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FEEDING ECOLOGY OF SMALL DEEP-WATER LANTERNSHARKS (*ETMOPTERUS SPINAX* AND *ETMOPTERUS PUSILLUS*) OFF THE ALGARVE COAST

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Declaro ser autor deste trabalho, que é original e inedito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluida.

"Ignorance more frequently begets confidence than does knowledge: it is those who know little, not those who know much, who so positively assert that this or that problem will never be solved by science."

Charles Darwin, The Descent of Man.

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RESUMO

Os eslasmobrânquios, um dos principais predadores do oceano, desempenham um papel bastante importante nos ecossistemas marinhos. Eles exercem um controle no tamanho e na dinâmica de muitas espécies, no sentido dos níveis tróficos mais baixos para os mais altos, ajudando na gestão dos ecossistemas do oceano. Em resultado, o conhecimento da ecologia trófica de predadores marinhos torna-se crucial para compreender o importante papel que desempenham nos ecossistemas. Neste sentido, este estudo tem por objetivo estudar a ecologia alimentar de duas espécies de tubarões de profundidade, *Etmopterus spinax* (Linnaeus, 1758) (lixinha da fundura) e *Etmopterus pusillus* (Lowe, 1839) (xarinha preta), sobre as quais a informação é muito escassa ou até inexistente. Assim, neste estudo são descritos os hábitos alimentares de *Etmopterus spinax* e *Etmopterus pusillus*, na costa do Algarve, a sul de Portugal. A fim de conhecer a composição da dieta alimentar de cada espécie de tubarão de profundidade, efectuaram-se análises de acordo com o sexo,o estado de maturação dos indivíduos e as estações do ano para a espécie *E. spinax* e análises de acordo com o sexo para a espécie *E. pusillus*. A sua dieta foi determinada através da análise do conteúdo dos estômagos de 231 espécimes capturados acessoriamente por arrastos de crustáceos, ao longo da costa do Algarve e no período compreendido entre 1999-2000 e em 2015, tendo sido rejeitados devido ao seu baixo ou nulo valor comercial. Do total de espécimens analisados, 173 pertencem à espécie *E. spinax* e os 58 restantes à espécie *E. pusillus*. Os espécimes foram medidos (comprimento total) e pesados em laboratório, e o estado de maturação de cada um foi atribuido em funçãodo comprimento da sua primeira maturação . Os estômagos foram pesados e abertos e o estado de digestão dos seus conteúdos foram analisados seguindo uma escala com cinco níveis de digestão de acordo com o estado de decomposição em que as presas se encontravam. As presas ingeridas foram identificadas através de diferentes chaves taxonómicas até ao menor nível de classificação taxonómica possível. Além disso, as presas mais afetadas pelo processo de digestão foram identificadas pelas suas estruturas duras, como sejam os otólitos no caso dos peixes e os bicos no caso dos cefalópodes. Esta análise foi realizada através da aplicação de três tipos de análise, a saber: (1) análises quantitativas, como o método numérico (% N), o método gravimétrico (% W) e a frequência de ocorrência (% FO); (2) análise mista, como o Índice de Importância Relativa (IRI) e o Índice Alimentar Ponderado (IPO2); e (3) análise qualitativa, como a classificação das categorias de presas encontradas. De igual modo, também foram

aplicados seis tipos de análise complementar, como sejam os índices de diversidade (índice de Shannon), riqueza específica (índice de Margalef) e uniformidade de espécies (indice de Pielou), o índice de sobreposição trófica (índice de Schoener), o nível trófico e o índice de vacuidade (VI). Além disso, foi realizada uma análise estatística multivariada, mediante a utilização do software PRIMER V6.0, recorrendo ao uso de diferentes testes (CLUSTERS, MSD, ANOSIM e SIMPER). Mediante estas análises, este estudo vem demonstrar a existência de uma sobreposição trófica na dieta de *E. spinax* e *E. pusillus*, onde foi observado que a diversidade das espécies de presas encontradas nos conteúdos dos estômagos foi baixa, não existindo diferencas significativas entre as duas dietas (Mann-Whitney, $p > 0,05$), o que leva a sugerir que podem apresentar uma dieta seletiva. Portanto foi possível observar a existência de uma certa homogeneidade na dieta alimentar entre as duas espécies de tubarões de profundidade. Alem disso, os resultados obtidos neste estudo permitiram observar evidências de que ambas as espécies partilham os mesmos recursos se bem que em proporções diferentes. Ambas as espécies alimentaram-se principalmente de três grupos de presas: crustáceos, peixes e cefalópodes. As principais presas encontradas na dieta dos tubarões de profundidade em estudo consistiram em organismos da subordem Natantia (presas preferenciais), peixes não identificados (presas secundárias) e cefalópodes (presas acessórias) no caso de *E. spinax*. Enquanto que as principais presas encontradas na dieta de *E. pusillus* foram peixes não identificados (presas preferenciais), organismos da subordem Natantia (presas secundárias) e cefalópodes (presas acessórias). Para além disso, encontrou-se um número elevado de estômagos vazios que se veio a reflectir num elevado valor de vacuidade em ambos os tubarões, podendo este facto estar relacionado, com a duração dos arrastos (de várias horas), na medida em que o período de longas horas em que a embarcação vai arrastando no fundo pode ser que seja o suficiente para que haja uma completa digestão dos estômagos, tornando os seus conteúdos não identificáveis. Não houve diferenças significativas na composição da dieta de *E. pusillus* de acordo com o sexo (ANOSIM, $p>0,05$). Para *E. spinax* tambem não foram encontradas diferenças de acordo com o sexo e a estação do ano (ANOSIM, $p>0,05$). Além disso, também não houve diferenças de acordo com o estado de maturação , apresentando a dieta entre espécimes imaturos e maturos uma elevada variabilidade (ANOSIM, $R=0,05$, $p<0.05$). No entanto, no caso dos espécimes maduros de ambos os sexos de *E. spinax* foi possível observar uma ligeira variação da dieta alimentar. A dieta consistiu principalmente de pequenos crustáceos para os espécimes imaturos, mudando a sua composição para presas potencialmente maiores, como peixes, para espécimes maduros. Esta mudança na dieta alimentar pode ser causada por variações ontogénicas, uma vez que a alimentação está relacionada com o tamanho da boca, a capacidade de armazenamento do estômago e a capacidade de natação de cada indivíduo; e também pela necessidade de obter a energia necessária de acordo com o ciclo reprodutivo em que se encontra. Consequentemente, o fato de os espécimes maiores consumirem presas maiores pode ajudar a reduzir a competição entre indivíduos da mesma espécie ou de espécies diferentes. Por outro lado, em termos de sazonalidade, os crustáceos continuaram a fazer parte da dieta principal de *E. spinax*. O fato de não se terem encontrado diferenças significativas entre a sazonalidade e a composição da dieta de *E. spinax* pode ser devido à estabilidade da temperatura no meio. As variações de temperatura são geralmente grandes na superfície e mínimas a grandes profundidades, de modo que podem afectar na composição das presas, nas flutuações de abundância e/ou as alterações na distribuição na coluna de água. Finalmente, os resultados obtidos neste estudo foram comparados com os de outros autores, verificando-se algumas diferenças, no que respeita à dieta alimentar entre *E. spinax* e *E. pusillus*.

Abstract

The knowledge about the trophic ecology of marine predators is crucial to understand their ecological role in ecosystems. In this study, the feeding ecology of two small deep water Lanternsharks, *Etmopterus spinax* (Linnaeus, 1758) and *Etmopterus pusillus* (Lowe, 1839), from the Algarve coast, south Portugal, was analysed. In order to better understand their diet composition, this study included an evaluation by sex, maturity stage and seasonality. For this purpose, the stomach contents of 173 *E. spinax* and 58 *E. pusillus* individuals were analysed. Through univariate quantitative analysis as well as occurrence percent by number (%N), weight (%W) and frequency of occurrence (%FO) and mixed analysis as Relative Importance Index (%IRI) and Weighted Food Index (IPO2) of prey items were obtained for both deep-water shark species. Also, additional indices such as overlap index, trophic level, vacuity index, diversity, richness and evenness indices were used. Besides a statistical analysis was performed through multivariate analysis with Software PRIMER V6.0, using different tests (CLUSTERS, MSD, ANOSIM and SIMPER). Through the results obtained by these analysis there seems to exist a trophic overlap in the diet of these deep-water shark species, with a low diversity, richness and evenness of prey species in their stomach contents. Thereby, it is possible to suggest that they are selective predators. Mainly crustaceans, fish and cephalopods were found in their diets. In general, it was observed that *E. spinax* has a preference for crustaceans (mainly Natantia organisms), followed by fish (unidentified fish) and cephalopods (unidentified cephalopods) as accessory prey. However, in case of *E. pusillus* the preference for fish (mainly unidentified fish), followed by crustaceans (Natantia organisms) and cephalopods (unidentified cephalopods) as accessory prey. On the other hand, the sex, maturity and season variables were not significant sources of variation in the diet of *E. spinax* species. In the same way, no significant differences concerning sex was observed for *E. pusillus* diet. Finally, the results obtained in this study were compared with the some author studies verifying some differences with regard to the diet of *E. spinax* and *E. pusillus*.

Key words: *Etmopterus spinax, Etmopterus pusillus,* Algarve, feeding ecology, stomach contents analysis, trophic level.

Chapter 1 *<u>INTRODUCTION</u>*

1. INTRODUCTION

1.1. General introduction

There have been long, large changes on different time scales that have affected marine communities. In the case of benthic marine assemblages, they have experienced gradual long-term changes related to climate and sea level oscillations (Cartes *et al.*, 2013). These changes have not only affected various taxonomic levels but also trophic groups, causing changes in the habitat and the ecological dynamics of benthic communities. However, despite all these changes, the sharks have been swimming in the oceans of the world for more than 400 million years (Griffin, 2008).

The *Chondrichthyes* class, including cartilaginous fish, sharks, rays and chimaeras, is a very old and successful group of jawed fish, which currently contains between 900 and 1100 know living species (Compagno, 1990). They show a highly morphological diversity during most of their evolutionary development from the Paleozoic to the present days. It is important to know that they are relatively large predators which have remained in a competitive environment such as marine ecosystems. Cartilaginous fish are highly diverse and they show different life-history styles to which they have to adapt. Usually they are large migratory which are distributed in all types of marine environments with different characteristics (Compagno, 1990).

Elasmobranchs are top predators, playing an important role in the marine ecosystems with a top-down control on the size and dynamics of many species (Valls *et al.*, 2011). Top predators keep all species bellow them in the trophic chain, because they directly limit the populations of their prey and help to manage healthy ocean ecosystems, including seagrass beds and coral reefs (Griffin *et al.*, 2008). Furthermore, they exploit a wide range of habitats, from epipelagic to deep-sea benthic environments (Xavier *et al.*, 2012). Nowadays, populations of sharks are being decimated by commercial fishing, and some species are vulnerable to extinction (Lopez *et al.*, 2009), because sharks usually tend to have a slow growth rate, late age of maturity and low fecundity. This means that they follow a K-selected life-history strategy (Stevens *et al.*, 2000), so their recruitment is directly dependent on the size of stock. These reasons determine that some populations will probably not be able to recover quickly from overexploitation, thus harming the sustainability of fisheries (Stevens *et al.*, 2000).

The pressure of fisheries induces special vulnerability in species, and in low-productivity habitats such as deep-sea, mainly due to the fast growth of this industry and the by-catch. Each year, humans kill more than 100 million sharks worldwide, this includes the tens of millions of sharks that are caught annually for their fins, which are one of the most expensive seafood products of the world (Griffin *et al.*, 2008). On the other hand, sharks are important from an economical point of view for their flesh for human consumption, skin for making complement stuff like handbags, belts and also as sandpaper to pottery and wood, liver for oil extraction and for medical use, jaws as trophies and in aquariums for entertainment (García, 2008). Another important factor that may contribute to the decline of their populations is the scarceness of prey that may be threatened from over fishing and habitat loss due to coastal development and pollution (García, 2008). Additionally, all species are exposed to the risk of bioaccumulation of contaminants in their bodies because of their high rank position in the trophic chain (García, 2008). All these factors cause significant problems for the correct conservation and management of sharks.

On the other hand, to understand the biological interactions in animal communities and the dynamics of marine ecosystems it is essential to know the trophic relationships (Valls *et al*., 2011). Unfortunately, there is still little information about the feeding ecology of most elasmobranchs; only the species with important commercial value are studied. The diets of most top predators are normally quite varied. This allows them to change prey species when certain populations are low (Griffin *et al*., 2008), being an advantage since they allow prey species to persist. Moreover Pardo-Gandarillas *et al*., (2007) suggested that the size, the movement of prey and the contrast with the medium are commonly positive relationships between the size of predator fish and their prey. Besides, visual characteristics that may influence the selection of prey by predators are important in sharks feeding ecology.

As Fanelli *et al*. (2009) described, the segregation and competition among species may be the result of the combination of several niche aspects, one of which may be preponderant over the others. It is necessary to determine the food and feeding ecology of sharks to clarify the relationships between sharks, prey availability and fisheries (Xavier *et al*., 2012), thus the study of the stomach contents is necessary to contribute the analysis of bio-ecological aspects in ecosystems where the species are integrated.

1.2. Description of studied species

Compagno *et al.* (2005) reported that the family *Etmopteridae* is the largest squaloid shark family, with more than 50 species distributed in five genera. The genus

Etmopterus is the most diverse genus of this family, containing at least 31 described species. In Portugal, the genus *Etmopterus* is commonly caught as by-catch and discarded by several deep-water fisheries that operate in the area (Xavier *et al.*, 2012).

1.2.1. Taxonomic classification

Phylum CHORDATA

Class ELASMOBRANCHII

Order SQUALIFORMES

Family ETMOPTERIDAE Fowler, 1934

Genus ETMOPTERUS Rafinesque, 1810

1.2.2. *Etmopterus spinax* (Linnaeus, 1758)

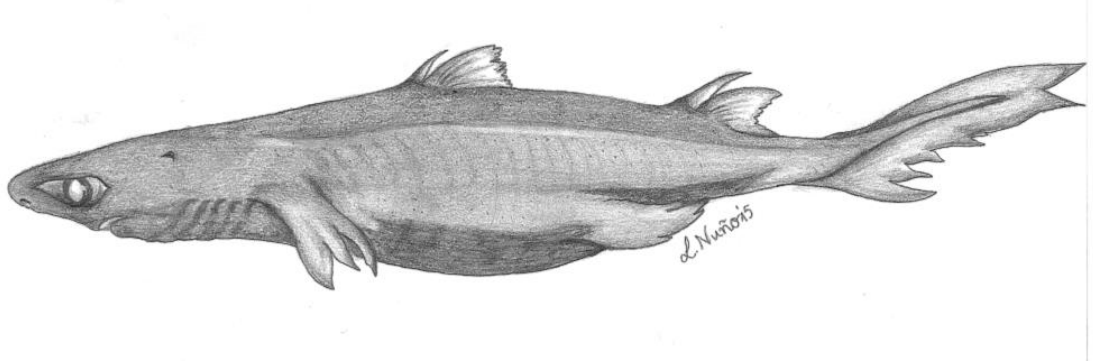


Figure 1.1: *Etmopterus spinax* (Linnaeus, 1758) (drawing by @ Laura Nuño Munõz).

1.2.2.1. General diagnosis

Etmopterus spinax (**Fig. 1.1**), commonly called Velvet belly, is a small sized deep-water squaliform shark, characterized by a fairly stout body, which is brown above and black below and has big eyes (Compagno *et al.*, 2005). It has a flattened snout, the upper teeth are small, with a narrow central cusp and usually less than three pairs of lateral cusplets. Lower teeth are much larger, strongly slanted, with blade-like cusps at the top and interlocked bases. On the other hand, the five pairs of gill slits are tiny compared with the size to the spiracles. Its main characteristic is the grooved spines that are located on both dorsal fins, with the second one much longer than the first one and curved, being perfectly visible. The anal fin is absent and the caudal fin is long. The Velvet Belly also possesses numerous photophores which emit a blue-green light visible from away and are distributed in a unique pattern for this species, as defined by. It can measure up to a maximum of 60.0 cm in total length for male or unsexed; its length is normally ~ 45.0 cm, however females are larger than males Compagno (1984).

1.2.2.2. Biological characteristics

The Velvet Belly Lanternshark has an aplacental viviparous reproduction, i.e. the embryos develop inside the eggs that are retained within the mother's body until they are ready to hatch. Females usually have litters of 6 or 7 hatchlings. Studies by Coelho and Erizini (2005) revealed that for *E. spinax* the size at first maturity is significantly higher for females (30.86 cm; 75.8% of the maximum observed size) than males, (25.39 cm; 75.1% of the maximum observed size).

Regarding their mating season, Aranha et al. (2009) reported that during the months of April and July it is possible to occur the breeding season of the females. However, in case of males, a higher percentage of active males was observed during May and June. The lifespan of *E. spinax* is estimated at 22 years for females and at 18 years for males (Gennari and Scacco, 2007).

1.2.2.3. Distribution and habitat

The Velvet belly is distributed from the eastern side of the Atlantic Ocean, extending from Iceland and Norway to Gabon, including the Mediterranean Sea, Archipelago of the Azores, Canary Islands and Cape Verde. The species has also been reported in the Cape Province in South Africa (**Fig. 1.2**). This shark species lives mainly in the outer continental and insular shelves and upper slopes, mostly at depths of 200 to 500 meters, near or well above the bottom. However the depth range for the species can range from 70 to 2000 meters, FishBase, (Froese and Pauly, 2015).

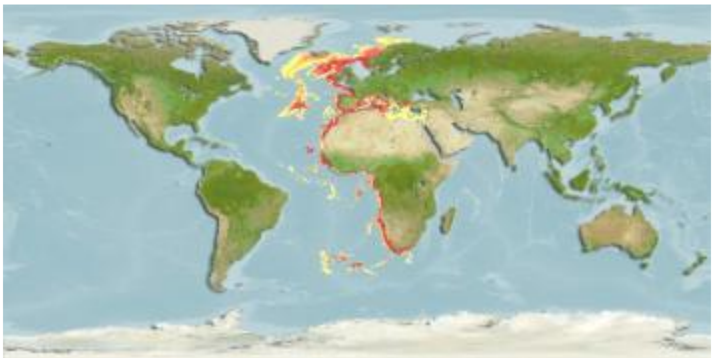


Figure 1.2. Geographical distribution of *Etmopterus spinax* (Linnaeus, 1758) (source: Global species database of fish species, FishBase (Froese and Pauly, 2015)).

1.2.3. *Etmopterus pusillus* (Lowe, 1839)

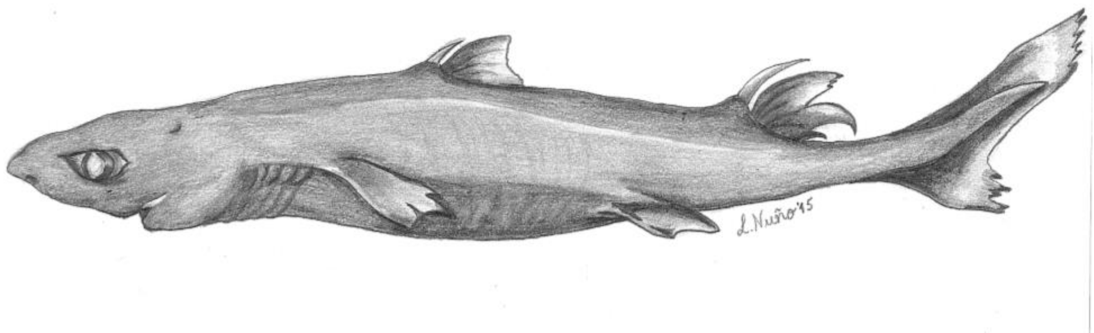


Figure 1.3. *Etmopterus pusillus* (Lowe, 1839) (drawing by @ Laura Nuño Munõz)

1.2.3.1. General diagnosis

Etmopterus pusillus (**Fig. 1.3**) also commonly called the Smooth lanternshark, is a small sized deep-water shark, globally widespread (Coelho and Erzini, 2007). It is characterized by a large head with a pointed snout and large oval eyes. This species has many rows of teeth on both jaws, where lower teeth are smooth, blade-like and with cusps, on the upper jaw they have cusplets (Compagno, 1984). On the other hand, the skin is covered by many widely spaced small blocky denticles not arranged in regular rows, giving it a smooth appearance. On its two dorsal fins there are two spines, being the one on the dorsal fin much larger than the one on the first. In addition, there is no anal fin, and the caudal fin is short and broad. The coloration is uniform dark brown along the dorsal side, with an obscure broad black mark running above, in front and behind pelvic fins (Compagno, 1984).

1.2.3.2. Biological characteristics

Coelho and Erzini (2005) reported that the Smooth lanternshark is an aplacental viviparous species which presents preliminary lengths at maturity, but there is no other biological information available. Generally the family Etmopteridae is characterized by having a very slow growth rate, late maturities and low reproductive potential (Fowler *et al.*, 2005). The size at first maturity for *E. pusillus* is significantly higher for females than for males, obtaining in this case, results where the size at first maturity was 43.60 cm (86.9% of the maximum observed size) for females and 38.10 cm for males (79.5% of the maximum observed size) as Coelho *et al.* (2005) reported.

Regarding their reproductive cycle, Coelho and Erzini (2007) stated that it is difficult to establish a definitive reproductive season. Nevertheless, both mature females with ripe oocytes and pregnant females were recorded mainly during the winter, from

November to April. However the active males appeared during the entire year, so it is not possible to define a reproductive season clearly.

1.2.3.3. Distribution and habitat

The Smooth lanternshark has a global distribution (**Fig. 1.4**), and can be found in the western Atlantic, in the northern area of the Gulf of Mexico and between southern Brazil and Argentina, but also in the eastern Atlantic from Portugal to Namibia. It has also been reported in the western Indian Ocean, South Africa and in the western Pacific near Japan. This species lives mainly on the continental and insular shelves, and upper slopes at depths from 274 to 1000 meters, but it has also been described in oceanic waters, between Argentina and South Africa, FishBase (Froese and Pauly, 2015).

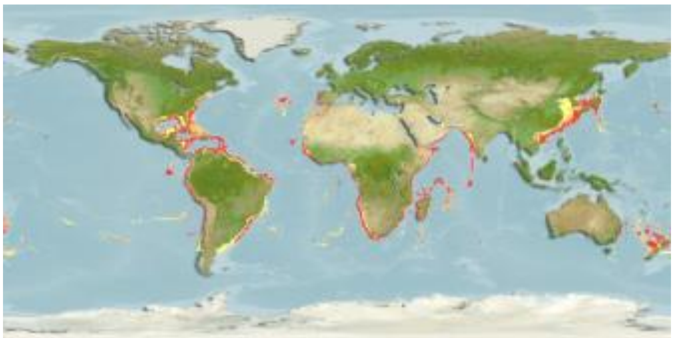


Figure 1.4. Geographical distribution of *Etmopterus pusillus* (Linnaeus, 1758) (source: Global species database of fish species, FishBase Froese and Pauly, 2015).

1.2.4. Feeding ecology of both species of lantern sharks

According to several authors, (Neiva *et al.*, 2006, Fanelli *et al.*, 2009 and Xavier *et al.*, 2012) both species of lantern sharks have similar feeding ecology. This is due to their main diet being based on Euphausiids, teleost fish, Natantid decapods and cephalopods, and many food items found in the stomach of both sharks belonging to the same species. The main species of prey found in *E. spinax* were *Meganyctiphanes norvegica* (Euphausiids), *Pasiphaea sivado* (Natantid decapods), *Micromesistius poutassou, Phycis blennoides* and myctophids family (teleosts fish) and some cephalopods like *Sepietta oweniana* (Neiva *et al.*, 2006) and *Histhioteuthis* spp. (Fanelli *et al.*, 2009). On the other hand the main prey species in the diet of *E. pusillus* were *Pasiphaea sivado, Micromesistius poutassou,* Myctiophids, Ommastrephidae and Sepiolidade families (Xavier *et al.*, 2012).

1.2.4.1. Main prey items

- Euphausiids:

- *Meganyctiphanes norvegica* (M. Sars, 1857):

Cruzin-Roudy *et al*. (2004) reported that the populations of this species of Crustacea, also called Northern krill, are adapted to very different environments and trophic conditions along the European coasts and adjacent seas. Krill perform vertical migrations from about 100 m in shallow environments reaching more than 500 m in the Ligurian Sea. Their reproductive season is long during spring and summer seasons for the northern sites and short during the late winter and early spring in the Ligurian Sea.

- Natantid decapod:

- *Pasiphaea sivado* (Risso, 1816):

This Crustacean of the family Pasiphaeidae is distributed along the eastern Atlantic, British Islands and Mediterranean Sea. It is a demersal species and it lives between 10 and 600 m deep. *P. sivado* is an ovigerous species and their reproductive cycle happens during the early summer (July) and early winter (December) (Falcai *et al.* 1995).

- Teleosts fish:

- *Micromesistius poutassou* (Risso, 1827)*:*

Blue whiting (*Micromesistius poutassou*) is a bathypelagic gadoid which is found along to the north-east Atlantic, from Spitsbergen (Greenland) in the north to Morocco (Africa) in the south (Heino *et al.*, 2008). Usually this species is distributed between 300 to 400 m from the surface but it can be found at depths of over 150-3000 m in midwaters, FishBase (Froese and Pauly, 2015). The reproduction of this species of gadoid is produced from February in the south to May in the north, at 180-360 m, on the edge of continental shelf (Marine Species Identification Portal, 2015).

- *Phycis blennoides* (Brünnich, 1768):

Phycis blennoides is another gadoid species, which is distributed from the eastern Atlantic to West Africa (Norway and Iceland to Cape Blanc), also the Mediterranean Sea. It is possible to found at a depth range of 10 to 800 m, over sand and mud bottoms (Cohen *et al.,* 1990). The spawning period observed in this species in the Mediterranean Sea occurs from January to March (Svetovidov, 1986; Cohen *et al.,* 1990; Massutí *et al.*, 1996 *in* Rotllant *et al.*, 2002).

- Family Myctophidae:

According to Nelson (2006) the Myctiophids are the most species-rich family of mesopelagic fishes with 235 species. They are possible to found in all oceans from near the surface to deep waters, because they have a diurnal migration of several hundred meters. During the daytime the peak abundance of most species is between 300 and 1200 m depth, while at night they are between 10 and 100 m. Their period of spawning is very variable (Marine Species Identification Portal, 2015).

- Cephalopods:

- *Sepietta oweniana* (d'Orbigny, 1841):

Sepietta oweniana is a cephalopod species, which belongs to the family Sepiolidae. It is a benthopelagic species that is found over muddy bottoms on the shelf and the upper slope, ranging in depth from about 50 to over 600 m. It has a geographical distribution from Northeast Atlantic (Norway), at depths of 50-100 m, to Morroco and the Mediterranean Sea, at 100-400 m. Their entire spawning period extends from March to November (Roper *et al.*, 1984).

-Family Histioteuthidae Verrill, 1881:

The species of this family of cephalopods are widespread from Eastern and western central Atlantic and western Mediterranean; southeast Atlantic, south Indian Ocean (limits undetermined); west of the Galapagos Islands off California, Hawaii and Japan; South Pacific. It is an oceanic species, which is reported to usually exist between depths of 500 and 1500 m, sometimes associated with the bottom (Roper *et al.*, 1984). According to Cuccu *et al.* (2014), these cephalopods have a single breeding period in their life, due to them being semelparous, but a great flexibility has been observed in their reproductive strategies, which range from simultaneous terminal spawning over a short period at the end of the animal’s life to continuous spawning over a long period of their life.

- *Ommastrephes bartramii* d’Orbigny, 1835:

Ommastrephes bartramii has a wide and discontinuous distribution in subtropical and temperate ocean waters and it is absent from equatorial waters (Boschi, 1998). As Watanabe *et al.* (2004) reported, this species are mainly distributed in the 300 to 600 m layer during the daytime and migrate to the 0 to 100 m layer at night in transitional waters of the central North Pacific. The period of spawning for *Ommastrephes bartramii* is rather extended (January to May in Japan) (Roper, 1984).

1.2.5. Economic and conservation values in fisheries

As explained above, *Elasmobranch* fishes are generally highly susceptible to fishing mortality. According to Coelho and Erzini (2008) the genus *Etmopterus* is caught in deep-water fisheries (mainly bottom trawl) that operate in Portuguese waters. In these fisheries *Etmopterus pusillus* is caught in large quantities as a by-catch species but, due to its low or insignificant commercial value, it is more often discarded (Coelho and Erzini, 2008). The Algarve is the sixth area with the highest fish discharge (by biomass) using crustacean trawl fishery (Neiva *et al*., 2006). In the semi-pelagic and near-bottom longline fishery for hake (*Merlucciidae*) *Etmopterus pusillus* is the fourth most abundant species caught. However, the catch of this species is not recorded in the fishery statistics of Portugal making it difficult to obtain any information about its biology, feeding preferences and ecology (Xavier *et al*., 2012). This also seems to be the case with catches of *Etmopterus spinax* in the coast of southern Portugal.

The two studied species are exposed to high levels of fishing mortality, long life cycles with slow growth rates, late maturity and low fecundities (Coelho *et al*., 2010). The intense fishing activities combined with the biological factors of these species will probably contribute for their decline in a very near future, and it could take decades for them to recover.

1.3. Objectives of this study:

The main aim of this study was to determine the feeding composition of two species of small deep-water Lanternsharks (*Etmopterus spinax* and *Etmopterus pusillus*). Secondary objectives were:

1) Evaluating the diet of each species by sex and maturity stage;

2) Evaluating the influence of seasonality into the diet of each species;

3) Determining the trophic level of each species;

4) Comparing the diets of the two species of lanternsharks.

Despite being widespread along the Algarve coast, there is still not much information on the feeding ecology of these two species. Consequently, studying the feeding habits of these species is important to reach a better knowledge of their behaviour and their position in the trophic level. Studying their biology and gathering information about their suitable habitats may be an important step towards the conservation of these vulnerable species or contribute to reduce their discards and/or increase their economic value. For these reasons, this study has a social, economic and especially biological interest.

Chapter 2 *MATERIALS AND METHODS*

2. MATERIALS AND METHODS

2.1. Geographic localization of the studied area

Stomachs from *E. spinax* and *E. pusillus* were taken from specimens fished in the Atlantic Ocean off the Algarve coast. This region is located in the south of Portugal, where Faro is the administrative centre (37^0 01’ N, 7^0 56’ W). It has a surface area of 4960 km^2, and around 200 km of coast, from Odeceixe in the west to Vila Real do Santo António close to the Spanish border in the southeast (Monteiro, 1987). The west coast is characterized by a rugged coastline with cliffs, and the south coast is mainly distinguished by its sandy beaches and limestone caves (Monteiro, 1987). There are also particular geological features that generate specific conditions for the sea environment or the current flow patterns, and consequently for the ecological biodiversity and trophic relations in the natural wildlife. The continental shelf has a variable width, from 8 km in Cabo de Santa Maria to 30 km near the locality of Portimão, whereas the depth is limited from about 110 to 150 meters (Magalhães, 2001).

As for the geological features, the current flow is normally weak and the main drift current follows the coastline at a bathymetry about 30 meters (Magalhães, 2001). In addition, the Algarve coast is also under the influence of the Mediterranean Sea because its water, denser and warmer, flows through the Gibraltar Strait into the Atlantic Ocean and affects it at a hydrological and biological level (Monteiro, 1987). Thereby, the sea surface temperature oscillates between 17^0 C in winter and 21^0 C in summer, occurring a stratification during this period. Furthermore, the temperature at a depth about 50 m is established at around 15^0 C during the whole year (Sanchez and Relvas, 2003).

2.2. Characterization of sample

The biological material used to develop the present study was collected from accidental catches, called bycatch, and discards of crustaceans and fish deep-water commercial trawlers throughout the south coast of Algarve (Portugal). These captures were carried out during two scientific projects concerning bycatch and discards (BYDISCARD, CE DGXIV-99/058; DISCALG, CE DGXIV-97/0087) (Costa *et al.*, 2002), which began in February 1999 and ended in September 2000. Fish samples also come from a campaign for evaluating bycatch of the crustacean trawl fishery in the Algarve, which lasted for five days, this began on the 27th of April 2015 and ended on the 3rd of May 2015. In the Algarve coast bycatch can exceed the catch of target species with this type of fishing gear (Campos and Fonseca, 2004), similar to what happens in many crustacean trawl fisheries worldwide.

During the two scientific projects concerning bycatch and discards, previously specified, 173 *Etmopterus spinax* specimens were randomly collected (out of 629) from 297 m to 754 m deep (**Annex 4**). On the other hand, during the sampling/campaign of 2015, 58 individuals of *Etmopterus pusillus* were collected. In this last case, the trawl was working at depths ranging from 160 m to 490 m off the coast of Olhão (Algarve, Portugal) (**Annex 5).** More lately, the individuals were stored in the freezer so they would not deteriorate for further analysis in the laboratory.

Length, sex, maturity and season data were taken from the above mentioned project's database. Individuals from the second campaign were measured (Total length, cm and weight, g) and sexed at the laboratory. The total length (cm) was used for each specimen of each species in order to obtain the state of maturity for each shark species (**Tab. 2.1**). The broad maturity stage (juvenil vs adult) of each individual was determined by confronting each individual length with the length at first maturity according to data reported by Coelho and Erzini (2005) for both species of lantern sharks. In the case of *E. spinax,* the length at 50% maturity was 30.86 cm for females and 25.39 cm for males, while the length at 50% maturity for *E. pusillus* was higher than for *E. spinax,* obtaining a length of 43.60 cm for females and 38.10 cm for males.

Table 2.1: Characterization of the samples of each species, *E. spinax* (above) and *E. pusillus* (below) in respect to the total length (cm) and total weight (g), where it was calculated; n (number of specimens), % (percentage number), Min. (Minimum size/weight), Max. (Maximum size/weight), Mean of the size/weight range, Stdv(±) (standard deviation) and CV (coefficient of variation) for the female (♀), male (♂) and the total specimens (♀+♂) of both deep-water shark species.

***E. spinax* specimens**			**Total length (cm)**					**Total weight (g)**				
Sex	**n**	**%**	**Min.**	**Max.**	**Mean**	**Stdv(±)**	**CV**	**Min.**	**Max.**	**Mean**	**Stdv(±)**	**CV**
♀	89	51.4	9.3	38.6	23.7	9.67	0.41	2.7	301.1	86.4	81.12	0.94
♂	84	48.6	9.1	37.7	20.1	7.63	0.38	2.4	264.1	44.3	44.77	1.01
♀ + ♂	173	100	9.1	38.6	22.0	8.90	0.41	2.4	301.1	66.0	69.12	1.05

***E. pusillus* specimens**			**Total length (cm)**					**Total weigth (g)**				
Sex	**n**	**%**	**Min.**	**Max.**	**Mean**	**Stdv(±)**	**CV**	**Min.**	**Max.**	**Mean**	**Stdv(±)**	**CV**
♀	12	20.7	22.0	40.5	29.9	6.74	0.23	31.9	270.9	115.1	79.70	0.69
♂	46	79.3	18.9	42.5	29.4	5.40	0.18	20.2	276.2	98.6	60.83	0.62
♀ + ♂	58	100	18.9	42.5	29.5	5.64	0.19	20.2	276.2	101.9	64.49	0.63

In the case of season analysis, the samples were grouped in two different categories according to the month in which the specimens were caught. This fact was due to the samples shortage of some seasons. Then, the specimens obtained during the warm months were grouped into the *hot season category* (spring and summer), and the specimens caught during the cold months were grouped into the *cold season category* (winter and autumn).

2.3.Laboratory procedure

The stomach samples of *E. spinax*, were kept in formalin for a time. Therefore the stomachs were removed from formalin plastic containers where they were individually preserved in 4% buffered formalin and subsequently immersed in 70% ethanol in marked containers for further analysis (Costa *et al.,* 2002). The use of 4% formalin allows the fixation of tissues and also minimizes the digestion process when the individual is caught (Bowen, 1983 *in* Gonçalves, 2000). Once the remaining contents of formalin disappeared, each stomach was weighed, considering the result as wet weight. Subsequently the sample was opened and the content was placed on a Petri plate using small ethanol jets, then the remaining liquid was removed with an absorbent paper (**Fig. 2.1**). On the other hand the stomach wall was weighed (wet weight) to have a more accurate value of the weight of the contents in the stomach. Later, the individuals ingested were identified using a lens and sorted into different categories (cephalopods, crustaceans and fish). Furthermore, the category of not identified preys was assigned in the case of prey items really affected by the digestion process (**Fig. 2.2**). After this procedure, individuals of each taxon were weighed (total weight) separately using scales (± 0.01 g). Once the stomach contents of all individuals were weighed, they were transferred to small plastic containers with ethanol 70% for further taxonomic analysis.

Figure 2.1: Laboratory procedure carried out for the stomach contents analysis of *E. spinax* and *E. pusillus* (photos by @ Laura Nuño Muñoz).

For the identification procedure different taxonomic keys were used depending on the phyllum of each organism found (Zariquiey, 1968; Roper *et al.*, 1984; Falciai and Minervini, 2005). On the other hand, it is also important to know that all preys were digested and the identification process was complicated. In the cases in which the stomachs were full of sediment and/or unidentified bulk, the data was not taken into account for further analysis. A digestion key was used to identify the digested rank according to the decomposition state of the preys (Aloncle and Delaporte, 1970). This key describes the degradation state for fishes, crustaceans, bivalves and polychaetes. The rank ranges from 1 to 4 for fish, crustaceans and polychaetes and from 1 to 3 for bivalves, being 1 the state in which the preys have been digested recently and therefore they have a firm consistency, in the 2 state the prey begin to decompose, and finally 3 and/or 4 the state in which only the hard parts of individuals will be preserved. In order to avoid digestion biases, prey items were identified mainly based on fresh hard parts, such as fish otoliths, cephalopod beaks, crustacean carapaces or appendices, reaching the lowest taxonomic level possible. So as for our case, the fish otoliths and cephalopod beaks were treated in order to reach each ingested prey's type.

Figure 2.2: Prey items (cephalopods, crustaceans and fish) found in the stomach contents of *E. spinax* and *E. pusillus* in different digestion stages (photos by @ Laura Nuño Muñoz).

The otoliths were identified observing their morphologic descriptions according to otolith shapes, outline and groove features using different taxonomic keys such as Tuset *et al*. (2008) and Ramos (1999).

The cephalopods beaks were identified according to different measures on the upper and lower beak, where both beak measures were used as independent variables in a linear regression model with mantle length and body weight (log transformed) of different cephalopod species (Wolff, 1984) (**Fig. 2.3**).

In our case only the beak measures reported by Wolff (1984) were taken for the identification of each cephalopod species by lens. The upper beak measures were: Length of the rostrum (RL), rostral tip to inner margin of wing (RW), length of hood (HL), width of the wing (WW), wing to crest length (WCL), jaw angle width (JW), and length of the crest (CL). Similarly the lower beak measures were: Rostral tip to inner posterior corner of the lateral wall (RC), rostral tip to inner margin of wing (RW), length of the rostrum (RL), length of the wing (WL) and jaw angle width (JW).

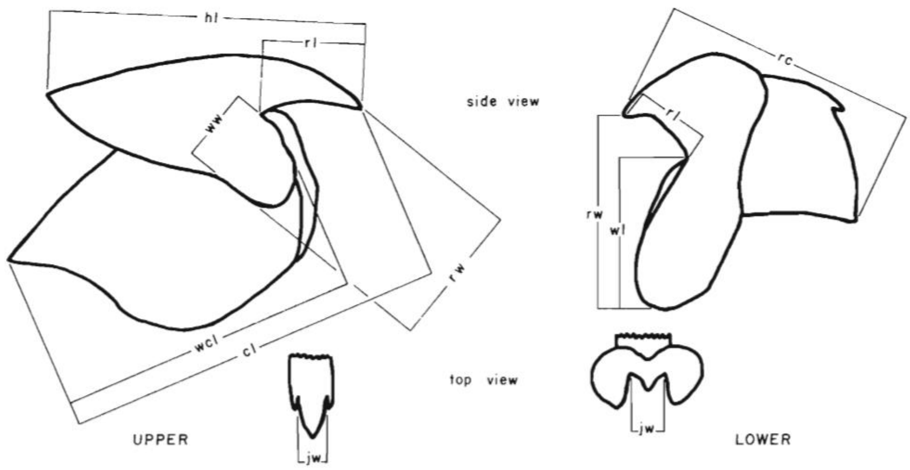


Figure 2.3: Side and top view of upper part and lower part of cephalopod beak with their different measures (Wolff, 1984).

Finally, the data obtained was stored in a data base to proceed to perform calculations with different statistical analysis types.

2.4. Representativeness of the sample

Calculating the trophic diversity by Shannon index or calculation of the cumulative number of dietary items are the main methods used to determine the number of predators which are necessary to describe their diet, for this purpose the number of individuals analysed must be random (Hurtubia, 1973; Cailliet, 1977 *in* Gonçalves, 2000). To consider a sample representative, it needs to reach a point in which the curve defined by each method, begins to stabilize. This point of stabilization is the point at which the probability of not considering an important item in the diet is quite low (Gonçalves, 2000).

In this study, the cumulative number of prey species found in the stomachs analysed for both species of sharks , which contained some prey type, was used, considering all individuals identified to the lowest taxonomic level.

2.5. Univariate statistical analyses

2.5.1. Quantitative analyses

Diet quantity data was analysed by Relative Measures of Prey Quantities (RMPQs) using the following indices: percentage of occurrence by number (%N), percentage of frequency of occurrence (%F), and percentage of occurrence by weight (%W) of prey items (Preti *et al*., 2008).

2.5.1.1. Numeric method:

The value %N is the number of individuals of a specific taxon (n) found in all stomachs divided by the total number of all prey found into the stomach which contained prey (N) and multiplied by 100 (Hyslop, 1980).

$$\%N = \frac{n}{N} \times 100$$

Normally the digestion process causes the fractionation of prey species, so it was necessary to establish some criteria for accounting for prey individuals for further analyses (Gonçalves, 2000). In the case of the prey that were most affected by the digestion process and which were identified by their distinctive parts. However some prey species could not be quantified, thus it was considered an amount of 1 individual (e.g., a group of similar fish scales). However, in the case of the prey that were least affected by the digestion process their quantification could be performed.

2.5.1.2. Gravimetric method:

The gravimetric method or percentage by weight (%W) is obtained as the total weight of all remains of a specific taxon (w) divided by the total weight of all prey remains found (W) multiplied by 100 (Hyslop, 1980).

$$\%W = \frac{w}{W} \times 100$$

2.5.1.3. Frequency of occurrence:

This quantitative index (%F) is represented as the number of stomachs containing prey of a specific taxon (f) divided by the total number of stomachs containing prey multiplied by 100 (Hyslop, 1980).

$$\%FO = \frac{f}{Total\ number\ of\ stomachs\ with\ prey} \times 100$$

It is possible that the sum of the frequency of occurrence values could exceeded 100%, because more than one different item can be found in the same stomach (Gonçalves, 2000). Different categories of prey can be distinguished according to the results obtained for %F (Cardoza Martínez *et al.*, 2011), which indicates the possible feeding preferences of an organism: accidental prey (%F < 10%), secondary prey (10% < %F < 50%) and preferential prey (%F > 50%).

In addition, to normalize the different indices and thereby compare them more easily (inter- and intraspecific comparisons), Rosecchi and Nouaze (1987) proposed altering its formula to transform the values of FO to percentage as occurs with %N and %W values, obtaining the corrected occurrence frequency (FOc) by modifying all indices in which it operates:

$$FOc = 100 \times \frac{\%FO}{\sum \%FO}$$

Regarding the calculation of this quantitative analyses it is important to highlight that the empty stomachs and stomachs with sediment content were not considered in the calculation of the different indices of this study.

2.5.2. Mixed analyses

In order to evaluate the main preys in the diet of *E. spinax* and *E. pusillus*, different mixed analysis were calculated, taking into account the quantitative methods (%N, %W and %F) aforementioned. The mixed indices analysed were: Index of Relative Importance (IRI) and a Weighted Food Index (IPO2) (Gonçalves, 2000).

2.5.2.1. Index of Relative Importance (IRI)

The Index of Relative Importance (IRI) is the result of the interaction of %N, %F and %V (Volumetric percentage), which was developed to assist in evaluating the relationship between the various food items found in stomachs (Pinkas *et al*., 1971). In our study the %V parameter was amended by %W parameter as Capitoli *et al*. (1995) previously reported. This index can be calculated by adding the percentage of occurrence by number and percentage of occurrence by weight values and multiplying by the percentage of frequency of occurrence value, obtaining the following equation:

$$IRI = (\%N + \%W) \times \%FO$$

In order to facilitate the inter- and intraspecific comparisons among studies (Preti *et al*., 2008), the IRI value was converted into the percentage to keep the same scale as other indices. Index values are sorted into decreasing order according to their %IRI contribution (Carrassón *et al*., 1992) for further calculation of cumulative %IRI.

2.5.2.2. Weighted Food Index (IPO2):

The Weighted Food Index IPO2, assigned high weight values according to their nutritional importance (Mattson, 1981; Bowen, 1983 *in* Gonçalves, 2000). The weighted value "x", which corresponds to a value of 0.25, the sum of %N and %FO. Moreover it is also attributed the value "y" to %W, which corresponds to 0.5, causing the value of the weight component to have a higher importance, due to the addition of these three parameters (Gonçalves, 2000):

a) IPO2 = (%N + %FO) x "x" + %W x "y" (x = 0.25 and y = 0.5)

2.5.3. General classification methods prey

The prey were classified into different categories according to classification methods of Rosecchi and Nouaze (1987), in which all indices, obtained in percentage, were arranged in descending order. This was followed by the sum of the values of the first category with the second category and so on, reaching 50% of the value of the total index (preferential prey). Then the addition of the index values for the same cumulative order to reach 75% of the total index, corresponds to secondary prey, considering the remaining prey with values reaching 100%, as accessory prey.

This general classification method allows a comparison between the diets calculated through the different indices, and also indicates the existing food selectivity by the number of preferential prey groups (Gonçalves, 2000).

The IRI, MFI, and IPO2 indices were calculated to obtain the principal, secondary and accessory prey items (Annex 4-19) according to Gonçalves, (2000) (**Annex 4**).

2.5.4. Vacuity Index (VI)

The Vacuity Index (VI) was used to determinate the filling state of stomachs (Cardoza Martínez *et al*., 2011), so that it could be indicators of the feeding activity of fishes. Thus, to evaluate the percentage of empty stomachs the Vacuity Index (VI) was used, considering VI as the number of empty stomachs divided by the total number of examined stomachs multiplied by 100. This index provides information about the physical conditions and the feeding activity periods of individuals (fasting or end of digestive process) (Simões de Sá, 2008).

2.5.5. Comparing the diets between species

2.5.5.1. Feeding overlap (Schoener Index)

The Schoener Index was used to evaluate dietary affinities between the different sexes, size classes, and seasons, in terms of absolute prey abundance (Cartes and Sardà, 1989). According to Brulé and Canché (1993), the feeding overlap index can be calculated by the following equation:

$$\propto = 1 - 0.5 \left(\sum_{i=1}^{n} \left| p_{xi} - p_{yi} \right| \right)$$

As Morte *et al.*, (2002) determined, n is the number of food organisms, p_{xi} and p_{yi} are the numerical composition indices of prey (i) in the diets of species x and y, respectively. This index has a minimum value of 0 when the overlap feeding is non-existent, and a maximum value of 1 when all prey have the same proportion inside the stomachs. There are three categories established according to the results of Schoener index values: i) low level (0 - 0.29), ii) mean level (0.30 – 0.60), and iii) high level (> 0.60), which indicates a trophic overlap (Simões de Sá, 2008). However, if the overlap index value is below 0.6 it is normally because diets of different species are significantly different (Cartes and Sardà, 1989).

2.5.5.2. Trophic level:

In order to determine the position in the trophic chain of deep-water shark species the trophic levels (TL) based on diets of *E. spinax* and *E. pusillus* were estimated. For the TL calculation for each shark species was taken into account the average trophic level value for each prey category (TL_k) according to Cortés (1999) methodology using standardized IRI:

$$TL_k = 1 + \left(\sum_{j=1} P_j \times TL_j \right)$$

Where j is the number of prey categories, P_j is the proportion of the IRI index of the prey "j" and TL_j is the trophic level of each prey category "j".

2.5.5.3. Diversity, Richness and Evenness Indices

The diversity indices are composed by two different components, the total number of species and the evenness; in other words, how the abundance data is distributed among the species. Although they are easy to use, there are many problems due to certain limitations (Ludwing and Reynolds, 1988). The reason is the confusion between these two variables when trying to combine them. Moreover, the species richness is the oldest and the simplest concept, since it is the number of species in the community (Krebs, 1999). It is also easy to use but, in the same as diverse indices, this index has a problem in the measurement, owing to it is often impossible to enumerate all of species in a natural community.

Hence, in order to evaluate the diversity, richness and evenness of species in the diet of *E. spinax* and *E. pusillus* species, Shannon (H'), Margalef (R_1) and Pielou (J') indices were applied (Haidari *et al*., 2012) using PRIMER-E (Clarke and Warwick, 2001). In this study, the identified prey items were used to the calculation of each of these indices (Gonçalves, 2000). In addition to the comparison between sexes, maturity stages and seasons variables of each deep-water shark species an analysis based on these three diversity indices was also carried out. With the purpose to know the existence or absence of significant differences in the diet diversity by sexes, maturity stage and season for both deep-water shark species, the diversity values were tested by a non-parametric Mann-Whitney Rank Sum Test.

a) Diversity Index (H')

The Shannon Index (H') is the most common index used to quantify the specific biodiversity (Pla, 2006). This index shows the heterogeneity of a community by the following factors: number of species found and their relative abundance. Shannon Index (H') is defined as:

$$H' = -\left(\sum_{i=1}^{n} p_i \ log_2 \, p_i\right)$$

Where *H'* is the diversity in the stomach; *n* corresponds to the total number of species in the sample, and p_i is the proportion of the species (*i*) in the stomach.

Conceptually it is a measurement of the uncertainty level associated with the random selection of an individual in the community (Pla, 2006); i.e. if a community of species is highly heterogeneous, it is due to the existence of a clearly dominant species and the remaining species are scarcely present. The uncertainty level will be lower if all species are equally abundant.

b) *Richness Index (R_1)*

To estimate the species richness in sampling units, the Margalef Index will be used (Gonçalves, 2000), which considers the numeric distribution of individuals for different species depending on the individual number existing in the analyzed sample. However, it does not consider the relative importance of species, because it is only based on the number of species found. The Margalef Index is calculated using the following equation (Gonçalves, 2000):

$$R_1 = \frac{S-1}{\ln(N)}$$

Where S is the number of species and N is the total number of individuals. If the results of the richness index are near to 0, there is lower species richness and consequently the diversity in sampling units is also low. However, if its values are near to 1, the species richness is higher and thus the diversity of sampling units is high.

c) *Evenness index (J')*

According to Magurran (1988), in order to know how the abundance data is distributed among the species, the Pielou's evenness index was used. This index is based in the relationship between the observed diversity, such as Shannon's diversity index (H'), and the maximum diversity (H_{max}). The maximum diversity could occur when all species have the same abundance, in other words if H'= H_{max} = ln S. Hence, the ratio of observed diversity to maximum diversity can be taken as a measure of evenness (*E*):

$$E = \frac{\mathrm{H}'}{\mathrm{H}_{\mathrm{max}}} = \frac{\mathrm{H}'}{\ln \mathrm{S}}$$

The *E* value is within the range between 0 and 1, where the value of 1 represents a situation in which all species are equally abundant (Magurran, 1988).

2.6. Multivariate statistical analyses

The software PRIMER V6.0 (Plymouth Routines In Multivariate Ecological Research) was used for multivariate statistical analyses (Clarke and Gorley, 2006). These analyses were used to compare two or more samples which share particular species at a comparable level of abundance (Clarke and Warwick, 2001). In this case, this method was used to obtain information about the diet composition of *E. spinax* and *E. pusillus*, to compare their diets and to test the overall differences in general prey types. Comparisons were done through different tests such as CLUSTER, MDS and SIMPER and a statistical permutational test ANOSIM.

The starting point for many of the analyses is normally linked to the concept of similarity between samples, in terms of biological communities. There are many ways to define similarity, usually depending on the weight it has on different aspects of the community. In order to increase the abundance of minority species and adjust the similarity level of the communities, it is necessary to replace the original data matrix (species / samples) for a transformed data matrix. Therefore, in the present study, the statistical analyses of the samples will be performed with a fourth root of the original data matrix (Clarke and Warwick, 2001). The data matrix was formed by the most representative index, i.e. relative importance index corrected (IRIc).

2.6.1. CLUSTER analysis

In order to determine similarities and differences in the diet of *E. spinax* and *E. pusillus*, a matrix of similarities (calculated from original data matrix) between each pair of individuals was calculated using the Bray-Curtis similarity coefficient. This coefficient is necessary for the Cluster analysis and it was calculated with data transformed to fourth root. The aim of Cluster analysis is to find “natural groupings” of those samples that are more similar to each other within a group, than other samples in different groups. Samples are “mapped” in such a way that distances between pairs of samples show the relative dissimilarity of the species composition in the diet (Clarke and Warwick, 2001)

2.6.2. Multidimensional Scaling analysis (MDS)

The Multidimensional Scaling analysis (MDS) is a spatial representation technique which reflects the particular aspects of community structure (Clarke, 1993). The values were represented graphically on a map in order to analyse the similarity or dissimilarity in the diet of these two species according to the distance between each represented point on the multidimensional space. Thus, if the distance between two points is large, the diet composition will have a low similarity and if this distance is smaller, the similarity in the diet composition will be (Clarke and Warwick, 2001)

2.6.3. ANOSIM analysis

ANOSIM test is an analysis used to evaluate statistical differences in the diet composition between the elasmobranchs groups of *E. spinax* and *E. pusillus* and within each species in relation to sex, maturity state and season. This multivariate analysis (González *et al*., 2013) performs a null hypothesis of similarity of the parameters that describe the behaviour of the variables in the different species according to their type of diet. The value of ANOSIM-R indicates the extent of the difference between groups (R > 0.75: well separated groups; R = 0.50-0.75: separated but overlapping groups; R = 0.25-0.50: separated but strongly overlapping groups; and R < 0.25: barely separated groups) (Pethybridge *et al*., 2011).

2.6.4. SIMPER analysis

The Similarity Percentage analysis (SIMPER) procedure was used in order to calculate the value of similarity (Krebs, 1999) according to the main species of both elasmobranch diets depending on different parameters (sex, maturity state and season) (Fanelli *et al*., 2009). Each community sample must be standardized in terms of percentages, so the relative abundances reach 100% in each sample. So it is possible to know what prey is typical of the diet depending on the degree of similarity in the dendrogram, and thus it is possible to identify the main prey *taxon*. (Krebs, 1999).

Chapter 3 ***RESULTS***

3. RESULTS

3.1. *Etmopterus spinax* (Linnaeus, 1758)

3.1.1. Representativeness of the sample

The representativeness of the sample was obtained from the relationship between the cumulative number of prey species and cumulative number of stomachs with content of *E. spinax* (n=97). The cumulative prey curves described an increasing relationship which reached asymptotic stabilization with approximately 59 stomachs analyzed and 22 species of prey, where the sample starts to be representative. (**Fig. 3.1**).

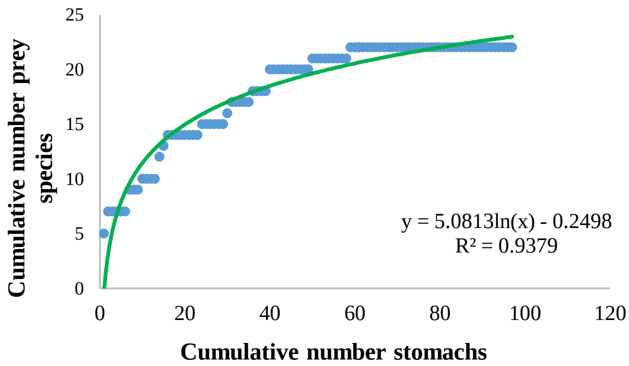


Figure 3.1: Relationship between cumulative number of prey species identified in the stomach contents and cumulative number of stomachs analyzed of *E. spinax.*

3.1.2. General composition of the diet

In the feeding study of *E. spinax* 173 stomachs were considered, of which 76 stomachs were empty, resulting in a vacuity index of 43.9%, regarding the total number of stomachs. The average number of prey per stomach was 1.6 with an average weight of 1.16 g. The percentages for stomachs filled with unidentified bulk and with sediments (sand and mud) regarding the total number of stomachs, were obtained: 19.1% and ~11%, respectively, resulting in 97 stomachs with contents for further analysis.

In the stomach contents of *E. spinax*, 19 different prey species were identified. They belong to three groups of prey - cephalopods, crustaceans and fish - in which different prey items were identified (9, 5 and 5, respectively) (**Tab. 3.1**). The rest of prey items were considered as unidentified items. The systematic classification of all prey items are represented in the **Annexes 1, 2 and 3**.

The quantitative indices (%N, %W, %FO and %IRI) of the three main groups of prey were taken into account to represent the diet of *E. spinax*. The crustaceans were the most representative group in terms of IRI (70.3%), %N (70%) and %FO (54.4%). In terms of %W, the fish were the most representative group (46%), followed by the cephalopods group (21%), which was the least representative one regarding all indices (**Fig. 3.2**) (**Annex 6**).

Table 3.1: List of prey found in the stomach contents of *E. spinax.* Where SbO: Suborder, O: Order and F: Famlily.

PREY GROUPS	PREY ITEMS
CEPHALOPODS	O. Oegopsida
	F. Sepiolidae
	Sepia orbignyana [1]
	Ommastrephes bartramii [1]
	Pterygioteuthis giardi [1]
	Histioteuthis heteropsis [1]
	Liocranchia reinhardtii [1]
	Onychoteuthis banksii [1]
	Abraliopsis affinis [1]
	N.i. Ceph
CRUSTACEANS	O. Euphausiacea
	SbO. Natantia [2]
	F. Pandalidae [2]
	Pasiphaea spp. [2]
	Pasiphaea sivado [2]
	N.i. Crust
FISH	F. Gonostomatoidae [3]
	Symphurus spp.[4]
	Arnoglossus spp. [4]
	Phycis blennoides [5]
	Micromesistius poutassou [5]
	N.i. Fish

[1]Oegospsida; [2]Decapoda; [3]Stomiiformes; [4] Pleuronectiformes; [5] Gadiformes

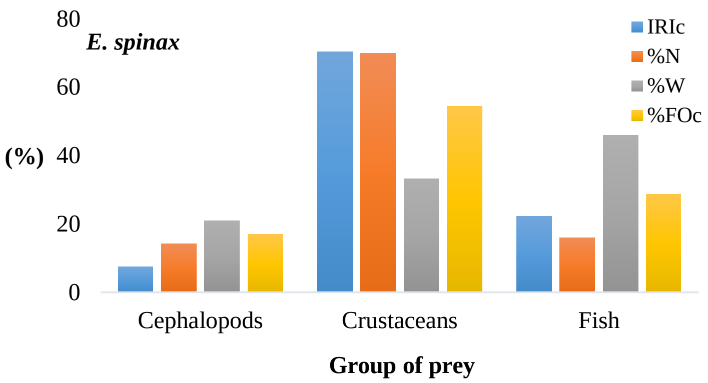


Figure 3.2: Representation of quantitative methods (IRI, %N, %Wand %FO) of the three main prey groups in the diet of *E. spinax.*

According to the numerical method, 276 prey were counted. The most important prey group in the diet of *E. spinax* were the crustaceans (69.9%), being the decapods (44.6%) and Euphausiacea orders (17.8%) the most abundant, followed by the fish group (15.9%), mostly represented by unidentified (N.i.) Fish (13%). The cephalopods were the least representative group in terms of number (14.1%), with Oegopsida and unidentified cephalopods (N.i. Ceph) in the same proportion (6.5%) (**Fig. 3.3**) (**Annex 18**).

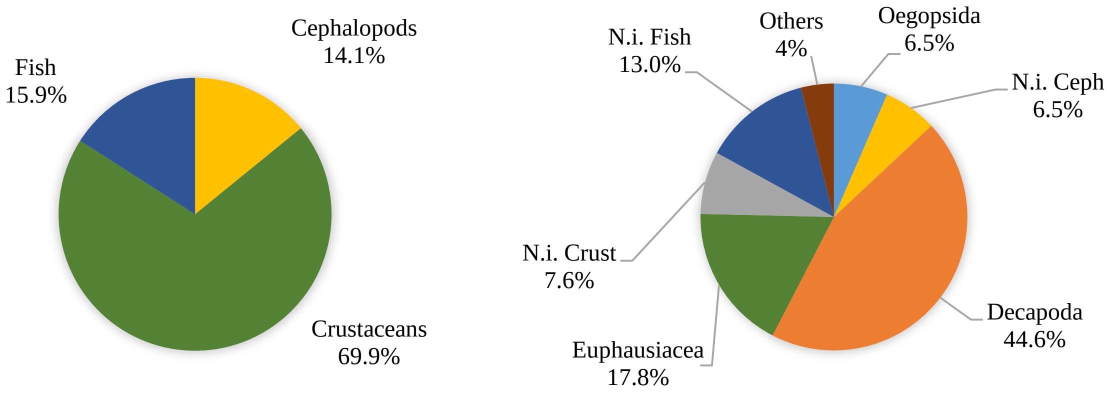


Figure 3.3: Relative importance by number (%N) of the main groups of prey (left) and of the different orders of prey (right) in the diet of *E. spinax* (N=97).

Concerning the gravimetric method, the total weight obtained for all prey was 201.25 g. The fish group was the most representative (46%) of which the N.i. Fish attained the largest proportion (40.5%), followed by the crustaceans group (33.2%) represented mostly by the decapods (25%). In terms of weight (%W), cephalopods continued to be the least representative group of prey (20.8%), with unidentified cephalopods (N.i. Ceph) contributing with 7.9% (**Fig. 3.4**) (**Annex 18**).

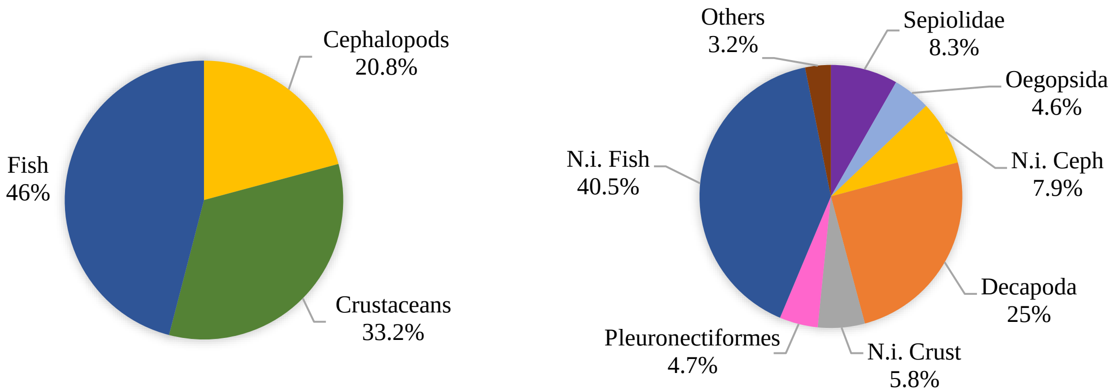


Figure 3.4: Relative importance by weight (%W) of main groups of prey (left) and the different orders of prey (right) in the diet of *E. spinax* (N=97).

In terms of frequency of occurrence (%FO), the most frequent group were the crustaceans (54.4%), of which the major proportion were decapods (42.9%). The fish group was the least frequent (28.7%), 20.2% of which corresponds to N.i. Fish. The least frequent group were the cephalopods (16.9%), where the N.i. Ceph (10.4%) and the Oegopsida order (8.6%) were the most representative (**Fig. 3.5**) (**Annex 18**).

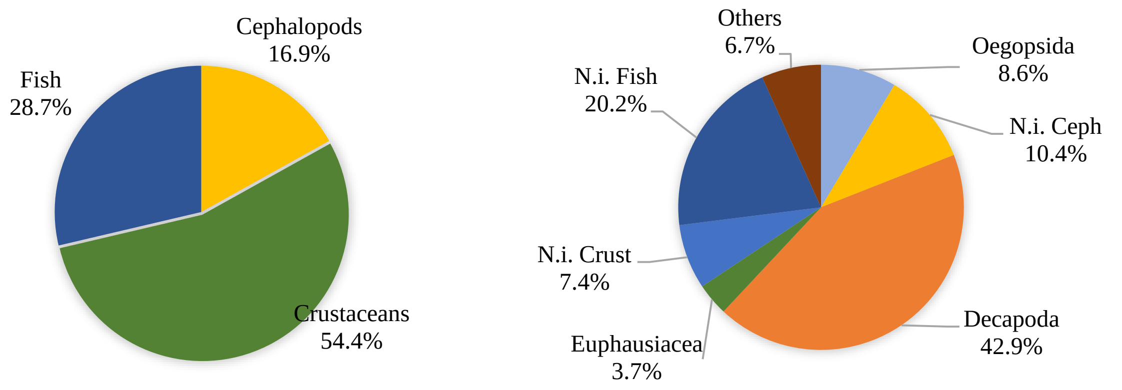


Figure 3.5: Frequency of occurrence (%FO) of main groups of prey (left) and the different orders of prey (right) in the diet of *E. spinax* (N=97).

Regarding the relative importance index (IRI) the crustaceans were the most important group (70.3%), mainly composed by decapods (67.1%). The fish was the second most important group (22.2%), being the N.i. Fish the dominant prey (24.3%). Stomiiformes and Gadiformes fish had a residual importance in the diet (0.04% and 0.02%, respectively). The least important group was the cephalopods (7.4%), with the N.i. Ceph as the most important prey (3.4%) (**Fig. 3.6**) (**Annex 18**).

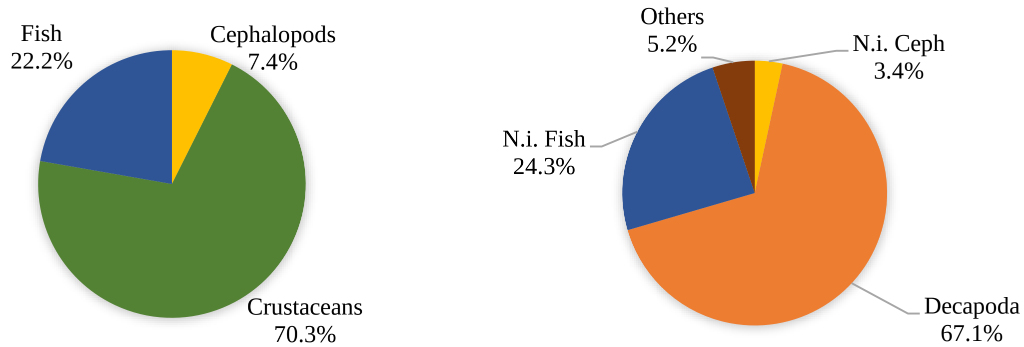


Figure 3.6: Relative importance (%IRI) of main groups of prey (left) and the different orders of prey (right) in the diet of *E. spinax* (N=97).

3.1.2.1. Prey classifications

The indices calculated for the diet of *E. spinax* were pooled into different groups according to the order of importance for each type of prey and, in this case, three groups were obtained for each index. The first group (G.I) consisted in the IRI index and the second one (G.II) was composed by the IPO2 index. According to the different indices analysis it was possible to verify that the main prey items in the diet of *E. spinax* were the Suborder (SbO) Natantia, N.i. Fish, N.i. Ceph and N.i. Crust (**Tab. 3.2**).

The groups of indices are fairly homogenous until the 6th category, where these groups begin to have slight variations according to the normal order. Both groups (G.I and G.II) were similar in respect to the main order.

Table 3.2: Ranking of prey groups according to the indices used for *E. spinax.*

MAIN ORDER	PREY ITEMS	G. I	G. II
		IRI	IPO2
1	SbO. Natantia	1	1
2	N.i. Fish	2	2
3	N.i. Ceph	3	3
4	N.i. Crust	4	4
5	*Pasiphaea sivado*	5	5
6	O. Euphausiacea	6	6
7	*Symphurus* spp.	7	10
8	*Pterygioteuthis giardi*	8	7
9	O. Oegopsida	9	9
10	*Sepia orbignyana*	10	8
11	*Phasiphaea* spp.	11	12
12	F. Sepiolidae	12	11
13	F. Gonostomatidae	13	13
14	*Liocranchia reinhardtii*	14	15
15	*Ommastrephes bartramii*	15	14
16	*Abraliopsis affinis*	16	16
17	F. Pandalidae	17	17
18	*Histioteuthis heteropsis*	18	18
19	*Physcis blennoides*	19	19
20	*Micromesistius poutassou*	20	20
21	*Onychoteuthis banksii*	21	21
22	*Arnoglossus* spp.	22	22

N.i.: Not identified

When applying the classification of prey by IRI values according to Rosecchi and Nouaze (1987), it can be observed that the G.I followed the same ranking as the normal order (**Tab. 3.2**) without variations, i.e., SbO. Natantia which is the 1st in the ranking order is the preferential prey, N.i. Fish in the 2nd rank is the secondary prey and the rest of prey are accessory prey (**Tab. 3.3**) (**Annex 30**).

Table 3.3: Classification of prey by IRI values according to Rosecchi and Nouaze (1987) for *E. spinax*.

Prey items	%IRI values	Cumulative %IRI values	Rosecchi and Nouaze (1987) classification
SbO. Natantia	54.27%	54.27%	Preferential prey
N.i. Fish	32.27%	86.54%	Secondary prey
N.i. Ceph	4.48%	91.03%	Accessory prey
The rest of prey	2.93%-0.01%	93.96%-100%	

Using the classification of prey by IPO2 index it was observed that G.II showed a similar pattern of G.I (**Tab. 3.2**). According to this classification it was possible to verify that from 2 preferential prey, the SbO. Natantia (27.5%) and N.i. Fish (27.37%). Regarding secondary prey, N.i. Ceph (8.52%) and N.i. Crust (6.73%) were the most relevant secondary prey. *Sepia orbignyana* (3.33%) was the most representative of the other prey items (**Tab. 3.4**) (**Annex 31**).

Table 3.4: Classification of prey by IPO2 values for *E. spinax.*

Group of prey	IPO2 values (%)	Cumulative IPO2 values (%)	Classification of prey
SbO. Natantia	27.47%	27.47%	Preferential prey
N.i. Fish	27.37%	54.83%	
N.i. Ceph	8.52%	63.36%	Secondary prey
N.i. Crust	6.73%	70.09%	
Sepia orbignyana	3.33%	86.12%	Accessory prey
The rest of prey	0.87%-0.30%	95.91%-100%	

3.1.2.2. Diversity indices

Concerning the results obtained of species diversity in the diet composition of *E. spinax* was possible to observe that this species present similar and very low values of each index, some of them near 0 and other being 0. Therefore resulting values of 0.3 ± 0.47; 0.5 ± 0.72 and 0.4 ± 0.45 for diversity, richness and evenness indices, respectively.

3.1.3. Relationship between sex and diet composition

In the analysis of the relationship between sex and diet of *E. spinax* species only the index of relative importance (IRI) was taken into account in this study. The IRI index was used because it combines all quantitative indices (N, FO, and W) that while giving more importance to the frequency of occurrence, it also discriminates better between values of the index allowing an effective evaluation of the importance of each type of prey to predator diet.

The vacuity index (VI) and the different percentages of unidentified bulk and sediment obtained for the sex variable of this species was calculated, taking into account the total number of stomachs of each sex. These results are shown in the following table (**Tab. 3.5**).

Table 3.5: Vacuity Index (VI) and percentages of unidentified bulk (N.i. Bulk) and sediments obtained for females (♀) and males (♂) of *E. spinax* species.

	T_N stomachs	N^0 empty stomachs	VI (%)	N.i. Bulk (%)	Sediments (%)
♀	89	38	42.7%	22.5%	8.9%
♂	84	38	45.2%	15.5%	13.1%

Females

In the analysis of the relationship between sex and diet composition for the female specimens of *E. spinax* and taking into account the empty stomachs, 51 stomach were analyzed. Considering the overall quantitative analysis (**Annex 7**), it is possible to verify that crustaceans are the most abundant and important group of prey in the diet of the Velvet belly females, representing 74.6% in terms of IRI. Decapods were the crustacean prey items which contributed most to IRI (72.6%) (**Fig. 3.7**) (**Annex 19**).

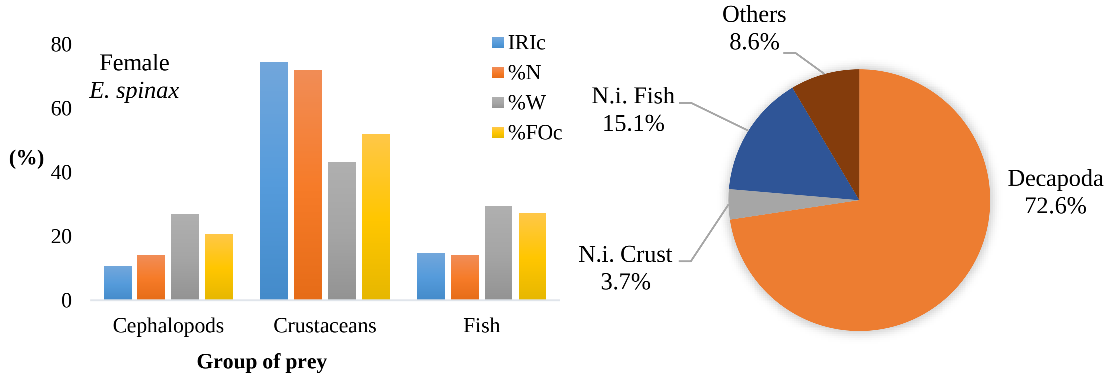


Figure 3.7: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* male specimens (N=51).

Males

For the relationship between sex and diet for the male specimens of *E. spinax* and considering the empty stomachs, 46 stomachs were analyzed. According to the quantitative analysis (**Annex 8**), crustaceans were the main component in the diet of Velvet belly male specimens and in terms of IRI (62.7%), as in the case of females, the decapods was the prey items which contributed most to IRI (56.5%) (**Fig. 3.8**) (**Annex 20**).

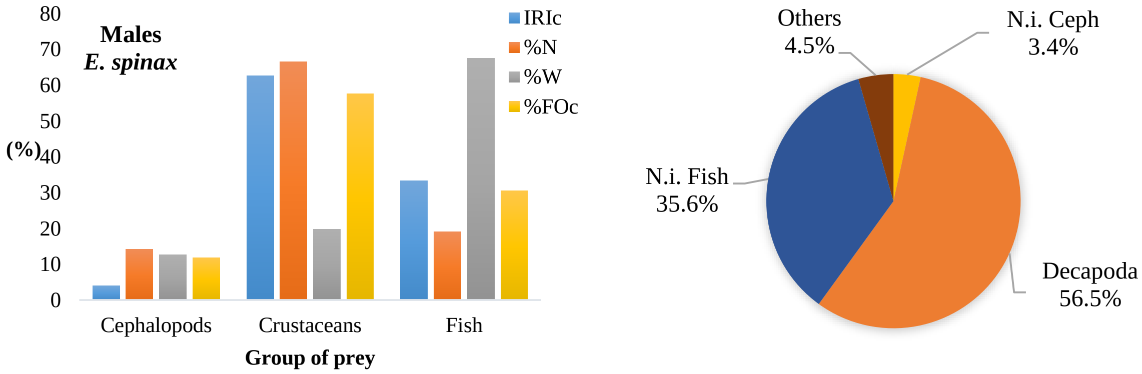


Figure 3.8: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* male specimens (N=46).

3.1.3.1. Diversity indices

Concerning the results obtained of species diversity in the diet composition between sexes for *E. spinax* species, was possible to observe that both females and males present similar and low values of each index. Hence resulting diversity values of 0.6 ± 0.75 for female and 0.6 ± 0.72 for male specimens. In addition, the values obtained of species richness for females and males were 0.6 ± 0.75 and 0.6 ± 0.72, respectively. Finally, in case of species evenness, the values obtained were 0.4 ± 0.46 for females and 0.4 ± 0.48 for males. The Mann-Whitney Rank Sum Test showed that there were not a statistically significant differences between sexes for any index, obtaining the followed *p-values:* $p = 0.624$, $p = 0.820$ and $p = 0.853$.

3.1.3.2. Multivariate statistical analysis:

The classification of the Index of Relative Importance (IRI) of the prey items found in the diet of both sexes (female and male) of *E. spinax* specimens indicated that, at the 17% similarity level, the 97 specimens' combination (only specimens with prey items into the stomachs) falls into eight groups of clusters, of which two clusters were isolated. The main six groups of clusters were composed of different combinations between both sexes of *E. spinax* specimens. The last two groups (isolated clusters) consisted in two mature males captured in summer (**Fig. 3.9**).

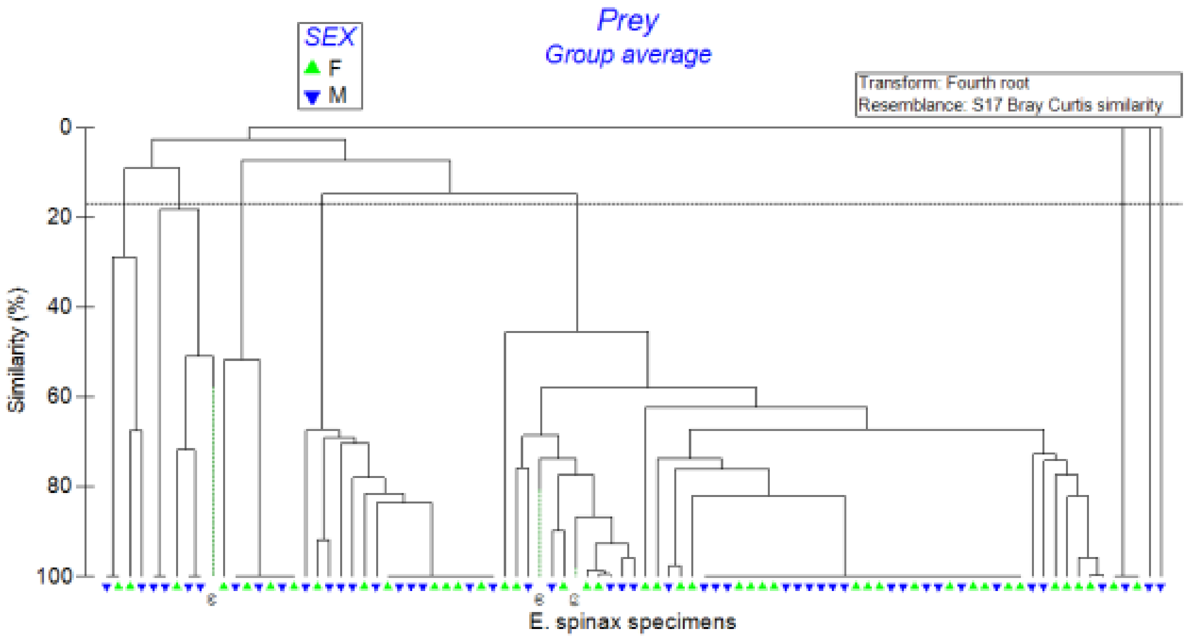


Figure 3.9: Dendogram representation obtained by CLUSTERS analysis of the relative importance in the diet for both sexes of *E. spinax* specimens. The eight groups defined at similarity level of 17% are indicated by the dashed line. (▲: A, B and C).

The result of MDS analysis also corresponds to the main five groups of CLUSTERS analysis. For this analysis it is important to note that the isolated clusters were not taken into account, because in these specimens were only found one prey item with very low weight. Hence it was done the MDS subset in the groups of clusters with high percentage of similarity. The stress value obtained for the MDS plot was 0.07, which indicates a good representation of the ordination in a plane (2D) (**Fig. 3.10**).

In addition, the ANOSIM statistical analysis shows that there are not significant differences in the diet composition of both sexes of *E. spinax* specimens (ANOSIM test, R= -0.005, p > 0.05).

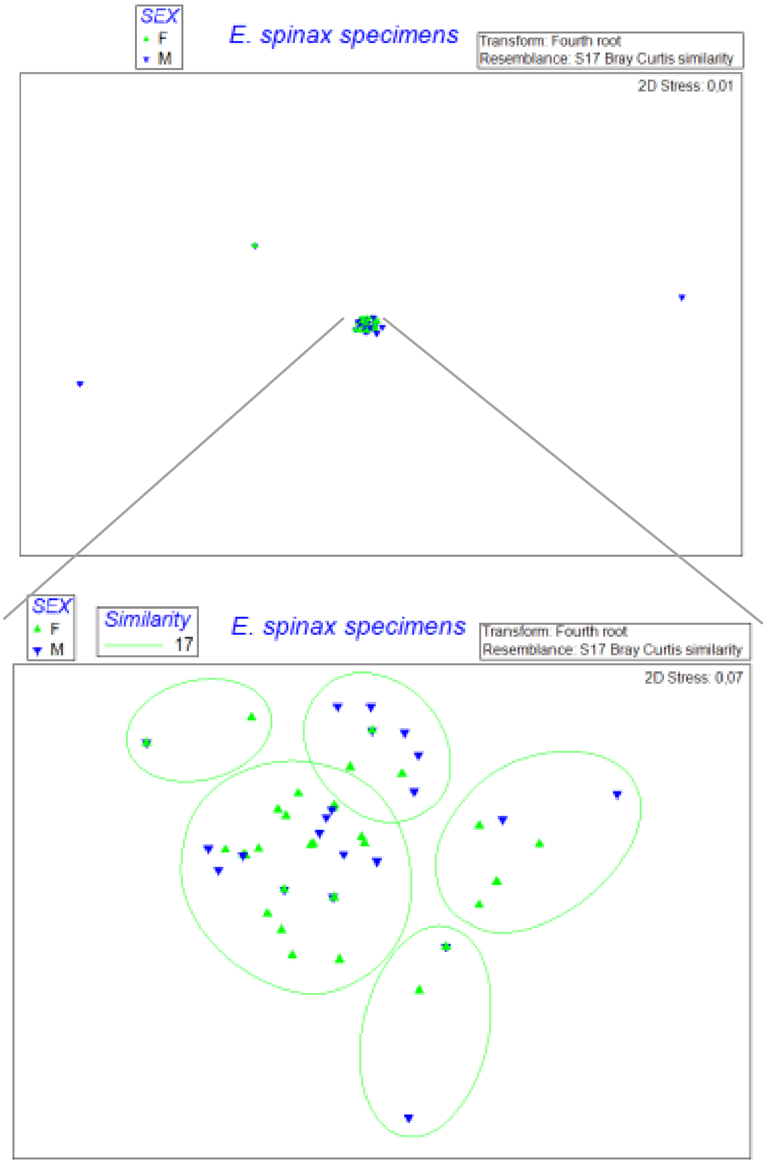


Figure 3.10: MDS plot of relative importance in the diet for both sexes of *E. spinax* specimens. The main five groups defined at similarity level of 17% with superimposed clusters are indicated by the dashed line.

Concerning to SIMPER analysis, it is possible to observe that the average contribution of the prey items to the similarity in the diet of both sexes of *E. spinax* specimens were 35.40% for females and 34.11% for male specimens. In terms of relative importance, SbO. Natantia and N.i. Fish were the prey items most responsible for the similarity in the diet between both sexes of *E. spinax.* For female specimens the contribution percentages of SbO. Natantia and N.i. Fish were 67.04% and 25.92%, respectively, and for male specimens were 81.40% and 11.85%, respectively (**Tab. 3.6**).

Table 3.6: SIMPER results for the relative importance of prey items between female (F) and male (M) specimens of *E. spinax*. Parameters include: average contribution to relative importance (Av. IRI) and to similarity (Av. Sim), similarity/standard deviation (Sim/SD), contribution of the dominant species to the similarity % (Contrib%) and cumulative similarity % (Cum.%).

Species	Group F Av.IRI	Av.Sim 35.40%	Sim/SD	Contrib%	Cum.%
SbO.Natn	1.85	23.73	0.73	**67.04**	67.04
N.i. Fish	1.13	9.18	0.41	**25.92**	92.96
Species	**Group M Av.IRI**	**Av.Sim 34.11%**	**Sim/SD**	**Contrib%**	**Cum.%**
SbO.Natn	1.88	27.77	0.72	**81.40**	81.40
N.i. Fish	0.80	4.04	0.28	**11.85**	93.26

3.1.4. Relationship between maturity and diet:

In the analysis of the relationship between maturity stages and diet of each deep-water shark species only the index of relative importance (IRI) was taken into account in this study. The IRI index was used due to its adequate combination of quantitative indices (N, FO and W) and better discrimination power.

The vacuity index (VI) and the different percentages of unidentified bulk and sediment obtained for the maturity variable of this species was calculated, taking into account the total number of stomachs of each maturity for each sex are shown in the following table (**Tab. 3.7**).

Table 3.7: Vacuity Index (VI) and percentages of unidentified bulk (N.i. Bulk) and sediments obtained for immature and mature females (♀) and immature and mature males (♂) of *E. spinax* species.

	T_N stomachs	N^0 empty stomachs	VI (%)	N.i. Bulk (%)	Sediments (%)
Immature ♀	59	29	49.10%	20.30%	5.10%
Mature ♀	30	9	30%	26.70%	16.70%
Immature ♂	54	31	57.40%	9.30%	9.30%
Mature ♂	30	7	23.30%	26.70%	20%

Immature females

In the analysis of the relationship between maturity and diet for the immature female specimens of *E. spinax* and taking into account the empty stomachs, 30 stomachs were analyzed. Regarding the overall quantitative analysis (**Annex 9**), it is possible to verify that crustaceans, in respect to all indices, are the most abundant and important group of prey in the diet of the *E. spinax* immature females. In addition, in terms of IRI, the decapods were the crustacean prey items which contributed most (67.1%) (**Fig. 3.11**) (**Annex 21**).

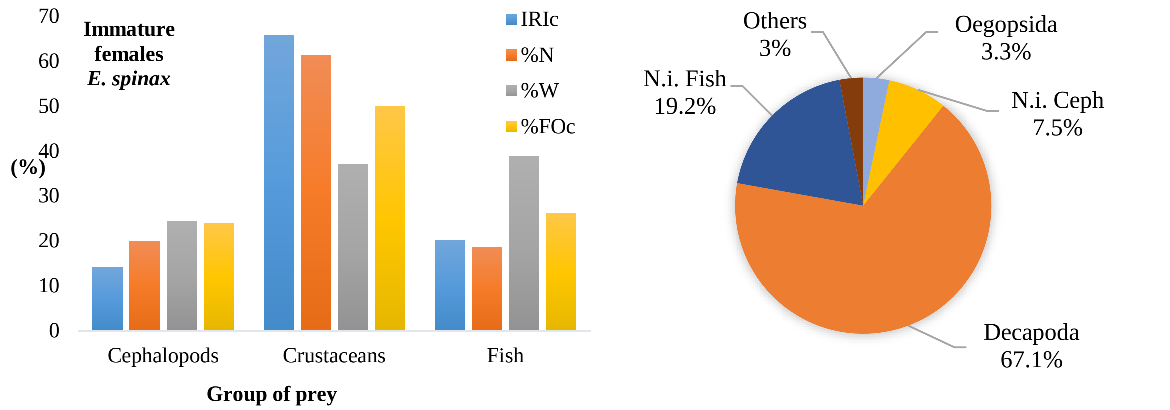


Figure 3.11: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of immature female specimens of *E. spinax* (N= 30).

Mature females

In the case of the analysis of the relationship between maturity and diet for the mature female specimens of *E. spinax* and considering the empty stomachs, 21 stomachs were analyzed. Concerning the overall quantitative analysis (**Annex 10**), it is possible to verify that crustaceans are also, by far, the most abundant and important group of prey in the diet of the *E. spinax* mature females. Decapods were the crustacean prey items which contributed most to IRI (73.4%) (**Fig. 3.12**) (**Annex 22**).

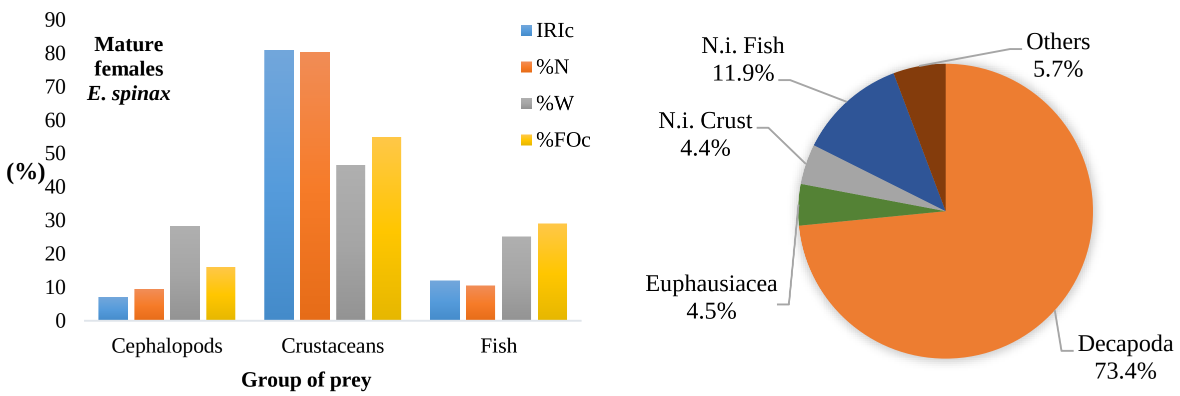


Figure 3.12: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* mature female specimens (N=21).

Immature males

For the analysis of the relationship between maturity and diet for the immature male specimens of *E. spinax* and taking account the empty stomachs, 23 stomachs were analyzed. As regard the overall quantitative analysis (**Annex 11**), it is possible to verify that crustaceans are the most abundant and important group of prey, by far, in the diet of the *E. spinax* immature males. In terms of IRI, the decapods were the crustacean prey items which most contributed to this index, with a value of 87.3% (**Fig. 3.13**) (**Annex 23**).

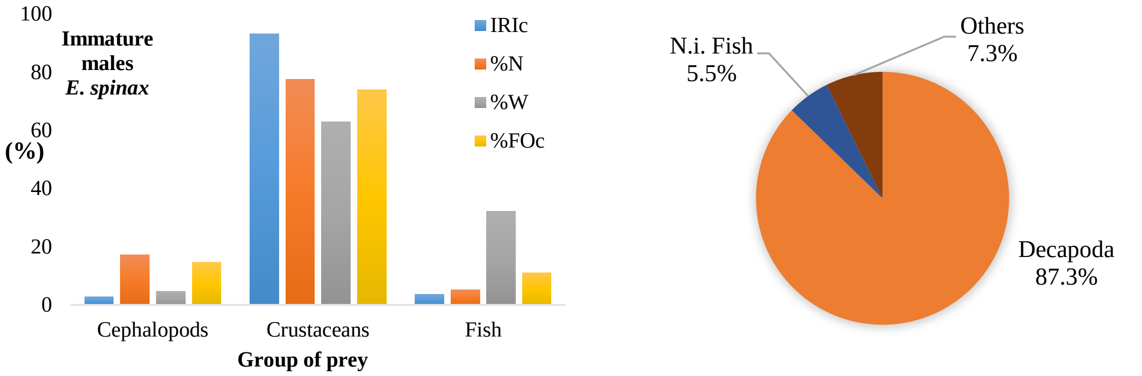


Figure 3.13: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* immature female specimens (N=23).

Mature males

In the analysis of the relationship between maturity and diet for the mature male specimens of *E. spinax* and considering the empty stomachs, 23 stomachs were analyzed. Concerning the overall quantitative analysis (**Annex 12**), it is possible to verify that fish are the most abundant and important group of prey in the diet of the *E. spinax* mature males. Furthermore, the N.i. Fish were the fish prey items which contributed most to IRI (60%) (**Fig. 3.14**) (**Annex 24**).

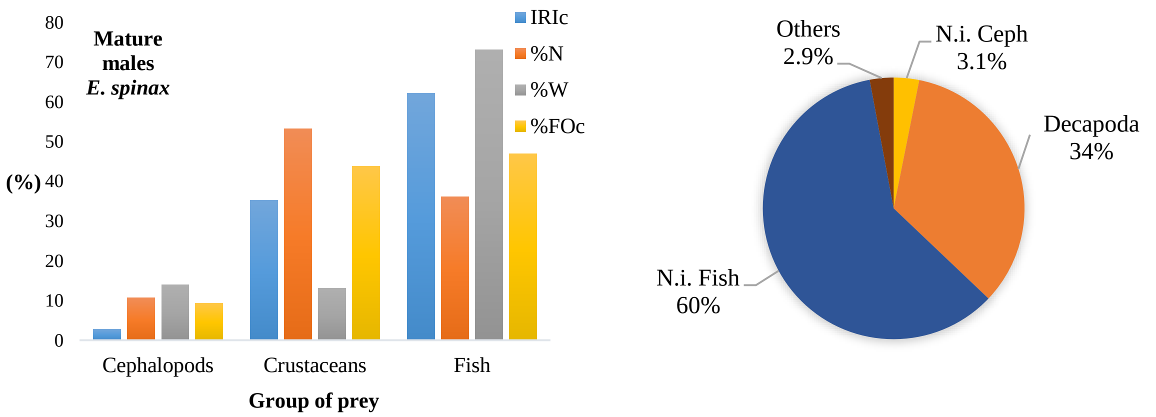


Figure 3.14: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* mature male specimens (N=23).

3.1.4.1. Diversity indices

Regarding the results obtained of species diversity in the diet composition between maturity stages for *E. spinax* species, was possible to observe that both immature and mature specimens present similar and very low values of each index. Thus resulting diversity values of 0.3 ± 0.46 for immature and 0.4 ± 0.47 for mature specimens. In the same way happened with the values of richness of species, obtaining of 0.4 ± 0.68 for immature and 0.6 ± 0.75 for mature specimens. Finally, in case of species evenness, the values obtained were 0.3 ± 0.43 for immature and 0.5 ± 0.47 for mature specimens. The Mann-Whitney Rank Sum Test showed that there were not a statistically significant differences between sexes for any index, obtaining the followed *p-values* $p = 0.131$, $p = 0.107$ and $p = 0.055$.

3.1.4.2. Multivariate statistical analysis

The classification of the Index of Relative Importance (IRI) of the prey items found in the diet of immature and mature specimens of *E. spinax* indicated that, at the 17% similarity level, the 97 specimens' combination (only specimens with prey items into the stomachs) falls into eight groups of clusters, of which two clusters were isolated. The main six groups of clusters were composed of different combinations between both maturity stages of *E. spinax* specimens. The two isolated clusters consisted in two mature males captured in summer (**Fig. 3.15**).

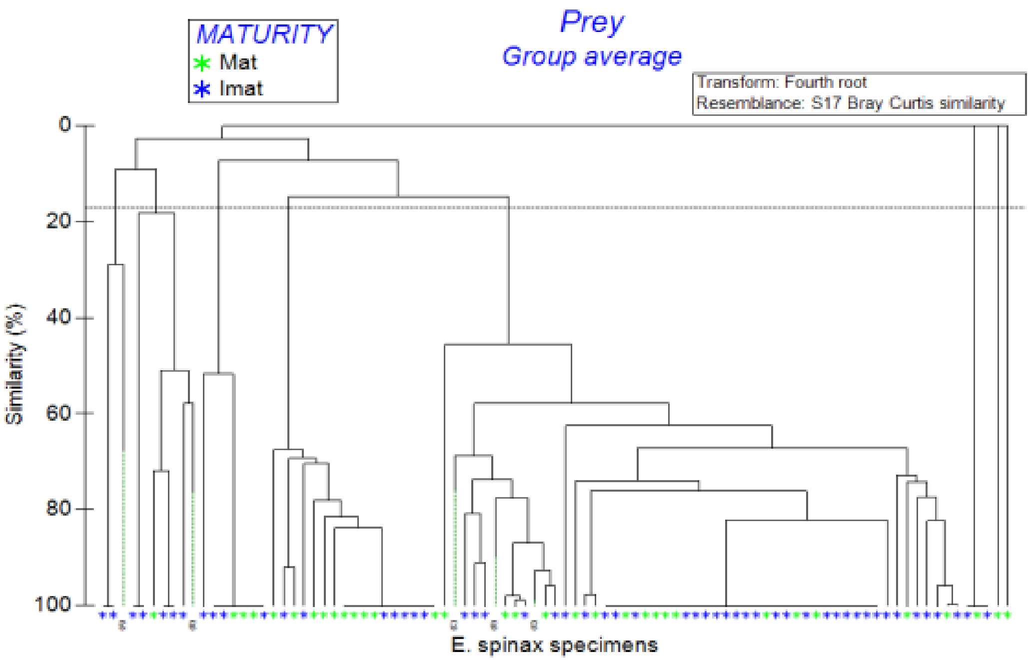


Figure 3.15: Dendogram representation obtained by CLUSTERS analysis of the relative importance in the diet for both maturity stages of *E. spinax* specimens. The eight groups defined at similarity level of 17% are indicated by the dashed line.

The result of MDS analysis corresponds to the first five group of CLUSTERS analysis. In addition it is important to note that the isolated clusters were not taken into account, because in these specimens were only found one prey item with very low weight. Hence it was done the MDS subset in the groups of clusters with high percentage of similarity. The stress value obtained for the MDS plot was 0.07, which indicates a good representation of the ordination in a 2-dimensional graphic (**Fig. 3.16**).

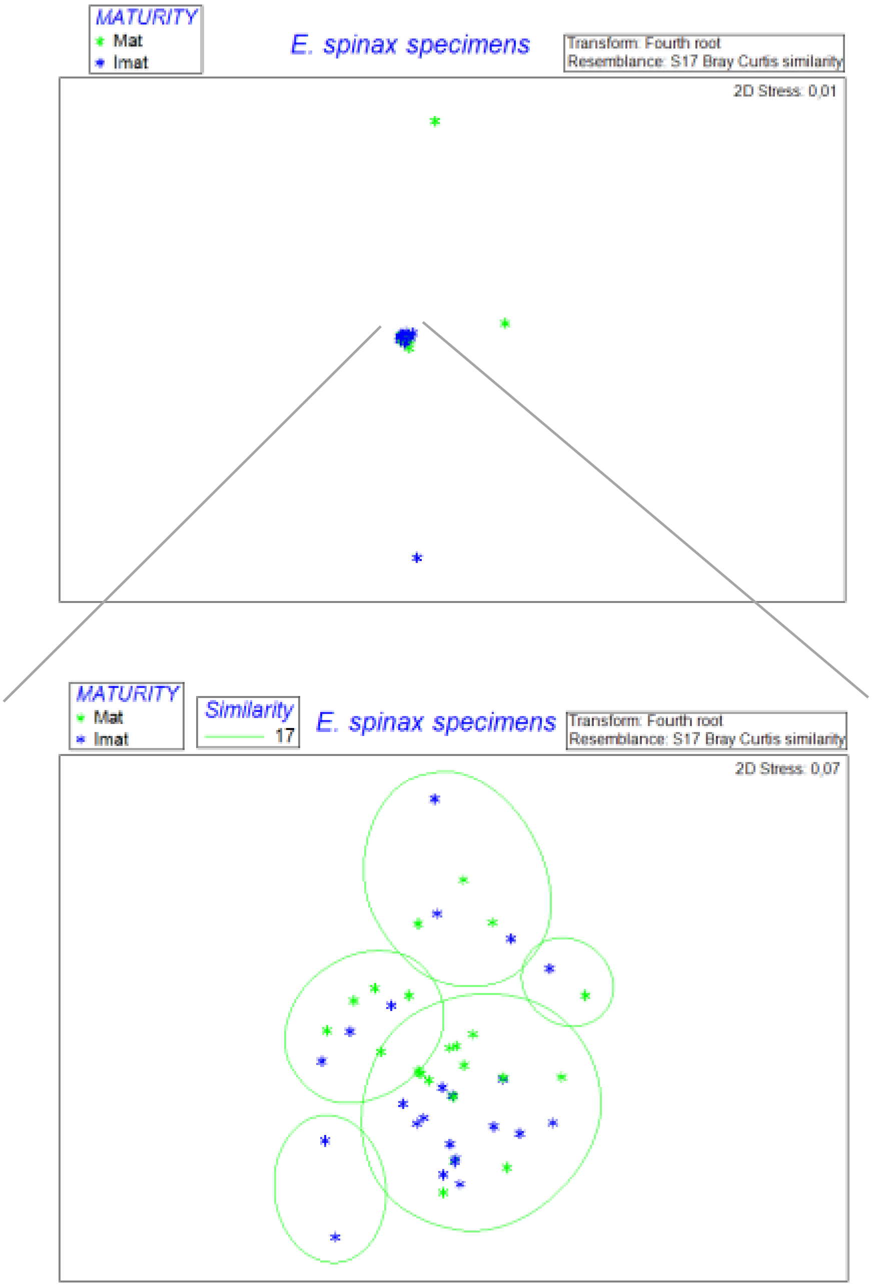


Figure 3.15: MDS plot of relative importance in the diet for both maturity stages of *E. spinax* specimens. The main five groups defined at similarity level of 17% with superimposed clusters are indicated by the dashed line.

Furthermore, the ANOSIM statistical analysis shows that there are not differences between the diet composition of immature and mature specimens of *E. spinax*, although the p value is less than 0.05 (ANOSIM test, R=0.05, p<0.05). This fact mean that there is a great variability in the diet composition between both maturity stages of *E. spinax*.

Regarding SIMPER analysis, it is possible to observe that the average contribution of the prey items to the dissimilarity in the diet between both maturity stages of *E. spinax* specimens was 66.74%. In terms of relative importance SbO. Natantia (29.26%), N.i. Fish (24.38%) and N.i. Cephalopods (N.i. Ceph) (12.73%) were the prey items most responsible for the dissimilarity in the diet between immature and mature specimens of *E. spinax*. In addition the differences in the IRI average of the most contributors prey items in the diet are very consistent between both immature and mature specimens (**Tab. 3.8**).

Table 3.8: SIMPER results for the relative importance of prey items between immature (Imat) and mature (Mat) specimens of *E. spinax*. Parameters include: average contribution to relative importance (Av. IRI) and to dissimilarity (Av. Diss), dissimilarity/standard deviation (Diss/SD), contribution of the dominant species to the dissimilarity % (Contrib%) and cumulative dissimilarity % (Cum.%).

	Group Mat Av.IRI	Group Imat Av.IRI	Av.Diss			
Species			**66.74%**	**Diss/SD**	**Contrib%**	**Cum.%**
SbO.Natn	1.53	2.15	19.53	1.01	**29.26**	29.26
N.i.Fish	1.22	0.75	16.27	0.92	**24.38**	53.64
N.i.Ceph	0.43	0.41	8.50	0.57	**12.73**	66.37
PasipSiv	0.39	0.20	5.91	0.49	8.85	75.22
N.i.Crust	0.36	0.23	5.53	0.50	8.29	83.51
OrdEuph	0.25	0.06	3.56	0.34	5.34	88.84
FamSymp	0.14	0.02	1.94	0.23	2.90	91.74

3.1.5. Relationship between season and diet

In the analysis of the relationship between sex and diet of each deep-water shark species only the index of relative importance (IRI) was taken into account in this study. The IRI index was used due to its apropriate combination of quantitative indices (N, FO and W) and better discrimination capacity.

The vacuity index (VI) and the different percentages of unidentified bulk and sediment obtained in the stomachs of this species according to the season variable was calculated, taking into account the total number of stomachs of each season category (hot season and cold season). These results are shown in the following table (**Tab. 9**).

Table 9: Vacuity Index (VI) and percentages of unidentified bulk (N.i. Bulk) and sediments obtained for females (♀) and males (♂) of *E. spinax* species.

	T_N stomachs	N⁰ empty stomachs	VI (%)	N.i. Bulk (%)	Sediments (%)
Hot season	130	65	50%	19.2%	9.2%
Cold season	43	11	25.6%	18.1%	16.3%

Hot season

For the analysis of the relationship between seasons and diet for the specimens of *E. spinax* in hot seasons (spring and summer), considering the empty stomachs, 65 stomachs were analyzed. Concerning the overall quantitative analysis (**Annex 13**), it is possible to verify that crustaceans are the most abundant and important group of prey in the diet of the *E. pusillus* during hot season. Furthermore, the decapods were the crustacean prey items most contributors, with IRI value of 66.5% (**Fig. 3.16**) (**Annex 25**).

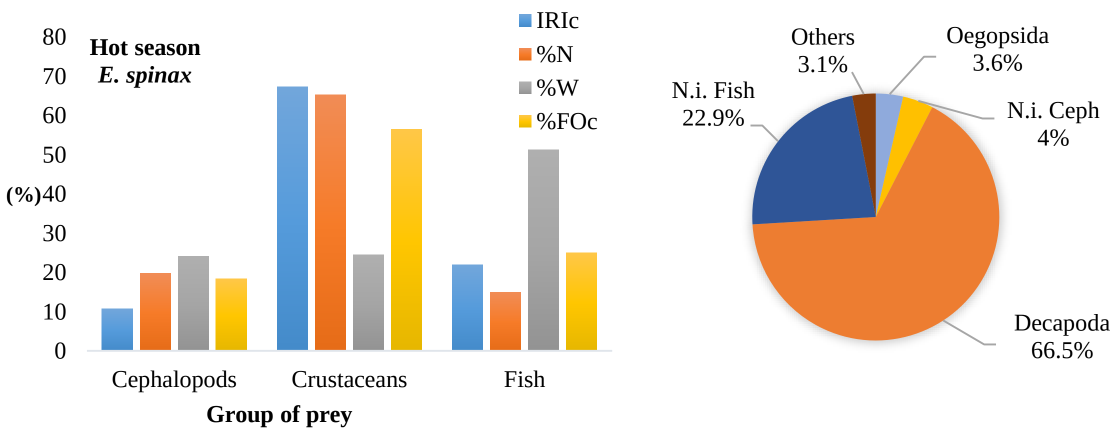


Figure 3.16: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* specimens in hot season (N=65).

Cold season

For the analysis of the relationship between seasons and diet for the specimens of *E. spinax* in cold seasons (winter and autumn) and taking account the empty stomachs, 32 stomachs were analyzed. Regarding the overall quantitative analysis (**Annex 14**), it is possible to verify that crustaceans are the most abundant and important group of prey in the diet of the *E. spinax* during cold season, also the contribution to IRI index of Sepiolidae prey item was very low (0.09%). Decapods were the crustacean prey items which contributed most to IRI (63.7%) (**Fig. 3.17**) (**Annex 26**).

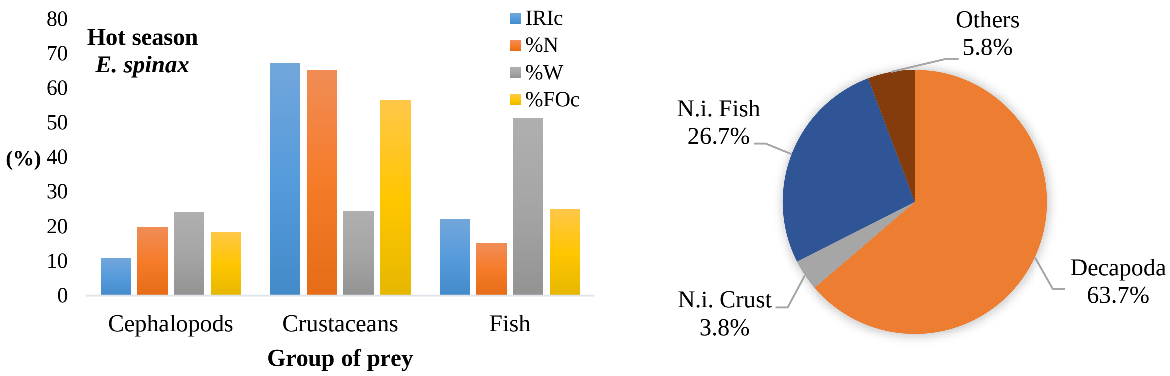


Figure 3.17: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. spinax* specimens in cold season (N=32).

3.1.5.1. Diversity indices

Regarding the results obtained of species diversity in the diet composition between two different season categories (hot and cold seasons) for *E. spinax* species, was possible to observe that the values of both season categories presented, for each index, almost the same values and also very low. Hence resulting diversity values of 0.3 ± 0.48 for hot season and 0.3 ± 0.43 for cold season. Furthermore, the values obtained of species richness for hot season were 0.5 ± 0.74, whereas for cold season were 0.5 ± 0.66. Finally, in case of species evenness, the values obtained were 0.4 ± 0.46 and 0.4 ± 0.45 for hot and cold season, respectively. The Mann-Whitney Rank Sum Test showed that there were not a statistically significant differences between sexes for any index, obtaining the followed *p-values* p = 0.062, p = 0.107 and p = 0.952.

3.1.5.2. Multivariate statistical analysis

The classification of the Index of Relative Importance (IRI) of the prey items found in the specimens diet of *E. spinax* in spring, summer, winter and autumn indicated that, at the 17% similarity level, the 97 specimens' combination (only specimens with prey items into the stomachs) falls into eight groups of clusters, of which two clusters were isolated. These clusters consisted in two mature males captured in summer (**Fig. 3.18**).

The result of MDS analysis corresponds to the first five groups of CLUSTERS analysis. Furthermore it is important to note that the isolated clusters were not taken into account, because in these specimens were only found one prey item with very low weight. Hence it was done the MDS subset in the groups of clusters with high percentage of similarity. The stress value obtained for the MDS plot was 0.07, which indicates a good ordination with less misrepresentative interpretation (**Fig. 3.19**).

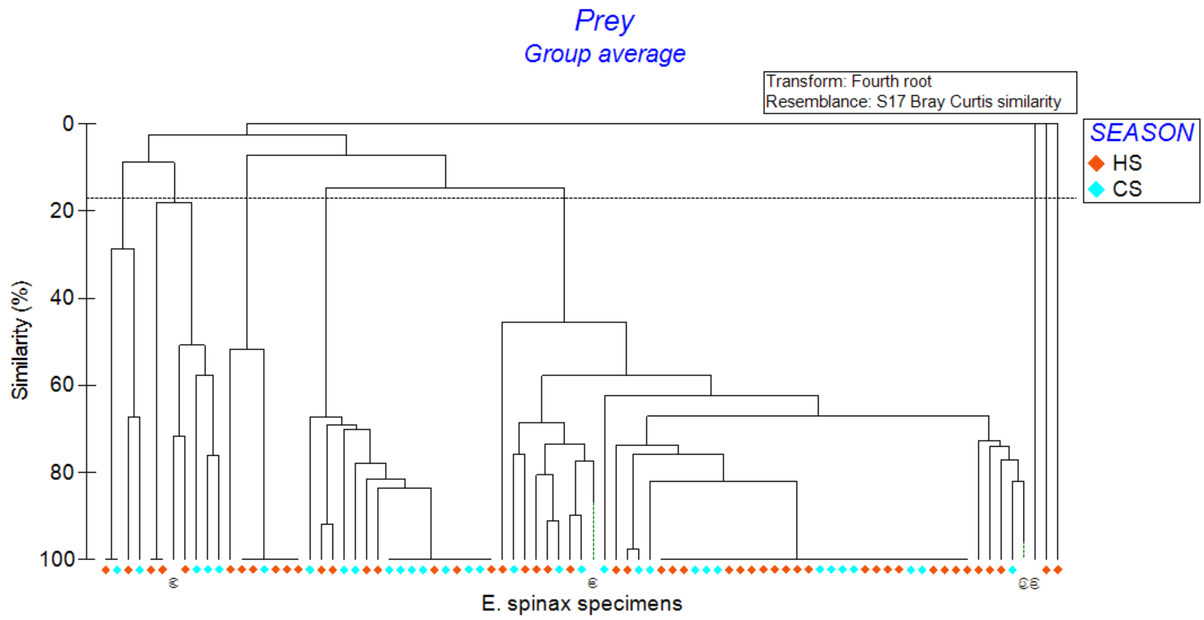


Figure 3.18: Dendogram representation obtained by CLUSTERS analysis of the relative importance in the diet for specimens of *E. spinax* captured in hot season and cold season. The eight groups defined at similarity level of 17% are indicated by the dashed line. (♦ : A, B, C and D).

Furthermore, the ANOSIM statistical analysis shows that there are not significant differences between the diet composition of *E. spinax* specimens captured during both season categories (ANOSIM test, R=0.044, p > 0.05).

Concerning the SIMPER analysis, it is possible to observe that the averages contribution of the prey items to the similarity in the diet of *E. spinax* specimens captured during hot and cold seasons were 38.79% and 26.29%, respectively. In terms of relative importance SbO. Natantia and N.i. Fish were the main prey items that contributed to the similarity during hot season. Nevertheless during cold season the main prey items with high contribution to the similarity were SbO. Natantia (SbO.Natn), N.i. Fish and *P. sivado* (*PasipSiv*) (**Tab. 3.10**).

Table 3.10: SIMPER results for the relative importance of prey items between specimens of *E. spinax* captured during hot season (HS) and cold season (CS). Parameters include: average contribution to relative importance (Av. IRI) and to similarity (Av. Sim), similarity/standard deviation (Sim/SD), contribution of the dominant species to the similarity % (Contrib%) and cumulative similarity % (Cum.%).

Species	Group HS Av.IRI	Av.Sim 38.79%	Sim/SD	Contrib%	Cum.%
SbO.Natn	1.97	28.53	0.81	73.56	73.56
N.i.Fish	1.1	8.33	0.41	21.48	95.03
Species	**Group CS Av.IRI**	**Av.Sim 26.29%**	**Sim/SD**	**Contrib%**	**Cum.%**
SbO.Natn	1.55	18.3	0.54	69.6	69.6
N.i.Fish	0.78	4.08	0.25	15.53	85.13
PasipSiv	08.59	2.11	0.22	8.03	93.17

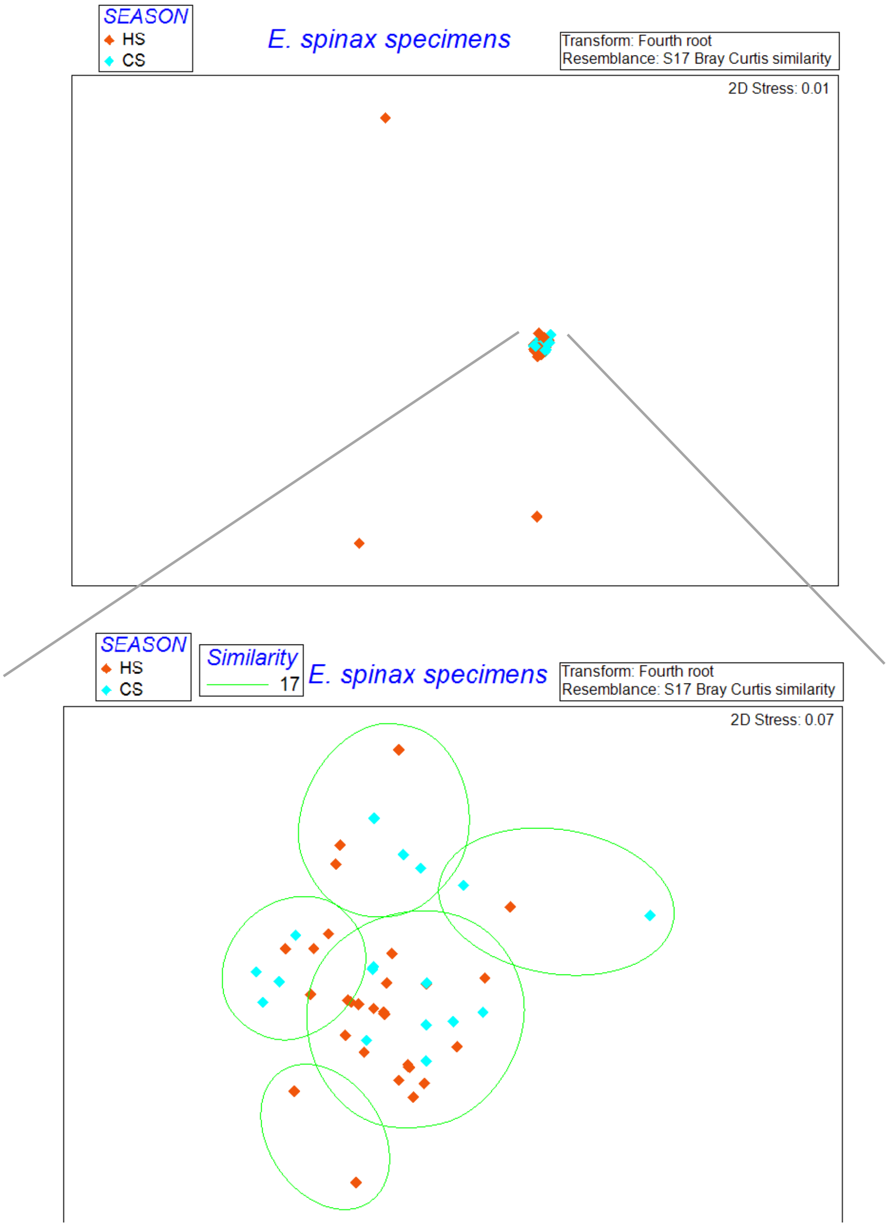


Figure 3.19: MDS plot of relative importance in the diet of *E. spinax* specimens in the different season categories. The main five groups defined at similarity level of 17% with superimposed clusters are indicated by the dashed line.

3.2. *Etmopterus pusillus* (Lowe, 1835)

3.2.1. Representativeness of the sample

The curve obtained from the relationship between the cumulative number of prey species and cumulative number of stomachs with analyzed content of *E. pusillus* (n=32). The cumulative prey curves also described an increasing relationship, but in this case did not reach a full asymptotic stabilization (**Fig. 3.20**). This could be to the fact that the stomachs amount of *E. pusillus* may not have been sufficient to describe the overall trophic diversity in the diet of this species.

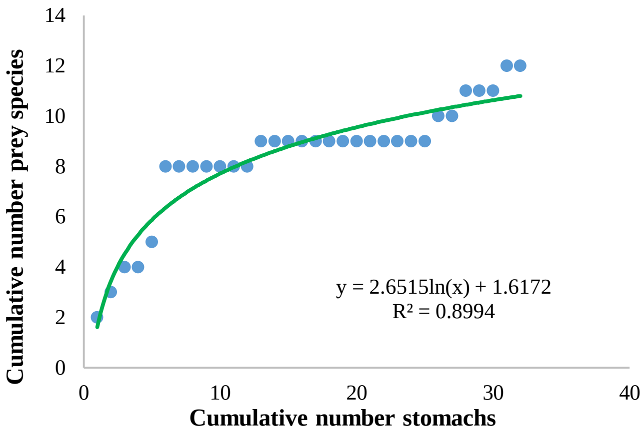


Figure 3.20: Relationship between cumulative number of prey species identified in the stomachs content and cumulative number of stomachs analyzed of *E. pusillus.*

3.2.2. General composition of the diet

For the feeding study of *E. pusillus* 58 stomachs were considered of which 26 stomachs were found to be empty, resulting in a vacuity index of 44.8%, regarding the total number of stomachs. The average number of prey per stomach was 1.15 with an average weight of 0.72 g. In addition, 57% of the stomachs were filled with unidentified bulk, resulting in x stomachs with contents for further analysis.

In the stomach contents of *E. pusillus* 10 different prey species were identified. They belong to the same three groups of prey found for *E. spinax* - cephalopods, crustaceans and fish - in which also different prey items were identified (3, 5 and 2, respectively) (**Tab. 3.11**) The rest of prey items found they were composed by unidentified items. The systematic classification of all prey items are represented in the **Annexes 1, 2 and 3**.

Table 3.11: List of prey found in the stomach contents of *E. spinax*. Where SbO: Suborder, O: Order and F: Famlily.

PREY GROUPS	PREY ITEMS
CEPHALOPODS	*Liocranchia reinhardi* [1]
	Onychoteuthis banksii [1]
	Abraliopsis affinis [1]
	N.i. Ceph
CRUSTACEANS	O. Euphausiacea
	SbO. Natantia [2]
	F. Pandalidae [2]
	Pasiphaea spp. [2]
	Pasiphaea sivado [2]
	N.i. Crust
FISH	O. Pleuronectiformes
	Phycis blennoides
	N.i. Fish

[1] Oegopsida; [2] Decapoda

Regarding the diet of *E. pusillus*, the quantitative indices (%N, %W, %FO and IRI) for the three main groups of prey were taken into account. In terms of IRI, %N and %FO, the most representative group was the crustaceans (50.4%, 59.7% and 53.5%, respectively) and in terms of %W the most representative group was the fish (76.1%). The cephalopods group was the least representative of all main groups of prey regarding all indices (**Fig. 3.21**) (**Annex 15**).

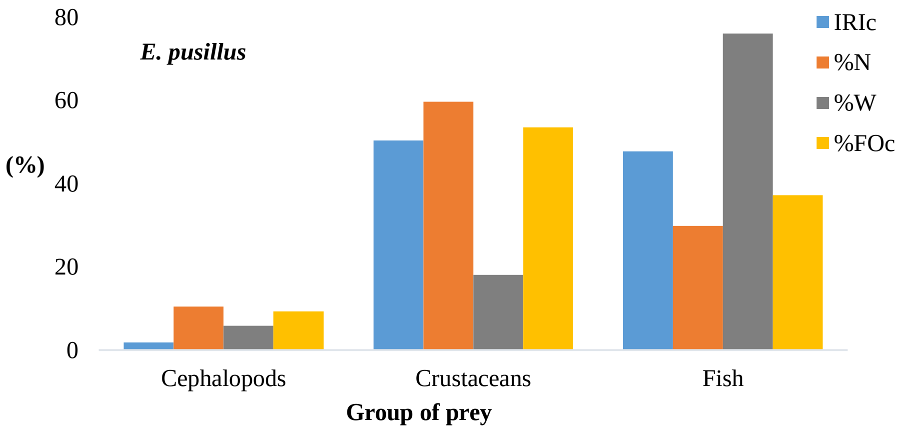
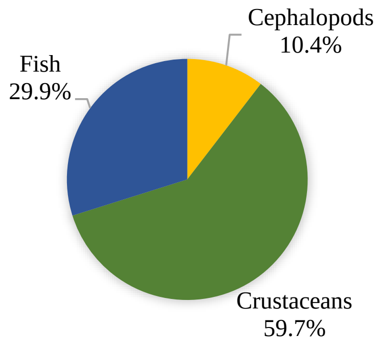


Figure 3.21: Representation of quantitative methods (IRI, %N, %Wand %FO) of the three principal groups of prey in the diet of *E. pusillus.*

According to the numerical method, 67 prey were counted. The most important prey group in the diet of *E. pusillus* were the crustaceans (59.7%), being the Euphausiacea (26.9%) and the Decapoda orders (25.4%) the most abundant, followed by the fish group (29.9%), with N.i.Fish in greater proportion (25.4%). In terms of number (%N), the least important group was the cephalopods (10.4%), although being mostly represented by the Oegopsida (6%) (**Fig. 3.22**) (**Annex 15**).



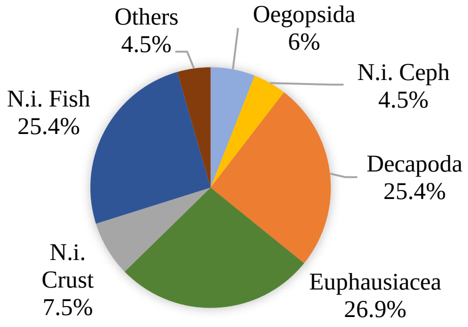
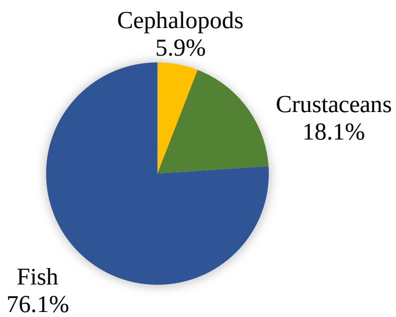


Figure 3.22: Relative importance by number (%N) of the main groups of prey (left) and of the different orders of prey (right) in the diet of *E. pusillus* (N=32).

Regarding the gravimetric method, the total weight obtained from all prey was 41.68 g. The most representative group of prey was the fish (76.1%), in which the N.i. Fish contributed to the largest proportion (75.9%). The next most abundant group in terms of weight was the crustaceans (18.1%), of which 10.3% corresponded to the decapods. Cephalopods remains the least representative group in terms of %W (5.9%) mainly represented by N.i. Ceph (5.5%) (**Fig. 3.23**) (**Annex 15**).



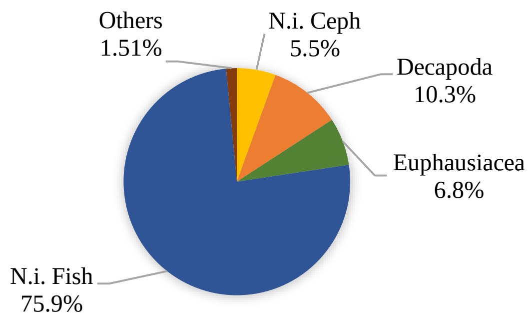
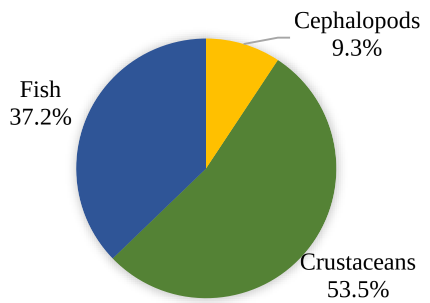


Figure 3.23: Relative importance by weight (%W) of the main groups of prey (left) and of the different orders of prey (right) in the diet of *E. pusillus* (N=32).

In terms of frequency of occurrence (%FO), the most frequent group were the crustaceans (53.5%), mostly represented by the decapods (34.8%). The fish group was less frequent (37.2%), 32.6% of which corresponding to N.i. Fish. The least frequent group was the cephalopods (9.3%), where the N.i. Ceph and the Oegopsida order had the same frequency of occurrence (6.5%) (**Fig. 3.24**) (**Annex 15**).



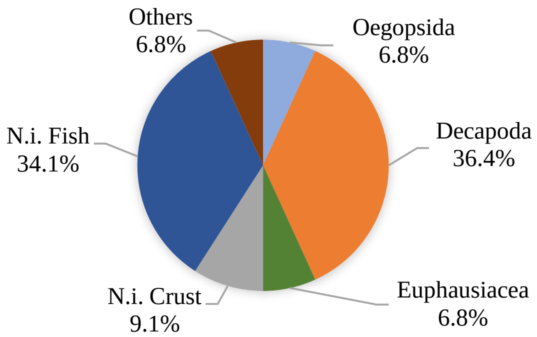
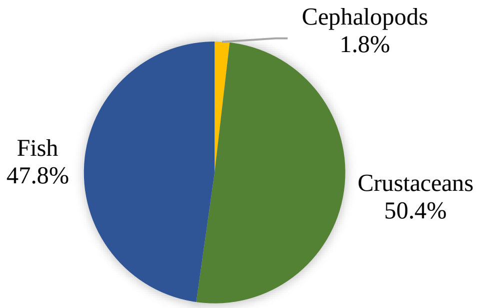


Figure 3.24: Frequency of occurrence (%FO) for the different prey groups (left) and for the orders of prey (right) in the diet of *E. pusillus* (N=32).

Regarding the relative importance index (IRI), the crustaceans and fish groups were nearly of equal importance (50% and 48%, respectively). However, the most important prey was the N.i. Fish (67%), followed by the decapods (25%). The least important group as a whole was the cephalopods (2%), with the N.i. Ceph (1.3%) and the order Oegopsida (0.8%) as prey items contributors (**Fig. 3.25**) (**Annex 15**).



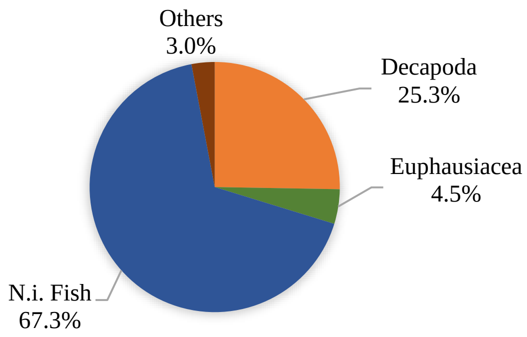


Figure 3.25: Relative importance (%IRI) of the main groups of prey (left) and of the different orders of prey (right) in the diet of *E. pusillus* (N=32).

3.2.2.1. Prey classifications

As happened for *E. spinax*, the indices calculated for the diet of *E. pusillus* were divided into different groups according to the order of importance for each type of prey, obtaining also three groups for each index. The first group (G.I) consisted in the IRI index, and the second one (G.II) was composed by the IPO2 index (**Tab. 3.12**).

In respect to the different indices analysis it was possible to verify that the principal preys in the diet of *E. pusillus* were the N.i. Fish group, SbO. Natantia and O. Euphausiacea. The groups of indices are fairly homogenous until 3th category, where these groups begin to have slight variations according to the principal order. Both groups (G.I and G.II) were similar in terms of main order (**Tab. 3.12**).

Table 3.12: Management of prey groups according to the indices used for *E. pusillus.*

MAIN ORDER	TAXONOMIC GROUP	G. I	G.II
		IRI	IPO2
1	N.i. Fish	1	1
2	SbO. Natantia	2	2
3	O. Euphausiacea	3	3
4	N.i. Custaceans	4	5
5	*Pasiphaea sivado*	5	6
6	N.i. Cephalopods	6	4
7	*Onychoteuthis bankssi*	7	9
8	*Physcis blennoides*	8	7
9	*Pasiphaea* spp.	9	8
10	O. Pleuronectiformes	10	10
11	*Liocranchia reinhardtii*	11	11
12	*Abraliopsis affinis*	12	12

N.i.: Not identified

When applying the classification of prey by IRI values according to Rosecchi and Nouaze (1987), it can be observed that the G.I followed the same ranking as the main order (**Tab. 3.13**) without variations, i.e., N.i. Fish, the 1st in the ranking order, is the preferential prey, SbO. Natantia ranking 2nd is already an accessory prey, being the O. Euphausiacea the next prey item which presented a higher percentage (5.1%) (**Tab. 3.12**) (**Annex 32**).

Table 3.13: Classification of prey by IRI values to Rosecchi and Nouaze (1987) classification for *E. pusillus*.

Group of prey	IRI values (%)	Cumulative IRI values (%)	Rosecchi and Nouaze (1987) classification
N.i. Fish	75.69%	75.69%	Preferential prey
SbO. Natantia	13.69%	89.38%	Accessory prey
O. Euphausiacea	5.12%	94.49%	
The rest of prey	1.72%-0.08%	96.21%-100%	

Using the classification of prey by IPO2 index it was observed that N.i. Fish was the preferential prey (50.1%), following SbO. Natantia as secondary prey (64.5%) and O. Euphausiacea also as a secondary prey (11.3%) (**Tab. 3.14)** (**Annex 33**).

Table 3.14: Classification of prey by IPO2 values for *E. pusillus.*

Group of prey	IPO2 values (%)	Cumulative IPO2 values (%)	Classification of prey
N.i. Fish	50.12%	50.12%	Preferential prey
SbO. Natantia	14.36%	64.48%	Secondary prey
O. Euphausiacea	11.29%	75.77%	
The rest of prey	5.68%-1.07%	81.65%-100%	Accessory prey

3.2.2.2. Diversity indices

Concerning the results obtained of species diversity in the diet composition of *E. pusillus* was possible to observe that this species present similar and very low values of each index, some of them near 0 and other being 0. Therefore resulting values of 0.2 ± 0.39; 0.4 ± 0.68 and 0.3 ± 0.45 for diversity, richness and evenness indices, respectively.

3.2.3. Relationship between sex and diet

In the analysis of the relationship between sex and diet of each deep-water shark species only the index of relative importance (IRI) was taken into account in this study. The IRI index was used because it combines all quantitative indices (N, FO, and W) that while giving more importance to the frequency of occurrence, it also discriminates better between values of the index allowing an effective evaluation of the importance of each type of prey to predator diet.

The vacuity index (VI) and the different percentages of unidentified bulk obtained for the sex variable of *E. pusillus* was calculated, taking into account the total number of stomachs of each sex. These results are shown in the following table (**Tab. 3.15**).

Table 3.15: Vacuity Index (VI) and percentages of unidentified bulk (N.i. Bulk) and sediments obtained for females (♀) and males (♂) of *E. spinax* species.

	T_N stomachs	N^0 empty stomachs	VI (%)	N.i. Bulk (%)
♀	12	5	41.7%	58.3%
♂	46	21	45.6%	13.1%

Females

In the analysis of the relationship between sex and diet for the female specimens of *E. pusillus* and considering the empty stomachs, 7 stomachs were analyzed. Considering the overall quantitative analysis (**Annex 16**), it is possible to verify that both crustaceans and fish are the most abundant and important group of prey in the diet of the Smooth Lanternshark females. In terms of IRI, these groups of prey represented 44.1% and 50.8%. Decapoda and Euphausiacea orders were the crustaceans prey items which contributed most to IRI (5.5% and 11%, respectively) and also the N.i. Fish (IRI=72.8%) (**Fig. 3.26**) (**Annex 28**).

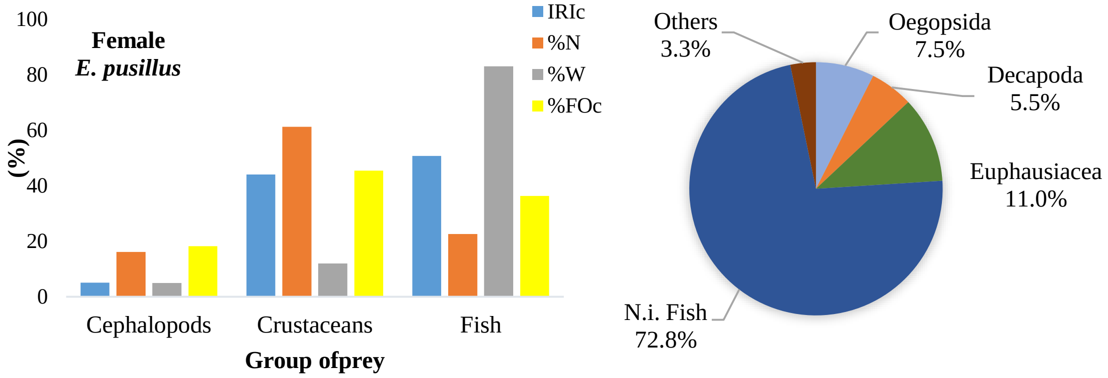


Figure 3.26: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of the female specimens of *E. pusillus* (N=7).

Males

In the analysis of the relationship between sex and diet for the male specimens of *E. pusillus* and taking into account the empty stomachs, 25 stomachs was analyzed. According to the quantitative analysis (**Annex 17**), crustaceans were the main component in the diet of Smooth Lanternshark male specimens. The prey items of crustaceans which contributed most to IRI were the decapods (39.9%), but the N.i. Fish had also an elevated relative importance (55.7%) (**Fig. 3.27**) (**Annex 29**).

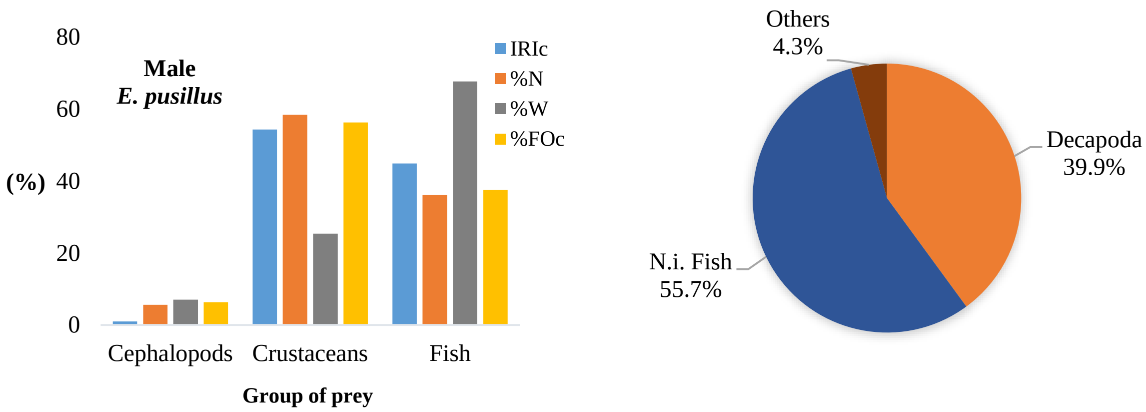


Figure 3.27: Representation of the quantitative methods of the main groups of prey (left) and the relative importance (IRI) of prey items (right) in the diet of *E. pusillus* male specimens (N=25).

3.2.3.1. Diversity indices

Concerning the results obtained of species diversity in the diet composition between sexes for *E. pusillus* species, was possible to observe that both females and males present low values of each index. Hence resulting diversity values of 0.5 ± 0.59 for female and 0.2 ± 0.31 for male specimens. In addition, the values obtained of species richness for females and males were 0.7 ± 0.94 and 0.4 ± 0.61, respectively. Finally, in case of species evenness, the values obtained were 0.5 ± 0.50 for females and 0.2 ± 0.42 for males. The Mann-Whitney Rank Sum Test showed that there were not a statistically significant differences between sexes for any index, obtaining the followed *p-values* p = 0.143, p = 0.224 and p = 0.111.

3.2.3.2. Multivariate statistical analysis

The classification of the Index of Relative Importance (IRI) of the prey items found in the diet of female and male specimens of *E. pusillus* indicated that, at the 17% similarity level, the 32 specimens' combination (only specimens with prey items into the stomachs) falls into four groups of clusters, of which three clusters were isolated. The main group of clusters was composed of different combinations between both sexes of *E. spinax* specimens. The last three isolated groups of clusters consisted in two immature males captured in spring and one immature female capture in spring (**Fig. 3.28**)

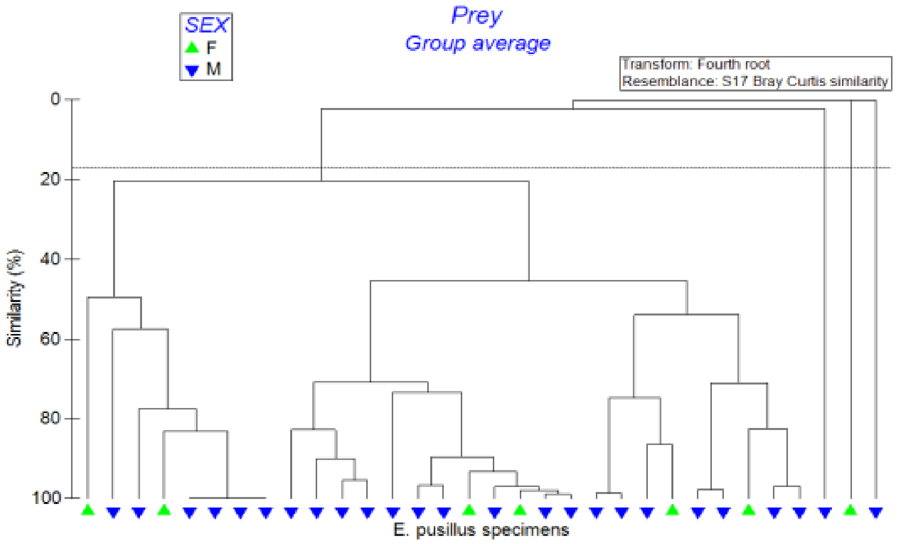


Figure 3.28: Dendogram representation obtained by CLUSTERS analysis of the relative importance in the diet for both sexes of *E. pusillus* specimens. The four groups defined at similarity level of 17% are indicated by the dashed line.

The result of MDS analysis corresponds to the main group of CLUSTERS analysis. Then it is important to note that the isolated clusters were not taken into account, because in these specimens were only found one prey item with very low weight. Hence it was done the MDS subset in the groups of clusters with high percentage of similarity. The stress value obtained for the MDS plot was 0.08, which indicates a good representation of the ordination in a 2D graphic (**Fig. 3.29**).

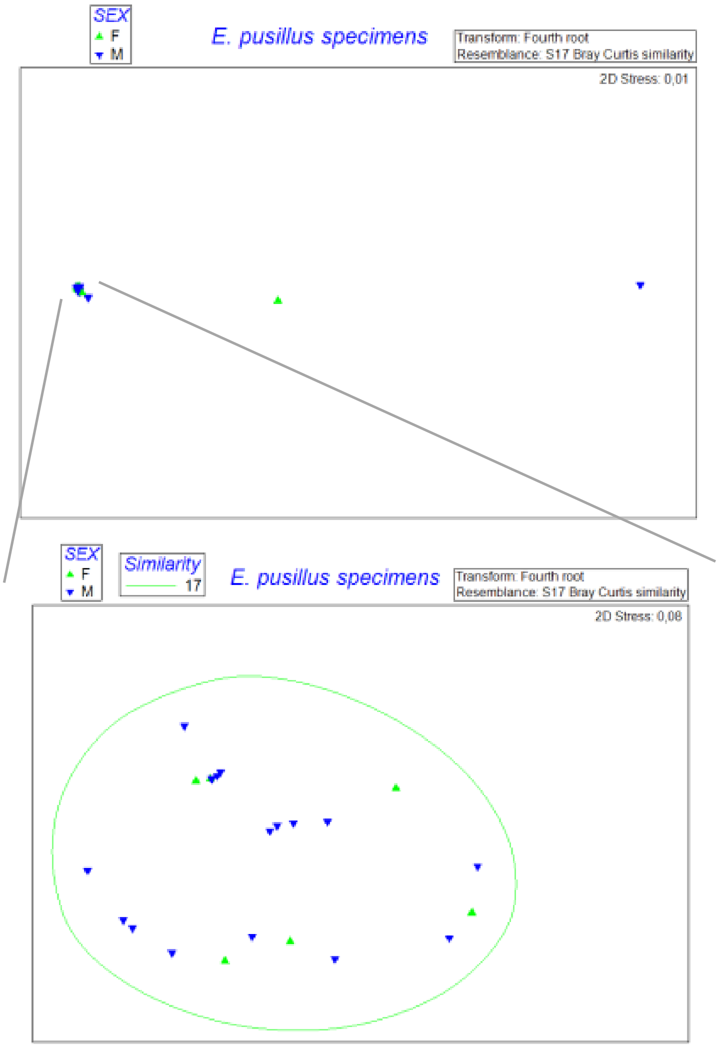


Figure 3.29: MDS plot of relative importance in the diet for both sexes of *E .pusillus* specimens. The main group defined at similarity level of 17% with superimposed clusters are indicated by the dashed line.

In addition, the ANOSIM statistical analysis shows that there are not significant differences in the diet composition of female and males specimens of *E. pusillus* (ANOSIM test, R=0.021, $p > 0.05$).

Concerning to SIMPER analysis, it is possible to observe that the average contribution of the prey items to the similarity in the diet of both sexes of *E. pusillus* specimens were 28.56% for females and 39.97% for male specimens. In terms of relative importance, N.i. Crustaceans, N.i. Fish and SbO. Natantia were the prey items most responsible for the similarity in the diet between females and males of *E. pusillus.* In the case of female specimens, the contribution percentages of N.i. Crustaceans, N.i. Fish and

SbO. Natantia were 50.40%, 34.19% and 11.84%, respectively, and for male specimens were 56.02%, 25.12% and 12.73%, respectively (**Tab. 3.16**).

Table 3.16: SIMPER results for the relative importance of prey items between female (F) and male (M) specimens of *E. pusillus*. Parameters include: average contribution to relative importance (Av. IRI) and to similarity (Av. Sim), similarity/standard deviation (Sim/SD), contribution of the dominant species to the similarity % (Contrib%) and cumulative similarity % (Cum.%).

Species	Group F Av.IRI	Av.Sim 28.56%	Sim/SD	Contrib%	Cum.%
N.i. Crust	1.72	14.39	0.74	**50.40**	50.40
N.i. Fish	1.56	9.76	0.58	**34.19**	84.58
SbO.Natn	0.69	3.38	0.39	**11.84**	96.42
Species	**Group M Av.IRI**	**Av.Sim 39.97%**	**Sim/SD**	**Contrib%**	**Cum.%**
N.i. Crust	1.99	22.39	1.04	**56.02**	56.02
N.i. Fish	1.25	10.04	0.45	**25.12**	81.14
SbO.Natn	0.87	5.09	0.43	**12.73**	93.87

3.3. Comparison *Etmopterus spinax* and *Etmopterus pusillus*

Regarding the comparison between both diets, and in terms of feeding overlap, it was verified that the diet of both shark species are subjected to a high level of overlap, obtaining values above α=0.6. The overlap values obtained for the %N, %W, %FOc and IRIc indices were α=0.64, α=0.60, α=0.62, α= 0.53, respectively.

Concerning the position in the trophic chain, the trophic levels of each deep-water shark was calculated. Therefore, values of 3.8 for *E. spinax* and 4.1 for *E. pusillus* were obtained.

3.3.1. Diversity

Concerning the results obtained of species diversity in the diet composition between both deep-water sharks species, was possible to observe that all indices present low values for each predator species, obtaining the following results. In case of the diversity index, the value obtained for *E. spinax* was somewhat higher (0.3 ± 0.47) than for *E. pusillus* (0.2 ± 0.39). In addition, the Mann-Whitney Rank Sum Test was calculated and there was not a statistically significant difference (P = 0.451).

On the other hand, similar values with the richness of species were observed in both diets. Thus the values of average and standard deviation obtained for *E. spinax* were 0.5 ± 0.72, while the values for *E. pusillus* were 0.4 ± 0.68. Hence, there was not a statistically significant difference (P = 0.573) in the species richness values for both diets.

According the species evenness index (J'), the values of average and standard deviation obtained for *E. spinax* were 0.4 ± 0.45, while the values for *E. pusillus* were 0.3 ± 0.45. Then, there was not statistically significant difference (P = 0.864) in the species evenness index values for both diets.

3.3.2. Multivariate statistical analysis

The classification of the Index of Relative Importance (IRI) of the prey items found in the diet of both *Etmopterus* species indicated that, at the 17% similarity level, the 129 specimens' combination (only specimens with prey items into the stomachs) falls into eight main groups or clusters, of which four clusters was isolated. Two of the main clusters consisted of the *E. spinax* specimens, another cluster was formed by *E. spinax* (n=7) and *E. pusillus* (n=1; immature female captured in spring) specimens and the third one was composed of different combinations between both deep-water shark species. The first and third clusters isolated consisted in one specimen of *E. spinax* in each one (both corresponded to mature male specimens captured in summer), the second one and the fourth one cluster consisted in one specimen of *E. pusillus* in each one (2nd: immature female captured in spring and 4th: immature male captured in spring) (**Fig. 3.30**).

The result of MDS analysis also corresponds to the first four groups of CLUSTERS analysis. For this analysis it is important to note that the isolated clusters were not taken into account, so it was done the MDS subset in the groups of clusters with high percentage of similarity. The stress value obtained for the MDS plot was 0.08, which indicates a good 2D ordination with less probabilities of misrepresentative interpretation (**Fig. 3.31**).

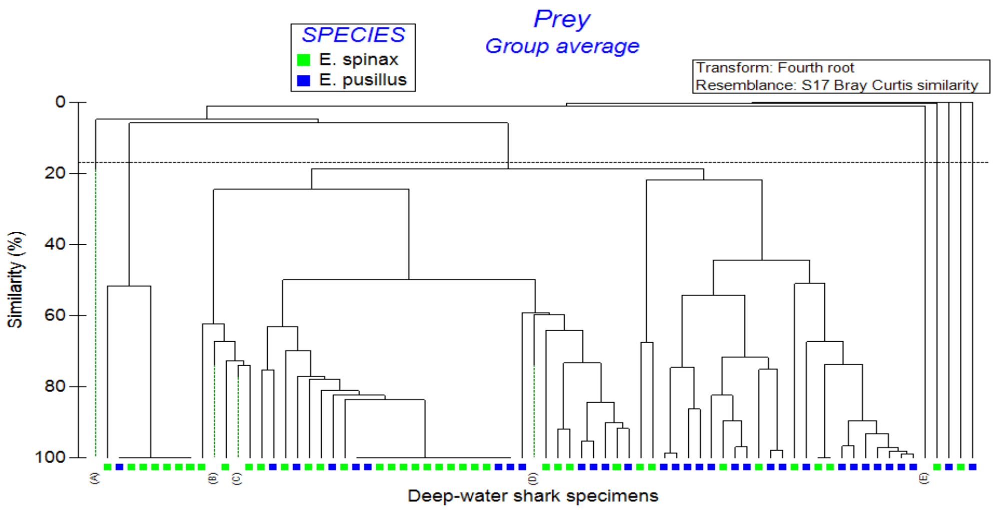


Figure 3.30: Dendogram representation obtained by CLUSTERS analysis of the relative importance in the diet of *E. spinax* and *E. pusillus* specimens. The eight groups defined at similarity level of 17% are indicated by the dashed line. (■ : A, B, C, D and E).

In addition, the ANOSIM statistical analysis shows that there are slight differences between the diet composition of both *E. spinax* and *E. pusillus*, but these differences are significant (ANOSIM test, R=0. 156, p < 0.05).

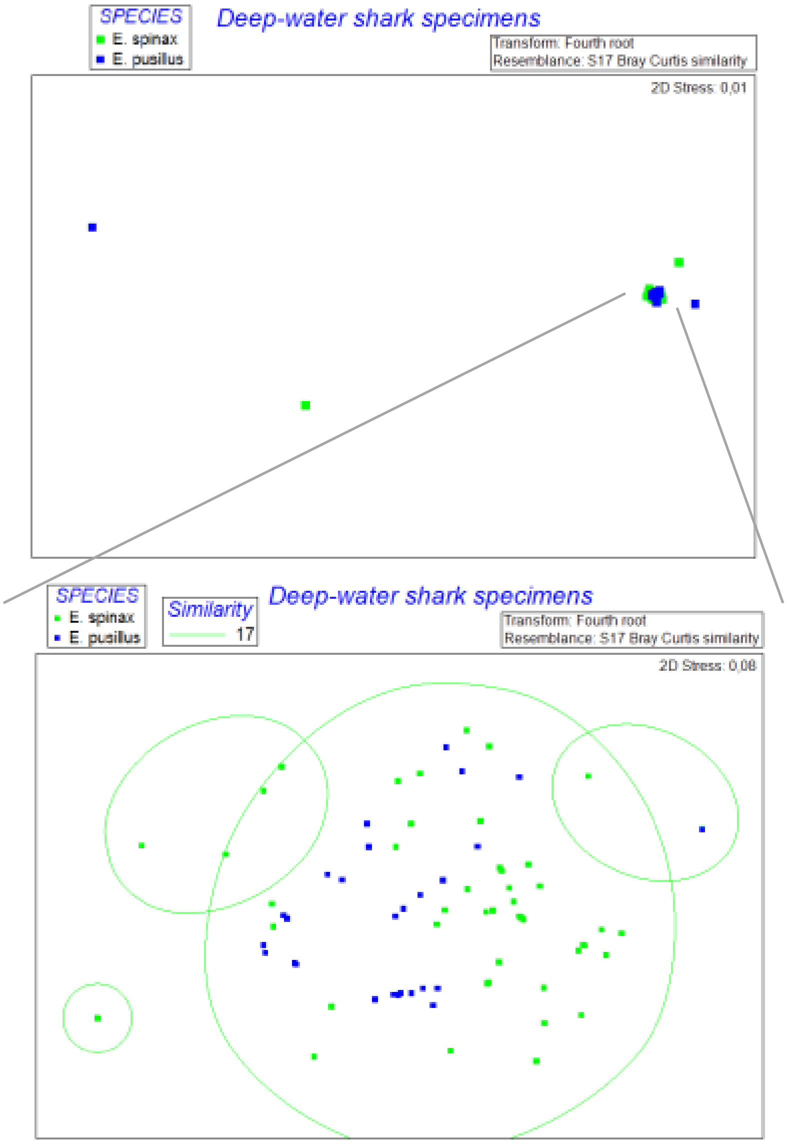


Figure 3.31: MDS plot of relative importance in the diet of *E. spinax* and *E. pusillus* specimens. The main four groups defined at similarity level of 17% with superimposed clusters are indicated by the dashed line.

Concerning to SIMPER analysis, it is possible to observe that the average contribution of the prey items to the dissimilarity in the diet between both deep-water species of sharks was 74.82%. In terms of relative importance N.i. Crustaceans (N.i. Crust) (25.87%), SbO. Natantia (SbO. Natn) (23.08%) and N.i. Fish (20.89%) were the prey items most responsible for the dissimilarity in the diet between both deep-water shark species. In addition the differences in the IRI average are very consistent between both deep-water shark species for most contributors prey items in their diets (**Tab. 3.17**).

Table 3.17: SIMPER results for the relative importance of prey items between *E. spinax* and *E. pusillus*. Parameters include: average contribution to relative importance (Av. IRI) and to dissimilarity (Av. Diss), dissimilarity/standard deviation (Diss/SD), contribution of the dominant species to the dissimilarity % (Contrib%) and cumulative dissimilarity % (Cum.%).

Species	Group *E. spinax* Av.IRI	Group *E. pusillus* Av.IRI	Av.Diss 74.82%	Diss/SD	Contrib%	Cum.%
N.i.Crust	0.29	2.06	17.99	1.49	**25.87**	25.87
SbO.Natn	1.86	0.88	17.55	1.25	**23.08**	48.95
N.i. Fish	0.97	1.41	15.36	0.98	**20.89**	69.85
PasipSiv	0.29	0.48	5.81	0.60	8.21	78.05
OrdEuph	0.15	0.47	5.75	0.56	7.30	85.35
N.i.Ceph	0.42	0.11	5.10	0.48	6.47	91.82

Chapter 4 *DISCUSSION*

4. DISCUSSION

This research is focused on the diet study of two species of small deep-water sharks, the Velvet belly (*Etmopterus spinax)* and Smooth lanternshark (*Etmopterus pusillus),* along the Algarve coast and taking into account different variables (sex, maturity stage and season), which influence diet composition of both species.

Despite the considerable number of samples of *E. spinax*, the number of stomachs analysed from *E. pusillus* was lower and come from different times of sampling and for this reason, a comprehensive analysis of the presented results should be done with caution.

4.1. General diet composition

In the northeast Atlantic, especially along the Algarve coast, the diets of *E. spinax* and *E. pusillus* are based primarily on organisms with pelagic and demersal affinities, such as crustaceans, teleost fish and cephalopods. The present study is in accordance with Xavier *et al*. (2006), who analyzed the diet composition of deep-water shark species and found that *Etmopterus* spp. feed primarily on crustaceans, fish and cephalopods. Moreover, a significant component of their diets are organisms with diel vertical migrations such as *Pasiphaea sivado* and euphausiids, but others showing demersal distribution (e.g. *Micromesistius poutassou*) were also observed (Neiva *et al*., 2006; Xavier *et al*., 2012). Pelagic organisms represent one of the main food sources for many demersal communities (Mauchline *et al*., 1991 *in* Xavier *et al*., 2012; Blaber *et al*., 2002). Thereby, according to Neiva *et al*. (2006), Sims *et al*. (2006) and Xavier *et al*. (2012), it can be suggested that *E. spinax* and *E. pusillus* could perform vertical feeding migrations, which is a common behavior in sharks. However, it is important to note that further evidences are required to confirm this behavior pattern in the genus *Etmopterus* (Xavier *et al.,* 2012).

Regarding the diet composition of both deep-water sharks species studied it is interesting to point out that usually all species of prey consumed by *E. spinax* and *E. pusillus* are bioluminescent (Neiva *et al.*, 2006). Therefore it is possible that these predators have a visual adaption that plays an important role in detecting and capturing this type of prey, as referred to by Neiva *et al*. (2006).

In terms of relative importance (IRI), crustaceans were the main group in the diet of *E. spinax* (IRI=70.3%), particularly representatives of the Order Decapoda, followed by teleost fishes (IRI=22.2%) and cephalopods (IRI=7.4%). Decapods were dominant both in number (44.6%), frequency of occurrence (42.9%) and relative importance (67.1%), but fishes (mainly unidentified) were the most abundant in terms of weight (40.5%): these results are consistent with values reported by Costa *et al.* (2002). In the same way, concerning the relative importance of prey found in the *E. pusillus* diet, it was observed that crustaceans and fishes were the principal groups of prey (IRI=50.4% and 47.8%, respectively), (particularly the decapods (25.1%)), followed by cephalopods (IRI=1.8%). The similar importance to both groups (crustaceans and fish) in the diet could be due to the fact that composition diet of *E. pusillus* varies according to different factors, such as the need to consume preys which provide the highest amount of energy possible, as long as these prey are available. In addition, in respect to the taxonomic orders, the Order Euphausiacea dominated in terms of numbers (26.9%), whilst the unidentified fish group was the most relevant in terms of weight and relative importance (%W=75.9% and IRI=66.7%).

Regarding the Relative Importance Index (IRI), and according to Clarke *et al.* (1995), it is important to note that the determination of relative importance for cephalopods and fish in the diets can raise some problems. The identification of cephalopods and fishes from remaining hard structures (beaks and otoliths) can be complicated and, most of the times, does not allows a reliable identification of the prey. In contrast, identification of crustaceans present in the gut is facilitated by the fact that their structures remain almost intact. Consequently, IRI values obtained for fish and cephalopods were low (and underestimated), due to the weight of the hard structures being very low (0.01 g approximately), influencing the index final estimations.

In contrast with other studies (Neiva *et al.*, 2006; Valls *et al.*, 2011), in this research the diet of *E. spinax* was not dominated by teleost fish, such as *Micromesistius poutassou* (IRI=0.01%), nor by cephalopods such as Sepiolids (IRI=0.4%). Moreover the previous two preys were absented in the diet of *E. pusillus.* Nevertheless, the fish group had an important contribution in terms of weight for both deep-water species, 40% for *E. spinax* and 76% for *E. pusillus*, particularly with unidentified fish. This is in accordance with what was reported by Bergstad *et al*. (2003) in their study. In addition, there are three main factors that suggest that *E. pusillus* species feeds intermittently, in accordance with the study carried out by Xavier *et al*. (2012): the frequent observation of a limited amount of food items in their stomachs, the presence of many prey in advanced digestion stages, and the high number of empty stomachs (overall vacuity index=44.8%).

Otherwise the benthic organisms such as reptantid decapods, polychaetes and echinoderms were not part in the diet of both species studied, except the Order Pleuronectiformes with a %FO of 3% in *E. spinax* and 2% in *E. pusillus,* respectively. Furthermore, an 11% of sediment (%FO) was found in the stomach contents of *E. spinax*. Usually, the ingestion of small amounts of sediment can suggest that this species is also foraged near the bottom (Costa *et al.*, 2002). In contrast, sediments were not found in the stomach contents of *E. pusillus*. Thus, these results suggests that *E. pusillus* feeds mainly on benthopelagic prey, as suggested Neiva *et al.* (2006), but also prey with epibenthic affinities.

Concerning the statistical analysis used to know the existence of significant differences, the ANOSIM test showed that there were slight different in the diet compositions between both species of deep-water sharks, and these differences were significant. These differences could be related with the more importance the fish species have in the *E. pusillus* diet and with the differences in feeding behaviour raised above.

On the other hand, the results obtained in this study about the diet overlap between *E. spinax* and *E. pusillus* indicated that, despite the existence of slight differences in their feeding habits, the diet overlap was high ($\alpha > 0.6$). This index was estimated in order to understand how species that utilize common resources, such as food or space, coexist. This fact is due to that each species in a community has a determined position in the trophic chain, including its use of food resources, activity time, location, form of interaction with other species, among other factors (Ludwing and Reynolds, 1988). Regarding the trophic level, the values obtained for these species are in agreement with Cortés (1999), where both species are in the same trophic level, corresponding TL= 3.8 (*E. spinax*) and TL= 4.1 (*E. pusillus*). Furthermore, their trophic level values are similar to the values obtained for species with the same distribution, such as *Etmopterus princeps* (TL= 4.2), *Scyliorhinus canicula* (TL= 3.6) and *Galeus melastomus* (TL= 3.7) (Cortés, 1999), being this fact probably related with their benthopelagic life style. Cortés (1999) and Neiva *et al.* (2006) stated that the body length and trophic level were moderately correlated, suggesting that larger species have a higher trophic level than smaller species, due to larger species can also consume cetaceans and other elasmobranchs. In the same way, Gamito *et al.* (2005) suggested for species, such as *Spondyliosoma cantharus* and *Sparus auratus,* that the elevated trophic level can be related with the consumed amount of teleost fish. Hence, and according to those authors, the species with a diet rich in teleost have a higher trophic level values than species with a poor diet in teleost.

The degree of overlap found in the diets of both deep-water shark species can indicate competition or resource partitioning. Results presented in this study allowed observing that both species use the same feeding resources, although in different proportions. In the case of *E. spinax* the preferential prey was the crustacean group, specifically the Natantia order. However, the preferential prey for *E. pusillus* was the fish group, specifically the N.i. Fish. This can indicate a resource partitioning between both shark species. Preliminary analysis (in progress and unpublished) of the stable isotopic composition of carbon and nitrogen in muscle of *E. spinax* and *E. pusillus* support feeding overlap but different foraging strategies, providing further evidence of resource partitioning among these species. Moreover, this fact can also suggest that both deep-water shark species feeding on locally abundant resources off the Algarve coast (Xavier *et al.*, 2012). Here the Euphausiids, *P. sivado* and *M. poutassou* are key *taxa* in the regional marine food web (Santos *et al*., 2001). The variation of the diets can be related to the abundance or scarcity of food, so the description of their feeding ecology just present a momentary image of available resources during the performance of this study.

When compared with other studies it could be seen that in the present study there were a lower number of prey items, namely consumed by *E. pusillus* (10 vs 26 prey *taxa*, Xavier *et al.*, 2012) and by *E. spinax* (19 vs 29 prey *taxa* Neiva *et al.* 2006). However, the values of diversity, richness and evenness obtained for the diets of *E. spinax* and *E. pusillus*, were low and very similar (*E. spinax* H'= 0.3; R_1= 0.5; J'= 0.4 and *E. pusillus* H'=0.2; R_1= 0.4; J'= 0.3). One reason of obtaining low values of species diversity, richness and evenness in their diets can be associated to the low number of specimens caught for the analysis, as was in case of *E. pusillus,* and/or the advanced stage of digestion of many of the prey items that did not allow identification to the species level for some taxonomic groups.

The diversity and richness of items consumed is indicative of the ecological specialization of their predators, particularly being designated as specialist, opportunist or generalist feeders. Lower values of these indices suggest that this predator species might have a strict diet, i.e it is relatively specialist, while higher values suggest a possible generalist diet, where the predator shows a rich and diverse consumption of prey available. Also, the low values of species evenness in their diets can suggest that the prey items consumed have not a uniformly distribution in their diets. Moreover, it is important to note that, as mentioned by Mauchline & Gordon (1985), the diversity indices applied to the feeding ecology have a high degree of subjectivity. For this reasons, the results here presented should be interpreted with caution.

The ecological specialization can be assigned from the diagram (Fig. 4.1) adapted from Costello (1990), which suggests that *E. spinax* and *E. pusillus* have a specialist diet, also reported in Neiva *et al.* (2006) and Xavier *et al.* (2012), and eventually opportunistic diet. Despite the observations, allowed by the stomach content analysis, are not entirely conclusive, one can assume that the studied sharks are not generalist-feeders. It is important to note that the differences in the proportions of the diversity values in respect to several authors, it may be due to the quantitative and qualitative variations in the benthic and ichthyologic populations to the local study, in this case the coast of Algarve.

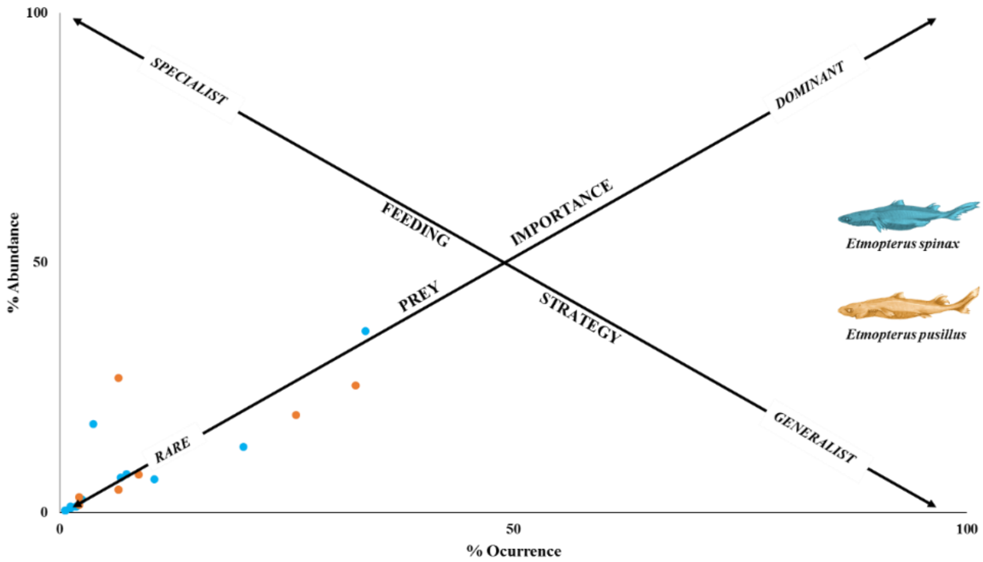


Figure 4.1: Explanatory diagram of the proposed method of diet analysis by Costello (1990).

Xavier *et al.* (2012) recorded that the vacuity index may be related with the fishing gear used and its selectivity. Usually the specimens caught by trawling have fuller stomachs than those caught by longline. In the case of longline captures the specimens are hooked on bait so their stomachs sometimes contain the bait remains and it is assumed that when a fish is hooked is because it was hungry and thus with a higher probability of having an empty stomach. On the contrary, in the captures by trawling the specimens can have their stomachs full and even eat while they are within the net. In contrast to Xavier *et al.* (2012), in this study there seems to be no influence of the fishing gear/selectivity on the vacuity index. This was reflected on the high values of vacuity presented by the specimens caught by trawling, both for *E. spinax* (43.9%) and for *E. pusillus* (44.8%). This highest vacuity values can be influenced by the trawling time, because this fishing method can take several hours and the stomachs may become completely digested and non-identifiable, as Costa *et al.* (2002) suggested.

4.2. Diet composition according to sex and maturity stages

The segregation of the sexes between the species is a general behavior phenomenon observed in terrestrial and aquatic ecosystems (Sims, 2006). Sharks can be an appropriate model to test theories on the mechanisms underlying sexual segregation. This is a common characteristic in shark populations (Sims, 2006), where juveniles as well as male and female adults begin to separate into different groups (Springer, 1967).

Concerning their diet compositions and corroborating the authors Neiva *et al.* (2006) and Xavier *et al.* (2012) findings, this study showed that the diet composition between sexes of each deep-water shark species did not present significant differences. Although the diet of *E. spinax* male specimens showed a higher heterogeneity diet than females. In the same way the maturity was not a significant source of variation in the diet of *E. spinax*. Furthermore and in terms of diversity, richness and evenness of species in their diets no statistically significant differences between sexes were also obtained for each species in study.

For the same species, the sizes between mature females and males are usually different. This seems to be related with the reproduction mode (Sims, 2006). Most shark species present larger females than males, this could mean that sexual segregation plays an important role in the different energy requirements between males and females (Sims, 2006). Regarding their length at first maturity, it was also significantly higher for females than males. According to Coelho *et al.* (2005), *E. spinax* first maturity was 30.86 cm for females and 25.39 cm for males, while for *E. pusillus* was 43.60 cm for females and 38.10 cm for males. So both *E. spinax* and *E. pusillus* have a low growth rate and late maturing, which makes these populations extremely vulnerable to increasing fishing mortality, as suggested the Coelho *et al*. (2005) study.

Regarding the diet compositions of both studied species and in terms of IRI, the crustaceans were the most important group of prey for immature females and males in *E. spinax* species. Nevertheless the fish were the most important group in the diet composition of mature males of *E. spinax*, but not for the mature females, whose diet was composed mainly by crustaceans. This suggests that the ontogenetic shift in the diet of *E. spinax* is probably due to the low selectivity of increasingly larger specimens in the selection of the prey size (Neiva *et al*., 2006). On the other hand, Xavier *et al.* (2012)

suggest that in the *E. pusillus* diet, the fish increase in importance as the specimens increase in size. However, in spite the same has been observed, due to the low number of *E. pusillus* specimens obtained to carry out the present study, it was not possible to prove this evidence. Furthermore, Jakobsdóttir (2001) studied the diet of the shark *Centroscyllium fabricii,* which is in the same order as *E. spinax* and *E. pusillus and* she verified that the diet of the small/immature specimens of this species was based mainly on crustaceans and some fish (opportunistically), while diet of larger/mature specimens was mainly composed of fish. On the other hand, the amount of prey items in terms of number and weight (especially for the fish group), was usually higher in mature than in immature specimens of *E. spinax*. This can be affected by their reproductive cycle, as the fish group provides a large energetic value for overcoming this period, which requires a lot of energy. The study of Lopez *et al*. (2009) for the shark, *Isurus oxyrinchus,* also suggested that the higher consumption of fish is due to the large migrations of this species, which uses a large amount of energy.

It is also interesting to suggest that the preference of the specimens for smaller or larger prey may be related with size of the predator's mouth (Erzini *et al.,* 1997). The mouth size of the individuals is related to their total length, therefore it is also connected to the size of the dominant prey found in their diets. It is also related with the stomach storage capacity, the swimming capability and energetic requirements, which accompany the growth of the predator and are important characteristics for the prey selection (Neiva *et al*. (2006). Thus it is probable that with the increase of specimen size the diet becomes more diverse in terms of prey size (Neiva *et al*., 2006). Consequently, the fact that larger specimens feed on larger species of prey may reduce the competition between both intra- and inter- species, just as Fanelli *et al*. (2009) suggested for *E. spinax* and *G. melastomus*.

4.3. Diet composition according to the season

The analysis of the diet of both deep-water shark species for the different seasons, and separating them between warm season (summer and spring) and cold season (winter and autumn) showed that the crustaceans remain the main prey items in the diet composition during all seasons.

The diet was richest during warm season for *E. spinax*, obtaining larger amount of items prey in warm season (n=167) than cold season (n=109). Generally, this study showed that *E. spinax* does not change diet seasonally. This means that they feed on the same prey groups along the year, although slight differences in their abundance, frequency of occurrence and relative importance were observed. This result contrast with conclusions obtained by Anastasopoulou *et al*. (2013), who found seasonal shifts on the diet of another deepwater shark, namely *Galeus melastomus*, in the eastern Ionian Sea. In terms of IRI, %N and %FO, the diet composition for *E. spinax* during all year was composed mainly by crustaceans, being the decapods the most important type of prey. In the same way occurred for *E. pusillus,* but during the warm season. Otherwise, in terms of %W the fish group was most representative during hot season (51% for *E. spinax* and 76.1% for *E. pusillus*).

In contrast to some studies (Fanelly *et al*., 2009, Anastasopoulou *et al*., 2013 and Costa *et al.,*2002), this research did not show significant differences in the ANOSIM analysis of the diet composition of *E. spinax* in different season categories. In addition, no statistically significant differences between seasons were also obtained for *E. spinax* and for each index of diversity, richness and evenness.

These facts could be explain by many factors which can affect to the diet composition of the predators (Cailliet (1977) *in* Gonçalves (2000)). Shifts in diets can be a result of the amount of food available, migrations of prey and/or variations in water temperature (Gonçalves, 2000). The absence of a seasonal variation in the diet can be related to a relative constancy in values of temperature in the deep sea. Usually temperature variations are more evident in shallow waters than at greater depths, with effects on prey composition, fluctuations in abundance, and/or changes in distribution in the water column. Finally, high fishing pressure can have an impact on the prey communities, leading to the disruption of the food web with unpredictable effects on top predators.

4.4. General conclusions:

The ecological importance and pressure that deep-water communities are subject in the context of human activities and climate change make basic biology studies very important in the understanding of the ecology of still considerably unknown species.

This work is a valid contribution for the evaluation of the trophic relationships of two deepwater shark species from the Portuguese south coast and a common component of the crustacean fishery in the region as a result of bycatch.

The results here presented indicated that *Etmopterus spinax* and *Etmopterus pusillus* are benthopelagic feeders, with a certain degree of specialization and eventually opportunistic behavior. The diet composition of both sharks is dominated by crustaceans, fish and cephalopods, suggesting a high degree of overlap between species. This indicates they are sharing prey resources, but also suggesting that they feed on abundant species present off the Algarve coast. Furthermore, there were no differences in the diet composition diversity, richness and evenness of prey species, between sexes, maturity stages and seasons for each species, which reveals common patterns and strategies in relatively stable environment. The slight differences found between the diets of the two lantern sharks were probably due to a higher preference for fish and for preys less dependent on the bottom by the *E. pusillus*. The variability in the diet of *E. spinax* showed a preference of immature specimens for crustaceans and the mature specimens for fish. This is probably related with their mouth sizes, stomachs storage capacity, swimming capacity and their energy needs.

Finally, differences found between the present study and previously reported diet composition for these species might be related with changes in the community structure of the prey-components caused both by natural variation and bottom trawling. However, more evidences are needed to draw further conclusions.

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ANNEXES

ANNEX 1: Systematic classification of the different cephalopods prey items found in the stomach contents of both deep-water shark species (*Etmopterus spinax* and *Etmopterus pusillus).*

Kingdom Animalia

Phyllum Mollusca

Class Cephalopoda

Subclass Coleoidea

Superorder Decapodiformes

Order Sepiida

Family Sepiidae ***Sepia orbugnyana*** **Férussac, 1826**

Order Oegopsida

Family Ommastrephidae ***Ommastrephes bartramii*** **(Lesueur, 1821)**

Family Pyroteuthidae ***Pterygioteuthis giardi*** **P. Fischer, 1896**

Family Cranchiidae ***Liocranchia reinhardti*** **(Steenstrup, 1856)**

Family Onychoteuthidae ***Onychoteuthis banksii*** **(Leach, 1817)**

Family Enoploteuthidae ***Abraliopsis affinis*** **(Pfeffer, 1912)**

ANNEX 2: Systematic classification of the different crustaceans prey items found in the stomach contents of both deep-water shark species (*Etmopterus spinax* and *Etmopterus pusillus).*

- Kingdom Animalia
  - Phylum Arthropoda
    - Subphylum Crustacea
      - Class Malacostraca
        - Subclass Eumalacostraca
          - Superorder Eucarida
            - Order Decapoda
              - **Suborder Natantia**
              - Suborder Pleocyemata
                - Infraorder Caridea
                  - Family Pasiphaeidae
                    - **Genus *Pasiphaea*** **   *Pasiphaea sivado* (Risso, 1816)**
                  - **Family Pandalidae**
            - **Order Euphausiacea**

ANNEX 3: Systematic classification of the different fish prey items found in the stomach contents of both deep-water shark species (*Etmopterus spinax* and *Etmopterus pusillus*) (*i*n bold are represented the prey items found in the stomachs contents).

Kingdom Animalia

Phylum Chordata

Subphylum Vertebrata

Class Actinopterygii

Order Pleuronectiformes

Family Cynoglossidae

Genus *Symphurus* spp.

Family Bothidae

Genus *Arnoglossus* spp.

Order Gadiformes

Family Phycidae **_Phycis blennoides_ (Brünnich, 1768)**

Family Gadidae **_Micromesistius poutassou_ (Risso, 1827)**

Order Stomiiformes

Family Gonostomatidae

ANNEX 4: Geographical coordinates of scientific projects concerning bycatch and discards (BYDISCARD, CE DGXIV-99/058; DISCALG, CE DGXIV-97/0087) carried out from February 1999 to September 2000 throughout the south coast of Algarve (Portugal), where (^{i}) is the initial latitude and longitude of the throw and (^{f}) is the final latitude and longitude of the throw.

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
06/02/1999		6	598 m - 680 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 45.540 N	$^{i}7^{0}$ 50.290 W	640 m
	36^{0} 45.400 N	7^{0} 46.580 W	620 m
	36^{0} 44.330 N	7^{0} 43.790 W	635 m
	36^{0} 43.430 N	7^{0} 42.320 W	630 m
	36^{0} 42.230 N	7^{0} 41.240 W	650 m
	36^{0} 41.830 N	7^{0} 42.200 W	680 m
	$^{f}36^{0}$ 49.800 N	$^{f}7^{0}$ 48.380 W	598 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
23/02/1999		12	300 m - 655 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 45.950 N	$^{i}7^{0}$ 55.080 W	650 m
	36^{0} 44.880 N	7^{0} 44.460 W	630 m
	36^{0} 42.190 N	7^{0} 42.050 W	655 m
	36^{0} 48.140 N	7^{0} 48.100 W	595 m
	36^{0} 49.456 N	7^{0} 48.352 W	323 m
	36^{0} 53.920 N	7^{0} 47.230 W	390 m
	36^{0} 55.360 N	7^{0} 43.240 W	315 m
	36^{0} 55.970 N	7^{0} 40.550 W	300 m
	$^{f}36^{0}$ 54.590 N	$^{f}7^{0}$ 45.810 W	330 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
19/03/1999		6	308 m - 346 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 46.035 N	$^{i}7^{0}$ 50.093 W	346 m
	36^{0} 52.07 N	7^{0} 42.730 W	308 m
	36^{0} 52.920 N	7^{0} 43.140 W	325 m
	36^{0} 51.804 N	7^{0} 45.569 W	321 m
	$^{f}36^{0}$ 49.08 N	$^{f}7^{0}$ 48.18 W	324 m

E. spinax			
DATA		N^0 SPECIMENS	DEEP RANGES
11/05/1999		11	324 m - 670 m
COORDINATES			
THROW	LATITUDE	LONGITUDE	DEPTHS
1	$^{i}36^0$ 53.99 N	$^{i}7^0$ 39.83 W	355 m
	36^0 52.28 N	7^0 45.44 W	343 m
	36^0 51.52 N	7^0 46.77 W	327 m
	$^{f}36^0$ 46.34 N	$^{f}7^0$ 53.92 W	640 m
2	$^{i}36^0$ 46.31 N	$^{i}7^0$ 53.66 W	640 m
	36^0 50.67 N	7^0 47.44 W	325 m
	36^0 52.32 N	7^0 44.95 W	329 m
	$^{f}36^0$ 52.320 N	$^{f}7^0$ 45.340 W	650 m
3	$^{i}36^0$ 53.09 N	$^{i}7^0$ 42.28 W	324 m
	36^0 51.35 N	7^0 48.44 W	363 m
	36^0 48.38 N	7^0 50.88 W	345 m
	$^{f}36^0$ 45.308 N	$^{f}7^0$ 54.451 W	670 m

E. spinax			
DATA		N^0 SPECIMENS	DEEP RANGES
28/05/1999		5	297 m - 754 m
COORDINATES			
THROW	LATITUDE	LONGITUDE	DEPTHS
1	$^{i}36^0$ 46.56 N	$^{i}7^0$ 53.40 W	349 m
	36^0 50.10 N	7^0 42.03 W	297 m
	36^0 51.56 N	7^0 41.86 W	570 m
	36^0 50.80 N	7^0 47.64 W	600 m
	36^0 52.05 N	7^0 48.48 W	321 m
	36^0 46.32 N	7^0 51.96 W	626 m
	36^0 47.47 N	7^0 49.96 W	582 m
	36^0 48.74 N	7^0 49.20 W	663 m
	36^0 49.89 N	7^0 48.26 W	590 m
	36^0 52.56 N	7^0 44.45 W	700 m
	$^{f}36^0$ 53.87 N	$^{f}7^0$ 39.62 W	754 m

E. spinax			
DATA		**N^0 SPECIMENS**	**DEEP RANGES**
10/06/1999		25	440 m - 485 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 48.246 N	$^{i}7^{0}$ 58.452 W	446 m
	36^{0} 47.760 N	8^{0} 05.140 W	480 m
	36^{0} 47.380 N	8^{0} 08.750 W	455 m
	36^{0} 47.160 N	8^{0} 08.470 W	485 m
	36^{0} 47.460 N	8^{0} 06.820 W	450 m
	36^{0} 48.240 N	8^{0} 03.480 W	480 m
	36^{0} 49.270 N	7^{0} 58.940 W	440 m
	36^{0} 50.021 N	7^{0} 56.419 W	474 m
	36^{0} 50.020 N	7^{0} 55.700 W	455 m
	36^{0} 49.190 N	7^{0} 58.590 W	465 m
	36^{0} 48.380 N	8^{0} 02.400 W	480 m
	36^{0} 48.430 N	8^{0} 02.460 W	465 m
	$^{f}36^{0}$ 49.025 N	$^{f}8^{0}$ 59.471 W	474 m

E. spinax			
DATA		**N^0 SPECIMENS**	**DEEP RANGES**
15/06/1999		31	420 m - 480 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 49.990 N	$^{i}7^{0}$ 56.760 W	465 m
	36^{0} 48.660 N	8^{0} 02.670 W	445 m
	36^{0} 47.060 N	8^{0} 12.160 W	450 m
	36^{0} 47.510 N	8^{0} 08.140 W	445 m
	36^{0} 48.590 N	8^{0} 03.210 W	440 m
	$^{f}36^{0}$ 50.070 N	$^{f}7^{0}$ 56.730 W	460 m
2	$^{i}36^{0}$ 50.110 N	$^{i}7^{0}$ 56.820 W	450 m
	36^{0} 49.350 N	7^{0} 59.060 W	425 m
	36^{0} 48.620 N	8^{0} 02.980 W	420 m
	36^{0} 47.730 N	8^{0} 07.470 W	420 m
	36^{0} 47.150 N	8^{0} 09.040 W	480 m
	36^{0} 47.420 N	8^{0} 07.840 W	465 m
	$^{f}36^{0}$ 49.879 N	$^{f}7^{0}$ 57.279 W	421 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
20/07/1999		9	305 m - 655 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 47.102 N	$^{i}7^{0}$ 54.55 W	366 m
	36^{0} 47.090 N	7^{0} 52.278 W	340 m
	36^{0} 47.320 N	7^{0} 47.240 W	590 m
	36^{0} 46.380 N	7^{0} 40.450 W	575 m
	36^{0} 45.070 N	7^{0} 34.000 W	580 m
	$^{f}36^{0}$ 44.390 N	$^{f}7^{0}$ 30.250 W	305 m
2	$^{i}36^{0}$ 44.850 N	$^{i}7^{0}$ 29.970 W	306 m
	36^{0} 45.690 N	7^{0} 32.700 W	555 m
	36^{0} 46.560 N	7^{0} 37.670 W	555 m
	36^{0} 46.850 N	7^{0} 40.170 W	570 m
	36^{0} 50.020 N	7^{0} 49.310 W	655 m
	$^{f}36^{0}$ 51.100 N	$^{f}7^{0}$ 47.650 W	625 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
12/08/1999		12	450 m - 645 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 50.301 N	$^{i}7^{0}$ 47.623 W	587 m
	36^{0} 45.980 N	7^{0} 52.280 W	645 m
	36^{0} 46.190 N	7^{0} 47.110 W	605 m
	36^{0} 46.190 N	7^{0} 48.880 W	615 m
	$^{f}36^{0}$ 46.690 N	$^{f}7^{0}$ 53.480 W	625 m
2	$^{i}36^{0}$ 49.340 N	$^{i}7^{0}$ 56.680 W	570 m
	36^{0} 48.440 N	8^{0} 02.300 W	458 m
	36^{0} 47.210 N	8^{0} 09.850 W	450 m
	36^{0} 47.770 N	8^{0} 05.110 W	480 m
	$^{f}36^{0}$ 48.810 N	$^{f}8^{0}$ 00.530 W	460 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
19/08/1999		5	563 m - 650 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 46.594 N	$^{i}7^{0}$ 53.768 W	650 m
	36^{0} 46.781 N	7^{0} 47.724 W	595 m
	36^{0} 43.941 N	7^{0} 33.997 W	575 m
	36^{0} 41.934 N	7^{0} 30.822 W	570 m
	$^{f}36^{0}$ 41.848 N	$^{f}7^{0}$ 30.669 W	563 m

E. spinax			
DATA		**N^0 SPECIMENS**	**DEEP RANGES**
07/09/1999		13	475 m - 640 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^i 36^0$ 46.100 N	$^i 7^0$ 52.620 W	640 m
	36^0 44.900 N	7^0 45.010 W	625 m
	36^0 42.920 N	7^0 42.850 W	640 m
	36^0 45.930 N	7^0 49.190 W	595 m
	36^0 46.756 N	7^0 51.988 W	624 m
	36^0 49.410 N	7^0 50.950 W	492 m
	36^0 48.840 N	7^0 56.460 W	475 m
	36^0 47.840 N	8^0 04.210 W	590 m
	36^0 47.090 N	8^0 07.910 W	550 m
	36^0 48.620 N	8^0 00.390 W	500 m
	$^f 36^0$ 49.708 N	$^f 7^0$ 56.601 W	494 m

E. spinax			
DATA		**N^0 SPECIMENS**	**DEEP RANGES**
04/11/1999		16	336 m - 635 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^i 36^0$ 46.630 N	$^i 7^0$ 51.270 W	336 m
	36^0 46.600 N	7^0 50.360 W	625 m
	36^0 45.040 N	7^0 44.440 W	620 m
	36^0 43.900 N	7^0 43.190 W	635 m
	36^0 46.930 N	7^0 49.540 W	610 m
	$^f 36^0$ 47.410 N	$^f 7^0$ 51.010 W	620 m

E. spinax			
DATA		**N^0 SPECIMENS**	**DEEP RANGES**
15/03/2000		7	390 m - 430 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^i 36^0$ 50.267 N	$^i 7^0$ 57.083 W	400 m
	36^0 48.805 N	8^0 02.794 W	420 m
	36^0 48.469 N	8^0 04.462 W	390 m
	36^0 47.243 N	8^0 11.375 W	425 m
	$^f 36^0$ 46.917 N	$^f 8^0$ 17.536 W	400 m
2	$^i 36^0$ 46.678 N	$^i 8^0$ 18.353 W	430 m
	36^0 47.980 N	8^0 07.187 W	390 m
	36^0 49.189 N	8^0 00.388 W	430 m
	$^f 36^0$ 50.010 N	$^f 7^0$ 57.502 W	410 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
30/05/2000		5	350 m - 460 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 51.002 N	$^{i}7^{0}$ 56.499 W	350 m
	36^{0} 47.242 N	8^{0} 10.436 W	460 m
	36^{0} 46.806 N	8^{0} 16.316 W	400 m
	$^{f}36^{0}$ 46.748 N	$^{f}8^{0}$ 21.353 W	425 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
08/06/2000		6	550 m - 620 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 53.359 N	$^{i}7^{0}$ 35.796 W	550 m
	36^{0} 53.547 N	7^{0} 40.622 W	600 m
	36^{0} 52.833 N	7^{0} 43.035 W	610 m
	36^{0} 51.329 N	7^{0} 44.928 W	580 m
	36^{0} 50.614 N	7^{0} 47.199 W	590 m
	$^{f}36^{0}$ 50.499 N	$^{f}7^{0}$ 48.453 W	620 m

E. spinax			
DATA		**N^{0} SPECIMENS**	**DEEP RANGES**
12/09/2000		4	550 m - 620 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	$^{i}36^{0}$ 46.91 N	$^{i}8^{0}$ 49.99 W	515 m
	36^{0} 47.39 N	8^{0} 46.66 W	480 m
	36^{0} 47.89 N	8^{0} 44.67 W	430 m
	36^{0} 46.29 N	8^{0} 43.34 W	550 m
	$^{f}36^{0}$ 46.36 N	$^{f}8^{0}$ 53.99 W	565 m

ANNEX 5: Geographical coordinates of campaign concerning bycatch and discards carried out from 27th of April 2015 to 3rd of May 2015 throughout the south coast of Algarve (Portugal), where (i) is the initial latitude and longitude of the throw and (f) is the final latitude and longitude of the throw.

E. pusillus			
DATA		**Nº SPECIMENS**	**DEEP RANGES**
27/04/2015		18	256 m - 490 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
1	i 36º 51.810 N f 36º 50.147 N	i 07º 56.124 W f 08º 04.549 W	256
2	i 36º 49.601 N f 36º 51.634 N	i 08º 03.096 W f 07º 53.546 W	329
3	i 36º 51.583 N f 36º 49.620 N	i 07º 54.561 W f 08º 03.383 W	320
4	i 36º 49.960 N f 36º 51.769 N	i 08º 02.294 W f 07º 54.587 W	285
5	i 36º 49.426 N f 36º 47.270 N	i 07º 57.045 W f 08º 07.075 W	490

E. pusillus			
DATA		**Nº SPECIMENS**	**DEEP RANGES**
28/04/2015		6	180 m - 300 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
6	i 36º 48.087 N f 36º 49.632 N	i 08º 03.078 W f 07º 55.990 W	275
7	i 36º 49.231 N f 36º 47.979 N	i 07º 56.903 W f 08º 02.257 W	300
8	i 36º 48.262 N f 36º 47.313 N	i 08º 08.878 W f 08º 17.049 W	190
9	i 36º 47.367 N f 36º 48.122 N	i 08º 16.949 W f 08º 08.449 W	184
10	i 36º 48.262 N f 36º 49.656 N	i 08º 08.282 W f 08º 02.005 W	180
11	i 36º 49.788 N f 36º 51.427 N	i 08º 01.458 W f 07º 54.676 W	180
12	i 36º 49.220 N f 36º 47.130 N	i 07º 56.860 W f 08º 06.870 W	295

E. pusillus			
DATA		**Nº SPECIMENS**	**DEEP RANGES**
29/04/2015		12	175 m - 270 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
13	i 36º 46.699 N f 36º 46.320 N	i 08º 09.460 W f 08º 18.150 W	270
14	i 36º 47.727 N f 36º 48.250 N	i 08º 17.140 W f 08º 07.060 W	190
15	i 36º 47.798 N f 36º 47.120 N	i 08º 08.050 W f 08º 17.010 W	195
16	i 36º 47.690 N f 36º 49.150 N	i 08º 12.880 W f 08º 04.140 W	175
17	i 36º 49.600 N f 36º 51.400 N	i 08º 02.770 W f 07º 54.730 W	175

E. pusillus			
DATA		**Nº SPECIMENS**	**DEEP RANGES**
02/05/2015		4	160 m - 275 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
18	i 36º 49.446 N f 36º 48.490 N	i 07º 57.045 W f 08º 00.677 W	265
19	i 36º 48.446 N f 36º 49.586 N	i 08º 00.778 W f 07º 56.145 W	275
20	i 36º 51.444 N f 36º 49.894 N	i 07º 57.502 W f 08º 03.223 W	160
21	i 36º 49.589 N f 36º 51.354 N	i 08º 02.716 W f 07º 54.970 W	180
22	i 36º 51.460 N f 36º 49.898 N	i 07º 55.425 W f 08º 01.502 W	166
23	i 36º 50.180 N f 36º 51.496 N	i 08º 01.188 W f 07º 54.527 W	160
24	i 36º 49.453 N f 36º 47.084 N	i 07º 56.794 W f 08º 08.058 W	275

E. pusillus			
DATA		**Nº SPECIMENS**	**DEEP RANGES**
03/05/2015		18	160 m - 280 m
COORDINATES			
THROW	**LATITUDE**	**LONGITUDE**	**DEPTHS**
25	^{i}36º 47.880 N ^{f}36º 49.591 N	^{i}08º 03.962 W ^{f}07º 56.097 W	280
26	^{i}36º 49.490 N ^{f}36º 47.996 N	^{i}07º 56.600 W ^{f}08º 02.782 W	275
27	^{i}36º 49.896 N ^{f}36º 51.636 N	^{i}08º 02.479 W ^{f}07º 54.580 W	160
28	^{i}36º 51.489 N ^{f}36º 49.923 N	^{i}07º 53.328 W ^{f}08º 01.690 W	162
29	^{i}36º 49.904 N ^{f}36º 51.676 N	^{i}08º 01.847 W ^{f}07º 54.807 W	160
30	^{i}36º 51.485 N ^{f}36º 49.507 N	^{i}07º 54.560 W ^{f}08º 04.098 W	177

ANNEX 6: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	14.13	20.83	23.71	16.91	828.95	7.41	18.06
Sepia orbignyana	0.36	7.09	1.03	0.61	7.68	0.14	3.33
O. Oegopsida	0.72	3.78	2.06	1.23	9.28	0.16	2.21
F. Sepiolidae	0.72	1.17	2.06	1.23	3.91	0.07	1.10
Ommastrephes bartramii	0.72	0.30	2.06	1.23	2.12	0.04	0.73
Pterygioteuthis giardi	2.54	0.47	4.12	2.45	12.38	0.22	1.62
Histioteuthis heteropsis	0.36	0.05	1.03	0.61	0.43	0.01	0.32
Liocranchia reinhardti	1.09	0.02	2.06	1.23	2.29	0.04	0.68
Onychoteuthis banksii	0.36	>0.01	1.03	0.61	0.38	0.01	0.30
Abraliopsis affinis	0.72	0.01	2.06	1.23	1.51	0.03	0.60
N.i. Cephalopods	6.52	7.92	17.53	10.43	253.20	4.48	8.53
Crustaceans	69.93	33.21	76.29	54.41	7868.41	70.34	48.31
SbO. Natantia	36.23	17.81	56.70	33.74	3064.16	54.27	27.47
Phasiphaea spp.	1.09	0.45	3.09	1.84	4.74	0.08	1.08
Pasiphaea sivado	6.88	6.20	11.34	6.75	148.39	2.63	6.54
O. Euphausiidae	17.75	2.44	6.19	3.68	124.91	2.21	18.06
F. Pandalidae	0.36	0.55	1.03	0.61	0.98	0.02	3.33
N.i. Crustaceans	7.61	5.77	12.37	7.36	165.50	2.93	2.21
Fish	15.94	45.96	40.21	28.68	2488.75	22.25	1.10
Symphurus spp.	1.09	4.72	3.09	1.84	17.98	0.32	0.73
F. Gonostomatidae	0.72	0.64	2.06	1.23	2.82	0.05	1.62
Physcis blennoides	0.36	0.05	1.03	0.61	0.43	0.01	0.32
Micromesistius poutassou	0.36	0.02	1.03	0.61	0.39	0.01	0.68
Arnoglossus spp.	0.36	>0.01	1.03	0.61	0.38	0.01	0.30
N.i. Fish	13.04	40.51	34.02	20.24	1821.98	32.27	0.60

ANNEX 7: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by female specimens of *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	14.04	27.02	31.37	20.78	1288.11	10.62	22.05
Sepia orbignyana	0.59	12.47	1.96	1.06	25.6	0.45	5.68
O. Oegopsida	1.17	6.64	3.92	2.13	30.63	0.54	3.79
F. Sepiolidae	1.17	2.06	3.92	2.13	12.68	0.22	1.9
Ommastrephes bartramii	1.17	0.53	3.92	2.13	6.68	0.12	1.27
Pterygioteuthis giardi	2.34	0.79	3.92	2.13	12.26	0.22	1.62
Histioteuthis heteropsis	0.59	0.1	1.96	1.06	1.34	0.02	0.57
Abraliopsis affinis	0.59	0.01	1.96	1.06	1.16	0.02	0.53
N.i. Cephalopods	6.43	4.42	21.57	11.7	234.13	4.1	7.61
Crustaceans	71.93	43.42	78.43	51.95	9046.97	74.58	52.6
SbO. Natantia	34.5	23.96	60.78	32.98	3553.89	62.29	29.57
Phasiphaea spp.	1.17	0.4	3.92	2.13	6.16	0.11	1.22
Pasiphaea sivado	8.19	7.97	11.77	6.38	190.09	3.33	7.41
O. Euphausiidae	19.3	2.86	3.92	2.13	86.89	1.52	5.98
N.i. Crustaceans	8.77	8.22	17.65	9.57	299.93	5.26	8.85
Fish	14.04	29.56	41.18	27.27	1795	14.8	25.35
Symphurus spp.	1.17	6.64	3.92	2.13	30.63	0.54	3.79
F. Gonostomatidae	0.59	0.3	1.96	1.06	1.73	0.03	0.65
Arnoglossus spp.	0.59	0.01	1.96	1.06	1.16	0.02	0.53
N.i. Fish	11.7	22.61	35.29	19.15	1210.79	21.22	19.04

ANNEX 8: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by male specimens of *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	14.29	12.67	15.22	11.86	410.17	4.03	12.81
Sepia orbignyana	-	-	-	-	-	-	-
O. Oegopsida	-	-	-	-	-	-	-
F. Sepiolidae	-	-	-	-	-	-	-
Ommastrephes bartramii	-	-	-	-	-	-	-
Pterygioteuthis giardi	2.86	0.05	4.35	2.9	12.62	0.22	1.62
Histioteuthis heteropsis	-	-	-	-	-	-	-
Liocranchia reinhardti	2.86	0.06	4.35	2.9	12.67	0.22	1.63
Onychoteuthis banksii	0.95	0.01	2.17	1.45	2.1	0.04	0.7
Abraliopsis affinis	0.95	0.01	2.17	1.45	2.1	0.04	0.7
N.i. cephalopods	6.67	12.54	13.04	8.7	250.54	4.37	9.95
Crustaceans	66.67	19.76	73.91	57.63	6388.26	62.71	42.06
SbO. Natantia	39.05	9.7	52.17	34.78	2543.2	44.38	24.58
Phasiphaea spp.	0.95	0.51	2.17	1.45	3.17	0.06	0.92
Pasiphaea sivado	4.76	3.87	10.87	7.25	93.82	1.64	5.19
O. Euphausiidae	15.24	1.89	8.7	5.8	148.93	2.6	6.16
F. Pandalidae	0.95	1.27	2.17	1.45	4.82	0.08	1.26
N.i. crustaceans	5.71	2.53	6.52	4.35	53.79	0.94	3.85
Fish	19.05	67.57	39.13	30.51	3389.34	33.27	45.14
Symphurus spp.	0.95	2.2	2.17	1.45	6.85	0.12	1.67
F. Gonostomatidae	0.95	1.09	2.17	1.45	4.45	0.08	1.18
Physcis blennoides	0.95	0.13	2.17	1.45	2.35	0.04	0.75
Micromesistius poutassou	0.95	0.05	2.17	1.45	2.17	0.04	0.72
Arnoglossus spp.	-	-	-	-	-	-	-
N.i Fish	15.24	64.1	32.61	21.74	2587.19	45.15	39.12

ANNEX 9: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by immature females of *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	20	24.24	36.67	23.91	1622.16	14.16	23.19
Sepia orbignyana	-	-	-	-	-	-	-
O. Oegopsida	1.33	10.21	3.33	1.85	38.49	0.52	5.23
F. Sepiolidae	1.33	3.17	3.33	1.85	15.02	0.2	2.29
Ommastrephes bartramii	1.33	0.03	3.33	1.85	4.53	0.06	0.98
Pterygioteuthis giardi	1.33	0.3	3.33	1.85	5.43	0.07	1.1
Histioteuthis heteropsis	1.33	0.3	3.33	1.85	5.43	0.07	1.1
Liocranchia reinhardti	-	-	-	-	-	-	-
Onychoteuthis banksii	-	-	-	-	-	-	-
Abraliopsis affinis	1.33	0.03	3.33	1.85	4.53	0.06	0.98
N.i. Cephalopods	12	10.21	30	16.67	666.37	9	13.01
Crustaceans	61.33	36.98	76.67	50	7537.3	65.79	46.76
SbO. Natantia	48	30.07	60	33.33	4684.35	63.23	35.03
Phasiphaea spp.	2.67	1.24	6.67	3.7	26.02	0.35	2.46
Pasiphaea sivado	1.33	2.02	3.33	1.85	11.16	0.15	1.81
O. Euphausiidae	-	-	-	-	-	-	-
F. Pandalidae	-	-	-	-	-	-	-
N.i. Crustaceans	9.33	3.65	16.67	9.26	216.47	2.92	6.94
Fish	18.67	38.78	40	26.09	2297.86	20.06	30.05
Symphurus spp.	1.33	1.21	3.33	1.85	8.48	0.11	1.48
F. Gonostomatidae	1.33	0.91	3.33	1.85	7.49	0.1	1.35
Physcis blennoides	-	-	-	-	-	-	-
Micromesistius poutassou	-	-	-	-	-	-	-
Arnoglossus spp.	1.33	0.03	3.33	1.85	4.53	0.06	0.98
N.i. Fish	14.67	36.63	33.33	18.52	1709.89	23.08	25.26

ANNEX 10: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by mature females of *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	9.38	28.36	23.81	16.13	898.55	7.09	20.09
Sepia orbignyana	1.04	18.48	4.76	2.5	92.97	1.75	8.72
O. Oegopsida	1.04	4.92	4.76	2.5	28.4	0.54	3.19
F. Sepiolidae	1.04	1.53	4.76	2.5	12.24	0.23	1.81
Ommastrephes bartramii	1.04	0.78	4.76	2.5	8.66	0.16	1.5
Pterygioteuthis giardi	3.13	1.02	4.76	2.5	19.75	0.37	2.03
Histioteuthis heteropsis	-	-	-	-	-	-	-
Liocranchia reinhardti	-	-	-	-	-	-	-
Onychoteuthis banksii	-	-	-	-	-	-	-
Abraliopsis affinis	-	-	-	-	-	-	-
N.i. cephalopods	2.08	1.63	9.52	5	35.38	0.67	3.03
Crustaceans	80.21	46.52	80.95	54.84	10259.16	80.91	56.79
SbO. Natantia	23.96	21.02	61.9	32.5	2784.41	52.56	26.08
Phasiphaea spp.	-	-	-	-	-	-	-
Pasiphaea sivado	13.54	10.84	23.81	12.5	580.53	10.96	12.04
O. Euphausiidae	34.38	4.24	9.52	5	367.72	6.94	10.68
F. Pandalidae	-	-	-	-	-	-	-
N.i. crustaceans	8.33	10.43	19.05	10	357.32	6.75	9.83
Fish	10.42	25.11	42.86	29.03	1522.71	12.01	23.12
Symphurus spp.	1.04	9.26	4.76	2.5	49.06	0.93	4.96
F. Gonostomatidae	-	-	-	-	-	-	-
Physcis blennoides	-	-	-	-	-	-	-
Micromesistius poutassou	-	-	-	-	-	-	-
Arnoglossus spp.	-	-	-	-	-	-	-
N.i. Fish	9.38	15.85	38.1	20	961.06	18.14	16.14

ANNEX 11: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by immature males of *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	17.24	4.74	17.39	14.81	382.31	2.92	10.57
Sepia orbignyana	-	-	-	-	-	-	-
O. Oegopsida	-	-	-	-	-	-	-
F. Sepiolidae	-	-	-	-	-	-	-
Ommastrephes bartramii	-	-	-	-	-	-	-
Pterygioteuthis giardi	5.17	0.34	8.7	6.45	47.98	0.73	3.35
Histioteuthis heteropsis	-	-	-	-	-	-	-
Liocranchia reinhardti	3.45	0.34	4.35	3.23	16.49	0.25	1.95
Onychoteuthis banksii	-	-	-	-	-	-	-
Abraliopsis affinis	1.72	0.09	4.35	3.23	7.87	0.12	1.44
N.i. Cephalopods	6.9	3.97	13.04	9.68	141.68	2.15	6.41
Crustaceans	77.59	63.1	86.96	74.07	12233.88	93.37	69.66
SbO. Natantia	44.83	37.5	65.22	48.39	5369.19	81.35	42.56
Phasiphaea spp.	-	-	-	-	-	-	-
Pasiphaea sivado	5.17	11.64	13.04	9.68	219.27	3.32	9.54
O. Euphausiidae	20.69	9.31	8.7	6.45	260.87	3.95	11.04
F. Pandalidae	-	-	-	-	-	-	-
N.i. Crustaceans	6.9	4.66	4.35	3.23	50.22	0.76	4.73
Fish	5.17	32.16	13.04	11.11	486.88	3.72	19.77
Symphurus spp.	-	-	-	-	-	-	-
F. Gonostomatidae	-	-	-	-	-	-	-
Physcis blennoides	-	-	-	-	-	-	-
Micromesistius poutassou	-	-	-	-	-	-	-
Arnoglossus spp.	-	-	-	-	-	-	-
N.i. Fish	5.17	32.16	13.04	9.68	486.88	7.38	18.98

ANNEX 12: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by mature males of *Etmopterus spinax*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	10.64	13.89	13.04	9.38	319.94	2.79	11.72
Sepia orbignyana	-	-	-	-	-	-	-
O. Oegopsida	-	-	-	-	-	-	-
F. Sepiolidae	-	-	-	-	-	-	-
Ommastrephes bartramii	-	-	-	-	-	-	-
Pterygioteuthis giardi	-	-	-	-	-	-	-
Histioteuthis heteropsis	-	-	-	-	-	-	-
Liocranchia reinhardti	2.13	0.01	4.35	2.63	9.31	0.13	1.4
Onychoteuthis banksii	2.13	0.01	4.35	2.63	9.31	0.13	1.4
Abraliopsis affinis	-	-	-	-	-	-	-
N.i. Cephalopods	6.38	13.86	13.04	7.89	264.09	3.74	10.14
Crustaceans	53.19	13.08	60.87	43.75	4033.91	35.15	31.93
SbO. Natantia	31.91	5.41	39.13	23.68	1460.54	20.67	17.6
Phasiphaea spp.	2.13	0.58	4.35	2.63	11.79	0.17	1.64
Pasiphaea sivado	4.26	2.67	8.7	5.26	60.24	0.85	3.93
O. Euphausiacea	8.51	0.74	8.7	5.26	80.48	1.14	4.02
F. Pandalidae	2.13	1.46	4.35	2.63	15.61	0.22	2.02
N.i. Crustaceans	4.26	2.21	8.7	5.26	56.19	0.8	3.73
Fish	36.17	73.03	65.22	46.88	7121.71	62.06	56.35
Symphurus spp.	2.13	2.54	4.35	2.63	20.29	0.29	2.48
F. Gonostomatidae	2.13	1.26	4.35	2.63	14.74	0.21	1.93
Physcis blennoides	2.13	0.15	4.35	2.63	9.89	0.14	1.45
Micromesistius poutassou	2.13	0.05	4.35	2.63	9.48	0.13	1.41
Arnoglossus spp.	-	-	-	-	-	-	-
N.i. Fish	27.66	69.03	52.17	31.58	5044.59	71.39	46.84

ANNEX 13: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by specimens of *Etmopterus spinax* in hot season (spring and summer), where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	19.76	24.2	26.15	18.48	1149.72	10.76	21.36
Sepia orbignyana	0.6	13.02	1.54	0.91	20.95	0.35	6
O. Oegopsida	0.6	3.47	1.54	0.91	6.25	0.11	1.93
F. Sepiolidae	0.6	1.08	1.54	0.91	2.58	0.04	0.91
Ommastrephes bartramii	1.2	0.56	3.08	1.82	5.4	0.09	1.15
Pterygioteuthis giardi	3.59	0.85	4.62	2.73	20.5	0.35	2.11
Histioteuthis heteropsis	0.6	0.1	1.54	0.91	1.08	0.02	0.5
Liocranchia reinhardti	1.8	0.05	3.08	1.82	5.67	0.1	1.06
Onychoteuthis banksii	0.6	0.01	1.54	0.91	0.94	0.02	0.46
Abraliopsis affinis	1.2	0.02	3.08	1.82	3.74	0.06	0.92
N.i. Cephalopods	8.98	5.06	21.54	12.73	302.5	5.11	8.66
Crustaceans	65.27	24.59	80	56.52	7188.9	67.31	44.04
SbO. Natantia	43.71	15.74	60	35.45	3567.39	60.26	28.81
Pasiphaea spp.	1.8	0.82	4.62	2.73	12.08	0.2	1.72
Pasiphaea sivado	3.59	3.88	7.69	4.55	57.46	0.97	4.06
O. Euphausiacea	9.58	1.5	6.15	3.64	68.16	1.15	3.99
F. Pandalidae	-	-	-	-	-	-	-
N.i. Crustaceans	6.59	2.65	10.77	6.36	99.52	1.68	4.83
Fish	14.97	51.21	35.38	25	2341.71	21.93	34.6
Symphurus spp.	0.6	6.52	1.54	0.91	10.95	0.19	3.24
F. Gonostomatidae	0.6	0.87	1.54	0.91	2.25	0.04	0.82
Physcis blennoides	0.6	0.1	1.54	0.91	1.08	0.02	0.5
Micromesistius poutassou	0.6	0.04	1.54	0.91	0.98	0.02	0.47
Arnoglossus spp.	-	-	-	-	-	-	-
N.i. Fish	12.57	43.68	30.77	18.18	1731.02	29.24	27.86

ANNEX 14: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by specimens of *Etmopterus spinax* in cold season (winter and autumn), where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	5.5	16.8	18.75	13.64	418.17	3.62	13.22
Sepia orbignyana	-	-	-	-	-	-	-
O. Oegopsida	0.92	4.15	3.13	1.89	15.83	0.29	2.65
F. Sepiolidae	0.92	1.29	3.13	1.89	6.89	0.13	1.42
Ommastrephes bartramii	-	-	-	-	-	-	-
Pterygioteuthis giardi	0.92	0.01	3.13	1.89	2.9	0.05	0.87
Histioteuthis heteropsis	-	-	-	-	-	-	-
Liocranchia reinhardti	-	-	-	-	-	-	-
Onychoteuthis banksii	-	-	-	-	-	-	-
Abraliopsis affinis	-	-	-	-	-	-	-
N.i. Cephalopods	2.75	11.35	9.38	5.66	132.22	2.45	7.48
Crustaceans	77.06	43.53	68.75	50	8290.69	71.69	53.23
SbO. Natantia	24.77	20.28	50	30.19	2252.5	41.73	24.77
Pasiphaea spp.	-	-	-	-	-	-	-
Pasiphaea sivado	11.93	8.98	18.75	11.32	392.05	7.26	10.45
O. Euphausiacea	30.28	3.57	6.25	3.77	211.53	3.92	9.38
F. Pandalidae	0.92	1.2	3.13	1.89	6.62	0.12	1.38
N.i. Crustaceans	9.17	9.5	15.63	9.43	291.72	5.4	9.4
Fish	17.43	39.67	50	36.36	2855.3	24.69	33.55
Symphurus spp.	1.83	2.58	6.25	3.77	27.57	0.51	2.84
F. Gonostomatidae	0.92	0.37	3.13	1.89	4.03	0.07	1.03
Physcis blennoides	-	-	-	-	-	-	-
Micromesistius poutassou	-	-	-	-	-	-	-
Arnoglossus spp.	-	0.01	3.13	1.89	2.9	0.05	0.87
N.i. Fish	13.76	36.72	40.63	24.53	2050.68	37.99	27.45

ANNEX 15: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by *Etmopterus pusillus*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	10.45	5.88	12.50	9.30	204.07	1.84	7.99
Onychoteuthis bankssi	2.99	0.29	3.13	2.17	10.23	0.16	1.51
Liocranchia reinhardti	1.49	0.02	3.13	2.17	4.74	0.08	1.05
Abraliopsis affinis	1.49	0.02	3.13	2.17	4.74	0.08	1.05
N.i. Cephalopods	4.48	5.54	9.38	6.52	93.94	1.50	5.62
Crustaceans	59.70	18.07	71.88	53.49	5589.55	50.41	38.61
SbO. Natantia	19.40	3.07	37.50	26.09	842.78	13.50	14.21
Pasiphaea spp.	1.49	1.10	3.13	2.17	8.11	0.13	1.54
Pasiphaea sivado	4.48	6.12	9.38	6.52	99.33	1.59	5.88
O. Euphausiacea	26.87	6.79	9.38	6.52	315.52	5.05	11.23
N.i. Crustaceans	7.46	0.98	12.50	8.70	105.58	1.69	4.94
Fish	29.85	76.06	50.00	37.21	5295.32	47.75	53.40
O. Pleuronectiformes	1.49	0.05	3.13	2.17	4.81	0.08	1.06
Physcis blennoides	2.99	0.14	3.13	2.17	9.78	0.16	1.44
N.i. Fish	25.37	75.86	46.88	32.61	4745.48	75.99	50.47

ANNEX 16: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by female specimens of *Etmopterus pusillus*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	16.13	4.92	28.57	18.18	601.37	5.06	11.93
Onychoteuthis bankssi	6.45	0.53	14.29	7.14	99.69	1.35	4.36
Liocranchia reinhardti	3.23	0.04	14.29	7.14	46.71	0.63	3.52
Abraliopsis affinis	3.23	0.04	14.29	7.14	46.71	0.63	3.52
N.i. Cephalopods	3.23	4.3	14.29	7.14	107.57	1.46	5.22
Crustaceans	61.29	12.03	71.43	45.45	5237.41	44.11	34.3
SbO. Natantia	9.68	0.35	42.86	21.43	429.8	5.82	10.65
Pasiphaea spp.	-	-	-	-	-	-	-
Pasiphaea sivado	-	-	-	-	-	-	-
O. Euphausiacea	48.39	11.42	14.29	7.14	854.37	11.57	17.1
N.i. Crustaceans	3.23	0.26	14.29	7.14	49.85	0.68	3.61
Fish	22.58	83.05	57.14	36.36	6035.92	50.83	53.77
O. Pleuronectiformes	-	-	-	-	-	-	-
Physcis blennoides	6.45	0.26	14.29	7.14	95.93	1.3	4.25
N.i. Fish	16.13	82.78	57.14	28.57	5652.19	76.56	47.77

ANNEX 17: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent frequency of occurrence (%FO), percent occurrence by weight (%W) and Mixed Analysis as the relative importance index (%IRI) and Weighted Food Index (IPO2) values of the groups of prey and the main prey consumed by male specimens of *Etmopterus pusillus*, where: O: Order; SbO: Suborder; F: Family; G: Genus and N.i.: Not identified.

ITEM	%N	%W	%FO	%FOc	IRI	IRIc	IPO2
Cephalopods	5.56	7.03	8	6.25	100.71	0.91	6.45
Onychoteuthis bankssi	-	-	-	-	-	-	-
Liocranchia reinhardti	-	-	-	-	-	-	-
Abraliopsis affinis	-	-	-	-	-	-	-
N.i. Cephalopods	5.56	7.03	8	6.25	100.71	1.6	6.45
Crustaceans	58.33	25.33	72	56.25	6023.8	54.25	42.29
SbO. Natantia	27.78	6.35	36	28.13	1228.45	19.52	17.87
Pasiphaea spp.	2.78	2.43	4	3.13	20.84	0.33	2.72
Pasiphaea sivado	8.33	13.48	12	9.38	261.82	4.16	11.05
O. Euphausiacea	8.33	1.22	8	6.25	76.4	1.21	4.38
N.i. Crustaceans	11.11	1.85	12	9.38	155.54	2.47	6.26
Fish	36.11	67.64	48	37.5	4979.87	44.85	51.26
O. Pleuronectiformes	2.78	0.11	4	3.13	11.53	0.18	1.63
Physcis blennoides	-	-	-	-	-	-	-
N.i. Fish	33.33	67.53	44	34.38	4438	70.52	49.62

ANNEX 18: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in *E. spinax* diet.

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	1.09	8.26	3.09	1.84	28.92	0.39
	Oegopsida	6.52	4.64	14.43	8.59	28.40	0.50
	N.i. Ceph	6.52	7.93	17.53	10.43	253.20	3.39
CRUSTACEANS	Decapoda	44.57	25.00	72.16	42.94	5020.44	67.18
	Euphausiacea	17.75	2.44	6.19	3.68	124.91	1.67
	N.i. Crust	7.61	5.77	12.37	7.36	165.50	2.21
FISH	Pleuronectiformes	1.45	4.73	4.12	2.45	25.48	0.34
	Stomiiformes	0.72	0.64	2.06	1.23	2.82	0.04
	Gadiformes	0.72	0.07	2.06	1.23	1.65	0.02
	N.i. Fish	13.04	40.51	34.02	20.25	1821.98	24.38

ANNEX 19: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of female specimens of *E. spinax.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	1.75	14.53	5.88	3.19	95.82	1.19
	Oegopsida	5.85	8.07	15.69	8.51	218.27	2.72
	N.i. Ceph	6.43	4.42	21.57	11.70	234.13	2.92
CRUSTACEANS	Decapoda	43.86	32.34	76.47	41.49	5826.80	72.62
	Euphausiacea	19.30	2.86	3.92	2.13	86.89	1.08
	N.i. Crust	8.77	8.22	17.65	9.57	299.93	3.74
FISH	Pleuronectiformes	1.75	6.65	5.88	3.19	49.44	0.62
	Stomiiformes	0.58	0.30	1.96	1.06	1.73	0.02
	Gadiformes	-	-	-	-	-	-
	N.i. Fish	11.70	22.61	35.29	19.15	1210.79	15.09

ANNEX 20: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of male specimens of *E. spinax.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	-	-	-	-	-	-
	Oegopsida	7.62	0.13	13.04	8.70	101.03	1.39
	N.i. Ceph	6.67	12.54	13.04	8.70	250.54	3.44
CRUSTACEANS	Decapoda	45.71	15.34	67.39	44.93	4114.55	56.55
	Euphausiacea	15.24	1.89	8.70	5.80	148.93	2.05
	N.i. Crust	5.71	2.53	6.52	4.35	53.79	0.74
FISH	Pleuronectiformes	0.95	2.20	2.17	1.45	6.85	0.09
	Stomiiformes	0.95	1.09	2.17	1.45	4.45	0.06
	Gadiformes	1.90	0.17	4.35	2.90	9.03	0.12
	N.i. Fish	15.24	64.10	32.61	21.74	2587.19	35.56

ANNEX 21: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of immature females of *E. spinax.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	1.33	3.17	3.33	1.85	15.02	0.17
	Oegopsida	6.67	10.86	16.67	9.26	292.07	3.28
	N.i. Ceph	12.00	10.21	30.00	16.67	666.37	7.48
CRUSTACEANS	Decapoda	52.00	33.32	70.00	38.89	5972.71	67.06
	Euphausiacea	-	-	-	-	-	-
	N.i. Crust	9.33	3.65	16.67	9.26	216.47	2.43
FISH	Pleuronectiformes	2.67	1.24	6.67	3.70	26.02	0.29
	Stomiiformes	1.33	0.91	3.33	1.85	7.49	0.08
	Gadiformes	-	-	-	-	-	-
	N.i. Fish	14.67	36.63	33.33	18.52	1709.89	19.20

ANNEX 22: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of mature females of *E. spinax.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	2.08	20.01	9.52	5.00	210.42	2.60
	Oegopsida	5.21	6.72	14.29	7.50	170.43	2.10
	N.i. Ceph	2.08	1.63	9.52	5.00	35.38	0.44
CRUSTACEANS	Decapoda	37.50	31.86	85.71	45.00	5945.24	73.43
	Euphausiacea	34.38	4.24	9.52	5.00	367.72	4.54
	N.i. Crust	8.33	10.43	19.05	10.00	357.32	4.41
FISH	Pleuronectiformes	1.04	9.26	4.76	2.50	49.06	0.61
	Stomiiformes	-	-	-	-	-	-
	Gadiformes	-	-	-	-	-	-
	N.i. Fish	9.38	15.85	38.10	20.00	961.06	11.87

ANNEX 23: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of immature males of *E. spinax.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	-	-	-	-	-	-
	Oegopsida	10.34	0.78	17.39	12.90	193.40	2.18
	N.i. Ceph	6.90	3.97	13.04	9.68	141.68	1.59
CRUSTACEANS	Decapoda	50.00	49.14	78.26	58.06	7758.62	87.26
	Euphausiacea	20.69	9.31	8.70	6.45	260.87	2.93
	N.i. Crust	6.90	4.66	4.35	3.23	50.22	0.56
FISH	Pleuronectiformes	-	-	-	-	-	-
	Stomiiformes	-	-	-	-	-	-
	Gadiformes	-	-	-	-	-	-
	N.i. Fish	5.17	32.16	13.04	9.68	486.88	5.48

ANNEX 24: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of mature males of *E. spinax.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	-	-	-	-	-	-
	Oegopsida	4.26	0.03	8.70	5.26	37.23	0.44
	N.i. Ceph	6.38	13.86	13.04	7.89	264.09	3.14
CRUSTACEANS	Decapoda	40.43	10.13	56.52	34.21	2857.43	33.96
	Euphausiacea	8.51	0.74	8.70	5.26	80.48	0.96
	N.i. Crust	4.26	2.21	8.70	5.26	56.19	0.67
FISH	Pleuronectiformes	2.13	2.54	4.35	2.63	20.29	0.24
	Stomiiformes	2.13	1.26	4.35	2.63	14.74	0.18
	Gadiformes	4.26	0.20	8.70	5.26	38.74	0.46
	N.i. Fish	27.66	69.03	52.17	31.58	5044.59	59.96

ANNEX 25: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in *E. spinax* diet during hot season (spring and summer).

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	1.20	14.09	3.08	1.82	47.05	0.62
	Oegopsida	9.58	5.04	18.46	10.91	270.00	3.57
	N.i. Ceph	8.98	5.06	21.54	12.73	302.50	4.00
CRUSTACEANS	Decapoda	49.10	20.44	72.31	42.73	5028.51	66.48
	Euphausiacea	9.58	1.50	6.15	3.64	68.16	0.90
	N.i. Crust	6.59	2.65	10.77	6.36	99.52	1.32
FISH	Pleuronectiformes	0.60	6.52	1.54	0.91	10.95	0.14
	Stomiiformes	0.60	0.87	1.54	0.91	2.25	0.03
	Gadiformes	1.20	0.14	3.08	1.82	4.11	0.05
	N.i. Fish	12.57	43.68	30.77	18.18	1731.02	22.88

ANNEX 26: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in *E. spinax* diet during cold season (winter and autumn).

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	IRIc
CEPHALOPODS	Sepiolidae	0.92	1.29	3.13	1.89	6.89	0.09
	Oegopsida	1.83	4.16	6.25	3.77	37.46	0.49
	N.i. Ceph	2.75	11.35	9.38	5.66	132.22	1.72
CRUSTACEANS	Decapoda	37.61	30.46	71.88	43.40	4893.07	63.73
	Euphausiacea	30.28	3.57	6.25	3.77	211.53	2.76
	N.i. Crust	9.17	9.50	15.63	9.43	291.72	3.80
FISH	Pleuronectiformes	2.75	2.59	9.38	5.66	50.05	0.65
	Stomiiformes	0.92	0.37	3.13	1.89	4.03	0.05
	Gadiformes	-	-	-	-	-	-
	N.i. Fish	13.76	36.72	40.63	24.53	2050.68	26.71

ANNEX 27: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in *E. pusillus* diet.

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	%IRIc
CEPHALOPODS	Oegopsida	5.97	0.34	9.38	6.82	59.12	0.84
	N.i. Ceph	4.48	5.54	3.13	2.27	31.31	0.44
CRUSTACEANS	Decapoda	25.37	10.29	50.00	36.36	1783.29	25.28
	Euphausiacea	26.87	6.79	9.38	6.82	315.52	4.47
	N.i. Crust	7.46	0.98	12.50	9.09	105.58	1.50
FISH	Pleuronectiformes	1.49	0.05	3.13	2.27	4.81	0.07
	Gadiformes	2.99	0.14	3.13	2.27	9.78	0.14
	N.i. Fish	25.37	75.86	46.88	34.09	4745.48	67.27

ANNEX 28: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of female specimens of *E. pusillus.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	%IRIc
CEPHALOPODS	Oegopsida	12.90	0.61	42.86	21.43	579.35	7.46
	N.i. Ceph	3.23	4.30	14.29	7.14	107.57	1.38
CRUSTACEANS	Decapoda	9.68	0.35	42.86	21.43	429.80	5.53
	Euphausiacea	48.39	11.42	14.29	7.14	854.37	11.00
	N.i. Crust	3.23	0.26	14.29	7.14	49.85	0.64
FISH	Pleuronectiformes	-	-	-	-	-	-
	Gadiformes	6.45	0.26	14.29	7.14	95.93	1.23
	N.i. Fish	16.13	82.78	57.14	28.57	5652.19	72.75

ANNEX 29: Relative Measures of Prey Quantities (RMPQs), percent occurrence by number (%N), percent occurrence by weight (%W), percent frequency of occurrence (%FO), corrected occurrence frequency (%FOc) and Mixed Analysis as the relative importance index (%IRI) and corrected relative importance index (IRIc) of the different taxonomic orders of prey items found in the diet of male specimens of *E. pusillus.*

GROUP	ORDER	%N	%W	%FO	%FOc	IRI	%IRIc
CEPHALOPODS	Oegopsida	-	-	-	-	-	-
	N.i. Ceph	5.56	7.03	8.00	6.25	100.71	1.26
CRUSTACEANS	Decapoda	38.89	22.26	52.00	40.63	3179.92	39.94
	Euphausiacea	8.33	1.22	8.00	6.25	76.40	0.96
	N.i. Crust	11.11	1.85	12.00	9.38	155.54	1.95
FISH	Pleuronectiformes	2.78	0.11	4.00	3.13	11.53	0.14
	Gadiformes	-	-	-	-	-	-
	N.i. Fish	33.33	67.53	44.00	34.38	4438.00	55.74

ANNEX 30: Classification of prey by IRI values according to Rosecchi and Nouaze (1987) for *E. spinax*.

Prey items	IRI	Cumulative IRI	Rosecchi and Nouaze (1987) classification
SbO. Natantia	54.27	54.27	Preferential prey
N.i. Fish	32.27	86.54	Secondary prey
N.i. Cephalopods	4.48	91.03	Accessory prey
N.i. Crustaceans	2.93	93.96	Accessory prey
Pasiphaea sivado	2.63	96.59	Accessory prey
O. Euphausiacea	2.21	98.80	Accessory prey
Symphurus spp.	0.32	99.12	Accessory prey
Pterygioteuthis giardi	0.22	99.34	Accessory prey
O. Oegopsida	0.16	99.50	Accessory prey
Sepia orbignyana	0.14	99.64	Accessory prey
Phasiphaea spp.	0.08	99.72	Accessory prey
F. Sepiolidae	0.07	99.79	Accessory prey
F. Gonostomatidae	0.05	99.84	Accessory prey
Liocranchia reinhardti	0.04	99.88	Accessory prey
Ommastrephes bartramii	0.04	99.92	Accessory prey
Abraliopsis affinis	0.03	99.95	Accessory prey
F. Pandalidae	0.02	99.96	Accessory prey
Histioteuthis heteropsis	0.01	99.97	Accessory prey
Phycis blennoides	0.01	99.98	Accessory prey
Micromesistius poutassou	0.01	99.99	Accessory prey
Onychoteuthis banksii	0.01	99.99	Accessory prey
Arnoglossus spp.	0.01	100.00	Accessory prey

ANNEX 31: Classification of prey by IPO2 values for *E. spinax*.

Prey items	IPO2	Cumulative IPO2	Prey classification
SbO. Natantia	27.47	27.47	Preferential prey
N.i. Fish	27.37	54.83	Preferential prey
N.i. Cephalopods	8.52	63.36	Secondary prey
N.i. Crustaceans	6.73	70.09	Secondary prey
Pasiphaea sivado	6.54	76.63	Secondary prey
O. Euphausiacea	6.16	82.79	Accessory prey
Sepia orbignyana	3.33	86.12	Accessory prey
Symphurus spp.	2.91	89.03	Accessory prey
O. Oegopsida	2.21	91.24	Accessory prey
Pterygioteuthis giardi	1.62	92.86	Accessory prey
F. Sepiolidae	1.10	93.96	Accessory prey
Phasiphaea spp.	1.08	95.04	Accessory prey
F. Gonostomatidae	0.87	95.91	Accessory prey
Ommastrephes bartramii	0.72	96.64	Accessory prey
Liocranchia reinhardti	0.68	97.32	Accessory prey
Abraliopsis affinis	0.60	97.92	Accessory prey
F. Pandalidae	0.53	98.45	Accessory prey
Histioteuthis heteropsis	0.32	98.77	Accessory prey
Physcis blennoides	0.32	99.09	Accessory prey
Micromesistius poutassou	0.31	99.40	Accessory prey
Onychoteuthis banksii	0.30	99.70	Accessory prey
Arnoglossus spp.	0.30	100.00	Accessory prey

ANNEX 32: Classification of prey by IRI values to Rosecchi and Nouaze (1987) classification for *E. pusillus*.

Prey items	IRI	Cumulative IRI	Rosecchi and Nouaze (1987) classification
N.i. Fish	75.69	75.69	Preferential prey
SbO. Natantia	13.69	89.38	Secondary prey
O. Euphausiacea	5.12	94.49	Accessory prey
N.i. Ceph	1.72	96.21	Accessory prey
Pasiphaea sivado	1.59	97.80	Accessory prey
N.i. Crust	1.51	99.31	Accessory prey
Onychoteuthis bankssi	0.17	99.48	Accessory prey
Physcis blennoides	0.16	99.64	Accessory prey
Phasiphaea spp.	0.13	99.77	Accessory prey
O. Pleuronectiformes	0.08	99.85	Accessory prey
Liocranchia reinhardti	0.08	99.92	Accessory prey
Abraliopsis affinis	0.08	100.00	Accessory prey

ANNEX 33: Classification of prey by IPO2 values for *E. pusillus.*

Prey items	IPO2	Cumulative IPO2	Prey classification
N.i. Fish	50.12	50.12	Preferential prey
SbO. Natantia	14.36	64.48	Secondary prey
O. Euphausiacea	11.29	75.77	Secondary prey
Pasiphaea sivado	5.88	81.65	Accessory prey
N.i. Ceph	5.62	87.27	Accessory prey
N.i. Crust	5.00	92.26	Accessory prey
Phasiphaea spp.	1.54	93.81	Accessory prey
Onychoteuthis bankssi	1.52	95.33	Accessory prey
Physcis blennoides	1.46	96.79	Accessory prey
O. Pleuronectiformes	1.08	97.87	Accessory prey
Liocranchia reinhardti	1.07	98.93	Accessory prey
Abraliopsis affinis	1.07	100.00	Accessory prey