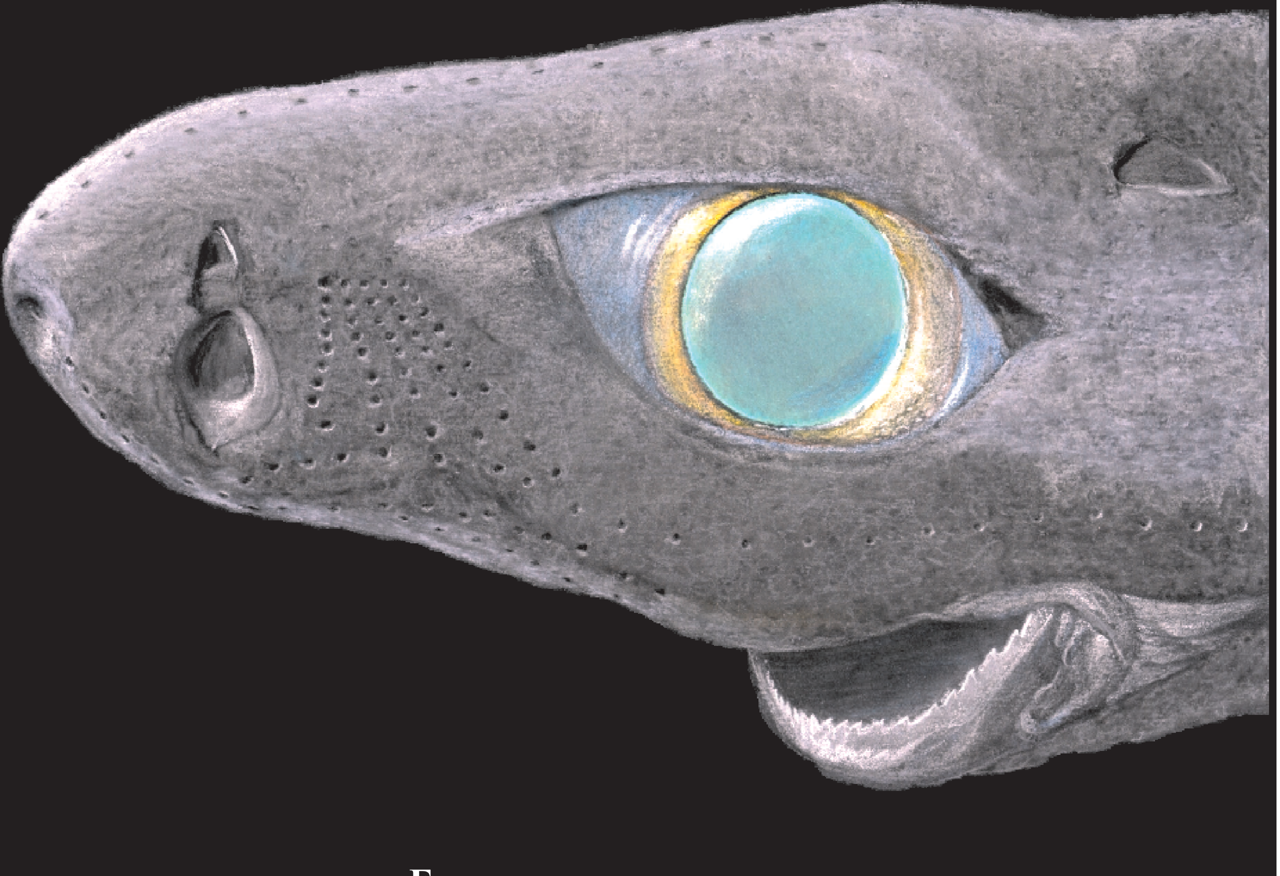
UNIVERSIDADE do ALGARVE
Faculdade de Ciências do Mar e do Ambiente

Biology, population dynamics, management and conservation of deep water lantern sharks, *Etmopterus spinax* and *Etmopterus pusillus* (Chondrichthyes: Etmopteridae) in southern Portugal (northeast Atlantic).

(Doutoramento em Ciências e Tecnologias das Pescas, especialidade de Biologia Pesqueira)

(Thesis for the degree in Doctor of Philosophy in Fisheries Sciences and Technologies, specialty in Fisheries Biology)

RUI PEDRO ANDRADE COELHO



Faro

(2007)

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FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE

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FARO
(2007)

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DATE: November 2007
THESIS TITLE: Biology, population dynamics, management and conservation of deep water lantern sharks, *Etmopterus spinax* and *Etmopterus pusillus* (Chondrichthyes: Etmopteridae) in southern Portugal (northeast Atlantic).

Abstract

Deep water sharks are particularly vulnerable to fishing mortality with population collapses occurring even at low levels of fishing. Two lantern sharks species, *Etmopterus spinax* and *E. pusillus,* are commonly caught as by-catch in commercial fisheries that operate in southern Portuguese waters. Little is known about the fisheries biology of these two species that have low or null commercial value and are usually discarded. The present study focused on several aspects of these two species, including fisheries, morphology, life history, distribution patterns and population dynamics. Commercial fisheries are having an impact on these species, with different gears affecting different components of the populations, as well as critical areas such as mating and nursery areas. Multivariate analysis was used to efficiently separate these two very similar and easily mistakable species, and models were created for future studies to more easily discriminate these species. Life history parameters, specifically age, growth, fecundity, maturity and reproductive seasonality, were determined and incorporated in mortality and demographic models. The results indicate that the current fishing pressures in the south of Portugal are not sustainable and the populations are declining. Urgent conservation measurements are therefore needed.

Key-words: deep-water; fisheries; life-history; management; population dynamics; sharks.

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ORIENTADOR: Prof. Dr. Karim Erzini

DATA: November de 2007

TÍTULO DA TESE: Biologia, dinâmica populacional, gestão e conservação de tubarões lanterna de profundidade, *Etmopterus spinax* e *Etmopterus pusillus* (Chondrichthyes: Etmopteridae), na costa sul de Portugal (Atlântico nordeste).

Resumo

Os tubarões de profundidade são especialmente susceptíveis à sobre-pesca, com colapsos populacionais a puderem ocorrer mesmo com níveis baixos de pesca. Duas espécies de tubarão lanterna são normalmente capturadas nas pescarias comerciais que operam na costa sul de Portugal, nomeadamente *Etmopterus spinax* e *E. pusillus*. Devido ao seu reduzido ou nulo valor comercial, estas espécies são normalmente rejeitadas, existindo assim uma lacuna nos conhecimentos relativos à sua pesca e biologia. O presente estudo focou aspectos relativos à pesca, morfologia, ciclo de vida, distribuição e dinâmica populacional destas espécies. As pescarias comerciais estão a ter um impacto significativo nestas espécies, com diferentes artes a afectarem de modo diferente as várias componentes das populações, incluindo áreas criticas como zonas de acasalamento e de nascimento. Pela análise multi-variada foi possível separar estas espécies muito semelhantes, sendo criado um modelo que poderá ser utilizado em estudos futuros para mais facilmente separar as espécies. Os parâmetros do ciclo de vida, especificamente idade, crescimento, fecundidade, maturidade e sazonalidade reprodutiva foram estimados e incorporados em modelos de mortalidade e de análise demográfica. Os resultados indicam que a pressão pesqueira actual na costa sul de Portugal não é sustentável e que as populações estão em declínio. Medidas urgentes de conservação são necessárias.

Palavras-chave: ciclo de vida; dinâmica populacional; gestão; pescas; águas profundas; tubarões.

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CHAPTER 1: General introduction

(Sampling deep water sharks aboard the research vessel "Cornide de Saavedra" in the Mediterranean Sea)

1.1. General characteristics of elasmobranch fishes

Chondrichthyan fishes are probably some of the most successful of all fishes, having survived and evolved for the last 400 million years, and surviving through major mass extinction episodes (Compagno, 2001). This group seems to have first appeared during the Devonian, with the earliest unquestionable evidence in the fossil record dating from this Period (409 – 363 million years ago (mya)), while the modern chondrichthyans living today derived from the forms present in the Mesozoic Period (245 – 65 mya) (Grogan and Lund, 2004).

Chondrichthyan fishes are characterized by an internal skeleton formed by flexible cartilage, without true bone present in their skeletons, fins or scales. Unlike the jawless lampreys, that also have cartilage skeletons, chondrichthyan fishes have true jaws and nostrils below their heads (Compagno *et al.*, 2005). Other characteristic that further separates chondrichthyans from all other fish groups is the presence of claspers in males (secondary sexual organs used to inseminate females) that are formed by the mineralization of the endoskeletal tissue along with the modification of the pelvic fins (Grogan and Lund, 2004).

It is accepted that the class Chondrichthyes is a monophyletic group (Compagno *et al.*, 2005) that is divided into two sister taxa: the subclass Elasmobranchii, that groups sharks and rays and the subclass Holocephali that groups the chimaeras (Table 1.1). The elasmobranchs are easily recognized from their multiple (5 to 7) paired gill openings on the sides of the head, while the holocephalans have a soft gill cover with just a single opening on each side of the head that protects the 4 pairs of gill openings (Compagno *et al.*, 2005).

Table 1.1. Extant orders of the class Chondrichthyes, according to Compagno (2001) and Compagno *et al.* (2005).

Subclass	**Superorder**	**Order**	**Common name**
Holocephali		Chimaeriformes	Chimaeras
Elasmobranchii	Squalomorphii	Hexanchiformes	Cow and frilled sharks
		Squaliformes	Dogfish sharks
		Squatiniformes	Angel sharks
		Pristiophoriformes	Saw sharks
		Rajiformes	Batoids
	Galeomorphii	Heterodontiformes	Bullhead sharks
		Orectolobiformes	Carpet sharks
		Lamniformes	Mackerel sharks
		Carcharhiniformes	Ground sharks

Including the species currently described, there are around 1180 chondrichthyan species, including 480 species of sharks, 650 species of batoids and 50 species of chimaeras, but the taxonomic resolution in chondrichthyans is low and problematic in many cases, with many known species still waiting for a formal description while others are in doubt regarding their validity (B. Séret, pers. comm.[1]). A characteristic example of such identification problems that still occur nowadays, even with abundant and well know species, is the case of the catcharks of the *Galeus* genus in the NE Atlantic and the Mediterranean, one of the most abundant elasmobranch groups in the continental shelves and slopes of that area, and that up until recently have been misidentified as belonging to only one species, *Galeus melastomus*. Muñoz-Chápuli and Ortega (1985) recently suggested that there were in fact two very

---

[1] B. Séret: Muséum National d'Histoire Naturelle, Département Systématique et Evolution, Paris, France.

similar species being grouped together (*G. melastomus* and *G. atlanticus*), which was corroborated by Rey *et al.* (2006) and finally resolved by Castilho *et al.* (2007) through genetic analysis.

Chondrichthyan fishes occupy a wide range of habitat types, including freshwater rivers and lake systems, inshore estuaries and lagoons, coastal waters, the open sea, and the deep ocean. Although sharks are generally thought of as wide-ranging, only a few (including some commercially important species) make oceanic migrations. Overall, some 5% of chondrichthyan species are oceanic (found offshore and migrating across ocean basins), 50% occur in shelf waters down to 200 m depth, 35% are found in deeper waters, from 200 to 2000 m, 5% occur in fresh water, and 5% have been recorded from several of these habitats (Camhi *et al.*, 1998).

Although it is strictly inaccurate, it is commonly accepted to use the general term “elasmobranch” to refer to all chondrichthyan fishes, including sharks, rays and chimaeras. This type of generalization has been used both in the scientific literature (e.g. Bonfil, 1994) and also by international organizations such as the International Council for the Exploration of the Sea (ICES) (e.g. ICES, 2003; 2004; 2005; 2006) or The World Conservation Union (IUCN) (e.g. Fowler *et al.*, 2005). The same generalization was used in this study, where the general term “elasmobranch” often refers to all chondrichthyan fishes.

1.2. The vulnerability of elasmobranch fishes to exploitation, with comments on deep water species

In recent years, elasmobranch fishes have become important fisheries resources, with a substantial increase in fishing effort worldwide (Vannuccini, 1999; Barker and Schluessel, 2005). However, traditionally, elasmobranch fishes have not been highly priced products, and their economic value ranks low among other marine commercial fisheries species (Bonfil, 1994). The most notable exception to this general low commercial value are the fins of some species, which are marketed at extremely high prices in oriental markets for shark fin soup (Fowler *et al.*, 2004).

The exploitation of elasmobranch resources has been attributed not only to fisheries specifically targeting elasmobranchs (e.g. Campbell *et al.*, 1992; Castillo-Geniz *et al.*, 1998; Chiaramonte, 1998; Francis, 1998; Holts *et al.*, 1998; Hurley, 1998; McVean *et al.*, 2006; Bizzarro *et al.*, 2007) but perhaps more importantly due to the by-catch of fisheries targeting other species (e.g. Stevens, 1992; Amorim *et al.*, 1998; Buencuerpo *et al.*, 1998; McKinnell and Seki, 1998; Van Der Molen *et al.*, 1998; Francis *et al.*, 2001; Stobutzki *et al.*, 2002; Beerkircher *et al.*, 2003; Carbonell *et al.*, 2003; Cedrola *et al.*, 2005; Megalofonou *et al.*, 2005; Zeeberg *et al.*, 2006). Game fishing also has some impact on elasmobranch fishes, especially on the large pelagic species (e.g. Stevens, 1984; Pepperell, 1992; Campana *et al.*, 2006b).

Most elasmobranch fishes are predators at, or near, the top of the marine food chains (Cortés, 1999). These animals are extremely important for the entire ecosystems balance, by regulating not only their direct main preys, but also second and third degree non-prey species through trophic linkages (Schindler *et al.*, 2002). The effect of the removal of such predators from the marine ecosystems is difficult to foresee, but may be ecologically and economically significant, and may persist over long time periods (Stevens *et al.*, 2000).

Even though there are fisheries directly targeting elasmobranchs, and many where they are caught as by-catch, there is still limited information about these fishes' life cycles and biological parameters. Elasmobranch fishes in general have K-strategy life cycles characterized by slow growth rates (e.g. Francis and Mulligan, 1998;

Francis and Ó-Maolagáin, 2000; Coelho and Erzini, 2002; Conrath *et al.*, 2002; Driggers *et al.*, 2004; Carlson and Baremore, 2005; Campana *et al.*, 2006a; Francis *et al.*, 2007) and reduced progeny (e.g. Guallart and Vicent, 2001; Hazin *et al.*, 2001; Jones and Ugland, 2001; Jensen *et al.*, 2002) with maturity occurring late in their life cycle (e.g. Pratt and Casey, 1990; Oddone and Velasco, 2004; Capape *et al.*, 2005; Coelho and Erzini, 2005; Chen and Liu, 2006; Coelho and Erzini, 2006; Hazin *et al.*, 2006). This low fecundity and relatively high survival rate of newborns suggests that there is a strong relationship between the number of mature females in a population and the new recruits for the next cohort, meaning that the success of the future generation is mainly dependant on the present mature population abundance (Ellis *et al.*, 2005).

These life cycle characteristics make these fishes extremely vulnerable to fisheries mortality, with overexploitation occurring even with relatively low levels of fishing (Smith *et al.*, 1998; Baum *et al.*, 2003). Once these populations start to decline, it can take several decades before recovery can take place (Stevens *et al.*, 2000). Bonfil (1994) and Shotton (1999) provided reviews of world elasmobranch fisheries and have included examples of situations where commercial catches have been declining, such as in the northeast Atlantic (Pawson and Vince, 1999) and in Japan (Nakano, 1999), and examples of situations of possible concern, such as in India (Hanfee, 1999). Baum *et al.* (2003) stated that the northwest populations of large pelagic sharks including the scalloped hammerhead, *Sphyrna lewini*, and the thresher sharks, *Alopias vulpinus* and *A. superciliousus*, have declined by more than 75% over the last 15 years. Although the values presented by Baum *et al.* (2003) seem to be overestimated (Burgess *et al.*, 2005), there is no doubt that there are causes for concern.

Even though all elasmobranch fishes are facing possible threats of population declines in the near future, some groups are considered to be particularly vulnerable, and have raised particular concern amongst scientists. One of these elasmobranch groups that is believed to be amongst the most vulnerable of all elasmobranchs are deep water sharks (Fowler *et al.*, 2005). In fact these species have life cycle characteristics than make then even more vulnerable than most other elasmobranchs, and because of this, the ICES Working Group on Elasmobranch Fishes has been recommending a zero catch policy for deep water elasmobranchs since 2005 (ICES, 2005, 2006). Examples of extreme life cycles characteristics of deep water sharks include the case of the gulper shark, *Centrophorus granulosus*, that has a fecundity of one descendent in each two year pregnancy cycle (Guallart and Vicent, 2001), or the case of the leafscale gulper shark, *Centrophorus squamosus*, that lives for at least 70 years and matures at more than 30 years of age (Clarke *et al.*, 2002). An example of a deep water squalid shark that has declined severely due to excessive fishing, is the picked dogfish, *Squalus acanthias*, whose northeastern population has been depleted to about 5% of the virgin biomass (Hammond and Ellis, 2005).

1.3. The status of worldwide elasmobranchs, with comments on Portuguese fisheries

While worldwide general marine fisheries landings seem to have reached a plateau in the late 1980's, elasmobranch catches have been increasing progressively since the 1950's, with decreases occurring only in the last few years, specifically 2004 and 2005 (Figure 1.1). However, worldwide marine fisheries have shifted in these last decades from catching long lived high trophic level fishes toward catching mainly short lived, low trophic level invertebrates and planktivorous pelagic fish (Pauly *et al.*, 1998; Pauly and Palomares, 2005). This effect, originally called “fishing down the food web” by Pauly *et al.* (1998) shows that the marine ecosystems top predators are the first ones to suffer from overfishing, with unsustainable exploitation patterns occurring presently. The fisheries, in order to compensate for the faster declines of these top predators, have to rely increasingly on fish and invertebrates that are of lower trophic levels.

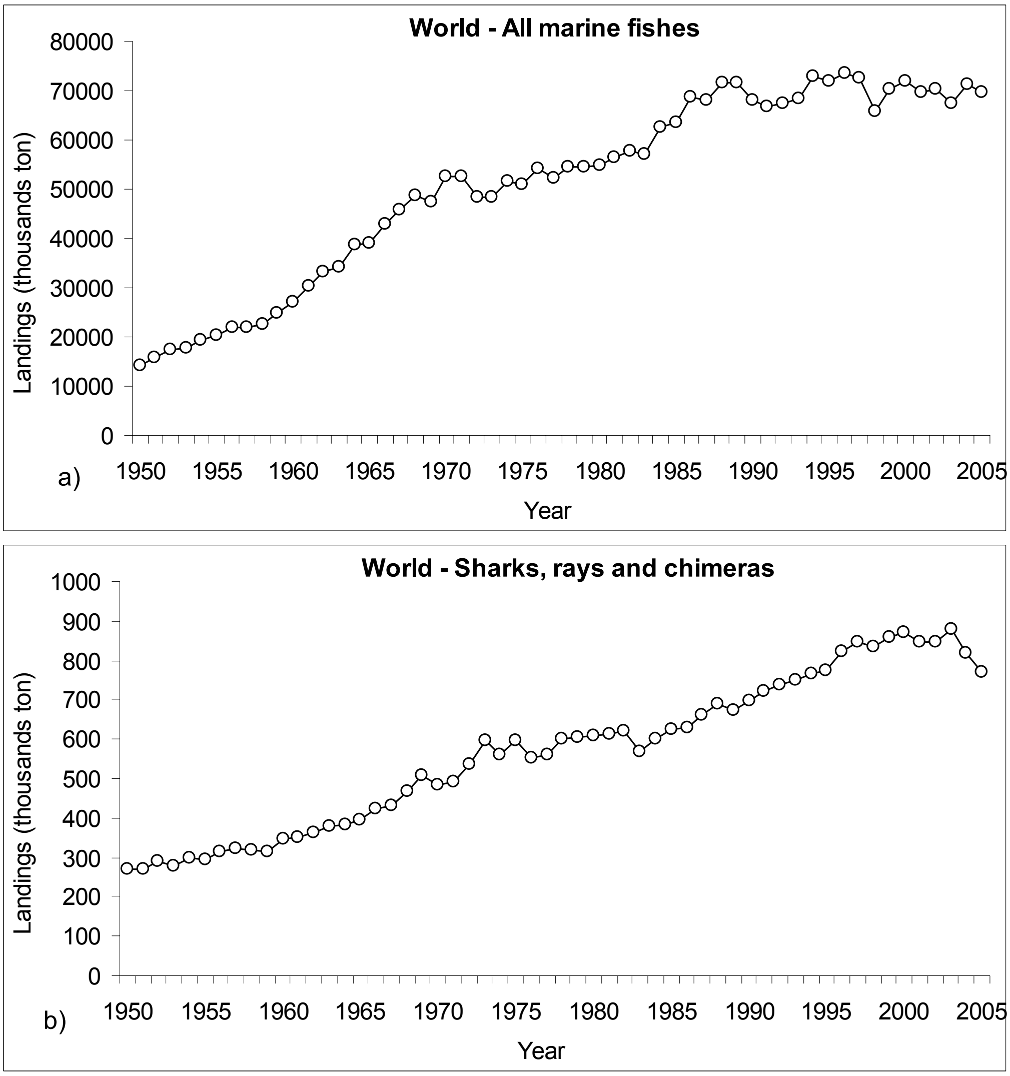

Figure 1.1. World landings of all marine fishes (a) and sharks, rays and chimeras (b), from 1950 to 2005. Data from FAO FIGIS data collection (FAO, 2007).

Up until the 1980's, elasmobranch fisheries were generally unimportant, small fisheries, with very little commercial value worldwide. Traditionally, these elasmobranch fisheries of the past were multi-specific fisheries that caught several species of elasmobranchs depending on the region and the season of the year. There was little or no interest in these fisheries, mainly due to their relatively small size and the low commercial value of elasmobranchs. Bonfil (1994) reported that cartilaginous fishes were a minor group which contributed with an average of 0.8% of the total world fishery landings between 1947 and 1985, while bony fishes such as clupeoids, gadoids and scombroids, accounted for 24.6%, 13.9% and 6.5%, respectively. In the last decades however, the declining catches per unit effort (CPUE) and rising prices of traditional food fishes, along with the growing market for shark fins for oriental markets, have made the previously under utilized elasmobranchs increasingly important resources. During the 1980's, elasmobranch fisheries throughout the world were growing at a rapid pace and by the mid 1990's, the high value of dry shark fins provided sufficient incentive to harvest sharks, even when the meat was not marketable (Castro *et al.*, 1999).

The history of elasmobranch fisheries worldwide indicates, however, that these resources are usually not sustainable. Most elasmobranch targeted fisheries have been characterized by a "boom and burst" scenario, where an initial rapid increase of the exploitation and catches is followed by a rapid decline in catch rates and eventually a complete collapse of the fishery (Stevens *et al.*, 2000). This situation seems to be particularly common in deep water species, including not only elasmobranchs but also the bony fishes (Haedrich *et al.*, 2001). However, and even though overexploitation and population collapses is the most common scenario in elasmobranch fisheries, Walker (1998) demonstrated that elasmobranch stocks of some species can be harvested sustainably and provide for stable fisheries when carefully managed. Some species such as the tope shark, *Galeorhinus galeus*, the sandbar shark, *Carcharhinus plumbeus*, the great white shark, *Carcharodon carcharias* and several species of dogfishes (order Squaliformes) have low productivity and cannot withstand high levels of fishing, whereas other species such as the gummy shark, *Mustelus antarcticus*, the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, the bonnethead, *Sphyrna tiburo* and the blue shark, *Prionace glauca* have higher productivity and can support higher levels of fishing mortality (Walker, 1998).

A huge problem with many current fisheries that is generally not reflected in landings databases is by-catch and especially discards (Zeller and Pauly, 2005). Most fisheries operating around the world catch elasmobranchs as by-catch and although some species are retained and utilized in some of these fisheries, others are discarded, sometimes after their fins have been removed. The survival of discarded elasmobranchs may vary depending on the species, the type of gear used and the depth where they have been caught, but there is a consensus that trawls, gill nets and purse seines cause high mortalities while longlines may allow for better survival rates (Bonfil, 1994). The by-catches and discards of elasmobranchs in large scale fisheries around the world are large and the associated unreported catches may represent as much as 50% of the total reported elasmobranch catch (Bonfil, 1994; Stevens *et al.*, 2000).

In Portugal, landings of marine fishes from 1950 to 2005 were characterized by a peak during the 1960's and a smaller peak in the mid 1980's, but in general have been deceasing (Figure 1.2). Specifically, total Portuguese landings peaked at 560 thousand ton in 1964 and decreased by some 66% down to 188 thousand ton in 2005. On the other hand, elasmobranch fisheries remained relatively unimportant until 1990, when substantial increases were reported for a few years, but have decreased sharply since then (Figure 1.2). However, the relative importance of elasmobranch fisheries has been increasing substantially: the elasmobranch landings represented on average 1.0% of all Portuguese marine fish landings between 1950 and 1989 but this value increased in recent years with elasmobranch landings between 1990 to 2005 representing an average of 7.4% of all marine fish landings.

Elasmobranch fisheries in Portugal are not regulated, and thus there are no established minimum landing sizes or maximum catch quota limits for any species. According to Correia and Smith (2003), current Portuguese elasmobranch landings come mainly from five distinct fisheries, 1) targeted deep-sea elasmobranch longlining, 2) targeted pelagic elasmobranch surface longlining; 3) by-catch of deep water elasmobranchs from deep sea longlining; 4) by-catch of pelagic elasmobranchs from teleost gill-netting, purse seining, and bottom trawling, and 5) by-catch of deep water elasmobranchs from crustacean bottom trawling.

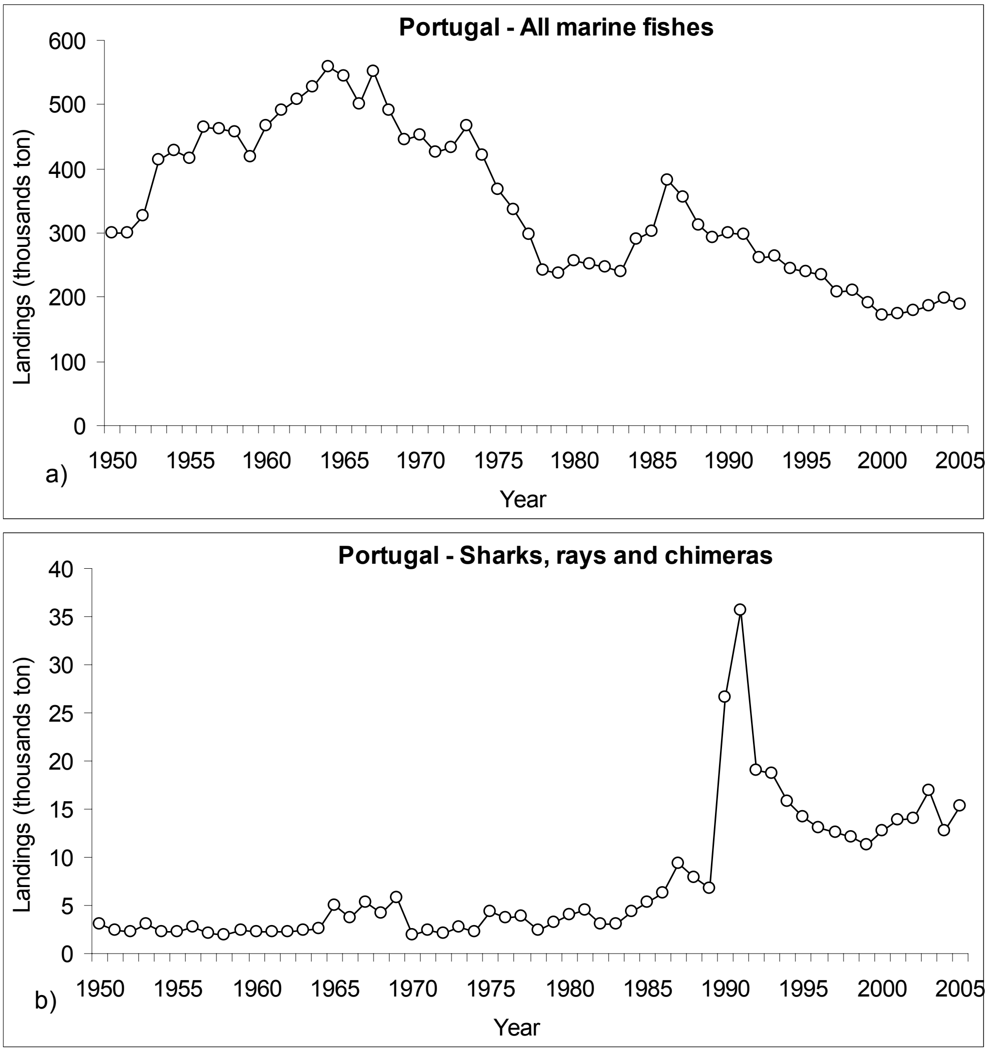

Figure 1.2. Portuguese landings of all marine fishes (a) and sharks, rays and chimeras (b), from 1950 to 2005. Data from FAO FIGIS data collection (FAO, 2007).

The Algarve region does not differ much from the national scenario, with records from 1988 to 2005 showing a trend of a general decline in landings in this region. Specifically, landings of elasmobranchs in 1988 in this region peaked at 888 ton and declined by some 38.1% to 550 ton in 2005 (Figure 1.3). On the other hand the commercial value of these resources has been increasing progressively. The average value of elasmobranchs sold at auction in the Algarve increased by 133% from 1988 (0.81 Euros per Kg) to 2005 (1.90 Euros per Kg) (Figure 1.3).

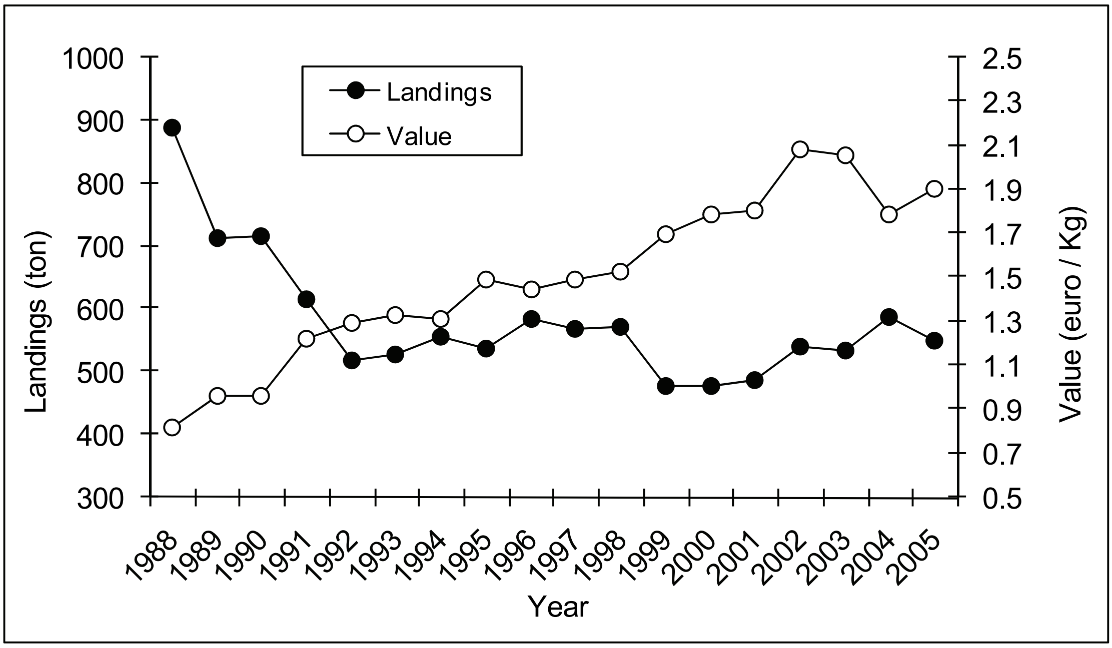

Figure 1.3. Landings and average value of elasmobranchs landed in the Algarve region from 1988 to 2005. Data from DGPA (GEPP, 1988; 1989; 1990; 1991; 1992; 1993; DGP, 1994; 1995; 1996; DGPA, 1997a; b; 1999; 2000; 2001; 2002; 2003; 2004; 2005; 2006)

One of the major problems with these large statistical databases is that data is not usually specified down to species level, and there is no way to discriminate between species, with the analysis usually having to be performed at higher taxonomic levels such as genus or even family. Most deep water species are even more problematic, given that due to their low or even null commercial value they are often discarded at sea and there is no way to account for fishing mortality in these landings statistics. That is the case of most catches of lantern sharks worldwide, where due to the small size of most species, specimens are usually discarded. Nonetheless, the FAO databases (FAO, 2007) does have two categories to list lantern sharks: the general “lanternshark NEI” group that refers to *Etmopterus* spp. and the “velvet belly (ETX)” group that refers specifically to *Etmopterus spinax*. Up until 1995 no recordings appeared for either of these categories, but from 1995 to 2005 some catches were recorded. Specifically, between those years, there were 953 ton of

Etmopterus spp. landed worldwide, of which 871 ton were recorded in Europe. The 75 ton of *E. spinax* landed in Europe accounted for the entire catch reported worldwide for this species (Figure 1.4). Portugal does not have records in any of these two categories.

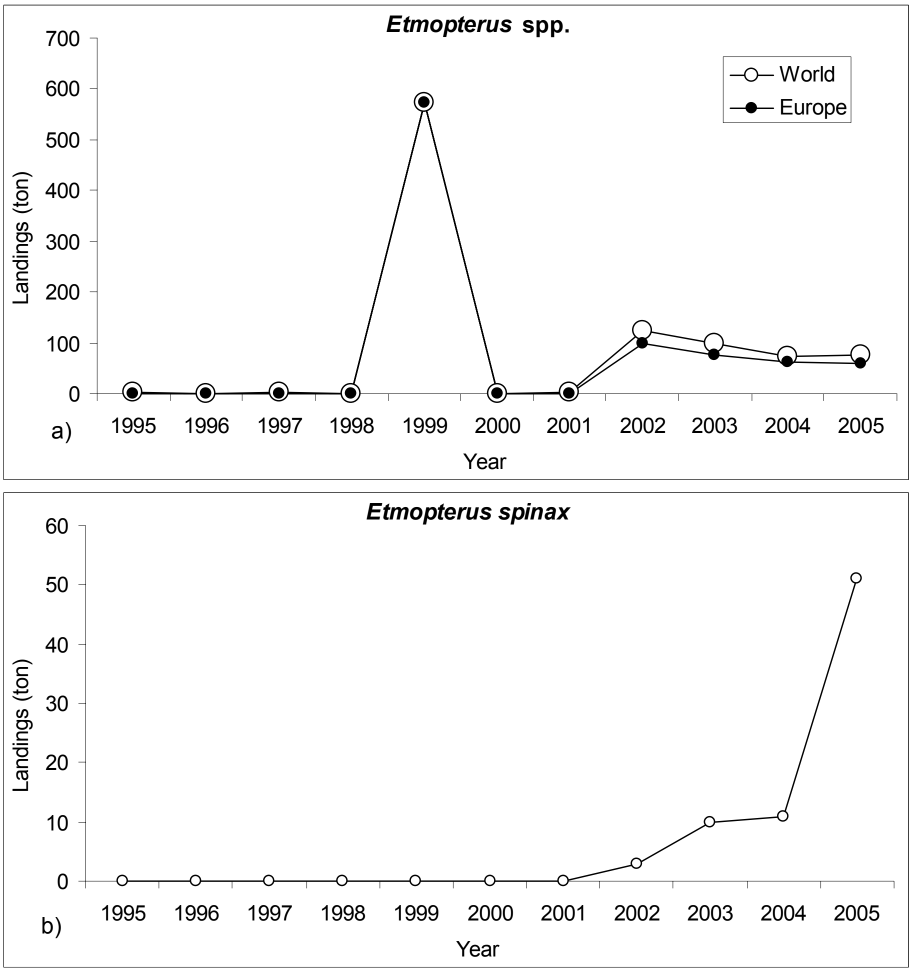

Figure 1.4. *Etmopterus* spp. (a) and *Etmopterus spinax* (b) worldwide and European landings from 1995 to 2005. On *E. spinax*, the world and European landings are the same. Data from FAO FIGIS data collection (FAO, 2007).

The rapid growth in the size and value of elasmobranch fisheries in the last few decades, the alarming increase of elasmobranch by-catch of other fisheries and the fact that we now know that these resources are highly vulnerable to overfishing, has created a worldwide concern and the management and conservation of these resources is now a clear objective.

In 1999, the FAO developed the International Plan of Action for the Conservation and Management of Sharks (IPOA Sharks) (FAO, 1999). This tool was a voluntary instrument that applied to all States where elasmobranch fisheries take place and that set out guidelines that the implementing States were encouraged and expected to carry out in order to protect and manage these resources. Such measurements included highly general goals such as making sure that elasmobranch catches were sustainable, and other more direct goals such as monitoring elasmobranch catches, encouraging the full use of dead elasmobranch and reporting species specific biological and trade data. So far only a few countries have effectively developed their respective National Plans of Action and even fewer are implementing them, so the progress made is clearly insufficient (Fowler *et al.*, 2004).

On the other hand, the Shark Specialist Group (SSG) of the World Conservation Union (IUCN) has, since 1991 when it was first established, been making a huge effort to assess all elasmobranch species described worldwide, in order to include them in the IUCN Red List of Threatened Species (IUCN, 2006a). This list is widely recognized as the most comprehensive source of information on the global conservation status of plant and animal species and can therefore be used as a tool for measuring and monitoring changes in the status of elasmobranch biodiversity. The assessments presented there are an essential baseline which evaluates the conservation status of individual species, identifies threats affecting them and if necessary, proposes recovery objectives. Up until the end of 2006, 547 chondrichthyan species had been assessed under the IUCN Red List criteria (IUCN, 2001), of which some 20% were listed under one of the threatened categories (IUCN, 2006b).

These IUCN SSG assessments have also been used to advise on the inclusion of elasmobranchs in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The CITES has been playing an important role against over exploitation of wild fauna and flora species by regulating international trade. The species can be listed in one of three different Appendices: Appendix I lists the most threatened species and international trade is entirely prohibited unless exceptional circumstances are demonstrated (e.g. for scientific research); Appendix II lists species where trade may be authorized if it is demonstrated that it will not be detrimental to the survival of the species in the wild and Appendix III list species already regulated by one or some countries and that need cooperation of other countries to prevent unsustainable or illegal exploitation. As a consequence of the assessments that have been carried out by the IUCN SSG, three chondrichthyan species, namely the whale shark, *Rhincodon typus*, the basking shark, *Cetorhinus maximus* and the great white shark have already been included in CITES under Appendix II, and two more species namely the piked dogfish and the porbeagle, *Lamna nasus*, and an entire family (the sawfishes, family Pristidae) have been proposed to be included this year during the 2007 Conference of the Parties (CoP 14) (Fowler *et al.*, 2004; Lack, 2006).

1.4. Sharks fisheries products

Elasmobranch fishes provide multiple products that are commercialized for different usages. The most common products taken from these animals include meat, fins, liver, skin, cartilage, and jaws (Musick, 2004).

Even though the study of elasmobranch remains in archeological sites is problematic (Rick *et al.*, 2002), it seems that shark meat has been used as food in coastal regions for at least 5000 years, when the Cretans and the Persians caught and sold sharks in the Persian Gulf and the Mediterranean (Vannuccini, 1999). A major problem when it comes to consumption of shark meat is that sharks retain urea in their blood and tissues as part of their osmoregulatory physiology (Evans *et al.*, 2004), and when the shark dies the urea breaks down into ammonia which releases a strong smell from the flesh, which may even be toxic in high concentrations (Musick, 2004). Since the late 1950's, shark meat started to be more favorably accepted, mainly due to the better handling associated with the use of ice and freezing for processing the flesh. Nowadays, the flesh of some species such as the threshers (*Alopias* spp.) the shortfin mako (*Isurus oxyrinchus*) and the porbeagle, whose meat is particularly appreciated fresh in the USA and Europe can reach relatively high prices, similar to swordfish (Vannuccini, 1999).

While the livers of deep water squalid sharks are usually kept and commercialized, the rest of the animal has a low or null commercial value and is usually discarded. Some exceptions do however occur, such as the case of the flesh of *C. squamosus* and the Portuguese dogfish, *Centroscymnus coelolepis* that in France are commercialized under the generic common name "siki" and whose landings in recent years have been increasing (Girard *et al.*, 2000).

The most valuable product obtained from sharks, that is in fact one of the most valuable fish products in the world are the fins of some species (Camhi *et al.*, 1998) that are marketed in the oriental markets, especially Hong Kong and Singapore, where they are used to make the traditional Chinese shark fin soup (Vannuccini, 1999). Even thought the fins of all shark species larger than 1.5m are commercially valuable, some species are considered excellent, namely the blue shark, the dusky shark (*Carcharhinus obscurus*), the giant guitarfishes (*Rhynchobatus* spp.), the hammerheads (*Sphyrna* spp.), the shortfin mako, the oceanic whitetip shark (*Carcharhinus longimanus*) and the sandbar shark (Vannuccini, 1999). In most sharks, the first dorsal, the pectorals and the lower lobe of the caudal fins are the most valuable and these are usually sold as a set from each shark (Musick, 2004). The high value leads to the slaughter of tens of millions of sharks every year for their fins, and once these structures are removed the rest of the carcasses is usually discarded overboard (Fordham, 2006). This finning practice represents a considerable waste of resources, as the fins on average make up only about 5% of the total weight of sharks (Vannuccini, 1999).

The liver of sharks, apart from being the organ for storing energy and conserving energy, is also the hydrostatic organ. In deep sea sharks, buoyancy is achieved by means of a large liver, which contains large quantities of uncommon low density oils, mainly squalene and diacyl glyceryl ether (DAGE) (Deprez *et al.*, 1990). Deep water sharks have long been caught for their livers, and more specifically for the oil extracted from them. One of the first commercial uses of this oil was during the XIX century when it was used as fuel for street lamps (Vannuccini, 1999). During the 2nd world war, squalid shark livers were an important source for vitamin A, but more recently this vitamin started to be artificially synthesized and no longer extracted from shark liver oil (Vannuccini, 1999). In the last decades, squalid shark livers started to be commercialized for the lipids present in high quantities in their livers, specifically the squalene and the DAGE (Deprez *et al.*, 1990). The relative weight of the liver in deep water squalid sharks can range from 15% to 26% of the total body weight (Hernández-Pérez *et al.*, 1997) and squalene typically represents 50 to 82% of the oil in the liver (Bakes and Nichols, 1995). Squalane ($C_{30}H_{50}$) is a colorless, odorless, tasteless, stable, transparent and inert oil that is readily miscible with the human skin lipids. Squalane aids the absorption of pharmaceuticals through the skin and reduces water loss, so it is an excellent cosmetic base, lubricant and emollient (Deprez *et al.*, 1990). Squalene has also been experimentally used for tumor growth inhibition, and it seems especially efficient when combined with conventional chemotherapeutic agents (Cho and Kim, 2002). DAGE seems to help reducing the severity of certain types of cancer, promote formation of blood cells, and provide protection against radiation injury (Wetherbee and Nichols, 2000).

Over the last decades there has been a false popular belief that shark cartilage, after been dried and pulverized into a powder can prevent and treat cancer. This controversy come from the false belief that elasmobranchs, with skeleton made of cartilage, do not develop cancer and that solid forms of shark cartilage may cure cancer in animals and humans (Gingras *et al.*, 2000). The market for shark cartilage pills expanded greatly in recent years, but there is now evidence that the ingestion of cartilage powder pills is worthless for treating cancer in humans (Gingras *et al.*, 2000; Cho and Kim, 2002). However, shark cartilage has been used to extract some biologically active substances, that have been shown to have antiangiogenic and antitumor properties, by retarding the development of blood vessels necessary for the development of the cancer, and that are being tested for the treatment of some forms of cancers (Gingras *et al.*, 2000; Cho and Kim, 2002).

1.5. The species studied

The order of Squaliformes sharks, commonly named dogfishes, are geographically widely distributed and occurs in a wide range of marine habitats and depth ranges in all oceans. However, the greatest diversity of these sharks occurs in deep waters, with many species occurring nowhere else (Compagno *et al.*, 2005). The definition of deep water elasmobranch varies between authors, with some defining deep water below the 200 m depth (Camhi *et al.*, 1998; Kyne and Simpfendorfer, 2007) and others defining it below depths of 400 m (Gordon, 1999). Either way, authors seem to agree that the pelagic species that occasionally go down to those depths should not be included in the deep water species definition and this definition is therefore restricted to demersal species that spend most of their life cycles in these deep waters.

Squaliformes sharks differ from the others by having two dorsal fins, lacking an anal fin, having a cylindrical body section and a ventral mouth, and with most species having dorsal spines. Worldwide, the order Squaliformes has at least 130 species, with some very recently described (Last *et al.*, 2007) and is divided in 7 families (Echinorhinidae, Squalidae, Centrophoridae, Etmopteridae, Somniosidae, Oxynotidae and Dalatiidae).

Among these, the family Etmopteridae, commonly named lantern sharks, is by far the most numerous, with at least 50 species divided in five genera (*Aculeola*, *Centroscyllium*, *Etmopterus*, *Miroscyllium* and *Trigonognathus*), and among these, the genus *Etmopterus* is the most numerous accounting for 31 species worldwide (Compagno *et al.*, 2005). Most of the species in this family occur in deep waters and many are endemic, although some are wide ranging. Lantern sharks often have light organs (photophores) on the underside and hence the common name lantern shark.

In Portugal, there are three species described for the *Etmopterus* genus: *E. spinax*, *E. pusillus* and *E. princeps*. However, even though *E. princeps* in the eastern Atlantic is known to occur from Greenland and Iceland to northwest Africa, this species has not been recorded by any recent studies that have surveyed the outer shelf and upper slopes of the southwest and southern Portuguese coasts (e.g. Erzini *et al.*, 1999; Borges *et al.*, 2001; Erzini *et al.*, 2001; Monteiro *et al.*, 2001; Coelho *et al.*, 2003; Coelho and Erzini, 2005; Coelho *et al.*, 2005). Therefore, this study focused on the other two species of lantern sharks that are commonly caught in the southern Portuguese continental shelf and upper slope, *E. spinax* and *E. pusillus*.

Biological data for these species is either scarce (*E. spinax*) or almost non-existent (*E. pusillus*). Furthermore, most literature mentioning these species comes from general elasmobranch distribution books and usually reports biological observations rather than population parameters (e.g. Compagno, 1984; Bauchot, 1987; Notarbartolo di Sciara and Bianchi, 1998; Hennemann, 2001; Compagno *et al.*, 2005; Serena, 2005). Biological data for other Etmopteridae sharks worldwide is also very scarce and little or nothing at all is known for most species (Kyne and Simpfendorfer, 2007).

1.5.1. Velvet belly lantern shark

The velvet belly lantern shark, *Etmopterus spinax*, is a small sized deep water lantern shark (order Squaliformes, family Etmopteridae) (Figure 1.5). It has been described for the eastern side of the Atlantic Ocean, from Iceland and Norway (Compagno *et al.*, 2005) to South Africa (Compagno, 1984), including the Azores (Santos *et al.*, 1997), the Canaries (Brito *et al.*, 2002) and the Cape Verde Islands (Reiner, 1996). This species also occurs in the western and central Mediterranean (Bauchot, 1987; Serena, 2005), including the Ionian, the lower Adriatic and Aegean Seas (Notarbartolo di Sciara and Bianchi, 1998) (Figure 1.6).

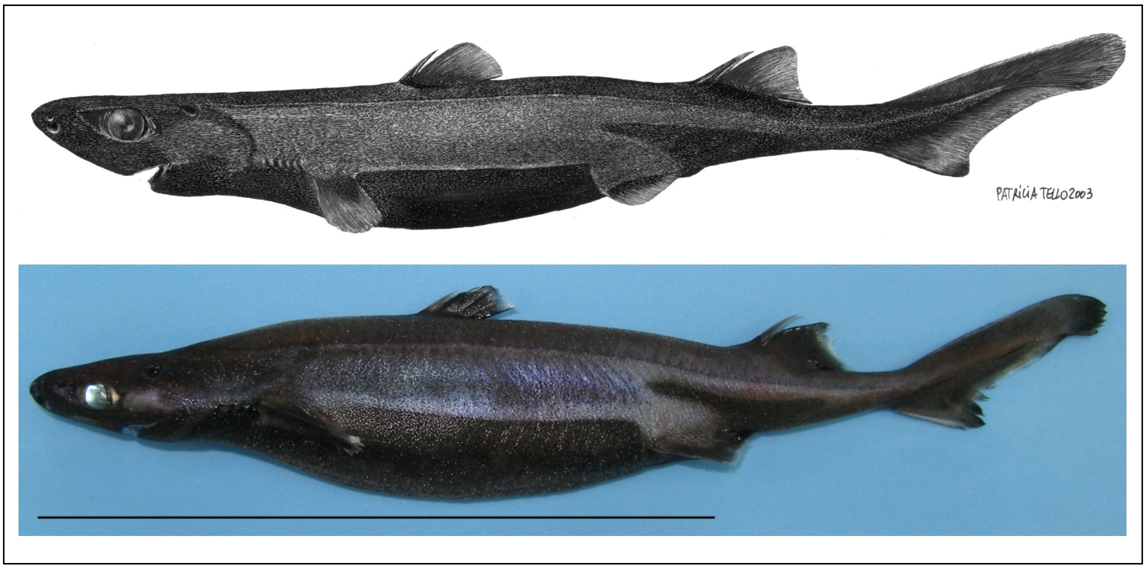

Figure 1.5. The velvet belly lantern shark, *Etmopterus spinax*, from the south coast of Portugal. Above is an original scientific drawing (reproduced with permission of the author Patrícia Tello) and below a photograph. The scale bar in the photograph represents 20 cm.

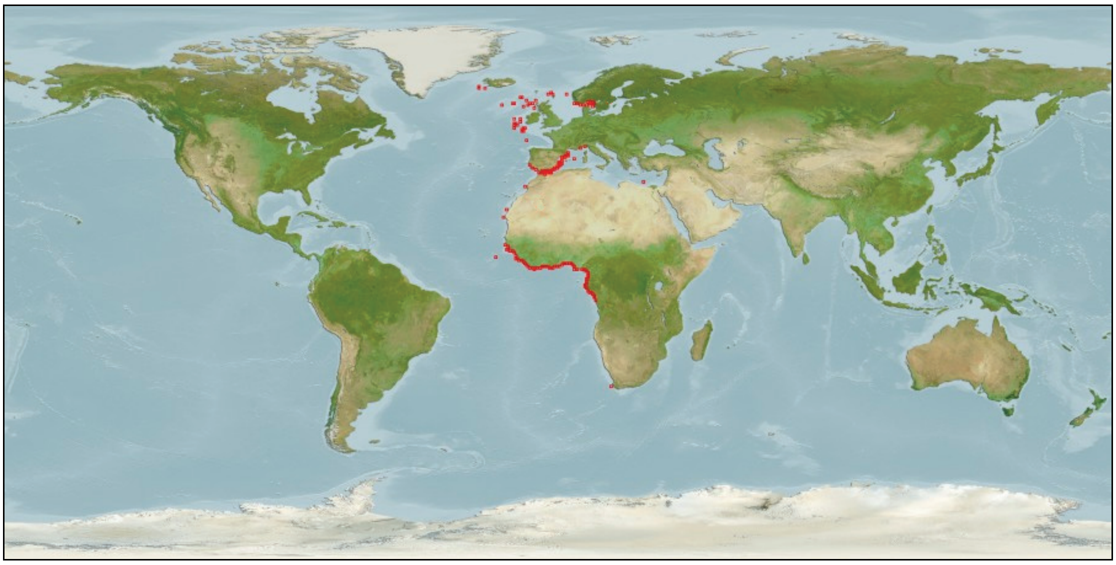

Figure 1.6. Geographical distribution of *Etmopterus spinax* (adapted from Fishbase (Froese and Pauly, 2006)).

E. spinax lives near the bottom mainly on the outer continental and insular shelves and upper slopes, at depths from 70 to 2000 m, but mostly between 200 and 500 m (Compagno *et al.*, 2005). In the Mediterranean (Catalan Sea) it has been found down to 1800 – 1900 m (Stefanescu *et al.*, 1992) and in the Adriatic Sea, down to 1200 m (Ungaro *et al.*, 2001). In the northeast Atlantic *E spinax* has been caught at depths down to 1600 m along the Reykjanes Ridge (southwest of Iceland) (Hareide and Garnes, 2001). One of the shallowest records of this species was at 80 m depth, where a live specimen was photographed by scuba divers in a Norwegian Fjord (Hennemann, 2001). In the northwest African region, this species has been captured at depths from 400 to 800 where it is either a dominant or sub-dominant species (Yu *et al.*, 2006).

The maximum size recorded for *E. spinax* was a female of 60 cm total length (TL) but specimens are rarely larger than 45 cm (Compagno *et al.*, 2005). According to Compagno (1984) this species matures between 33 and 36 cm TL, while Bauchot (1987) reported 28 to 36 cm TL. However, neither of these authors specified sizes of maturation for sexes separately. On the other hand, Coelho and Erzini (2005) presented sizes at first maturity (L_{50}) estimates of 30.86 cm TL for females and 25.39 cm TL for males from Southern Portugal.

E. spinax is an aplacental viviparous shark, producing 6 to 20 pups in each litter (Compagno, 1984; Bauchot, 1987). Compagno (1984) reports that pups are born between 12 and 14 cm TL while in the Mediterranean pups seem to born at somewhat smaller sizes, specifically between 9 and 11 cm TL according to Serena (2005) and between 10 and 11 cm TL according to Bauchot (1987).

E. spinax is a predatory shark and several studies have focused on its feeding strategy. Bello (1998) referred that 74.8% of the diet consisted of crustaceans, 16.9% fishes, 6.9% cephalopods, 0.9% polychaetes and 0.5% other prey categories in the Adriatic Sea. In South Portugal a significant ontogenic variation in feeding ecology was observed, with specimens smaller than 17 cm TL feeding mainly on euphausiids, specimens from 17 to 27 cm TL feeding mainly on euphausiids and natant decapods and specimens larger than 27 cm TL feeding also on teleosts and cephalopods (Neiva *et al.*, 2006). In Angolan waters, off the southwest African coast, its diet seems to be very different from the northern populations, consisting mainly of cephalopods (63%), 33% of crustaceans and 4% of teleosts (Zaera, 2005).

No complete age and growth estimates are known for this species. The only known study is a preliminary work by Sion *et al.* (2002), who made a first attempt to age the Ionian Sea (Mediterranean) population based on dorsal spines. Sion *et al.* (2002) estimated, without any validation, maximum ages of 7 years and maturity ages of 5 years for that population.

E. spinax is a common by-catch species caught by the deep water fisheries that operate in the areas where they occur, especially by bottom trawls (Monteiro *et al.*, 2001; Carbonell *et al.*, 2003; Abella and Serena, 2005). In general, *E. spinax* occurs within the depth ranges where these bottom trawl fisheries operate, but the ban on bottom trawling deeper than 1000 m in the Mediterranean, adopted by all members of the General Fisheries Commission for the Mediterranean (GFCM) and that came into force during 2005, may afford *E. spinax* some protection in this area.

This species in not currently listed in the IUCN Red List of endangered species (IUCN, 2006a), but discussions during IUCN assessment meetings where the author participated have shown that this species should be categorized as “Near Threatened” for the northeast (NE) Atlantic, “Least Concern” for the Mediterranean and “Data Deficient” for the West African region.

1.5.2. Smooth lantern shark

The smooth lantern shark, *Etmopterus pusillus* is also a small sized lantern shark (order Squaliformes, family Etmopteridae) (Figure 1.7). However, unlike *E. spinax* that is restricted to the eastern Atlantic and Mediterranean, *E. pusillus* is a globally widespread species, having been recorded from the Atlantic, the Pacific and the Indian Oceans. In the western Atlantic it has been recorded in the northern area of the Gulf of Mexico and between southern Brazil and Argentina (Compagno, 1984), while in the eastern Atlantic it has been recorded from Portugal (Saldanha, 1997) to South Africa (Compagno, 1984). In the western Indian Ocean it has been described for South Africa and in the western Pacific from Japan (Compagno, 1984). *E. pusillus* is found on or near the bottom of continental and insular slopes at depths from 274 to 1000 m (possibly to 2000 m), but has also been described in oceanic waters (Compagno *et al.*, 2005) (Figure 1.8).

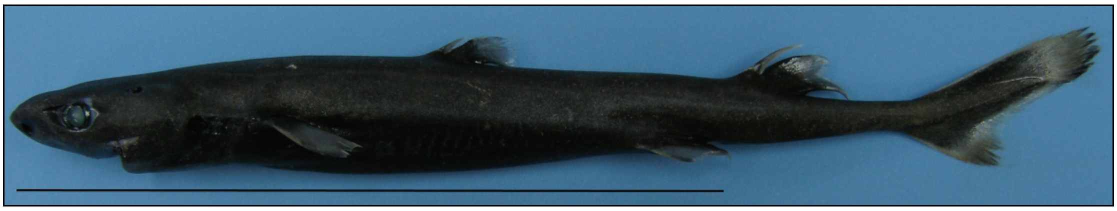

Figure 1.7. A smooth lantern shark, *Etmopterus pusillus*, from the south coast of Portugal. The scale bar represents 20 cm.

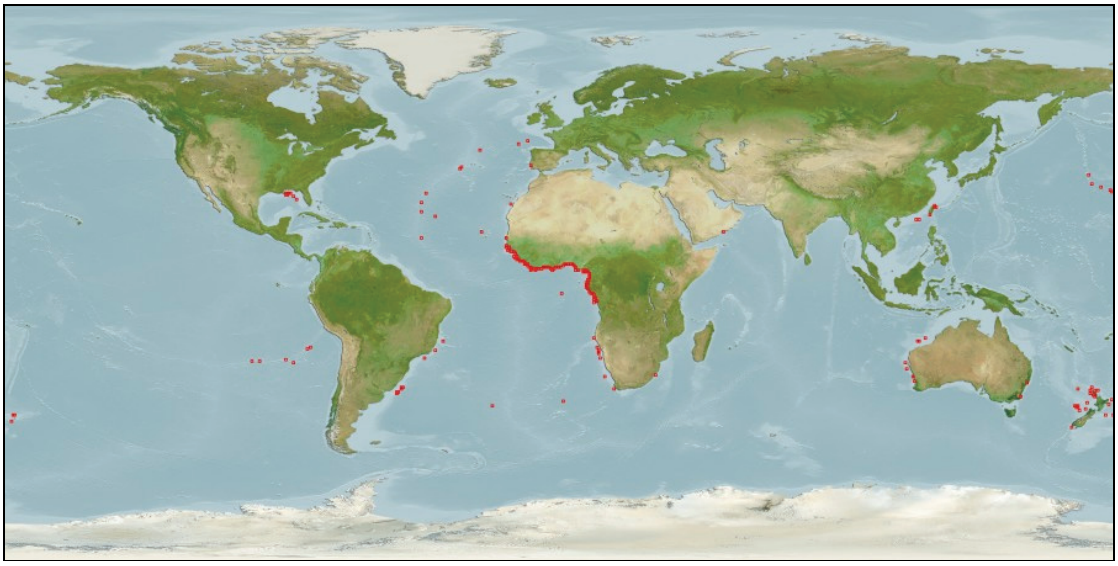

Figure 1.8. Geographical distribution of *Etmopterus pusillus* (adapted from Fishbase (Froese and Pauly, 2006)).

Even though this is a globally widespread species, almost nothing is known about this species habitat, ecology and biology. Compagno (1984) reported a maximum recorded size of 47 cm TL but suggested that it can probably grow to larger sizes. It is known to be an aplacental viviparous species, and sizes at maturity for the southern Portuguese population have been estimated to be 38.1 cm TL for males and 43.6 cm for females (Coelho and Erzini, 2005). At this stage, no other biological studies focusing on this species are known.

As is the case of *E. spinax*, this species is likely to be caught by most deep water commercial fishing operating in the areas where they occur. Such catches have been reported mostly for the NE Atlantic area both for longlines (Erzini *et al.*, 2001; Coelho *et al.*, 2005) and trawls (Monteiro *et al.*, 2001). Yu *et al.* (2006) detected huge declines in the catches of this species between 2005 and historical 1970's and 1980's catches along the west African coast records, and attributed these declines to environmental changes.

This species in not currently listed in the IUCN Red List of endangered species (IUCN, 2006a). However, discussions during IUCN assessment meetings where the author participated lead to the classification of “Least Concern”, given that it has a widespread distribution and occurs in some areas where fishing pressure is low or inexistent.

1.6. General objectives of this study, with notes on thesis style

Given the lack of biological, life history, mortality and fisheries information on the two lantern shark species, both being important discard components of the by-catch of deep water fisheries, and that as top predators they are probably highly vulnerable to fishing mortality and are thus a cause for conservation concern, the objectives of the present study were:

1) Characterize the commercial deep water fisheries that affect these lantern sharks in the southern Portuguese area, (Chapter 2);

2) Investigate if multivariate analysis is adequate to morphologically separate these two similar lantern sharks (Chapter 3);

3) Study the life history of each species, with regards to age, growth, reproductive cycle, maturity and fecundity (Chapters 4 and 5);

4) Determine the depth distribution patterns of *E. spinax*, and correlate these distributional patterns with growth and reproductive cycle (Chapter 6);

5) Determine if density dependant mechanisms occur in these species, by comparing life history parameters in a heavily fished and a non fished population of *E. spinax* (Chapter 7) and;

6) Determine mortality and demographic parameters of the two species and assess if the current levels of fishing mortality are sustainable (Chapter 8).

Each chapter on this thesis has been written in a paper-style format, suitable and appropriate to be published in a scientific journal. Each chapter constitutes a complete study (although in some cases references to previous chapters are included), and can be read independently of others. At the beginning of each chapter, information regarding that particular chapter publication status is given, listing all the co-authors and including the complete reference to the journal where it has been published or submitted to. Some chapters are still not ready to be submitted to scientific journals and are therefore classified as “in preparation”. Tables and figures appear in the text inside each chapter, but all acknowledgements have been compiled at the beginning of the thesis and all references compiled at the end of the thesis.

CHAPTER 2: A comparative study of size distributions, maturity distributions and sex ratios of four deepwater shark by-catch species caught by longline and trawl off southern Portugal [*]

(Aboard the commercial bottom trawler "Gamba", off the Algarve coast)

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[*] S*ubmitted*: Coelho, R. & Erzini, K. Effects of fishing methods on deep water shark species caught as by-catch off southern Portugal. *Hydrobiologia*.

2.1. Abstract

Deep water sharks are commonly caught as by-catch of longlines targeting bony fishes and trawlers targeting crustaceans in deep water off the southern Portuguese coast. Due to low or no commercial value, these species are most of the times discarded at sea, with only the larger specimens of some species commercialized at very low prices. In this study we present size distributions, maturity distributions and sex ratios of 2138 specimens belonging to four different species, namely the lantern sharks *Etmopterus pusillus* and *Etmopterus spinax* and the catsharks *Galeus melastomus* and *Galeus atlanticus*, caught with these two gears. Trawls generally caught smaller sized specimens, in a wider length range than longlines. Trawls caught mostly immature specimens of all species, namely 83.7% immature of *E. pusillus*, 84.3% of *E. spinax*, 89.5% of *G. melastomus* and 95.5% of *G. atlanticus*, while longlines caught mostly immature *E. pusillus* (69.2%) and *G. melastomus* (78.6%) and mostly mature *E. spinax* (88.2%) and *G. atlanticus* (87.2%). Trawl tended to catch more males than females of all species except *E. spinax*, while longlines caught more females than males of *E. spinax* and *G. melastomus* and more males than females of the other two species. The data presented here have significant implications for the conservation of these shark populations since sizes, sexes and the immature and mature components of the populations are being affected differently by these two fishing gears.

2.2. Introduction

Due to their typically vulnerable life cycle, characterized by slow growth rates, late maturity and low fecundity (Cortés, 2000), elasmobranch fishes are highly susceptible to fishing mortality. In these animals, overexploitation can occur even with low levels of fishing mortality (Stevens *et al.*, 2000), and once they start to decline, it can take decades for populations to recover (Anderson, 1990). Examples of well documented collapses in elasmobranch populations throughout the world include pelagic sharks in the northwest Atlantic (Baum *et al.*, 2003) and several skate species in the northeast Atlantic (Dulvy *et al.*, 2000).

Deep water species are usually even less resilient to fisheries pressure than coastal and epipelagic oceanic species, since productivity is among the lowest of all elasmobranchs (Gordon, 1999). Several deep water elasmobranch population have been severely depleted, such as the picked dogfish, *Squalus acanthias*, whose northeastern population has been depleted to about 5% of the virgin biomass (Hammond and Ellis, 2005), or even locally extinct such as the blue skate, *Dipturus batis* in the Mediterranean (Abdulla, 2004).

In southern Portuguese waters, several deep water shark species are commonly caught as by-catch by both longliners targeting bony fishes and trawlers targeting crustaceans (Monteiro *et al.*, 2001; Coelho *et al.*, 2005). Although several species are present in this by-catch, two species of lantern sharks (*Etmopterus pusillus* and *Etmopterus spinax*) and two species of catsharks (*Galeus atlanticus* and *Galeus melastomus*) account for the majority of this shark by-catch. Most of this catch is discarded, given that these species have little or no commercial value, with only the larger catshark specimens (mostly *G. melastomus*) commercialized at very low prices.

Since most of this shark by-catch is discarded and is never accounted for in the official fisheries statistics, it is very difficult to gather information on the impact that these commercial fisheries are having on these shark populations. One additional difficulty is that there are still problems with the correct identification of these less known species. As an example, the official fisheries statistics only identifies one species of the *Galeus* genus in Portuguese waters (*G. melastomus*) while there is now evidence that another species (*G. atlanticus*) also occurs (Rey *et al.*, 2006) and is probably being misidentified.

Several previous studies have examined or mentioned by-catch of non commercial elasmobranch species in Portuguese waters. Monteiro *et al.* (2001) looked into general discards from the trawl fishery, Erzini *et al.* (2001) looked into the hake deep water longline fishery and Coelho *et al.* (2005) analyzed elasmobranch by-catch from coastal trammel nets and semi-pelagic deep water longlines. These studies presented quantitative values of the by-catch in number or biomass, but did not explore intra-specific characteristics of the by-catch such as length distribution, maturity distribution or sex ratios of the species caught with the different fishing gears.

Therefore, the objectives of the this study are to present and compare size distributions, maturity distributions and sex ratios of four commonly discarded deep water shark species, namely the lantern sharks *E. spinax* and *E. pusillus*, and the catsharks *G. melastomus* and *G. atlanticus* caught by deep water longlines and deep water trawls off the south and southwestern coasts of Portugal.

2.3. Material and methods

Specimens were caught aboard commercial fishing vessels operating off the south and southwestern coasts of Portugal. Overall, data from 46 fishing days, 9 with trawls (corresponding to 29 hauls) and 37 with longlines, from February 2003 to October 2004 were analyzed for this study. Longline catches came from a commercial longliner targeting wreckfish, *Polyprion americanus* and European conger, *Conger conger* (Figure 2.1). This fishing vessel usually uses SIAPAL brand number 6 hooks (gape = 17.5 mm, SD = 1.3, n = 6), and some 3200 to 3600 hooks are set each fishing day. The catch from the deep water trawls came from the commercial fisheries targeting crustaceans such as Norway lobster, *Nephrops norvegicus*, deep water rose shrimp, *Parapenaeus longirostris*, and blue and red shrimp, *Aristeus antennatus* (Figure 2.1). The minimum codend mesh size for that fishery is 55mm, and the hauls analyzed in this study ranged in duration from 2h50min to 10h20min, with boat speed varying from 2.5 to 3 knots. Trawl fishing took place at depths from 199 to 641 m mostly on sandy and muddy bottoms, and longline fishing at depths from 458 to 787 m, mostly near rocky bottoms.

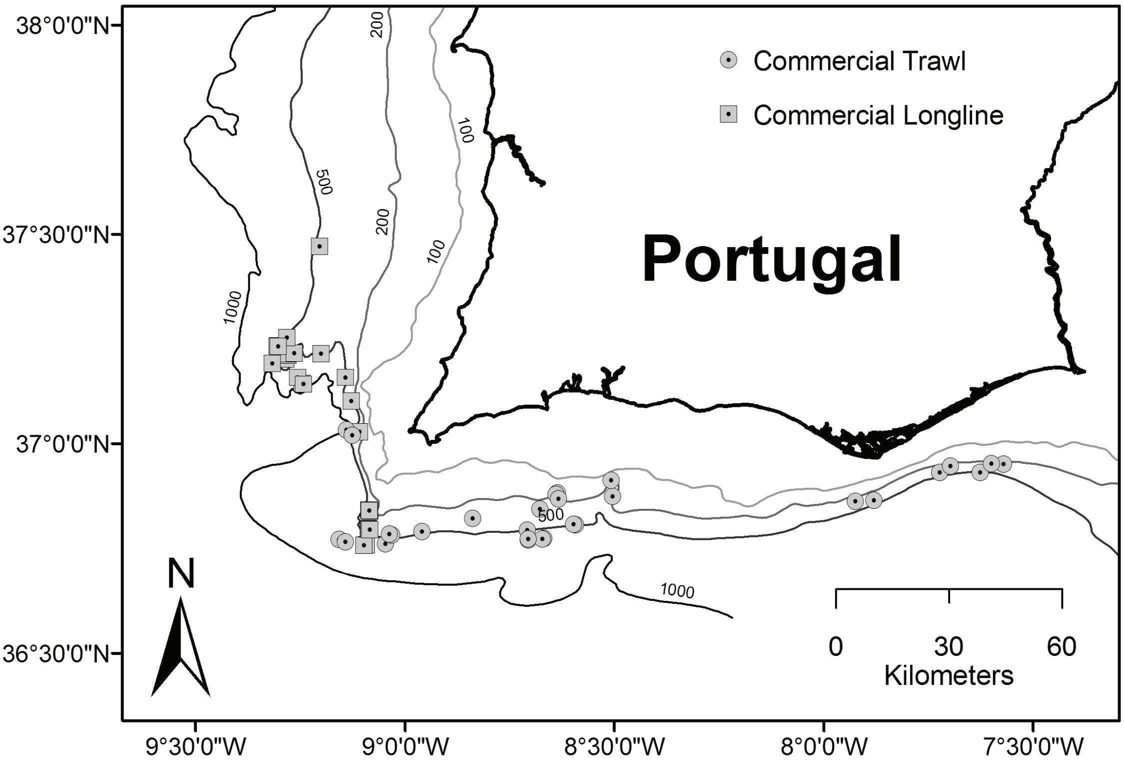


Figure 2.1. Map of the south and southwestern coasts of Portugal with the location of the coastline, the bathymetric lines (100, 200, 500 and 1000m depth) and the sampling points. Bathymetric lines and coastline adapted from "Atlas do Ambiente Digital – Instituto do Ambiente".

Specimens were taken to the University of the Algarve laboratory, where they were identified to species level, sexed, measured for total length (TL) in a straight line from the tip of the snout to the tip of the upper lobe of the caudal fin in a natural position (nearest lower millimeter) (Compagno, 2001) and the total weight (W) was recorded (nearest lower centigram). The gonads of both males and females were observed macroscopically and classified according to the maturity stages proposed by Costa *et al.* (2005) for the oviparous species (in this study *Galeus* spp.) and by Clarke *et al.* (2001) for the aplacentary viviparous species (in this study *Etmopterus* spp.). Specimens were then divided in mature or immature, with mature specimens defined as specimens that are ready to produce progeny or have done so in the past.

The Kolmogorov-Smirnov test was used to find differences in the general shape, dispersion and skewness of the length distributions of the catches between fishing gears, while the Mann-Whitney statistical test was used to compare catches in length and weight between fishing gears (StatSoft, 2004). The null hypothesis tested in both cases was that in each species, specimens caught by the different fishing gears came from the same part of the population. Each species sex ratio per fishing gear was compared by the Chi^2 observed vs. expected test (StatSoft, 2004), testing the null hypothesis that males and females of each species and in each fishing gear were caught in similar ratios.

2.4. Results

A total of 2138 specimens were caught during this study. In general, trawls caught smaller and lighter specimens than longlines (Table 2.1), and the observed differences, both in average length and weight were statistically significant for all species (Mann-Whitney: P-values < 0.05).

Table 2.1. Number of specimens (n), mean and ranges of total length (TL) and weight (W) of the four shark species caught by longline and trawl.

		E. pusillus	*E. spinax*	*G. atlanticus*	*G. melastomus*
Longline	n	514	279	99	462
	Mean TL (cm)	38.1	33.4	41.8	49.3
	Range TL (cm)	25.3 - 50.2	23.8 - 40.7	31.6 - 45.8	23.2 - 75.1
	Mean W (g)	230.88	165.88	196.20	352.22
	Range W (g)	41.61 - 605.00	42.78 - 304.33	79.91 - 298.94	35.14 - 1219.63
Trawl	n	98	407	22	257
	Mean TL (cm)	31.1	23.0	21.0	23.5
	Range TL (cm)	15.8 - 48.2	9.1 - 39.6	14.7 - 41.4	12.5 - 65.8
	Mean W (g)	138.31	56.69	33.48	58.32
	Range W (g)	14.44 - 512.40	2.85 - 367.05	8.43 - 198.40	4.66 - 889.21

Size distributions by fishing gear showed that trawls tended to catch specimens in a wider length range while longline catches tended to be more concentrated in the larger size classes (Figure 2.2). For all species, the size distribution of the catches between the two fishing gears was significantly different (Kolmogorov-Smirnov: P-values < 0.05).

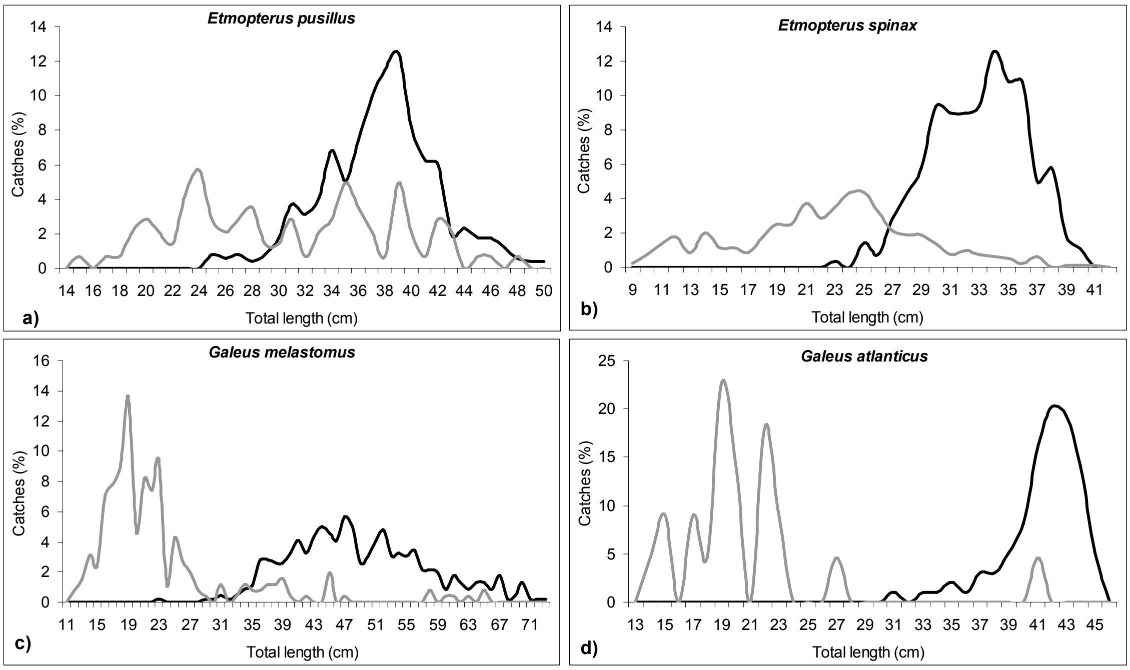


Figure 2.2. Size distributions (1cm total length size classes) in percentage of catches per fishing gear of *Etmopterus pusillus* (a), *E. spinax* (b), *Galeus melastomus* (c) and *G. atlanticus* (d) caught both by longline (black line) and trawl (grey line).

Trawls tended to catch more males than females of all species except *E. spinax*, while longlines caught more females than males of *E. spinax* and *G. melastomus* and more males than females of the other two species. Both trawls and longlines caught more male *E. pusillus* than females, although in trawls the differences were more noticeable. On the other hand, both gears caught more females of *E. spinax* than males and this difference was most noticeable in longlines. For *G. atlanticus* the situation was similar to *E. pusillus*, with more males being caught than females by both gears, while relatively more female *G. melastomus* were caught on longlines and more males were caught with trawls (Figure 2.3). The observed vs. expected sex ratio frequencies were significantly different for all species and for both gears (Chi^2 Observed vs. Expected: P-values < 0.05), except for the case of *G. atlanticus* caught with trawl where no significant differences were detected (Chi^2 Observed vs. Expected: P-value > 0.05).

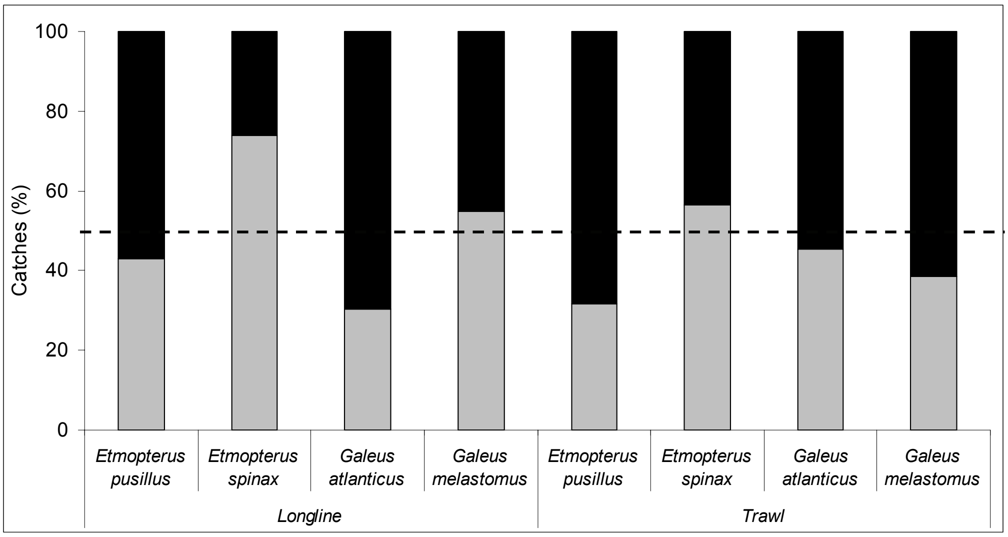


Figure 2.3. Sex ratios (females =grey bars and males = black bars) for each species caught with each fishing gear. The dotted line represents 50% of the catches.

In general, more immature than mature specimens were caught. In *E. pusillus*, immature specimens, independently of sex, were always caught in higher percentages than mature specimens, both by trawl (83.7%) and by longline (69.2%). In *E. spinax* there was a discrepancy between fishing gears, with longlines catching mostly mature specimens (88.2%), and trawls mostly immature specimens (84.3%). Immature specimens of both male and female *G. melastomus* were always caught in higher quantities than mature specimens, both by longline (78.6%) and trawl (89.5%), while longlines caught mostly mature *G. atlanticus* (87.2%) and trawls mostly immature specimens (95.5%), independently of sex (Figure 2.4).

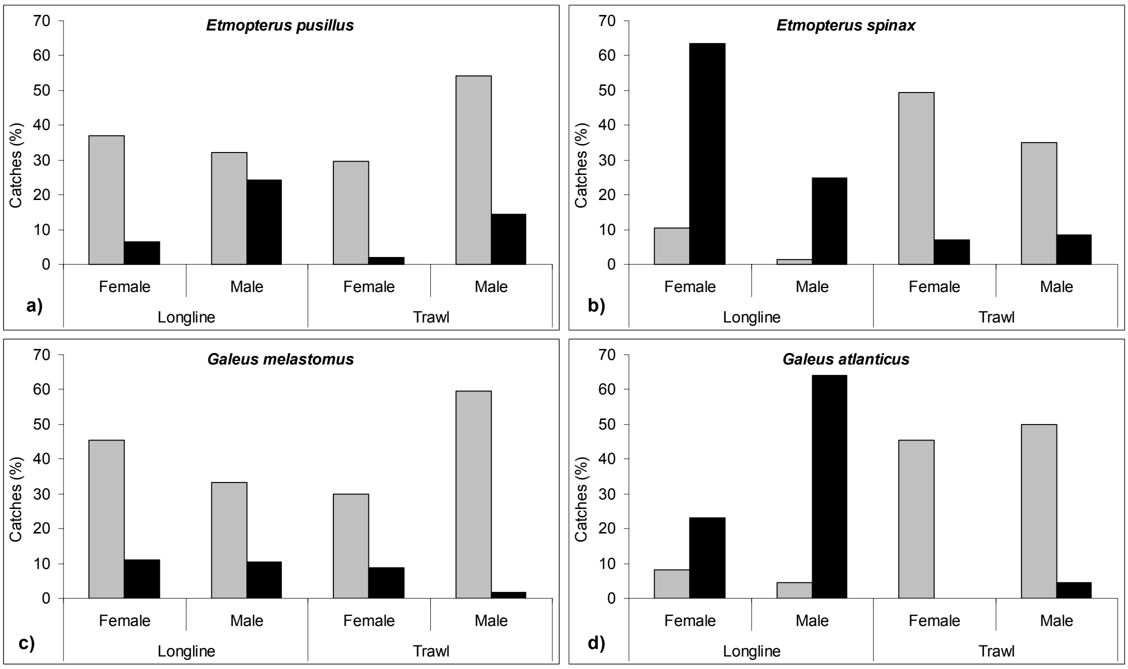


Figure 2.4. Catches, in percentage per fishing gear, of immature (grey bars) and mature (black bars) males and females of *Etmopterus pusillus* (a), *E. spinax* (b), *Galeus melastomus* (c) and *G. atlanticus* (d).

2.5. Discussion

Three of the studied species, *E. pusillus*, *E. spinax* and *G. melastomus* are very frequent and are probably the most common elasmobranch species at the depths fished in this study (Monteiro *et al.*, 2001; Coelho *et al.*, 2005), while the fourth species, *G. atlanticus* is a relatively rare species, that given its similarity to *G. melastomu*s can easily be confounded. In fact this species has only recently been redescribed based on exterior field marks (Rey *et al.*, 2006), and is still not recognized by the official Portuguese fisheries statistics where it is mixed with *G. melastomus*.

Since these species are mostly discarded, only a very small portion is accounted for in the official fisheries statistics. Nonetheless, due to the high fishing mortalities, these populations are suffering severe impacts from these fisheries. In fact, even though most of these specimens are discarded, they are returned to sea either dead or with severe injuries that probably affect their survival. Specifically, specimens caught with trawls tend to arrive dead on board, probably due to the trauma of being towed for several hours, while specimens caught with longlines are often still alive, but with injuries caused by the hooks and by the sudden changes in pressure and temperature that might impair their survival. One possible way to effectively reduce by-catch in longlines is to increase the distance for setting the hooks above the seabed (Coelho *et al.*, 2003).

Although longline fishing effort cannot be compared with that of trawls, the data presented in this study can give some idea on the catchability of the studied species. Regarding the *Etmopterus* genus, *E. pusillus* seems to be mostly caught by longlines, while *E. spinax* is mostly caught by trawl. At this point we can put forward some tentative explanations for this observation: 1) these species have different habitats in terms of water column placement, with *E. spinax* being a more benthic species that is more easily caught by trawl, while *E. pusillus* is a more bentho-pelagic species not easily caught by trawl but that is attracted to the baited longlines or 2) these species have different substrate preferences, with *E. spinax* more common on sandy and muddy bottoms, where the trawls operate and *E. pusillus* more associated with rocky bottoms, near where longlines operate. While at this point we can only speculate on the true causes for this observation, Compagno *et al.* (2005) reports that *E. pusillus* has been recorded in oceanic waters in the southern Atlantic, which might indicate vertical migrations that make them less vulnerable to bottom trawls. For the *Galeus* genus, given that *G. atlanticus* is a rare species, caught in very low numbers by both gears we cannot forward such hypothesis.

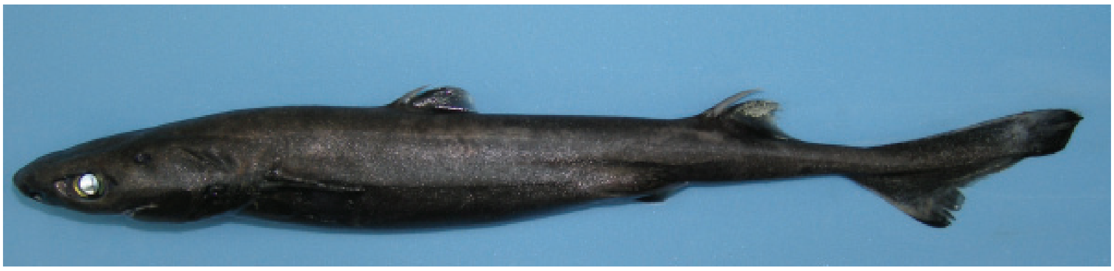
Significant differences in sex ratios were detected for all species and for all gears except *G. atlanticus* caught with trawl. For this species, the low sample size may have biased this particular analysis. The differences found in the sex ratios may be correlated with the differences found in the size distribution, since there are differences in maximum sizes of males and females. In fact, in all these species, females tend to grow to larger sizes than males, and given that longlines catch mostly large specimens it would be expected that most of them would be females. This hypothesis seems to hold true for the case of *G. melastomus* where longlines catch more females and trawls catch more males, but not for the other species. In *E. spinax*, although trawls catch smaller sized specimens than longlines, both gears catch mainly females, while in *E. pusillus* the opposite situation occurs with more males than females caught with both gears. As stated before, the fact that these gears are operating in different habitats might be the cause for the different sex composition of the catches, implying that these species are aggregating in different habitats by sex. Although there are no specific studies for these species, there are examples from another squaloid shark, *S. acanthias* that has for long been known to aggregate by size and sex (Ford, 1921).

Trawls are catching mostly immature specimens of all species while longlines are catching mostly immature *E. pusillus* and *G. melastomus*, and mature *E. spinax* and *G. atlanticus*. Although there is no stock assessment based evidence (Hilborn and Walters, 1992), the persistent capture of large quantities of small sized and immature sharks is likely to lead to growth overfishing (Bonfil, 1997). In this case, the larger sized species (*E. pusillus* and *G melastomus*), that are caught largely as juveniles by both gears, are probably more affected by fishing than the smaller sized species (*E. spinax* and *G. atlanticus*).

This work has presented information on the differential population catches of four by-catch and frequently discarded deep water elasmobranch species in southern

Portugal. As a conclusion, we showed that when compared to longlines, trawls are catching smaller sized and mostly immature specimens. Continuous fishing pressure on these populations may lead to their collapse. The use of hooks fished off the bottom (Coelho *et al.*, 2003) would greatly reduce the catch of discarded deepwater sharks. Reduction of shark by-catch in the deepwater crustacean trawl fisheries could be achieved through the use of by-catch reduction devices (BRDs), such as a rigid grill combined with an escape window. Such BRDs have been tested in Portuguese trawl fisheries with mixed results (Campos and Fonseca, 2004).

CHAPTER 3: Identification of deep water lantern sharks (Chondrichthyes: Etmopteridae) using morphometric data and multivariate analysis [*]



(The two lantern sharks,*Etmopterus spinax* and *E. pusillus*, common in southern Portugal)

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[*] *In Press*: Coelho, R. & Erzini, K. Identification of deep water lantern sharks (Chondrichthyes: Etmopteridae) using morphometric data and multivariate analysis. *Journal of the Marine Biological Association of the United Kingdom*. DOI: 10.1017/S0025315408000532.

3.1. Abstract

Taxonomic distinction to species level of deep water sharks is complex and often impossible to achieve during fisheries related studies. The species of the genus *Etmopterus* are particularly difficult to identify, so they often appear without species assignation as *Etmopterus* sp. or spp. in studies even those focusing on elasmobranches. During this work, the morphometric traits of two species of *Etmopterus*, *E. spinax* and *E. pusillus* were studied using 27 different morphological measurements, relatively easily to obtain even in the field. These measurements were processed with multivariate analysis in order to find out the most important ones likely to separate the two species. Sexual dimorphism was also assessed using the same techniques, and it was found that it does not occur in these species. The two *Etmopterus* species presented in this study share the same habitats in the overlapping ranges of distribution and are caught together on the outer shelves and slopes of the northeastern Atlantic.

3.2. Introduction

The correct taxonomic identification of species provides a critical baseline that supports the rest of the biological research (Last, 2007). Elasmobranchs in general have suffered major taxonomic constraints that have lead to identification problems when by-catch and fisheries data is analysed, usually solved by grouping the data in higher (genus or family) taxonomic levels (e.g. Girard *et al.*, 2000; Zeeberg *et al.*, 2006).

The identification of the deep water elasmobranchs is even more problematic because their morphological similarities and the lack of studies on these groups. Among these, the lantern sharks (genus *Etmopterus*), is particularly problematic because the small size of most species and lack of commercial interest, and even specific elasmobranch studies where most *taxa* were identified to species level have failed to identify lantern sharks to species level (e.g. McKinnell and Seki, 1998). In the FAO official fisheries data (FAO, 2007) there are only two categories to list lantern sharks (*Etmopterus* spp. and *Etmopterus spinax*) indicating that identification problems occur in this group.

Elasmobranch fishes are vulnerable to exploitation, due to their slow growth rates, late maturity and low fecundities (Cortés, 2000), with the potential for overexploitation even at low levels of fishing mortality (Stevens *et al.*, 2000). Commercially exploited deep water shark species are amongst elasmobranchs with the highest risks of extinction (Fowler *et al.*, 2005), and implementing fisheries management or species conservation programs is impossible when identification is still an issue.

Two species of lantern sharks of the genus *Etmopterus* are common in southern Portuguese waters: the velvet belly lantern shark, *Etmopterus spinax* and the smooth lantern shark, *Etmopterus pusillus*. A third species, *Etmopterus princeps*, has been recorded in these waters but it has not been recorded in any recent studies that have surveyed the area (Borges *et al.*, 2001; Erzini *et al.*, 2001; Monteiro *et al.*, 2001; Coelho *et al.*, 2005).

Both these species occur in deep waters, on or near the bottoms of the outer continental shelves and slopes and have same habitats in their overlapping ranges of distribution; *E. spinax* has been recorded between 70 and 2000 m depth (mostly between 200 and 500 m) and *E. pusillus* between 274 and 1000m depth (Compagno *et al.*, 2005). *E. spinax* is restricted to the eastern Atlantic Ocean, including the Mediterranean Sea, whereas *E. pusillus* has a worldwide distribution, having been recorded on both sides of the Atlantic, the western and southeast Pacific, and the western Indian Oceans (Compagno *et al.*, 2005).

Even thought both species of lantern shark species presented in this study are valid, the morphological differences between them are not very evident and there is a need to determine which morphometric measurements are most useful for researchers to quickly distinguish between them. The use of multivariate analysis allows to process entire set of morphometric measurements. Furthermore, when each morphometric measurement is contributing very little to the overall differences, multivariate analysis allows for those small differences to be added and analysed as a whole (Clarke and Warwick, 2001). The objectives of the present study were to: 1) determine if the two *Etmopterus* species could be separated using multivariate analysis based on morphological features, 2) determine the most important morphometric measurements for separating the two species and 3) determine if sexual dimorphism occurs in each species.

3.3. Material and methods

For this work, a total of 104 specimens were sampled, corresponding to 69 *E. spinax* and 35 *E. pusillus*. Both species had samples from both sexes and covered a wide range of lengths (Table 3.1). The samples were collected between February and June 2003 by commercial fishing vessels using deep water trawls and longlines and operating off the southern Portuguese coast.

Table 3.1. Size (n) and total length (TL, in mm) range of the sample used in this study, for each sex and each species.

Sex	*Etmopterus spinax*			*Etmopterus pusillus*		
	n	Min. TL	Max. TL	n	Min. TL	Max. TL
Males	27	143	335	24	223	430
Females	42	120	365	11	231	455
Total	69	120	365	35	223	455

Once in the laboratory, specimens were sexed and measured for a total of 27 different morphometric characteristics. These measurements covered all the general body areas of these sharks, including general length measurements, body heights and widths and measurements on each of the fins (Table 3.2). Most measurements were taken with a digital calliper with 0.01 mm precision, except the largest body measurements that were taken with a 1 mm precision. All measurements throughout this study refer to millimetres and names and abbreviations were defined according to Compagno (2001). All measurements were expressed in % of total length (TL).

Table 3.2. Morphometric measurements used in this study, with the corresponding abbreviations (Compagno, 2001). All measurements were taken with a precision of 0.01 mm except for measurements marked with an asterisk (*), that were taken with 1 mm precision.

Place of measurement	Abbreviation	Name
Body length	TL*	Total length
	FL*	Fork length
	PCL*	Pre caudal-fin length
	GIR*	Girth
Body height	HDH	Head height
	TRH	Trunk height
	ABH	Abdomen height
	TAH	Tail height
	CPH	Caudal-fin peduncle height
Body width	HDW	Head width
	TRW	Trunk width
	ABW	Abdomen width
	TAW	Tail width
	CPW	Caudal-fin peduncle width
Head	HDL	Head length
	POB	Pre-orbital length
	POR	Pre-oral length
	EYL	Eye length
	ING	Inter-gill length
Caudal fin	CDM	Dorsal caudal-fin margin
	CPV	Pre-ventral caudal-fin margin
	CFL	Caudal-fin fork length
Dorsal fins	D1A	1st dorsal-fin anterior margin
	D2A	2nd dorsal-fin anterior margin
	IDS	Inter-dorsal space
Pectoral fin	P1A	Pectoral-fin anterior margin
Pelvic fin	P2A	Pelvic-fin anterior margin

For the paired structures, such as the pectoral and pelvic fins, both the left and the right side structures were measured and compared with analysis of covariance (ANCOVA), using TL as the covariate. Once it was determined that no differences occurred between the two sides, only the left side structure was used for the multivariate analysis.

Multivariate analysis was carried out with the PRIMER 6 software (Clarke and Gorley, 2006). A matrix of the morphometric data as percentages of TL was created, and the variables species (*E. spinax* or *E. pusillus*) and sex (male or female) added as factors to test. This matrix was then used to build a similarity matrix, using Euclidean distances. Non-metric multidimensional scaling (MDS) of distances in this similarity matrix was used to visually evaluate differences between the two species and between sexes in each species. Analysis of similarities (ANOSIM) statistical tests were used to assess if the differences observed in the MDS plots were significant, both between species and between sexes. This statistic tests the null hypothesis that no differences occur between groups. In the cases where differences were found, a SIMPER (similarities of percentages) analysis was carried out, in order to determine the percentage of contribution of each morphological measurement to the overall differences. This analysis was carried out until the cumulative differences were higher than 50%.

In order to create a model capable of correctly separating these two species, a discriminant analysis was carried out with the STATISTICA 6 software (StatSoft, 2004). All the morphometric measurements (as percentages of TL) were used and the model was constructed using a backward stepwise approach, where all variables are included in the initial model and variables that contribute least to the prediction of group membership are sequentially removed. Thus, only the most important variables (those that contribute the most to the discrimination of the groups) are kept (StatSoft, 2004).

The coefficients of the discriminant analysis were calculated by determining optimal variable combinations in different functions (canonical analysis). Given that in this case there were only two species (groups), only one canonical function was calculated, given by:

$$Group = a + b_1 x_1 + b_2 x_2 + ... + b_m x_m$$

where a is the constant and b_1 through b_m are the canonical coefficients of the morphometric variables x_1 through x_m. The correct identification to the species level of an *Etmopterus* specimen can be obtained by applying this formula.

3.4. Results

The complete morphometric characterization of the two *Etmopterus* species is presented in Table 3.3. Some measurements such as PCL, FL or CDM were fairly different between species, with a very slight overlapping of the ranges whilst others, such as the P1A or the P2A were very similar, with the ranges of values mostly overlapped. For some measurements such as ABW, there were large differences between males and females, probably due to the fact that the abdomen width in females increases proportionally more than in males once maturity is achieved.

In both species, no differences were detected for the measurements taken for the paired structures, namely the left and right side P1A (ANCOVA $_{E. spinax}$: F = 1.01; P-value = 0.32; ANCOVA $_{E. pusillus}$: F = 0.08; P-value = 0.78) and the left and right side P2A (ANCOVA $_{E. spinax}$: F = 1.36; P-value = 0.25; ANCOVA $_{E. pusillus}$: F = 0.64; P-value = 0.43). Therefore, for the remaining multivariate analysis only the left side structures were considered.

Table 3.3. Morphometric characterization of males and females of *Etmopterus spinax* and *E. pusillus*. All values are presented as percentage of total length (TL), except TL that is given in mm. The mean values and ranges (between brackets) are given.

	Etmopterus pusillus		*Etmopterus spinax*	
	Female	Male	Female	Male
TL	365 (231 - 455)	345 (223 - 430)	268 (120 - 365)	239 (143 - 335)
FL	89.0 (87.7 - 89.9)	89.4 (88 - 90.9)	86.4 (83.6 - 88.6)	86.2 (85.3 - 87.7)
PCL	80.7 (79.7 - 82.4)	81.1 (79.5 - 83.3)	77.2 (72.1 - 80.1)	76.9 (73.6 - 78.7)
GIR	32.2 (29.2 - 36.5)	31.8 (27.5 - 36.5)	34.7 (29.1 - 43.0)	33.4 (29.0 - 39.1)
HDL	23.7 (21.6 - 25.2)	23.6 (21.5 - 25.5)	22.5 (20.7 - 24.7)	22.9 (20.9 - 25.6)
POB	7.0 (5.7 - 7.9)	7.1 (6.5 - 8.3)	7.1 (5.1 - 8.6)	7.3 (6.2 - 8.6)
POR	9.9 (9.2 - 10.7)	9.6 (8.4 - 11.4)	10.2 (8.5 - 11.9)	10.3 (9.0 - 11.7)
EYL	3.6 (3.0 - 4.1)	3.7 (2.8 - 4.2)	4.3 (3.3 - 5.5)	4.1 (3.1 - 5.2)
ING	5.3 (4.2 - 6.2)	5.5 (4.6 - 7.1)	4.9 (3.2 - 5.9)	5.2 (4.2 - 7.2)
CDM	18.8 (17.4 - 19.6)	18.7 (16.0 - 21.2)	22.5 (20.2 - 25.9)	22.6 (19.4 - 24)
CPV	9.8 (8.8 - 10.8)	9.4 (8.4 - 10.7)	10.4 (8.9 - 14.3)	10.3 (8.6 - 11.7)
CFL	10.4 (9.6 - 11.4)	10.0 (8.5 - 11.8)	11.4 (10.4 - 13.6)	11.5 (10.3 - 13.7)
D1A	5.5 (5.1 - 5.8)	6.0 (5.0 - 9.1)	6.5 (4.7 - 8.8)	6.3 (5.1 - 7.6)
D2A	6.8 (5.9 - 7.3)	7.0 (5.7 - 9.5)	8.5 (6.2 - 11.2)	8.5 (7.1 - 11.2)
IDS	24.2 (22.2 - 26.5)	24.3 (21.8 - 26.8)	23.0 (21.0 - 25.9)	22.8 (16.3 - 25.9)
P1A	8.4 (6.8 - 9.9)	8.1 (6.4 - 9.2)	8.4 (6.5 - 10.1)	8.6 (6.5 - 10.2)
P2A	6.3 (5.3 - 7.6)	6.1 (5.2 - 7.2)	6.4 (4.9 - 8.3)	6.6 (5.1 - 8.6)
HDH	8.2 (7.0 - 9.4)	8.4 (6.6 - 10.2)	8.0 (6.9 - 9.6)	7.4 (6.1 - 9.1)
TRH	11.0 (8.8 - 12.2)	10.5 (8.2 - 12.2)	11.4 (7.1 - 14.8)	10.4 (6.7 - 13)
ABH	11.2 (8.5 - 13.5)	10.1 (7.2 - 12.3)	13.0 (6.5 - 17.4)	11.9 (9.0 - 14.6)
TAH	5.1 (4.2 - 6.0)	5.1 (4.3 - 5.8)	6.5 (4.6 - 9.2)	5.7 (4.8 - 6.6)
CPH	2.1 (1.7 - 2.5)	2.1 (1.9 - 2.5)	2.7 (2.2 - 3.3)	2.6 (2.2 - 2.9)
HDW	9.6 (8.6 - 10.6)	9.0 (7.9 - 11.1)	10.0 (8.2 - 11.9)	9.4 (8.0 - 12.6)
TRW	10.9 (9.5 - 13.3)	10.2 (8.6 - 13.5)	11.1 (6.6 - 14.6)	10.2 (8.5 - 12.4)
ABW	10.2 (8.2 - 11.6)	8.9 (6.9 - 11.3)	11.6 (6.9 - 15.3)	10.6 (8.3 - 13.5)
TAW	4.2 (3.2 - 5.2)	4.5 (3.4 - 5.7)	5.0 (3.5 - 6.9)	4.4 (3.6 - 5.7)
CPW	1.8 (1.6 - 2.4)	1.8 (1.4 - 2.4)	1.9 (1.1 - 2.4)	1.8 (1.4 - 2.7)

In both *Etmopterus* species, no sexual dimorphism was detected (Figure 3.1). Statistically, the visual analyses made with the MDS were corroborated by ANOSIM tests, with no differences detected between sexes: ANOSIM $_{E.\ spinax}$: R = 0.055; P-value = 0.078 and ANOSIM $_{E.\ pusillus}$: R = 0.057, P-value = 0.220.

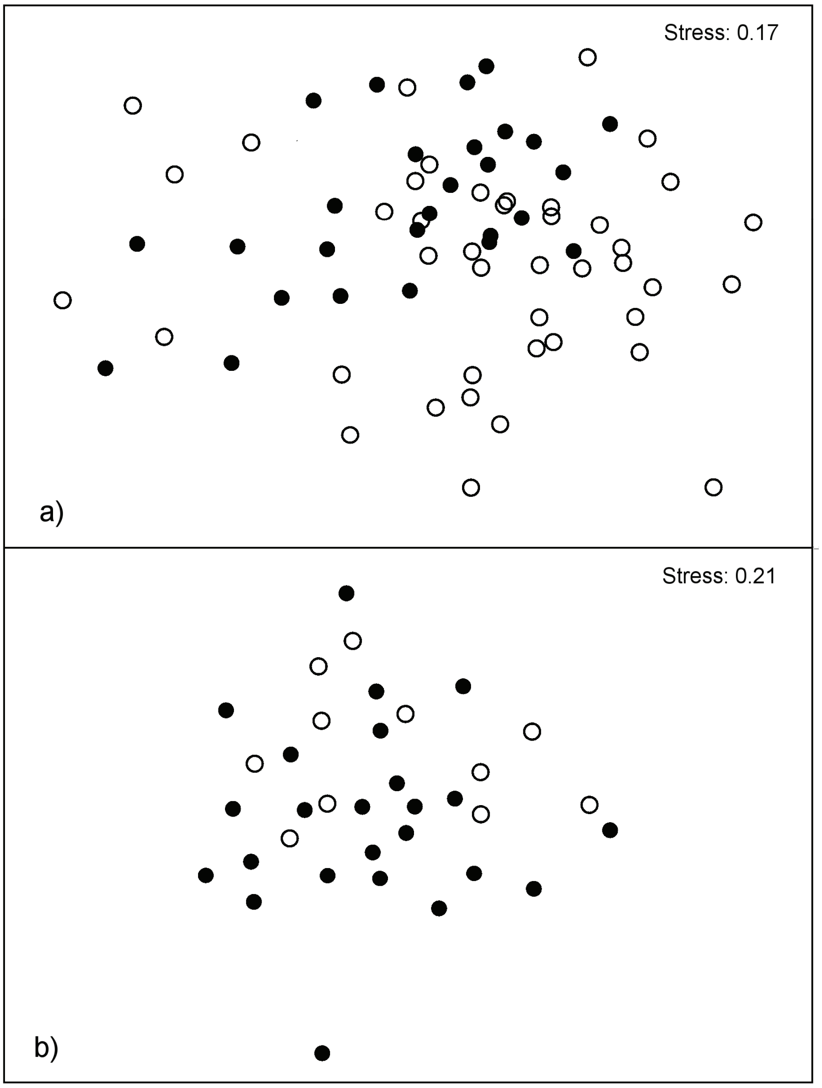


Figure 3.1. Non-metric multidimensional scaling of the morphometric differences between males (black dots) and females (white dots) of *Etmopterus spinax* (a) and *Etmopterus pusillus* (b). The stress value refers to the error that is created when multidimensional data are plotted in two dimensions.

Given that no sexual dimorphism was detected, the comparison between the two species was made using the sexes combined. The multivariate visualization of the data with a MDS plot produced a clear separation between the species (Figure 3.2). Statistically, these differences were significant (ANOSIM: R = 0.491, P-value < 0.01).

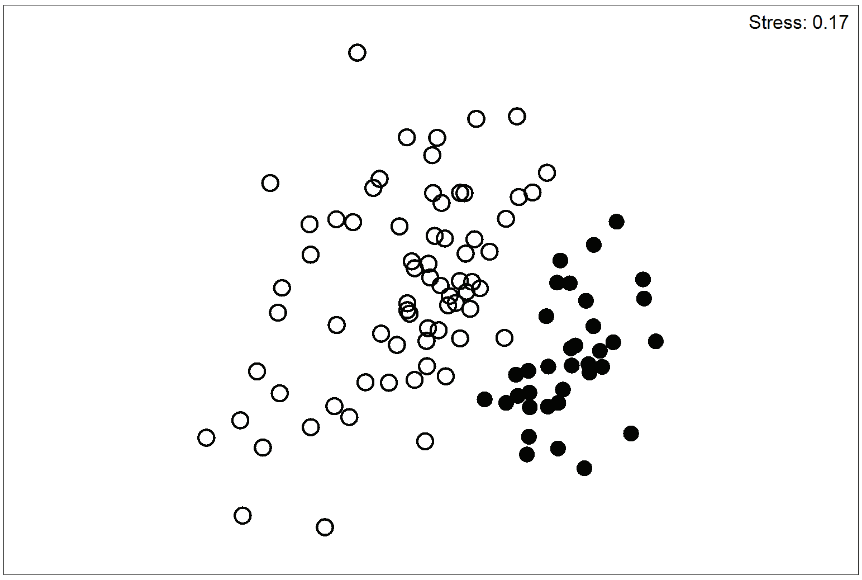


Figure 3.2. Non-metric multidimensional scaling of the morphometric differences between the two *Etmopterus* species. White dots refer to *E. spinax* and black dots refer to *E. pusillus*. The stress value refers to the error that is created when multidimensional data is plotted in two dimensions.

The SIMPER analysis evidenced the morphometrics that contributed most to the species separation (Table 3.4). A > 50% cumulative difference was achieved when 11 morphometric traits were considered: the measurement that individually contributed more for the species separation was the FL, and accounted for 5.94% of the differences, followed by the CDM with 5.84% and PCL with 5.64%.

Table 3.4. Morphometric measurements that contributed most to the differences found between the two species. Only the morphometrics up to a cumulative difference of > 50% are indicated.

Measurement	Average values (% TL)		Differences (%)	
	E. spinax	*E. pusillus*	Contribution	Cumulative
FL	86.30	89.26	5.94	5.94
CDM	22.25	18.73	5.84	11.78
PCL	77.07	80.97	5.64	17.43
TRW	10.75	10.44	5.22	22.65
CFL	11.43	10.13	4.52	27.17
D2A	8.51	6.93	4.51	31.68
HDW	9.75	9.23	4.20	35.89
EYL	4.23	3.63	3.86	39.75
HDL	22.69	23.63	3.80	43.55
IDS	22.89	24.28	3.79	47.34
CPH	2.65	2.11	3.79	51.13

With the backward stepwise discriminant analysis a model using only 6 variables (morphometrics) was created that adequately explains the variability between the two species (Wilks' Lambda = 0.077; approx. F = 178.69; P-value < 0.001). Given that in this case only two groups (species) were being separated, only one canonical function was needed to separate the data. With these canonical coefficients, an equation for identifying the species was created and defined as:

$$Group = -25.51 + 0.52PCL - 0.44CDM + 0.71HDH - 0.75ABH - 2.89CPH + 0.44TRW$$

The centroids of this model were respectively -2.176 for *Etmopterus spinax* and 5.362 for *Etmopterus pusillus* when applied to the original data (Figure 3.3). The best cutting point is half way between these values (1.593) and in this way it is possible to determine the category (species) of a new observation (specimen) depending on the value obtained. For values greater than 1.593 the specimen is probably *E. pusillus* and if it is lower it is probably *E. spinax*. When a *post hoc* prediction was run on all specimens observed during this study, 100% accuracy was obtained between the model estimated and the actual observed species.

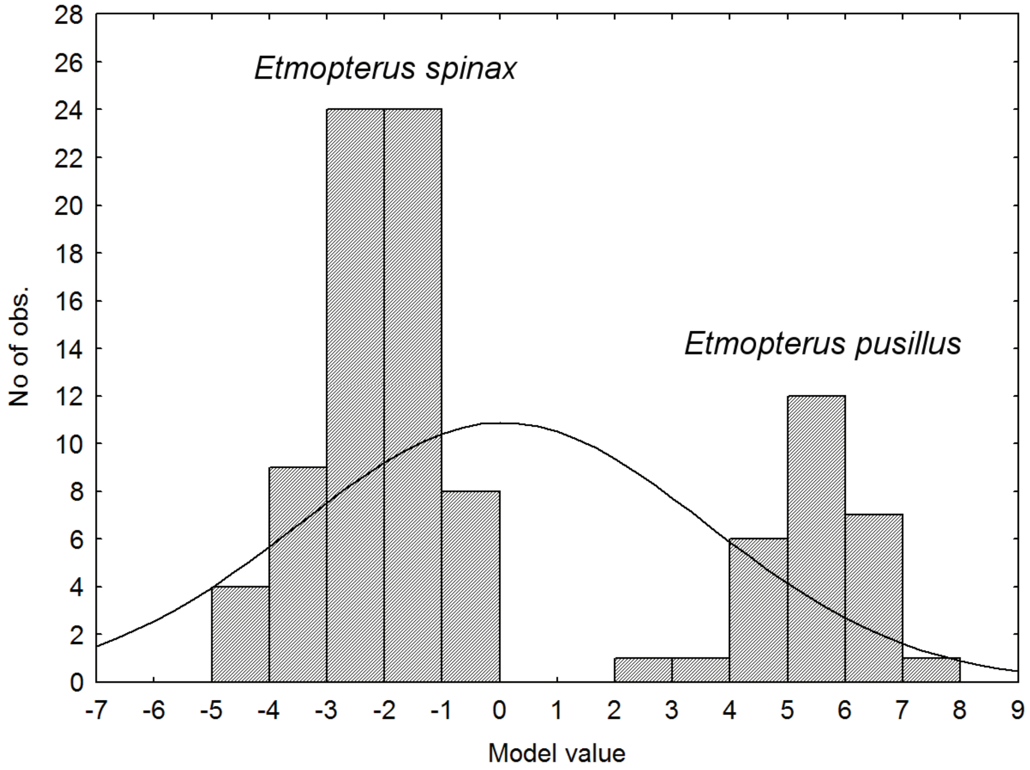


Figure 3.3. Histogram of the values obtained when applying the model to the specimens used in this study. The expected normal curve, if only one group occurred, is also given.

3.5. Discussion

This work showed both the importance and difficulty of separating some morphologically similar deep water shark species, in this case from the *Etmopterus* genus. Difficulties in separating elasmobranch catches to species level is common in fisheries and discards studies, and many authors have had to analyse the data considering a higher taxonomic level such as genus or even family. Examples of such cases are the deep water squaloid fishery in northern France where commercialised squaloid sharks are all identified under the same common name (Girard *et al.*, 2000) and the analysis of by-catch from pelagic trawlers in western Africa (Zeeberg *et al.*, 2006). In Portugal, the official fisheries statistics have most of the elasmobranch catch listed under a general “unidentified sharks and rays” group (DGPA, 2006).

The particular case of the genus *Etmopterus* seems to be even more problematic and there are some studies, where most elasmobranch catches have been identified to species level except for the genus *Etmopterus* where specimens were only identified to genus level. Examples of such cases are the studies by Anderson and Clark (2003) on the by-catch of the orange roughy, *Hoplostethus atlanticus*, in New Zealand or the work of McKinnell and Seki (1998) on shark by-catch in a Japanese squid fishery.

However, even though these species are morphologically very similar, they have different ecological life cycles and in order to have accurate fisheries management and conservation programs, there is a need to correctly discriminate them to species level. In fact, deep water elasmobranch fishes are amongst the most vulnerable marine organisms (Fowler *et al.*, 2005) and there is an urgent need for the implementation of efficient management and conservation programs in the short term that will require fisheries and by-catch data specified to species level.

Even though lantern sharks are mainly discarded species, part of the catch may sometimes be landed and should therefore be recorded by the countries fisheries statistics. The FAO fisheries statistics (FAO, 2007) has two categories to list lantern sharks: the general “lantern sharks” group that refers to *Etmopterus* spp. and the “velvet belly (ETX)” group that refers specifically to *Etmopterus spinax*. Up until

1994 no recordings appeared for either of these groups, meaning that before this date these species were being identified only to family level. Starting in 1995, some catches started being recorded but most of the problems remained. In the case of Europe, from 1999 to 2005, the *Etmopterus* spp. group recorded 871 tonnes while the *E. spinax* group only recorded 75 tonnes, and this evidences clear limitations of these data sets at two levels: 1) most *E. spinax* are not being correctly identified to species level and therefore are being placed in the general *Etmopterus* spp group and 2) the relatively low biomass of these groups indicates that most data is not even being identified to genus level and is probably being placed in the Squalidae family group.

In this work the two *Etmopterus* species studied were well separated by multivariate analysis. Each morphometric measurement contributed relatively little for the overall differences, but when all these small differences were considered together it was possible to separate the species.

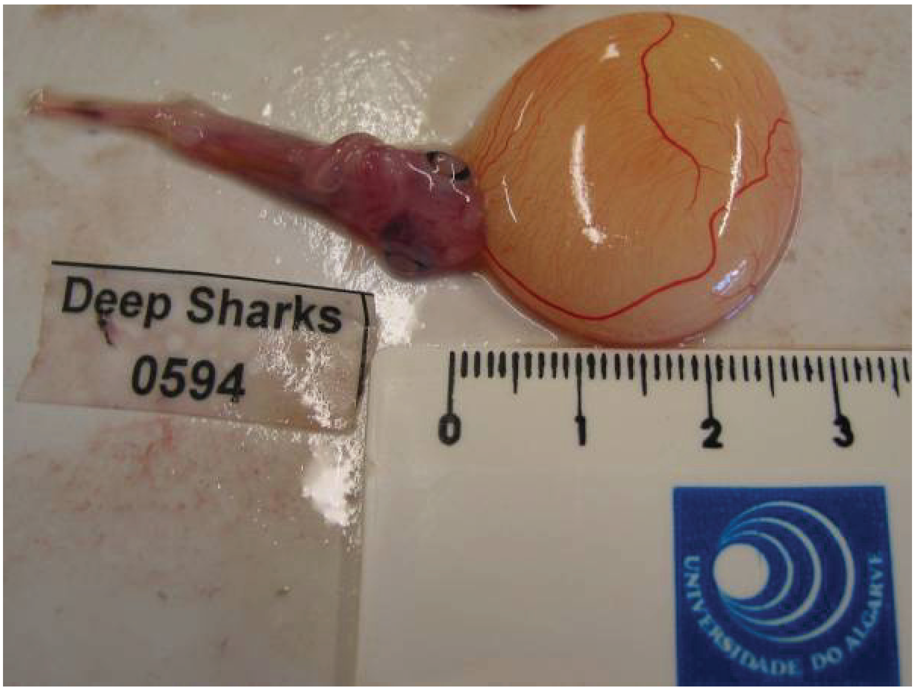
The discriminant analysis carried out is typically used to determine which variables discriminate between two or more naturally occurring groups (StatSoft, 2004). In this study, it was possible to create a model that could effectively separate between the two species, in this case using a backward stepwise approach that used only 6 of the 27 possible morphometrical measurements. The *post hoc* tests, used to compare what was observed to what was being predicted by the model showed an accuracy of 100%, meaning that the model created accurately identified all the specimens used for this study. Four morphometric measurements were common both to the SIMPER and to the discriminant analysis and that two of them relate the proportion between the specimen length and the caudal fin length. This fact is important since these measurements are relatively easy to obtain: even in photographed specimens, these measurements can be estimated with relative ease and eventually allow for a posterior identification or confirmation of the species.

Some measurements, such as the abdomen width were clearly not adequate for separating these species. For these particular measurements, there is large intra-specific variability that is mostly related with sex and maturity stage and not so much with species characteristics. Once maturity is achieved, the abdomen width of females increases proportionally more than that of males due to the presence of large oocytes or embryos, and this intra-specific variability prevents these measurements from reflecting specific species characteristics.

No sexual dimorphism was detected in these species. Sexual dimorphism in terms of maximum sizes of Squalidae sharks is common with females usually attaining larger sizes than males and heavier weights for the same size (e.g. Ford, 1921; Wetherbee, 1996; Girard and Du Buit, 1999; Coelho and Erzini, 2005; McLaughlin and Morrissey, 2005). However, the results presented here evidence that even though females might reach larger sizes than males, the body proportions are maintained and therefore no sexual dimorphism is observed, once the effect of growth is removed.

The two *Etmopterus* species studied have overlapped distributions, both in terms of depth and geographic areas, so most fisheries and by-catch studies carried out in the outer shelves and slopes of the eastern Atlantic Ocean are likely to catch both. With this work, a relatively easy way to separate both species based on morphometric traits that can inclusively be applied after the biological sampling procedures, using detailed photographs of the specimens, was demonstrated.

CHAPTER 4: Population parameters of the smooth lanternshark, *Etmopterus pusillus*, in southern Portugal (NE Atlantic) [*][†]



(Lantern shark embryo)

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[*] Coelho, R. & Erzini, K. 2007. Population parameters of the smooth lanternshark, *Etmopterus pusillus*, in southern Portugal (NE Atlantic). *Fisheries Research*, 86 (1): 42-57. DOI:10.1016/j.fishres.2007.04.006

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4.1. Abstract

Etmopterus pusillus is a deep water lantern shark with a widespread global distribution that is caught in large quantities in some areas, but is usually discarded due to the low commercial value. In this work, the population biology was studied and life history parameters determined for the first time in this species. Age was estimated from sections of the 2nd dorsal spine and validated by marginal increment analysis. Males attained a maximum age of 13 years while 17 year old females were found. Several growth models were fitted and compared for both size and weight at age data, showing that even though this is a small sized species, it has a relatively slow growth rate. This species matures late and at a relatively large size: at 86.8% (38.0 cm) and 79.4% (43.6 cm) of the maximum observed sizes and at 58.0% (7.1 yr) and 54.4% (9.9 yr) of the maximum observed ages for males and females respectively. It has a low fecundity, with a mean ovarian fecundity of 10.44 oocytes per reproductive cycle. The estimated parameters indicate that this species has a vulnerable life cycle, typical of deep water squalid sharks. Given the high fishing pressures that it is suffering in the NE Atlantic, this smooth lanternshark may be in danger of severe declines in the near future.

4.2. Introduction

The smooth lantern shark, *Etmopterus pusillus* is a small sized, globally widespread, deep water squalid shark. In the western Atlantic it has been recorded in the northern area of the Gulf of Mexico and between southern Brazil and Argentina (Compagno, 1984). In the eastern Atlantic it has been recorded from Portugal (Saldanha, 1997) to Namibia. In the western Indian Ocean it has been described for South Africa and in the western Pacific in Japan (Compagno, 1984). This species lives mainly in the continental and insular shelves and upper slopes, at depths from 274 to 1000m, but has also been described in oceanic waters, between Argentina and South Africa (Compagno, 1984).

The family Etmopteridae is the largest squaloid shark family, with more than 50 species in 5 genera. The *Etmopterus* genus is the most speciose genus in this family, currently with 31 valid species (Compagno *et al.*, 2005). Most of the species in this genus are either endemic or very limited in their distribution, and *E. pusillus* is one of the few with a cosmopolitan distribution (Compagno *et al.*, 2005). However, it is particularly abundant only in some areas, such as off the south and southwestern coasts of Portugal. Here, this species, along with *Etmopterus spinax*, comprise the two most abundant lantern sharks (Coelho *et al.*, 2005).

In Portugal, *E. pusillus* is commonly caught as by-catch and discarded by several deep water fisheries that operate in the area, namely the deep bottom trawl fishery that targets Norway lobster, *Nephrops norvegicus*, deepwater rose shrimp, *Parapenaeus longirostris*, and red shrimp, *Aristeus antennatus*, (Monteiro *et al.*, 2001) and the deep water longline fishery that targets wreckfish, *Polyprion americanus*, European-conger, *Conger conger*, and European hake, *Merluccius merluccius* (Coelho *et al.*, 2005). However, even though *E. pusillus* is caught in large quantities, it has a very low or no commercial value and is therefore usually discarded (Monteiro *et al.*, 2001; Coelho *et al.*, 2005). Therefore, this species is never landed and is never accounted for in the official fisheries statistics, limiting the availability of data for monitoring its fisheries mortality and assessing its population status.

Although *E. pusillus* is widespread, little biological information exists on this species. Coelho and Erzini (2005) report that this is an aplacental viviparous species, and present preliminary lengths-at-maturity, but no other biological information is available. We believe that this is the first study that focuses on detailed life history parameters of this species.

Given the relatively high levels of fishing mortality that this species is currently suffering and that this is a deep water squalid shark, a family of sharks generally characterized by very vulnerable life cycles, with very slow growth rates, late maturities and low reproductive potential (Fowler *et al.*, 2005), there is an urgent need for biological studies of this species. The objectives were to study various aspects of the population biology of *E. pusillus*, especially growth, maturity and fecundity. The data presented here will be useful for modeling purposes (e.g. risk analysis), for monitoring this population's evolution in the future and may serve as a basis for comparison with other studies on this species in other areas.

4.3. Material and methods

4.3.1. Biological sample

Specimens were caught on a monthly basis, from February 2003 to June 2004, except for March 2003, as by-catch of commercial fishing vessels, namely deep water trawlers and deep water longliners. The commercial longliners usually operated on rocky bottoms to catch demersal bony fishes such as *P. americanus* and *C. conger*, while the commercial trawlers operated on muddy and sandy bottoms, targetting crustaceans such as *N. norvegicus*, *P. longirostris* and *A. antennatus*. Additionally, specimens were obtained from the Portuguese Fisheries Institute (INIAP – IPIMAR) deep water trawl research cruise during the summer of 2003. Samples came from fishing that took place at depths from 245m to 745m (Figure 4.1).

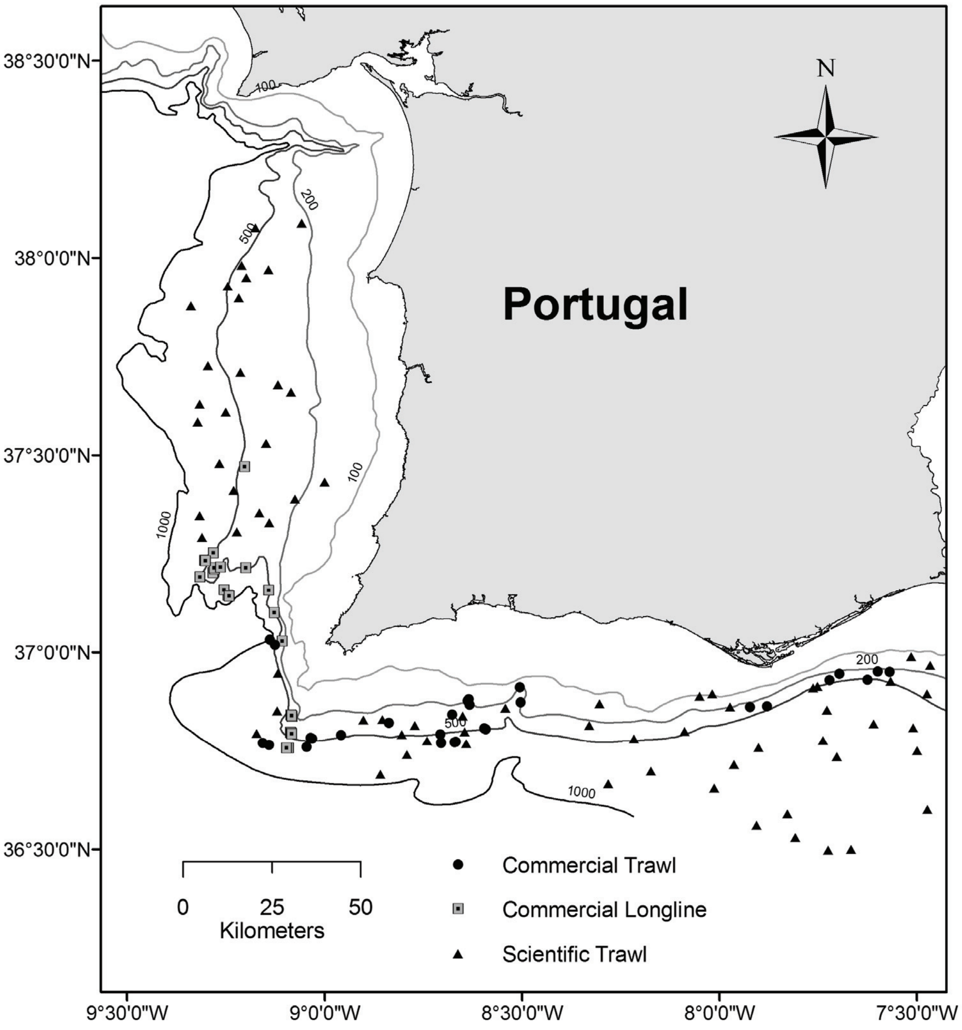

Figure 4.1. Map of the south and southwestern coasts of Portugal with the location of the coastline, the bathymetric lines (100, 200, 500 and 1000m depths), the commercial fishing operations and the research survey tows. Bathymetric lines and coastline adapted from "Atlas do Ambiente Digital – Instituto do Ambiente".

All specimens caught were brought to the laboratory where a series of external body measurements were taken to the nearest lower millimetre, namely the total length (TL), measured in a straight line from the tip of the snout to the tip of the caudal fin in its natural position, the fork length (FL), measured form the tip of the snout to the caudal fin fork, the pre-caudal length (PCL), measured from the tip of the snout to the beginning of the upper lobe of the caudal fin and the body girth (GIR) measured around the body at its widest area. Total weight (W) and eviscerated weight

(Wev) were recorded to the nearest centigram. After dissection, the gonads and the liver were weighed to the nearest centigram. Additionally, electronic callipers were used to measure the inner clasper lengths in males, from the vent opening to the tip of the claspers, and the uterus diameter of females in its widest area, to the nearest 0.01 mm. The diameter of ripe oocytes in mature females and the length and weight of embryos in pregnant females were also measured, using a digital caliper with 0.01 mm precision and a digital scale with 0.01g precision.

4.3.2. Morphometric relationships

The relationships between the explanatory variable TL (in cm) and each of the dependant variables FL, PCL and GIR (in cm) without any data transformation, and between the explanatory variable TL and the dependant variables W and Wev (in g) with natural logarithm transformed data were explored by linear regression. Standard errors were calculated for all the estimated parameters, along with the coefficient of determination (r^2) of each regression. Linear regressions were carried out for males and females separately, and analysis of covariance (ANCOVA), using TL as the covariate, used for comparing the two sexes (homogeneity of the regressions).

4.3.3. Age estimation and validation

Linear regression was used to assess if there were relationships between specimen growth and dorsal spines growth, an assumption needing verification if age is to be determined in these structures. Linear models were fitted with TL (cm) as the explanatory variable and various variables representing the growth of both the 1st and the 2nd dorsal spines, namely total spine length (TSL, in mm), measured from the spine tip to the anterior side of the spine base and the spine base width (SBW, in mm), measured as the diameter of the spine at its base (Clarke and Irvine, 2006). Linear regression was also used to explore the relationship between natural logarithm transformed TL and spine weight (SW, in mg). Standard errors were calculated for all the estimated parameters and r^2 values determined. Linear regressions were carried out for males and females separately, with analysis of covariance (ANCOVA), using TL as the covariate for comparing the two sexes (homogeneity of the regressions). All spine measurements were taken with digital calipers with 0.01 mm precision and weights recorded using electronic precision (0.1 mg) scales.

Age was estimated by direct counting of the bands formed in the inner dentine layer of the 2nd dorsal spines. A band was defined as a pair made by an opaque and a translucent band. The spines were initially cleaned by removing most of the organic tissue with scalpels and scissors. After, the spines were immersed in a sodium hypochlorite (10%) solution, between 2 to 10 minutes, depending on the size of the structure and until all organic tissue was cleaned. The spines were then rinsed in tap water for 1 minute and left immersed in distilled water for 30 minutes to remove all traces of sodium hypochlorite. The cleaned spines were then stored dry until ready to be processed.

Once dry, the spines were embedded in epoxy resin, in individual plastic moulds. After a 24 hour period for complete drying of the epoxy, these moulds were placed in a Buehler Isomet low speed sawing machine using a series 15LC diamond blade to cut 3 transverse 500 μm sections. Spines were sectioned on the exterior spine region (Irvine *et al.*, 2006a), with the distance of the section to the spine tip varying with spine length. The sections were mounted in microscope glass slides with DPX, and observed in a Zeiss Axiolab binocular microscope with 100x amplification under transmitted white light.

The sharpest section of each specimen was micro-photographed with a digital Canon G2 photographic machine mounted in the microscope. All photographs were taken in the aperture priority mode, with this value set manually to the lowest possible aperture (highest F value, in this case F8.0), to increase the depth of field and decrease the possibility of blurring the photographs. To reduce photo noise, the ISO value was set manually to ISO50 and a manual light exposition compensation of -2/3 EV was set to compensate for possible over-exposure.

All photos were processed and analyzed in the Image Pro Plus 4.5 software. Measurements of the spine radius in the area where the bands were observed were taken and linear regressions established. These regressions were carried for each sex separately and compared with ANCOVA, using TL as the covariate.

Preliminary tests were made on vertebrae to assess their possible use for age confirmation, but given that no bands were visible, even after using the alizarin red S band enhancing technique (La Marca, 1966), no further processing or analysis of these structures was carried out.

Age was estimated by a single reader who made three independent readings of each structure. To reduce possible sources of bias, these readings took place at least one month apart and no information regarding specimen characteristics or previous readings was available during each reading. An age class was only attributed to a specimen if at least 2 of the 3 age estimations were concordant.

The precision of the age estimates, defined as the reproducibility of repeated measurements on a given structure (Campana, 2001) was determined by several different techniques. The percent agreement, a technique that determines the percentage of age estimations that agree entirely, that agree within ±1 year, ± 2 years, ± 3 years and so on was used. Given that this technique is dependant on the age estimate, meaning that similar values of percent agreement can have different meanings depending on the range of the age estimates, alternative techniques were also used, namely the average percent error (APE) defined by Beamish and Fournier (1981) and the coefficient of variation (CV) and the index of precision (D) defined by Chang (1982).

The periodicity of the formation of band patterns was validated by using the relative marginal increment analysis, expressed as:

$$MIR = \frac{(R - R_n)}{(R_n - R_{n-1})}$$

where *MIR* is the marginal increment ratio; *R* is the radius of the structure, *Rn* is the distance to the outer edge of the last complete band and *Rn−1* is the distance to the outer edge of the next-to-last complete band.

The monthly evolution of the mean MIR was plotted to determine trends in band formation throughout the year. An analysis of variance (ANOVA) was used to test for differences in the MIR values along the year and the multiple comparisons Tukey pairwise test used to assess differences between pairs of months.

4.3.4. Growth modelling

Modeling of growth in length was based on four relatively commonly used models, namely the Von Bertalanffy Growth Function (VBGF), a modified version of the VBGF with known size at birth, the Gompertz model and the logistic equation.

The VBGF is one of the most used models to explain fish growth that can also be applied to other organisms such as cephalopods. The principle underlying this model is that growth rate of fishes tends to decrease linearly with size and it can be expressed as:

$$Lt = L_{\inf}(1 - e^{-k(t-t0)})$$

where Lt is the total length at age t, L_{inf} is the maximum asymptotic length, k is the growth coefficient and t_0 is the theoretical age when Lt =0

As an alternative to the traditional VBGF, a model with a fixed intercept of the length axis (known size at birth (L_0)) was used:

$$Lt = L_{\inf}(1 - be^{-kt})$$

where b = ($L_{\inf}$-L_0) / $L_{\inf}$

and L_0 is the size at birth, that in this species was estimated to be 12.8cm TL (SD = 0.6cm; n = 5), based on observations of totally formed embryos present in late term (stage 6) pregnant females.

The Gompertz growth model is a sigmoidal growth curve that assumes an exponential decrease of the growth rate with size and can be expressed as:

$$Lt = L_{\inf} e^{-e^{(-g(t-t0))}}$$

where g is the Gompertz growth coefficient.

The logistic equation can be expressed as:

$$Lt = L_{\inf} / (1 + ((L_{\inf} - L_0) / L_0)(e^{(-rt)}))$$

where L_0 is the theoretical length at birth and r is the logistic growth coefficient.

Weight-at-age data were modelled using the VBGF and the Gompertz model. The VBGF used with weight data can be expressed as:

$$Wt = W_{\inf}(1 - e^{-k(t-t0)})^b$$

where Wt is total weight at age t; W_{inf} is the maximum asymptotic weight and b is the allometric growth coefficient from the TL-W relationship (3.345 and 3.431 for males and females, respectively). The Gompertz model for weight-at-age data is the same as the one used with length-at-age.

Each model was fitted by non-linear least squares regression, with iterations by the Levenberg-Marquardt algorithm in the STATISTICA 6 software (StatSoft, 2004) and the r^2 value calculated. For each model, growth was estimated for males and females separately and compared using the maximum likelihood test (Kimura, 1980).

Model comparison and selection was based on the small sample corrected form of the Akaike information criterion (AICc) (Shono, 2000), that for the least squares fit can be expressed as:

$$AICc = \frac{RSS}{n} + \frac{2k(k+1)}{n-k-1}$$

where *RSS* is the residual sum of squares, *n* is the number of observations and *k* is total number of estimated regression parameters that equals the number of parameters in the model + 1.

The model with the smallest AICc value was selected as the “best” model (AICc, min) and the differences between this “best” model and all others expressed as:

$$\Delta i = AICc,i - AICc,\ min$$

4.3.5. Reproductive cycle

Maturity stages were defined for both males and females based on the macroscopic observations of the reproductive organs of the specimens. Males were divided in 4 stages, where stage 1 and 2 represent immature specimens and stage 3 and 4 represent mature specimens (Table 4.1). Females were divided in 7 stages, where stages 1 and 2 represent immature specimens and stages 3 to 7 represent mature specimens. In females, stages 1 to 3 represent the ovarian stages and stages 4 to 7 the uterine stages (Table 4.1). In this species, ovarian and uterine phases of mature females are independent and do not occur at the same time. Although no stage 4 females were found, we assume that it is part of this species life cycle given that it has been described for other aplacental viviparous squalid sharks (Clarke *et al.*, 2001; Jakobsdottir, 2001).

Table 4.1. Macroscopically defined maturity stages for males and females of *Etmopterus pusillus*.

Males	
Stage 1: immature	Underdeveloped and soft claspers, shorter than the pelvic fins. Small whitish threadlike gonads. Sperm duct narrow and straight.
Stage 2: maturing	Claspers developing, longer than the pelvic fins, but still soft. Gonads enlarged. Sperm duct starting to coil.
Stage 3: mature	Claspers fully formed, and stiff. Large and rounded gonads, full of sperm. Sperm ducts coiled.
Stage 4: active	Claspers stiff and swollen. Large and rounded gonads, full of sperm. Sperm flowing under pressure.
Females	
Ovarian phases	
Stage 1: immature	Small ovaries without differentiated oocytes. Uterus threadlike.
Stage 2: maturing	Ovaries a little larger, with oocytes starting to differentiate, but still small in size. Uterus a little wider, but still narrow.
Stage 3: mature	Large ovaries, with large and well differentiated ripe oocytes, orange-yellowish in colour.
Uterine phases	
Stage 4: early pregnancy	Uterus filled with non-segmented and undifferentiated yolky content.
Stage 5: middle pregnancy	Uterus with small embryos, with yolk sacs attached and filled with yolky content.
Stage 6: late pregnancy	Uterus with fully formed embryos, with reduced or inexistent yolk soc.
Stage 7: resting	Ovaries resting with immature oocytes, similar to stage 2. Uterus empty but considerably dilated, sometimes with blood traces from the parturition.

The percentage of each maturity stage throughout the year for both males and females was plotted, in order to assess if different stages were occurring predominantly during a specific season or period.

The gonadosomatic index (GSI) and the hepatosomatic index (HSI) were calculated for all specimens and the means for each maturity stage in each sex plotted. These indices were calculated as:

$$\mathrm{GSI} = \frac{\text{Gonad weight (g)}}{\text{Wev (g)}} \times 100$$

$$\mathrm{HSI} = \frac{\text{Liver weight (g)}}{\text{Wev (g)}} \times 100$$

Kruskal–Wallis and pairwise Dunn tests were used to test if significant differences occurred between the different maturity stages.

4.3.6. Maturity

For the purpose of the maturity estimations, the reproductive stages of the specimens were grouped in either mature or immature. Considering that a mature specimen is a specimen that is able to reproduce or has done so in the past (Conrath, 2004), stages 1 and 2 in both males and females were considered immature and the following stages considered mature. Box and whiskers plots were used to plot the means, standard deviations and ranges for both size and age of mature and immature specimens of each sex. A two way ANOVA was used to test for differences in these mean sizes and ages.

The proportion of mature individuals by 1 cm TL size classes was used to fit length based maturity ogives and to estimate the size at maturity (TL at which 50% of the individuals are mature). The logistic curve was fitted by non-linear least squares regression, using the Levenberg-Marquardt algorithm, in the STATISTICA 6.0 software (StatSoft, 2004) by:

$$P_{Li} = \frac{1}{1 + e^{-b(L_i - L_{50})}}$$

where P_{Li} is the proportion of mature individuals in the size class Li, b is the slope and L_{50} is the size where 50% of the individuals are mature.

The same procedure was followed to estimate age at maturity (age at which 50% of the individuals are mature, using the equation:

$$Page_i = \frac{1}{1 + e^{-b(age_i - age_{50})}}$$

where $Page_i$ is the proportion of mature individuals in age class age_i, b is the slope and age_{50} is the age where 50% of the individuals are mature.

The standard errors and the lower and upper limits of the 95% confidence intervals were calculated for each estimated parameter. Length and age based maturity ogives were fitted to males and females separately, and the maximum likelihood test (Kimura, 1980) used to test for differences between sexes.

Sexual characters such as clasper length in males and uterus width in females were used to confirm the maturity estimated by the ogives. Given that both are paired structures, both the left and the right side structures were measured and compared with ANCOVA tests, using TL as the covariate. Once it was determined that there were no differences between the structures of the two sides, a mean clasper length and uterus width was calculated respectively for each male and female and plotted against TL so that relative growth with size of the structure could be observed.

4.3.7. Fecundity

Total fecundity was estimated by direct methods, by counting both the number of oocytes in mature stage 3 females and the number of mid-term embryos in pregnant females in stage 5 (Conrath, 2004). Pregnant females in stage 6, with near-term embryos were not considered for this part of the study, given the possibility that some of the pups may have already been born at the time of capture, resulting in underestimation of fecundity.

4.4. Results

4.4.1. Biological sample

A total of 614 specimens (252 females and 362 males) was caught for this study during the sampling period, with 571 specimens caught by the commercial fisheries and 43 specimens caught during the INIAP – IPIMAR research cruise. A sub-sample of 546 specimens was used for the age and growth study, while for some morphometric relationships 16 additional specimens caught outside the sampled period were also used. Both male and female samples had a wide length range, covering most of the length range described for this species. Females attained slightly larger sizes than males. Specifically, female lengths varied from 15.9 to 50.2 cm TL while males ranged in length from 15.8 to 47.9 cm TL (Figure 4.2).

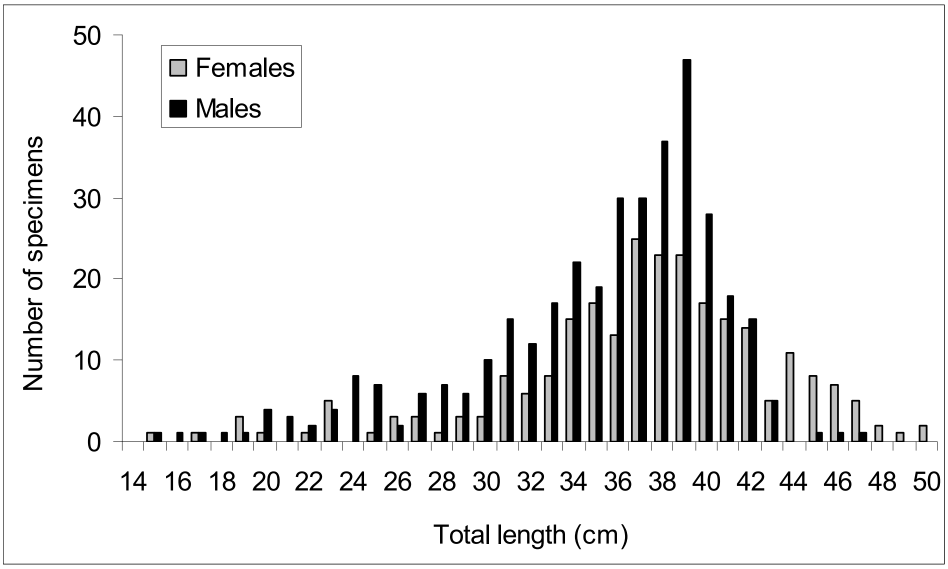

Figure 4.2. Size distribution, by 1cm TL size class, of the sample of *Etmopterus pusillus* used in this study.

4.4.2. Morphometric relationships

The morphometric relationships are presented in Table 4.2. No significant differences between sexes were detected for the TL-FL (ANCOVA: F = 0.02; P-value = 0.884) and TL-PCL (ANCOVA: F = 0.15; P-value = 0.701) relationships, and therefore regressions for both sexes combined were carried out. For all other regressions significant differences were detected between sexes ($\text{ANCOVA}_{\text{TL-GIR}}$: F = 17.20; P-value < 0.001; $\text{ANCOVA}_{\text{TL-W}}$: F = 94.52; P-value < 0.001; $\text{ANCOVA}_{\text{TL-Wev}}$: F = 91.22; P-value < 0.001).

Table 4.2. Linear regressions between TL and FL, PCL, GIR, W and Wev in *Etmopterus pusillus*, indicating the total sample (n), the type of data transformation, the range of the explanatory variable (cm) and the intersect (a) and the slope (b) of the linear regression, with the respective standard errors (SE). The coefficient of determination (r^2) of each regression is also given.

Relation	Transf.	Sex	Sample characteristics		Parameters of the relationship				
			n	Range (exp. var.)	a	b	$SE_{(a)}$	$SE_{(b)}$	r^2
TL - FL	None	Males	288	15.8 - 46.3	-0.474	0.907	0.131	0.004	0.995
		Females	221	19 - 50.2	-0.553	0.907	0.153	0.004	0.996
		Combined	509	15.8 - 50.2	-0.475	0.906	0.098	0.003	0.996
TL - PCL	None	Males	283	15.8 - 46.3	-0.909	0.825	0.170	0.005	0.991
		Females	215	19 - 50.2	-1.123	0.828	0.240	0.006	0.988
		Combined	498	15.8 - 50.2	-0.932	0.825	0.139	0.004	0.989
TL - GIR	None	Males	287	15.8 - 46.3	-2.834	0.393	0.367	0.010	0.841
		Females	221	19 - 50.2	-5.098	0.462	0.528	0.014	0.839
TL - W	Nat. Log	Males	370	15.8 - 46.3	-6.793	3.345	0.109	0.031	0.970
		Females	260	15.9 - 50.2	-7.071	3.431	0.146	0.040	0.966
		Combined	630	15.8 – 50.2	-6.956	3.394	0.088	0.024	0.968
TL - Wev	Nat. Log	Males	369	15.8 - 46.3	-6.321	3.121	0.096	0.027	0.973
		Females	258	15.9 - 50.2	-6.500	3.180	0.112	0.031	0.976

4.4.3. Age estimation and validation

There is a clear relation between the growth in weight and size of the spines with TL (Table 4.3). No differences between sexes were detected for the relations between TL and TSL and between TL and SBW for both the first and the second spines (ANCOVA $_{TL\text{-}TSL1}$: F < 0.01; P-value = 0.968; ANCOVA $_{TL\text{-}SBW1}$: F = 0.65; P-value = 0.421; ANCOVA $_{TL\text{-}TSL2}$: F = 0.52; P-value = 0.470; ANCOVA $_{TL\text{-}SBW2}$: F = 0.60; P-value = 0.439), so measurements for both sexes were combined. Differences between sexes were detected for both spines for the regressions between TL and SW, (ANCOVA $_{TL-SW1}$: F = 9.16; P-value = 0.003; ANCOVA $_{TL-SW2}$: F = 8.39; P-value = 0.004). The relationship between TL and the radius of the spine section where age was estimated was also linear. Given that no differences were detected between males and females (ANCOVA: F < 0.001; P-value = 0.945), a regression for both sexes combined was estimated (Figure 4.3).

Table 4.3. Linear regressions between TL and total spine length (TSL), spine base width (SBW) and spine weight (SW) for both 1st and 2nd dorsal spines of *Etmopterus pusillus*. The total sample size (n), the type of data transformation, the range of the explanatory variable (cm) and the intersect (a) and the slope (b) of the linear regression, along with the respective standard errors (SE) and the coefficient of determination (r^2) of each regression are given.

Relation	Transf.	Sex	Sample characteristics		Parameters of the relationship				
			n	Range (exp. var.)	a	b	$SE_{(a)}$	$SE_{(b)}$	r^2
TL – TSL1	None	Males	285	15.8 - 46.3	0.570	0.489	0.577	0.016	0.764
		Females	196	15.9 - 48.5	0.837	0.490	0.823	0.022	0.722
		Combined	481	15.8 - 48.5	0.541	0.494	0.473	0.013	0.752
TL – SBW1	None	Males	289	15.8 - 47.9	-0.012	0.085	0.118	0.003	0.701
		Females	200	15.9 - 48.5	-0.056	0.089	0.147	0.004	0.727
		Combined	489	15.8 - 48.5	-0.079	0.088	0.092	0.003	0.719
TL – SW1	Nat. Log.	Males	288	15.8 - 46.3	-6.520	2.670	0.206	0.058	0.881
		Females	199	15.9 - 48.5	-6.670	2.725	0.284	0.079	0.859
TL – TSL2	None	Males	308	15.8 - 47.9	3.222	0.680	0.636	0.018	0.827
		Females	226	15.9 - 50.2	3.142	0.699	0.813	0.021	0.827
		Combined	534	15.8 - 50.2	2.825	0.698	0.500	0.014	0.832
TL – SBW2	None	Males	311	15.8 - 47.9	0.376	0.080	0.113	0.003	0.676
		Females	229	15.9 - 50.2	0.255	0.084	0.127	0.003	0.738
		Combined	540	15.8 - 50.2	0.317	0.082	0.083	0.002	0.712
TL – SW2	Nat. Log.	Males	310	15.8 - 46.3	-5.436	2.663	0.170	0.048	0.909
		Females	232	15.9 - 48.5	-5.387	2.659	0.220	0.061	0.892

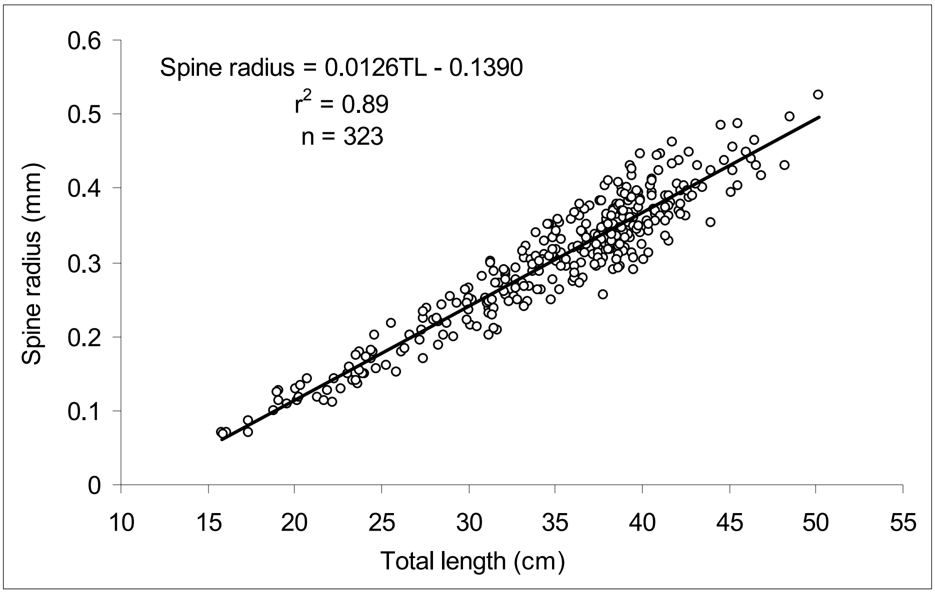

Figure 4.3. Relationship between total length of specimens and the radius of the spine sections where age was estimated for both sexes of *Etmopterus pusillus* combined. The regression equation with the respective coefficient of determination (r^2) and the sample size (n) are also given.

A clear pattern of alternating translucent and opaque band formation was visible on the spine sections (Figure 4.4). Age was determined successfully for 523 of 546 specimens (95.8%), with poor band discrimination (12 specimens) and lack of concordance on at least 2 of the 3 readings (11 specimens) accounting for the remainder. The percent of concordant ages in 0, ± 1, ± 2 and ± 3 years was 61.5%, 28.8%, 7.9% and 1.8% for males and 62.9%, 28.8%, 7.6% and 1.5% for females. The APE, V and D precision indices obtained were respectively 18.33, 19.79 and 11.43 for males and 11.41, 12.79 and 7.39 for females.

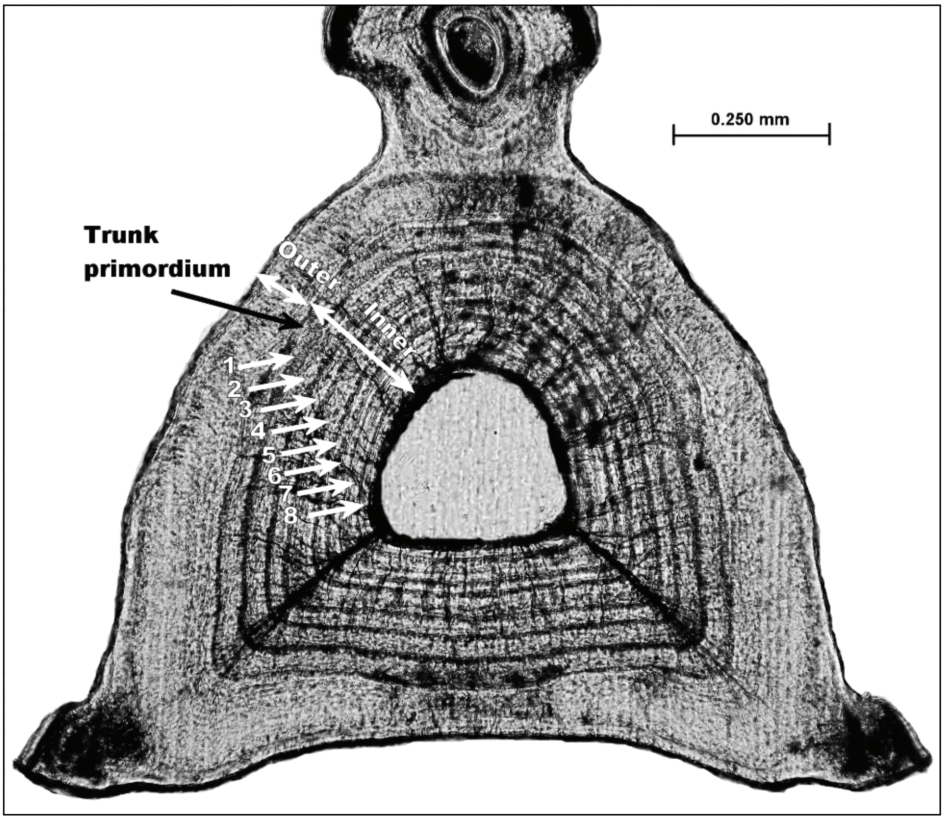

Figure 4.4. Microphotograph of a sectioned dorsal spine of a female *Etmopterus pusillus* with 40.4 cm total length and an estimated age of 8 years. It is possible to distinguish the inner trunk layer where the annual bands were counted and the outer trunk layer (without any growth bands and already present in late term embryos), as well as the trunk primordium.

Females had a wider age range than males. Estimated ages of females varied from 0 to 17 years while males ranged from 0 to 13 years. Overall, the female component of this population was older than the male component, since most of the females (87.1%) were between 4 and 11 years old while most of the males (92.0%) were between 2 and 9 years old (Figure 4.5). From 1 to 5 years of age there were no significant differences in the mean total length-at-age between sexes, while the older age classes showed significant differences, specifically for age class 5 and age classes 8 to 10 (Table 4.4). Differences for older specimens were not tested due to small sample sizes, especially for males.

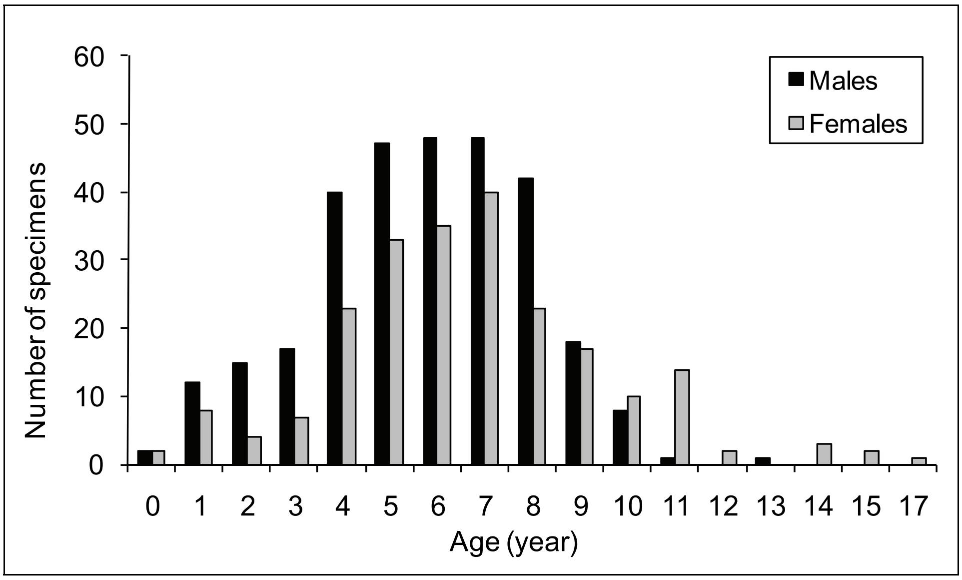

Figure 4.5. Age distributions of male and female *Etmopterus pusillus*.

Age was validated by the marginal increment analysis. There was a clear annual pattern of band formation, with the marginal increment showing progressively higher values from April to November. Between December and March, there was a sharp decrease of the MIR, indicating that the new band is formed during this season of the year. This pattern was visible for all age classes where the MIR was plotted, namely age classes 4, 5, 6, 7 and 8 (Figure 4.6). For the other age classes, this type of graph was not plotted due to the low sample size. Statistically significant differences were found between mean MIR values along the different months (ANOVA: F = 11.48; P-value < 0.001). The Tukey multiple comparison pairwise test showed that there were differences between only some of the possible pairs of months, specifically between some months with the lowest MIR values (February to March) and the months with the highest MIR values (June to November) (Tukey: P-values < 0.05 in all possible pairwise comparisons). The relatively low January MIR value also differed significantly from the highest MIR values of September and November (Tukey: P-value < 0.05 for all possible pairwise comparisons). No significant differences were detected between the other pairwise comparisons, involving months with mostly intermediate MIR values (Tukey: P-value > 0.05 for all possible pairwise comparisons).

Table 4.4. Comparison of the mean total length (TL, cm) between male and female *Etmopterus pusillus* for each age group (year). n refers to the sample size and SD to the standard deviation. The t-statistic value, the degrees of freedom (df) and the P-value are given.

Age	Females			Males			t - student test		
	n	Mean TL	SD	n	Mean TL	SD	t	df	P-value
0	2	16.65	1.06	2	15.95	0.21			
1	8	21.35	2.19	12	20.56	1.47	-0.97	18	0.344
2	4	24.26	1.35	15	24.51	0.89	0.45	17	0.658
3	7	28.59	1.85	17	28.00	1.61	-0.78	22	0.445
4	23	32.49	1.91	40	31.64	1.90	-1.70	61	0.094
5	33	35.41	2.01	47	34.47	1.79	-2.20	78	0.030
6	35	37.04	1.59	48	37.06	1.50	0.07	81	0.948
7	40	39.08	1.42	48	38.73	1.10	-1.29	86	0.200
8	23	40.81	1.32	42	40.13	1.21	-2.08	63	0.042
9	17	42.40	1.18	18	41.45	1.10	-2.46	33	0.019
10	10	44.47	1.29	8	42.55	0.72	-3.75	16	<0.01
11	14	45.98	0.81	1	41.50				
12	2	45.90	0.57						
13				1	46.30				
14	3	48.67	1.36						
15	2	48.80	0.85						
16									
17	1	50.20							

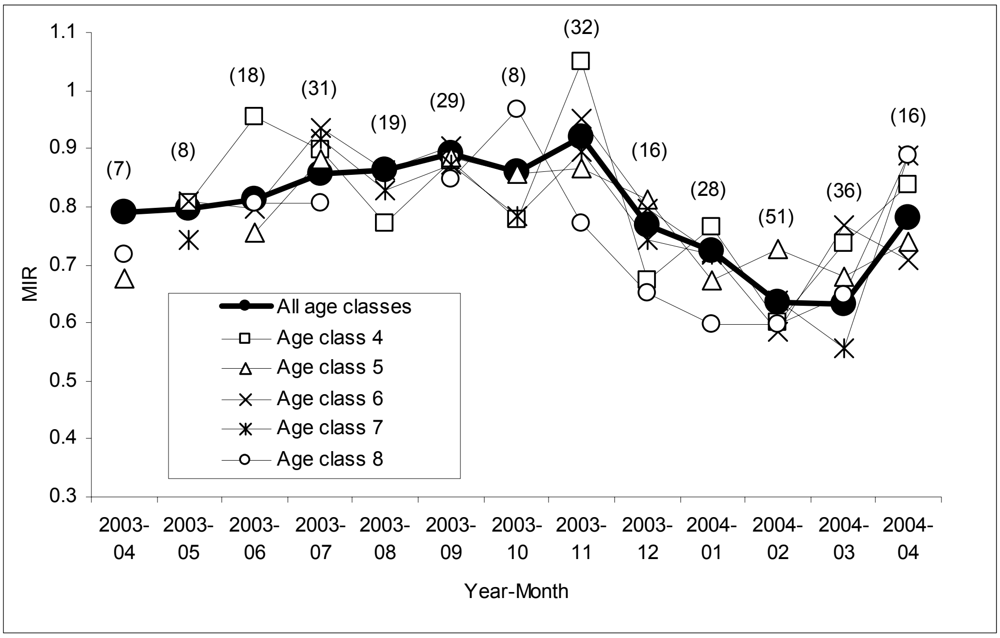

Figure 4.6. Monthly evolution of the marginal increment (MIR) both for all age classes combined and for each age class separately. The values in brackets refer to the total sample size (n) in each month.

4.4.4. *Growth modelling*

All four models gave good fits to the length at age data and produced very similar curves, both in the case of males and females. The VBGF curve with fixed L_0 intersected the TL axis at values lower than the others where this parameter was estimated (Figure 4.7). For weight based data, both curves were very similar, with the VBGF producing slightly higher estimates for older age classes (Figure 4.8).

Between sexes comparisons for each length based model showed significant differences between male and female growth curves (Max. Likelihood $_{\text{VBGF}}$: χ^2 = 52.13; P-value < 0.05; Max. Likelihood $_{\text{VBGF known L0}}$: χ^2 = 43.36; P-value < 0.05; Max. Likelihood $_{\text{Logistic}}$: χ^2 = 47.26; P-value < 0.05; Max. Likelihood $_{\text{Gompertz}}$: χ^2 = 47.07; P-value < 0.05). Between sexes comparisons for each weight based model also showed significant differences between male and female growth curves (Max. Likelihood

$_{\text{VBGF}}$: $\chi^2 = 161.02$; P-value < 0.05; Max. Likelihood $_{\text{Gompertz}}$: $\chi^2 = 157.50$; P-value $<$ 0.05).

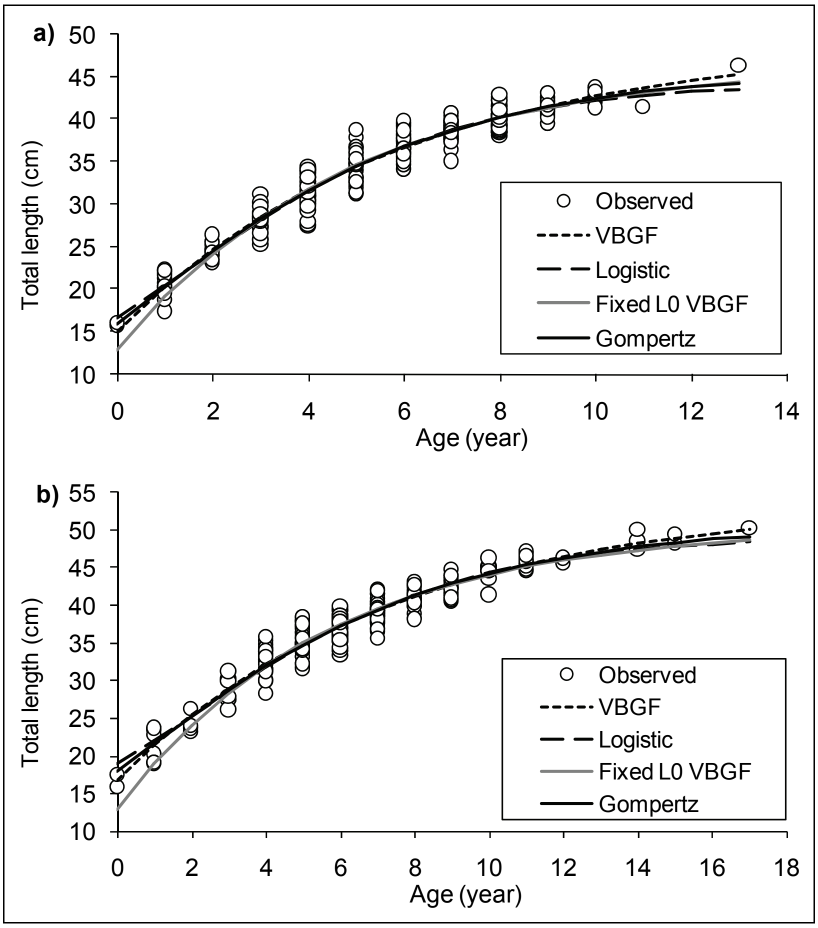

Figure 4.7. Length at age data for males (a) and females (b) of *Etmopterus pusillus*, with the respective fitted growth models.

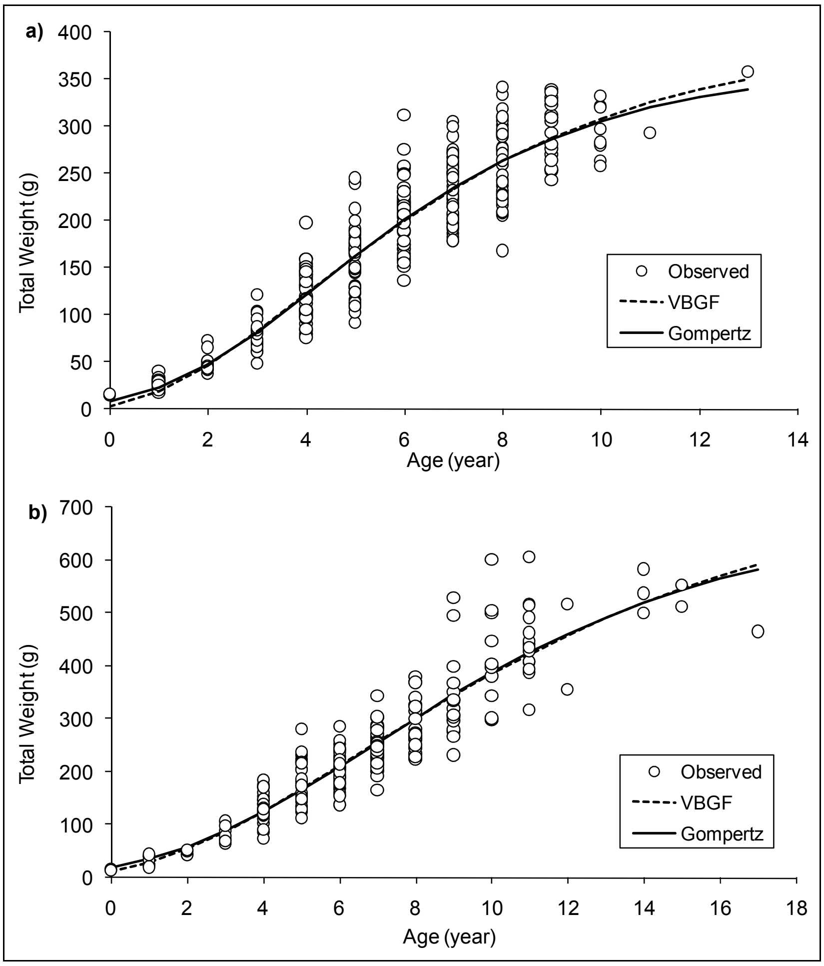

Figure 4.8. Weight at age data for males (a) and females (b) of *Etmopterus pusillus*, with the respective fitted growth models.

For both length at age and weight at age models, females had higher maximum asymptotic sizes and weights and lower growth coefficients than males. For length at age models, the logistic equation produced the lowest maximum asymptotic sizes and the VBGF the highest values, while for weight at age data, the fitted Gompertz model gave lower maximum asymptotic weights and higher growth rates than the VBGF for both sexes (Table 4.5).

Table 4.5. Comparison of parameters estimated with the different models for length at age and weight at age data, for male and female *Etmopterus pusillus*. The maximum asymptotic size is indicated in cm for the length at age models and g for the weight at age models. The growth coefficient refers to the parameters k (VBGF models), g (Gompertz model) and r (logistic model).

Data set	Sex	Model	Maximum asymptotic size (cm)				Growth coefficient (year $^{-1}$)			
			Estimated	SE	Lower 95% CI	Upper 95% CI	Estimated	SE	Lower 95% CI	Upper 95% CI
Length at age	Males	VBFG	49.01	0.99	47.07	50.96	0.17	0.01	0.15	0.19
		VBGF Fixed L0	46.81	0.56	45.71	47.91	0.20	0.01	0.19	0.22
		Logistic	44.30	0.47	43.38	45.22	0.35	0.01	0.32	0.38
		Gompertz	45.91	0.62	44.69	47.14	0.26	0.01	0.24	0.29
	Females	VBFG	54.04	1.16	51.75	56.34	0.13	0.01	0.12	0.15
		VBGF Fixed L0	50.51	0.67	49.19	51.83	0.18	0.01	0.16	0.19
		Logistic	49.25	0.65	47.96	50.53	0.27	0.01	0.24	0.29
		Gompertz	50.88	0.80	49.30	52.46	0.20	0.01	0.18	0.22
Weight at age	Males	VBGF	394.51	24.84	345.62	443.40	0.24	0.02	0.19	0.28
		Gompertz	363.39	18.18	327.60	399.17	0.31	0.03	0.26	0.36
	Females	VBGF	756.82	78.52	601.27	910.76	0.14	0.02	0.10	0.17
		Gompertz	679.28	54.90	571.08	787.48	0.18	0.02	0.15	0.22

For length at age data, all models fitted the data with high values of r^2 and $\Delta i < 2$ for all cases and both sexes. For weight based data, the r^2 values were similar for both models and sexes, but according to the *AICc*, the Gompertz model was more adequate for males while the VBGF model gave a better fit for females (Table 4.6).

Table 4.6. Values of the coefficient of determination (r^2), the small-sample corrected form of Akaike's information criterion (*AICc*) and the Akaike's differences (Δi) for each growth model, both in length and weight and for each sex. In each case, models are sorted from best to worst according to the *AICc*.

Data set	Sex	Model	r^2	*AICc*	Δi
Length at age	Males	Gompertz	0.938	2.22	0.00
		Logistic	0.938	2.23	0.01
		VBFG	0.937	2.26	0.03
		VBGF known L_0	0.934	2.33	0.10
	Females	VBFG	0.935	2.69	0.00
		Gompertz	0.933	2.78	0.09
		Logistic	0.929	2.93	0.24
		VBGF known L_0	0.923	3.08	0.39
Weight at age	Males	Gompertz	0.860	961.08	0.00
		VBFG	0.859	963.82	2.74
	Females	VBFG	0.848	2278.93	0.00
		Gompertz	0.845	2321.34	42.42

4.4.5. Reproductive cycle

The annual variation of the percentage of occurrence of the different maturity stages showed that mature females with ripe oocytes (stage 3) and pregnant females (stage 5 and 6) only occurred from November to April. During the rest of the year, only immature specimens (stages 1 and 2) or specimens in the resting phases (stage 7) were caught. In males, all the four stages occurred during the entire year, but the relative percentage of active males (stage 4) was a little higher during August and then from November to April (Figure 4.9).

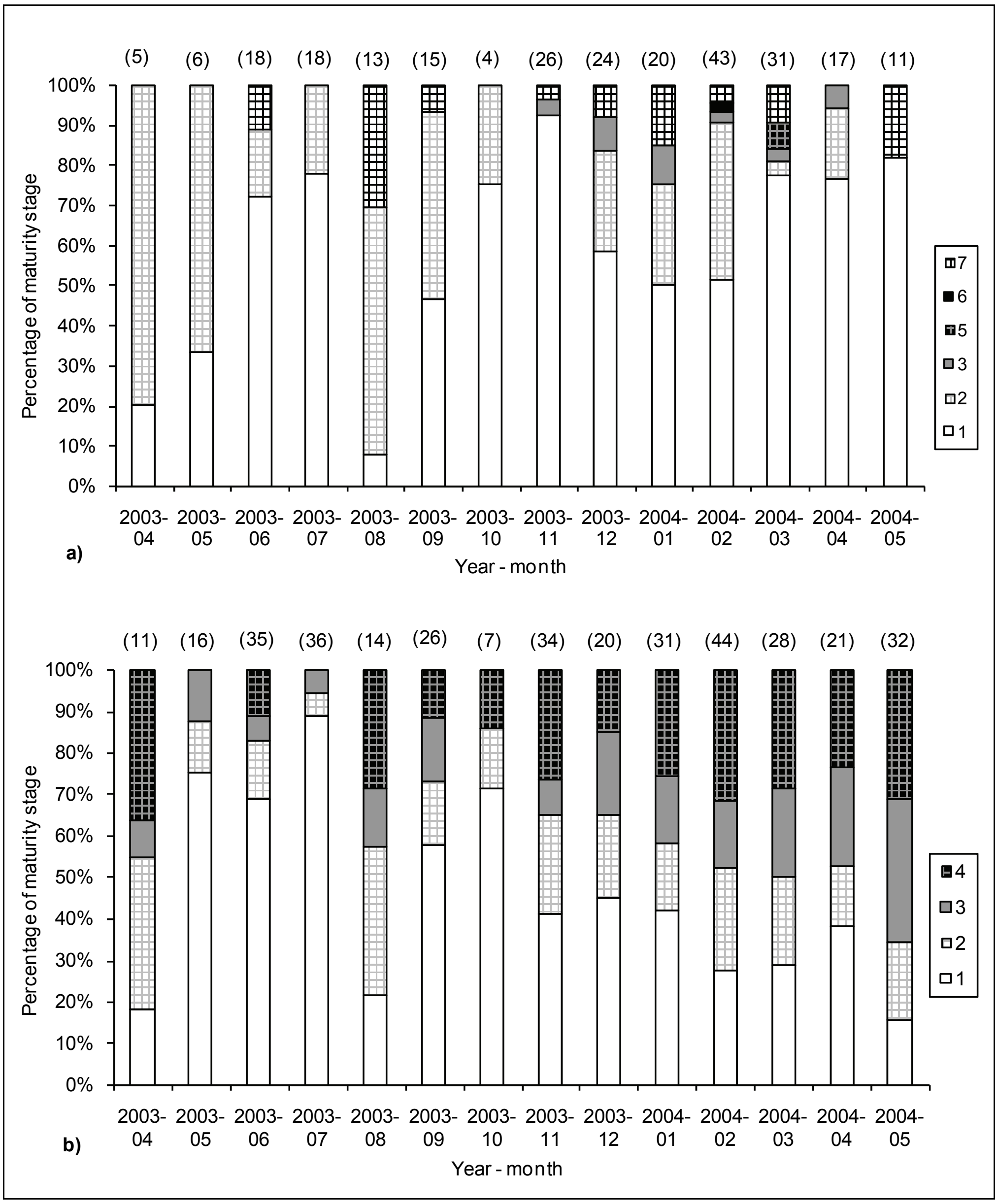

Figure 4.9. Annual variation of the percentage of occurrence of the different maturity stages in female (a) and male (b) *Etmopterus pusillus*. The values above each point are the sample sizes (n)

A clear evolution of the GSI was observed as the maturity stages of both males and females developed (Figure 4.10). In females, this index is very low while specimens are immature, due to the fact that the gonads have a very low relative weight. In stage 3, this index increases to the highest values, given that during this stage the gonads contain ripe oocytes. In pregnant females, this index falls again to values similar to those of immature specimens, indicating that this species has an alternate reproductive cycle, with the ovarian and the uterine phases occurring at separate times. In this type of reproductive cycle strategy, while females are pregnant the oocytes remain immature and the gonads do not develop, remaining relatively small. In stage 7 females, there is a slight increase of the GSI, probably due to the fact that in some specimens the oocytes are already starting to develop, in order to start a new ovarian cycle. Significant differences were found between the GSI values of the different maturity stages (Kruskal-Wallis: H= 105.29, P-value < 0.001). The pairwise Dunn test showed that significant differences occurred between stage 3 and all others (Dunn: P-values < 0.05), but not between the other possible pair wise combinations (Dunn: P-values > 0.05). In males, there is a progressive increase of this index with the evolution of the maturity stages. Significant differences were found (Kruskal-Wallis: H = 283.23; P-value < 0.001), and according to the pair wise multiple comparison test, significant differences occurred between all possible pairs (Dunn: P-values < 0.05 in all cases) except between stages 2 and 3 and between stages 3 and 4 (Dunn: P-values > 0.05).

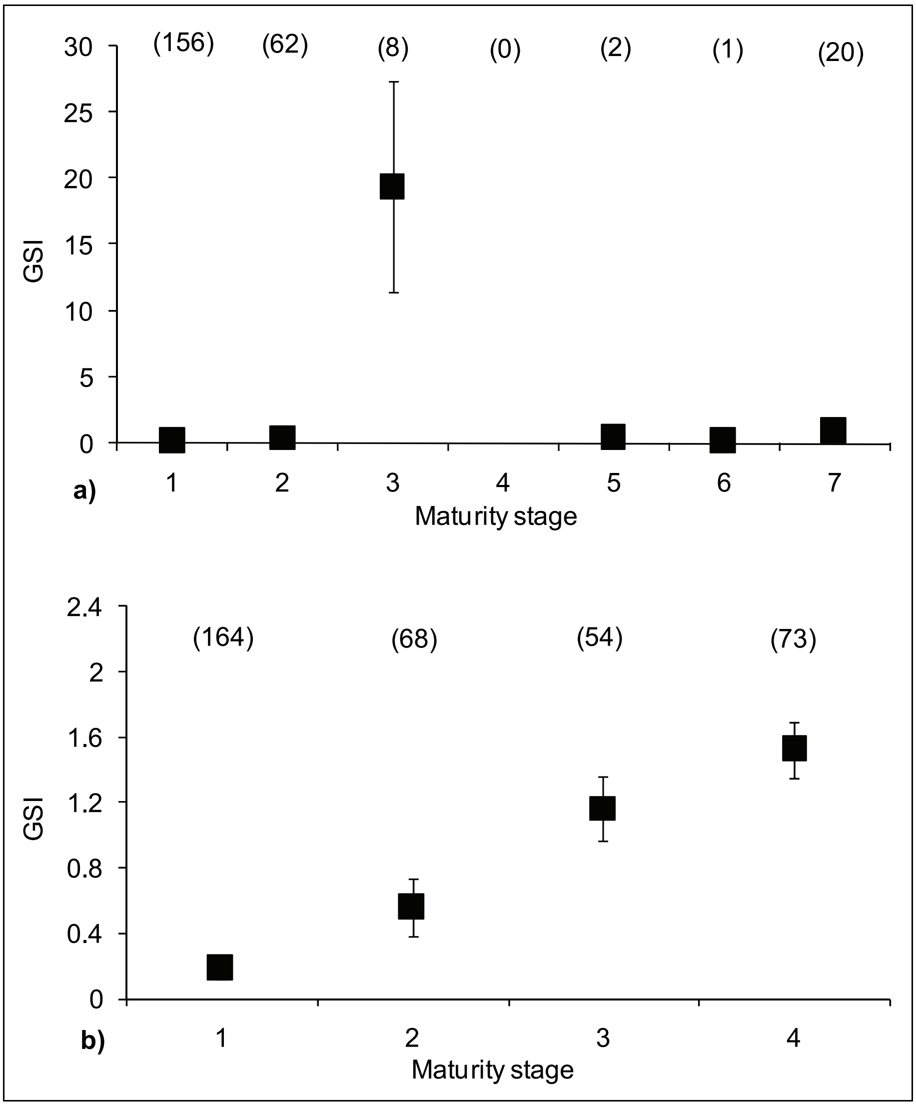

Figure 4.10. Evolution of the Gonadosomatic Index (GSI) for both female (a) and male (b) *Etmopterus pusillus*. Error bars represent ± 1 standard deviation. The values above each point are the sample sizes (n).

A clear evolution of HSI is also observed with the evolution of maturity stages in both males and females (Figure 4.11). It is possible to observe an increase in this index as females mature until they reach stage 3. While females are pregnant there is a decrease in this index, probably due to the high energy demand during this phase. In the resting phase, the index increases again, probably due to fact that the specimens are again starting to accumulate energy for the next reproductive cycle. The variations in HSI were significant (Kruskal-Wallis: H = 62.40; P-value < 0.001), with the pair wise tests showing differences between stage 1 and stage 3 and between stage 1 and stage 7 (Dunn: P-values < 0.05), but not for the other possible pairs (Dunn: P-value > 0.05). In males, there is a progressive increase of the HSI with the evolution of the maturity stage, with the differences more accentuated between stages 1 and 2 and more progressive for the other stages. Significant differences were found (Kruskal-Wallis: H = 172.07; P-value < 0.001), with significant differences between all possible pairs (Dunn: P-values < 0.05) except between pairs 2 and 3 and between pairs 3 and 4 (Dunn: P-values > 0.05).

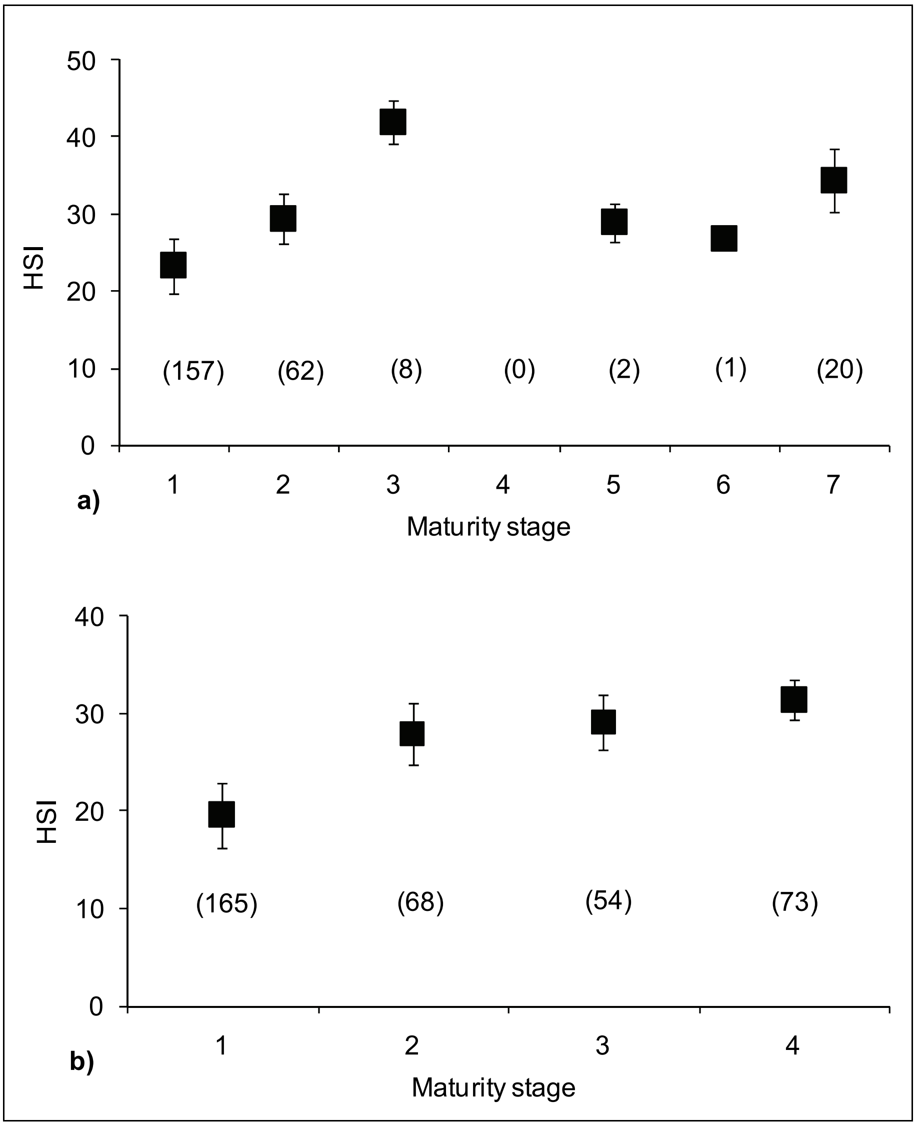

Figure 4.11. Evolution of the Hepatosomatic Index (HSI) for female (a) and male (b) *Etmopterus pusillus*. Error bars represent ± 1 standard deviation. The values below each point is the sample size (n).

4.4.6. Maturity

The maximum size of immature males was 41.7 cm while the smallest mature male was 35.4 cm in TL. The largest immature female was 47.0 cm TL and the smallest mature female had a TL of 41.9 cm (Figure 4.12). In terms of age, the oldest immature males were 9 years old and the oldest immature females were 11 years old. On the other hand, the youngest mature males and females were only 5 and 8 years old respectively (Figure 4.13). The 2 way ANOVA showed significant differences between sexes and for mature or immature condition, for both length (2 way ANOVA $_{\text{Sex}}$: F = 63.77; P-value < 0.001; 2 way ANOVA $_{\text{Maturity}}$: F = 199.49; P-value < 0.001) and age (2 way ANOVA $_{\text{Sex}}$: F = 103.07; P-value < 0.001; 2 way ANOVA $_{\text{Maturity}}$: F = 305.74; P-value < 0.001).

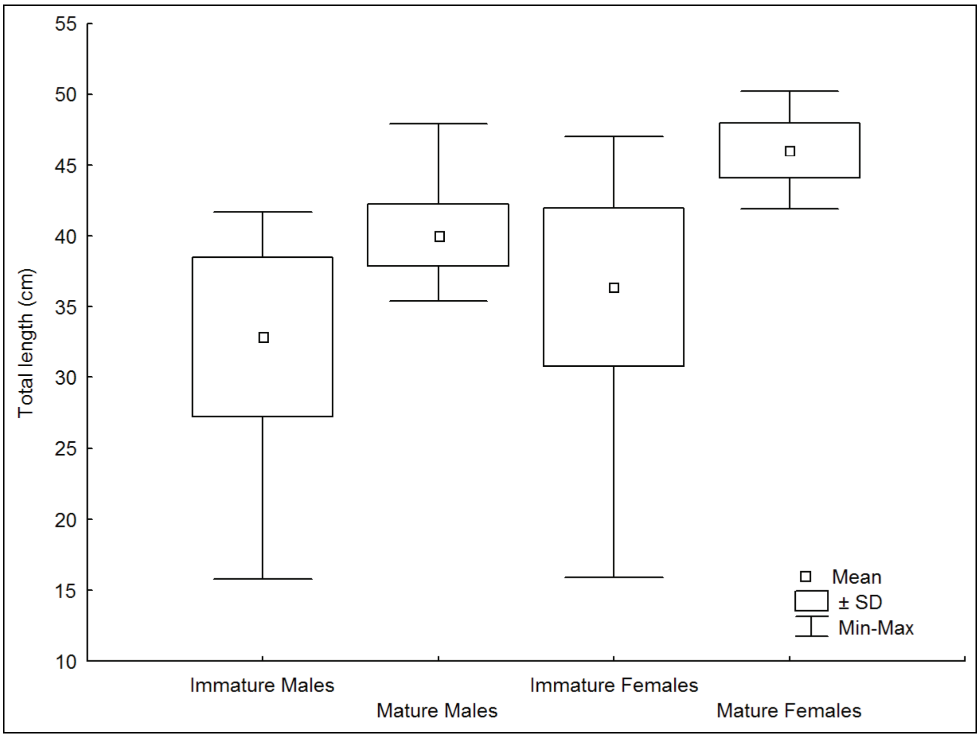

Figure 4.12. Box and whiskers plot with the mean, standard deviation and size ranges for mature and immature males and females of *Etmopterus pusillus*.

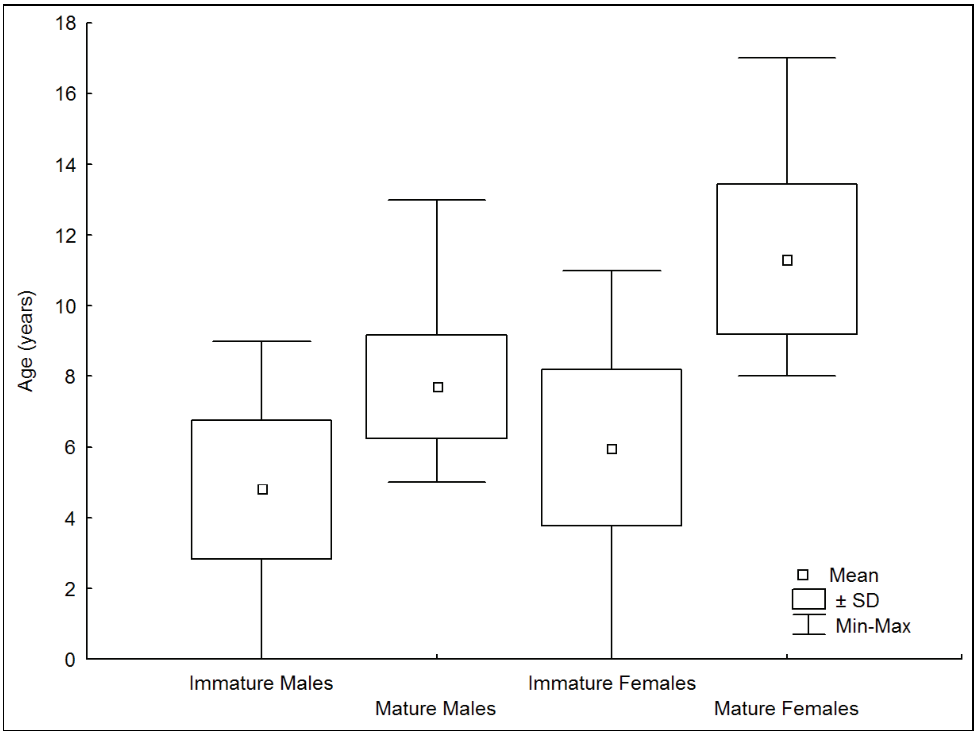

Figure 4.13. Box and whiskers plot with the mean, standard deviation and age ranges for mature and immature males and females of *Etmopterus pusillus*.

Both size based and age based maturity ogives produced good fits to the observed data. The values of r^2 were high; 0.985 and 0.990 for female and male length based ogives and 0.981 and 0.991 for female and male age based ogives.

Females matured at larger sizes than males, with estimated sizes at first maturity of 43.58 cm TL for females and 38.03 cm TL for males (Figure 4.14). Females also matured at later ages than males, with estimated ages of first maturity of 7.13 years for males and 9.86 years for females (Figure 4.15). There were significant differences between sexes in terms of the parameters of both the size (Max. Likelihood: χ^2 = 189.82; P-value < 0.05) and the age-based (Max. Likelihood: χ^2 = 65.01; P-value < 0.05) maturity ogives.

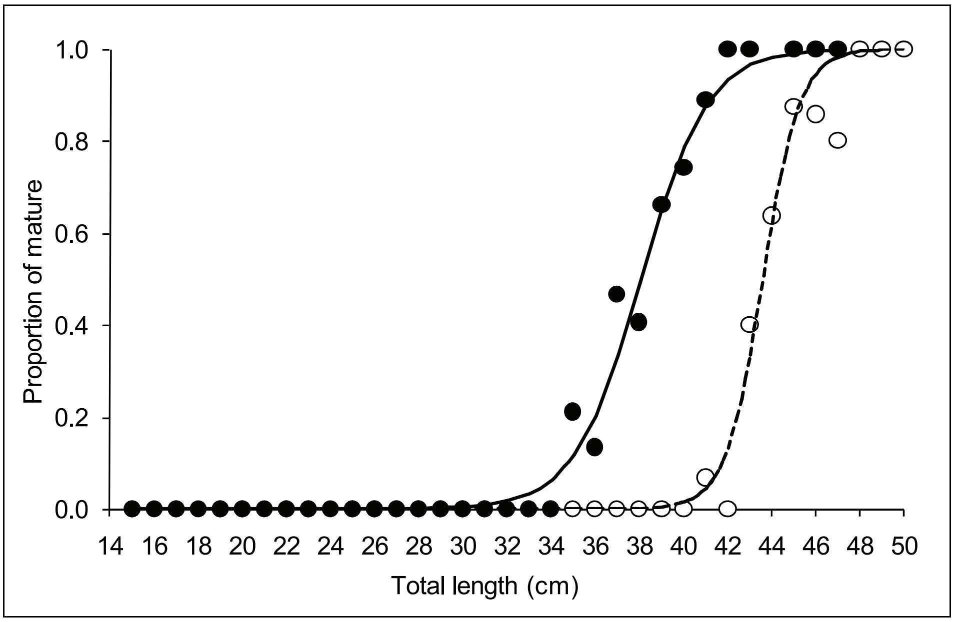

Figure 4.14. Size based maturity ogives for *Etmopterus pusillus*. Dark and white points represent the proportion of mature males and females in each 1 cm TL interval class, while the solid and dotted lines represent the corresponding fitted logistic curves.

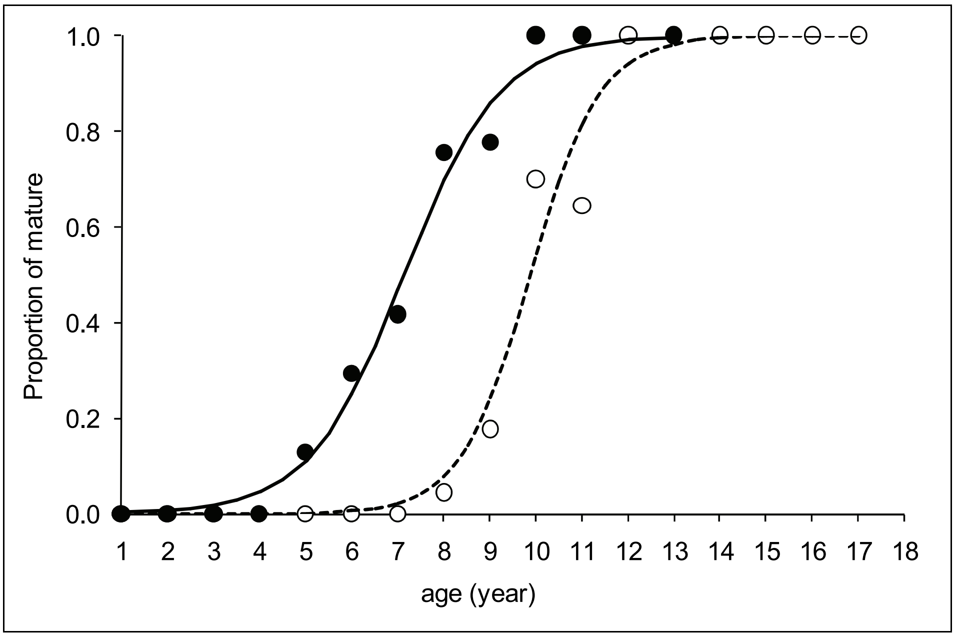

Figure 4.15. Age based maturity ogives for *Etmopterus pusillus*. Dark and white points represent the proportion of mature males and females in each age class, while the solid and dotted lines represent the corresponding fitted logistic curves.

Since no significant difference was found between left and right side claspers (ANCOVA: F = 0.10; P-value = 0.754), the mean value was calculated for each specimen and plotted against total length. It is clear that there is a relationship between clasper length and TL, with an accentuated increase once the specimens achieved maturity (Figure 4.16).

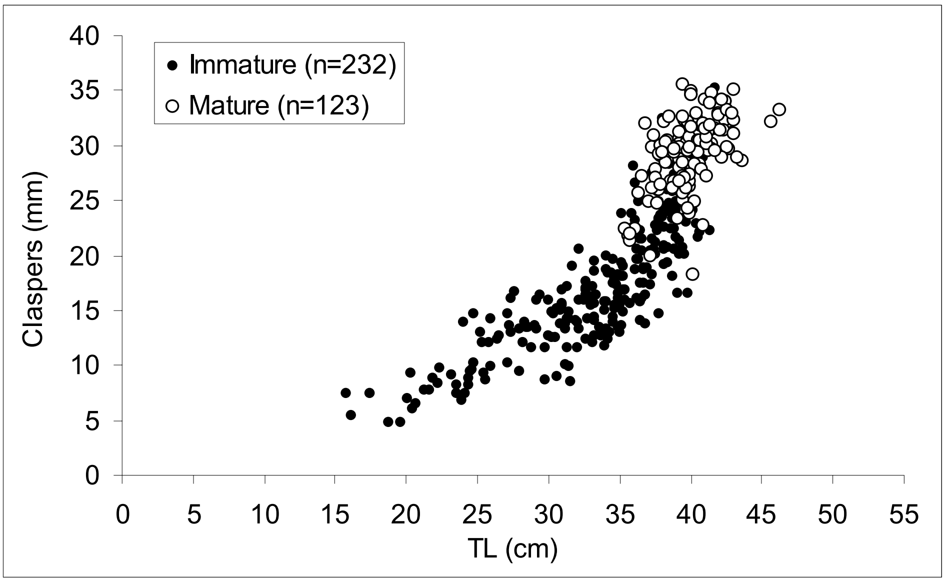

Figure 4.16. Relation between total length (TL) and clasper length in males *Etmopterus pusillus*.

Likewise, since no significant differences between left and right side uterus (ANCOVA: F = 0.52; P-value = 0.471), a mean value was calculated for each female and plotted against TL. Although a clear relationship can be observed between uterus width and TL, this relation is not as progressive as that of the claspers in males. In this case, immature specimens have a relatively narrow uterus independently of TL, and once specimens achieved maturity, the uterus either increases substantially (in the case of pregnant females) or remains relatively narrow (mature but not pregnant females) (Figure 4.17).

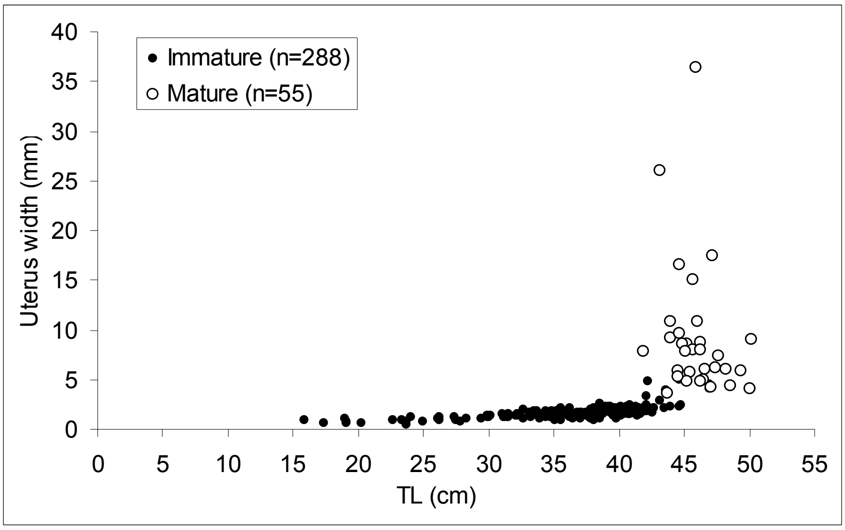

Figure 4.17. Relation between total length (TL) and uterus width in females of *Etmopterus pusillus*.

Fifty percent of the females and the males in this population are mature respectively at 86.81% and 79.40% of the maximum observed size. In terms of age, 50% of the females are mature at 58.02% and 50% of the males are mature at 54.86% of the maximum observed ages (Table 4.7).

Table 4.7. Estimated parameters for size (L50) and age (Age50) at maturity, with the respective 95% confidence intervals (CI) for males and females of *Etmopterus pusillus*.

		Females	Males
Size range (cm)	Immature	15.9 - 47.0	15.8 - 41.7
	Mature	41.9 - 50.2	35.4 - 47.9
L_{50} (cm)	Estimate	43.58	38.03
	Lower 95% CI	43.38	37.78
	Upper 95% CI	43.78	38.29
Age range (years)	Immature	0 - 11	0 - 9
	Mature	8 - 17	5 - 13
Age_{50} (years)	Estimate	9.86	7.13
	Lower 95% CI	9.57	6.89
	Upper 95% CI	10.16	7.37
L_{50} / L_{max} (%)		86.81	79.4
Age_{50} / Age_{max} (%)		58.02	54.86

4.4.7. Fecundity

The ovarian fecundity in mature (stage 3) females varied from 2 to 18 ripe oocytes, with an average of 10.44 (SD = 3.65; n = 16), while the uterine fecundity in mid term pregnant females (stage 5) varied from 1 to 6 embryos with an average of 3.50 (SD = 3.54; n = 2). Only one pregnant female in stage 6 (final pregnancy), with 5 totally developed and ready to be born embryos was caught.

Although the relationship between ovarian fecundity (number of ripe oocytes) and the female TL (ANOVA: F = 1.05; P-value = 0.323) (Figure 4.18) was not significant, this may be due to the relatively small sample size (n = 16) and the very restricted size range of stage 3 females.

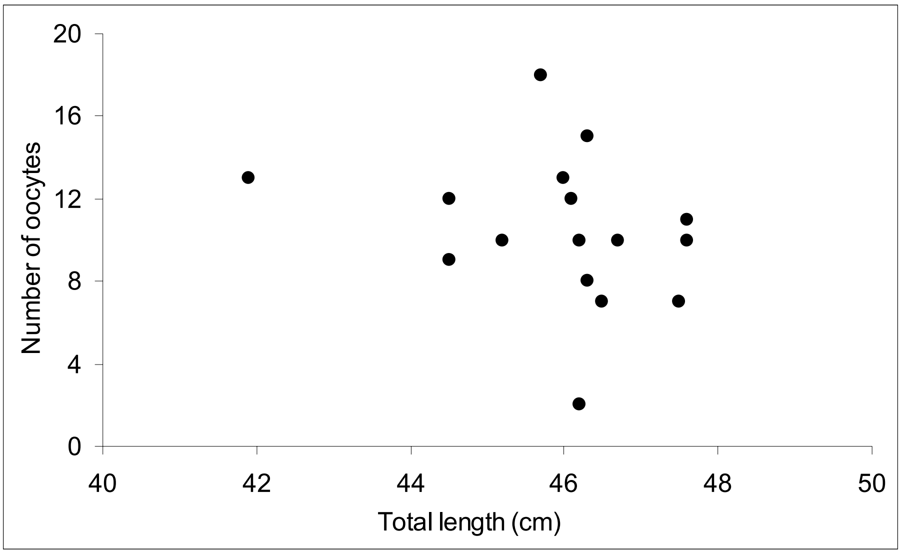

Figure 4.18. Relationship between the total length in cm and the number of ripe oocytes present in mature (stage 3) females.

4.5. Discussion

Even though *E. pusillus* is a widespread and at least in some areas a very common deep water lantern shark, it has never been thoroughly studied and this is probably the first study to explore aspects of the population biology of this species.

Catches of this species can be very high in the fisheries of the south and south-west coasts of Portugal, and although most of the specimens are discarded, they are returned to sea either dead or with severe injuries that probably affect their survival. Specimens caught with trawls tend to arrive dead on board, probably due to the trauma of being towed for several hours, while specimens caught with longlines are often still alive, but with injuries caused by the hooks and by the sudden changes in pressure and temperature. The survival rates of *Scyliorhinus canicula* discarded by commercial trawlers seems to be very high, ranging from 78% (Rodriguez-Cabello *et al.*, 2005) to 98% (Revill *et al.*, 2005). However, these experiments were conducted in waters much shallower than those where *E. pusillus* is caught and no similar studies are known for *Etmopterus* species. A small survivorship experiment where *E. pusillus* caught by longlines were kept in a temperature controlled tank was carried out during the course of the present study and 100% mortality was achieved within 48 hours in all cases, suggesting that the survival of these sharks fished at considerable depths and released at the surface is probably zero. Therefore, alternative by-catch reduction strategies such as the one proposed by Coelho *et al.* (2003) might be more adequate to prevent excessive fishing related mortality on this species.

The sizes of both males and females caught during this study covered most of the length ranges described for this species (Compagno *et al.*, 2005), so we consider that the sample size and range used were adequate. The fact that different fishing gears were used was an advantage, given that a wide range of depths, and habitats (muddy / sandy habitats fished with trawls and near rocky habitats fished with longlines), were sampled. The use of several fishing gears further diminishes the selectivity effect of particular fishing gears that can lead to a skewed distribution of the sample.

As stated by Clarke and Irvine (2006), prior to the examination of growth bands, spine growth should be investigated by measuring spine morphometrics and comparing these with the individual total length. In this work significant relationships were found between specimen length and several spine morphometrics. Therefore, prior to determination of age, there was already evidence that the growth of spines is proportional to specimen growth and therefore spines could be suitable for estimating age. In addition, relationships between specimen length and spine weight were also investigated and again positive and significant relationships were found, thus confirming the relationship between spine and specimen growth.

During this study, age was estimated based only on the inner dentine layer of the 2nd dorsal spine. The reason for choosing this spine instead of the 1st dorsal spine is that preliminary investigations showed that the 1st dorsal spines were often more damaged than the 2nd. This same situation has been observed in other deep water squalid sharks, such as *Centroselachus crepidater* in Tasmanian waters (Irvine *et al.*, 2006b), with these authors choosing the 2nd dorsal spine for the same reason. Irvine *et al.* (2006a) estimated ages of *Etmopterus baxteri* off Tasmanian waters based on both the inner dentine layer and on the exterior bands of the spines, with significant discrepancies found between the two techniques. Irvine *et al.* (2006a) hypothesized that the inner dentine bands may underestimate age of the older specimens. In the present case, given that the relationship between spine radius, where the dentine is deposited, and specimen growth are linear for the entire length distribution of the species, we do not believe that the deposition of this layer stops in the older specimens. Therefore, only the inner dentine layer was used for age estimation. Nevertheless, future work should also investigate the exterior enamel of the spines.

The validation of age estimates is a fundamental aspect of age and growth studies (Cailliet *et al.*, 1986; Cailliet, 1990; Campana, 2001; Cailliet and Goldman, 2004; Cailliet *et al.*, 2006). In this study, age estimations were validated by the MIR analysis, which is one of the most commonly used techniques for validating annual ages in elasmobranch fishes, including sharks (e.g. Simpfendorfer, 1993; Conrath *et al.*, 2002; Carlson and Baremore, 2005) and rays (Neer and Thompson, 2005b; Smith *et al.*, 2007).

According to Campana (2001), the MIR analysis is not one of the most accurate for validating ages and this author only classified it in 7th place among 16 possible techniques. However, the techniques that Campana (2001) recommends over the MIR analysis are not applicable to *E. pusillus*. Examples of such techniques are the release of tagged fishes of known age, which implies that the species must be bred in captivity; bomb radiocarbon validation, which implies that at least some specimens must have been born before the 1960's when the ^{14}C in the world oceans increased significantly; or tagging fishes with oxitetracycline, which implies that specimens must be caught, tagged and released without significant mortality. Given that this deep water species can not be bred in captivity, that there are no individuals born before the 1960's and that catch related mortality is high, it is not possible to use capture/tag/recapture or bomb radio-carbon techniques. Thus, we think that MIR analysis is the best available technique for this species.

However, Campana (2001) also recognized that the MIR analysis can be used successfully if some assumptions are respected, namely 1) measuring blindly the structures, without knowledge of the date of capture, 2) observing at least two complete band forming cycles, 3) making an objective interpretation of the results, ideally with the resource of statistics and 4) analysing few age groups at a time. In this work, we effectively respected three of these assumptions, with the only shortcoming being the fact that due to sampling restrictions we analyzed only one instead of two complete cycles. However, given that all other assumptions were respected, we are relatively sure that the age validation procedure used in this study is robust and effectively proves that in this species one pair of bands (one opaque and one translucent) is formed each year. The only study known at this point where age validation was accomplished for dorsal spines based on bomb radiocarbon is a recent study by Campana *et al.* (2006a) where age was validated for the external enamel bands of *Squalus acanthias*.

Although the von Bertalanffy growth curve is the most widely used to model the growth of fishes (Katsanevakis, 2006), several authors have shown that alternative models can provide better fits to age and growth data for some elasmobranch species. In this study, and even though the VBGF produced good fits and biologically sound results, additional growth curves were used for comparison purposes. Examples of successful alternative growth models applied to elasmobranch fishes include the logistic model applied to the big skate, *Raja binoculata*, the longnose skate, *Raja rhina* (McFarlane and King, 2006) and the spinner shark, *Carcharhinus brevipinna* (Carlson and Baremore, 2005), the Gompertz model applied to the cownose ray, *Rhinoptera bonasus* (Neer and Thompson, 2005b) and the VBGF with known size at birth applied to the bull shark, *Carcharhinus leucas* (Neer and Thompson, 2005a).

The selection of the most adequate model to explain the growth of a species varies between studies. Some authors use the coefficient of determination or parameters such as the lowest mean square error of the regressions as measures of the adequacy of the models (Carlson and Baremore, 2005). Katsanevakis (2006) suggested the use of the Akaike information criterion (Shono, 2000) as the most efficient model selecting tool and presented several examples where the most adequate model according to this criterion is not always the model with the largest coefficient of determination.

Therefore, the Akaike information criterion was used to evaluate the information that each model was contributing and the Akaike differences used to assess the extent of the contribution of the alternative models. According to Katsanevakis (2006), the lower the value of the Δi the more support an alternative model has, with models with $\Delta i > 10$ having essentially no support, and models with $\Delta i < 2$ having substantial support. In this case, all size at age based models, both in males and females produced values of $\Delta i < 2$, meaning that every model tested in this study fits and can support the data. Although the VBGF with fixed size at birth proved to be the worst model in every case, the Akaike differences were minimal, indicating that the data set had enough small and young specimens, and that the size at birth observed during the surveys and used in this model is probably adequate. For weight at age data, the Gompertz model was the best for male growth while the VBGF model was better for female growth. However, in the case of males, the VBGF model also proved to be relatively good, only exceeding the optimum value of Δi (2) by a small amount, while the Δi value showed no support for the Gompertz model for females.

Even though this is a relatively small species, it is noteworthy that it is long lived and has a relatively slow growth rate. In the only other study on age and growth of another Etmopteridae shark, Irvine *et al.* (2006a) also reports that the giant lanternshark, *Etmopterus baxteri* in Tasmanian waters, is long lived and slow growing. Specifically, Irvine *et al.* (2006a) reported values of L_{inf} and k of 60.6 cm and 0.082 year^{-1} for males and 68.1 cm and 0.040 year^{-1} for females based on the external dorsal spine bands and of 59.6 cm and 0.163 year^{-1} for males and 69.3 cm and 0.116 year^{-1} for females based on data from dorsal spine sections.

Since in mature females ovarian and uterine stages did not occur at the same time in pregnant females, we can conclude that this species needs a resting phase after parturition, during which the oocytes in the gonads mature for the next cycle. This situation has significant implications for management and conservation, since this species needs two reproductive seasons to complete one reproductive cycle: one season for the development of the oocytes in the gonads followed by one season for the development of the embryos in the uterus. Other deep water squalid sharks have been described to have long gestation periods such as the *Centroscymnus coelolepis* (Clarke *et al.*, 2001), the *Squalus megalops* (Watson and Smale, 1998; Braccini *et al.*, 2006; Hazin *et al.*, 2006) and the *Centrophorus* cf. *uyato* (McLaughlin and Morrissey, 2005).

Uterine reproductive stages were rarely found during this study. Several authors have found similar patterns of rare pregnant females during their surveys and suggestions have been made that pregnant females of some deep water squalids may move into nursery areas, probably in deeper waters, for parturition. This has been hypothesized for *Centroscymnus owstoni* and *C. coelolepis* in Japan (Yano and Tanaka, 1988) and *Centroscyllium fabricii* and *Etmopterus princeps* in Iceland (Jakobsdottir, 2001), and we think that a similar situation occurs with *E. pusillus* off southern Portugal.

During this study it was difficult to establish a definitive reproductive season for *E. pusillus*. It is worth noting that both mature females with ripe oocytes and pregnant females occurred mostly during the winter, specifically from November to April, but the low sample size of both mature and pregnant females may have influenced this analysis. On the other hand, active males were recorded throughout the year, suggesting there is no clearly defined reproductive season. Other authors have found a lack of seasonal reproductive pattern in deep water squalid sharks, including *Centroscyllium fabricii* and *Etmopterus princeps* in Iceland (Jakobsdottir, 2001) and *Etmopterus granulosus* in New Zealand (Wetherbee, 1996). On the other hand, (Flammang *et al.*, 2008) found seasonal reproductive patterns in several oviparous deep water Scyliorhinidae sharks, based on gonadosomatic indices variations throughout the year.

E. pusillus in Portuguese waters matures relatively late in its life cycle. Coelho and Erzini (2005) presented preliminary results regarding size at maturity for this species that are very similar to the final results now presented in this work. This information is also now complemented with age at maturity estimates not previously reported. Cortés (2000) examined 164 species of sharks and concluded that on average, maturity occurs at around 75% of the maximum size and around 50% of the maximum age. The values obtained during this study were a little higher, namely 86.81% for females and 79.40% on males for size based data and 58.02% for females and 54.86% for males for age based data. The size at maturity estimated by the maturity ogives resulted in maturity estimates similar to what was observed by the growth of the sexual characters, specifically clasper length in males and the uterus width in females. In this study, females matured at significantly larger sizes and older ages than males. Sexual dimorphism in terms of size-at-maturity is common in elasmobranch fishes, with females usually maturing later and at larger sizes than males. This sexual dimorphism has been described previously for the *Etmopterus* genus by Jakobsdottir (2001) for *Etmopterus princeps* and by Irvine *et al.* (2006a) for *Etmopterus baxteri*. Ebert (2005) studied reproductive patterns of several deep water skates along the Bering Sea and concluded that size at maturity occurred at > 80% of their TL.

E. pusillus is an aplacental viviparous shark with a relatively low fecundity. The differences observed between the ovarian and the uterine fecundities may be explained by two hypotheses: 1) that part of the ripe oocytes present in stage 3 females never develop into embryos or, 2) that since this is an aplacental species, without an umbilical cord connecting the mother to the embryos, it is possible that the stress produced during the fishing process leads to the release of some of the embryos in the uterus of pregnant females. During the sampling process aboard the fishing boats, and while the specimens were deposited in boxes for later processing, it was common to observe middle term embryos in the middle of the catch. This observation supports the second hypothesis, indicating that there is indeed a loss of embryos by pregnant females during the fishing process. Therefore, fecundity in this species should be estimated by the ovarian fecundity and not by uterine fecundity, since the latter may tend to underestimate this parameter.

In this species, no significant relationship was observed between the female total length and the number of ripe oocytes in the gonads. However, we must emphasize that this particular analysis may have been conditioned by the small sample size and the limited size distribution of mature females studied. Other species of deep water viviparous sharks such as *Centroscymnus owstoni* and *C. coelolepis* in Japan (Yano and Tanaka, 1988) have significant relationships between female size and fecundity. Morphologically, these relationships are sound since in viviparous species the number of oocytes in the gonads and, after fecundation, the number of embryos in the uterus, are limited by the size of the abdominal cavity, which increases with increasing specimen size.

In conclusion, this study suggests that *E. pusillus* in the NE Atlantic has a vulnerable life cycle, a situation previously described for several other deep water squalids. Several deep water fisheries operate in the area and there are no perspectives of a decrease of effort or a reduction of the discards in the near future. Even if effective management and conservation plans are implemented for deep water shark species in the future, discarded species such as *E. pusillus*, where accurate catch data is extremely difficult to obtain, will still remain a problem for management and conservation.

CHAPTER 5: Life history of a wide ranging deep water lanternshark in the NE Atlantic, *Etmopterus spinax* (Chondrichthyes: Etmopteridae), with implications for conservation.*

(Viscera details of *Etmopterus spinax*)

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* *Submitted*: Coelho, R. & Erzini, K. Life history of a wide ranging deep water lanternshark in the NE Atlantic, *Etmopterus spinax* (Chondrichthyes: Etmopteridae), with implications for conservation. *Journal of Fish Biology*.

5.1. Abstract

Etmopterus spinax is a deep water lantern shark occurring in the eastern Atlantic and Mediterranean that is caught in large quantities in some areas where deep water commercial fisheries occur, and is usually discarded due lack of commercial value. The population biology was studied and life history parameters determined for the first time for this species. Age was estimated from sections of the 2nd dorsal spine and validated by marginal increment analysis. Males attained a maximum age of 8 years while 11 year old females were found. Several growth models were fitted and compared for both size- and weight-at-age data, showing that even though this is a small sized species, it has a relatively slow growth rate. This species matures late and at a relatively large size: at 76.5% (25.9cm) and 74.7% (30.7cm) of the maximum observed sizes and at 49.6% (3.9 yr) and 42.5% (4.7 yr) of the maximum observed ages for males and females respectively. It has a low fecundity, with a mean ovarian fecundity of 9.94 oocytes and a mean uterine fecundity of 7.59 embryos per reproductive cycle. Given that in this species the oocytes take one year to develop, that uterine and ovarian phases do not occur at the same time, and that a resting phase of one year was observed, a three year reproductive cycle is probable. The estimated parameters indicate that this species has a vulnerable life cycle, typical of deep water squalid sharks. Given the high fishing pressures that it is suffering in the NE Atlantic, this lanternshark may be already facing severe declines or in risk of facing them in the near future.

5.2. Introduction

The velvet belly lantern shark, *Etmopterus spinax* is a small sized deep water squalid shark, that occurs in the eastern side of the Atlantic Ocean, from Iceland and

Norway (Compagno *et al.*, 2005) to South Africa (Compagno, 1984), including the Azores (Santos *et al.*, 1997), the Canaries (Brito *et al.*, 2002) and the Cape Verde Islands (Reiner, 1996). It also occurs in the western and central Mediterranean (Serena, 2005), including the Ionian, the lower Adriatic and the Aegean seas (Notarbartolo di Sciara and Bianchi, 1998). This species lives mainly in the outer continental and insular shelves and upper slopes, at depths from 70 to 2000m, but mostly between 200 and 500m, near or well above the bottom (Compagno *et al.*, 2005).

In Portugal, the *Etmopterus* genus is a common caught by-catch and discard of several deep water fisheries that operate in the area, namely the bottom trawl fishery targeting Norway lobster, *Nephrops norvegicus*, deepwater rose shrimp, *Parapenaeus longirostris*, and red shrimp, *Aristeus antennatus* (Monteiro *et al.*, 2001) and the deep water longline fishery targeting wreckfish, *Polyprion americanus*, European-conger, *Conger conger* and European hake, *Merluccius merluccius* (Coelho *et al.*, 2005). However, even though these species are caught in large quantities, their commercial value is small or none and they are therefore usually discarded (Monteiro *et al.*, 2001). Thus, this species is never landed and is never accounted for in the official fisheries statistics, limiting the availability of data for monitoring its fisheries mortality and assessing its population status.

Although *E. spinax* is a relatively common species, information on the biology is limited. Coelho and Erzini (2005) report that this is an aplacental viviparous species, and present preliminary lengths-at-maturity, while Sion *et al.* (2002) made a first attempt at ageing the Mediterranean population from dorsal spines. Neiva *et al.* (2006) studied the feeding ecology of the Atlantic population and Atkinson and Bottaro (2006) correlated the ampullatory distribution of the pores with the predatory lifestyle. Other studies that mention this species deal mainly with species distribution (e.g. Capape *et al.*, 2000; Massuti and Moranta, 2003) or focus on fisheries aspects (e.g. Carbonell *et al.*, 2003).

Given the relatively high levels of fishing mortality that this species is currently suffering, the lack of previous studies on this species and the fact that deep water squalid sharks in general are characterized for having vulnerable life cycles and are amongst the most vulnerable of elasmobranchs, there is a need for population dynamics studies on this species. The objectives of this study were to estimate various population dynamics aspects of *E. spinax*, specifically regarding age, growth, maturity, reproduction and fecundity. The data presented here will be useful for modelling purposes, for monitoring this population's evolution in the future and may serve as a basis for comparison with other studies on this species in other areas. We believe that this is the first in depth study on the life history parameters of the velvet belly lantern shark.

5.3. Material and methods

5.3.1. Biological sample

Specimens were caught in all months except March 2003 from February 2003 to April 2004, as by-catch of deep water trawlers and longliners. The commercial longliners usually operated near rocky bottoms to catch demersal bony fishes such as *P. americanus* and *C. conger*, while the commercial trawlers fished on muddy and sandy bottoms, targeting crustaceans such as *N. norvegicus*, *P. longirostris* and *A. antennatus*. In addition, some specimens were obtained during the Portuguese Fisheries Institute (INIAP – IPIMAR) deep water demersal trawl survey in the summer of 2003. Individuals were caught at depths from 245m to 745m (Figure 5.1).

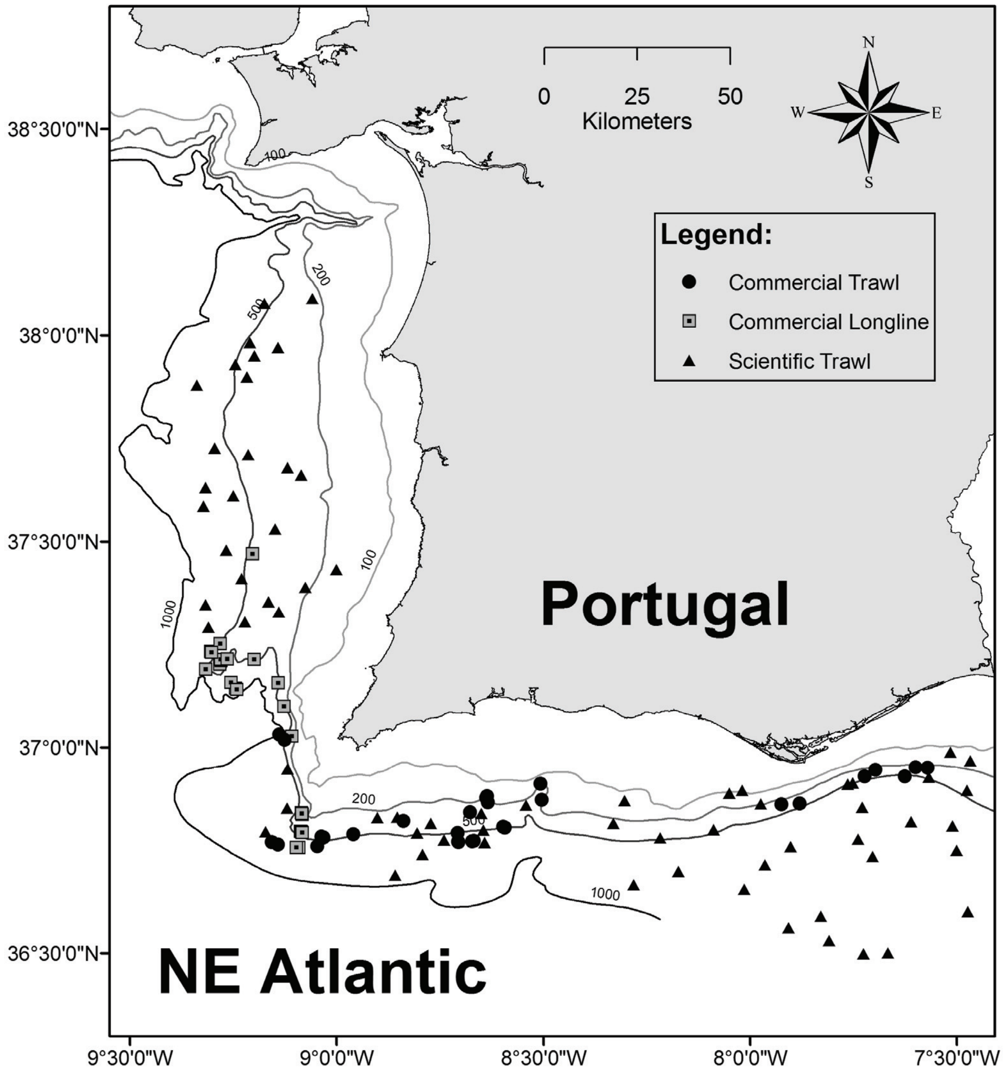

Figure 5.1. Map of the south and southwestern coasts of Portugal with the locations of the coastline, the 100, 200, 500 and 1000m isobaths and the commercial and research survey fishing operations. Isobaths and coastline adapted from "Atlas do Ambiente Digital – Instituto do Ambiente".

In the laboratory, total length (TL), fork length (FL), pre-caudal length (PCL) and body girth (GIR) were recorded to the nearest lower mm. Total weight (W), eviscerated weight (Wev) and the weights of the gonads and the liver were recorded to the nearest centigram. Male clasper length, the diameter of female uterus, the diameter of ripe oocytes in mature females and the length and weight of embryos in pregnant females were recorded to the nearest 0.01 mm using a digital caliper and a digital scale with 0.01 g precision. All morphometric measurements were taken according to the specification presented in Coelho and Erzini (2007).

5.3.2. Morphometric relationships

Linear regression was used to explore the relationships between the explanatory variable TL (in cm) and each of the dependant variables FL, PCL and GIR (in cm) without any data transformation, and between the explanatory variable TL and the natural logarithm transformed variables W and Wev (in g). Standard errors were calculated for all the estimated parameters, along with the coefficient of determination (r^2) of each regression. Linear regressions were carried out for males and females separately, with analysis of covariance (ANCOVA), using TL as the covariate used to compare the two sexes (homogeneity of the regressions)

5.3.3. Age estimation and validation

Preliminary tests on vertebrae showed that no bands were visible, even after using the alizarin red S band enhancing technique (La Marca, 1966). Thus, these structures were abandoned in favour of the dorsal spines. The relationship between individual growth in length (TL cm) and 1st and the 2nd dorsal spine growth namely total spine length (TSL, in mm), measured from the spine tip to the anterior side of the spine base and the base spine width (SBW, in mm), measured as the diameter of the spine at its base (Clarke and Irvine, 2006), was explored by linear regression. Linear regression was also used to explore the relationship between natural logarithm transformed TL and spine weight (SW, in mg). Standard errors were calculated for all the estimated parameters and r^2 values determined. Separate analyses were carried out for males and females and analysis of covariance (ANCOVA) with TL as the covariate used to compare the sexes. All spine measurements were taken with a digital caliper with 0.01 mm precision and weights recorded using electronic precision (0.1 mg) scales.

Annual bands formed in the inner dentine layer of the 2nd dorsal spines and defined as pairs of opaque and translucent bands were counted in order to estimate age. The spines were cleaned, embedded in epoxy resin and cut in 500 μm sections with a Buehler Isomet low speed sawing machine with a series 15LC diamond blade. A Zeiss Axiolab binocular microscope was used to observe the sections mounted in microscope glass slides with DPX at 100x amplification under transmitted white light. The spine sections were digitally photographed and the software Image Pro Plus 4.5 used for image analysis. A complete protocol of spine cleaning, sectioning, photographing and visualization is described in Coelho and Erzini (2007). Spine radius in the area where the bands were observed was measured and linear regression used to explore the relationships between variables for each sex and ANCOVA, using TL as the covariate to compare sexes.

Three independent readings, at least one month apart, of each structure were made by a single reader who had no information regarding specimen characteristics or the results of previous readings. Age was attributed only when at least 2 of the 3 age readings were in agreement.

The precision of the age estimates (Campana, 2001) was evaluated by the percent agreement, the average percent error (APE) defined by Beamish and Fournier (1981) and the coefficient of variation (CV) and the index of precision (D) defined by Chang (1982).

Marginal increment analysis (MIR) was used to validate the periodicity of band pattern formation:

$$MIR = \frac{(R - R_n)}{(R_n - R_{n-1})}$$

where *R* is the radius of the structure, *Rn* is the distance to the outer edge of the last complete band and *Rn−1* is the distance to the outer edge of the next-to-last complete band. Monthly values of MIR were plotted to determine the annual pattern of band formation. Analysis of variance (ANOVA) was used to test for differences in the MIR values along the year and the multiple comparisons Tukey pairwise test used to assess differences between pairs of months.

5.3.4. Growth modelling

The Von Bertalanffy Growth Function (VBGF), a modified version of the VBGF with known size at birth, the Gompertz model and the logistic equation were used to model growth in length. The VBGF is expressed as:

$$Lt = L_{\inf}(1 - e^{-k(t-t0)})$$

where *Lt* is the total length at age *t*, L_{inf} is the maximum asymptotic length, *k* is the growth coefficient and t_0 is the theoretical age when *Lt* =0. The VBGF with a fixed intersect of the length axis (known size at birth (L_0)) is given by:

$$Lt = L_{\inf}(1 - be^{-kt})$$

where b = (L_{inf}-L_0) / L_{inf} and L_0 is the size at birth, that in this species was measured to be 10.7 cm TL (SD = 0.9cm; n = 34), based on observations of totally formed embryos present in late term pregnant females. The Gompertz growth model is expressed as:

$$Lt = L_{\inf} e^{-e^{(-g(t-t0))}}$$

where g is the Gompertz growth coefficient. The logistic equation can be expressed as:

$$Lt = L_{\inf} / (1 + ((L_{\inf} - L_0) / L_0)(e^{(-rt)}))$$

where L_0 is the theoretical length at birth and r is the logistic growth coefficient.

Weight-at-age data was modelled using the VBGF and the Gompertz models. While for the latter the same model is used for weight-at-age, the VBGF for weight is:

$$Wt = W_{\inf}(1 - e^{-k(t-t0)})^b$$

where Wt is total weight at age t; W_{inf} is the maximum asymptotic weight and b is the allometric growth coefficient from the L_T-W relationship (3.092 and 3.290 for males and females, respectively).

Parameters and associated standard errors of all the models were estimated for males and females separately by non-linear least squares regression with the STATISTICA 6 software (StatSoft, 2004). The maximum likelihood test (Kimura, 1980) was used to compare male and female growth parameters.

Model comparison and selection was based on the small sample corrected form of the Akaike information criterion (*AICc*) (Shono, 2000). For the least squares fit this is given by:

$$AICc = \frac{RSS}{n} + \frac{2k(k+1)}{n-k-1}$$

where RSS is the residual sum of squares, n is the number of observations and k is total number of estimated regression parameters. The smallest AICc value was the criteria used to select the "best" model (AICc, min) and the differences between this "best" model and all others expressed as:

$$\Delta i = AICc,i - AICc,\ min$$

5.3.5. Reproductive cycle

Macroscopic observations of the reproductive organs of the specimens were used to define male and female maturity stages according to the scale proposed by Coelho and Erzini (2007) for other *Etmopterus* species. According to that scale, 4 stages were used to describe males, where stages 1 and 2 represent immature, stage 3 mature and stage 4 active specimens, while females were divided in 7 stages, where stages 1 and 2 represent immature, stage 3 mature, stages 4 to 6 pregnant and stage 7 resting females. The percentage of each maturity stage throughout the year for both males and females was plotted, in order to assess if different stages were occurring predominantly during a specific season or period.

The gonadosomatic index (GSI) and the hepatosomatic index (HSI) were calculated for all specimens and the means for each maturity stage in each sex plotted. These indexes were calculated as:

$$\mathrm{GSI} = \frac{\text{Gonad weight (g)}}{\text{Wev (g)}} \times 100$$

$$\mathrm{HSI} = \frac{\text{Liver weight (g)}}{\text{Wev (g)}} \times 100$$

Kruskal–Wallis and pairwise Dunn tests were used to test if significant differences occurred between the different maturity stages.

5.3.6. Maturity

Box and whiskers plots were used to plot the means, standard deviations and ranges for both size and age of mature and immature specimens of each sex, with mature specimens considered to be those able to reproduce or who had already reproduced in the past (Conrath, 2004). A two way ANOVA was used to test for differences in mean sizes and ages of mature and immature males and females.

Size at maturity (TL at which 50% of the individuals are mature) was estimated by fitting maturity ogives to the proportion of mature individuals by 1 cm TL size classes. Non-linear least squares regression, implemented in the STATISTICA 6.0 software (StatSoft, 2004) was used to estimate the parameters and the associated standard errors and 95% confidence intervals of the logistic model:

$$P_{Li} = \frac{1}{1+e^{-b(L_i - L_{50})}}$$

where P_{Li} is the proportion of mature individuals in the size class Li, b is the slope and L_{50} is the size where 50% of the individuals are mature. The same procedure was followed to estimate age at maturity (age at which 50% of the individuals are mature). The maximum likelihood test (Kimura, 1980) was used to test for differences between sexes in the estimated parameters.

Sexual characters such as claspers length in males and uterus width in females were used to confirm the maturity estimated by the ogives. ANCOVA tests, using TL as the covariate, were used to assess if there were differences between these paired structures, and once it was determined that there were no differences, a mean clasper length and uterus width was calculated respectively for each male and female and plotted against TL.

5.3.7. Fecundity

The number of oocytes in mature stage 3 females and the number of mid-term embryos in stage 5 pregnant females were counted to determine total fecundity. Given the possibility that some of the pups may had already been born at the time of capture, pregnant females in stage 6, with near-term embryos were excluded from the fecundity study.

5.4. Results

5.4.1. Biological sample

A total of 795 specimens (485 females and 310 males) were caught and processed in the laboratory. Undamaged spines for age and growth were collected from 790 specimens. For some morphometric relationships, specifically for the length - weight relationships, 494 additional specimens (218 males and 276 females) caught outside the sampling period were also used. Both male and female samples had a wide length range, with females attaining substantially larger sizes than males. Specifically, female lengths varied from 9.1 to 41.1 cm TL while males ranged in length from 10.2 to 33.8 cm TL. Females had a wider age range than males. Estimated ages of females varied from 0 to 11 years while males ranged from 0 to 8 years (Figure 5.2).

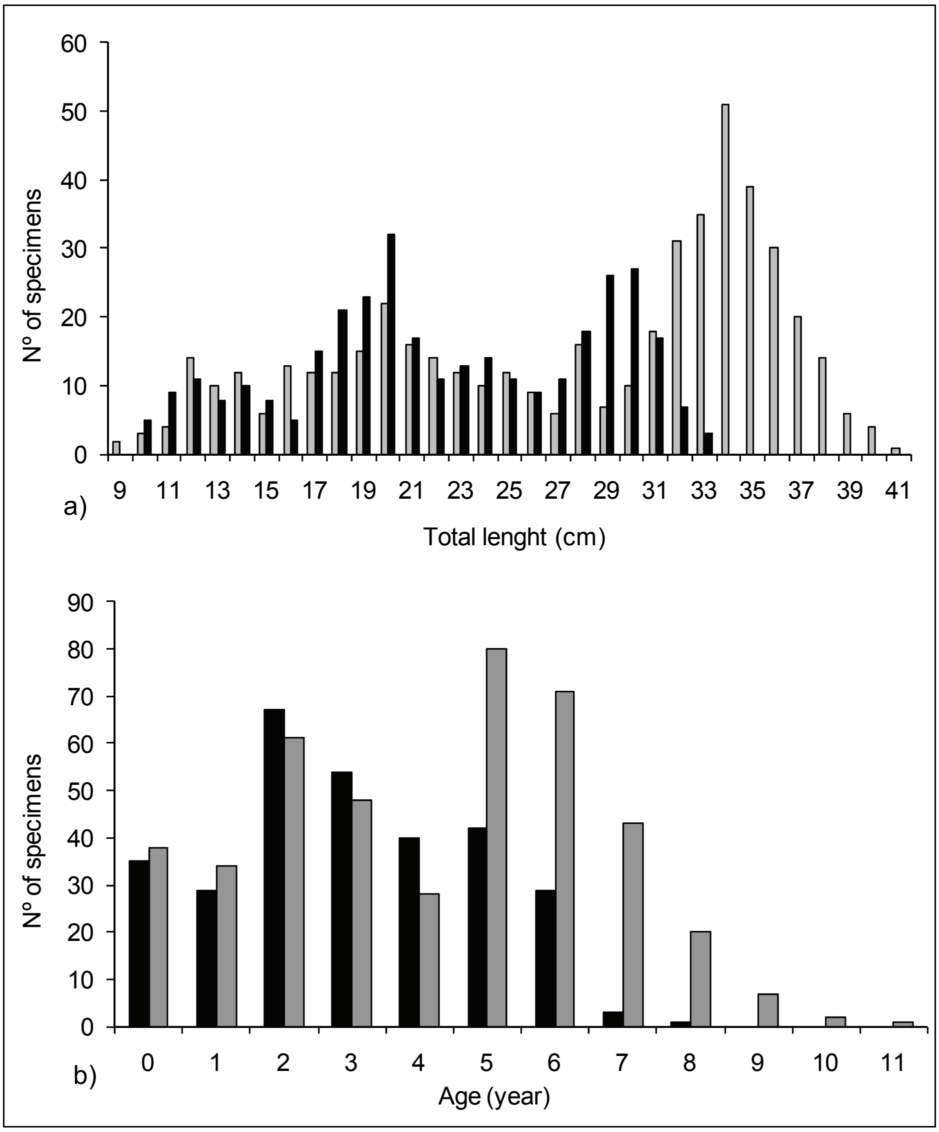

Figure 5.2. Size (a) and age (b) distribution of the male (black bars) and female (grey bars) sample of *Etmopterus spinax* used in this study.

5.4.2. Morphometric relationships

The morphometric relationships are presented in Table 5.1. No significant differences between sexes were detected for the TL-FL (ANCOVA: F = 0.02; P-value = 0.897) and TL-PCL (ANCOVA: F = 1.94; P-value = 0.164) relationships, and therefore regressions for both sexes combined were carried out. For all other regressions significant differences were detected between sexes (ANCOVA $_{\text{TL-GIR}}$: F = 36.77; P-value < 0.001; ANCOVA $_{\text{TL-W}}$: F = 361.61; P-value < 0.001; ANCOVA $_{\text{TL-Wev}}$: F = 199.86; P-value < 0.001).

Table 5.1. Linear regressions between TL and FL, PCL, GIR, W and Wev in *Etmopterus spinax*, indicating the total sample (n), the type of data transformation, the range of the explanatory variable (cm) and the intersect (a) and the slope (b) of the linear regression, with the respective standard errors (SE). The coefficient of determination (r^2) of each regression is also given.

Relation	Transf.	Sex	Sample characteristics		Parameters of the relationship				
			n	Range	a	b	$SE_{(a)}$	$SE_{(b)}$	r^2
TL - FL	None	Males	112	10.6 - 33.8	-0.101	0.865	0.115	0.004	0.997
		Females	255	9.1 - 40.7	-0.134	0.866	0.096	0.003	0.997
		All	367	9.1 - 40.7	-0.110	0.865	0.068	0.002	0.998
TL - PCL	None	Males	112	10.6 - 33.8	-0.157	0.762	0.204	0.008	0.989
		Females	255	9.1 - 40.7	-0.412	0.775	0.168	0.005	0.989
		All	367	9.1 - 40.7	-0.449	0.776	0.120	0.004	0.991
TL - GIR	None	Males	103	10.6 - 33.8	0.780	0.282	0.374	0.014	0.808
		Females	252	12.0 - 40.7	-2.003	0.418	0.409	0.012	0.820
TL - W	Nat. Log	Males	528	10.2 - 33.8	-5.904	3.092	0.062	0.020	0.979
		Females	761	9.1 - 41.1	-6.482	3.290	0.060	0.018	0.977
		All	1289	9.1 – 41.1	-6.336	3.240	0.044	0.014	0.997
TL - Wev	Nat. Log	Males	344	10.2 - 33.8	-5.952	3.038	0.054	0.017	0.989
		Females	513	9.1 - 41.1	-5.950	3.038	0.051	0.016	0.987

5.4.3. Age estimation and validation

A clear relationship between the growth in weight and size of the spines with TL of specimens was observed (Table 5.2). Differences between sexes were detected for most of the relations, namely for the TL-ESL1 (ANCOVA: F = 16.21, P-value < 0.001), TL-SBW1 (ANCOVA: F = 10.98, P-value = 0.001), TL-SW1 (ANCOVA: F =

6.03, P-value = 0.014), TL-TSL2 (ANCOVA: F = 7.36, P-value = 0.007), TL-SBW2 (ANCOVA: F = 8.15, P-value = 0.004), TL-ESW2 (ANCOVA: F = 7.65, P-value = 0.006) and TL-SW2 (ANCOVA: F = 11.96, P-value < 0.001). Differences between sexes were not detected in the TL-TSL1 (ANCOVA: F = 1.82, P-value = 0.178), TL-ESW1 (ANCOVA: F = 0.46, P-value = 0.499) and Tl-ESL2 (ANCOVA: F = 2.08, P-value < 0.150), and in these cases a regressions for sexes combined was carried out.

Table 5.2. Linear regressions between TL of specimens and several spine measurements and spine weight, for both 1st and 2nd dorsal spines of *Etmopterus spinax*. The total sample size (n), the type of data transformation, the range of the explanatory variable (cm) and the intersect (a) and the slope (b) of the linear regression, along with the respective standard errors (SE) and the coefficient of determination (r^2) of each regression are given.

Relationship	Transf.	Sex	Sample		Regression parameters				
			n	Range	a	b	SE (a)	SE (b)	r^2
TL - TSL1	None	Males	301	10.2 - 33.8	1.901	0.530	0.238	0.010	0.900
		Females	437	9.1 - 41.1	2.547	0.508	0.285	0.010	0.861
		Combined	738	9.1 - 41.1	2.269	0.517	0.185	0.007	0.886
TL - ESL1	None	Males	281	10.2 - 33.8	2.029	0.238	0.195	0.008	0.745
		Females	426	9.1 - 41.1	1.384	0.284	0.184	0.006	0.827
TL - SBW1	None	Males	302	10.2 - 33.8	-0.123	0.115	0.053	0.002	0.893
		Females	445	9.1 - 41.1	0.111	0.104	0.056	0.002	0.868
TL - ESW1	None	Males	281	10.2 - 33.8	0.334	0.112	0.077	0.003	0.803
		Females	433	9.1 - 41.1	0.353	0.115	0.072	0.002	0.834
		Combined	714	9.1 - 41.1	0.291	0.116	0.050	0.002	0.845
TL - SW1	Nat. Log.	Males	300	10.2 - 33.8	-4.230	2.141	0.087	0.028	0.951
		Females	439	9.1 - 41.1	-3.941	2.046	0.079	0.024	0.943
TL - TSL2	None	Males	307	10.2 - 33.8	2.887	0.773	0.259	0.011	0.941
		Females	454	9.1 - 41.1	3.930	0.729	0.275	0.009	0.929
TL - ESL2	None	Males	284	10.2 - 33.8	3.768	0.377	0.243	0.010	0.824
		Females	431	9.1 - 41.1	4.470	0.356	0.245	0.008	0.806
		Combined	715	9.1 - 41.1	4.136	0.365	0.167	0.006	0.830
TL - SBW2	None	Males	310	10.2 - 33.8	-0.152	0.108	0.050	0.002	0.893
		Females	460	9.1 - 41.1	0.010	0.101	0.039	0.001	0.923
TL - ESW2	None	Males	288	10.2 - 33.8	0.391	0.102	0.065	0.003	0.822
		Females	443	9.1 - 41.1	0.609	0.092	0.054	0.002	0.851
TL - SW2	Nat. Log.	Males	306	10.2 - 33.8	-4.090	2.346	0.082	0.027	0.962
		Females	456	9.1 - 41.1	-3.726	2.222	0.071	0.022	0.959

The linear relationships between TL and the radius of the spine section where age was estimated was significant for both males (ANOVA: F = 2695, P-value < 0.001) and females (ANOVA: F = 4898, P-value < 0.001) (Figure 5.3), with differences detected between sexes (ANCOVA: F = 9.54, P-value = 0.002).

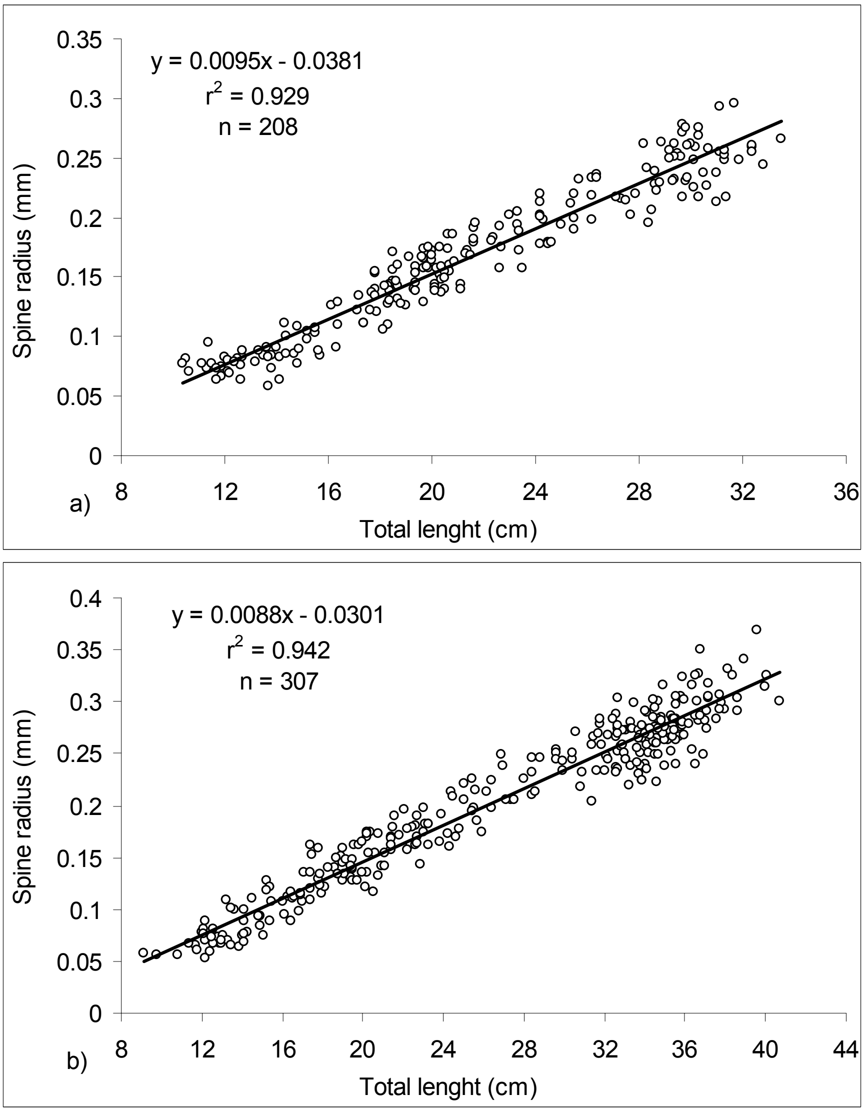

Figure 5.3. Linear relationships between total length of specimens and radius of the spine sections where age was estimated for both males (a) and females (b) of *Etmopterus spinax*. The regressions equations with the respective coefficients of determination (r^2) and sample sizes (n) are also given.

A clear pattern of alternating translucent and opaque band formation was visible on the spine sections (Figure 5.4). Age was determined successfully for 733 of the 790 specimens (92.8%), with poor band discrimination (32 specimens) and lack of concordance on at least 2 of the 3 readings (25 specimens) accounting for the remainder.

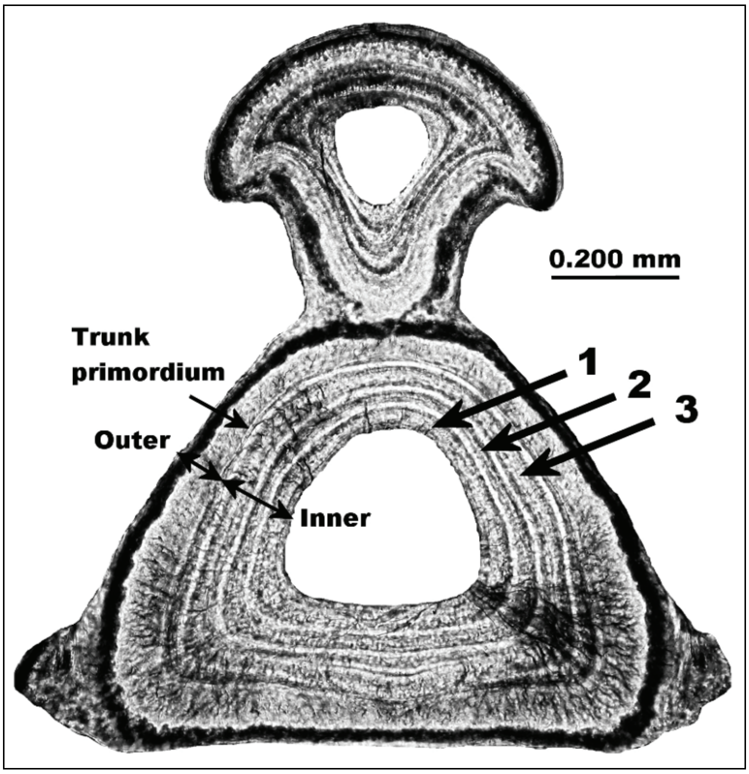

Figure 5.4. Microphotograph of a sectioned dorsal spine of a male *Etmopterus spinax* of 23.4 cm total length and an estimated age of 3 years. It is possible to distinguish the inner trunk layer where the annual bands were counted and the outer trunk layer (without any growth bands and already present in late term embryos), as well as the trunk primordium.

The percent of concordant ages in 0, ± 1, ± 2 and ± 3 years was 89.0%, 9.0%, 2.0% and 0.0% for males and 85.2%, 11.8%, 2.8% and 0.2% for females. The APE, V and D precision indexes obtained were respectively 9.93, 9.23 and 5.33 for males and 17.58, 18.22 and 10.52 for females.

In general, no significant differences in the mean total length-at-age between sexes were detected for the younger age classes while significant differences were detected for the older age classes. Specifically, while no differences were found for age classes 0, 1, 2 and 4, there were differences for age classes 3, 5, 6 and 7 (Table

5.3). Differences for older specimens were not tested due to small sample size (age class 8) or due to the complete lack of males (age classes 9, 10 and 11).

Table 5.3. Comparison of the mean total length (TL, cm) between male and female *Etmopterus spinax* for each age group. n refers to the sample size and SD to the standard deviation. The t-statistic value, the P-value and the decision (significant or not significant) are also given.

Age	Females			Males			t - student		
	n	Mean TL	SD	n	Mean TL	SD	t	P - value	Sig. Dif.
0	38	12.64	1.44	35	12.32	1.27	-1.01	0.319	No
1	34	16.34	1.62	29	16.00	1.46	-0.87	0.390	No
2	61	19.91	1.55	67	19.52	1.24	-1.55	0.120	No
3	48	23.47	1.80	54	22.38	1.71	-3.13	0.002	Yes
4	28	27.76	2.45	40	26.87	1.91	-1.67	0.099	No
5	80	32.42	2.03	42	29.51	1.33	-8.38	<0.001	Yes
6	71	34.55	1.45	29	31.07	1.02	-11.77	<0.001	Yes
7	43	36.09	1.24	3	31.10	0.72	-6.85	<0.001	Yes
8	20	37.25	1.26	1	33.50				
9	7	39.00	1.21						
10	2	39.60	2.12						
11	1	40.70							

A total of 395 specimens were used for age validation by marginal increment analysis. A clear annual pattern of band formation could be observed with this analysis, with the marginal increment showing higher values during the warmer months, from April to October and lower values during the colder months, from November to February (Figure 5.5). This pattern was observed in all age classes for which this analysis was performed (between ages 3 and 7), while for the other age classes this analysis was not performed due to low sample size. Significant differences were found between mean MIR values along the different months (ANOVA: F = 8.69; P-value < 0.001).

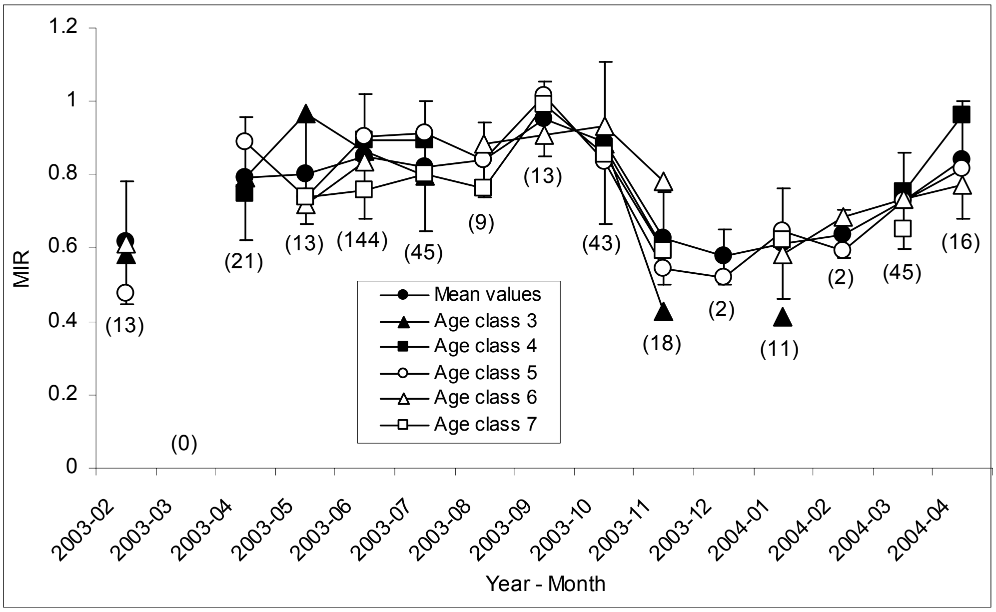

Figure 5.5. Monthly evolution of the marginal increment (MIR) both for all age classes combined and for each age class separately. The values in brackets refer to the total sample size (n) in each month.

5.4.4. Growth modelling

In general, the four models used gave good fits to the length-at-age data and produced relatively similar curves, both in the case of males and females. In all cases the estimated L_{inf} values were higher for females, except for the VBGF where the opposite situation was observed (Figure 5.6). For weight based data, the VBGF produced slightly higher estimates of growth for the older age classes (Figure 5.6).

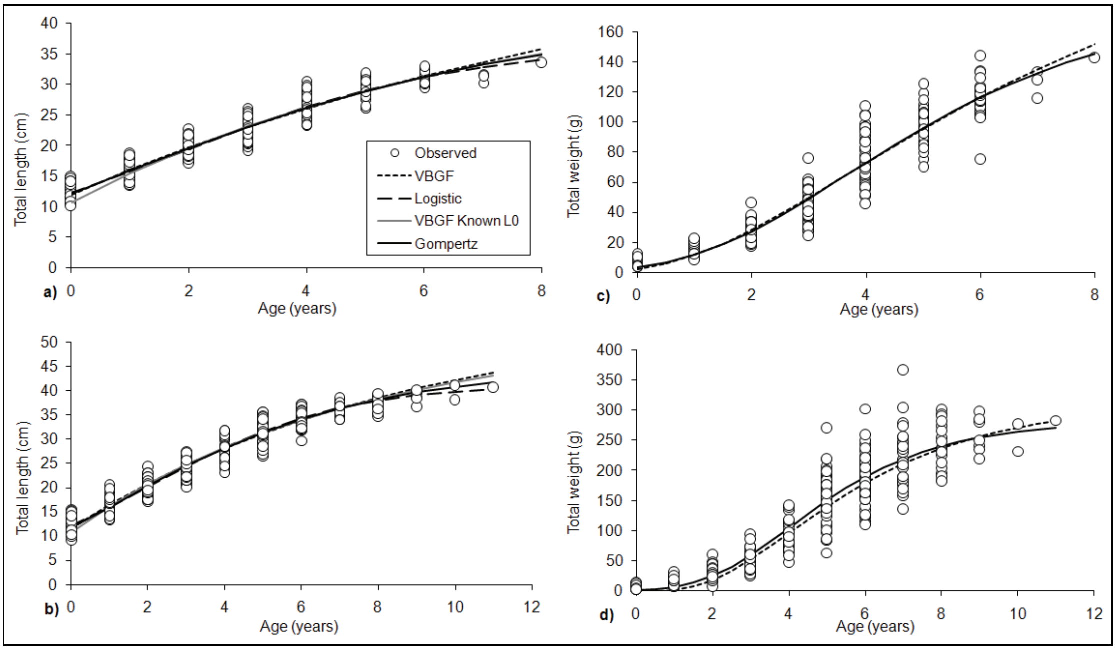

Figure 5.6. Length-at-age data (a, b) and weight-at-age (c, d) data for males (a, c) and females (b, d) of *Etmopterus spinax*, with the respective fitted growth models.

Between sexes comparisons for each length based model showed significant differences between male and female growth curves (Max. Likelihood $_{\text{VBGF}}$: $\chi^2 = 162$, P-value < 0.05; Max. Likelihood $_{\text{VBGF known L0}}$: $\chi^2 = 143$, P-value < 0.05; Max. Likelihood $_{\text{Logistic}}$: $\chi^2 = 182$, P-value < 0.05; Max. Likelihood $_{\text{Gompertz}}$: $\chi^2 = 172$, P-value < 0.05). Between sexes comparisons for each weight based model also showed significant differences between male and female growth curves (Max. Likelihood $_{\text{VBGF}}$: $\chi^2 = 640$, P-value < 0.05; Max. Likelihood $_{\text{Gompertz}}$: $\chi^2 = 635$, P-value < 0.05).

A considerable variability in L_{inf} and W_{inf} values was obtained depending on the growth model used. L_{inf} values for males varied from 36.83 to 57.96 cm TL and for females from 41.38 to 55.84 cm TL, with the VBGF producing the highest values and the logistic equation the lowest. W_{inf} values varied from 139.78 to 177.20 g W for males and from 249.58 to 283.19 g W for females, with the VBGF producing the lowest estimated values and the Gompertz the highest (Table 5.4).

Table 5.4. Comparison of parameters estimated for the different models for length-at-age and weight-at-age data, for male and female *Etmopterus spinax*. The maximum asymptotic size is indicated in cm for the length-at-age models and g for the weight-at-age models. The growth coefficient refers to the parameters k (VBGF models), g (Gompertz model) and r (logistic model).

Data set	Sex	Model	L_{inf} (cm)- W_{inf} (g)				Growth coefficient ($Year^{-1}$)			
			Estimate	SE	Lower 95% CI	Upper 95% CI	Estimate	SE	Lower 95% CI	Upper 95% CI
Length-at-age	Males	VBFG	57.96	5.94	46.28	69.64	0.09	0.02	0.06	0.12
		VBGF known L_0	46.50	2.23	42.10	50.90	0.14	0.01	0.12	0.17
		Logistic	36.83	0.87	35.11	38.55	0.40	0.02	0.37	0.44
		Gompertz	44.97	0.84	43.32	46.63	0.25	0.02	0.21	0.28
	Females	VBFG	55.84	2.38	51.15	60.53	0.12	0.01	0.10	0.14
		VBGF known L_0	52.42	1.54	49.40	55.44	0.14	0.01	0.12	0.15
		Logistic	41.38	0.51	40.38	42.39	0.41	0.01	0.39	0.43
		Gompertz	41.43	1.57	38.34	44.53	0.26	0.01	0.24	0.28
Weight-at-age	Males	VBGF	226.09	24.43	178.02	274.16	0.23	0.03	0.18	0.28
		Gompertz	177.20	11.75	154.07	200.33	0.37	0.03	0.32	0.43
	Females	VBGF	311.49	14.82	282.36	340.63	0.33	0.02	0.28	0.38
		Gompertz	283.19	10.11	263.31	303.07	0.45	0.03	0.40	0.51

For both sexes, the best model according both to the Akaike's information criterion and the coefficient of determination was the logistic model, followed by the Gompertz model, then the VBGF and finally the VBGF with known L_0. However, given the low values of Δi (< 2 in all cases) we assume all models are valid and provide useful information. For weight based data, the r^2 values were similar for both models and sexes, but according to the *AICc*, the VBGF was more accurate than the Gompertz model (Table 5.5).

Table 5.5. Values of the coefficient of determination (r^2), the small-sample corrected form of Akaike's information criterion (AICc) and the Akaike's differences (Δi) for each growth model, both in length and weight and for each sex. In each case, models are listed from best to worst according to the AICc.

Data set	Sex	Model	r^2	AIC_c	Δi
Length at age	Males	Logistic	0.941	2.39	0.00
		Gompertz	0.939	2.44	0.06
		VBFG	0.937	2.52	0.14
		VBGF known L_0	0.930	2.74	0.35
	Females	Logistic	0.956	3.16	0.00
		Gompertz	0.953	3.38	0.22
		VBFG	0.947	3.76	0.60
		VBGF known L_0	0.946	3.83	0.67
Weight at age	Males	VBFG	0.921	108.06	0.00
		Gompertz	0.924	110.25	2.19
	Females	VBFG	0.894	770.84	0.00
		Gompertz	0.896	774.50	3.66

5.4.5. Reproductive cycle

The annual variation of the percentage of occurrence of the different maturity stages showed that most of the mature females stages, namely mature females with ripe oocytes (stage 3), pregnant females (stage 4 and 5) and resting females (stage 7) occurred throughout the year. Late term pregnant females (stage 6) were only caught during June. For males, both mature stages (stages 3 and 4) also occurred throughout the year, although the percentages varied a little, with mature but not active males (stage 3) occurring in higher percentages during the summer months (Figure 5.7).

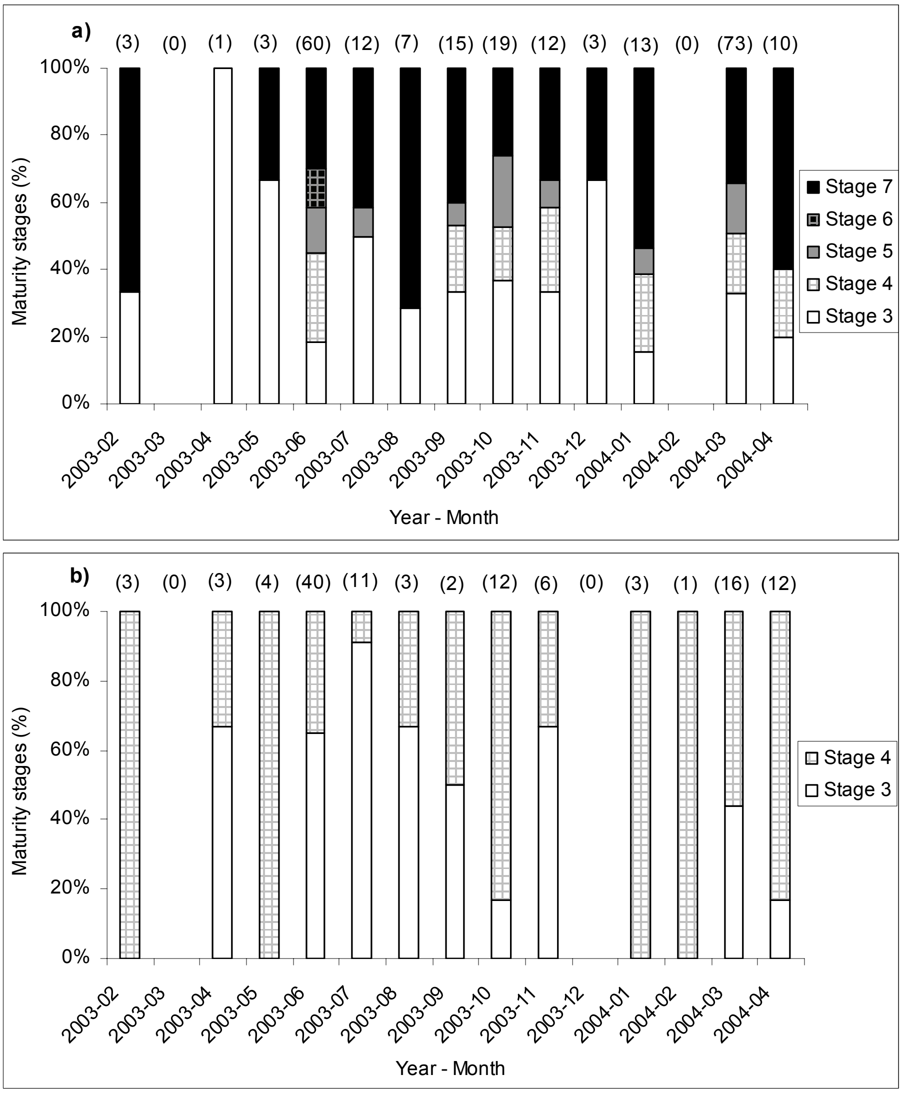

Figure 5.7. Monthly variation of the percentage of occurrence of the different mature stages in female (a) and male (b) *Etmopterus spinax*. The values between brackets represent sample size (n).

Even though mature females with ripe oocytes (stage 3) occur throughout the year, the variation of the gonadossomatic index of this component of the population shows a trend, with a progressive increase of this value starting in September, increasing during the autumn and winter and reaching the highest values during the summer (Figure 5.8). This pattern suggests that it takes one year for the oocytes do develop until they reach maturity during the summer, when they are ready to be fertilized and move into the uterus as embryos.

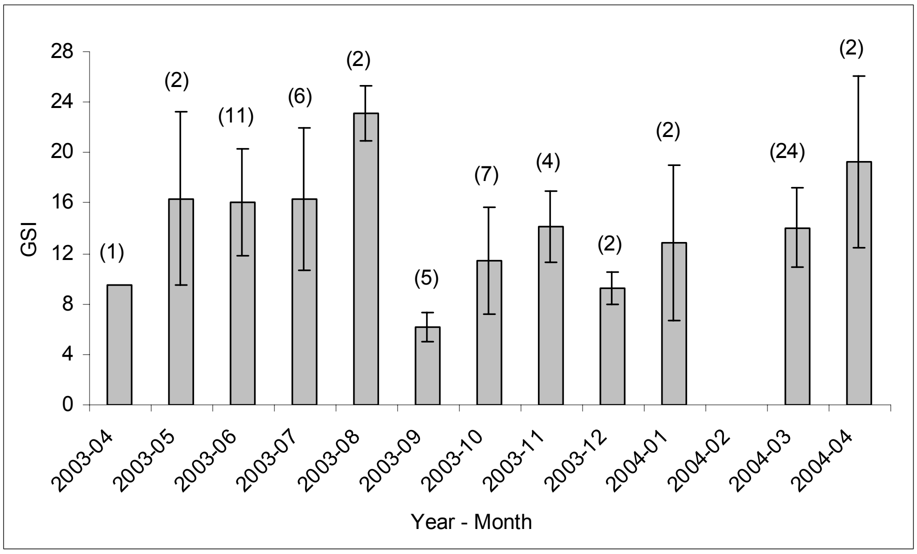

Figure 5.8. Monthly variation of the gonadosomatic index (GSI) for mature females with ripe oocytes (stage 3) *Etmopterus spinax*. The values between brackets represent sample size (n).

A clear evolution of the GSI was observed as the maturity stages of both males and females developed (Figure 5.9). In females, this index is very low while specimens are immature (stage 1 and 2) and increase to the highest value in stage 3. In pregnant females (stages 4 to 6) this index falls again to values similar to those of immature specimens, indicating that this species has an alternate reproductive cycle, with the ovarian and the uterine phases occurring separately. In this type of reproductive cycle strategy, while females are pregnant the oocytes remain immature and the gonads do not develop, remaining relatively small. In stage 7 females, there is a slight increase of the GSI, reaching values similar to stage 2, probably due to the fact that in some specimens the oocytes are already starting to develop, in order to start a new ovarian cycle. Significant differences were found between the GSI values of the different maturity stages (Kruskal-Wallis: H = 344, P-value < 0.001). The pairwise Dunn tests showed that significant differences occurred between stage 3 and all others (Dunn: P-values < 0.05) and between stage 1 and all others except stage 6 (Dunn: P-values < 0.05). No differences were detected between the other possible pairwise combinations (Dunn: P-values > 0.05). In males, there is an increase of this index with the evolution of the maturity stages, with the highest differences observed when specimens reach maturity (between stages 2 and 3). Significant differences were found (Kruskal-Wallis: H = 232; P-value < 0.001), and according to the pair wise multiple comparison test, there are significant differences between stage 1 and all other stages (Dunn: P-values < 0.05 in all cases) but not between the other pairwise possible combinations (Dunn: P-values > 0.05).

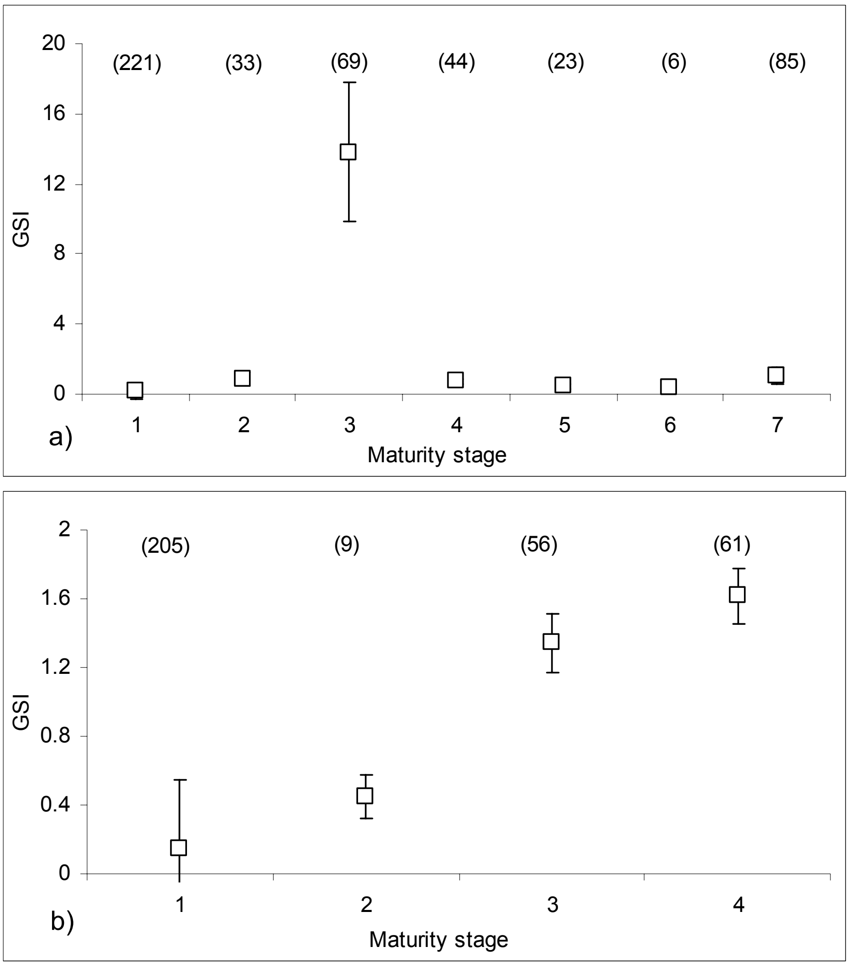

Figure 5.9. Evolution of the gonadosomatic index (GSI) for both female (a) and male (b) *Etmopterus spinax*. Error bars represent ± 1 standard deviation. The values above each point are the sample sizes (n).

A clear evolution of HSI is also observed with the evolution of maturity stages in both males and females (Figure 5.10). On females, this index increases until they reach stage 3 and then, during pregnancy (stages 4 to 6), it decreases probably due to the high energy demand for those stages. In the resting phase (stage 7), the index increases again, probably due to a stat of energy accumulation for the next reproductive cycle. The variations in HSI were significant (Kruskal-Wallis: H = 325; P-value < 0.001), with the pairwise tests showing differences between stage 1 and all other stages except stage 6 (Dunn: P-values < 0.05), but not for the other possible pairs (Dunn: P-value > 0.05). In males, there is a progressive increase of the HSI with the evolution of the maturity stage, with the differences more accentuated between stages 1 and 2 and more progressive for the other stages. Significant differences were found (Kruskal-Wallis: H = 176; P-value < 0.001) between stage 1 and all other stages (Dunn: P-values < 0.05) but there were no differences between all other possible pairs (Dunn: P-values > 0.05).

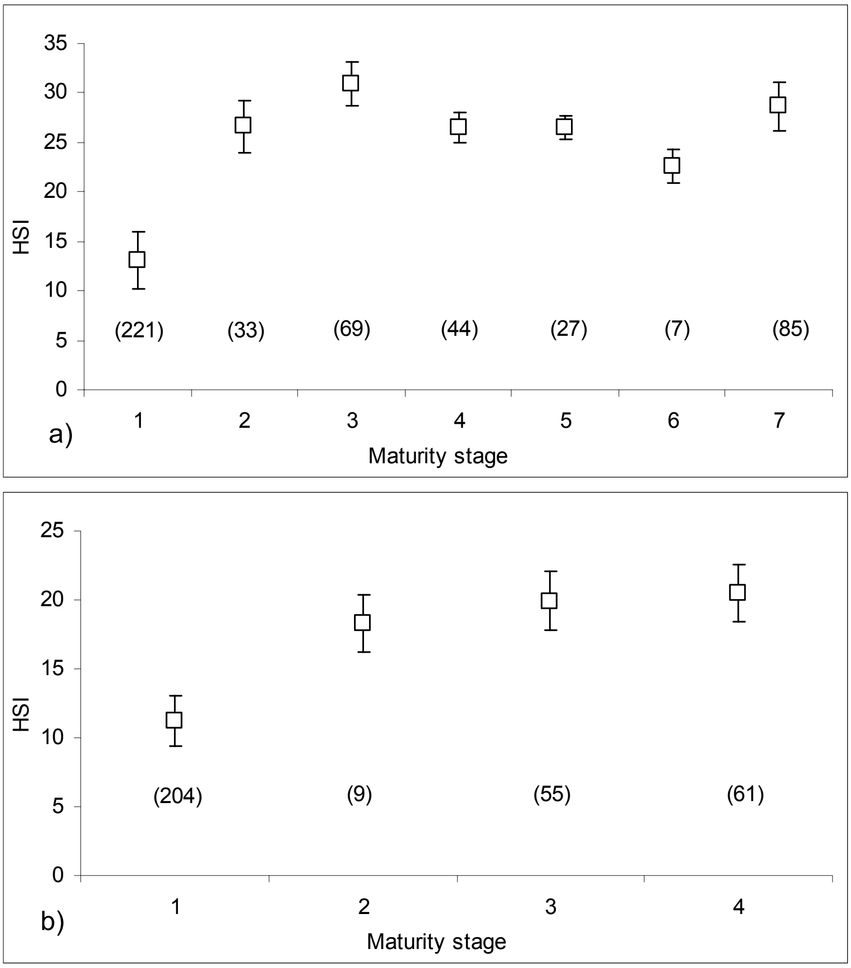

Figure 5.10. Evolution of the hepatosomatic Index (HSI) for female (a) and male (b) *Etmopterus spinax*. Error bars represent ± 1 standard deviation. The value below each point is the sample size (n).

5.4.6. Maturity

In general terms, females of *E. spinax* mature at larger sizes and older ages than males. The maximum size of immature males was 26.7 cm while the smallest mature male was 24.2 cm TL. The largest immature female was 36.2 cm and the smallest mature female had 30.5 cm TL (Figure 5.11). In terms of age, the oldest immature males were 4 years old and the oldest immature females were 8 years old. On the other hand, the youngest mature males and females were both 4 years old (Figure 5.11). The 2 way ANOVA showed significant differences between sexes and for mature or immature condition, for both length (2 way ANOVA $_{\text{Sex}}$: $F = 161$; $P\text{-value} < 0.001$; 2 way ANOVA $_{\text{Maturity}}$: $F = 1490$; $P\text{-value} < 0.001$) and age (2 way ANOVA $_{\text{Sex}}$: $F = 56$; $P\text{-value} < 0.001$; 2 way ANOVA $_{\text{Maturity}}$: $F = 1141$; $P\text{-value} < 0.001$).

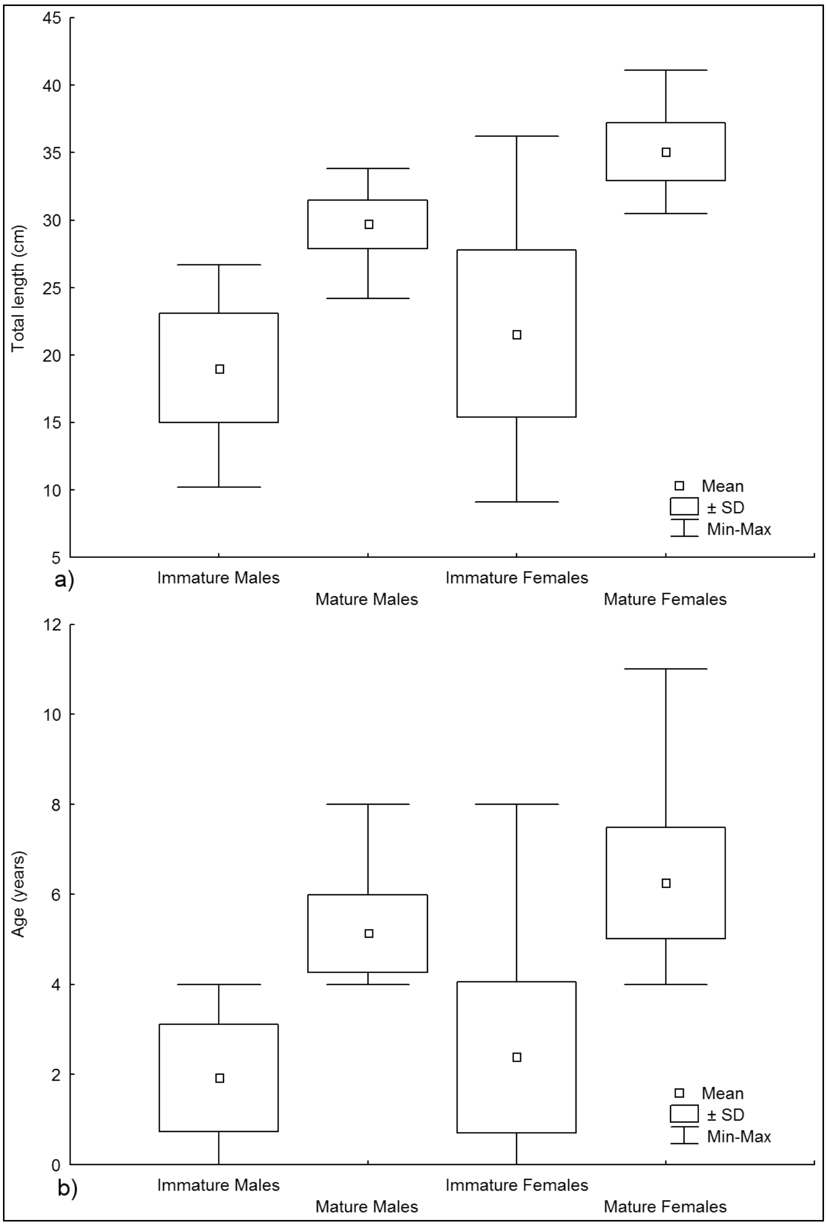

Figure 5.11. Box and whiskers plot with the mean, standard deviation ranges of size (a) and age (b) for mature and immature males and females *Etmopterus spinax*.

Both size based and age based maturity ogives produced good fits to the observed data, with high r^2 values, namely 0.997 and 0.992 for female and male length based ogives and 0.996 and 1 for female and male age based ogives. Females matured at larger sizes than males, with estimated sizes at first maturity of 30.69 cm TL for females and 25.85 cm TL for males (Figure 5.12). Females also matured at later ages than males, with estimated ages of first maturity of 3.97 years for males and

4.67 years for females (Figure 5.12). There were significant differences between sexes in terms of the parameters of both the size- (Max. Likelihood: $\chi^2 = 211.15$; P-value < 0.05) and the age-based (Max. Likelihood: $\chi^2 = 304.30$; P-value < 0.05) maturity ogives.

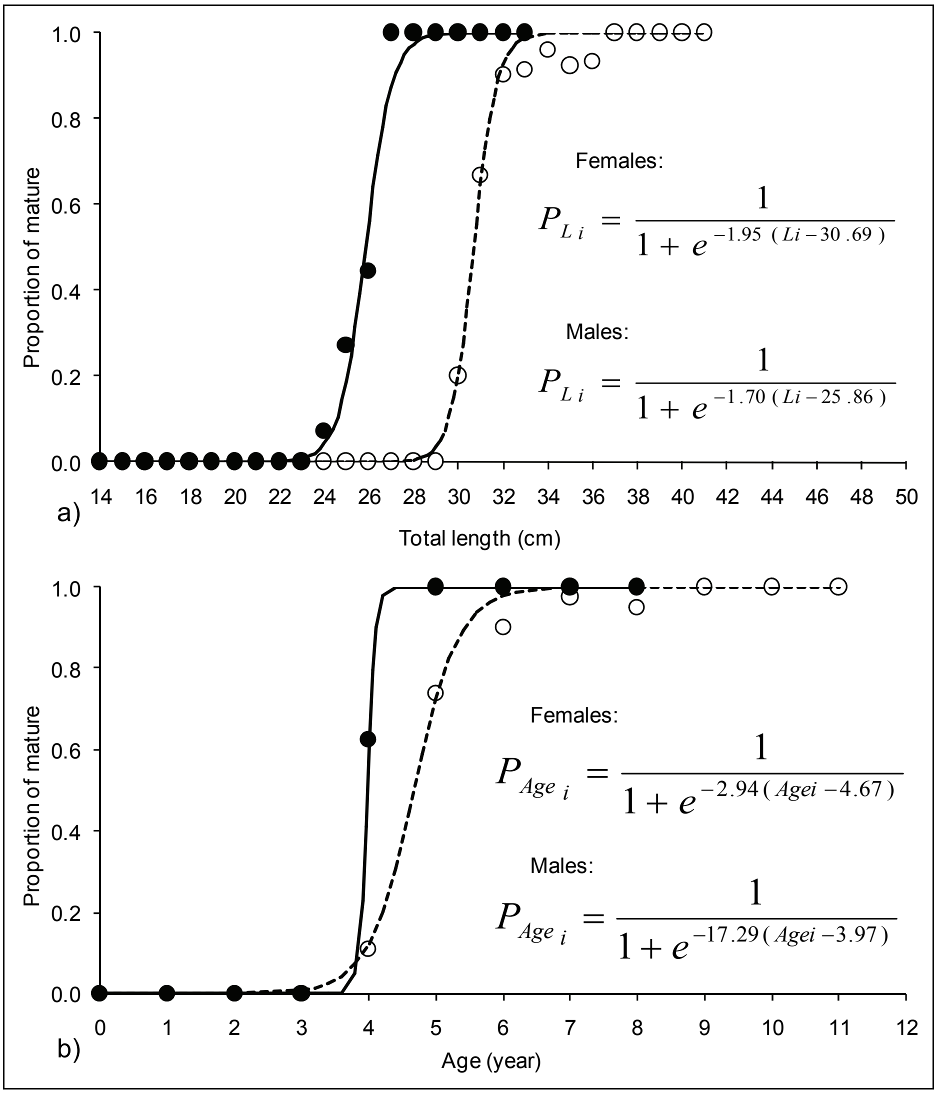

Figure 5.12. Size (a) and age (b) based maturity ogives for *Etmopterus spinax* with the respective equations. Dark and white points represent the proportion of mature males and females in each size or age class, while the solid (male) and dotted (female) lines represent the corresponding fitted logistic curves.

Since no significant differences were found between left and right side claspers (ANCOVA: F = 0.10; P-value = 0.756), a mean value was calculated for each specimen and plotted against total length. There is a clear relationship between clasper length and TL, with an accentuated increase once the specimens attained maturity (Figure 5.13). Likewise, no significant differences were found between left and right side uterus of females (ANCOVA: F = 0.16; P-value = 0.691). Immature specimens have a relatively narrow uterus independently of TL, while in mature specimens, the uterus can either appear substantially enlarged, in the case of pregnant females with embryos inside the uterus, or remain relatively narrow, in the case of mature specimens with ripe oocytes in the gonads. Resting specimens have uterus widths somewhat between the before mentioned conditions (Figure 5.13).

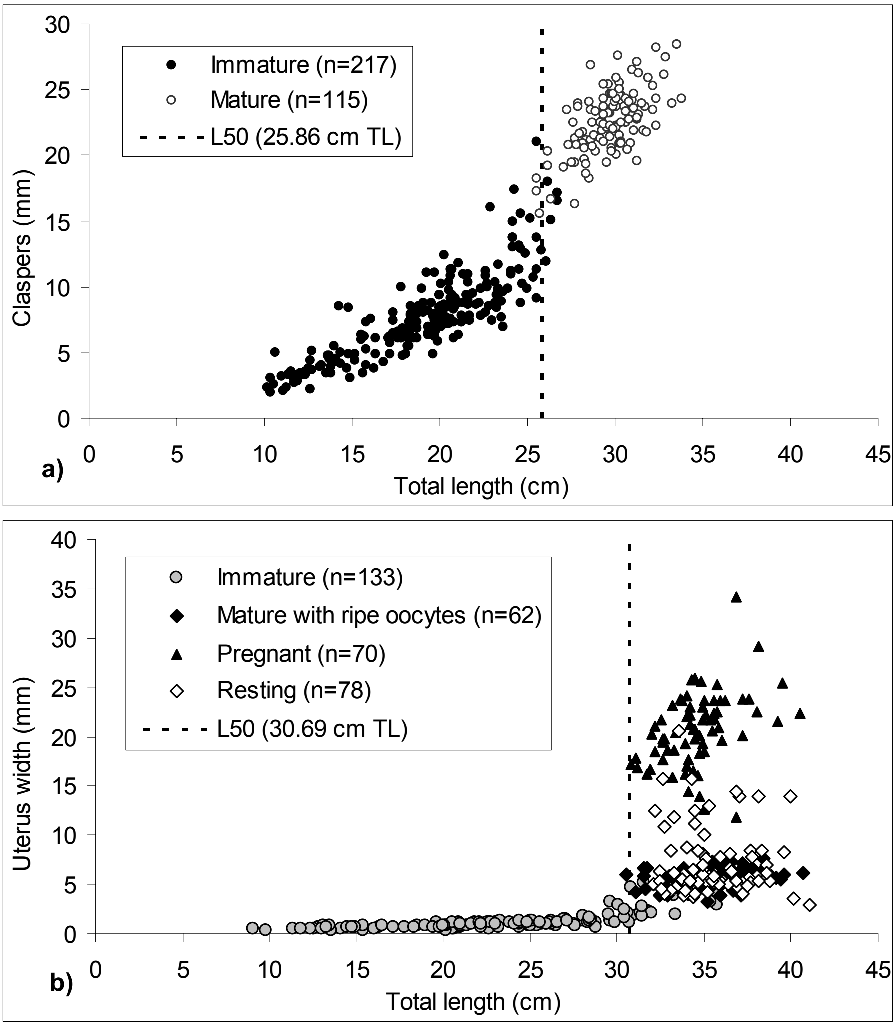

Figure 5.13. Relation between total length (TL) with clasper length in males (a) and uterus width on females (b) of *Etmopterus spinax*. It is also indicated the L_{50} value estimated by the maturity ogives.

Fifty percent of the females and the males in this population are mature respectively at 74.7% and 76.5% of the maximum observed size. In terms of age, 50% of the females are mature at 42.5% and 50% of the males are mature at 49.6% of the maximum observed ages (Table 5.6).

Table 5.6. Estimated parameters for size (L_{50}) and age (Age_{50}) at maturity, with the respective 95% confidence intervals (CI) for males and females of *Etmopterus spinax*.

		Females	Males
Size range (cm)	Immature	9.1 – 36.2	10.2 – 26.6
	Mature	30.5 – 41.1	24.2 – 33.8
L_{50} (cm)	Estimate	30.69	25.86
	Lower 95% CI	30.6	25.7
	Upper 95% CI	30.78	26.02
Age range (year)	Immature	0 – 8	0 – 4
	Mature	4 – 11	4 – 8
Age_{50} (year)	Estimate	4.67	3.97
	Lower 95% CI	4.57	3.97
	Upper 95% CI	4.78	3.97
L_{50} / L_{max} (%)		74.7	76.5
Age_{50} / Age_{max} (%)		42.5	49.6

5.4.7. Fecundity

The ovarian fecundity in mature (stage 3) females varied from 5 to 21 ripe oocytes, while the uterine fecundity in mid term pregnant females (stage 5) was a little lower and varied from 1 to 16 embryos (Table 5.7). Only six pregnant females in final pregnancy (stage 6) were caught, carrying from 1 to 9 completely formed embryos.

A linear relationship between the ovarian fecundity (number of ripe oocytes) and the female TL was observed (ANOVA: F = 14.67, P-value < 0.001), meaning that the fecundity in this species increases with increasing TL of the females (Figure 5.14).

Table 5.7. Ovarian and uterine fecundity of *Etmopterus spinax*, with the respective averages, standard deviations (SD), ranges and sample sizes both in number of specimens and either ripe oocytes or mid term embryos.

	Ovarian fecundity (stage 3)	Uterine fecundity (stage 5)
Average	9.94	7.59
SD	2.61	3.31
Range	5 – 21	1 – 16
$n_{\text{specimens}}$	83	34
$n_{\text{oocytes or embryos}}$	825	258

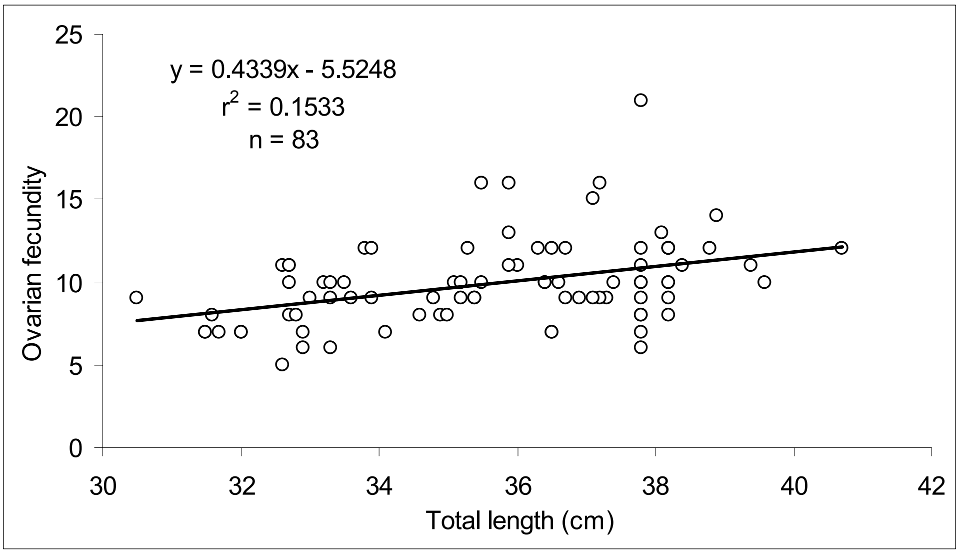

Figure 5.14. Relationship between the total length and the ovarian fecundity (number of ripe oocytes in mature stage 3 females), in *Etmopterus spinax*, with the respective linear regression, the coefficient of determination (r^2) and the sample size (n).

5.5. Discussion

E. spinax has a wide distribution, occurring throughout most of the eastern side of the Atlantic and the Mediterranean. Lacking any commercial value and commonly discarded in trawl and longline fisheries, it has been poorly studied. This is the first thorough study that focuses on the population dynamics and life history of this species.

Catches of this species can be very high in the commercial deep water fisheries operating in the area, and although caught specimens are mostly discarded, they are usually returned to sea either dead or with severe injuries that probably affect their survival. Specimens caught with trawls tend to arrive dead on board, while specimens caught with longlines are often still alive, but with injuries caused by the hooks and by the sudden changes in pressure and temperature. By-catch reduction strategies such as the one proposed by Coelho *et al.* (2003) might be the only possibility to prevent excessive fishing related mortality on this and other similar deep water squalid species.

Although this study included a relatively large sample in number with a wide size range, the maximum sizes caught were considerably smaller that the maximum sizes described by Compagno *et al.* (2005). Specifically, the largest females and males in this study with respectively 41.1 cm and 33.8 cm TL, were much smaller than the maximum generalist size of 60 cm TL (rare above 45cm) reported by Compagno *et al.* (2005). Given that during this study a wide variety of depths and habitats were surveyed, using different commercial and scientific survey fishing gears in different seasons of the year, we consider that the lack of larger specimens in the sample reflects a lack of specimens in the study area and not a shortcoming of the sampling strategy.

Prior to examination of growth bands, spine growth should be investigated by measuring spine morphometrics (Clarke and Irvine, 2006), and this was accomplished in this study when significant relationships were found between specimen length and several spine morphometrics. Therefore, prior to determination of age, there was already evidence that the growth of spines is proportional to specimen growth and therefore spines could be suitable for estimating age. In addition, relationships between specimen length and spine weight were also investigated and again, positive and significant relationships were found, thus confirming the relationship between spine and specimen growth.

Given that preliminary investigations showed that the 1st dorsal spine is often more damaged than the 2nd, age was estimated based only on the inner dentine layer of the 2nd dorsal spine. Irvine *et al.* (2006a) estimated ages of *Etmopterus baxteri* based on both the inner dentine layer and on the exterior bands of the spines and hypothesized that the inner dentine bands may underestimate age of the older specimens. In the present study, and due to the significant linear relationships established between spine radius, where the dentine is deposited, and specimen growth for the entire length distribution of the species, it was considered that the deposition of this layer did not cease for the older specimens, and therefore age estimations should not be underestimated. Nevertheless, future work should also investigate the exterior enamel of the spines (Irvine *et al.*, 2006b). Even thought *E. spinax* is a small sized species, it has a relatively slow growth rate. Only two other studies are known that have determined growth rates for *Etmopterus* species, specifically Ivine *et al.* (2006a) for *E. baxteri* and Coelho and Erzini (2007) for *E. pusillus* and on both cases slow growth rates were also observed.

Age validation is an essential aspect of age and growth studies (Cailliet *et al.*, 1986; Cailliet, 1990; Campana, 2001; Cailliet *et al.*, 2006). In the present study, age was validated by the MIR analysis, which is one of the most commonly used techniques (e.g. Simpfendorfer, 1993; Conrath *et al.*, 2002; Carlson and Baremore, 2005; Neer and Thompson, 2005b). Even thought Campana (2001) stated that the

MIR analysis is not one of the most accurate for age validation, it was considered that the techniques recommended by that author over the MIR analysis are not applicable to *E. spinax*. Such techniques include the release of tagged fishes of known age, which implies that the species must be bred in captivity; bomb radiocarbon validation, which implies that at least some specimens must have been born before the 1960's when the ^{14}C in the world oceans increased significantly; or tagging fishes with oxitetracycline, which implies that specimens must be caught, tagged and released without significant mortality. At this point, only one study is known to have validated age based on squalid spines bomb radiocarbon (Campana *et al.*, 2006a). However, Campana (2001) stated that the MIR analysis could be used successfully if some suppositions were guaranteed, specifically 1) measuring blindly the structures, without knowledge of the date of capture, 2) observing at least two complete band forming cycles, 3) making an objective interpretation of the results, ideally with the resource of statistics and 4) analysing few (ideally one) age groups at a time. Given that in the present study three of these suppositions were respected, with the only shortcoming being that only one, instead of two complete cycles were analyzed, it was considered that the age validation procedure used is robust and effectively proves that in this species one pair of bands (one opaque and one translucent) is formed each year.

Even thought the von Bertalanffy growth curve is the most widely used approach to model the growth of fishes (Katsanevakis, 2006), several authors have shown that alternative models have provided better fits to length-at-age data of some elasmobranch species. In this study, and even though the VBGF produced good fits, additional growth models were used for comparative purposes. It was concluded that for both sexes the best fit was achieved by the logistic equation, followed by the

Gompertz model, then the VBGF and finally the VBGF with known size at birth. Alternative growth models have been applied in elasmobranch fishes to both rays (e.g. Neer and Thompson, 2005b; McFarlane and King, 2006) and sharks (e.g. Carlson and Baremore, 2005; Neer and Thompson, 2005a). Growth model selection was based on the Akaike information criterion (Shono, 2000) as suggested by Katsanevakis (2006), and the Akaike differences (Δi) used to assess the extent of the contribution of the alternative models. All size-at-age based models, both for males and females produced values of $\Delta i < 2$, meaning that every model tested in this study can be used to explain and support the data. For weight-at-age data, the VBGF model was the best for both sexes and the Gompertz model produced values of $2 < \Delta i < 10$ meaning that it can be used but it is not the most adequate.

The evolution of the GSI in stage 3 females throughout the year suggested that it takes one year for females to develop the oocytes in the gonads until fertilization in the summer. The presence of active males mostly during the winter and spring suggests that mating occurs before the oocytes are totally mature and that females probably store sperm. If we add the fact that the ovarian and the uterine cycles do not occur at the same time in pregnant females, meaning that while females are pregnant the oocytes in the gonads remain immature and do not develop for the next cycle, and that resting females (with immature oocytes) were found throughout the year, then this species may reproduce only once every three years. The complete cycle may take one year for the oocytes to reach maturity, which occurs in the summer, one year for the embryos do develop in the uterus, culminating in June when late term pregnant females (stage 6) were found, and finally one year of resting, with both immature oocytes in the gonads and no embryos in the uterus. This situation has significant implications for management and conservation. Other deep water squalid sharks have been described to have long gestation periods, such as the cases of *Centroscymnus coelolepis* (Clarke *et al.*, 2001) and *Squalus megalops* (Braccini *et al.*, 2006) that have two year cycles or the case of *Centrophorus* cf. *uyato* (McLaughlin and Morrissey, 2005) that may also have a three year cycle.

E. spinax in Portuguese waters matures relatively late in its life cycle. Coelho and Erzini (2005) presented preliminary results regarding size at maturity for this species that are very similar to the final results now presented in this work. This information is also now complemented with age-at-maturity estimates not previously reported. Cortés (2000) examined 164 species of sharks and concluded that on average, maturity occurs at around 75% of the maximum size and around 50% of the maximum age and the values obtained during this study are very close to these general values. The size at maturity estimated by the maturity ogives resulted in maturity estimates similar to what was observed by the growth of the sexual characters, specifically clasper length in males and the uterus width in females. In this study, females matured at significantly larger sizes and older ages than males. Sexual dimorphism in terms of size-at-maturity is common in elasmobranch fishes and specifically for the *Etmopterus* genus, with females usually maturing later and at larger sizes than males (Jakobsdottir, 2001; Irvine *et al.*, 2006a).

This species is an aplacental viviparous shark with a relatively low fecundity. The differences observed between the ovarian and the uterine fecundities may be explained by two hypotheses: 1) that part of the ripe oocytes present in stage 3 females never develop into embryos or, 2) that since this is an aplacental species, without an umbilical cord connecting the mother to the embryos, it is possible that the stress produced during the fishing process leads to the release of some of the embryos in the uterus of pregnant females. During the sampling process aboard the fishing boats, and while the specimens were deposited in boxes for later processing, it was common to observe middle term embryos in the middle of the catch. This observation supports the second hypothesis, indicating that there is indeed a loss of embryos by pregnant females during the fishing process. Therefore, fecundity in this species should be estimated by the ovarian fecundity and not by uterine fecundity, since the latter may tend to underestimate this parameter.

In this species, a significant linear relationship was observed between the female TL and the number of ripe oocytes in the gonads. Morphologically, this relationship makes sense given that in viviparous species the number of oocytes in the gonads and, after fecundation, the number of embryos in the uterus, are limited by the size of the abdominal cavity, which increases with increasing specimen size. Other species of deep water viviparous sharks where such relationships were found include *Centroscymnus owstoni* and *C. coelolepis* (Yano and Tanaka, 1988) and *Centroscyllium fabricii* (Yano, 1995).

In conclusion, this study suggests that *E. spinax* in the NE Atlantic has a vulnerable life cycle, a situation previously described for several other deep water squalid sharks. In this particular species, a possible three year reproductive cycle may have even further conservation implications. In the NE Atlantic, several deep water fisheries are in operation and there are no perspectives of a decrease of effort or a reduction of the discards in the near future, meaning that presently, this species may already be threatened and facing severe declines in this area.

CHAPTER 6: Depth distribution of the velvet belly lantern shark, *Etmopterus spinax* in relation to growth and reproductive cycle[*]

(Aboard the research vessel "Cornide de Saavedra" in the Mediterranean Sea)

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[*] *In Preparation*: Coelho, R. & Erzini, K. Depth distribution of the velvet belly lantern shark, *Etmopterus spinax* in relation to growth and reproductive cycle.

6.1. Abstract

The velvet belly lantern shark, *Etmopterus spinax*, is a small sized squalid shark that is commonly found in deep water off the Portuguese coast, mainly on soft bottoms. In this study, 67 one hour research cruise bottom trawl tows were carried out during June 2003, off the southern and southwestern Portuguese coasts, at depths that ranged from 84 to 786 m. All *E. spinax* specimens caught were sexed, measured, aged and classified for maturity stage. A total of 396 specimens (192 males and 204 females) were caught, with total lengths and ages ranging respectively from 10.2 to 32.9 cm and 0 to 7 years for males and from 9.8 to 41.1 cm and 0 to 10 years for females. Size, age, sex and maturity stages were found to be correlated with depth, with the larger, older and mature specimens occurring predominantly at greater depths. There seems to be a depth related migration of pregnant females between the deeper mating grounds and the shallower nursery grounds. The sex ratios were relatively similar in the shallower end of the range, but females dominated at more than 600 m and were exclusive at more than 700 m. Fishing related mortality may have complex and significant repercussions on this species, given that commercial fisheries are impacting different segments of this population differently.

6.2. Introduction

The velvet belly lantern shark, *Etmopterus spinax* is a small sized deep water squalid shark that lives mainly on the outer continental and insular shelves and upper slopes (Compagno *et al.*, 2005). Geographically, this species occurs in the eastern Atlantic and the Mediterranean: in the eastern Atlantic Ocean it has been described from Iceland and Norway to South Africa (Compagno, 1984), including the Azores (Santos *et al.*, 1997), the Canaries (Brito *et al.*, 2002) and the Cape Verde Islands

(Reiner, 1996), and in the Mediterranean it has been described for the western and central areas (Serena, 2005), including the Ionian, the lower Adriatic and the Aegean seas (Notarbartolo di Sciara and Bianchi, 1998).

In Portugal, as in most areas of its distribution, this species is commonly caught as by-catch of several deep water fisheries and due to its low or null commercial value is usually discarded. An important Portuguese fishery that catches this species is the deep bottom trawl fishery that targets Norway lobster, *Nephrops norvegicus*, deepwater rose shrimp, *Parapenaeus longirostris*, and red shrimp, *Aristeus antennatus* (Monteiro *et al.*, 2001).

Although information on population structure, reproductive biology, and age and growth is essential for proper management and conservation of populations, this information is lacking for most of the deep sea shark species. For the particular case of the southern Portuguese population of *E. spinax* some data is already available: Coelho and Erzini (2005) report that this is an aplacentary viviparous species and presented preliminary lengths at maturity, Neiva *et al.* (2006) studied the feeding ecology, and more recently Coelho and Erzini (submitted-b) presented a comprehensive population dynamics study including age and growth and reproductive biology.

Previous studies have established relationships between depth of capture and size of specimens of deep water squalid sharks (e.g. Yano and Tanaka, 1988; Clarke *et al.*, 2001; Jakobsdottir, 2001). In the specific case of *E. spinax*, two studies are known to have described depth distribution patterns for the Mediterranean Sea (Massuti and Moranta, 2003; Sion *et al.*, 2004), but no studies are known for the eastern Atlantic populations. It is worth noting that most studies describe depth distribution in terms of abundance and size, but none have used age based data.

Given the high fishing mortality that this species is currently suffering in the Portuguese coast, there is a need to understand how this species is distributed throughout its depth range, in order to understand the ecological repercussions of these fisheries. The objectives of this study are to present information regarding the depth distribution of this shark, correlate it with growth (both in size and age) and reproductive cycle and discuss some of the impacts that commercial fisheries in the area are having on this species.

6.3. Material and methods

Biological sampling was carried out during the Portuguese Marine Research Institute (INIAP – IPIMAR) demersal trawl research survey off the southwestern and southern Portuguese coasts, during the summer of 2003. The gear used was a bottom trawl with a codend mesh size of 25 mm and horizontal and vertical openings of 30.2 m and 3.2 m (Leite *et al.* 1990). A total of 67 standardized 1 hour bottom trawls were carried out, with vessel speed ranging from 2.7 to 2.9 knots during trawling. The geographic position (longitude and latitude coordinates; reference system: WGS84) and the depth were recorded both at the initial and final positions of each trawl and a mean position and depth calculated. Trawls ranged from 84 to 786 m depth and were carried out mostly on sandy / muddy bottoms. Trawls were grouped in 100 m depth intervals, such that depth class 0 grouped trawls from 0 to 99 m, depth class 100 grouped trawls from 100 to 199 m and so on up to depth class 700, that grouped trawls from 700 to 799 m depth.

All *E. spinax* specimens caught were brought to the laboratory where the biological analysis took place. Specimens were measured for total length (TL, to the nearest lower millimetre) in a strait line from the tip of the snout to the tip of the caudal fin in its natural position, sexed and dissected. Maturity stages were defined for both males and females based on the macroscopic observations of the reproductive organs, according to the scales developed by Coelho and Erzini (2007) for *Etmopterus* species. According to these scales, males are classified in 4 stages, where stage 1 (immature) and 2 (maturing) represent immature specimens and stages 3 (mature) and 4 (active) represent mature specimens. Females are divided in 7 stages, where stages 1 (immature) and 2 (maturing) represent immature specimens and stages 3 (mature with ripe oocytes), 4 (early pregnancy), 5 (middle pregnancy), 6 (late pregnancy) and 7 (resting) represent mature specimens.

Age of each specimen was estimated by direct counting of the annual bands formed in the inner dentine layer of the 2nd dorsal spine and age validation accomplished by marginal increment analysis. The complete age determination and validation procedures are described in detail in a paper dealing with population dynamics of this species Coelho and Erzini (submitted-b). For the present study we used part of the same age estimates to investigate depth distribution in relation to age.

Catch data was analyzed in terms of catch (in numbers) per unit effort (CPUE), using duration of trawl tow as a measure of effort. Given that all trawl tows were a standard 1 hour, CPUE data in this study refers to catches in numbers per 1 hour of trawling.

Depth distribution of this species in relation to both size and age of the specimens caught was investigated. Sexes were analyzed separately, taking into consideration the fact that sexual segregation with depth could occur. To test the differences found both in the size and age throughout the depth classes Kruskal-Wallis test followed by Dunn pairwise tests were performed for each sex. Mann-

Whitney tests were carried out for each depth class to find differences in both sizes and ages between sexes.

To evaluate a possible sexual segregation with depth, sex ratios were calculated for each depth class. Furthermore, in order to evaluate possible depth segregation by maturity stage of the specimens, the proportion of both mature and immature specimens by sex was plotted, as well as the proportion of each mature stage.

6.4. Results

A total of 396 specimens (192 males and 204 females) were caught during the course of the surveys (Figure 6.1). Males ranged in size from 10.2 to 32.9 cm TL and in age from 0 to 7 years, while females ranged in size from 9.8 to 41.1 cm TL and in age from 0 to 10 years. Of the 396 specimens caught, age was only determined for 373. Age could not be determined on the remainder, either due to lack of valid age estimation (17 specimens) or due to the spines being damaged and not suitable for age reading (6 specimens).

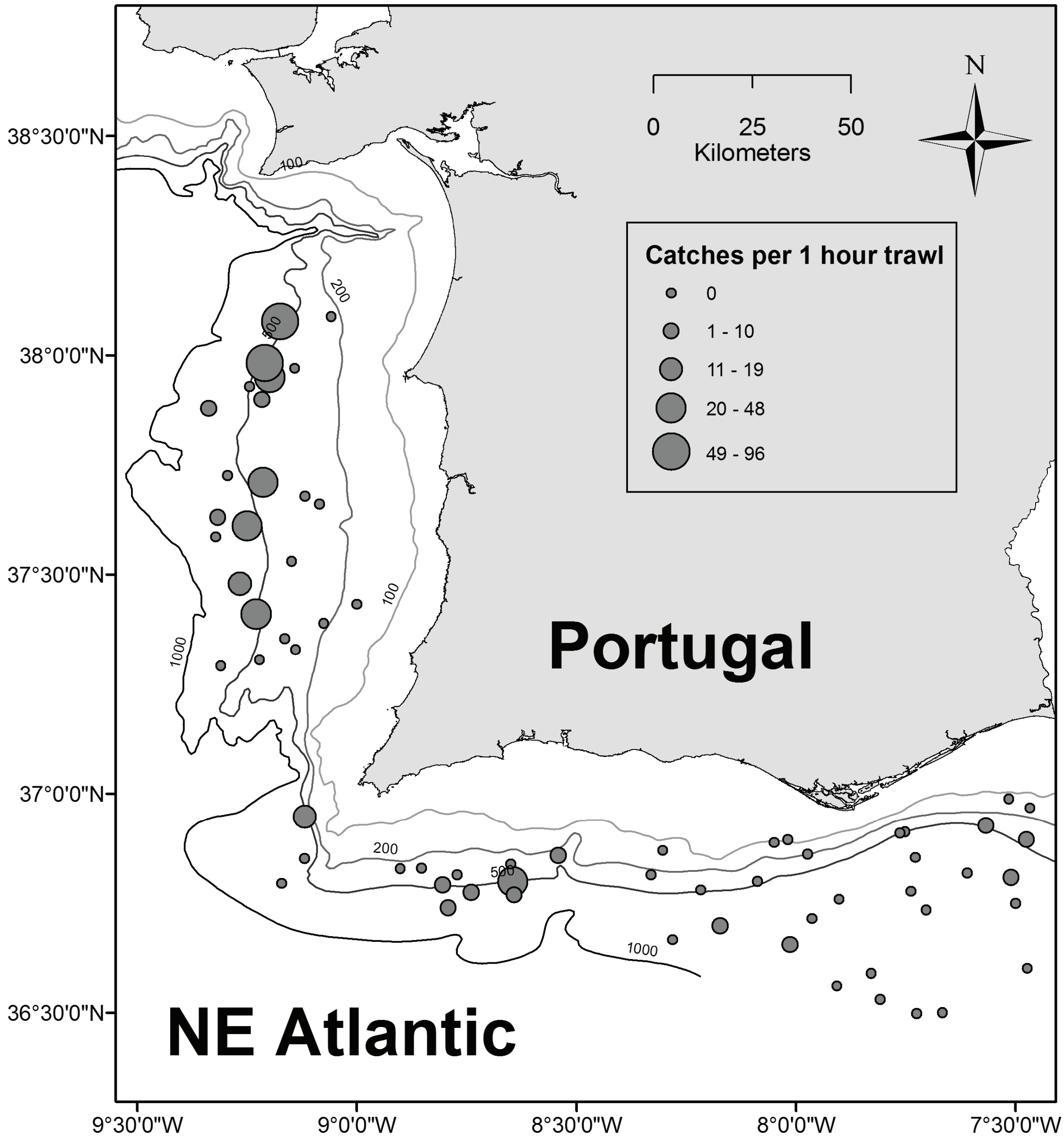

Figure 6.1. Map of the south and southwest coasts of Portugal with graduated symbols representing catches per 1 hour trawl at each of the surveyed sites. The coastline and the bathymetric lines (100, 200, 500 and 1000m depths) were adapted from "Atlas do Ambiente Digital – Instituto do Ambiente".

Both sexes had a similar CPUE distribution with depth, with no catches occurring shallower than 300 m. Average CPUE values per depth class were highest in the 500 m depth class, with 14.88 specimens caught during 1 hour of trawling (7.24 males + 7.65 females). These values decreased progressively in the adjacent shallower and deeper depth classes (Table 6.1).

Table 6.1. Number of trawls, catches (in number) and CPUE (means and standard deviation (SD)) for males and females *Etmopterus spinax* by depth class. The last line refers to total values for trawls and catches, and mean values for CPUE, calculated considering only the depths where catches occurred (300 to 700 m).

Depth class (m)	Trawls	Catches (n)			CPUE (catches / hour trawl)					
					Males		Females		Total	
		Males	Females	Total	Mean	SD	Mean	SD	Mean	SD
0	1	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
100	6	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
200	6	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
300	11	21	20	41	1.91	6.01	1.82	6.03	3.73	12.03
400	10	40	31	71	4.00	8.87	3.10	5.86	7.10	14.47
500	16	123	130	253	7.24	13.74	7.65	14.21	14.88	27.40
600	9	8	21	29	0.89	1.17	2.33	3.24	3.22	3.96
700	8	0	2	2	0.00	0.00	0.22	0.67	0.22	0.67
Total / Mean	67	192	204	396	2.78	8.28	2.96	8.19	5.74	16.14

Specimen size increased with depth, for both male and female *E. spinax.* In the shallower depth classes, from 300 to 500 m, the size distribution of males and females was very similar, but at the deeper end of the distribution (600 m), females tended to be larger than males (Figure 6.2). Significant differences were found in the sizes of both males (Kruskal-Wallis: H = 57.36; P-value < 0.001) and females (Kruskal-Wallis: H = 76.82; P-value < 0.001) found in the different depth strata. No differences in sizes between sexes were detected at each of the various depth classes (Mann-Whitney: P-values > 0.05 on all cases) except in the 600m strata (Mann-Whitney: P-value < 0.001).

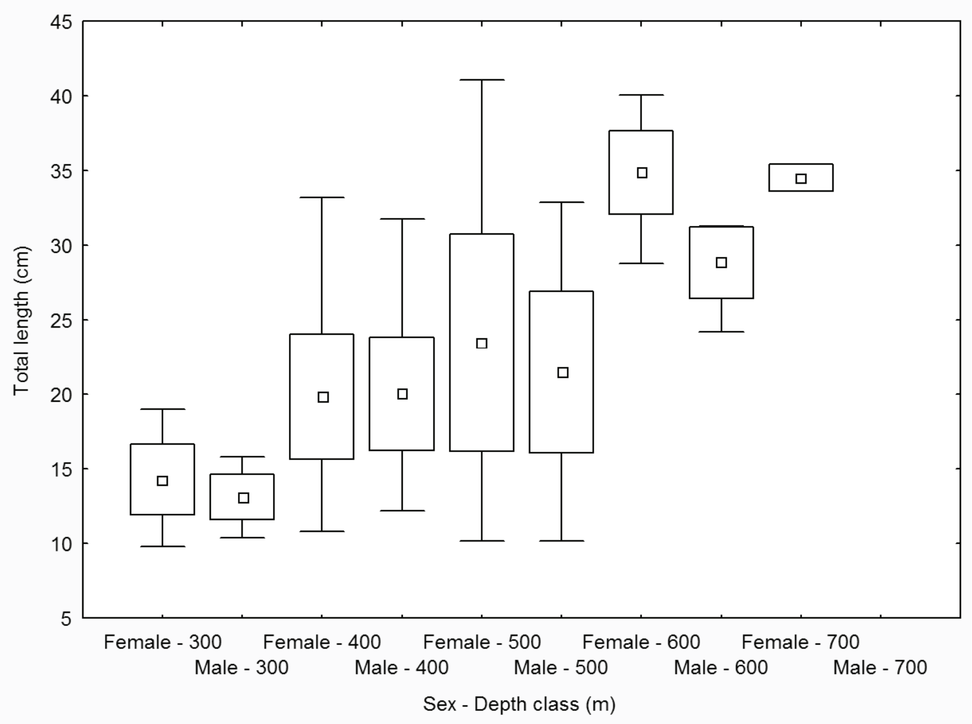

Figure 6.2. Size distributions of male and female *Etmopterus spinax* in the different depth strata. The middle points refer to the mean, the boxes to the standard deviations and the whiskers to the range.

Likewise, there is also an increase in specimen age with increasing depth, for both males and females. In the shallower depth classes, from 300 to 500 m, both the mean age and the maximum specimen age increased but young of the year age 0 specimens were also found. On the other hand, in the deeper depth classes (600 and 700 m) the youngest specimens were 4 years old (Figure 6.3). Significant differences were found in the sizes of both males (Kruskal-Wallis: H = 46.56; P-value < 0.001) and females (Kruskal-Wallis: H = 76.82; P-value < 0.001) from the different depth classes. For both males and females, the pairwise tests showed that significant differences occurred between all possible depth classes (Dunn: P-value < 0.05), except between the 400 and 500 m depth classes (Dunn: P-value > 0.05). No differences in ages between sexes were detected in any of the various depth classes

(Mann-Whitney: P-values > 0.05 on all cases) except for 600m depth class (Mann-Whitney: P-value = 0.017).

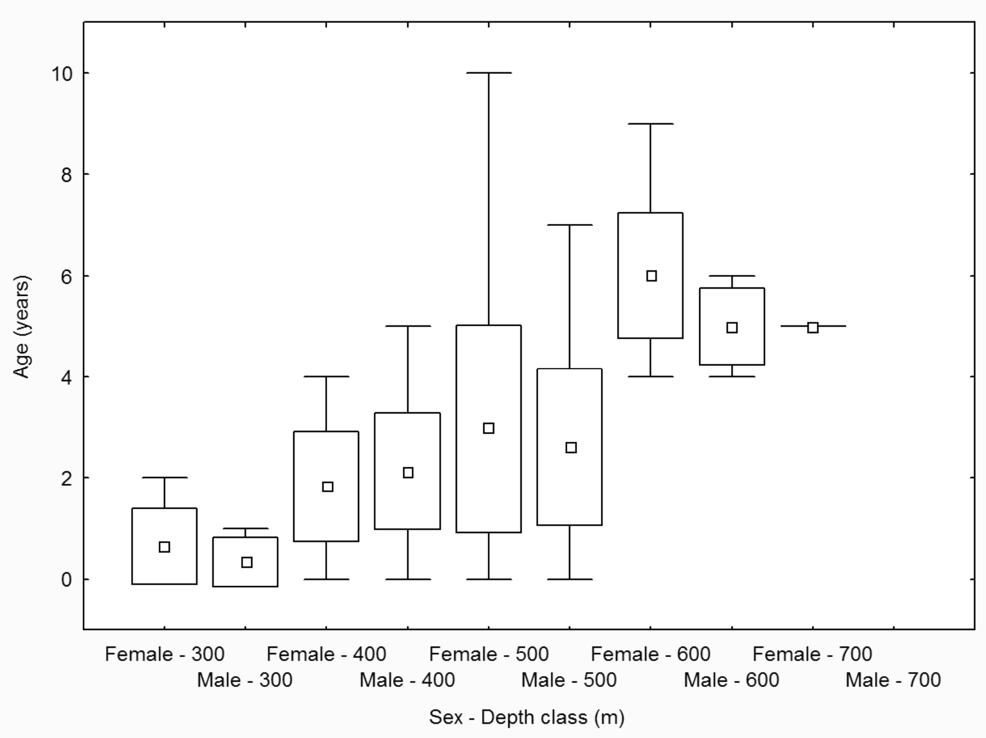

Figure 6.3. Age distribution of male and female *Etmopterus spinax* in each of the depth strata. The middle points refer to the mean, the boxes to the standard deviation and the whiskers to the range.

Females were caught in a wider depth range (from 300 to 700 m) than males (from 300 to 600m). However, in the only depth class where only females occurred (700 m), only 2 specimens were caught. The male to female sex ratio was approximately 50:50 for the shallower depth classes (300 to 500 m), with slightly more males than females in the 300 and 400m depth classes and more females than males in the 500m stratum. At the deeper range of the distribution, the sex ratio favored females (Figure 6.4).

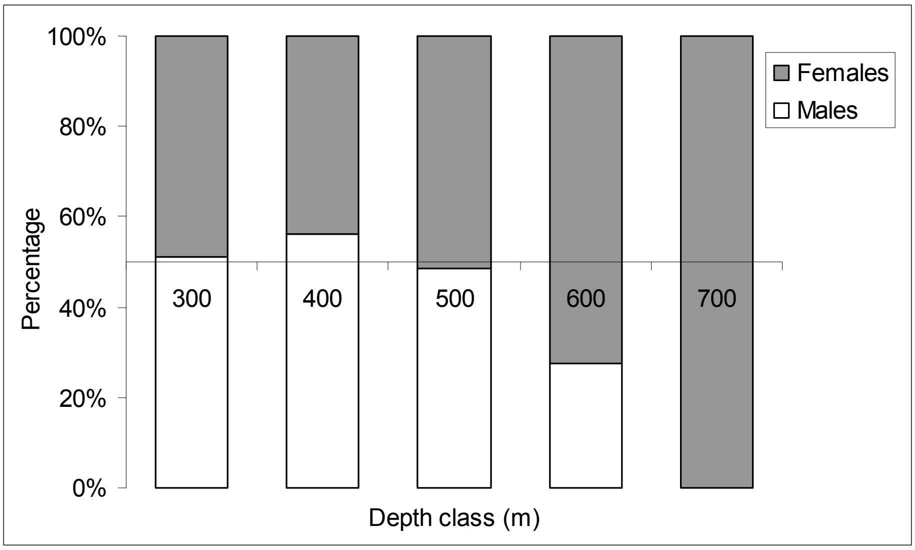

Figure 6.4. Percentages of male and female *Etmopterus spinax* by depth class.

A clear depth distribution pattern of maturity stage can be observed, with immature specimens occurring mainly in shallower waters, and mature specimens in deeper waters (Figure 6.5). Mature females with ripe oocytes and the early stages of pregnancy occur mainly in deeper waters (≥ 500 m), while late pregnancy stages occur in shallower waters (≤ 500m) (Figure 6.6). Mature but not active males were more frequent in the shallower depth classes while the proportion of active males increased with depth (Figure 6.6).

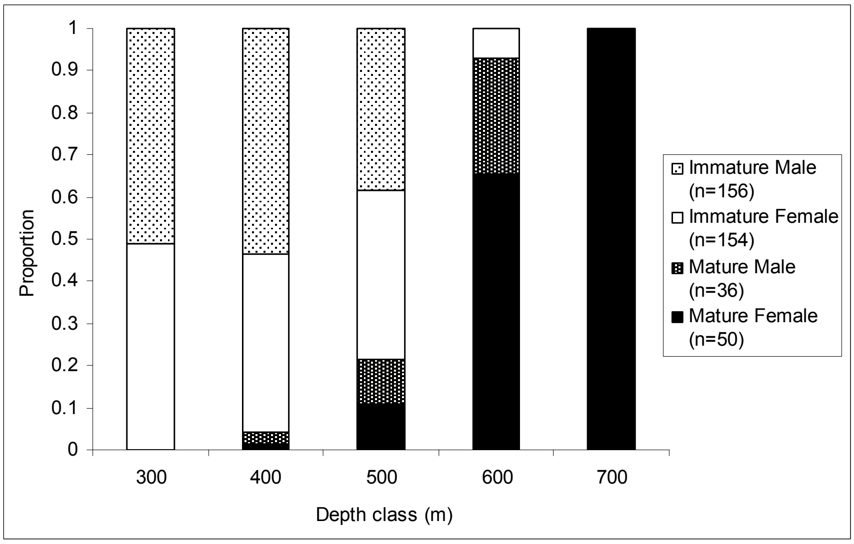

Figure 6.5. Proportion of mature and immature male and female *Etmopterus spinax* in each depth class. The numbers between brackets in the legend refer to the sample size (n).

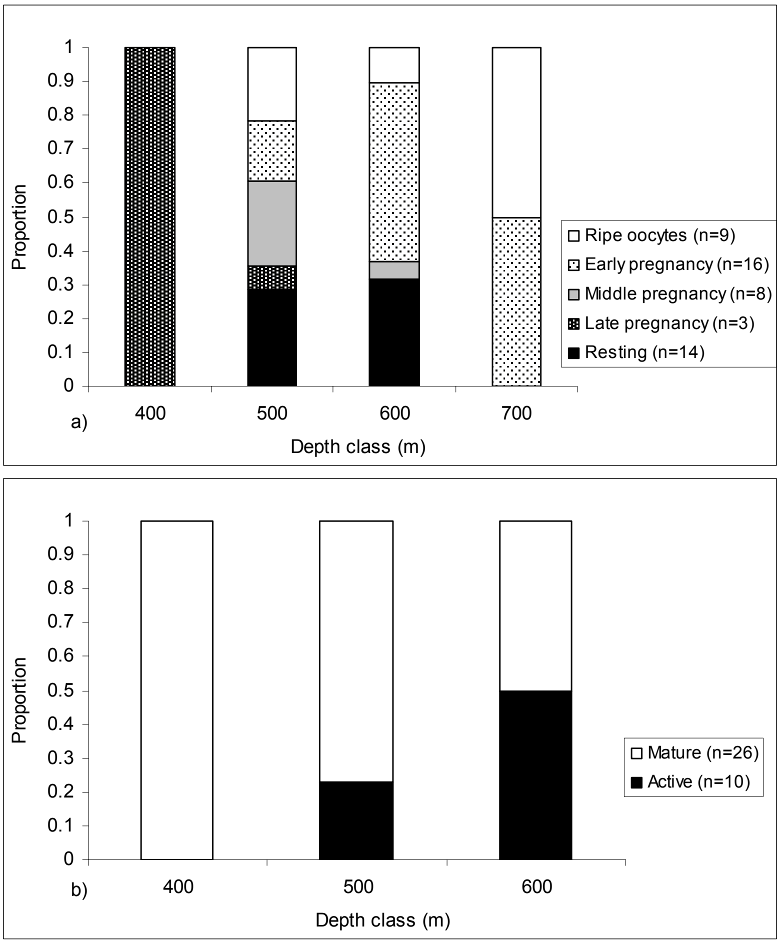

Figure 6.6. Proportion in each maturity stage for mature female (a) and male (b) *Etmopterus spinax* along the depth gradient. The numbers between brackets in the legend refer to the sample size (n).

6.5. Discussion

This study evidenced the differential depth distribution of *E. spinax* throughout its life cycle. The larger and older specimens occur predominantly in deeper waters, while the smaller and younger specimens occur mainly in shallower waters. Size segregation with depth is common in deep water squalid sharks (Muñoz-Chápuli, 1984), but the patterns seem to differ according to species: in the Mediterranean population of *E. spinax* a similar pattern to the one described in this study has been reported (Massuti and Moranta, 2003; Sion *et al.*, 2004), while for *Etmopterus princeps* from Icelandic waters the opposite situation has been described, with the size of specimens decreasing with increasing depth (Jakobsdottir, 2001).

In *E. spinax* the size and age distribution patterns seems to be mainly correlated with the species reproductive cycle, with immature specimens occurring mainly in shallower waters while mature specimens are largely found in deeper waters. Analysis based only on the mature component of the population, revealed interesting differences between mature specimens, which are probably related to movements between the mating and nursery grounds. The presence of mature females with ripe oocytes (that are ready for fecundation), early pregnant females (where fecundation has occurred recently) and active males in deeper waters indicates that the mating grounds are located in deeper waters. On the other hand, the presence of late term gravid females and the immature (age 0) specimens in shallower water suggests that during pregnancy, gravid females migrate to shallower waters for parturition, probably to leave the offspring in these shallower waters nursery grounds.

Other authors have described reproduction related to distributional patterns with regards to depth. Sion *et al.* (2004) observed a similar pattern in the Mediterranean *E. spinax* population, and also hypothesized that an inshore migration of adults to nursery grounds during spawning could be occurring. The opposite situation has, however, been more frequently observed. In squalid sharks such as *Centroscymnus owstoni* and *C. coelolepis* in Japan, the young, sub-adults and adults (except late term gravid females) move to shallower waters and gravid females may go to nursery grounds in deeper waters (Yano and Tanaka, 1988). Segregation patterns by sexual stage of development seems to occur in *Centroscymnus coelolepis* and *Centrophorus squamosus* off the British Isles, with immature specimens generally found at greater depths than adults (Girard and Du Buit, 1999). Finally, Clarke *et al.* (2001) reported gravid females of *Centroscymnus coelolepis* in shallower waters off Ireland and Scotland, although in this case post natal specimens were more numerous in the deeper segments of the depth range.

In terms of sex ratio, this study showed no clear differences between males and females at the shallower depths, but in deeper waters females were more numerous than males. This situation might be correlated with the fact that there is a size dimorphism in this species, with females growing to larger sizes than males. In this study the maximum attained sizes of 41.1 cm TL for females and 32.9cm for males is similar to what has been previously reported for the area (Coelho and Erzini, submitted-a). Similar sex ratio distributions have been described for *E. princeps*, with females more numerous than males at the deeper end of their depth range (Jakobsdottir, 2001). For *Etmopterus granulosus*, off New Zealand, twice as many females as males were caught, but no significant differences were detected between depths (Wetherbee, 1996). On carcharhinid sharks, multiple paternity has been observed for some species (Daly-Engel *et al.*, 2006), and this reproductive strategy seems to presents numerous potential advantages, such as maintaining the species genetic diversity (Newcomer *et al.*, 1999).

Ecologically, some hypothesis might be forwarded to explain these depth distributional patterns. One possibility is that juvenile development occurs on shallower waters in order to avoid feeding competition with the larger specimens. This hypothesis seems to be corroborated by the study of Neiva *et al.* (2006) where ontogenic changes in the feeding patterns for *E. spinax* were described. Another possibility is that these migrations may serve to avoid cannibalism, even though this situation has never been observed for this species (Neiva *et al.*, 2006).

The use of a fishing gear such as the bottom trawl seems to be the most adequate for this type of study. Yano and Tanaka (1988) suggested that gravid females of the *Centroscymnus* genus stopped feeding during pregnancy, which implies that a distributional pattern study using baited gear, such as longline, might be biased. Yano and Tanaka (1988) also recognized that the use of bottom gillnets, which are not baited, was more adequate for sampling that part of the population. One limitation of this study is that only the summer period was sampled, and at this stage there is no way to verify if the depth distribution patterns described here are the same throughout the year or if seasonal changes occur. However, the presence of all maturity stages, including females with ripe oocytes and females in late term pregnancy stages, seems to indicate that both mating and parturition is occurring during the summer period, probably with a one year interval (Coelho and Erzini, submitted-b). This study has therefore identified the depth ranges of both the mating and the nursery grounds for this species.

Deep water squalid sharks have particularly vulnerable life history characteristics, due to their slow growth rates, late maturity, prolonged gestation and small progeny, and are amongst the most vulnerable of all elasmobranchs (Fowler *et al.*, 2005). Deep water trawls targeting crustaceans have large by-catches of deep water squalid sharks, including *E. spinax* (Monteiro *et al.*, 2001), and since the 1980's have been fishing intensively in the area. Although this fishery operates off both the southern and southwestern Portuguese coasts, much more fishing effort is presently being expended off the southern coast, with around 30 trawlers accounting for 21.5% of the national trawl landings in 2005. Off the southwest coast the number of trawlers is usually less than five, with catches in that area accounting for only 6.9% of the national trawl landings in 2005 (DGPA, 2006). The lower CPUE values of *E. spinax* observed for the southern coast during this fishery independent study might be correlated with this differential fishing effort over the last decades and a sign that over-fishing is occurring in the southern area.

The commercial trawlers operate at different depths depending on the targeted crustacean species, with fishing taking place mainly between 150 to 400 m when targeting deepwater rose shrimp and deeper than 500 m depth when targeting Norway lobster and red shrimp. In the late 1990's fishing pressure was more intensive at the shallower end of the *E. spinax* range, with the commercial trawlers mainly targeting deepwater rose shrimp, while in more recent years the fishing grounds at greater depths have been increasing in area and more effort has been put into these greater depths with the commercial trawlers mainly targeting Norway lobster and red shrimp (Afonso-Dias, pers. comm[3].).

The depth migrations of *E. spinax* during its life cycle are complex, with this species using different depth ranges during its life to complete specific phases. With this work, we identified risk areas to this population where key parts of their life cycle, namely mating, parturition and first life-history stages occur. In order to protect this species, using an ecosystem-based management of fisheries approach (Frid *et al.*,

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[3] Manuel Afonso-Dias:, University of Algarve, FCMA, Faro, Portugal.

2006), the entire depth range including both the mating and the nursery grounds would have to be protected and that is naturally incompatible with the commercial fisheries that are taking place in the area (Barker and Schluessel, 2005). Possible ways to protect such a species might include the implementation of by-catch reducing devices or strategies, such as the one proposed by Coelho *et al.* (2003) to reduce elasmobranch by-catch in deep water longlines, or devices already developed to reduce trawl by-catch (Campos and Fonseca, 2004; Fonseca *et al.*, 2005). Selected risk areas can then act as no take zones, providing refuge to specimens during life-stages when vulnerability to exploitation is higher.

CHAPTER 7: Comparing biological parameters of the NE Atlantic and Mediterranean populations of a small sized viviparous shark, *Etmopterus spinax*: evidence for and efficiency of density dependant compensatory mechanisms.*

(The deep water longliner "Branca de Sagres")

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[*] *In Preparation*: Coelho, R., Rey, J., Gil de Sola, L. & Erzini, K. Comparing biological parameters of the NE Atlantic and Mediterranean populations of a small sized viviparous shark, *Etmopterus spinax*: evidence for and efficiency of density dependant compensatory mechanisms.

7.1. Abstract

Etmopterus spinax is a small sized deep water lantern shark that occurs in the eastern Atlantic and the Mediterranean. Differences in depth distribution, catch per unit effort (CPUE), size at maturity and fecundity were compared between a population that has suffered high levels of fishing mortality during the last decades (southern Portugal in the northeast Atlantic) and a population where low fishing pressure below 500m occurs at present or has occurred in the last decades (Alboran Sea in the western Mediterranean). The research survey CPUE in the northest Atlantic is substantially lower than in the Mediterranean throughout the entire depth range. The northest Atlantic population is maturing at smaller sizes than the Mediterranean population and has a lower mean fecundity. Specifically, sizes at maturity for the northest Atlantic and the Mediterranean were respectively 25.86 and 28.31 cm TL for males and 30.69 and 34.18 cm TL for females, while mean fecundities for the northest Atlantic and the Mediterranean were respectively 9.94 and 11.06 oocytes per mature female. This work evidenced the possible presence of a density dependant mechanism in the northest Atlantic population of *E. spinax* that has lowered the size at maturity as a result of excessive fishing mortality. However, given that this is an aplacentary viviparous shark, where fecundity is dependant on female size, this compensatory mechanism seems to be less efficient than what would be expected.

7.2. Introduction

The velvet belly lantern shark, *Etmopterus spinax* is a small sized deep water squalid shark, living in the outer continental and insular shelves and upper slopes (Compagno *et al.*, 2005) of the eastern Atlantic ocean and the Mediterranean Sea. In the eastern Atlantic it has been described from Iceland and Norway to South Africa

(Compagno, 1984), including the Azores (Santos *et al.*, 1997), the Canaries (Brito *et al.*, 2002) and the Cape Verde Islands (Reiner, 1996), while for the Mediterranean it has been described for the western and central areas (Serena, 2005).

Elasmobranch fishes in general are highly susceptible to fishing mortality. In these animals, overexploitation is known to occur even with low levels of fishing mortality (Stevens *et al.*, 2000), and once they start to decline it can take decades for depleted populations to start to recover (Anderson, 1990). Among these, and due to their life cycle characteristics that include slow growth rates, late maturity and low fecundities, deep water squalid sharks seem to be even more vulnerable (Gordon, 1999) and are amongst the most vulnerable of all marine species (Fowler *et al.*, 2005). A well documented case of a deep water squalid species that has been severely depleted is the picked dogfish, *Squalus acanthias* whose NE Atlantic population has been reduced to about 5% of the virgin biomass (Hammond and Ellis, 2005).

The outer shelves and upper slopes of the southern Portuguese (northeast Atlantic) and southern Spanish (western Mediterranean) coasts have had different historical fisheries pressures over the last decades. Off the southern Portuguese coast, an intensive deep water bottom trawl fishery started in the 1980's with as many as 30 commercial vessels continuously fishing in this area (Campos *et al.*, 2007). The fishery mainly targets crustaceans such as Norway lobster, *Nephrops norvegicus*, deepwater rose shrimp, *Parapenaeus longirostris*, and red shrimp, *Aristeus antennatus* (Monteiro *et al.*, 2001), at depths from 200 to 800 m, depending on the specific targeted species. *E. spinax* is a common by-catch of this fishery, and even though specimens are almost always discarded (Monteiro *et al.*, 2001), they are usually either dead or with severe injuries that make fisheries mortality very high (Coelho and Erzini, submitted-a). On the other hand, the western Mediterranean waters where this study was carried out does not have significant deep water (below 500m) fisheries operating at this time or over the last decades. The only exception is the Alboran Island area and some canyons in the northern Alboran Sea, where there is an intensive deep water bottom trawl fishery targeting crustaceans, especially *Aristeus antennatus* (Rey *et al.*, 2005), and where currently 35 to 40 boats have official licences to operate along the year.

Even though *E. spinax* is a relatively common species, information on the biology is still limited. In the northeast Atlantic area, the population biology and feeding ecology were studied by Coelho and Erzini (2005; submitted-b) and Neiva *et al.* (2006), respectively. For the Mediterranean area, Sion *et al.* (2002) made a first attempt at studying the age and growth, while Atkinson and Bottaro (2006) correlated the ampullatory distribution of the pores with the species predatory lifestyle.

The objectives of this work were to explore differences in depth distribution, catch per unit effort (CPUE), and population dynamics characteristics of two populations of *E. spinax*, one in the northeast Atlantic (southern Portugal) and another in the Alboran Sea (western Mediterranean, southern Spain). These two populations have been affected by very different fisheries pressures over the last decades, and we correlated the differences found between CPUE and sizes at maturity with this differential fisheries pressure. Furthermore, we compare the fecundities of the two populations and discuss the possible limitations that small sized viviparous sharks might have when density dependent compensatory mechanisms are present.

7.3. Material and methods

Etmopterus spinax specimens were sampled both in the northeast Atlantic and the Mediterranean. For the northeast Atlantic, specimens were caught in the southern and southwestern Portuguese coasts from February 2003 to April 2004, and came from both commercial fisheries using deep water trawls and longlines and from a scientific INIAP - IPIMAR research survey using a deep water demersal trawl. In the Mediterranean, specimens were caught during the summer of 2006 off the southern Spanish coast and around the Alboran Island, during a MEDITS scientific cruise using a demersal bottom trawl (Figure 7.1).

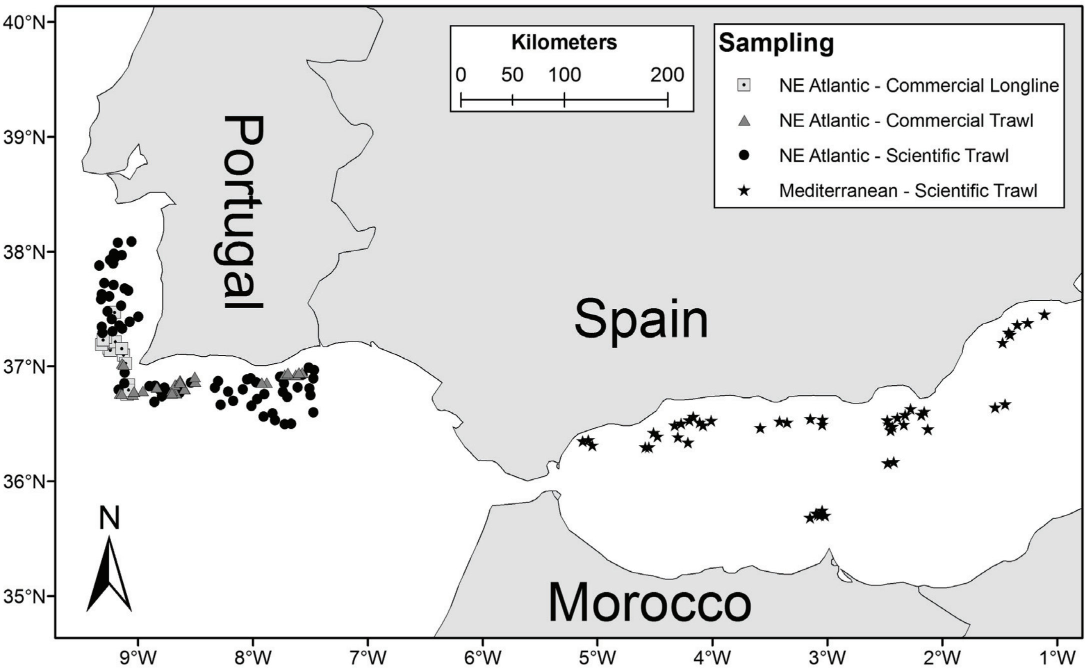

Figure 7.1. Map of the *Etmopterus spinax* sampling sites in the NE Atlantic and the Mediterranean.

The trawl used during the MEDITS surveys was a type GOC 73 gear, with horizontal and vertical openings of 16.4 and 2.8 m respectively, and a codend mesh size of 20 mm (Bertrand *et al.*, 2002). The gear used by IPIMAR was a bottom trawl with a codend mesh size of 25 mm and horizontal and vertical openings of 30.2 m and 3.2 m (Leite *et al.*, 1990). Boat speed during the scientific trawling operations, both in the northeast Atlantic and the Mediterranean, varied from 2.6 to 3.0 knots and trawls were standardized to last one hour. In 15 hauls in the northeast Atlantic and in all the MEDITS hauls, a conductivity, temperature and depth (CTD) recorder was attached to the fishing gear and data recorded during the sampling / fishing operations.

Specimens were sexed and total length (TL) recorded in a straight line from the tip of the snout to the tip of the caudal fin in its natural position. Each specimen was then dissected and the reproductive stages defined according to the macroscopic scales defined by Coelho and Erzini (2007) for other *Etmopterus* species. According to these scales, males were classified in 4 stages, where stage 1 (immature) and 2 (maturing) represent immature specimens and stages 3 (mature) and 4 (active) represent mature specimens. Females were classified in 7 stages, where stages 1 (immature) and 2 (maturing) represent immature specimens and stages 3 (mature with ripe oocytes), 4 (early pregnancy), 5 (middle pregnancy), 6 (late pregnancy) and 7 (resting) represent mature specimens.

Trawls were grouped in 100 m depth intervals, such that depth class 0 grouped trawls from 0 to 99 m, depth class 100 grouped trawls from 100 to 199 m and so on up to depth class 700, that grouped trawls from 700 to 799 m depth. CPUE was calculated for each region and for each depth strata, using only data taken from the scientific cruises. Given that these cruises used bottom trawls, catch per unit effort was expressed as the number of specimens caught per one hour of trawling.

Size at maturity (TL at which 50% of the individuals are mature) has been previously determined for the NE Atlantic population (Coelho and Erzini, submitted-b). For this work, and for comparative purposes, we applied the same procedure to the Mediterranean population. Therefore, size at maturity was estimated by fitting maturity ogives to the proportion of mature individuals in each 1 cm TL size class. Non-linear least squares regression, implemented in the STATISTICA 6.0 software

(StatSoft, 2004), was used to estimate the parameters and associated standard errors and 95% confidence intervals of the logistic model:

$$P_{Li} = \frac{1}{1 + e^{-b(L_i - L_{50})}}$$

where P_{Li} is the proportion of mature individuals in the size class Li, b is the slope and L_{50} is the size where 50% of the individuals are mature. The maximum likelihood test (Kimura, 1980) was used to test for differences between sexes in the estimated parameters, as well as between the two populations.

Fecundity was estimated by counting the number of ripe oocytes in maturity stage 3 females. The number of mid-term and late term embryos in stages 5 and 6 pregnant females were not used to estimate fecundity, given the possibility that some embryos may be lost during the catching and hauling process (Coelho and Erzini, submitted-b). Fecundity for the northeast Atlantic population has already been calculated (Coelho and Erzini, submitted-b) and for the present study we used the same procedure and criteria to estimate the fecundity of the Mediterranean population. The mean fecundity of the two populations was compared by means of a t-student test and linear regressions used to investigate the relationship between fecundity and female total length. Analysis of covariance (ANCOVA), using the TL as the covariate, was used to compare fecundity between populations after removing the effect of female size.

7.4. Results

A total of 1610 specimens (797 in the Mediterranean and 813 in the northeast Atlantic) were used in this study. All specimens from the Mediterranean came from scientific trawling while specimens in the NE Atlantic came from both commercial fisheries with trawl and longlines and from demersal research surveys. The total length range of specimens was very similar in both regions. Specifically, the northeast Atlantic specimens ranged from 9.1 to 41.1 cm TL and the Mediterranean ones from 10.1 to 41.0 cm TL (Table 7.1).

Table 7.1. *Etmopterus spinax* sample size (n) and total length range for each fishing / sampling gear in each region.

Region	Sampling		Sample	
	Gear	Depth range (m)	n	Size range (cm)
NE Atlantic	Commercial trawl	199 – 641	180	9.1 – 39.6
	Commercial longline	458 – 787	237	25.5 – 40.7
	Scientific trawl	84 – 786	396	9.8 – 41.1
Mediterranean	Scientific trawl	37 – 785	797	10.1 – 41.0

In the NE Atlantic, specimens were caught from 300m to 700m depths, with highest CPUE in the 500m strata, and decreasing progressively in the adjacent shallower and deeper classes. In the Mediterranean, specimens occur in deeper waters and were caught from 500m depth, with the CPUE values increasing with increasing depth (Table 7.2). Comparatively, the Mediterranean CPUE values were always higher than those of the northeast Atlantic.

Water temperature at the bottom depths where the specimens were caught was relatively similar for the two regions. Specifically, bottom water temperature in the northeast Atlantic ranged from 12.2 to 14.3ºC and in the Mediterranean from 12.9 to 13.3ºC.

Table 7.2. Catch per unit effort (CPUE) in number of specimens caught per 1 hour of trawling for both the NE Atlantic and the Mediterranean populations of *Etmopterus spinax*. The table presents mean values, with the respective standard deviations between brackets.

Depth class (m)	CPUE (catches / hour trawl)					
	Mediterranean			NE Atlantic		
	Males	Females	Total	Males	Females	Total
0	0.0	0.0	0.0	0.0	0.0	0.0
100	0.0	0.0	0.0	0.0	0.0	0.0
200	0.0	0.0	0.0	0.0	0.0	0.0
300	0.0	0.0	0.0	1.9 (6.0)	1.8 (6.0)	3.7 (12.0)
400	0.0	0.0	0.0	4.0 (8.9)	3.1 (5.9)	7.1 (14.5)
500	12.6 (14.8)	11.6 (12.5)	24.2 (27.2)	7.2 (13.7)	7.6 (14.2)	14.9 (27.4)
600	16.0 (13.8)	22.5 (19.8)	38.5 (33.3)	0.9 (1.2)	2.3 (3.2)	3.2 (4.0)
700	28.8 (10.4)	51.4 (27.0)	80.2 (35.5)	0.0	0.2 (0.7)	0.2 (0.7)
Mean	19.1 (13.0)	28.5 (19.8)	47.6 (32.0)	2.8 (6.0)	3.0 (8.2)	5.8 (16.1)

Differences in the maturity ogives showed that for both sexes the Mediterranean population is maturing at smaller sizes than the northeast Atlantic population (Figure 7.2). Northeast Atlantic and Mediterranean males are maturing respectively at 25.86 cm and 28.31 cm TL, while females in the same regions are maturing respectively at 30.69 and 34.18 cm TL (Table 7.3). Differences between male and female sizes at maturity were significant for both the northeast Atlantic (Max. likelihood: χ^2 = 211.15; P-value < 0.05) and the Mediterranean populations (Max. likelihood: χ^2 = 288.44; P-value < 0.05). Differences were also significant when each sex was compared between the two regions (Max. likelihood $_{\text{males}}$: χ^2 = 172.29; P-value < 0.05 and Max. likelihood $_{\text{females}}$: χ^2 = 226.37; P-value < 0.05).

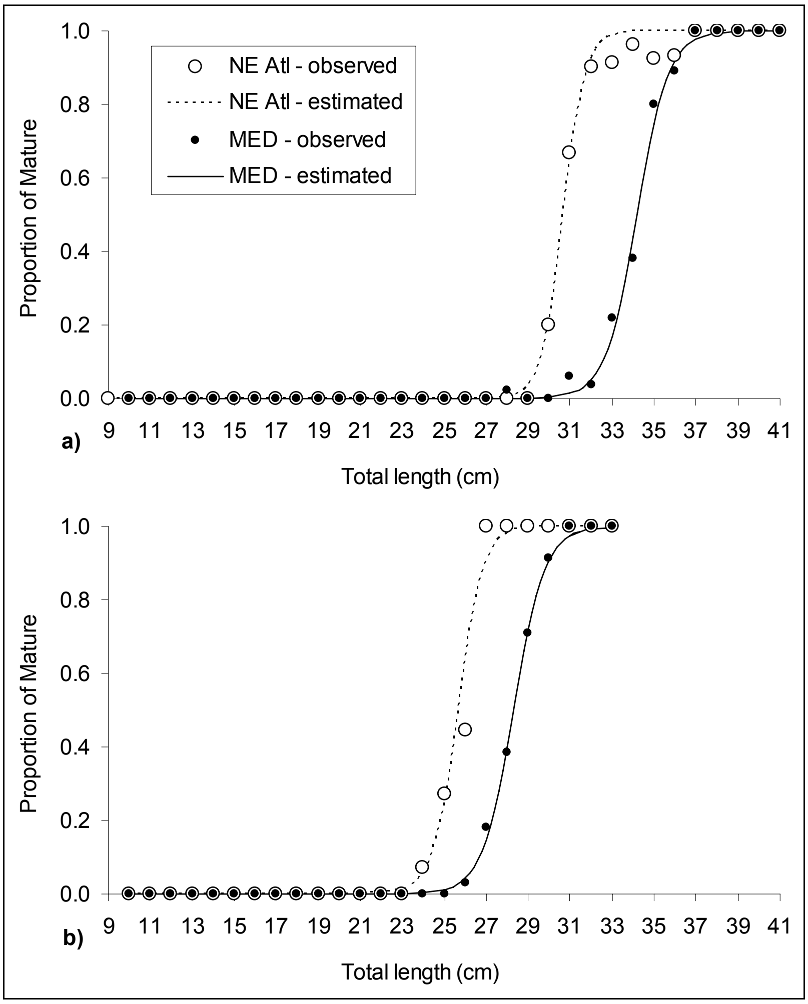

Figure 7.2. Maturity ogives for females (a) and males (b) of the NE Atlantic and the Mediterranean populations of *Etmopterus spinax*.

Table 7.3. Length at maturity (L_{50}) estimates for the NE Atlantic and the Mediterranean populations of *E. spinax* with the respective standard errors and the 95% confidence interval limits. NE Atlantic data from Coelho and Erzini (submitted-b).

Sex	Population	TL range (cm)		L_{50} (cm)	95% CI Interval		L_{50} / TL max. (%)
		Immature	Mature		Lower	Upper	
Males	NE Atlantic	10.2 – 26.7	24.2 – 33.8	25.86	25.70	26.02	76.50
	W Mediterranean	10.1 – 30.2	26.9 – 33.9	28.31	28.26	28.35	83.50
Females	NE Atlantic	9.1 – 36.2	30.5 – 41.1	30.69	30.60	30.78	74.67
	W Mediterranean	10.4 – 36.8	28.5 – 41.0	34.18	34.09	34.27	83.37

In both populations, a positive linear relationship was found between total length and fecundity, meaning that fecundity increases with increasing total length of the females (Figure 7.3). Mean ovarian fecundity for the northeast Atlantic population is lower than the mean ovarian fecundity for the Mediterranean (Table 7.4), and these differences are statistically significant (t-student: t = 2.134, P-value = 0.035). However, considering the linear relationship between total length and fecundity, no differences between regions were detected (ANCOVA: F = 2.15, P-value = 0.146) after removing the effect of female total length.

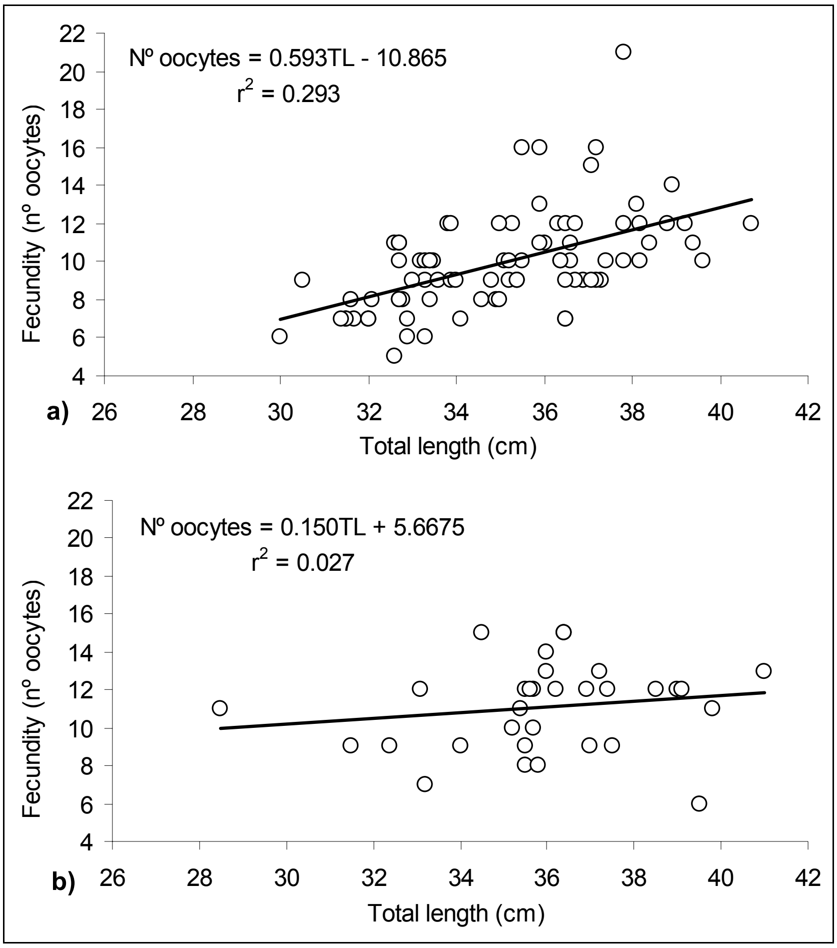

Figure 7.3. Relationship between total length of females and ovarian fecundity for the northeast Atlantic (a) and the Mediterranean (b) populations of *E. spinax*. The linear regression, with the respective equation and the value of the coefficient of determination (r^2) is also given.

Table 7.4. Ovarian fecundity estimated based on mature females with ripe oocytes (stage 3) in the northeast Atlantic and the Mediterranean populations of *E. spinax*. Data from the NE Atlantic from Coelho and Erzini (submitted-b).

	Ovarian Fecundity			Sample	
	Mean	St. dev.	Range	$n_{\text{specimens}}$	n_{oocytes}
NE Atlantic	9.94	2.61	5 – 21	83	825
W Mediterranean	11.06	2.29	6 – 15	32	354

7.5. Discussion

This study presents for the first time a comparison between population dynamics characteristics of this important demersal predator of the outer shelves and upper slopes of the northeast Atlantic and the western Mediterranean, the deep water lantern shark *E. spinax*. Deep water elasmobranchs are known to be amongst some of the most endangered and vulnerable elasmobranchs (Fowler *et al.*, 2005), and in the particular case of *E. spinax*, a previous study that focused on the northeast Atlantic population dynamics established that this is in fact a vulnerable species with relatively slow growth, low fecundity, late maturity and a long reproductive cycle that can take up to three years to complete (Coelho and Erzini, submitted-b).

Differences in CPUE based exclusively on research trawl surveys in both regions were found, with the northeast Atlantic population having lower CPUE values than the Mediterranean population. Although these two research trawls cannot be directly compared due to some small gear specifications differences, the huge CPUE differences found are probably due to more than just these small different gear characteristics and most likely related to the different historical and current fisheries pressure in the two areas. In fact, the southern Portuguese area has been fished intensively by some 30 bottom trawlers targeting crustaceans for the past 30 years

(Campos *et al.*, 2007), while in the Alboran Sea (with an exception of the Alboran Island area), at the depths where this species occurs, there has been no significant fishing pressure over the last decades. Thus, the NE Atlantic population of *E. spinax* seems to be an example of a species where substantial population changes in terms of abundance has occurred due to intensive fisheries induced mortality.

In terms of depth distribution, the Mediterranean population of *E. spinax* occurs in deeper waters than the NE Atlantic population and this fact seems to be correlated with water temperature. In southern Portugal, specimens start to occur at the 300 m depth class and that is the depth where water temperature stabilizes between the 12 and 14ºC, while in the Mediterranean the same temperature ranges are obtained in deeper waters, starting at the 500m depth class. The depth distribution of this species seems to be much more correlated with bottom water temperature than with water pressure caused by depth. This fact can in part explain why this species has been recorded at much shallower depths in more northern latitudes such as at around 80 m depth in a Norwegian fjord (Hennemann, 2001), where the water temperature is much lower than water temperature in the study areas of the present work.

Significant differences were observed between male and female size at maturity in each of the studied regions, with females maturing at larger sizes than males. Sexual dimorphism in terms of size at maturity is common in elasmobranch fishes and has been described before for other species of the *Etmopterus* genus, such as *Etmopterus princeps* (Jakobsdottir, 2001), *Etmopterus baxteri* (Irvine *et al.*, 2006a) and *Etmopterus granulosus* (Wetherbee, 1996). For the NE Atlantic this situation has been previously described for this species (Coelho and Erzini, 2005) and during this work we verified that the same was occurring for the Mediterranean population.

When sizes at maturity were compared between regions, we observed that the NE Atlantic population of *E. spinax* is maturing at smaller sizes than the Mediterranean population. Specifically, males in the NE Atlantic are maturing at sizes 2.5 cm smaller than in the Mediterranean and females at sizes 3.5 cm smaller. Given the very different CPUE values obtained between the two areas, which are most likely related with different fisheries pressures, we think that this reduction in size at maturity is a density dependant mechanism that has reduced size at maturity as a consequence of the decrease in population density that is in turn the consequence of excessive fisheries induced mortality.

Given that this is a small sized species, where maximum sizes of males and females are only 33 and 41 cm TL respectively, these relatively small differences in size represent large differences in terms of percentage of total length. It is interesting to note that the reductions in sizes at maturity of the two sexes as a percentage of the total length were similar. For the Mediterranean population, both sexes are maturing at around 83% of the maximum observed size while in the NE Atlantic population both sexes are maturing at around 75% of the maximum observed size, giving a reduction of around 8% in size at maturity for both. For the northeast USA population of *S. acanthias*, Sosebee (2005) reported that female mean size at maturity decreased from 85 to 79 cm TL over a 20 year period due to excessive fishery mortality. If we consider that females *S. acanthias* can reach up to 120 cm TL (Compagno, 1984) this reduction represents only a 5% difference. On the other hand, Henderson *et al.* (2002) also reported for *S. acanthias* that neither growth, size at maturity, nor fecundity had significantly changed due to fishing pressure off the coast of Ireland, and the only population change observed that was attributed to fishing by this author was the lower age range compared to North American populations.

Significant differences in mean fecundities were observed between the two regions, with the NE Atlantic population having a lower mean fecundity than the Mediterranean population. This fact may be correlated with the lower size at maturity of the NE Atlantic population, given that there is a relation between size of the female and fecundity. In placentary viviparous sharks or, as in the present case, aplacentary viviparous sharks, such relationships are to be expected given that the number of oocytes and embryos that females can carry is dependant on their size. The smaller the female, the smaller the abdominal cavity will be and less area will consequently be available for carrying the oocytes and the embryos. This type of relationship has been described for other deep water squalid sharks such as *Centroscymnus owstoni* and *Centroscymnus coelolepis* (Yano and Tanaka, 1988), *Squalus acanthias* (Henderson *et al.*, 2002; Chatzispyrou and Megalofonou, 2005), and also for other viviparous elasmobranchs such as *Mustelus canis* (Conrath and Musick, 2002) and even for oviparous sharks such as *Scyliorhinus canicula* (Ellis and Shackley, 1997).

Given that these biological relationships between female size and fecundity were established, we further investigated if the differences between fecundities in the two regions would still persist after removing the effects caused by female size. We concluded that after removing the effect of growth, no differences were found between the fecundities of the two populations, so the differences that we were observing were indeed related with the fact that maturity in the NE Atlantic specimens is occurring at smaller sizes. Therefore, there seems to be a trade off going on in this species (and probably in all other viviparous sharks), where due to density dependant mechanisms the exploited populations start to mature at smaller sizes, but with the consequence that the fecundity on these smaller mature females is lower.

In conclusion, this study has shown the presence of a density dependant mechanism in a small sized aplacentary viviparous shark that has decreased the size at maturity as a consequence of excessive fisheries related mortality. However, and even though the exploited population is maturing at smaller sizes, the fact that there is a direct relationship between female size and fecundity causes the smaller sized mature females to have lower fecundities than the average fecundity of the species. Therefore, this mechanism is not as efficient for viviparous shark as it would be for other fish species, making these sharks even more vulnerable to fisheries mortality than originally expected. A precautionary approach is therefore suggested when assessing this species vulnerability.

CHAPTER 8: Mortality and demographic analysis of deep water lantern sharks (Chondrychthyes: Etmopteridae) caught by trawl and longline in southern Portugal (NE Atlantic).*

(Sampling lantern sharks at the University of the Algarve)

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* *In Preparation*: Coelho, R. & Erzini, K. Mortality and demographic analysis of deep water lantern sharks (Chondrychthyes: Etmopteridae) caught by trawl and longline in southern Portugal (NE Atlantic).

8.1. Abstract

Elasmobranch fishes are in general highly vulnerable to fishing mortality. Two deep lantern shark species (*Etmopterus spinax* and *Etmopterus pusillus*) occur in the southern Portuguese area (NE Atlantic) and both are currently and commonly caught as by-catch of deep water commercial fisheries, specifically by deep water trawls and deep water longlines. In the present work, mortality and demographic analyses were carried out in order to assess if the current levels of fishing mortality are sustainable. Natural mortality was estimated by indirect empirical methods, total mortality by age structured catch curve analysis and fishing mortality was estimated separately for each of the fishing gears. Demographic analysis was carried out for different scenarios with and without fishing mortality. The intrinsic rates of population increase obtained for the different scenarios revealed that both lantern shark species are currently being affected by these commercial fisheries and that the populations are declining. In the case of *E. spinax* both fisheries are affecting the population with the trawl having a greater impact, while only the longline fishery is having a detrimental effect on the *E. pusillus* population. The results presented here support the need for urgent conservation measurements to be implemented for deep water sharks.

8.2. Introduction

Elasmobranch fishes in general are considered to be highly vulnerable to fishing mortality because of their life history characteristics that include slow growth rates, late maturities and low fecundities (Hoenig and Gruber, 1990; Cortés, 2000). Overexploitation in these animals can occur even with relatively low levels of fishing mortality (Stevens *et al.*, 2000). Deep water elasmobranch species are usually even less resilient to fisheries mortality than the coastal species (Gordon, 1999), and are currently amongst the groups that present the highest risks of population declines (Fowler *et al.*, 2005). An example where the lack of fisheries management lead to the collapse of a deep water sharks is the case of the picked dogfish, *Squalus acanthias*, whose northeastern Atlantic population has been depleted to about 5% of the virgin biomass (Hammond and Ellis, 2005).

In Portuguese waters, large numbers of deep water velvet belly lantern sharks, *Etmopterus spinax*, and smooth lantern sharks, *Etmopterus pusillus* are commonly caught as by-catch by both longliners targeting bony fishes and trawlers targeting crustaceans (Monteiro *et al.*, 2001; Coelho *et al.*, 2005). Most of this catch is discarded, due to the null or very low commercial value of these species, making it very difficult to gather information on the impact of these commercial fisheries on these shark populations. Both species occur in deep waters, on or near the bottoms of the outer continental shelves and slopes (Compagno *et al.*, 2005), and both are wide ranging in geographic distribution. Specifically, *E. spinax* occurs in most of the eastern Atlantic Ocean, including the Mediterranean Sea, whereas *E. pusillus* has a worldwide distribution, having been recorded on both sides of the Atlantic, the western and southeast Pacific, and the western Indian Oceans (Compagno *et al.*, 2005).

Mortality rates are some of the most important parameters for fisheries biology and stock assessment studies (Vetter, 1988), and even though several techniques have been developed, they still remain some of the most difficult parameters to estimate (Hewitt and Hoenig, 2005; Cortés, 2007). Understanding mortality is very important because of the need to know how fast individuals are removed from the population in order to model the population dynamics and estimate sustainable rates of exploitation (Simpfendorfer *et al.*, 2004). Demographic analysis is useful to determine the probability of decline in a population and to diagnose population susceptibility to fishing pressure (Simpfendorfer, 2004). This type of approach can be used to analyze the status of the populations, given that the intrinsic rate of increase of the populations decreases when fishing mortality increases.

Due to the generally low commercial value of most elasmobranch fishes and the consequent difficulty in gathering fisheries dependent data, demographic analysis that is based on life history parameters has become widely used for modeling this group (e.g. Cortés, 1995; Cortés and Parsons, 1996; Márquez-Farias and Castillo-Geniz, 1998). However, most of the species and populations studied are commercially important coastal or pelagic elasmobranchs, and the only known deep water shark where demographic parameters have been estimated is *S. acanthias* (Chen and Yuan, 2006). At this stage we know of no demographic analysis studies of deep water Etmopteridae sharks.

The two lantern sharks assessed in this study are caught in large quantities as by-catch of deepwater commercial fisheries, with high discard mortality rates (Borges *et al.*, 2001; Monteiro *et al.*, 2001). However, the question of how much these fisheries are affecting these populations remains unsolved. Therefore, the objectives of the present study were to: 1) estimate the natural, total and fishing mortality of these shark populations, 2) perform a demographic analysis on these populations considering scenarios with and without fisheries mortality, 3) determine the current population trends, and 4) assess if the current fisheries levels are sustainable.

8.3. Material and methods

8.3.1. Mortality

Population parameters of both lantern shark species have been previously estimated and were used for the present work. Specifically, the life history parameters of *E. pusillus* came from Coelho and Erzini (2007) and those of *E. spinax* from Coelho and Erzini (submitted-b) (Table 8.1).These include von Bertalanffy growth function (VBGF) parameters, size-at-age data, maximum age, age at maturity, fecundity and mature female gonadosomatic indices (GSI).

Table 8.1. Biological parameters used in this study and obtained from Coelho and Erzini (submitted-b) for *E. spinax* and from Coelho and Erzini (2007) for *E. pusillus*. L_{inf} and k are von Bertalanffy growth parameters, Age_{50} is the age at maturity, Age_{max} is the maximum age and *GSI* is the mature female average gonadosomatic index.

Parameter	*Etmopterus spinax*		*Etmopterus pusillus*	
	Male	Female	Male	Female
L_{inf} (cm)	57.96	55.84	49.01	54.04
k (year^{-1})	0.14	0.14	0.17	0.13
Age_{50} (year)	3.97	4.67	7.13	9.86
Age_{max} (year)	8	11	13	17
Fecundity		9.94		10.44
GSI		13.81		19.43
Reproductive periodicity	every 3 years		uncertain (assumed every 3 years)	

Both lantern sharks species are regularly caught by deep water commercial fisheries operating in the area, specifically bottom trawls and bottom longlines. Both these by-catches have been previously analysed and the fisheries data used in this study has been reported by Coelho and Erzini (submitted-a).

Natural mortality (M) and total mortality (Z) were estimated by indirect empirical techniques that use equations that correlate different life history parameters with mortality. In this study, and for comparison purposes, several different indirect methods for estimating mortality were used (Table 8.2). For the Pauly (1980) equation, the water temperature used was 13.0ºC (sd = 0.35, n_{hauls} = 15, $n_{data\ points}$ = 210), based on recordings made with a conductivity, temperature and depth recorder (CTD) in 15 commercial bottom trawl tows.

Table 8.2. Equations used to estimate natural mortality (M) and total mortality (Z). L_{inf} and k are von Bertalanffy growth parameters, T is the average water temperature (ºC), GSI is the mature female average gonadosomatic index, Age_{max} is the maximum age and Age_{50} is the age at maturity.

Method	Equation
Hoenig (1983) (teleosts)	$\ln Z = 1.46 - 1.01 \ln(Age_{\max})$
Hoenig (1983) (cetaceans)	$\ln Z = 0.941 - 0.873 \ln(Age_{\max})$
Hoenig (1983) (teleosts, cetaceans and molusks)	$\ln Z = 1.44 - 0.982 \ln(Age_{\max})$
Pauly (1980)	$\log M = -0.0066 - 0.279 \log L_{\inf} + 0.6543 \log k + 0.4634 \log T$
Gunderson and Dygert (1988)	$M = 0.03 + 1.68 GSI$
Jensen (1996) (age data)	$M = {}^{1.65}\!/_{Age_{50}}$
Jensen (1996) (growth data)	$M = 1.5k$

Given that age structured catch data was available for both species, total mortality was also estimated based on an age structured catch curve analysis. In this analysis, the logarithm of the catch in number was plotted by age class and a linear regression carried out using only the data on the descending part of the plot. The initial age classes are not used in these regressions, given that they correspond to age classes not fully recruited to the respective fisheries. This analysis was carried out separately for each sex and for each fishing gear.

Given that for any fish population total mortality is described as the sum of the natural mortality and the fishing mortality (F), the trawl and longline fishing mortality rates were estimated separately for each sex of each species with:

$$F = Z - M$$

Fishing mortality was estimated using the total mortality values obtained from the age structured catch curve analysis and from both the maximum and minimum natural mortality values obtained using the indirect methods. Therefore, both maximum and minimum fishing mortality values for each sex of each species and for each fishing gear were calculated.

8.3.2. Demographic analysis

Demographic parameters were estimated based on life history table analysis. Since only females produce offspring, the demographic analysis was carried out exclusively for the female components of the two populations (Simpfendorfer, 2004).

Age-specific female pup natality (m_x) was derived from the total litter size (fecundity) multiplied by 0.5 (the proportion of female embryos in each litter) and divided by 3 (assumed number of years between litters for both species). The m_x values were then further corrected taking into account the relationship between female age and fecundity and the proportion of females that are effectively mature at each age class. Since there is a linear relationship between size of the female and fecundity of *E. spinax* (Coelho and Erzini, submitted-b) relationships between age of the females and fecundity were derived. The proportion of the females mature in each age class (maturity ogives) previously reported by Coelho and Erzini (2007) for *E. pusillus* and by Coelho and Erzini (submitted-b) for *E. spinax* were used to assess the proportion of females in each age class that were effectively mature.

The proportion of the females surviving at the beginning of each age class (l_x) was calculated as the negative exponential relationship derived from a mortality measurement (Simpfendorfer, 2004), and given by:

$$l_x = l_{x-1} e^{-Z}$$

where Z is the mortality and the initial value of l_x was set to 1.

Life tables were then constructed taking into account several scenarios, based on the different mortality estimates. Scenarios without fishing mortality were considered by using only the natural mortality (Z = M) and in this case the uncertainty of the model was evaluated by using all values of natural mortality (M) estimated with the different indirect methods. Scenarios accounting for fishing mortality were also considered and in these cases the total mortality values (Z = F + M) obtained with the age structured catch curves were used.

In the scenarios with fishing mortality, and given that not all age classes are fully or equally recruited for each of the fishing gears, the total mortality value obtained through the catch curve analysis was corrected in order to take into account the percentage of the females effectively recruited to each of the fishing gears. Therefore, for the age classes on the descending part of the catch curves the full Z value was used, but for the initial ascending part of the catch curves, a proportion between the expected value and the real observed value was determined and used to correct the Z value. For the specific case of the catches with longline, the same correction was applied to the age classes partially recruited to the gear, but only natural mortality was used for age classes that were never caught, specifically ages 0 to 3 for *E. spinax* and ages 0 to 2 for *E. pusillus*.

Once the life tables were constructed, a series of demographic parameters were calculated. The main parameter estimated was the intrinsic rate of population increase (*r*), which measures the potential for growth in the population. This value was estimated by iteratively solving the Euler-Lotka equation (Krebs, 1985):

$$\sum_{x=Age_{50}}^{Age_{\max}} l_x m_x e^{rx} = 1$$

A number of other life history parameters were calculated, specifically the net reproductive rate (R_0) that is the total number of female offspring produced per individual in a single cohort, the generation time (*G*) that is the mean period between birth of a parent and the birth of their offspring and the population doubling time (t_{x2}). Simpfendorfer (2004) and Chen and Yuan (2006) provide complete formulas for calculating these parameters from the life tables that were used in this study.

8.4. Results

8.4.1. Mortality

Differetn natural mortality values according to the different indirect methods used were estimated. Specifically, M varied from 0.210 to 0.416 yr^{-1} for *E. spinax* males, from 0.210 to 0.353 yr^{-1} for *E. spinax* females, from 0.231 to 0.342 yr^{-1} for *E. pusillus* males and from 0.167 to 0.356 yr^{-1} for *E. pusillus* females (Table 8.3). The total mortality values estimated indirectly were relatively similar independently of the equation used. Specifically, Z varied from 0.417 to 0.548 yr^{-1} for *E. spinax* males, from 0.316 to 0.401 yr^{-1} for *E. spinax* females, from 0.273 to 0.340 yr^{-1} for *E. pusillus* males and from 0.216 to 0.261 yr^{-1} for *E. pusillus* females (Table 8.3).

Table 8.3. Indirect estimates of natural mortality (M) and total mortality (Z) coefficients for male and female *E. spinax* and *E. pusillus*.

Method	Mortality estimated	*E. spinax*		*E. pusillus*	
		Male	Female	Male	Female
Hoenig (1983) (teleosts)	Z	0.527	0.382	0.323	0.246
Hoenig (1983) (cetaceans)	Z	0.417	0.316	0.273	0.216
Hoenig (1983) (teleosts, cetaceans and molusks)	Z	0.548	0.401	0.340	0.261
Pauly (1980)	M	0.288	0.291	0.342	0.280
Gunderson and Dygert (1988)	M		0.262		0.356
Jensen (1996) (age data)	M	0.416	0.353	0.231	0.167
Jensen (1996) (growth data)	M	0.210	0.210	0.255	0.195

Given that age structured catch data was available for both species, an age structured catch curve analysis was also performed and the plots resulting from this analysis are presented in Figure 8.1 for *E. spinax* and in Figure 8.2 for *E. pusillus*. The estimated Z values varied significantly between sexes and between fishing gears. In general, Z values were higher for the stocks exploited with longlines than for the stocks exploited with trawl and in general higher for males than for females (Table 8.4).

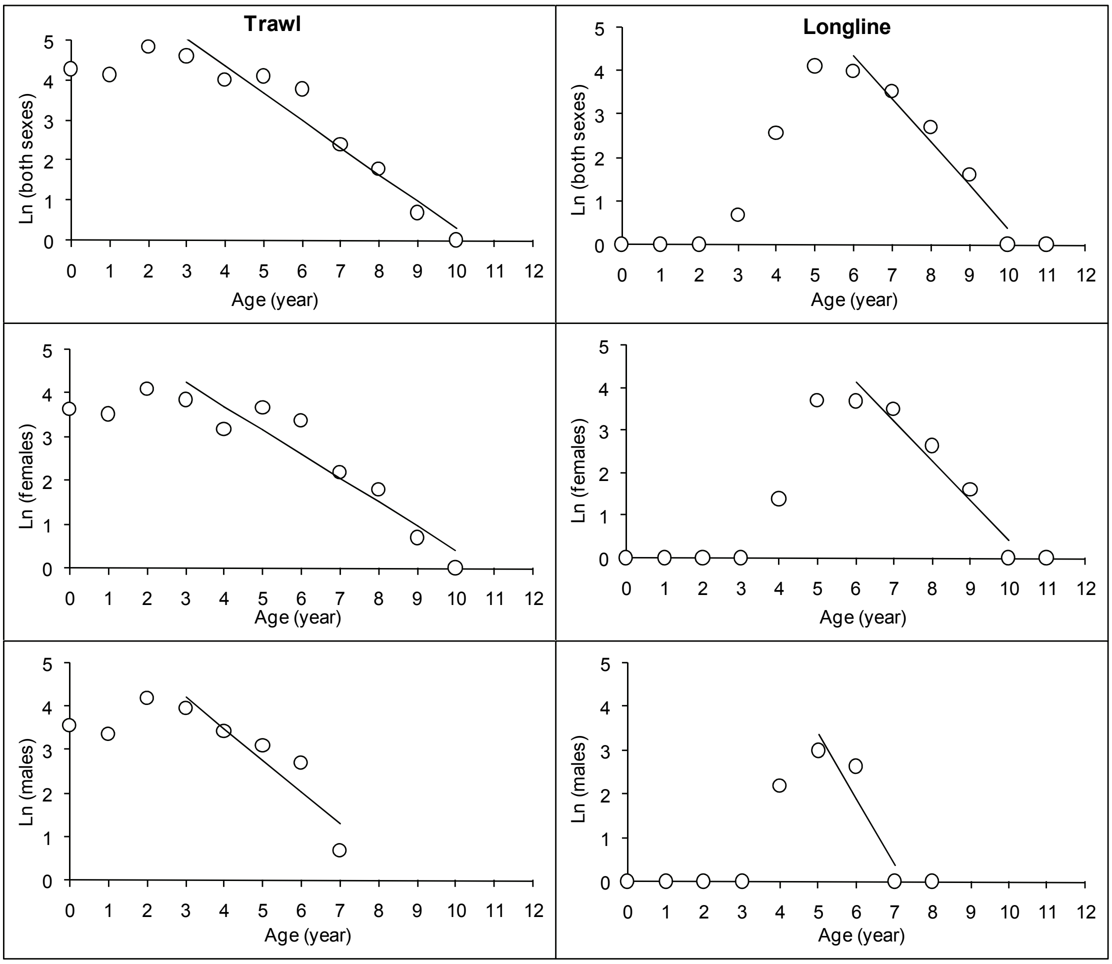

Figure 8.1. Age structured catch data for both sexes of *E. spinax* caught with trawl and longline, with the respective linear regression for the fully recruited age classes (descending part of the plot).

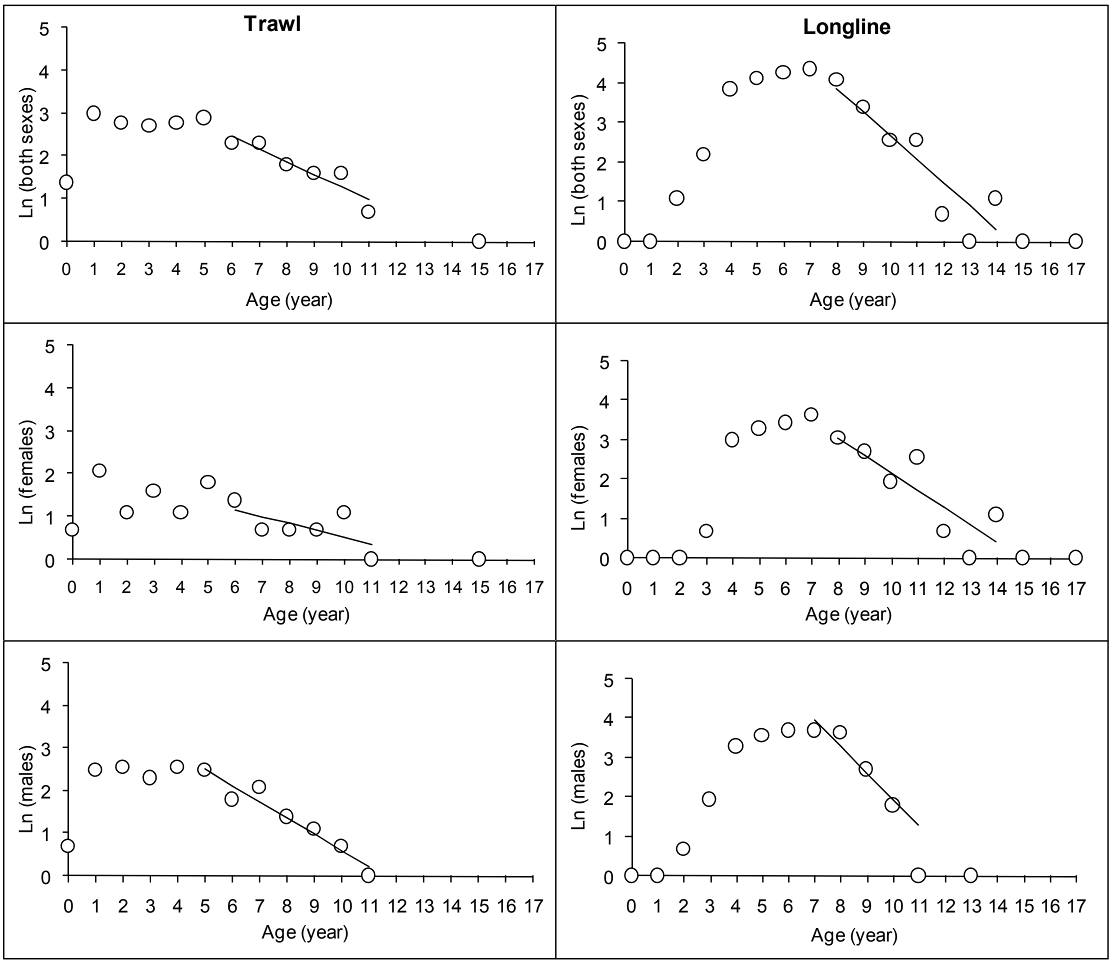

Figure 8.2. Age structured catch data for both sexes of *E. pusillus* caught by trawl and longline, with the respective linear regression for the fully recruited age classes (descending part of the plot).

Using the maximum and minimum M values estimated by indirect methods and the Z values calculated from the age structured catch curve, minimum and maximum F values for each sex and for each fishing gear were calculated. In general, F values for longline fishing were higher than for trawl and F values for males were higher than for females (Table 8.4).

Table 8.4. Total mortality coefficients (Z) estimated from the catch curve analysis and fishing mortality (F) for male, female and both sexes combined of *Etmopterus spinax* and *Etmopterus pusillus* by fishing gear.

Species	Gear	Sex	Z (yr^{-1})	F (yr^{-1})	
				Min.	Max.
Etmopterus spinax	Trawl	M	0.728	0.312	0.518
		F	0.550	0.196	0.340
		All	0.680	0.264	0.470
	Longline	M	1.498	1.082	1.288
		F	0.926	0.573	0.716
		All	0.989	0.573	0.779
Etmopterus pusillus	Trawl	M	0.380	0.037	0.148
		F	0.163	-0.193	-0.004
		All	0.295	-0.061	0.128
	Longline	M	0.662	0.320	0.431
		F	0.441	0.085	0.274
		All	0.591	0.235	0.424

8.4.2. Demographic analysis

For the life tables, linear relations were established between fecundity and female size (Figure 8.3). This relation was significant for *E. spinax* (ANOVA $_{\text{regression}}$: F = 7.07; P-value = 0.01) but not significant for *E. pusillus* (ANOVA $_{\text{regression}}$: F = 0.69; P-value = 0.45), possibly due to the reduced sample size (n = 7). This relationship for *E. spinax*, along with the proportion of mature females in each age class, was used to calculate age-specific female natality. For *E. pusillus*, only the proportion of mature females in each age class was used to calculate the age-specific female natality (Figure 8.4).

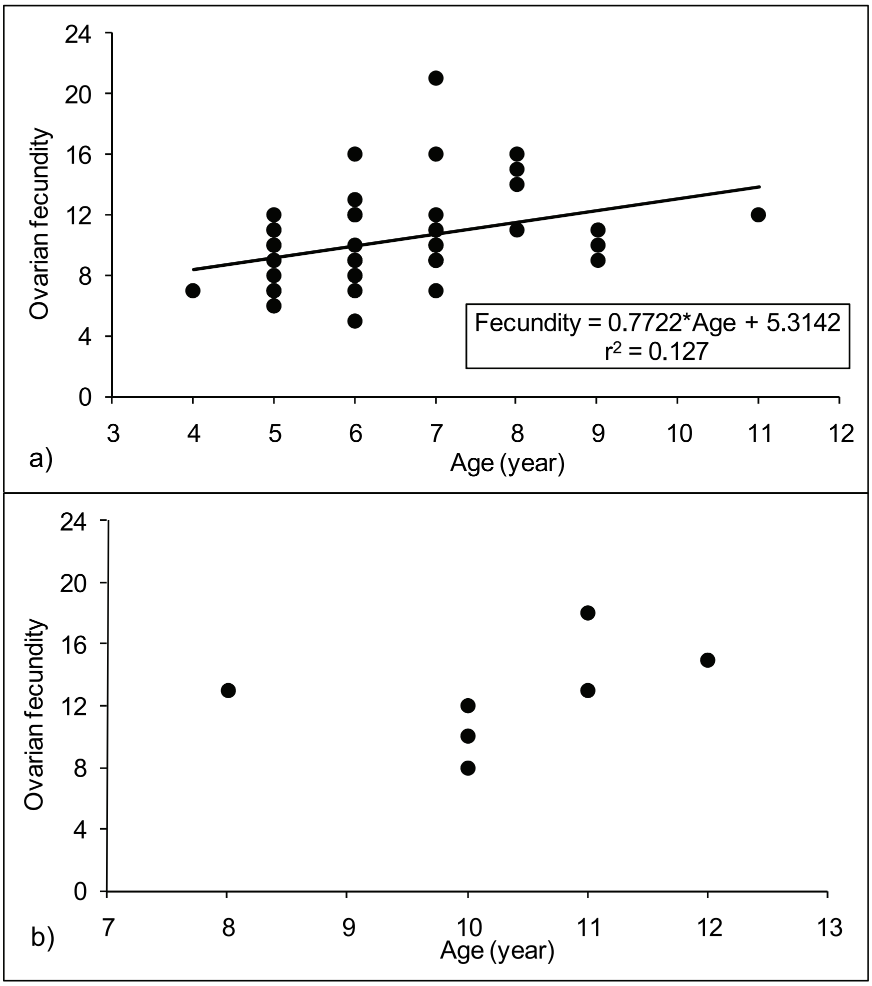

Figure 8.3. Relationship between ovarian fecundity and age for females of *Etmopterus spinax* (a) and *E. pusillus* (b). A significant linear regression, with the respective equation and coefficient of determination (r^2) value is given for *E. spinax*.

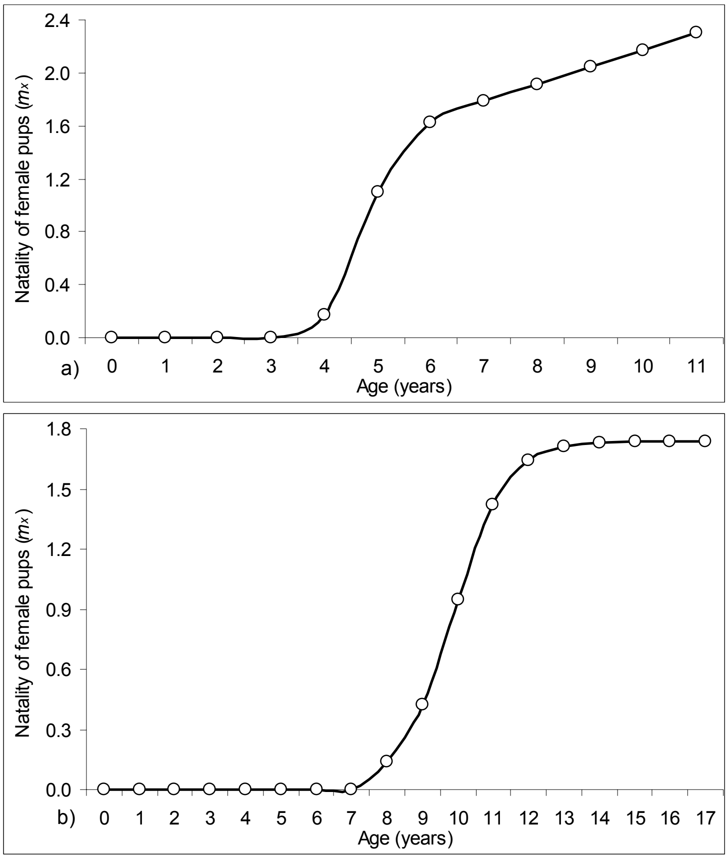

Figure 8.4. Age-specific natality of female pups (m_x) for *E. spinax* (a) and *E. pusillus* (b).

A relatively large variability was observed in survivorship when using the different natural mortality rates estimate. With the scenarios including fishing mortality, it was possible to observe initial descending phases of the survivorship curves that are less accentuated, given that the earlier age classes are not fully recruited to the fisheries (Figure 8.5). These two different phases are especially significant in the longline fishery, given that the fishing mortality caused by longlining is high, but the recruitment to the fishery is at a relatively late age. The age structured reproductive rates of the stock exploited by both fisheries are lower than in the scenarios without fisheries. The only exception is the case of *E. pusillus* exploited exclusively by trawl, where the reproductive rate is very similar to the case when the lowest natural mortality was considered (Figure 8.5).

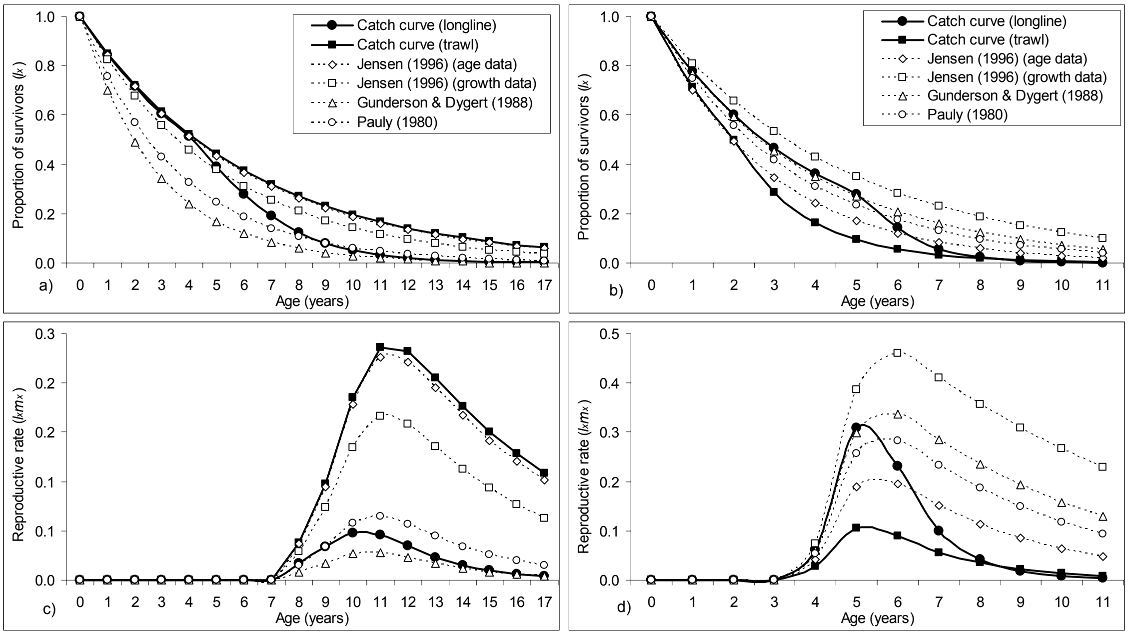

Figure 8.5. Survivorship of *E. pusillus* (a) and *E. spinax* (b) and reproductive rate of *E. pusillus* (c) and *E. spinax* (d) based on different scenarios with (solid lines and black symbols) and without fishing mortality (dotted lines and open symbols).

A relatively large variability was obtained in the different demographic parameters estimated, depending on the scenario considered. In the case of *E. spinax*, all scenarios based on natural mortality values produced reasonable results except when the Jensen (1996) model for age data was considered. When fishing mortality was added, both *r* estimates were negative, meaning negative population growth (Table 8.5). In the case of *E. pusillus*, all scenarios accounting only for natural mortality produced unreasonable results except when the Jensen (1996) model for age data was considered. The inclusion of the trawl fishing mortality produced similar results to the Jensen (1996) model for age data, with positive *r* values, but including longline fishing mortality resulted in negative *r* values (Table 8.5).

Table 8.5. Demographic parameters for *E. spinax* and *E. pusillus* based on life tables for the different scenarios with and without fisheries mortality. R_0 is the net reproductive rate, G is the generation time, r is the intrinsic rate of population increase and t_{x2} is the population doubling time.

Species	Scenarios		Demographic parameters			
	Fishing Mortality	Mortality coefficient estimate	R_0	G	r	t_{x2}
E. spinax	No	M (Pauly (1980))	1.375	7.024	0.045	15.478
		M (Gunderson and Dygert (1988))	1.696	7.024	0.074	9.394
		M (Jensen (1996) (age))	0.887	7.024	-0.017	-40.261
		M (Jensen (1996) (growth))	2.491	7.024	0.126	5.511
	Yes	Z (trawl catch curve)	0.357	6.824	-0.156	-4.432
		Z (longline catch curve)	0.774	5.880	-0.044	-15.823
E. pusillus	No	M (Pauly (1980))	0.369	12.452	-0.082	-8.495
		M (Gunderson and Dygert (1988))	0.150	12.452	-0.158	-4.398
		M (Jensen (1996) (age))	1.483	12.452	0.031	22.071
		M (Jensen (1996) (growth))	1.043	12.452	0.003	203.580
	Yes	Z (trawl catch curve)	1.560	12.452	0.035	19.578
		Z (longline catch curve)	0.237	11.820	-0.125	-5.549

8.5. Discussion

The natural mortality of a population is defined as the sum of all the possible causes of dead excluding fishing. Estimating natural mortality is very difficult, especially because of the dependence on age-specific abundance. In general, natural mortality rates are higher for the younger life stages, stabilize during adulthood and increase again during the older stages (Vetter, 1988). In order to facilitate the estimation of natural mortality, it is usually assumed that this parameter remains constant over the lifetime of an adult individual. A series of empirical equations for estimating mortality have been developed based on previously studied population dynamics parameters.

Given the difficulty in determining natural mortality, several indirect methods were used during this study and the results compared. Each of these methods has its own advantages and disadvantages, given that it has been developed for specific *taxa* groups and environments and based on different ecological theories. The Pauly (1980) equation was based on 175 fish stocks, most of them from warm waters, and included only two shark species, the basking shark, *Cetorhinus maximus*, and the porbeagle, *Lamna nasus*. This relationship, based on von Bertalanffy growth function parameters and mean environmental temperature, assumes that animals living in warmer water will have higher mortality rates than equivalent animals living in colder water (Pauly, 1980). The Gunderson and Dygert (1988) model uses r-K selection theory and is based on the relationship between the amount of energy that a female invests in reproduction (GSI) and natural mortality of the females. This model incorporates data from 20 fish species, including one shark, *S. acanthias*. One possible limitation of the Gunderson and Dygert (1988) model is that it assumes that female GSI is a proxy for reproductive investment, and as pointed out by Simpfendorfer (1999), in placentary viviparous sharks, not all the reproductive investment is included in the ripe oocytes (that produce maximum values of GSI), but part of the investment is made later, when a placental connection is made between the female and the embryos. Therefore, as concluded by Simpfendorfer (1999), this method seems to be more appropriate for aplacentary viviparous sharks, such as the presently studied Etmopteridae species. Jensen (1996) used the Beverton and Holt life history invariants to determine simple relationships between life history parameters and natural mortality. Two of the methods presented by Jensen (1996) were used during the present work, one using the optimal trade off between reproduction and survival (using age at maturity) and the other using a relationship between mortality and growth rate.

The natural mortality values estimated in this study with these different methods have a relatively large variability. Others authors have noted the importance of using different indirect methods to estimate mortality in order to get an idea of the error associated with them. For example Simpfendorfer (1999) used seven methods to calculate natural mortality of the *R. taylori* and obtained values ranging from 0.56 to 1.65, including some clearly unreasonable values.

For the total mortality estimation, both a direct method based on the age structured catch curve analysis and indirect methods based on the Hoenig (1983) equations were used. When it is possible and there is data available, it is always better to calculate total mortality from the age structured catch curve method as this technique uses real catch data and age characteristics of the populations rather than the general values estimated by the indirect methods (Simpfendorfer *et al.*, 2004). Nonetheless, the Hoenig (1983) equations are still some of the most widely used indirect methods to estimate mortality in shark species, and they predict total mortality based on the maximum observed age, assuming that longer lived species will die at a slower rate than short-lived species. Hoenig (1983) developed four different relationships but in this study only three that could sensibly be applied to elasmobranch fishes were used: one developed for teleost fishes (84 stocks), one developed for cetaceans (22 stocks) and one that grouped data from teleosts, cetaceans and mollusks. Mollet and Cailliet (2002) stated that mortality models developed by Hoenig (1983) that included cetaceans might be more adequate for elasmobranch fishes, given that like cetaceans, elasmobranch fishes are long lived. One limitation of the Hoenig (1983) equations is that it calculates total mortality and will only provide an accurate estimate of natural mortality if fishing mortality is close to zero, given that fishing may alter the age structure of the population (Hilborn and Walters, 1992). Since in the present study fishing activity is affecting most *Etmopterus* age classes, the mortality estimates based on the Hoenig (1983) models are probably biased, not giving a reliable estimate of natural mortality, and were therefore not further used.

Calculating survivorship based on both natural mortality and total mortality from the catch curve analysis allowed us to calculate demographic parameters with and without considering fishing mortality. On the other hand, the use of different indirect methods to estimate natural mortality and incorporating these different estimates into the demographic models allowed us to have an idea of the uncertainty of the method.

The most important parameter calculated by demographic analysis is the intrinsic rate of population increase: a positive *r* value reflects a population that is growing, if *r* is positive but close to 0 the population is fully developed in the current fishing pattern, while a negative *r* value means that the population has been over exploited and is declining (Simpfendorfer, 2004). The *r* values obtained when considering only natural mortality are important baseline parameters given that under these scenarios fishing mortality is not being accounted for. It was possible to observe that some natural mortality estimates were clearly unreasonable, given that they were producing negative *r* values, which is unrealistic for a population that is not being exploited. In such cases, the conclusion is that the particular indirect method is not adequate for estimating natural mortality: in the specific case of *E. spinax* only the Jensen (1996) model based on age data produced unreasonable results, while for *E. pusillus* only this same equation produced reasonable results. This kind of approach of testing several indirect methods is very important, as demonstrated by Simpfendorfer

(1999) who calculated demographic parameters for *R. taylori* and verified that only two of the seven indirect methods provided reasonable demographic results.

On the other hand, the use of catch curve derived total mortality allowed the evaluation of the impact of each fishing gear on the population. For *E. spinax* it was interesting to note that even though longline fishing mortality is higher than that of trawling, the demographic analysis indicates that the effects of trawling are more detrimental than longlining. This is due to the fact even though longline fishing mortality is higher, this gear only affects part of the population, specifically the older specimens, and has therefore a lower impact on the general population intrinsic increase rate. On the hand, trawling seems to have a much lower effect than longlining for *E. pusillus*, and in fact the *r* values calculated when accounting for trawl are similar to the best scenario using only natural mortality. This can be explained by the much lower catch rates of this species by trawls. Coelho and Erzini (submitted-b) hypothesized that *E. pusillus* is less caught by trawls than by longline because it is usually not present in trawled areas, either due to a more bentho-pelagic behaviour or greater affinity for rocky bottoms.

Demographic analysis studies in deep water sharks are scarce, and we believe that this is the first study that has performed such an analysis on non-commercial but fisheries impacted Etmopteridae sharks. The values obtained seem to corroborate what has been foreseen when population parameters of these species were studied, i.e., that that these deep water sharks are highly vulnerable to fishing mortality and that population decreases can occur rapidly once the populations start to be fished (Coelho and Erzini, 2007, submitted-b). Chen and Yuan (2006) estimated demographic parameters for a deep water species, *S. acanthias* and reported values of *r* varying from 0.059 to 0.118 and t_{x2} varying from 11.74 to 5.87 years depending on the population, which, like the presently studied species, also reflect relatively low intrinsic rates of population increases.

One shortcoming of this study, common in most demographic analysis studies, is that the analysis performed is static in time and reflects exclusively the period when both biological and fisheries data was collected, in this case from February 2003 to October 2004 (Coelho and Erzini, 2007, submitted-a, submitted-b). In order for a more robust analysis to be performed, a time series analysis would have to be carried out in order to determine if the population declines predicted by the current study are indeed occurring, and if eventual density dependant mechanisms, such as lowering size at maturity, increasing fecundity or accelerating growth could came into play and produce less dramatic scenarios. However, and given that the lantern shark species focused on in the present work have no commercial interest, it is highly unlikely that monitoring data will be collected on a regular basis in the future.

The most striking conclusion of this study is that the populations of both lantern shark species of the southern Portuguese shelf and upper slopes are probably declining due to excessive fishing mortality. Regarding *E. spinax*, both longlining and trawling are implicated in the population decline, with results suggesting that trawling is having a more detrimental effect than longling and that this species cannot withstand the current levels of fishing. For *E. pusillus,* the longline fishery does not seem to be sustainable, with trawling apparently not having much of an impact on this species.

If these species are to be protected, urgent management measures are needed. Regulating these fisheries based on conservation issues of these non commercial discarded sharks does not seem to be a realistic scenario but some measures, such as the use of devices already developed to reduce trawl by-catch (Campos and Fonseca, 2004; Fonseca *et al.*, 2005) or strategies proposed to reduce elasmobranch by-catch in deep water longlines (Coelho *et al.*, 2003) should be implemented.

CHAPTER 9: General discussion and conclusions

(Some deep water shark species caught by commercial fisheries off the Algarve:

Galeus melastomus, *Etmopterus spinax* and *Deania calcea*)

Due to the increasing fishing mortality of deep water sharks, and the lack of both fisheries and biological data on most affected species, there is a need for life history information and analyses on the impact of deepwater fisheries on these species. This thesis provides for the first time information on both the life cycle and fisheries catches of two common deep water lantern shark species commonly caught in southern Portugal. This life history and fisheries information allowed a complete population dynamics study and the assessment of the current impacts of these commercial fisheries on these populations.

This final chapter presents a general discussion and a summary of the key findings and conclusions presented in the various sections of this thesis.

9.1. Morphological characteristics

The correct taxonomic identification of species is very important and provides the critical baseline that supports the rest of all other biological research (Last, 2007). Due to their morphological similarities and to the fact that, as a whole, they are a less known group, deep water sharks still face relatively large problems when species specific identification is needed. The case of lantern sharks seems to be particularly problematic amongst the deep water elasmobranchs, mainly due to their small size, lack of commercial interest and endemic distribution of many species. The FAO database (FAO, 2007) currently only has two categories to list lantern sharks, specifically *Etmopterus* spp. and *Etmopterus spinax*, and given the very low quantities that these categories present, even for grouped worldwide data, there are clear identification problems in this group.

In southern Portugal, two species of lantern sharks are common, the velvet belly lantern shark, *E. spinax* and the smooth lantern shark, *Etmopterus pusillus*. Both these species have overlapped distributions, occurring in deep waters of the outer continental shelves and slopes (Compagno *et al.*, 2005). Commercial fisheries operating in the area commonly capture both these species as by-catch (e.g. Erzini *et al.*, 2001; Monteiro *et al.*, 2001; Coelho *et al.*, 2005) and if monitoring is to be implemented, there is a need for an easy way to identify easily confounded species.

During this study, it was demonstrated that the use of multivariate analysis is effective for separating the species and the most important morphometric traits when it comes to separating the species were determined. Most morphometric characteristics, when analysed individually, contributed very little to the overall differences, and only when multivariate analysis was carried out did the differences become more evident. Discriminant analysis was used to create an equation that can now be used to separate the two species, and this equation can be relatively easily implemented given that it is based on only 6 morphometric measurements. This equation may be an important aid in discriminating these species, given that it can inclusively be applied to specimens after the laboratory sampling procedures, using detailed photographs of the specimen’s body.

The implementation of routine identification methodologies for both fisheries data and during scientific cruises is critical for these poorly known lantern sharks, and with the present study this is now more easy to accomplish.

9.2. Commercial fisheries catches

Several deep water shark species are commonly caught as by-catch and discarded by commercial fisheries operating in southern Portuguese waters. Such fisheries comprise both longliners targeting bony fishes (Erzini *et al.*, 1999; 2001; Coelho *et al.*, 2005) and trawlers targeting crustaceans (Monteiro *et al.*, 2001). Some of the most common deep water elasmobranchs caught in this by-catch are the lantern sharks, *Etmopterus pusillus* and *Etmopterus spinax*, and the catsharks, *Galeus atlanticus* and *Galeus melastomus*.

Since most of this shark by-catch is discarded, and never accounted for in the official fisheries statistics, there was a need to gather information on the impact that these commercial fisheries are having on these shark populations. In this study length distribution, maturity distribution and sex ratios of each sex of both species caught as by-catch in the two commercial fisheries are presented. The most important findings were that compared to longlines, trawls catch smaller sized and mostly immature specimens, and that both fishing gears catch more female *E. spinax* than males, with the opposite situation observed for *E. pusillus*, with more males caught by both gears. This results show that different components of these shark populations are being impacted differently by the two main deepwater fishing gears in this region.

9.3. Life history parameters

Although the two lantern shark species are widespread in their distribution (Compagno *et al.*, 2005) and are important predators on the outer shelf and upper slope areas (Neiva *et al.*, 2006), the lack of commercial interest explains the lack of biological studies. For both species only some preliminary population dynamics data were available prior to this study, specifically age estimation of the Mediterranean population of *E. spinax* (Sion *et al.*, 2002) and size-at-maturity estimations for *E. spinax* and *E. pusillus* in southern Portugal (Coelho and Erzini, 2005), but no comprehensive studies focusing and integrating all life history aspects had ever been carried out.

Given that the life history parameters are the basic data needed for population modeling purposes, there was a need to perform a complete life history study for both species, including age, growth, maturity, fecundity, and reproductive seasonality. Only with this data, could further population dynamics assessments be carried out in order to determine, for example, if the current fishing pressures are sustainable.

The results show that both these lantern sharks are relatively small sized species, characterized by slow growth rates, low fecundities and late maturities. In the specific case of *E. spinax*, a 3 year reproductive cycle was observed, and a similar situation (or eventually a 2 year cycle) may be the case for *E. pusillus*. These extremely long reproductive cycles, along with their very low fecundities and late maturities make these species very vulnerable to fishing mortality. Such characteristics seem to be common for deep water squalid sharks in general and are some of the reasons why this is nowadays one of the elasmobranch groups that causes most concern among scientists (Fowler *et al.*, 2005).

Population parameters studies on Etmopteridae species are scarce, not only for the presently studied species but also for other species of the same genus. Besides the two species studied and presented in this work, only two other species are known to have been studied in relation to the reproductive aspects and only one in relation to age and growth. A list of population parameters estimated for Etmopteridae species worldwide is compiled in Table 9.1 and Table 9.2.

Table 9.1. Reproductive data available from the scientific literature for *Etmopterus* sharks worldwide. TL_{max} is the maximum observed size (cm) and L_{50} the size at first maturity (cm).

Species	Location	Sex	TL_{max}	L_{50}	Mean fecundity	Reproductive periodicity	Reference
E. baxteri	SE Australia (SW Pacific)	Male	72	54			(Irvine, 2004)
		Female	87	63	8.8		
E. granulosus	New Zealand (SW Pacific)	Male	69.7	55-58			(Wetherbee, 1996)*
		Female	78.8	64-69	12.7		
E. princeps	Iceland (NE Atlantic)	Male		57.3			(Jakobsdottir, 2001)
		Female	89	62.2			
E. pusillus	Portugal (NE Atlantic)	Male	47.9	38.03			Present study
		Female	50.2	43.58	10.44	Uncertain, but either 2 or 3 years	
E. spinax	Portugal (NE Atlantic)	Male	33.8	25.86			Present study
		Female	41.1	30.69	9.94	3 years	

* The species originally described by Wetherbee (1996) as *Etmopterus granulosus* is correctly *Etmopterus baxteri* (Kyne and Simpfendorfer, 2007).

Table 9.2. Age and growth data available from the scientific literature for *Etmopterus* sharks worldwide. TL_{max} is the maximum observed size (cm), Age_{max} is the maximum observed age (years), L_{inf}, k and t_0 are the von Bertalanffy growth function (VBGF) parameters and Age_{50} to the age at first maturity (years).

Species	Location	Method	Sex	TL_{max}	Age_{max}	VBGF parameters			Age_{50}	Reference
						L_{inf}	k	t_0		
E. baxteri	SE Australia (SW Pacific)	External dorsal spines bands	Male	74	48	60.6	0.08	-1.43	20	(Irvine *et al.*, 2006a)
			Female	80	57	68.1	0.04	-4.51	30	
		Internal dorsal spines sections	Male	74	22	59.6	0.16	-2.00	10.5	
			Female	80	26	69.3	0.12	-1.56	11.5	
E. spinax	Mediterranean	Internal dorsal spines sections	Both	40.0	7				5	(Sion *et al.*, 2002)
E. spinax	Portugal (NE Atlantic)	Internal dorsal spines sections	Male	33.8	8	57.96	0.09	-2.55	3.97	Present study
			Female	41.1	11	55.84	0.12	-1.99	4.67	
E. pusillus	Portugal (NE Atlantic)	Internal dorsal spines sections	Male	47.9	13	49.01	0.17	-2.15	9.86	Present study
			Female	50.2	17	54.04	0.13	-2.79	7.13	

9.4. Depth distribution

Several previous studies have established relationships between the depth and the size of specimens of some deep water squalid sharks species, such as *Centroscymnus owstoni* and *C. coelolepis* in Japan (Yano and Tanaka, 1988), *Centroscymnus coelolepis* and *Centrophorus squamosus* in the NE Atlantic (Clarke *et al.*, 2001) and *Cetroscyllium fabricii* and *Etmopterus princeps* in the NE Atlantic

(Jakobsdottir, 2001). With regards to the lantern sharks species in this study, two previous works have mentioned the depth distribution patterns of *E. spinax* in the Mediterranean Sea (Massuti and Moranta, 2003; Sion *et al.*, 2004), but no studies are known for the eastern Atlantic populations of this species, nor for any population of *E. pusillus*. The previous studies on *E. spinax* have, however, focused mainly on the description of the depth distribution of the specimens in relation to abundance and size, but no study is known to have described the age distribution or the reproductive cycle in relation to depth in these species.

Therefore, and given that different fishing gears have been demonstrated to be affecting different parts of the populations, there was a need to understand how these species are distributed throughout their depth range, in order to understand the ecological repercussions of these fisheries.

This particular study was only carried out for the species *E. spinax* and not for *E. pusillus*. The reason for this is that this particular study only used standardized fisheries independent catch data from scientific surveys and this type of data was only available for trawling. Given that *E. pusillus* is frequently caught with longlines but more rarely caught with trawls, an observation that was hypothesized to be because of either *E. pusillus* having a more bentho-pelagic behavior or being more associated with rocky bottoms, this analysis was not carried out for this species.

Size, age, sex and maturity stages were found to be correlated with depth, with the larger, older and mature specimens occurring predominantly at greater depths. A similar pattern of larger specimens more frequent at greater depths had been previously described for *E. spinax* in the Mediterranean (Massuti and Moranta, 2003; Sion *et al.*, 2004), but in the NE Atlantic, Jakobsdottir (2001) found a different pattern for *E. princeps*, with the size of specimens decreasing with increasing depth.

Furthermore, a depth related migration was observed in mature females, that seem to go to deeper waters for mating and once pregnant move to shallower waters to give birth.

The results of the depth distribution analysis have important and complex implications for the management of these vulnerable deep water sharks. The migrations of *E. spinax* during its life cycle are complex, with this species using different depth ranges during its life to complete specific phases, specifically deeper waters as mating grounds and shallower waters as nursery grounds. If an ecosystem-based management of fisheries approach is to be applied for this species, the entire depth range including both these important mating and the nursery grounds would have to be protected.

9.5. Density dependant mechanisms

Density dependant mechanisms refer to changes in life history parameters as a function of population density, with survivorship and fertility having an inverse relationship with population density. For deep water sharks, a decrease in the size at maturity had been previously reported for the northeast USA population of *S. acanthias*, due to a decrease in population density over a 20 year period due to excessive fishing mortality (Sosebee, 2005), but no such relationship had ever been reported for Etmopteridae sharks.

During the present work, and given that the *Etmopterus* species from the south of Portugal have been suffering high levels of fisheries pressure over the last 3 decades, there was a need to know if such mechanisms had been activated and how efficient they were. For this purpose, we compared size at maturity and fecundity between the southern Portuguese *E. spinax* population, where an intensive deep water bottom trawl fishery started in the 1980s' with as many as 30 commercial vessels continuously fishing in this area (Campos *et al.*, 2007), and a population of the Alboran Sea (western Mediterranean), where no significant deep water fisheries have operated for the last decades (J. Rey, Pers. comm.[4]).

It was observed that the northeast Atlantic population is maturing at significantly smaller sizes than the Mediterranean population. In the Mediterranean, both sexes are maturing at approximately 83% of the maximum observed size, while in the northeast Atlantic both sexes are maturing at approximately 75% of the maximum observed size. Given that the maximum observed sizes in both areas were approximately the same, we concluded that this 8% reduction in size at maturity of the southern Portuguese population could reflect a density dependant reduction as a consequence of a decrease in population density associated with excessive fisheries induced mortality.

However, a relationship between female size and fecundity is to be expected in both placentary and aplacentary viviparous species, given that the number of embryos that a female can carry in a litter is dependant on the available abdominal cavity space. Therefore, we compared mean fecundity between these two populations to try to assess how efficient this density dependant mechanism was. Relationships between female size and fecundity were established, with the NE Atlantic population having a lower mean fecundity than the Mediterranean population.

The conclusion is that there seems to be a trade off in this species (that is probably the same for all viviparous species), where due to density dependant mechanisms, the exploited populations start to mature at smaller sizes, but with the consequence that the fecundity of these smaller mature females is lower. These

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[4] Javier Rey, Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Spain.

mechanisms are therefore not as efficient in these sharks as they would be for oviparous species, which means that these deep water sharks are even more susceptible to overexploitation than initially expected.

9.6. Mortality rates and demographic analysis

The estimation of mortality rates and their use in demographic analysis is very important as it allows a better understanding of the dynamics of fish populations. The mortality parameters are important because there is a need to know how fast individuals are removed from the population in order to model the population dynamics and to estimate sustainable rates of exploitation (Simpfendorfer *et al.*, 2004), while demographic analysis is useful to determine the probability of decline in a population and to diagnose a population's susceptibility to fishing pressure (Simpfendorfer, 2004). Given that the intrinsic rate of increase of a population decreases when fishing mortality increases, demographic analysis is useful to predict how much fishing is affecting a population.

Most of the elasmobranch studies where this type of analysis has been carried out refer to commercially important coastal or pelagic elasmobranchs, with only one deep water squalid species, specifically the commercially important *S. acanthias* (Chen and Yuan, 2006) known to have been studied with regards to demographic parameters. No studies are known to have ever been carried out in Etmopteridae sharks.

Even though the two lantern sharks contemplated in this study are usually discarded due to their low or null commercial value, it was demonstrated that they are highly impacted by commercial fisheries and that they have vulnerable life cycles. For such reasons, there was a need to carry out a demographic analysis study and to determine if current fishing pressures in southern Portugal are sustainable.

Population demographic parameters were calculated and the age structured survivorship considered in scenarios with and without fishing mortality. In this way, it was possible to determine the impacts that the current fisheries pressures are having on these populations.

The most striking conclusion of this study is that both lantern shark species currently being affected by commercial fisheries that operate in the southern Portuguese shelf and upper slopes are declining. In the specific case of *E. spinax*, both longlining and trawling are resulting in population declines, meaning that this species cannot withstand the current levels of fishing, even though the trawl is having a more detrimental effect than the longline. Regarding *E. pusillus*, the longline fishery does not seem to be sustainable, but trawling seems to be having only a limited effect on this species.

9.7. Final considerations and future research

The final considerations and recommendations based on this PhD dissertation, along with a discussion of some possible future research perspectives are presented in this final chapter.

This study has presented comprehensive life history and population parameters for two lantern sharks from southern Portuguese waters, *Etmopterus spinax* and *E. pusillus*. Biological data and population parameters including age, growth and reproduction aspects are presented for both species along with bathymetric distribution patterns for one of the species, *E. spinax*. The feeding ecology of these species was not included as part of this thesis, but during the course of this work two parallel studies were carried out by two undergraduate students on this subject. In both cases, the supervisor of this PhD thesis, Dr. Karim Erzini, acted as main supervisor, and the author of this dissertation, Rui Coelho, acted as co-supervisor. Specifically, the feeding biology of *E. spinax* has been studied by an undergraduate student of the University of Évora, João Neiva, whose final report was defended in 2004 (Neiva, 2004) and subsequently published in a peer review journal in 2006 (Neiva *et al.*, 2006). The feeding ecology of the other species, *E. pusillus*, is currently being studied by an MSc student of the University of the Algarve, Marta Correia, with the MSc thesis defense expected in 2007.

Given their vulnerable life cycles and the fact that commercial fisheries are having clearly detrimental impacts on these populations, there is an urgent need to monitor the catches of these population in the future as well as the presence and effort of fishing activity at areas and depths important for these species. One possible and suggested alternative for controlling the spatial distribution of fishing effort of commercial vessels is the use of the MONICAP system, already in use by part of the commercial fishing fleet operating in Portugal. At this stage, this system can already filter the vessels activities, determining if they are navigating or fishing, but there is now a need to correlate the fishing grounds where specific vessels were fishing with actual catches. For commercial species that are landed and commercialized in fishing harbors, this will be possible by correlating the grounds where a vessel has been fishing with the species and respective quantities landed and registered by the DGPA, but for discarded species, such as these two lantern sharks, this will not be possible given that these species are never landed or commercialized. Alternative and suggested methods to monitor the fished quantities of these species include logbooks or the presence of regular fishing observers aboard commercial vessels.

Some issues have remained unsolved in this PhD and these may be addressed by further research in the near future. It was possible to determine bathymetric distributional patterns and migrations from mating to nursery areas in *E. spinax*, but it was not possible to determine if spatial migration patterns also occur. A possible approach for accomplishing this would be by using acoustic telemetry techniques, implying the need to capture and tag live specimens. At this stage, we can foresee three variables that might limit the survival of captured specimens, specifically the hauling speed, temperature and pressure changes. The use of a research vessel, where hauling speed can be controlled, and equipped with a hyperbaric chamber and temperature controlled tanks might make such studies possible. Another alternative might include the use of *in situ* external tagging with underwater tagging equipment already developed and tested for some bony fish species (Sigurdsson *et al.*, 2006).

During the present study the age of both species was verified by the use of marginal increment analysis but validation was not possible. If in the future a research vessel could be used to capture live specimens, these could be released after being injected with tetracycline and tagged with external tags. Eventually, future catches returned by fishermen could validate the ages estimated and verified at this stage. Other suggested future research includes the use of the biological parameters and fishing impacts that have been described at this stage to perform risk analysis on these species and to predict population declines or even extinction probabilities in the future in light of the current fishing activities that are impacting these populations (Gallucci *et al*., 2006).

Given that there are clear limitations to the knowledge of deep water sharks at this stage, conservative management measures should be implemented. A good example already implemented is the case of the Mediterranean Sea, where it is forbidden to trawl at depths deeper than 1000 m, and we suggest that a similar management measure should be implemented in Portuguese waters. Furthermore, the recommendations issued by both the IUCN and the ICES that currently recommend a zero catch for deep water sharks, should be enforced and strictly followed in national waters and eventually at a European level. Other management measures that should be enforced immediately are the use of by-catch reduction devices or strategies, such as the ones already developed for trawl (Campos and Fonseca, 2004; Fonseca *et al.*, 2005) and longline (Coelho *et al.*, 2003) that might reduce the by-catch of these species and limit the detrimental impacts that fisheries are currently having on these shark populations.

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