6.1 cm total length and 287 ± 98.3 g. *Etmopterus pusillus* had two adult males and three females (1 adult and 2 juveniles), one of the males was in a good condition and other individuals were dead exhibiting an average size (±SD) of 41.5 ± 3.2 cm and weighing 332 ± 74.4 g. *Galeus atlanticus,* like *D. profundorum*, also presented only females, although they were all composed of adult individuals (three dead and two in a poor condition) measuring an average (±SD) total length of 61.8 ± 5.6 cm and weighing an average (±SD) 678 ± 168.7 g. At last, *S. ringens* was the most representative species with four males and eight females- Eight individuals were in a poor condition and the rest of them were dead. The life stage of the individuals could not be specified since there is no information available in the literature regarding their life stage classification. They had on average (± SD) 57 ± 10.3 cm and 1.2 ± 0.8 kg and weighing on average (±SD) 678 ± 168.7 g (Table 3.1). At last, *S. ringens* was the most representative species with four males and eight females. Eight individuals were in a poor condition while the remaining were dead. The life stage of the individuals could not be specified since there is no information available. A complete list of the information obtained for each individual is provided in the ANNEX A Table 0.3.

Table 3.1: Shark species according with number of individuals (n), average (±SD) total length (TL, cm) and weight (g); gender (male or female); number of adults (A) and juveniles (J) and unknown life history (N/A); condition of the individuals: good (G), poor (P) and dead (D) is also presented.

Species	n		TL (cm)	Weight (g)	Gender (n)	Life stage (n)	Condition (n)
C. squamosus	2	Cs	101.5 ± 0.5	7675 ± 325	Males (2)	A (1)	P(1) D (1)
C. crepidater	2	Cc	61	1000	Males (1)	J (1)	D (1)
			85	2040	Females (1)	A (1)	D (1)
D. calcea	9	Dc	84.3 ± 2.5	2066.7 ± 124.7	Males (3)	A (2) J (1)	P (1) D (2)
			75.6 ± 16.9	2428.3 ±2237	Females (6)	J (6)	P (4) D (2)
D. profundorum	4	Dp	44.3 ± 6.1	287.5 ± 98.3	Females (4)	J (4)	P (1) D (3)
E. pusillus	5	Ep	41 ± 0	315 ± 35	Males (2)	A (2)	G (1) D (1)
			41.8 ± 4.1	343.3 ± 89.9	Females (3)	A (1) J (2)	D (3)
G. atlanticus	5	Ga	61.8 ± 5.6	678 ± 168.7	Females (5)	A (5)	P (2) D (3)
S. ringens	12	Sr	51.9 ± 2.3	957.5 ± 316.7	Males (4)	N/A	P (2) D (2)
			59.5 ± 11.7	1382 ± 958.7	Females (8)	N/A	P (6) D (2)

Most of the sharks collected during this study were females (69%) accounting for 27 individuals, while males comprised 31% of the sharks collected, corresponding to 12 individuals (Figure 3.2). The majority of the females were juveniles (44%), while 26% were adults, and 30% could not have its life stage determined (females of *Scymnodon ringens*). In contrast, the majority of the male sharks were adults (50%), a minority were juveniles (17%) and the males from *S.ringens* could not have the life stage determined (33%).

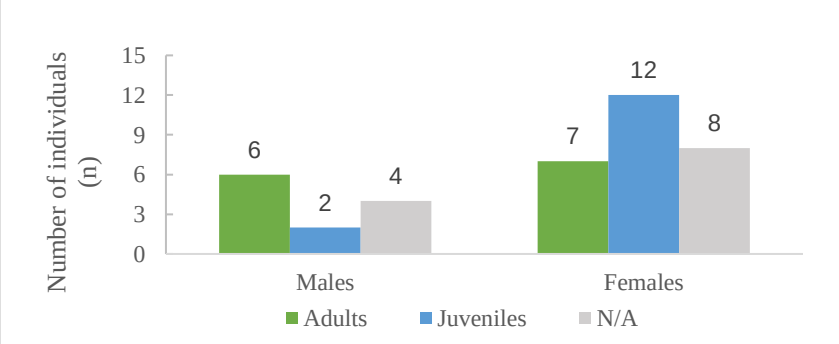

Figure 3.2: Number of adults and juvenile sharks collected in this study, according to gender (male and female) The life stage of individuals from the *S. ringens* could not be determined (N/A).

Four of the shark species in this study belong to the European Union (EU) list of deep-water sharks (EU Regulation, Nº: 1182/2013), which have a zero total allowance catch (TAC): *C. squamosus*, *C. crepidater*, *D.calcea*, and *S. ringens*; nonetheless, *C. crepidater* and *S.ringens* are on the European Red List of IUCN (Nieto *et al*. 2015) as 'Least concern'; *E. pusillus* and *D. profundorum* are 'Data deficient', *G. atlanticus* is 'Near threatened' and *D. calcea* and *C. squamosus* are already 'Endangered' (Figure 3.3).

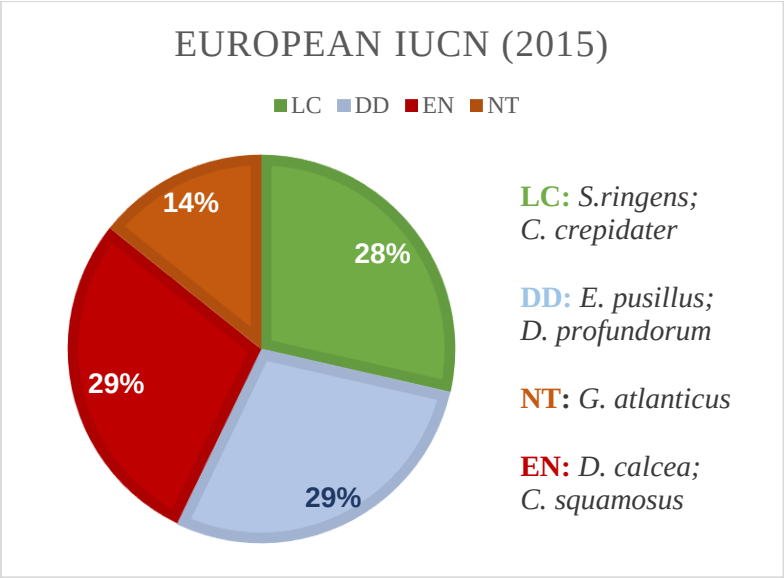

Figure 3.3: European IUCN status of the sharks collected in the present study. LC, least concern; DD, data deficient; NT, near threatened; EN, endangered.

3.2 Nutritional condition characterization

Nucleic acids were analyzed for 37 sharks' individuals. The values of RNA (mg), DNA (mg) and the standardized R/D (sRD) along with the size (total length in cm) for each individual are represented in Table 3.2. The species with the best nutritional condition was the species *Galeus atlanticus* with an average (±SD) R/D of 0.63 ± 0.15, followed by the species *Deania calcea* (0.44 ± 0.2) and *Etmopterus pusillus* (0.44 ± 0.11) (Table 3.2). The species with the lowest R/D ratios average was *D. profundorum* 0.26 ± 0.09 (Table 3.2). It is also possible to assess information of all sharks' individuals and parameters such as standardized R/D ratios in the Table 0.3 from the ANNEX A.

Table 3.2: Species size (average ± SD), average values for RNA and DNA given in mg also, average (±SD) for sRD.

Species	Size (cm)	RNA/mg	DNA/mg	sRD
Centrophorus squamosus	101.5 ± 0.5	1.47	2.48	0.38 ± 0.05
Centroselachus crepidater	73 ± 12	2.55	5.61	0.28 ± 0.09
Deania calcea	78.5 ± 14.5	2.20	3.33	0.44 ± 0.20
Deania profundorum	44.3 ± 6.1	1.52	4.07	0.26 ± 0.09
Etmopterus pusillus	41.6 ± 3.2	3.07	4.79	0.44 ± 0.11
Galeus atlanticus	61.8 ± 5.6	2.61	2.75	0.63 ± 0.15
Scymnodon ringens	57 ± 10.3	2.01	3.53	0.38 ± 0.21

The dataset of sharks' nucleic acids index with the size (total length, cm) is presented in the Figure 3.4 (A-C). It was possible to see that RNA/mg and DNA/mg decreased with size, and those trends indicate the normal decrease in growth and the increase in cell size with increase in size, although not significantly, $p = 0.4$ and $p = 0.8$ respectively. Also, the R/D ratios with size (cm), were not statistically significant ($p > 0.05$). This means that the size does not influence significantly the nucleic acids indices for this particular group of individuals sampled. The R/D analysis (Figure 3.4 C) allows the interpretation of the nutritional condition of each individual. Additional information on the sharks such as gender, size, and weight is present in the Table 0.3. Higher values of R/D indicate that sharks are in a better nutritional state. The individual 31 of *S. ringens*, a female, was in a better condition than all of the other individuals from the same species and all other species combined. Similar condition was observed for the individual 49 (adult female of *G. atlanticus*) and 28 (juvenile female of the species *D. calcea*). The individual with the lowest R/D was individual 30, a male from the species *S. ringens* (Figure 3.4 C). Likewise, none of the species presented a clear correlation between size and R/D as previously mentioned (Figure 3.4 C). For example, the smallest sharks from the species *D.calcea* such as the individual 28, was in a better condition than larger individuals from the same species like 24 (female juvenile), 37 (male adult) and 38 (female juvenile) (Figure 3.4 - check also Table 0.3 from ANNEX A).

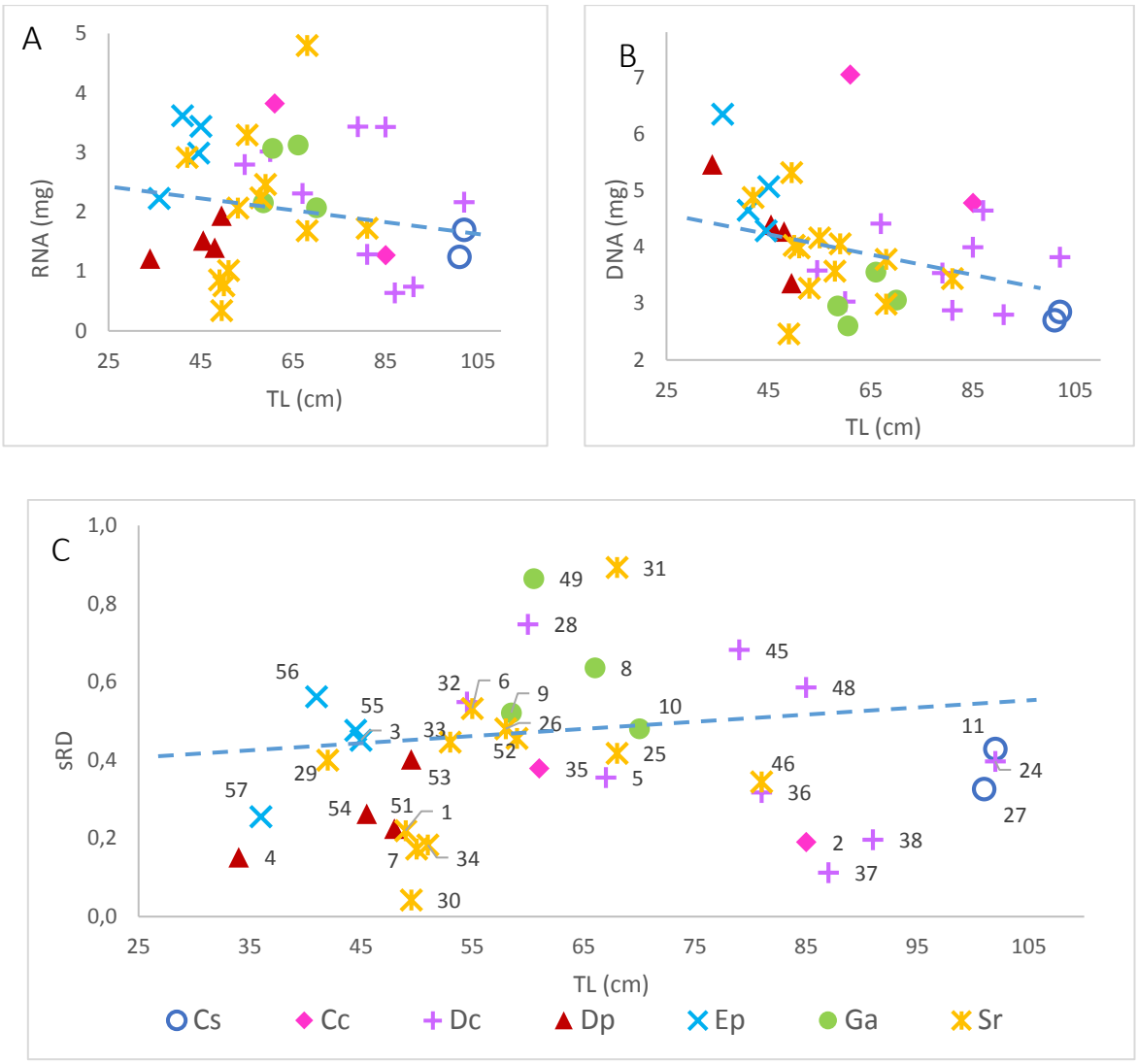

Figure 3.4: Scatter plot of individuals standardized nucleic acid ratios (sRD) with the animal's size. Observed values in relation to the animal's size (total length in cm), adjusted linear model (doted blue line). **A** RNA (mg); **B** DNA (mg); and **C** is the sRD. Cs, *Centrophorus squamosus*; Cc, *Centroselachus crepidater;* Dc, *Deania calcea*; Dp, *D.profundorum*; Ep, *Etmopterus pusillus*; Ga, *Galeus atlanticus* and Sr, *Scymnodon ringens*.

A critical step in the interpretation of consumers and preys' $\delta^{13}C$ values is whether to correct/extract lipids. If a significant ($p < 0.05$) inverse relationship between $C:N_{bulk}$ and $\delta^{13}C_{bulk}$ is found, than the $\delta^{13}C$ lipid corrected ($\delta^{13}C_{PTN}$) values have to be used instead of the $\delta^{13}C_{bulk}$ (DeNiro and Epstein 1977). Therefore, sharks' $\delta^{13}C$ values were plotted against C:N, to see if there was a potential effect of lipids on its values. The Figure 3.5 elucidates the results from the linear models $C:N_{bulk}$ and $\delta^{13}C_{bulk}$ for the sharks. There is an inverse trend for the species *E. pusillus* and *G. atlanticus*, although not significant ($p > 0.05$), and therefore, the $\delta^{13}C_{bulk}$ values were used in the following analysis.

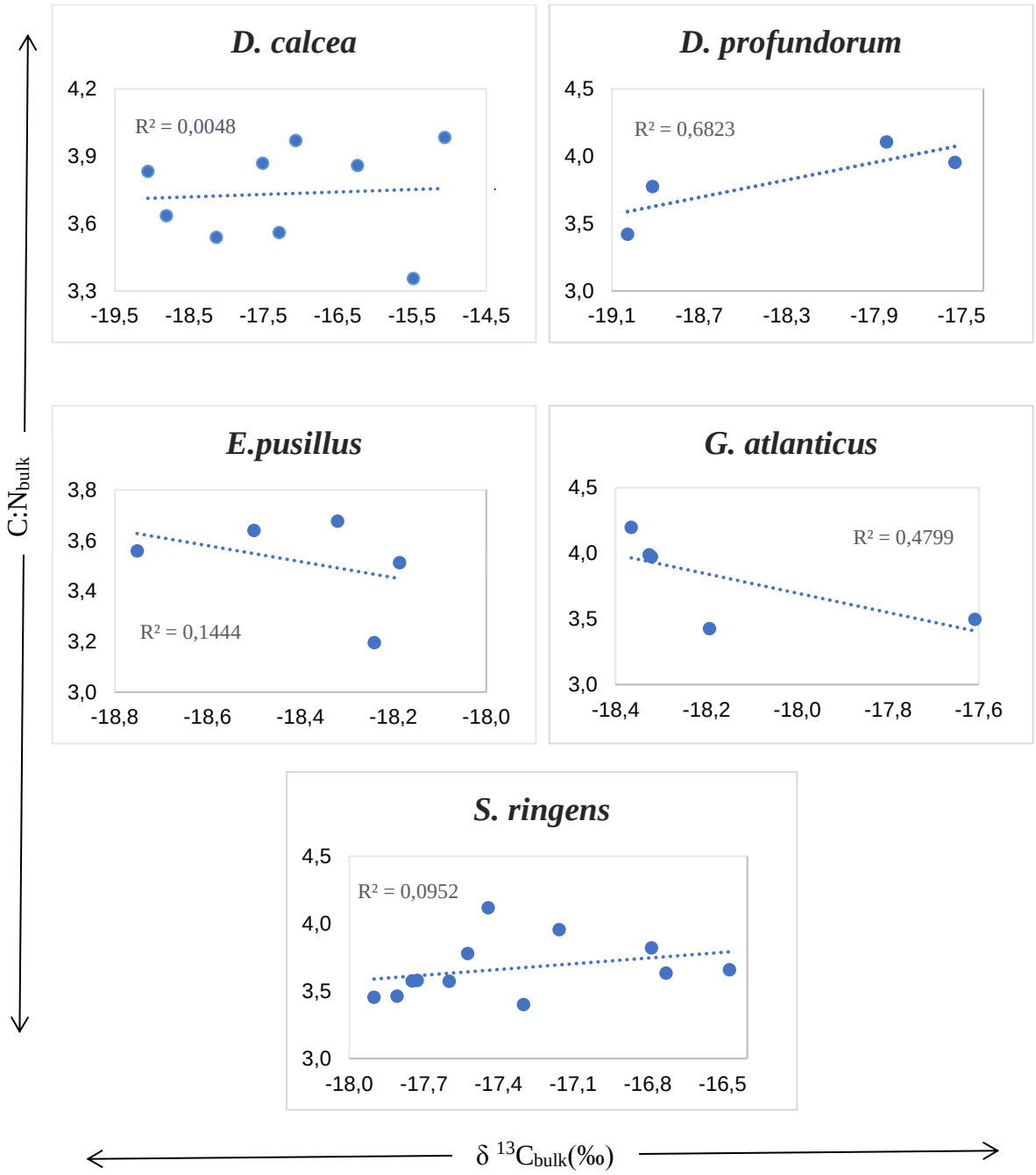

Figure 3.5: Linear regression between C:N_{bulk} and δ $^{13}C_{bulk}$(‰) for each shark species.

Table 3.3 shows the average (±SD) values of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰), the ratio C:N and the number of individuals (n) for each shark species. The $\delta^{13}C_{PTN}$ (‰) for each species are only displayed to indicate that these values are, in fact, pretty similar to the $\delta^{13}C_{bulk}$. The $\delta^{15}N$ values varied between 10.3 and 14.2‰ and $\delta^{13}C_{bulk}$ between - 19.1 and - 15.1‰ (Table 3.3). *Centrophorus squamosus* presented the highest average $\delta^{15}N$ values (13.6 ± 0.2‰) although one individual from *C. crepidater*, an adult pregnant female presented the highest of them all ($\delta^{15}N$: 14.2‰, Figure 3.3). *Deania profundorum* had the lowest averages of $\delta^{15}N$ 10.6 ± 0.3‰ (Table 3.3). The species with the highest $\delta^{13}C_{bulk}$ values was *C. squamosus* with an average

(±SD) of - 16.3±0.5‰, whereas the individual with the highest $\delta^{13}C_{bulk}$ value was the same pregnant female of *C. crepidater* with - 16‰. The species with the highest $C:N_{bulk}$ values were *C. squamosus, C.crepidater, D.profundorum,* and *G. atlanticus* which presented an average (±SD) 3.8 ± 0.3; on the other hand the species presenting the lowest values was *E. pusillus* with 3.5 ± 0.2 (Table 3.3).

Table 3.3: Number of individuals sampled from each shark species, average and standard deviation of isotopic values of $\delta^{15}N$ (‰) and $\delta^{13}C_{bulk}$ (‰) of the sharks' species. $\delta^{13}C_{bulk}$ (‰) is the ratio before lipid correction and $\delta^{13}C_{ptn}$ (‰) is the ratio after mathematical lipid correction.

Shark species	n	$\delta^{15}N$ (‰)	$\delta^{13}C_{bulk}$(‰)	C:N	$\delta^{13}C_{ptn}$(‰)
C. squamosus	2	13.6 ± 0.2	-16.3 ± 0.1	3.8 ± 0.3	-16.3 ± 0.5
C. crepidater	2	12.7 ± 1.5	-16.9 ± 0.9	3.8 ± 0.1	-16.9 ± 1.1
D. calcea	9	12.0 ± 0.8	-17.2 ± 1.3	3.7 ± 0.2	-17.2 ± 1.4
D. profundorum	4	10.6 ± 0.3	-18.3 ± 0.7	3.8 ± 0.3	-18.3 ± 1.0
E. pusillus	5	11.3 ± 0.1	-18.3 ± 0.7	3.5 ± 0.2	-18.9 ± 0.3
G. atlanticus	5	11.4 ± 0.2	-18.2 ± 0.3	3.8 ± 0.3	-18.1 ± 0.4
S. ringens	12	12.6 ± 0.3	-17.3 ± 0.5	3.7 ± 0.2	-17.5 ± 0.7

The shark with the highest $\delta^{15}N$ value was the number 2, belonging to *C. crepidater*, with 14.2‰ (Figure 3.6). The other individual collected from this species is represented by the number 35, and its $\delta^{15}N$ value is lower: 11.3‰ (Figure 3.6). The individual 2 ($\delta^{13}C$: - 16.0‰) was also ^{13}C- enriched in relation to 35 ($\delta^{13}C$: - 17.7‰) (Figure 3.6). While 2 represents an adult female (pregnant), 35 represents a juvenile male (Table 0.3, ANNEX A). The two individuals of *C. squamosus*, 11 and 27, showed similar isotopic values ($\delta^{15}N$: 13.3‰ and 13.8‰; $\delta^{13}C$: - 16.4‰ and - 16.2‰ respectively - Figure 3.6), both were adult males. The *D. calcea* presented the widest amplitude for $\delta^{13}C$ values varying between -15.1‰ and -19.1‰ (Figure 3.6). Also, this species presented the highest (- 15.1‰) and the lowest (- 19.1‰) $\delta^{13}C$ values among all the sharks sampled (Figure 3.6). The highest $\delta^{13}C$ value for *D. calcea* was from the individual 36, a juvenile male which also presented one of the highest $\delta^{15}N$ value (12.8‰) for this species, along with the juvenile female 24 (Figure 3.6). On the other hand, individual 32 from *D. calcea* (also a female juvenile) presented the lowest values of $\delta^{13}C$ and $\delta^{15}N$ (- 19.1‰ and 10.6‰ respectively). *Deania profundorum* presented a small variation in the $\delta^{15}N$ values varying between 10.3‰ and 11.1‰ (Figure 3.6). Nonetheless, the $\delta^{13}C$ values presented higher variations varying between - 17.5‰ and - 19.0‰ (Figure 3.6). The specimens belonging to this species were all juvenile females. The smallest individual, the individual 4, had 34 cm and weighted 120 g whilst the other females measured more than 45 cm and weighted more than 320 g (Table 0.3, ANNEX A). The individuals belonging to *E. pusillus* presented

very little variation in both $\delta^{15}N$ and $\delta^{13}C$ values although it included juveniles and adults males and females (Table 0.3, ANNEX A). A similar pattern was observed for *G. atlanticus* where all were adult females presenting little variation in the $\delta^{15}N$ and $\delta^{13}C$ values, with the exception of the individual 49 which was more ^{13}C- enriched than the others (- 17.6‰, Figure 3.6) . Despite having the highest number of individuals, *S. ringens* presented small variation in the $\delta^{15}N$ values, from 12.1‰ to 13‰, and in the $\delta^{13}C$ values, varying between - 17.9‰ and - 16.5‰ (Figure 3.6). The lowest $\delta^{13}C$ values was presented by the smallest individual (29) a female with 42 cm and a weight of 450 g (- 17.9‰) and the highest value (- 16.5‰) belonged to the largest and heavier individual (46) a female with 81 cm of length and 3700 g of weight (Table 0.3, ANNEX A).

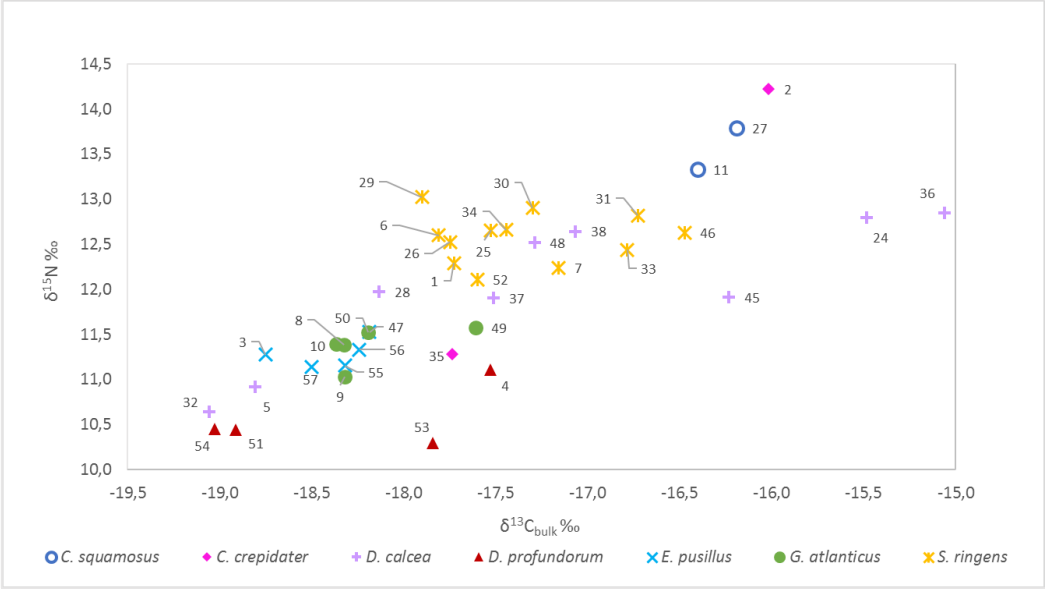

Figure 3.6: Sharks' $\delta^{13}C$ and $\delta^{15}N$ values . Numbers corresponds to the code of each individual which can be found in the Table 0.3 in ANNEX A.

PERMANOVA main test to the $\delta^{13}C$ and $\delta^{15}N$ values of all sharks collected indicate that the shark species are significantly different ($p < 0.05$, Table 3.4). Inter- specific differences in $\delta^{13}C$ and $\delta^{15}N$ values were found between *Centrophorus squamosus* and *Deania profundorum* (pMC = 0.002), *Etmopterus pusillus* (pMC = 0.0001), *Galeus atlanticus* (pMC = 0.0004), and *Scymnodon ringens* (pMC = 0.0434) (Table 3.4). *C. squamosus* presented significantly higher values than *D. profundorum* (Figure 3.7). *Deania calcea* differed from *D. profundorum* (pMC = 0.045) (Table 3.4) in terms of $\delta^{15}N$, nonetheless the $\delta^{13}C$ does not appear to have a significant variation between both species (Figure 3.7); *D. profundorum* was significantly different from *G. atlanticus* (pMC = 0.045) and *S. ringens* (pMC = 0.003) which presented lower values for both $\delta^{13}C$ and $\delta^{15}N$ although for the later this difference seems to be greater than with $\delta^{13}C$

which does not seems to exist in comparison with *G. atlanticus* Figure 3.7. Also, *S. ringens* was different than *E. pusillus* (pMC = 0.01) and *G. atlanticus* (pMC < = 0.03) (Table 3.4) in presenting higher values for both $\delta^{13}C$ and $\delta^{15}N$ (‰) (Figure 3.7).

Table 3.4: Results of PERMANOVA test for differences of $\delta^{13}C$ and $\delta^{15}N$ values between different shark species. Cs *Centrophorus squamosus*; Dc *Deania calcea*; Dp *Deania profundorum*; Ep *Etmopterus pusillus*; Ga *Galeus atlanticus*; Sr *Scymnodon ringens*. * species significantly different $p < 0.05$

	t	p(perm)	perm	p(MC)
MAIN TEST				
All Species		0.011*	9939	0.001*
PAIR-WISE				
Cs - Cc	0.575	1	3	0.628
Cs - Dc	1.551	0.167	55	0.140
Cs - Dp	5.747	0.067	15	0.002*
Cs - Ep	12.509	0.049	21	0.000*
Cs - Ga	9.200	0.048	21	0.000*
Cs - Sr	2.166	0.108	91	0.043*
Cc - Dc	0.612	0.596	55	0.613
Cc - Dp	2.159	0.070	15	0.081
Cc - Ep	2.249	0.047	21	0.070
Cc - Ga	1.964	0.094	21	0.110
Cc - Sr	0.803	0.335	91	0.471
Dc - Dp	2.185	0.044	714	0.045*
Dc - Ep	1.933	0.068	1985	0.072
Dc - Ga	1.583	0.133	1987	0.136
Dc - Sr	0.870	0.427	9775	0.422
Dp - Ep	1.901	0.051	126	0.052
Dp - Ga	2.073	0.056	126	0.045*
Dp - Sr	3.297	0.005	1807	0.003*
Ep - Ga	1.199	0.266	126	0.263
Ep - Sr	2.723	0.012	4960	0.010*
Ga - Sr	2.286	0.025	4929	0.030*

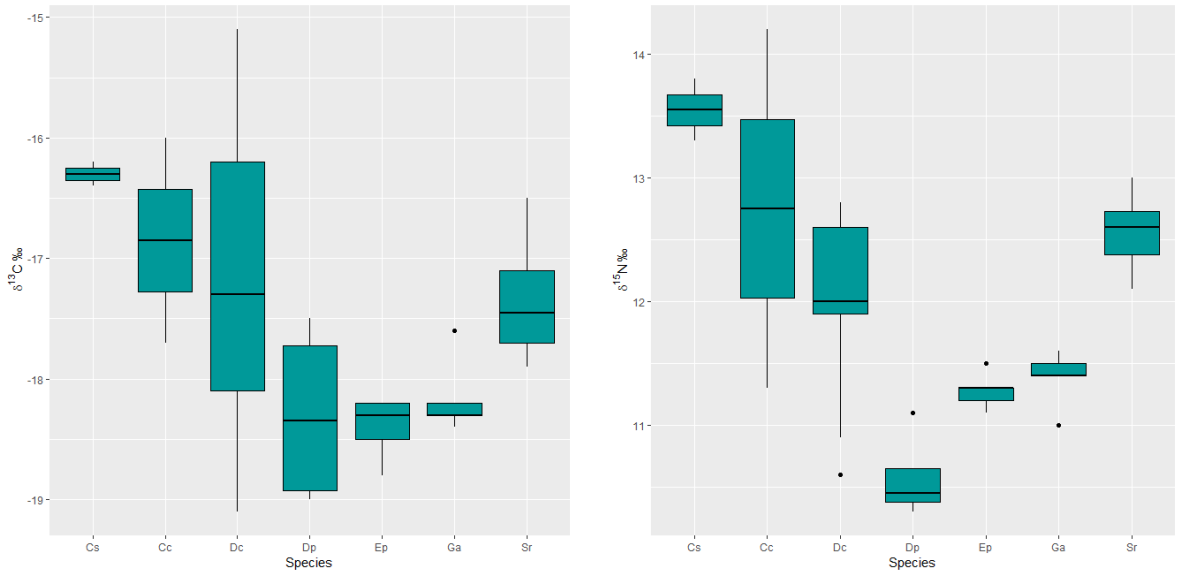

Figure 3.7: Boxplot with the isotopic ratios from all shark species. The horizontal black line inside the boxplot is the medium value, the standard deviation is represented by a vertical black line and inside the green bloxpot the minimum quartile is 25% and maximum quartile of 75%. **A**, $\delta^{13}C$ (‰) and **B**, $\delta^{15}N$ (‰). Cs, *Centrophorus squamosus*; Cc, *Centroselachus crepidater;* Dc, *Deania calcea*; Dp, *D.profundorum*; Ep, *Etmopterus pusillus*; Ga, *Galeus atlanticus* and Sr, *Scymnodon ringens*.

3.3 Food-web characterization

Identification and quantification of the main preys

A total of 53 potential preys were collected along with copepods (zooplankton) representing the baseline organisms for the characterization of the pelagic trophic web. The values of $\delta^{15}N$ varied between 8.9‰ and 14.1‰. The lowest value of $\delta^{15}N$ belongs to *Nephropsis atlantica* (Na) a crustacean species, with an average (±SD) of 8.9 ± 0.2‰. The highest $\delta^{15}N$ value belonged to *Gadomus arcuatus* (Gar) a Teleostei species, with an average (±SD) $\delta^{15}N$ value of 14.1 ± 0 ‰ (Figure 3.8). The $\delta^{13}C$ of the species varied between - 20.6‰ and - 17.5‰. The lowest $\delta^{13}C$ belongs to *Mastigoteuthis* sp. (Mm) a cephalopod species,with an average (±SD) $\delta^{13}C$ value of - 20.6 ± 0.3‰ (Figure 3.8). The Teleostei *Trachyrinchus scabrus* (Ts) presented the highest average (±SD) $\delta^{13}C$ value of -17.5 ± 0.7‰ (Figure 3.8). Those values were taken into consideration without the copepods (zoop) which presented an average (±SD) $\delta^{15}N$ and $\delta^{13}C$ of 4.8 ± 0.3‰ and - 20.7 ± 0.1‰ respectively (Figure 3.8). For a better understanding of this entire dataset, there is additional information about all the individuals at the Table 0.4 from the ANNEX A as well as the pictures of each species representative at the ANNEX B.

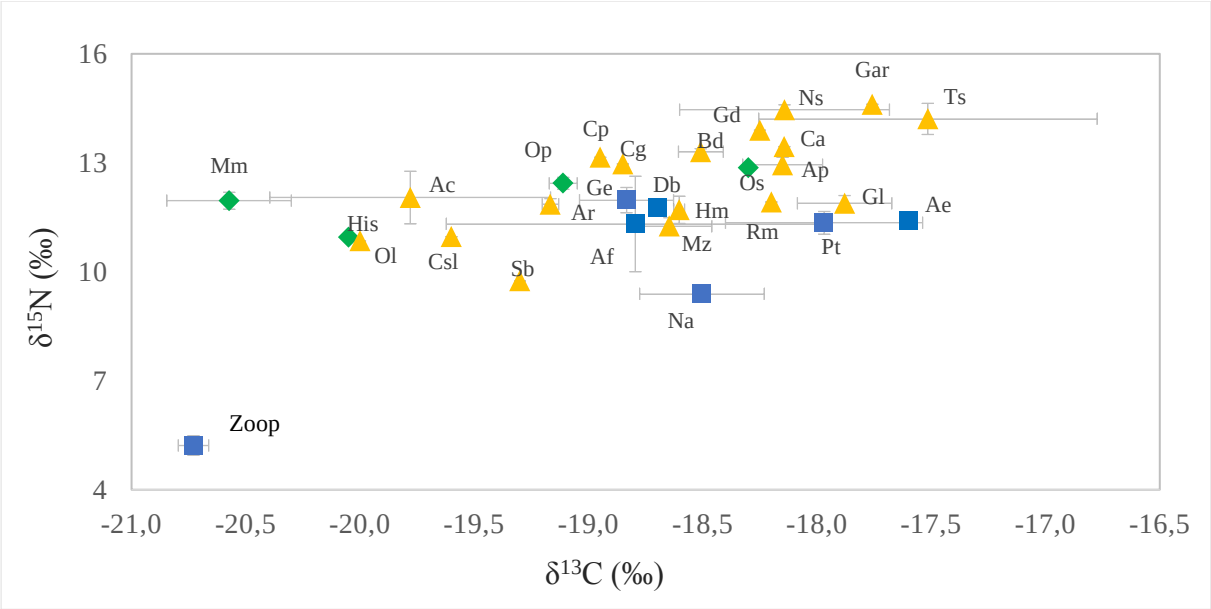

Figure 3.8: Potential preys average (±SD) $\delta^{13}C$ and $\delta^{15}N$ (‰) values for each species addressed by a code (check Table 0.2 from the ANNEX A). Teleosts (yellow triangles); crustacean (blue squares) and cephalopods (green diamond).

The sharks' potential sources were grouped into six major groups based on a cluster analysis (Figure 3.9) and according with taxa, habitat and isotopic variability. Teleosts, crustaceans and cephalopods presented a varied group of species with meso- and bathy -pelagic, bathy- demersal organisms presenting migratory and non-migratory habits and also organisms that perform diel vertical migrations.

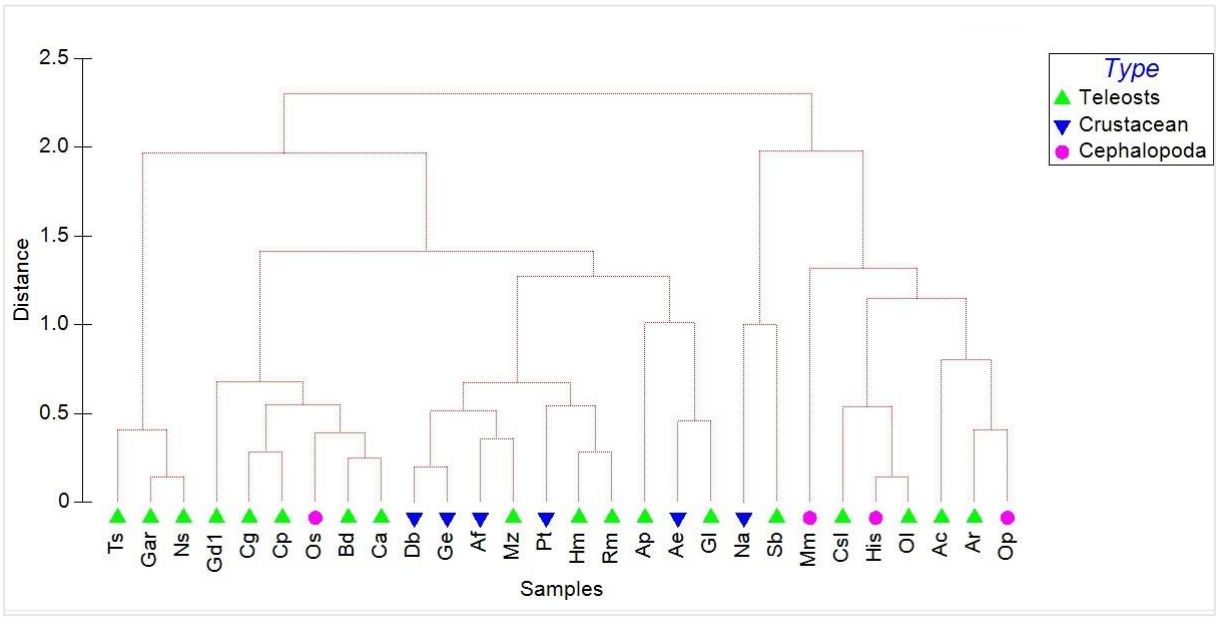

Figure 3.9: Cluster analysis of the potential sources of the deep-sea sharks from the SW coast of Portugal. Species names for the codes addressed here, can be found in the Table 0.2 ANNEX A

After that, it was possible to divide the teleosts into four major groups (T1, T2, T3 and T4) based mainly on the values of $\delta^{13}C$ and $\delta^{15}N$ (Table 3.5). Group T1 presents species of teleosts with higher average values (±SD) of both isotopes ($\delta^{13}C$ - 17.6 ± 0.1‰ and $\delta^{15}N$ 13.9 ± 0.2‰) and have non-migratory behavior being basically bathy- pelagic and -demersal (Table 3.5 and Figure 3.10). T2 as well as the crustacea presented a very similar signature on both isotopes $\delta^{13}C$ - 18.3 ± 0.1‰ and $\delta^{15}N$ 11.1 ± 0.3‰ and $\delta^{13}C$ -18.4 ± 0.5‰ and $\delta^{15}N$ 10.7 ± 0.9‰ respectively. Group T3 presented the lowest average values (±SD) for $\delta^{13}C$ - 19.6 ± 0.3‰ and $\delta^{15}N$ 10.6 ± 0.9‰ and have bathy- and meso- pelagic species, with some known to perform diel-vertical migrations such as Csl (*Chauliodus sloanii*) and Sb (*Serrivomer beanii*) (Froese and Pauly 2017). Group T4 was composed by organisms presenting higher average values (±SD) of $\delta^{15}N$, with $\delta^{13}C$ - 18.3 ± 0.5‰ and $\delta^{15}N$ 12.6 ± 0.6‰, bathy- pelagic and demersal habits. Crustaceans and cephalopods were not separated among their taxa and consequently, are represented by groups Cr and Cf respectively (Table 3.5 and Figure 3.10). Crustaceans presented a $\delta^{13}C$ with an average (±SD) of - 18.4 ± 0.5‰ and $\delta^{15}N$ 10.7 ± 0.9‰ and cephalopods $\delta^{13}C$ with an average (±SD) of - 19.0 ± 0.8‰ and $\delta^{15}N$ 11.6 ± 0.8‰. The species belonging to each of the groups and codes addressed at Table 3.5 are at the Table 0.4 from the ANNEX A.

Table 3.5: Group of sources and constituent species. T1, T2, T3 and T4 are groups of Teleostei; Cr stands for Crustacea and Cf stands for Cephalopoda with average (±SD) of $\delta^{13}C$ and $\delta^{15}N$ values of each group. The species code can be found in Table 0.4, ANNEX A.

Groups	Species code	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Habits *
T1	Gar; Ts; Ns	-17.6 ± (0.1)	13.9 ± (0.2)	NM; Bp-Bd
T2	Rm; Mz, Hm	-18.3 ± (0.1)	11.1 ± (0.4)	M; BMp-BMd
T3	Ar, Ac, Ol, Sb, Csl	-19.6 ± (0.3)	10.6 ± (0.9)	DV; BMp
T4	Ap, Bd, Cg, Cp, Gl, Gd1, Ca	-18.2 ± (0.5)	12.6 ± (0.6)	Bp-Bd
Cr	Ae, Af, Db, Ge, Na, Pt	-18.4 ± (0.5)	10.7 ± (0.9)	DV; BMp-BMd
Cf	His, Mm, Os, Op	-19.0 ± (0.8)	11.6 ± (0.8)	Bp - Bd

*Note: NM = non-migratory; M = Migratory; DV = diel vertical migration;
Bp = bathy-pelagic; Bd = bathy-demersal; BMp = bathy-meso pelagic; BMd = bathy-meso demersal

The distribution of each individual consumer and the potential sources based on the isotopic values (average ± SD) of $\delta^{13}C$ and $\delta^{15}N$ already divided by groups, are presented at the Figure 3.10. The most likely group of possible preys were selected based on the position of their $\delta^{13}C$ and $\delta^{15}N$ values of the sharks, after correcting for one trophic level (Figure 3.10).

Three groups of consumers were observed in Figure 3.10 based on the variability of the values $\delta^{13}C$: one group with $\delta^{13}C$ values varying between - 20‰ and - 19‰ with individuals from the

species *Deania calcea* and *D. profundorum* which were probably feeding on ^{13}C-depleted sources such as T3; another with intermediate $\delta^{13}C$ values (- 19‰ to - 18‰) with individuals from the species *D. calcea*, *D. profundorum*, *Etmopterus pusillus* and *Galeus atlanticus* which indicate they were likely feeding on Cr and/or T2; and a third group which presented $\delta^{13}C$ values outside the range of the sources collected ($\delta^{13}C$ > - 17.5‰) which it is the case of the species *Centrophorus squamosus*, *Centroselachus crepidater*, some individuals of *Scymnodon ringens* and one of *D. calcea*. The species *Deania calcea* presented a high variability in both $\delta^{15}N$ and $\delta^{13}C$ values. Nonetheless, they presented low $\delta^{15}N$ values, suggesting that they could be assimilating ^{15}N- depleted sources such as T3 and Cr. The same happened for *D. profundorum* which despite of low number of individuals presented a high variability of $\delta^{13}C$ suggesting that they could assimilate the preys with different values of $\delta^{13}C$ although with lower values for $\delta^{15}N$ which is the case of the T2, T3, Cr and Cf (Figure 3.10) *E pusillus* and *G. atlanticus* individuals presented little variability of $\delta^{15}N$ indicating that they could assimilate preys with low $\delta^{15}N$ values such as T3, and Cr (Figure 3.10). The individuals of *S. ringens* presented some variation of the $\delta^{13}C$ values, thus it seems likely that they could assimilate preys such as Cr and T3. *Centrophorus squamosus* and *C. crepidater* seemed to be out of range in comparison with the signatures of the group of preys presented, thus, are not analyzed by this model as for the individuals of *S. ringens* and *D. calcea* previously mentioned (Figure 3.10).

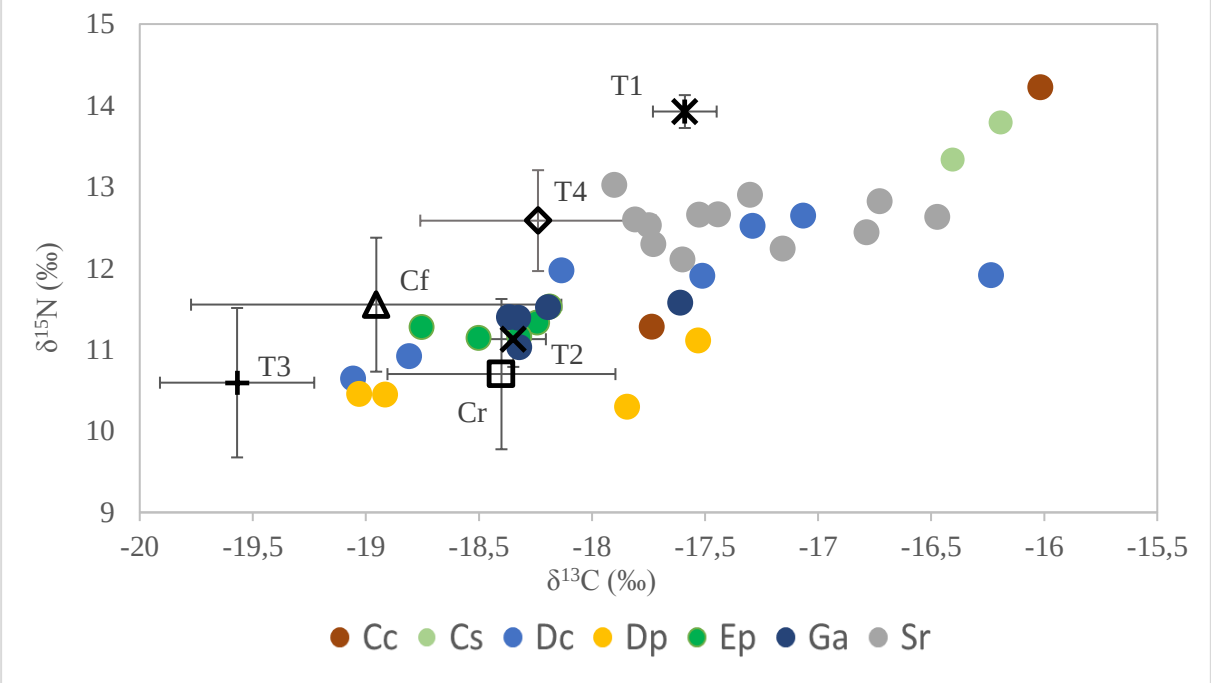

Figure 3.10: Average (± SD) $\delta^{15}N$ and $\delta^{13}C$ values of the main groups of sharks potential prey (T1, T2, T3, T4 for teleosts, Cr for crustaceans, and Cf for cephalopods) and individual sharks $\delta^{15}N$ and $\delta^{13}C$ values without corrected trophic fractionation. Cs, *Centrophorus squamosus*; Cc, *Centroselachus crepidater;* Dc, *Deania calcea*; Dp, *D.profundorum*; Ep, *Etmopterus pusillus*; Ga, *Galeus atlanticus* and Sr, *Scymnodon ringens*.

The isotopic mixing model (95% CI) indicates that crustacean were the main contributors (3-78%) to *D. calcea* biomass, followed by teleosts T2 (0-65%) (Figure 3.11). On the other hand, *D. profundorum* seemed to have a more generalist diet with a similar contribution from all the preys investigated T3 (01-55%), T2 (0-44%), crustacean Cr (0-47%) and cephalopods Cf (0-48%). *Etmopterus pusillus* presented major contributions from group T3 (21-83%), followed by crustacean (1-48%) and the cephalopods (0-49%). *Galeus atlanticus* had a higher contribution from T3 (11-69%), followed by crustacean (05-60%) and cephalopods (0-49%). *Scymnodon ringens* was one of the only species with an apparent preference for a specific group, in this case, the crustaceans (46-89%), and to a smaller degree teleosts from the group T3 (0-38%) and even less of cephalopods (0-31%) (Figure 3.11).

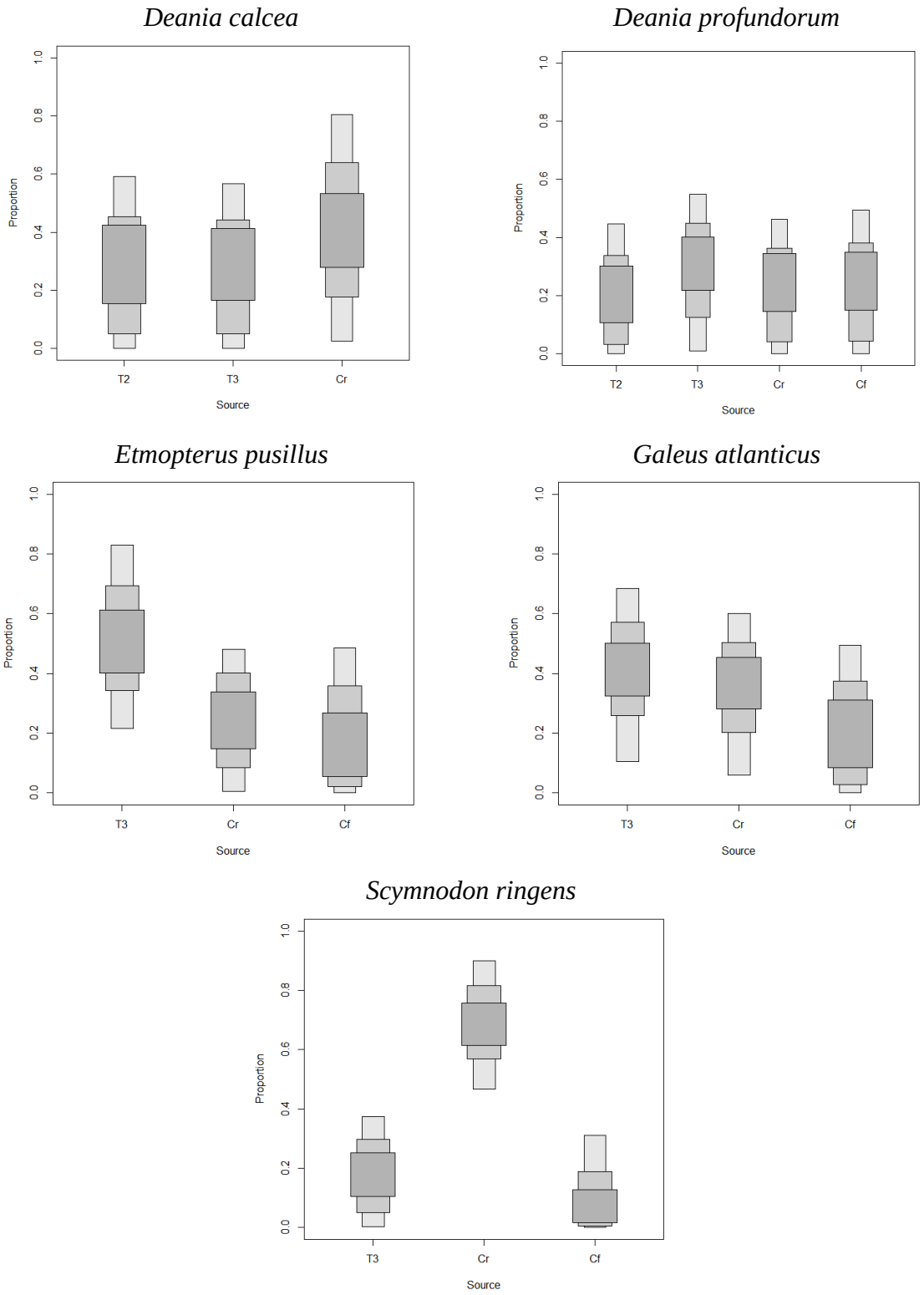

Figure 3.11: Proportion of each group of prey to the consumers biomass. Boxplot with the lowest to the highest density region (95%). T2 and T3 are groups of Teleostei; Cr is the contribution of the Crustacea and Cf is Cephalopoda (squids and octopus combined).

Trophic niche of sympatric sharks

The corrected standard ellipse areas (SEAc) and the niche overlap were determined for the sympatric sharks (i.e. sharks from the present study that share the same geographic area), using SIBER. Because SIBER only provide results when the number of individuals is equal to three or higher (Jackson *et al.* 2011), *C. squamosus* and *C. crepidater* were not included in this analysis.

The Figure 3.12 presents a visualization of the corrected standard ellipse areas which represents approximately 95% of the data and therefore represents the core niche or dietary isotopic space (Batschelet 1981). It is possible to see that the largest SEAc belongs to *Deania calcea* which overlaps in area with all the other species.

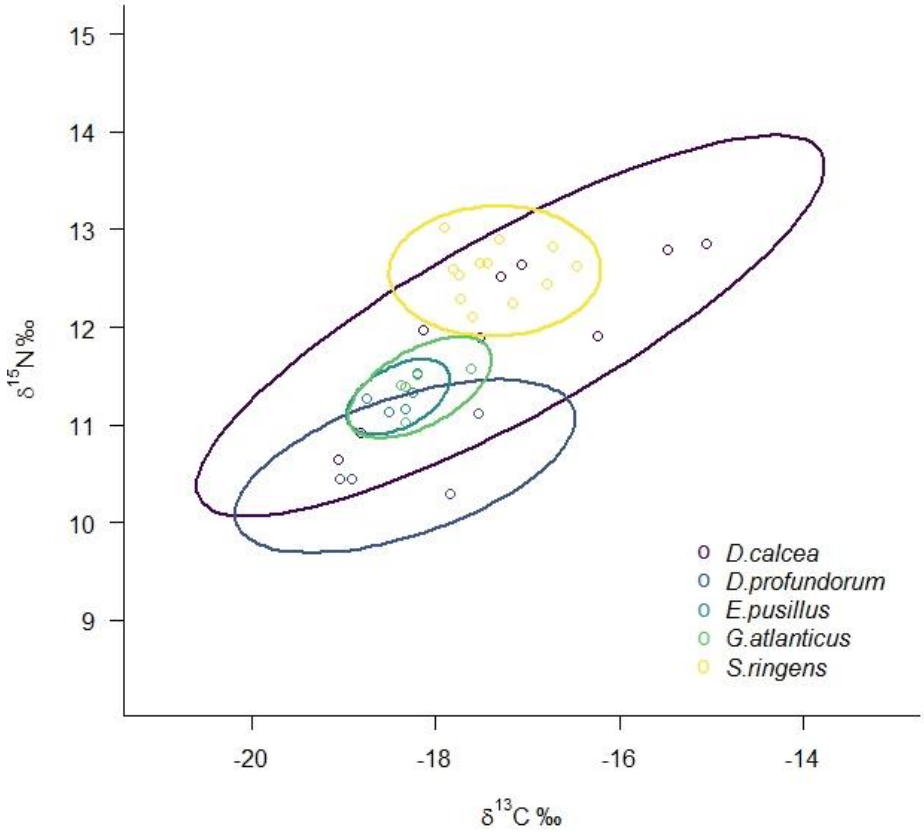

Figure 3.12: Corrected standard ellipse areas (SEAc) and overlap between shark species.

With the support from the Table 3.6 it is also possible to see the SEAc for each species in numbers and sustain the results from Figure 3.12. The values of the corrected standard ellipse area (SEAc) indicates a high trophic width where the species *D. calcea* presented the highest SEAc (2.15‰^{2}), followed *by D. profundorum* (1.07‰^{2}), *S. ringens* (0.44‰^{2}), *G. atlanticus* (0.23‰^{2}), and *E. pusillus* (0.13‰^{2}) (Table 3.6).

The degree of overlap with the species is presented in Table 3.6. *D. calcea* presented a high overlap with *D. profundorum* (3.6‰), *S. ringens* (2.15‰) and *G. atlanticus* (1.39‰). The trophic niche of the species *E. pusillus* and *G. atlanticus* overlap entirely with *D. calcea,* and *S. ringens* most entirely with *D. calcea*. (Table 3.6) *Scymnodon ringens* did not overlap its niche with neither *D. profundorum* and *E. pusillus* plus, the degree of overlap with *G. atlanticus* was minimal (0.04‰). *D. profundorum* trophic niche, overlapped with *E. pusillus* by 0.60‰ and *G. atlanticus* by 0.90‰. Lastly, *E. pusillus* niche overlapped entirely with *G. atlanticus* (0.79‰).

Table 3.6: Results from corrected standard area (SEAc ‰^{2}) total area (TA) and niche overlap (‰) between the species Dc, *Deania calcea*; Dp, *Deania profundorum*; Ep, *Etmopterus pusillus*, Ga, *Galeus atlanticus* and Sr, *Scymnodon ringens*.

	SEAc	**TA**	**Trophic Niche Overlap (‰)**					
			Dc	***Dp***	***Ep***	***Ga***	***Sr***	
Dc	2.15	2.79	12.87	3.60	0.79	1.39	2.15	***Dc***
Dp	1.07	0.50		6.42	0.60	0.90	0	***Dp***
Ep	0.13	0.10			0.79	0.79	0	***Ep***
Ga	0.23	0.17				1.39	0.04	***Ga***
Sr	0.44	0.76					2.64	***Sr***

Trophic Position

The trophic position (TP) with the average (±SD) for each shark species is presented at the Table 3.7 . One individual of the species *Centroselachus crepidater* (Cca – adult female) presented the highest value for the TP (6.3), followed by the species *Centrophorus squamosus* (6.1 ± 0.11), *S. ringens* (5.6 ± 0.12), *D. calcea* (5.4 ± 0.33), *C. crepidater* juvenile (Ccj), *E. pusillus* and *G. atlanticus* presented same TP (5.1) and, finally, *D. profundorum* presented the lowest values of TP (4.8 ± 0.14).

Table 3.7: Trophic position of shark species, average values (± SD) for each species.

Species	**Code**	**Trophic Position (TP)**
Centrophorus squamosus	Cs	6.1 ± 0.11
Centroselachus crepidater (adult)	Cc_a	6.3 ± 0
Centroselachus crepidater (juvenile)	Cc_j	5.1 ± 0
Deania calcea	Dc	5.4 ± 0.33
Deania profundorum	Dp	4.8 ± 0.14
Etmopterus pusillus	Ep	5.1 ± 0.06
Galeus atlanticus	Ga	5.1 ± 0.09
Scymnodon ringens	Sr	5.6 ± 0.12

The average values of the sharks' TP along with the group of potential preys (T1, T2, T3, T4, Cr and Cf) and the variation (±SD) of the TP within each group, is presented in the Figure 3.13. Sharks presented the highest values of TP among the dataset with the exception of *D. profundorum* which presented lower values than the T1 (5.0 ± 0.05). Variation of TP for *D. calcea* was the highest of all the sharks. For the sources, the highest variation was within the group T3 (4.0 ± 0.26), Cr (crustacean) presented a TP of 3.9 ± 0.25 and Cf (cephalopods) of 4.2 ± 0.21. T1 and T4 presented the highest TP values (5.0 and 4.6 respectively), although, as seen previously, they were not present in any of the sharks' diet, thus were not part of the isotopic models. Group T2 did not present a great variation of the TP values (4.2 ± 0.09).

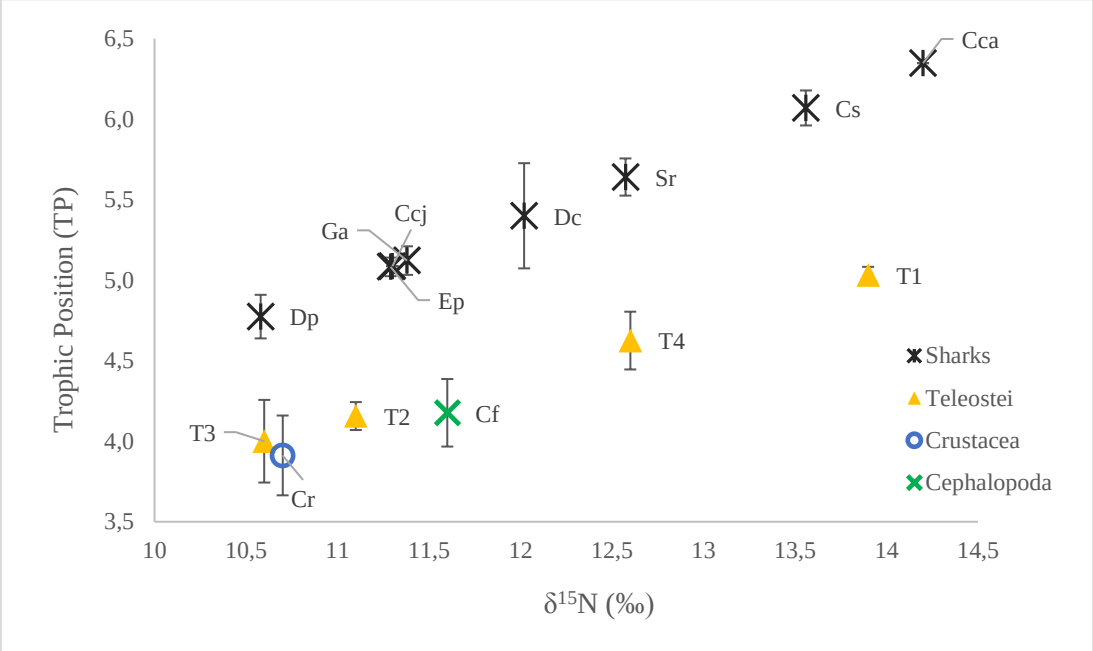

Figure 3.13: Trophic position of consumers and potential sources groups with standard deviation of each group. Cs, *Centrophorus squamosus*; Cca-j, *Centroselachus crepidater* adult and juvenile; Dc, *Deania calcea*; Dp, *D.profundorum*; Ep, *Etmopterus pusillus*; Ga, *Galeus atlanticus* and Sr, *Scymnodon ringens*. T1-T4 are groups of teleosts; Cr, are crustaceans and Cf, are cephalopods.

CHAPTER 4

DISCUSSION

4. DISCUSSION

The ecophysiological status, the diet and the trophic position of some of the deep-sea shark species from the southwest coast of Portugal were characterized for the first time. Deep-sea sharks analyzed in this study presented a wide size range, between 34 and 102 cm as well as weight which varied between 120 and 8000 g. The majority of the sharks collected were females (69%) with male sharks accounting for 31%. The juveniles were the majority (36%), adults (33%) although for the rest of the sharks (31%) the life stage information is absence. Little is known about the species *Scymnodon ringens* which was the most representative from this dataset (31%) thus, its life stage was reported as unknown (N/A) in this study.

The majority of the sharks were dead (54%) whilst 44% of them were in a very poor condition and only one shark presented a good condition in accordance with index provided by Benoît *et al*. (2010); Braccini *et al*. (2012) and Rodríguez-Cabello and Sánchez (2017). Even though all the live sharks were returned to sea (poor and good condition) after the data collection, their fate is uncertain since they came onboard with some injuries probably due to the trauma of being towed for several hours (Coelho and Erzini 2007). This is of great relevance taking into account that it is not allowed to catch the species *Centrophorus squamosus*, *Centroselachus crepidater*, *Deania calcea*, and *Scymnodon ringens* in European waters (list of deep-water sharks - EU Regulation, Nº: 1182/2013) adding to that, *Deania calcea* and *Centrophorus squamosus* are 'Endangered', *Galeus atlanticus* is 'Near threatened' and there is insufficient data to access the environmental status of *C. crepidater* and *S.ringens* (Data deficient) according with the European Red List (Nieto *et al*. 2015). This emphasize the fragility of these sharks and the urge to provide more information about those species.

4.1 Nutritional condition characterization

Although R/D ratios are widely tested for vertebrates such as fishes (Teleostei) and also, more recently, for sea turtles (Roark *et al*. 2009; Vieira *et al*. 2014) there is only one published study (Tavares *et al*. 2006) for R/D in sharks and elasmobranchs in general. In this study, Tavares *et al*. (2006) related R/D ratios with growth of an oceanodromous demersal shark, the smooth dogfish (*Mustelus canis*), in the coast of the Sucre State, Venezuela and they noted a decrease of the R/D in relation to size (total length in cm) of both genders (male and females). Despite the fact that this is the only study published with R/D and elasmobranchs, their results cannot be directly comparable with the present study because they did not calculate the ratios of the slope of the standard curve (sRD). R/D ratios are known to vary taxonomically and ontogenetically, as well in respect of environmental conditions, hence, in order to compare

results from different studies employing different methodologies, the ratios have to be standardized (Bulow 1987; Rooker *et al*. 1997; Foley *et al*. 2016).

Some of the sharks presented an improved condition amongst other individuals, for example the individual 31 (female from the species *Scymnodon ringens*), 49 (an adult female of *Galeus atlanticus*) and 28 (juvenile female *Deania calcea*) which is an indicative that they have been feeding more frequently than the rest of the sharks from this study. It was also possible to conclude that all the sharks had just fed over the last 1-3 days since their values were all above zero, being zero an indicative that sharks did not eat over the past days (Buckley *et al*. 1999). Likewise, the prey they ate at probably came from the study area due to the small window of time this analysis provide. There are only a few number of observations of sharks with relatively low R/D values which might not have been eating properly over the past days. This would be the case of the individual 30 (male from *Scymnodon ringens*), 4 (juvenile female of *Deania profundorum*) and also the individual 2, which was a pregnant female of *Centroselachus crepidater* that gave birth to five dead offsprings onboard.

Most sharks are sexually dimorphic, with females reaching larger sizes than males (Compagno *et al*. 2005) and this is known to be true for the species *Deania profundorum* (Sousa *et al*. 2009), *D. calcea* (Clarke *et al*. 2002), *Etmopterus pusillus* (Coelho *et al*. 2005) and *Centrophorus squamosus* (Girard and Buit 1998). This means that sexual segregation might play an important role between males and females in different energy requirements (Sims *et al*. 2006). Pregnant females are known to present a deprecated nutritional condition in comparison with males and females in the breeding season. During this season they require more nutritional reserves for reproduction and the development of the embryos thus presenting higher values for R/D, in contrast, during and after pregnancy this condition changes since the mother energetic reserves are transmitted to her litters (Chícharo *et al*. 2007). Thus, animals presenting lower R/D values, i.e. starving animals, will exhibit an elevated $\delta^{15}N$ (‰) (Hobson *et al*. 1993) presumably because animals catabolize their own body proteins, producing isotopic enrichment analogous to that for ingested food (Gannes *et al*. 1997). This might explain why the individual 2 from the species *Centroselachus crepidater*, the pregnant female, in comparison with the male of the same species, presented a lower value of R/D and an elevated $\delta^{15}N$ (‰) - the highest of all sharks. Globally, these results showed that the analyzed sharks were feeding in the last days, probably in the area as already mentioned. This allow us to further discuss the diet based on isotopic models.

Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ presented also some variation among the sharks' individuals ranging from - 19.1‰ to – 15.1‰ and 10.3‰ to 14.2‰ respectively and, according to statistical

analysis, there were significant differences between the stable isotope values of the different species. *Centrophorus squamosus* was significantly more enriched for both isotopes than *Deania profundorum*, *Etmopterus pusillus*, *Galeus atlanticus* and *Scymnodon ringens.* This could mean that *C. squamosus* do not share the same resources nor occupy the same trophic level as the above mentioned species and this may decrease the potential for resources competition. Significant differences were also found between the species *Deania calcea* and its congener *D. profundorum*, with *D. calcea* presenting higher $\delta^{15}N$ values, thus suggesting that *D. profundorum* was feeding at comparatively lower trophic levels.

The stable isotope analyses (SIA) indicated that there were three groups of consumers on this dataset: one composed by the species *Deania profundorum*, *Etmopterus pusillus* and *Galeus atlanticus* which presented low $\delta^{13}C$ and $\delta^{15}N$ values, indicating they were feeding on ^{13}C- and ^{15}N- depleted preys such as crustacean and the group T3 of teleosts; the other group of consumers is composed by the species *Scymnodon ringens* and *Centrophorus squamosus* with high $\delta^{13}C$ and $\delta^{15}N$ values, an indicative that they fed on ^{13}C- and ^{15}N- enriched preys such as teleosts of the group T2 and cephalopods and; finally the species *Deania calcea* and *Centroselachus crepidater* with a high isotopic variability suggesting that there might be intra-specific variation on the diet and/or that they are more generalists and present a wider trophic niche. Further the SIBER analysis showed that *D. calcea*, presented a wider trophic niche area than all the sharks although, ontogenic and gender were not tested in the present study they are not discarded.

The stable isotope mixing models supported this analysis. Overall, with the exception of *C. squamosus* and *C. crepidater* from which it was not possible to obtain results from the mixing models, these analyses indicated that the other five shark species were feeding on different proportions of the same groups of preys. From the results, it was clear that crustaceans were the major contributors to *Scymnodon ringens* biomass (46-89%) although, there is only one study that assessed the diet of *S. ringens* from Rockall Trough, Ireland, (Mauchline and Gordon 1983) where they found on the stomach of only two individuals fish bones and muscle tissue and some remains of crustaceans.

According with Mauchline and Gordon (1983), which analyzed the stomach contents from *Deania calcea* from the northeast Atlantic, the main preys were mesopelagic teleosts (myctophids), suggesting that they feed at some height above the bottom and also presented a contribution of demersal teleosts, supplementing their diet with crustaceans and squids. These findings were supported by other authors (e.g. Ebert *et al*. 1992; Daley *et al*. 2002). An additional study from the northeast Atlantic (Preciado *et al*. 2009) performed at the Cantabrian

Sea, Le Danois Bank also supported the preference of *D. calcea* for teleosts, mostly the blue whiting *Micromesistius poutassou* but also for cephalopods. At New Zealand, Dunn *et al*. (2013) also observed that the most frequent and numerous prey item in *D. calcea* stomach contents were myctophids along with mesopelagic and benthopelagic fishes, with some contribution of cephalopods and natant decapods. The results from this study agree with those above mentioned, because teleosts were also the major contributor to the diet of *D. calcea* T2 (CI: 0-65%) and T3 (0-50%) although no cephalopods were included in the model and also no myctophids were sampled due to the type of gear used. Myctophids are mesopelagic fishes and perform diel vertical migrations, and the net used to collect those fishes are pelagic trawl net with a 10 mm meshsize in the codend (Duhamel *et al*. 2000) which is different from the one used in this study (i.e. bottom trawler with a 70 mm meshsize in the codend). Additionally, according with a recent study where stable isotopes were used to identify the main prey assimilated by sharks from the Mediterranean (Barría *et al*. 2015) myctophids presented an average (±SD) $\delta^{13}C$ and $\delta^{15}N$ of about − 20.62 ± 0.83‰ and 8.41 ± 0.20‰ respectively. This indicate that this group should be taken into consideration in future food web studies in this area, because some shark species presented low $\delta^{15}N$ values, and close to those from myctophids after correcting from trophic fractionation (e.g. *G. atlanticus*, *E. pusillus*). In fact, some species presented $\delta^{15}N$ values lower than those from the potential sources collected, after correcting for trophic fractionation. That was the case of *Deania profundorum*, *Etmopterus pusillus*, *Galeus atlanticus* and some ^{15}N- depleted *D. calcea* individuals. This may be related to the absence of preys considered of relative importance to the diet of these species and which were not possible to be sampled due to the sampling methodology as mentioned previously. *Etmopterus pusillus* is known to feed almost entirely on mesopelagic and demersal prey. A significant component of the diet of *E. pusillus* was attributed to organisms that perform diel vertical migrations such as the mesopelagic crustacean *Pasiphaea silvado* and another with a demersal habit, the teleost *Microsistius potassiou,* along with myctophids and, to a smaller degree, cephalopods of the family Histioteuthidae (Santos and Borges 2001; Xavier *et al*. 2012; Muñoz 2015). However, none of the above mentioned species of preys were sampled in this study, with the exception of squids of the family Histioteuthidae. The absence of relevant preys such as *P. silvado* and *M. potassiou* in this study could be attributed to depth limits sampled since *P. silvado* are found between 10-600 m depth (Hayashi 1999) and *M. potassiou* is more frequent at 300-400 m depth though it might reach greater depths of up to 1000 m (Cohen *et al*. 1990). Although, it was possible to see a larger contribution of the group of teleosts T3 (21-83%) to the diet of *E. pusillus*, this group is composed of teleosts which are known to perform

diel vertical migrations such as the Sloane's viper fish *Chauliodus sloanii* (Gibbs 1984). In addition, Xavier *et al.* (2012), showed that this shark species presents ontogenic changes in dietary compositions and crustaceans decrease in importance with the size. It was noted a moderate contribution of crustaceans of up to 48% to the *E. pusillus* biomass in the present study, and this might be related either with the absence of natantiid decapods on the dataset such as the *P. silvado* or even with the age of the individuals since the majority were adults (60%). Adding to this *E. pusillus* is known to be oceanodromous and therefore its assimilated diet might not even be from the sampled region (Riede 2004).

Ebert *et al*. (1992) presented a study about the diet of *Deania profundorum* using stomach content analysis in South Africa, where it was found that its diet was based on myctophids (and other teleosts) and cephalopods; crustaceans were not identified as prey. Bass *et al*. (1975) and Compagno *et al*. (2005) indicated that there is also contribution of teleosts, squids and crustaceans to the diet of *D. profundorum*. In the present study the *D. profundorum* dietary model presented the most variable diet with similar contribution of the groups T2 (0-44%), T3 (1-55%), crustacea (0-47%) and cephalopods Cf (0-48%).

As with all the above mentioned species, the information available on *Galeus atlanticus* is scarce, and perhaps even scarcer since, before 2006, this species was grouped together with the blackmouth catshark (*Galeus melastomus*). Nonetheless it is clear now, due to genetic analyses, that they belong to different species (Rey *et al*. 2006; Castilho *et al*. 2007). Therefore, for this analysis it was taken into consideration data from *G. melastomus* prior to the year 2006 which might contain a contribution of both species. Santos and Borges (2001) described *G. melastomus* as a predator, feeding largely on teleosts and crustacean, including several penaeid and pandalid shrimps. These findings mirror the results from the present study which showed higher proportional contribution of teleosts (T3: 11-69%) and crustaceans (Cr: 5-60%) to their biomass. The contribution of cephalopods (0-49%) might be supported by another study (Velasco *et al*. 2001) which presented data from the Bay of Biscay, northeast Atlantic, where cephalopods were found in the stomachs of predators larger than 50 cm, including *G. melastomus*. That would be the case of *G. atlanticus* from this study which also presented individuals larger than 50 cm.

The apparent mismatch between some of the sharks and their potential prey could be due to differences in methods to assess the diet of these consumers since most of the above-mentioned articles presented diet composition based on stomach content analysis. The actual diet composition as derived from the stomach analyses might not reflect the total prey spectrum of a species, but, as stable isotopes mirror the feeding over time, dietary shifts might have occurred

previously to sampling (Denda *et al*. 2017), and in the case of sharks, the turnover in the white muscle tissue (i.e. the time it takes for the food ingested to be assimilated) might vary between 0.5 and 1.5 years (MacNeil *et al*. 2006; Caut *et al*. 2009; Hussey *et al*. 2010; Logan and Lutcavage 2010; Malpica-Cruz *et al*. 2012).

All of the five species presented some degree of niche overlap with the exception of *Deania profundorum* and *Etmopterus pusillus* which did not overlap with the niche of *Scymnodon ringens*, thus they might not be driven to change feeding habits in order to avoid competition. Actually, since *S. ringens* niche was narrow in comparison with the one from *Deania calcea* and *D. profundorum* for example, it is probable that *S. ringens* is likely either to have a more specialized diet or/and their preferred sources were abundant (Colwell and Futuyma 1971).

Etmopterus pusillus and *Galeus atlanticus* presented the narrowest trophic niche although they displayed a large niche overlap among each other in relation with their areas. The coexistence between species with similar trophic habits and a narrow niche breadth might be possible due to the abundance of food resources (Colwell and Futuyma 1971). Although the prey abundance was not determined during this study, the R/D analysis revealed that both species were in a good nutritional condition (R/D: 0.44 ± 0.11 and 0.63 ± 0.15 respectively) which suggest that they were not starving.

The high overlap of *E. pusillus* and *Galeus atlanticus* with *Deania calcea* trophic niche may imply that either they compete for the same resources, if resources are scarce (Macpherson 1981; Cartes 1998) either they coexist when resources are abundant (Colwell and Futuyma 1971). Their R/D values indicates that they had eaten in the last 1-3 days, and they were in a good nutritional condition, thus it is possible that these species have high food availability and that it may be enough to avoid competition for the resources. In addition, *D. calcea* could be feeding from other sources since its SEAc is the widest of all species (2.15‰^{2}). This could be explained by the fact that *D. calcea* was the second most representative species and presented individuals from different genders (males and females) and also ages (juveniles and adults) although only juvenile females.

On the other hand, *S. ringens* despite being the most representative species of this dataset, presented a low SEAc (0.44‰^{2}) in comparison with both *D. calcea* (2.15‰^{2}) and *D. profundorum* (1.04‰^{2}). Due to the wider niche breadth, *D. profundorum* seems to present a more generalist feeding behavior, in fact, along with its congener *D. calcea*, only juveniles females were collected, thus, leading to hypothesize that juvenile females from both species might present this type of generalist behavior.

Since all those species derived from the same geographical area, it seems that they share the same spatial area at least for some time and/or under specific circumstances; more sampling conducted on different seasons would be helpful to disclose when and for how long this occurs. Thus, some species likely compete for the same resources if they are scarce and some might coexist when resources are abundant (Colwell and Futuyma 1971; Macpherson 1981; Cartes 1998). Species can also adapt and modify their feeding behavior when sharing resources in a restricted environment in order to coexist in the same area (Lowe *et al*. 1996; Motta and Wilga 2001; Heithaus 2001). Because the shark species studied can feed on different prey groups, competition may be reduced when coexisting in the same areas (Carrassón and Cartes 2002; Heupel *et al*. 2007; Navarro *et al*. 2014) although, overall nutritional condition of these species indicates that the resources are enough to fulfill their nutritional needs.

4.2 Trophic position

Estimate the trophic position for deep-sea sharks with stable isotopes analysis (SIA), is perhaps, one of the greatest challenges faced by ecologists that venture in this area since crucial and complex factors have to be taken into consideration such as the right choice of the baseline $\delta^{15}N$ organism as well as the best trophic enrichment factor (TEF: Δ^{15} N).

To obtain proper TEF estimates, laboratorial experiments under controlled conditions are fundamental (Caut *et al*. 2009). However, TEF values vary highly as a function of the consumer's taxa, type of tissue sampled and even as a function of the type and quality of the food sources (Vanderklift and Ponsard 2003; Caut *et al*. 2009). Most organisms such as sharks feed on multiple prey items thus, it is highly recommended the use of different TEF for each group of prey (specially omnivores) such as teleosts, crustaceans, and cephalopods. The choice of the best TEF for interpreting stable isotope data, although of extreme relevance, remains very controversial (Logan *et al.* 2008; Caut *et al*. 2009; Hussey *et al.* 2010; Olin *et al.* 2013). The use of inappropriate TEF values when undertaking isotopic models or calculating trophic positions will result in erroneous interpretations regarding the reconstruction of a consumers diet and its role in a determined food-web (Perga and Grey 2010).

There are few studies with sharks where TEF were determined under controlled laboratorial conditions (MacNeil *et al*. 2005; Hussey *et al*. 2010; Logan and Lutcavage 2010; Kim *et al*. 2012b; Malpica-Cruz *et al*. 2012; Caut *et al*. 2013). Nevertheless, values of TEF tend to be variable among studies $\Delta^{15}N$ (2.3-5.5‰) and $\Delta^{13}C$ (0.9-3.5‰) varying with the species, type of diet and tissue sampled. Thus, many authors tend to use the TEF proposed by Post (2002) $\Delta^{15}N$ of 3.4‰ (Estrada *et al.* 2003, 2006; Kerr *et al.* 2006; Borrell *et al*. 2011), including those

focusing on the diet of elasmobranchs (Pethybridge *et al*. 2012; Colaço *et al.* 2013; Iitembu and Richoux 2015).This might not be the right approach since assuming a TEF of 3.4 for $\Delta^{15}N$ would imply assuming that sharks, teleosts, crustaceans and cephalopods have the same isotopic enrichment derived of their diet which is highly variable between taxa and even between shark species. If a fixed value of $\Delta^{15}N$ is used at every trophic position (TP), the assumed trophic structure of food webs is additive and may lead to potential biases underestimating top predators TP and compressing the length of the web (Hussey *et al*. 2014). Therefore, to compute the TP of each group in this study (sharks and potential preys), the approach was to assume different TEF per groups. The TEF for sharks ($\Delta^{15}N = 2.3$) was based on the most specific controlled study on muscle tissue of two shark species (*Negaprion brevirostris* and *Carcharias taurus*) fed a fish diet for over two years by Hussey *et al.* (2010). The TEF for teleostei ($\Delta^{15}N = 3.2$) was based on the study from Sweeting *et al.* (2007) the most specific study of TEF in teleostei muscle tissue. Finally, another value of TEF ($\Delta^{15}N = 3.4$) was used for the invertebrates (crustaceans and cephalopods), as recommended by Post (2002). This was the same process applied by Chouvelon *et al.* (2012) in the study of the trophic position of several sharks (including *Centroselachus crepidater*, *Deania calcea* and *Deania profundorum*), teleostei and invertebrates at the Biscay Bay, Spain (North East Atlantic).

The deep-sea shark species presented a higher TP than the other organisms at this study and this result was already expected since the choice of the sources was made based in literature (e.g. Compagno *et al*. 2005). The group of preys T1 and T4 (teleosts) were the most ^{15}N-enriched, naturally achieving the highest TP's among the potential preys, 5.0 and 4.6 respectively, revealing similar TP as *Etmopterus pusillus*, *Galeus atlanticus* and *C. crepidater* (male juvenile), which all presented a TP of 5.1. In fact, group T1 TP's value was a bit higher than the juvenile females of the species *D. profundorum* which was 4.8.

The overall findings of the present study regarding trophic position (TP) of the deep-sea sharks found some divergence with the study from Cortes (1999), based on stomach content analysis (SCA) where he stated that sharks are tertiary consumers (TP > 4) and that the family Squalidae - which at that time was composed also by *C. squamosus, C. crepidater, D. calcea, D. profundorum, E. pusillus* and *S. ringens* - presented a TP average (±SD) value of 4.1 ± 0.4 while the *G.atlanticus*, which was represented by its congener *G. melastomus* since by that time they were not separated yet, presented a TP average of 3.7. This indicates that the values achieved by the present study were two trophic levels higher in some cases. This difference might be related with the opportunistic behavior of some sharks (Wetherbee *et al*. 2012) which is not always captured by SCA.

In contrast, recent studies where TP were determined based on SIA presented results more similar to those from the present study. Barría *et al*. (2015) determined the TP of sharks and rays of the western Mediterranean through stomach content analysis (SCA) and SIA from muscle and cartilage samples, including from some species of Squaliformes. They found higher values of TP for SIA 5.6 ± 1.22 against 4.31 ± 0.61 of SCA.

Chouvelon *et al.* (2012) as explained above, used the same TEF as the present study to calculate the TP of a variety of organisms from different food webs of the Bay of Biscay. Even using the same TEF, the values of TP obtained were still lower than the ones described here for the species *C. crepidater* (TP = 4.3 against 6.3 and 5.1), *D. calcea* (TP = 4.3 against 5.4) and *D. profundorum* (TP = 4.5 against 4.8). For *D. profundorum* the values found were higher than for the other two species while the opposite occurred in the present study. This might be related with the choice of the baseline organism. They used filter-feeder bivalves with a TP = 2 and with the $\delta^{15}N$ of 9.8‰ and in the present study the baseline $\delta^{15}N$ value was 4.2‰ after correction for the ethanol preservation. Also, in the present study the copepods zooplankton were not individually identified to a species level and copepods are believed to have different diets depending on the species: herbivores, detritivores and even omnivores and carnivores (as reviewed by Denda *et al*. 2017). Therefore, results of the TP in the present study, should be interpreted with caution. For future studies, it is strongly recommended either the proper taxonomic identification of the zooplankton, or by choosing another baseline organism to represent the food-chain.

4.3 Limitations

Lipids have more negative $\delta^{13}C$ values relative to other biochemical compounds due to kinetic isotope effects (DeNiro and Epstein 1977). This might represent an issue since the variability in tissue lipid content can alter $\delta^{13}C$ values and easily could be incorrectly interpreted as dietary or habitat shifts (Focken and Becker 1998). Therefore, it is assumed that with a $C:N_{bulk} > 3.5$ lipid extraction is required, prior to stable isotope analysis (Post *et al*. 2007). One of the most commonly used methods to chemically extract lipids was developed by Bligh and Dyer (1959) where a polar organic solvent mixture of chloroform/methanol is used to extract both the simple lipid classes and the more complex polar lipids bound to other cellular constituents e.g. membrane proteins is used. This extraction may result in the loss of some non-lipid compounds which may alter values of $\delta^{15}N$ (Sweeting *et al*. 2006). Thus, when the analysis of both isotopes is necessary, an untreated subsample should be done to determine $\delta^{15}N$ values (Schlechtriem *et al*. 2003). Mathematic lipid corrections have been proposed to standardize $\delta^{13}C$ values using

$C:N_{bulk}$ ratios without the need for chloroform–methanol extraction (Sweeting *et al*. 2006; Post *et al.* 2007; Logan *et al.* 2008). Nonetheless, mathematical corrections of lipids are not encouraged to correct lipids for elasmobranchs tissue (Shipley *et al.* 2017) unless there is a negative relationship between C:N values and the $\delta^{13}C$ values (Post *et al*. 2007).

It was not possible to chemically extract the lipids from the samples collected during this study due to insufficient muscle tissue obtained for sharks' samples. So far, there is no suitable mathematical lipid correction developed for any of the shark species analyzed by this study. However, because the average C:N values obtained for sharks in this study (C:N range of 3.5 to 3.8) are not that distant from those from Reum (2011), and because it was not found any significant inverse relation between C:N and $\delta^{13}C$ values, it is likely that lipids were not affecting the sharks $\delta^{13}C$ values.

Most elasmobranchs store urea in their tissues for osmoregulatory purposes (Pang *et al*. 1977; Olson 1999; Ballantyne and Robinson 2010). The retention of urea, which is an isotopically lighter waste product (DeNiro and Epstein 1977), may result in lower ratio of heavy (^{15}N) to light (^{14}N) nitrogen isotopes which may underestimate the trophic position or impair food sources identification, if this extraction is neglected (Hussey *et al*. 2010; Borrell *et al*. 2011; Kim and Koch 2012; Carlisle *et al*. 2012). Thus, at present, the effect of urea in $\delta^{15}N$ elasmobranchs stable isotopes remains unclear, with some authors attesting that it may lower $\delta^{15}N$ values of muscle tissue like it was observed for the basking shark *Cetorhinus maximus* (Gunnerus, 1765) (Ostrom *et al*. 1993) and for seven other pelagic shark species (Li *et al*. 2016). Nonetheless, others showed that urea does not affect trophic level predictions nor nitrogen isotope composition in many other shark species (Estrada *et al*. 2003; Logan and Lutcavage 2010). This was the case of the study performed by Logan and Lutcavage (2010) in which under controlled laboratorial feeding regime, coastal skate species (*Leucoraja* spp.) and two shark species (*Carcharhinus plumbeus* and *Squalus acanthias*), urea retention did not affected nitrogen stable isotopes.

CHAPTER 5

CONCLUSION

5. CONCLUSION

Nucleic acids such as RNA and DNA were applied for the first time to deep-sea elasmobranch in which R/D ratios proved to be an important tool to evaluate the ecophysiological condition of deep-sea sharks. Also, this was the first study where R/D ratios and stable isotopes were combined to evaluate the condition and diet of elasmobranchs.

Although these tools have been contributing for the understanding of ecophysical and ecobiological traits of organisms, limitations are still a drawback for some studies, especially with elasmobranchs. For stable isotopes analysis (SIA) for example, many factors should be taken into consideration when using these analysis with such physiologically unique animals such as the turnover rates of the tissues analyzed, trophic enrichment factors (TEF) and a good choice of the baseline organism (Peterson and Fry 1987; Post 2002; Boecklen *et al*. 2011; Layman *et al*. 2012; Phillips *et al*. 2014).

Nevertheless, it was possible to draw some conclusions with the outcomes of this study:

- Sharks presented good nutritional condition overall, which means that they have been feeding within a time frame of 1-3 days prior to their collection, thus the food source were most likely to be from the study area;
- Sharks assimilated different proportions of the same groups of preys: teleosts (from group T2 and T3), crustacean, and cephalopods. *Scymnodon ringens* seems to have a preference for crustaceans which include commercial species of shrimps targeted by this fishery. This is of concern because very little biological information for this shark species is available;
- *Deania calcea* and *Deania profundorum* presented the widest niche and thus, *D. calcea* might have intra-specific variations and *D. profundorum* juvenile females appear to have an even more generalist diet. *Deania calcea* overlapped its niche with all the other species;
- *S*harks' trophic position varied between 4.8 and 6.3 indicating that they occupy high levels in the marine food web.

These results are of great relevance considering the lack of data for deep-sea elasmobranchs, especially from the SW coast of Portugal and also because of their vulnerability. Since the collection of a large sample size of rare and endangered elasmobranchs is difficult, even more for deep-sea taxa, using complementary approaches is probably the best way to advance our knowledge about these animals. Thus, for a more detailed quantification and qualification of

the diet composition and greater trophic resolution of deep-sea sharks, it is advised for future studies:

- Sampling different tissues to undertake SIA, over a long period of time to characterize the diet seasonally;
- Use of different trophic enrichment factor (TEF) for different types of species, according to its food preferences;
- A good choice of the baseline organisms which have to be in accordance with the trophic chain in study;
- A larger number of individuals in order to access dietary changes ontogenetically and within genders, coupled with collection over different bathymetric limits, since some species are believed to segregate by size and sex at different depths (Moura *et al*. 2014);
- Sampling with different types of gear to collect different groups of preys.

Despite of some limitations such as the small sample size of most of the species and the number of tissues analyzed, the data collected was satisfactory to achieve the overall goal of this study which was to improve the knowledge of ecophysiological condition, diet and trophic position of deep-sea sharks from the southwest coast of Portugal and this is believed to be another step forward to the understanding of the Selachii deep-sea community which, overall is very poorly studied, especially at this region.

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ANNEX A

Table 0.1: Classification of the taxa sampled with the respective order, family, species (whenever possible) and number of individuals of each species (n).

Taxa	Order	Family	Species	n
Selachii	Squaliformes	Squalidae	*Scymnodon ringens*	12
		Etmopteridae	*Etmopterus pusillus*	5
		Centrophoridae	*Deania profundorum*	4
			Deania calcea	9
			Centrophorus squamosus	2
		Somniosidae	*Centroscelachus crepidater*	2
	Carcharhiniformes	Scyliorhinidae	*Galeus atlanticus*	5
Cephalopoda	Octopoda	Opisthoteuthidae	*Opisthoteuthis sp.*	2
		Octopodidae	-	1
	Oegopsida	Mastigoteuthidae	*Mastigoteuthis sp.*	2
		Histioteuthidae	*Histioteuthis corona*	1
Teleostei	Osmeriformes	Alepocephalidae	*Alepocephalus rostratus*	4
			Rouleina maderensis	1
	Lophiiformes	Chaunacidae	*Chaunax pictus*	1
	Gadiformes	Macrouridae	*Gadomus longifilis*	2
			Gadomus arcuatus	1
			Cetonurus globiceps	1
			Trachyrinchus scabrus	2
			Nezumia sclerorhynchus	5
			Gadomus sp.	1
	Ophidiiformes	Bythitidae	*Cataetyx alleni*	1
		Melanonidae	*Melanonus zugmayeri*	5
	Aulopiformes	Ipnopidae	*Bathypterois dubius*	3
		Omosudidae	*Omosudis lowii*	1
	Stomiiformes	Stomiidae	*Chauliodus sloani*	1
	Anguiliformes	Serrivomeridae	*Serrivomer beanii*	1
	Beryciformes	Anoplogastridae	*Anoplogaster cornuta*	2
		Trachichthyidae	*Hoplostethus mediterraneus*	1
	Notacanthiformes	Halosauridae	*Aldrovandia phalacra*	3
Crustacea	Decapoda	Polychelidae	*Polycheles typhlops*	5
		Pandalidae	*Dichelopandalus bonnieri*	1
		Gerionidae	*Geryon longipes*	3

Contin. Table 0.1

	Nephropidae	*Nephropsis atlantica*	2
	Aristeidae	*Aristaeomorpha foliacea*	3
		Aristaeopsis edwardsiana	1
Copepoda			2*

* numbers of samples per taxa, each sample with more or less 15 individuals.

Table 0.2: Taxa of each source species and the code used.

Taxa	Species	Code
Teleostei	*Aldrovandia phalacra* (Vaillant, 1888)	Ap
Teleostei	*Alepocephalus rostratus* Risso, 1820	Ar
Teleostei	*Anoplogaster cornuta* (Valenciennes, 1833)	Ac
Crustacea	*Aristaeopsis edwardsiana* (Johnson, 1867)	Ae
Crustacea	*Aristeomorpha foliacea* (Risso, 1827)	Af
Teleostei	*Bathypterois dubius* Vaillant, 1888	Bd
Teleostei	*Cataetyx alleni* (Byrne, 1906)	Ca
Teleostei	*Cetonorus globiceps* (Vaillant, 1884)	Cg
Teleostei	*Chauliodus sloani* Bloch & Schneider, 1801	Csl
Teleostei	*Chaunax pictus* Lowe, 1846	Cp
Crustacea	*Dichelopandalus bonnieri* Caullery, 1896	Db
Teleostei	*Gadomus arcuatus* (Goode & Bean, 1886)	Gar
Teleostei	*Gadomus longifilis* (Goode & Bean, 1886)	Gl
Teleostei	*Gadomus sp.*	Gd1
Crustacea	*Geryon longipes* A. Milne Edwards, 1881	Ge
Cephalopoda	*Histioteuthis sp.* Voss & Voss, 1962	His
Teleostei	*Hoplostethus mediterraneus* Cuvier, 1829	Hm
Cephalopoda	*Mastigoteuthis sp.* (Joubin, 1916)	Mm
Teleostei	*Melanonus zugmayeri* Norman, 1930	Mz
Crustacea	*Nephropsis atlantica* Norman, 1882	Na
Teleostei	*Nezumia sclerorhynchus* (Valenciennes, 1838)	Ns
Cephalopoda	*Octopodidae*	Os
Teleostei	*Omosudis lowii* Gunther, 1887	Ol
Cephalopoda	*Opisthoteuthis sp.* Verril, 1883	Op
Crustacea	*Polycheles typhlops* Heller, 1862	Pt
Teleostei	*Rouleina maderensis* Maul, 1948	Rm
Teleostei	*Serrivomer beanii* Gill & Ryder, 1884	Sb
Teleostei	*Trachyrincus scabrus* (Rafinesque, 1810)	Ts
Crustacea	Copepod	Zoop

Table 0.3: Complete list of parameters from each individual of the consumers species. *Centrophorus squamosus*; *Centroscelachus crepidater*; *Deania calcea*, *D.profundorum*; *Etmopterus pusillus*, *Galeus atlanticus* and *Scymnodon ringens*. Individual species field code. Sex M, male or F female. TL total length in cm. Life Stage A, adult; J, juvenile or N/A unknown. Condition G, good; P, poor and D, dead. $\delta^{15}N$ and $\delta^{13}C$ in parts per mil (‰). sRD is the standardized RNA/DNA ratios.

Species	Spp. code	Sex	TL (cm)	Weight (g)	Life Stage	Condition	$\delta^{15}N$	$\delta^{13}C_{bulk}$	%N	%C	C:N	$\delta^{13}C_{PTN}$	sRD
C. squamosus	11	M	102	7350	A	D	13.3	-16.4	16.4	42.6	4.2	-15.8	0.43
C. squamosus	27	M	101	8000	A	P	13.8	-16.2	15.0	38.9	3.5	-16.7	0.33
C. crepidater	2	F	85	2040	A	D	14.2	-16.0	15.5	42.5	3.9	-15.8	0.19
C. crepidater	35	M	61	1000	J	D	11.3	-17.7	15.1	40.3	3.6	-18.0	0.38
D. calcea	5	F	67	1210	J	P	10.9	-18.8	15.3	39.8	3.6	-19.0	0.36
D. calcea	24	F	102	7000	J	P	12.8	-15.5	15.0	37.6	3.4	-16.3	0.40
D. calcea	28	F	60	720	J	P	12.0	-18.1	15.3	38.8	3.5	-18.5	0.75
D. calcea	32	F	54.5	540	J	P	10.6	-19.1	16.1	39.9	3.8	-18.9	0.55
D. calcea	36	M	81	2100	J	D	12.8	-15.1	15.8	42.3	4.0	-14.7	0.32
D. calcea	37	M	87	2200	A	D	11.9	-17.5	15.6	41.6	3.9	-17.3	0.11
D. calcea	38	F	91	3300	J	D	12.6	-17.1	16.0	41.6	4.0	-16.7	0.20
D. calcea	45	F	79	1800	J	D	11.9	-16.2	16.0	40.6	3.9	-16.1	0.68
D. calcea	48	M	85	1900	A	P	12.5	-17.3	15.4	38.9	3.6	-17.6	0.59
D. profundorum	4	F	34	120	J	D	11.1	-17.5	16.1	41.1	4.0	-17.2	0.15
D. profundorum	51	F	48	340	J	P	10.5	-19.0	14.9	38.6	3.4	-19.7	0.23
D. profundorum	53	F	49.5	370	J	D	10.3	-17.8	16.8	41.1	4.1	-17.3	0.40
D. profundorum	54	F	45.5	320	J	D	10.4	-18.9	15.9	40.0	3.8	-18.9	0.26
E. pusillus	3	F	45	450	J	D	11.3	-18.8	15.4	38.9	3.6	-19.1	0.45
E. pusillus	47	M	41	350	A	G	11.5	-18.2	15.0	39.4	3.5	-18.6	-
E. pusillus	55	F	44.5	350	A	D	11.2	-18.3	15.3	40.2	3.7	-18.5	0.48
E. pusillus	56	M	41	280	A	D	11.3	-18.2	13.7	39.2	3.2	-19.4	0.56
E. pusillus	57	F	36	230	J	D	11.1	-18.5	15.7	39.0	3.6	-18.7	0.26
G. atlanticus	10	F	70	960	A	D	11.4	-18.4	16.6	42.4	4.2	-17.7	0.48
G. atlanticus	8	F	66	690	A	D	11.4	-18.3	16.2	41.3	4.0	-18.0	0.64

Cont.
Table 0.3

G. atlanticus	9	F	58.5	580	A	D	11.0	-18.3	16.0	41.7	4.0	-18.0	0.52
G. atlanticus	49	F	60.5	710	A	P	11.6	-17.6	15.3	38.4	3.5	-18.1	0.86
G. atlanticus	50	F	54	450	A	P	11.5	-18.2	15.1	38.2	3.4	-18.8	-
S. ringens	25	F	68	1100	n/a	P	12.7	-17.5	15.9	40.1	3.8	-17.5	0.42
S. ringens	26	F	58	1300	n/a	P	12.5	-17.7	15.7	38.2	3.6	-18.1	0.48
S. ringens	29	F	42	450	n/a	P	13.0	-17.9	15.5	37.4	3.5	-18.5	0.40
S. ringens	30	M	49.5	760	n/a	P	12.9	-17.3	15.2	37.2	3.4	-18.0	0.04
S. ringens	31	F	68	1800	n/a	P	12.8	-16.7	15.8	38.6	3.6	-17.0	0.89
S. ringens	33	M	53	1500	n/a	P	12.4	-16.8	16.2	39.7	3.8	-16.7	0.45
S. ringens	34	F	51	880	n/a	D	12.7	-17.4	16.6	41.7	4.1	-16.9	0.18
S. ringens	46	F	81	3700	n/a	P	12.6	-16.5	15.7	39.0	3.7	-16.6	0.34
S. ringens	52	F	59	1200	n/a	P	12.1	-17.6	15.7	38.3	3.6	-17.9	0.46
S. ringens	1	F	49	630	n/a	D	12.3	-17.7	15.6	38.6	3.6	-18.1	0.22
S. ringens	6	M	55	850	n/a	D	12.6	-17.8	15.3	38.0	3.5	-18.4	0.53
S. ringens	7	M	50	720	n/a	D	12.2	-17.2	16.2	41.1	4.0	-16.8	0.17

Table 0.4: Complete list of sources individuals containing the species field code, length in cm (either total length for teleosts, carapace length for crustaceans and mantle length for cephalopods); the weight in grams, Nitrogen and Carbon values (%); $\delta^{15}N$ and $\delta^{13}C$ values from the bulk and after lipid correction ($\delta^{13}C_{PTN}$) for individuals with C:N > 3.5.

Species	Spp. Code	Length	Weight	$\delta^{15}N$	$\delta^{13}C_{bulk}$	N	C	C:N	$\delta^{13}C_{PTN}$
Aldrovandia phalacra	2.39 C1	35.0	13.6	12.3	-17.5	13.5	43.4	3.5	
	2.39 C2	34.4	15.8	12.7	-17.6	13.3	42.6	3.4	
	2.39 C3	33.5	17.0	12.3	-18.0	13.9	45.0	3.7	-18.1
Alepocephalus rostratus	1.16 A1	14.0	11.5	11.3	-19.2	12.7	41.2	3.1	
	1.16 A2	12.5	9.8	11.2	-19.2	12.8	41.3	3.1	
	3.42 C1	18.5	37.8	11.6	-19.1	11.4	39.4	2.7	
Anoplogaster cornuta	1.15 A1	11.0	22.1	10.8	-20.4	13.2	41.9	3.3	
	2.39 A1	19.5	103.0	12.3	-19.2	12.2	47.3	3.4	
Aristaeopsis edwardsiana	1.21 A2	13.0	10.8	10.9	-17.6	13.0	40.6	3.1	
Aristeomorpha foliacea	1.21 A1	15.0	18.9	10.1	-19.6	12.5	43.8	3.2	
	2.40 D1	23.0	87.1	12.7	-17.6	13.4	41.8	3.3	
	3.43 C1	13.0	19.5	9.7	-19.2	13.5	42.6	3.4	
Bathypterois dubius	1.14 A1	15.5	18.1	12.7	-18.4	14.1	44.4	3.7	-18.4
	1.14 A2	13.5	8.9	12.9	-18.6	14.1	44.7	3.7	-18.6
	1.14 A3	14.0	12.3	12.8	-18.5	14.2	44.7	3.8	-18.5
Cataetyx alleni	3.42 B1	13.5	17.1	12.9	-18.3	14.2	45.4	3.8	-18.1
Cetonurus globiceps	2.39 H1	48.0	127.5	12.5	-18.9	13.3	41.9	3.3	
Chauliodus sloani	2.39 F1	24.5	24.7	10.5	-19.6	12.3	44.0	3.2	
Chaunax pictus	6.58 A1	20.5	273.9	12.7	-18.7	13.9	43.9	3.6	-18.9
Dichelopandalus bonnieri	2.40 B1	10.0	5.1	11.3	-18.7	13.2	41.6	3.3	
Gadomus arcuatus	1.18 B1	23.8	82.1	14.1	-17.6	14.0	44.2	3.7	-17.8
Gadomus longifilis	1.18 A1	18.6	20.3	11.6	-17.0	13.4	42.5	3.4	
	3.42 A1	9.5	19.1	11.2	-17.9	13.8	44.0	3.6	-18.1
Gadomus sp.	1.18 C1	17.5	22.1	13.4	-18.3	13.7	43.3	3.5	
Geryon longipes	1.20 A1	5.0	56.8	11.6	-19.1	12.2	40.1	2.9	
	1.20 A2	6.5	44.1	11.0	-18.7	11.7	37.9	2.6	
	1.20 A3	8.0	69.8	11.8	-18.6	11.8	40.6	2.8	

Cont. Table 0.4

Histioteuthis sp.	6.58 B1	7.5	179.8	10.5	-20.1	13.3	37.6	3.0	
Hoplostethus mediterraneus	1.17 B1	15.5	52.7	10.8	-18.4	14.0	43.9	3.7	-18.6
	2.39 B1	18.2	92.7	11.6	-18.3	13.9	43.5	3.6	-18.6
Mastigoteuthis sp.	2.41 A1	8.0	36.3	11.2	-20.8	13.2	41.3	3.2	
	2.41 B1	10.0	43.1	11.7	-20.3	12.5	38.9	2.9	
Melanonus zugmayeri	1.12 A1	24.5	78.2	11.0	-18.6	13.9	44.1	3.6	-18.8
	1.12 A3	20.5	41.5	10.5	-18.1	13.7	44.1	3.6	-18.4
	1.12 A2	21.5	49.1	10.7	-18.8	14.1	45.1	3.8	-18.8
Nephropsis atlantica	2.40 A1	6.5	7.1	9.0	-18.8	13.0	40.7	3.1	
	3.43 B1	8.7	13.0	8.7	-18.2	12.4	39.0	2.9	
Nezumia sclerorhynchus	1.17 A1	13.5	18.1	14.2	-17.7	13.3	41.6	3.3	
	1.17 A2	15.5	14.9	13.9	-17.7	13.3	42.1	3.3	
	1.17 A3	17.5	17.7	13.8	-17.8	14.2	45.3	3.8	-17.7
	1.17 A4	19.0	17.4	14.0	-17.6	14.0	44.4	3.7	-17.7
Octopodidae	2.41 C1	9.0	95.9	12.4	-18.3	10.7	38.1	2.4	
Omosudis lowii	2.39 D1	11.0	3.3	10.4	-20.0	13.4	43.7	3.5	
Opisthoteuthis sp.	1.19 A1	3.5	109.8	11.8	-19.2	8.6	32.0	1.6	
	3.44 A1	2.0	20.0	12.1	-19.1	8.7	36.4	1.9	
Polycheles typhlops	2.40 C1	16.0	15.4	10.7	-17.4	12.0	35.6	2.5	
	3.43 A1	13.5	8.2	10.4	-17.8	14.1	42.3	3.5	
	3.43 A2	12.3	9.8	11.3	-18.1	12.4	38.9	2.9	
	3.43 A3	11.0	6.6	11.0	-18.6	11.9	37.6	2.7	
Rouleina maderensis	2.39 G1	30.5	141.3	11.4	-18.2	12.9	41.3	3.2	
Serrivomer beanii	2.39 E1	60.0	70.2	9.3	-19.3	10.5	38.7	2.4	
Trachyrincus scabrus	1.13 A1	35.5	155.0	14.1	-16.9	14.3	45.0	3.8	-16.8
	1.13 A2	14.5	17.7	13.3	-18.0	13.9	43.4	3.6	-18.3
Copepods*	B1			5.0	-20.8	10.3	36.0	2.2	
	B3			4.5	-20.7	10.4	37.8	2.3	

*Copepods are based in a sample value, each sample containing approximately 15 individuals

ANNEX B

Pictures of sharks and potential sources

Centrophorus squamosus

Centroscelachus crepidater

Deania calcea

Deania profundorum

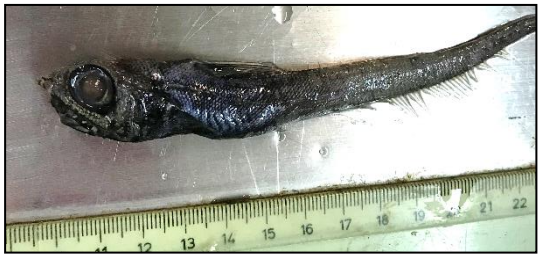
Etmopterus pusillus

Galeus atlanticus

Scymnodon ringens

Figure 0.1: Sharks species collected from the bathyal zone in the southwest coast of Portugal.

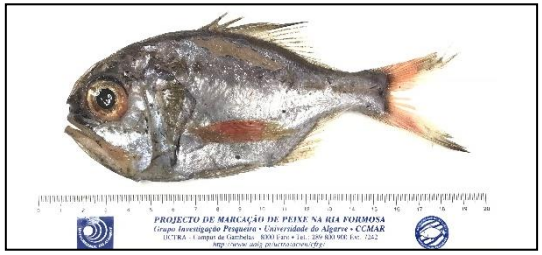
Gadomus arcuatus



Nezumia sclerorhynchus

Trachyrinchus scabrus

Figure 0.2: Teleosts from the group T1 with higher values of $\delta^{15}N$ and non-migratory behavior, collected from the bathyal zone in the southwest coast of Portugal.



Hoplosthethus mediterraneus

Melanonus zugmayeri

Rouleina maderensis

Figure 0.3: Teleosts from group T2 with migratory behavior, collected from the bathyal zone in the southwest coast of Portugal.

Alepocephalus rostratus

Anoplogaster cornuta

Chauliodus sloani

Omosudis lowii

Serrivomer beanii

Figure 0.4: Teleosts from the group T3 with species that performs diel migratory migrations and presents lower values of $\delta^{15}N$, from the bathyal zone in the southwest coast of Portugal.

Aldrovandia phalacra

Bathypterois dubius

Cataetyx alleni

Cetonurus globiceps

Chaunax pictus

Gadomus longifilis

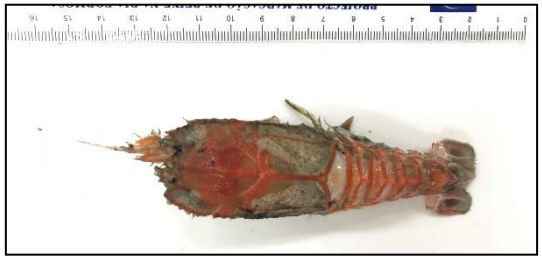
Gadomus sp.

Figure 0.5: Teleosts from the group T4 with average values of $\delta^{15}N$ from the bathyal zone in the southwest coast of Portugal.

Dichelopandalus bonnieri

Geryon longipes

Nephropsis atlantica

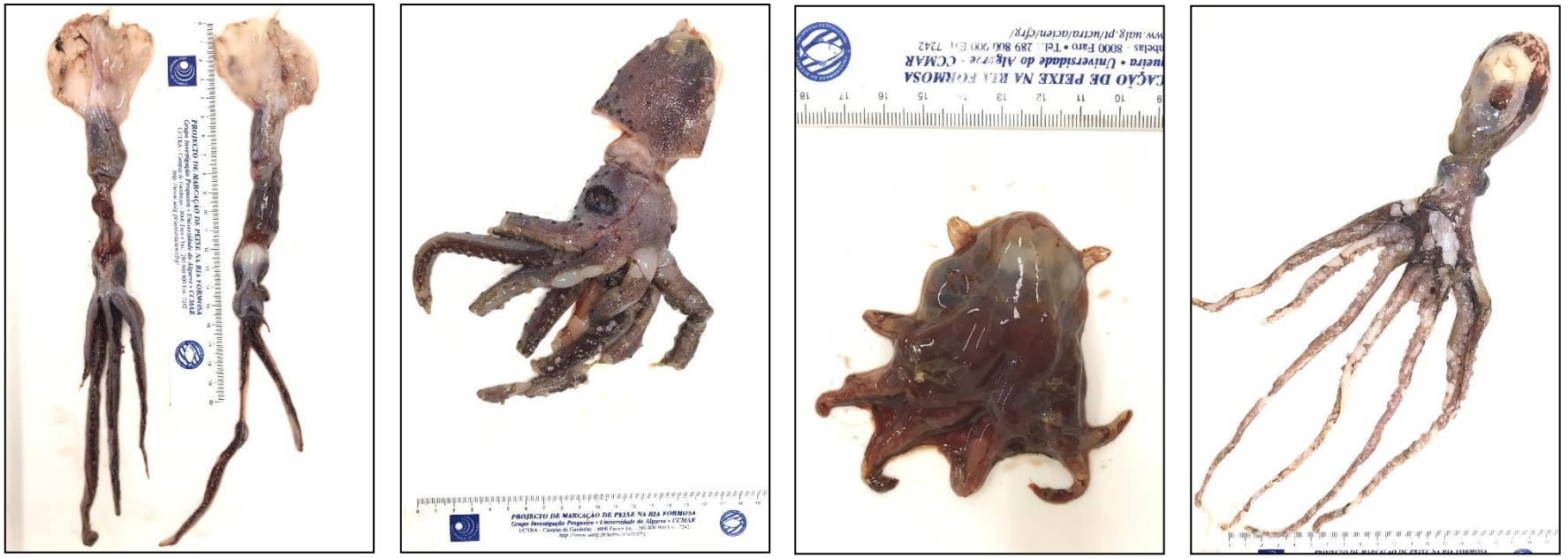


Polycheles typhlops

*Aristaemorpha foliacea**

*Aristaeopsis edwardsiana**

Figure 0.6: Crustaceans collected from the bathyal zone in the southwest coast of Portugal. Species with * are the target of this crustacean bottom trawler.



Mastigoteuthis sp. *Histioteuthis* sp. *Opisthoteuthis sp.* Octopodidae

Figure 0.7: Cephalopods collected from the bathyal zone of the southwest coast of Portugal.