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Faculty of Biosciences, Fisheries and Economics

Metazoan Parasites in deep-sea sharks

Part I. A Review of the Parasite Fauna of sharks of the genus *Etmopterus*

Part II. Metazoan gastrointestinal parasites of *Etmopterus spinax* (L., 1758) from southern Norwegian waters

Diogo Costa Ramos da Rocha Marques

FSK-3910 Master's Thesis in International Fisheries Management
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Front page photo of *Etmopterus spinax* (Velvet belly lanternshark)

By Rudolf Svensen

Acknowledgments

First and foremost, I would like to show my endless gratitude to my supervisor, Willy Hemmingsen (UiT). The guidance, working experience and the professional and knowledge input which he has provided me with throughout this process has been vital.

A huge thank you must also be addressed to both my advisors/mentors Kenneth MacKenzie (The University of Aberdeen), for his hospitality, help and for generously sharing his great academic and life knowledge, and Claudia Junge (IMR), an example of true passion for science and leadership who has been essential for the conclusion of this academic chapter of my life.

A big thank you must also be addressed to all my closest friends, especially the ones helping me with this thesis.

My friends all around the World and fellow classmates also deserve a thank you, for their love, for sharing life and knowledge perspectives and for the support I received.

Por fim queria dar um agradecimento da imensidão de um Oceano à minha família, especialmente à minha irmã, Mãe e Pai, pelo vosso apoio e o Amor. Vocês são as minhas raízes e tal e qual como uma árvore sem elas não consigo sobreviver.

Tromsø, 15th of November 2019

Diogo Rocha Marques

Abstract

Parasites play an important role in ecology due to their potential influence on the biodiversity and dynamics of ecosystems. The complexity of interaction between parasites and their hosts is crucial to understanding the host's populations dynamics, behaviour as well as interconnections between food chains in an ecosystem. Parasites have wide applications as bio-indicators for example to detect and monitor pollution and to provide additional information on the host's population connectivity. With increasing fishing pressures globally, an understanding of affected ecosystems including the species and their dynamics therein is crucial in order to implement effective management strategies. The velvet belly lanternshark (*Etmopterus spinax*) is a small deep-sea shark which is a common bycatch species in North Sea fisheries. There have been only few studies on the parasites of *E. spinax* in Norwegian waters and none of them included mature individuals studying the entire parasite community. Therefore, this study investigated a total of 115 *E. spinax* specimens from box sexes in different sexual stages were sampled at eight stations with different depths in the North Sea in January 2016. In addition, this thesis aimed to review all available literature on metazoan parasites within the entire genus *Etmopterus* to lay the basis for the expectations of the empirical study and to identify potential knowledge gaps. The literature review indicated 21 existing parasite species on 9 host species belonging to the *Etmopterus* genus, on 13 different sites of the host's body. The empirical data from eight stations in southern Norwegian waters revealed fairly low parasite prevalence and diversity on *E. spinax*. From a total of 115 studied shark specimen in this study only a total of four different parasite species (from four taxonomic groups) were recorded and only 18 host specimens were infected with at least one parasite species. The comparison of *E. spinax* individuals showed that larger sharks had a significantly higher prevalence of the parasite species *A. simplex* and *A. squalicola*, although the same could not be found for the other two parasite species. The generally lower values compared to the literature could be explained by e.g. parasite seasonality, diet shifts or methodological constraints, which are all discussed. The parasites species found here presented both strong and weak points for potential use as biological tags for host dynamics and food web interactions in the future.

Keywords: Metazoan parasites; population tag; *Etmopterus*; North Sea; management

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Introduction

Parasitism, from an evolutionary perspective, is defined as the relationship between species, where one organism, the parasite, lives on or in another organism, the host, causing it some harm, and is adapted structurally to this way of life (Poulin, 2007). The definitions for parasitism may differ significantly however, reflecting the research interests and backgrounds of academics, but one fact that is obvious and undisputed is that it is one of the most successful life strategies of all living systems (Poulin, 2007; Poulin & Morand, 2000; Rohde, 2015). This is evidenced by the diversity and absolute numbers of existing parasites (Poulin & Morand, 2000; Rohde, 2015). Parasites play an important role not only in commerce and economics (e.g. loss of quality of fish harvest and costs associated with the infection control and prophylaxis), but also in marine conservation (e.g. infection level of certain parasites species can provide information about host density) (Marcogliese, 2005; Sasal & Thomas, 2005; Catalano *et al.*, 2014; Shinn *et al.*, 2015). Parasites can consist of one single cell (eukaryote) or multiple cells (metazoan) (Loker & Hofkin, 2015). The group of metazoan parasites includes (i) roundworms (Nematoda), (ii) flatworms (Platyhelminthes) such as digeneas (endoparasitic flatworms), monogeneans (ectoparasitic flatworms) and cestodes (tapeworms), (ii) arthropods (Arthropoda) with crustaceans such as copepods and barnacles (*Cirripedia*) (Caira & Healy, 2004) and (iii) rotifers (Syndermata/Rotifera) like *Acanthocephala*.

The life cycle of parasites can be direct (i.e. implies only a single host to achieve their development and to reproduce) or indirect (i.e. requires at least two different hosts to complete their biological cycle) (Rohde, 2005; Loker & Hofkin, 2015). Metazoan parasites present numerous and diverge types of interaction with their host, which in some ecological cases are difficult to distinguish (Rohde, 2005). They include commensalism (i.e. parasites benefit from the host while the host is unaffected), phoresis (i.e. parasites use exclusively the host as a transport or shelter/support), mutualism (i.e. parasites and hosts benefit with the interaction, however is not obligatory), symbiosis (i.e. compulsory relationship between hosts and parasites) (Rohde, 2005; Loker & Hofkin, 2015).

Host-parasite interactions are crucial to our understanding of the host as an individual, within a population including its dynamics, and as part of an ecological community structure (Scott, 1988; Poulin, 2000; Marcogliese, 2004, Sasal & Thomas, 2005). Parasite species are often host-specific (i.e. with respect to their final host and/or the intermediate host) and within a host even

organ-specific (Caira *et al.*, 2012; Rohde, 2015), and the distributional patterns of that host will contribute to parasite distribution (Poulin & Morand, 2000; Marcogliese, 2004). The ecological study of parasites can therefore not only reveal crucial information about their role in food webs but also about the feeding habits and behaviour of the hosts (Marcogliese, 2004; Irigoitia *et al.*, 2017). The largest host species, the vertebrates, commonly populate the most abounding parasite fauna and provide a wide number of niches to parasites (Rohde, 1993). The physiological response by the host to parasitic infections may result in loss of energy (e.g. defence mechanism against parasitic infection), decrease of grown rate and may reduce host tolerance to stressors (Poulin, 2000; Marcogliese, 2004; Rynkiewicz *et al.*, 2015). Consequently it could induce competition for limited resources or deprivation of physiological responses such as reproduction and growth, and ultimately resulting in a pathological impact on host body and death of the host (Poulin, 2000; Rynkiewicz *et al.*, 2015). Hence, parasites indirectly have the ability to regulate and control populations densities and abundances (Dobson, 1988; Hatcher *et al.*, 2012, Catalano *et al.*, 2014). Parasites can play a negative role in biodiversity (e.g. causing local extinctions) (McCallum & Dobson, 1995). Under different conditions, they can fundamentally contribute to the stability of ecosystems, and consequently have been suggested as a proxy of ecosystem quality (Hudson *et al.*, 2006).

The complexity of interactions between parasites and their hosts can give promising information regarding the ecosystem that the parasite is inserted in (MacKenzie & Abaunza, 1998; Hermida *et al.*, 2013). Because of that, the use of parasites as indicators (or "tags") has gained wide acceptance in the last decades, for example as bio-indicators which are markers that are inside of an individual which can have physiological or pathological ethology and may give information about the organism, population and ecosystem surround (MacKenzie, 1998; MacKenzie, 1999; Catalano *et al.*, 2014) Such bio-indicators can be used as proxies to assess for example fish (host) population structure (i.e. identification, discrimination and evaluation of fish stocks) and migratory behaviour (Williams *et al.*, 1992; MacKenzie, 1998; MacKenzie, 1999; Mosquera *et al.*, 2003; Marcogliese, 2004; Catalano *et al.*, 2014). Parasites can also provide evidence of past movements of their hosts while eliminating concerns about the influence of abnormal behaviour of the tagged animal as one might expect from artificial tags (e.g. acoustic tags, coded wire tags) which could inhibit the host's movement (Mosquera *et al.*, 2003).

The diversity of parasites differs in different marine habitats, depending on biotic (e.g. species composition among the fish community) and abiotic factors (environmental conditions, as depth and habitat) (Willig, 2001; Rohde, 2005). Parasites have therefore been used as bio-indicators for abiotic conditions and for pollution (Williams *et al.*, 1992; MacKenzie, 1999; Marcogliese, 2004; Catalano *et al.*, 2014). Several parasites have delicate short-lived free-living transmission stages that consequently turn them highly sensitive to environmental conditions and their variations (Bush *et al.*, 1997; Hemmingsen & MacKenzie, 2001). So that each stage should be assessed individually, to ensure a higher number of potential indicators (Hemmingsen & MacKenzie, 2001). Often with increasing levels of pollution, infections of endoparasitic helminths (i.e. parasites which inhabit the interior of the host - cavities, ducts, organs and musculature) with complex life cycles tend to decrease. On the other hand, infections of ectoparasites (i.e. parasites which reside on the host's surface - exterior site or orifice) with single-host life cycle tend to increase (Gallagher *et al.*, 1994; Hemmingsen *et al.*, 1995; MacKenzie, 1999; Rohde, 2015). Despite this overall tendency, there are significant variations in the responses among different parasite species to different pollutants (Hemmingsen & MacKenzie, 2001).

For a parasite to be considered a good tag or bio-indicator, some recommendations should be taken into consideration (Timi, 2007; Catalano *et al.*, 2014; Timi & MacKenzie, 2015), see summary below:

1- The parasite species should have different levels of infection in the host at different geographical locations;
2- The life cycle of the parasite species should preferably involve only a single host as more information is needed on the biotic and abiotic factors influencing transmission between hosts for those parasite species with multi-host life cycles;
3- The life span of the parasite species in the host needs to cover the duration of the investigation as a minimum
4- The prevalence of the parasite species should remain relatively stable between seasons and years;
5- The parasite species should be easily detected, preferably by gross examination;
6- The parasite species should have no effects on the behaviour or survival of the host;

A potential candidate species for use as a population tag does not necessarily have to fulfil all these requirements (MacKenzie & Abaunza, 1998). However, parasites selected as tags need

to be correctly identified before they can be considered as a useful biological tag (Catalano *et al.*, 2014). The taxonomic identification of the parasite species involves morphological examination under a microscope and measurement of character traits in combination with a species identification key deciphering between those traits (Mosquera *et al.*, 2003; Catalano *et al.*, 2014; Irigoitia *et al.*, 2017). "Parasite tagging" can therefore be more time consuming (e.g. some of the larvae stage of certain parasites require a scanning electron microscopy due to their morphological complexity and size) and results could be biased due to uncertainty in the literature for the taxonomic identification and the used morphologic features of certain parasites species (Mosquera *et al.*, 2003; Catalano *et al.*, 2014). Nevertheless, tagging through parasites is becoming more widely used and does have a short-term economic advantage compared to molecular methods for identification and the added value of the possibility to also study host-parasite interactions (Mosquera *et al.*, 2003; Catalano *et al.*, 2014; Timi & MacKenzie, 2015). The combination of taxonomic identification and parasite genetics reveals to be most efficient, albeit most expensive (Mosquera *et al.*, 2003; Catalano *et al.*, 2014).

Extensive knowledge of the Earth's ecosystems, their habitat threats and the biology, ecology and population dynamics of key species is vital to ensure their future preservation (Hoggarth *et al.*, 2006). To achieve this, the establishment and implementation of effective and holistic management strategies and systems (e.g. marine protected area) are fundamental for promoting population recovery, and maintain and protect biological diversity, habitats and ecosystem functions (Aranha *et al.*, 2009; Rui Coelho & Erzini, 2010; Irigoitia *et al.*, 2017). To increase the quality of available biological data for such holistic approaches, a combination of various methods should therefore be applied, including the use of artificial as well as biological tags (Mosquera *et al.*, 2003; Irigoitia *et al.*, 2017).

Many marine environments around the world are experiencing an increase in fishing pressure due to the increasing world population and technological development of the harvest techniques of industrial fisheries (FAO, 2011; Martínez *et al.*, 2007). In addition, the unceasing increase of human population densities, mainly along world's coasts and the rapid advances in technology are actively contributing to alarming levels of anthropogenic effects on marine ecosystems (Martínez *et al.*, 2007; FAO, 2011; Brander, 2013; Dulvy *et al.*, 2014; Pendleton *et al.*, 2018). The consequences of these actions start to be more evident and alarming (e.g. species extinctions), and have become an essential issue of pressing social and political concern (Brander, 2013; Díaz *et al.*, 2019).

The North Sea is experiencing those anthropogenic pressures and is at the same time an important area for some important commercial species in Norwegian fisheries which include shrimps, *Pandalus borealis* and *Aristeus antennatu*s, Atlantic cod, *Gadus morhua*, saithe, *Pollachius virens*, North Sea herring, *Clupea harengus,* ling, *Molva molva*, and Norway pout, *Trisopterus esmarkii* (Coelho *et al.*, 2010; Isbert *et al.*, 2015; McMillan *et al.*, 2017; ICES, 2018). Some of these stocks have been strongly reduced by the intensive fishing pressure (McMillan *et al.*, 2017; ICES, 2018). The ICES (2018) annual report informed that several North Sea stocks (i.e. cod, haddock, mackerel, and blue whiting) have fishing mortality rates above the fishing mortality consistent with achieving maximum sustainable yield (= the highest possible annual catch that can be sustained over time, by keeping the stock at the level producing maximum growth). Additionally, not only target fishing species are affected (ICES, 2018), but certain fisheries may also catch protected, endangered, or threatened species as non-targeted bycatch (Lent & Squires, 2017; ICES, 2018). Some of these classic "bycatch-species" are sharks, skates, rays and chimaera, all belonging to the group of cartilaginous fishes (Klimpel *et al.*, 2003; Isbert *et al.*, 2015; ICES, 2018).

Many cartilaginous fishes are either considered top or meso-predators (i.e. on the top, or near the top, of the food chain within their ecosystem) and therefore have a fundamental role for the balance of the food webs and ecosystems which they are a part of (Heithaus *et al.*, 2008; Ferretti *et al.*, 2010; Roff *et al.*, 2016). Variations on shark abundance for example have been shown to cause changes in prey abundance or behaviour and consequently induce trophic cascades (Heithaus *et al.*, 2008; Ferretti *et al.*, 2010). This in return implies that increasing the catch rate of sharks may not only reflect changes on the population size, migration patterns, habitat expansions of this species but may also lead to variations in population density and behaviour of (other) intermediate predators, first order consumers and ultimately on primary consumers (i.e. algae and phytoplankton) (Ferretti *et al.*, 2010; Roff *et al.*, 2016). A significant decrease of high trophic level specimens, may induce changes in the ecosystem which may consequently decrease biodiversity (Simpfendorfer & Kyne, 2009; Więcaszek *et al.*, 2018). This can have an even stronger impact on marine areas which are hot spots of biodiversity and where food webs are incredibly complex as shown for example for coral reefs (Ruppert *et al.*, 2013; Roff *et al.*, 2016). Sharks also play an important selection role on the ecosystem because they remove the weak and diseased individuals by predation, therefore keeping certain diseases and populations under control (Heupel *et al.*, 2014; Roff *et al.*, 2016). Some shark species are acting as

scavengers, removing carcasses, which is an important function to maintain nutrient cycling dynamics (Techera & Klein, 2011; Roff *et al.*, 2016).

Deep-sea sharks are in various degrees affected by anthropogenic stressors like fisheries (including bycatch and illegal harvesting), pollution and habitat destruction (Dulvy *et al.*, 2014). In combination with some of their intrinsic biological features like slow growth rates, late maturity and low fecundity (compared to most teleosts), this leads to their very low recovery potential and high susceptibility to overexploitation (Klimpel *et al.*, 2003; Aranha *et al.*, 2009; Ferretti *et al.*, 2010; Dulvy *et al.*, 2014; Isbert *et al.*, 2015). In the North Sea, some shark species are some of the most affected bycatch species from commercial fisheries (McMillan *et al.*, 2017; ICES, 2018). The velvet belly lanternshark, *Etmopterus spinax* (L.1758), is a non-commercial deep-sea shark species, which is frequently captured as bycatch for Norway lobster, *Nephrops norvegicus*, deepwater rose shrimp, *Parapenaeus longirostris*, and red shrimp, *Aristeus antennatu*s from bottom trawlers and deep-water longliners in Norwegian waters (Monteiro *et al.*, 2001; Aranha *et al.*, 2009; Coelho *et al.*, 2010; Isbert *et al.*, 2015). When discarded after catching, the shark is usually either dead or has severe lesions (Aranha *et al.*, 2009). In order to reduce this detrimental bycatch some input measures were implemented to the fishing techniques for example the development of a selective grid for the bottom trawling nets and removing the hooks near the bottom for the long liners (Aranha *et al.*, 2009; Isbert *et al.*, 2015; Sistiaga *et al.*, 2019). The International Union for Conservation of Nature (IUCN) has categorized *E. spinax* as “least concern” overall (Coelho *et al.*, 2009). Although for deep-sea sharks, as for the velvet belly lanternshark, there is still a lack of data on the population structure and reproductive biology to inform the development of appropriate management and conservation strategies (Coelho & Erzini, 2010).

The velvet belly lanternshark (*Etmopterus spinax*) presents sexual dimorphism with females growing more and maturing at larger sizes than males (Coelho *et al.*, 2010; Porcu *et al.*, 2013). Females can reach a total body length of 60 centimetres (cm) (Compagno, 1984). Although it is a small-sized shark, it has a relatively slow growth rate and consequently matures relatively late in its life cycle (Coelho & Erzini, 2008; Isbert *et al.*, 2015). According to Coelho & Erzini (2008), the velvet belly lanternshark might only reproduce once every 2 to 3 years, suggesting a low fecundity rate. *E. spinax* is aplacental viviparous which means that embryos develop inside eggs that are retained within the mother's body until they are ready to hatch. *E. spinax* has a wide diet range (cephalopods, crustaceans and small fish), which is habitat-dependent

(Compagno, 1984; Klimpel *et al.*, 2003; Isbert *et al.*, 2015). With the increase of body length there is a tendency for it to become more piscivorous (mostly comprising meso, bentho- and bathypelagic fish species) (Klimpel *et al.*, 2003; Neiva *et al.*, 2006; Isbert *et al.*, 2015). It plays a valuable ecological role within the ecosystem helping to maintain food web balance (McMillan *et al.*, 2017).

Sharks (and also other cartilaginous fishes) in general are hosts to a number of metazoan endoparasites and ectoparasites which inhabit several organs and tissues (Caira *et al.*, 2012). The great diversity of parasites for which squaliform sharks, like *E. spinax*, present a suitable habitat includes Cestoda, Nematoda, Digenea, Monogenea, Cirripedia and Copepoda (Gallagher *et al.*, 1994; Caira *et al.*, 2012). Some parts of the sharks (e.g. skin and gastrointestinal system) have a tendency to have higher diversity of metazoan parasites (Caira *et al.*, 2012). The level of parasite diversity and load on sharks depends on numerous host variables, such as physiological condition, feeding behaviour, breeding behaviour, social interactions (e.g. schooling behaviour), diet (Benz & Bullard, 2004; Caira *et al.*, 2012; Isbert *et al.*, 2015). Cartilaginous fishes are frequently single host or final host for the majority of metazoan parasites which infect them, due to the higher trophic level (Benz & Bullard, 2004). Also, the older specimens of sharks tendentially have more parasites due to the long-term accumulation of parasites with a long life-cycle (Hemmingsen & MacKenzie, 2001; Benz & Bullard, 2004; Caira et al., 2012; Caira & Pickering, 2013).

There have been only very few studies on the parasites of *E. spinax* in the NE Atlantic, and only four of them were in Norwegian waters (Klimpel *et al.*, 2003, Rees et al., 2014; Ommundsen *et al.*, 2016, Rees *et al.*, 2019). The studies by Rees *et al.* (2014, 2019) and Ommundsen *et al.* (2016) focused only on the ectoparasite *Anelasma squalicola* and did not include any data or comparisons with endoparasites within *E. spinax*. Klimpel et al. (2003) provided good first insights into the endo- and ectoparasites in Norwegian *E. spinax*, however, the study focused only on juvenile specimen. There is to date no published information on the parasite load and composition of adult *E. spinax* and no data on any differences between sexes or different life history stages.

Motivation of this study was therefore to investigate the parasite diversity and load of the deep-sea shark *Etmopterus spinax* in the North Sea and to investigate the potential of using this type of data to inform management. This is the first study conducted in Norwegian waters analyzing the metazoan parasites (endo- and ectoparasites) of sexual immature as well as mature *E. spinax*

specimens, and the first one to investigate the North Sea. Due to the scarcity of published data on the species, I decided to conduct an extensive literature review comprising the entire genus *Etmopterus* as a first step of this thesis. This is followed by the empirical study of over 100 individuals of *E. spinax* from southern Norway with the aims to 1) investigate the level of infections of ectoparasites (on the skin and nostril) and gastrointestinal (endo)parasites and 2) to explore whether parasite communities differed between locations, sex and various life history traits of the host.

Informed by my literature review, I hypothesized that: (i) seven parasite species will be found, (ii) most sharks will have at least one endo- or ectoparasite, and (iii) parasite prevalence in *E. spinax* is positively correlated with the shark's total length.

Material and Methods

Part I. A Review of the Parasite Fauna of sharks of the genus *Etmopterus* - Literature Review

I conducted a systematic review of all published literature on the parasitic fauna of squaliform sharks of the genus *Etmopterus*. This review contains peer-reviewed journal articles, proceedings from conferences and book chapters, and contains to the best of my knowledge all available published information on the subject (up to 30.07.2019). I used the following search engines: google scholar, PubMed and ELVISIR, together with key word and project searches in ResearchGate.

Below is an introduction of the parasite groups found in marine vertebrates:

Digenea

Digeneans are one of the most diverse parasite group amid fish endoparasites. (Cribb, 2005). This parasite group belongs to the class Trematoda (Platyhelminthes) and as the Monogeneans, is frequently referred to as flukes (Cribb *et al.*, 2003; Cribb, 2005). Digeneans have a great plasticity and complexity range of life cycles. This characteristic makes it possible to find a great diversity of these flukes on several groups of invertebrate and vertebrate hosts (i.e. sexual adult digeneas infect all classes of marine vertebrates) (Cribb, 2005).

Diverse larval stages are included in this parasite group, including free-living and parasitic. Often its life cycle contains two intermediate hosts (Cribb *et al.*, 2002; Cribb *et al.*, 2003; Cribb, 2005). In the majority of the digenean species, the first host in the life cycle is a mollusc (first host), where asexual reproduction occurs. Followed by transmission to a definitive host (vertebrate) in which the parasite's sexual reproduction occurs (Cribb *et al.*, 2002).

Digeneans are mostly parasites that inhabit the gastrointestinal system, although they can also appear in the urinary system (i.e. urinary bladder), reproductive system (i.e. ovaries), circulatory system, musculature, and body cavity (Cribb, 2005). Morphologically, the majority of digeneans presents an oral sucker which opens into the gut, and a ventral sucker for attachment (Cribb *et al.*, 2002; Cribb, 2005).

Monogenea

Monogeneans are mainly ectoparasites on fish hosts (Cribb *et al.*, 2002; Hayward, 2005). Ecologically, this group presents a strong host-specificity (Rohde, 1993; Whittington *et al.*, 2000). This parasite group is usually found attached by a small flattened opisthaptor to the host gills, fins or skin (Rohde, 1993; Cribb *et al.*, 2002; Hayward, 2005). The life cycle of a monogenean is a direct cycle (i.e. there is exclusively a single host) (Rohde, 1993; Whittington *et al.*, 2000).

Cestoda

Cestodes, also known as tapeworms, are endoparasites, and the majority are hermaphrodites (Keneedy, 1965; Caira & Reyda, 2005). The cestode's adult form is commonly found in the digestive track (and sporadically in the associated organs of gastrointestinal tissues) in the definitive vertebrate host (Caira & Reyda, 2005; Pereira & Velloso, 2016). The *Archigetes* genus is the exception of the known species, in which adult forms occur in invertebrates (e.g. Polychaeta, Serpulidae) (Pereira & Velloso, 2016). One of the most singular characteristics of cestodes is their polyzoic condition (i.e. each segment has a complete male and female reproductive system) (Caira & Reyda, 2005; Pereira & Velloso, 2016). Also, most tapeworms undergo strobilation (i.e. external segmentation, called strobila, that separates the various sets of reproductive structures in adult forms, called the proglottid). In the proglottids, cestode eggs are stored for regular shedding into the host environment (Pereira & Velloso, 2016).

Tapeworms present a strong evolutionary specialization on their parasitic condition (e.g. absence of gastrointestinal tract) (Caira & Reyda, 2005; Pereira & Velloso, 2016). Anatomically they do not present gut or mouth (Caira & Reyda, 2005). Instead, cestodes have a cover of neodermal cuticles on their tegument, which allows them to absorb nutrients from the host's alimentary tract. (Keneedy, 1965; Pereira & Velloso, 2016). Tapeworm lifecycles are usually complex, including one to three intermediate hosts. In the marine environment, bony fishes are often hosts of larval forms of cestodes. The adult forms can be found in elasmobranchs, sea birds and mammals, which have fish as a part of their diet (Pereira & Velloso, 2016).

Copepoda

Copepods are the most abundant group among the parasitic crustaceans in the world's oceans (Boxshall, 2005; Gunn & Pitt, 2012). This large range size of parasites presents a diverse variety of hosts (i.e. practically every available phylum in the marine environment is a potential host) (Boxshall, 2005; Gunn & Pitt, 2012; Eiras & Castro, 2016). Also, their microhabitat range on the host is wide (i.e. they can be ectoparasites and endoparasites) (Boxshall, 2005, Eiras & Castro, 2016), although most species of this parasite group are free-living ectoparasites in the marine environment (Boxshall, 2005; Gunn & Pitt, 2012). Copepods represent a higher economic factor as vectors of diseases and pathomorphological effects in wild and aquaculture fish populations (e.g. salmon sea lice, *Lepeophtheirus salmonis* and Atlantic Salmon, *Salmo salar*) (Boxshall, 2005; Nekouei *et al.*, 2018).

Morphologically, like other crustacean groups, they have an exoskeleton, divided into two body plans (Boxshall, 2005; Gunn & Pitt, 2012) The first plan is gymnoplean plan, where the body is separated into two distinct tagmata: a prosome (on its anterior part) and urosome (on its posterior part) (Boxshall, 2005). The articulation between these parts there are five pedigerous (leg-bearing) and genital segments (somites) (Boxshall, 2005; Eiras & Castro, 2016) The second plan is the podoplean, where occur the articulation of prosome and urosome with one somite nearer to the cranial part of the parasite (usually between the fourth and fifth pedigerous somites) (Boxshall, 2005; Eiras & Castro, 2016). Its appendages contain five cephalic and seven thoracic limbs, also on the anal somite is located a pair of caudal rami (Boxshall, 2005).

Usually these ectoparasites are located on the skin, gills and tegument of the fish hosts (Eiras & Castro, 2016). The majority of marine parasitic copepods present sexual dimorphims (i.e body size/form and appendage assembly) (Boxshall, 2005). Basically the life cycle of this crustacean group is usally divided into naupliar and copepodid phases (in some cases it can have six naupliar stages and five copepodid stages) (Boxshall, 2005; Gunn & Pitt, 2012).

Nematodes

Marine nematodes are endoparasites, which frequently are found as adults or larval stages in the gut and musculature of the fish (Möller & Anders, 1986; McClelland, 2005). Nematoda present complex life cycles, involving at least three hosts (McClelland, 2005; Lamps & Lamps, 2009). Typically roundworms present a bilateral symmetry and commonly have an elongate

cylindrical form, tapered at both ends (McClelland, 2005; Roberts & Janovy, 2008b). Morphologically this phylum of parasites has a complete gut with a mouth, pharynx, intestine, and anus (McClelland, 2005; Janovy *et al.*, 2013). The body wall of a roundworm has a cuticle, hypodermis and a single layer of longitudinal musculature (Roberts & Janovy, 2008b). Commonly roundworms are dioecious (i.e. the male and female reproductive organs are in separate individuals), dimorphic and females are larger than males (McClelland, 2005; Roberts & Janovy, 2008b).

The roundworms present a short duration life cycle, consequently there can be a large variation in their population levels in a short period (i.e. weeks) (McClelland, 2005; Janovy *et al.*, 2013). Many nematodes are also able to utilise paratenic hosts (i.e. a host that harbors the sexually immature parasite but is not necessary for the parasite's development cycle to progress) to maximise transmission to the final hosts (which frequently is a bird or a marine mammal) (Lamps & Lamps, 2009). Few parasitic nematodes have successfully invaded the deep sea, although a significant number of parasites of this phylum normally occurs in shallower water, such as *Pseudoterranova* spp., *Contracaecum* spp. and *Anisakis* spp., are also found in deep water fish (Alioshkina *et al.*, 1985; Blaylock *et al.*, 2003; Lamps & Lamps, 2009).

Isopoda

Isopods are classic marine parasites and often inhabit warmer waters (Lester, 2005). Morphologically they have a carapace and the body is frequently dorsoventrally flattened (Lester, 2005; Roberts & Janovy, 2008a). They have antennules which are often uniramous (i.e. comprise of a single series of segments attached end-to-end), sometimes vestigial (i.e. rudimentary) (Roberts & Janovy, 2008a). The majority of Isopods are ectoparasites and have a short life cycle (Lester, 2005; Roberts & Janovy, 2008a). The larval stages cryptoniscus and praniza and the juvenile stage are the infective phases of Isopods to the hosts and the transmission mode is active by attachment (Roberts & Janovy, 2008a).

Thoracica

A small number of species of the Thoracica superorder are parasitic (Boxshall & Lützen, 2005). Actually, they are better known as conventional epizoic filter-feeding barnacles than parasites because the majority of them do not penetrate into their host (Boxshall & Lützen, 2005; Roberts & Janovy, 2008a; Eiras & Castro, 2016). Although there are some exceptions such as *Anelasma*

squalicola (e.g. Rees *et al.*, 2014; Eliassen, 2016). Generally, they have sixwell-developed pairs of thoracic appendages (Roberts & Janovy, 2008a).

Hirudinea

Commonly known as leeches, this diverse group inhabits different aquatic ecosystems, from seashore and deep ocean to rivers and lakes (Govedich *et al.*, 2005). Leeches can be predators (large range of invertebrate and vertebrate preys) or ectoparasites (often temporary) (Govedich, 2001; Davies & Govedich, 2001; Govedich *et al.*, 2005). Morphologically, their bodies do not have a complex division into different regions and are not externally divided into distinct regions (Govedich *et al.*, 2005). They lack chaetae bristle (chaetae) and they have on the anterior and posterior extremities of their bodies a sucker (oral and caudal sucker) (Govedich, 2001; Davies & Govedich, 2001; Govedich *et al.*, 2005).

Part II. Metazoan gastrointestinal parasites of *Etmopterus spinax* (L., 1758) from southern Norwegian waters – Empirical Study

The study species: *Etmopterus spinax*

The velvet belly lanternshark, *Etmopterus spinax* (L.1758) is a small-sized deep-water squaliform shark which inhabits predominantly the outer continental and insular shelves and upper to lower slopes near or at the bottom at depths of between 70 and 2,000 m (usually at 200–500m) (Compagno, 1984; Coelho & Erzini, 2008; Coelho *et al.*, 2010; Porcu *et al.*, 2013). *E. spinax* uses different depth ranges during its life cycle (Isbert *et al.*, 2015) and has a wide geographic distribution, from the eastern Atlantic Ocean (Iceland and Norway) to southern Africa (including the Azores, Madeira, Canaries and Cape Verde archipelagos) and in the Mediterranean Sea (western and central areas) (Compagno, 1984; Reiner, 1996; Klimpel *et al.*, 2003; Serena, 2005; Coelho & Erzini, 2010; Porcu *et al.*, 2013; Isbert *et al.*, 2015). Morphologically, it is characterized by having a compact body with a fairly long tail, very short gill slits, with brown coloration above and an abruptly black abdomen (Compagno *et al*, 2005; Aranha *et al.*, 2009). Like other species belonging to the genus *Etmopterus*, the velvet belly lanternshark is bioluminescent (Renwart et *al.*, 2015; Więcaszek *et al.*, 2018). Over its flanks and abdomen there are photophores (cup-shaped organs), composed of a protective layer of pigments and a reflector structure that encloses photocytes (light-emitting cells) (Renwart *et al.*, 2015).

The study area: The North Sea

The North Sea is one of the most extensive shallow shelf seas in the world (ICES, 1983; Huthnance, 1991; Ottesen, 2009). It is adjacent to the North Atlantic Ocean (Sündermann & Pohlmann, 2011). The North Sea presents a water volume of 40 300 km^3 and an area of 575 300 km^2 (ICES, 1983; Huthnance, 1991; Rodhe *et al.*, 2004). The average depth is 70 m, although in the Norwegian Trench it can increase until 700m (e.g. in Skagerrak) (Huthnance, 1991). The Norwegian Trench presents a fjord-like topography, and it cuts into the shelf along the Norwegian coast (Huthnance, 1991; Rodhe, 1998; Rodhe *et al.*, 2004).

The North Sea presents a cyclonic circulation, most of the time, which renews its water on a period of approximately one year. The largest amount of its water enters from the north and

flows through Norwegian Trench, in a cyclonic direction (Huthnance, 1991; Rodhe, 1998; Rodhe *et al.*, 2004). Along Norwegian channel, there is an all-year stratification by salinity, caused by an outflow from the local rivers and Baltic Sea. The salinity along this area is about 25–30 PSU (Rodhe *et al.*, 2004). On the western slope of the Norwegian Trench, there is an inflow of high-saline Atlantic water which renews the underlying water gradually in this section (Rodhe *et al.*, 2004).There is moderately constant upwelling from eastern Skagerrak (i.e. the deepest part of Norwegian Trench) and outwards along the Norwegian coast, which provide nutrient rich water for primary production (Rodhe, 1998; Rodhe *et al.*, 2004). The water temperature varies between 3°C and 18°C depending upon seasonally changes (Huthnance, 1991).

The North Sea presents a decreasing trend in biomass, in total biomass and in different taxonomic groups with increase of latitude (Heip *et al.*, 1992; Callaway, 2002). Phytoplankton, zooplankton and fish (pelagic and demersal) are key species on the North Sea waters (ICES, 2018).

Fish sampling and examination

This study benefited from the annual shrimp research survey conducted in southern Norwegian waters (57° 52' 30"-59° 39' 06" N; 3° 57' 24"-10° 36' 54" E) on board of F/F Håkon Mosby in January 2016 by the Institute of Marine Research. All stations were trawled with a bottom sampling trawl (Campelen 1800 Shrimp Survey Trawl) with mesh size 20 mm in the channel. The *E. spinax* sampled in each station were then frozen on board in blocks at −20◦C. Sharks were grouped per station. One hundred and fifteen (n=115) sharks from eight stations were selected to investigate the parasite prevalence and abundance at depths ranging from 173 m to 402 m (see Table **1**, Figure **1**).

Table 1 - Stations and sampling information. Shown are coding of stations, maximum trawl depth, geographic localization of the stations (lat/long), and number of sampled individuals of *E. spinax*.

Stations code	Depth (m)	Latitude	Longitude	Number of individuals
A	307	58° 36' 30" N	5° 23' 24" E	27
B	402	58° 46' 42" N	9° 45' 42" E	19
C	236	58° 17' 24" N	10° 36' 54" E	14
D	318	58° 03' 18" N	5° 59' 42" E	10
E	273	57° 55' 12" N	5° 58' 00" E	11
F	173	57° 52' 30" N	5° 37' 24" E	9
G	314	57° 57' 24" N	6° 22' 36" E	9
H	277	59° 39 06" N	3° 57' 24" E	16

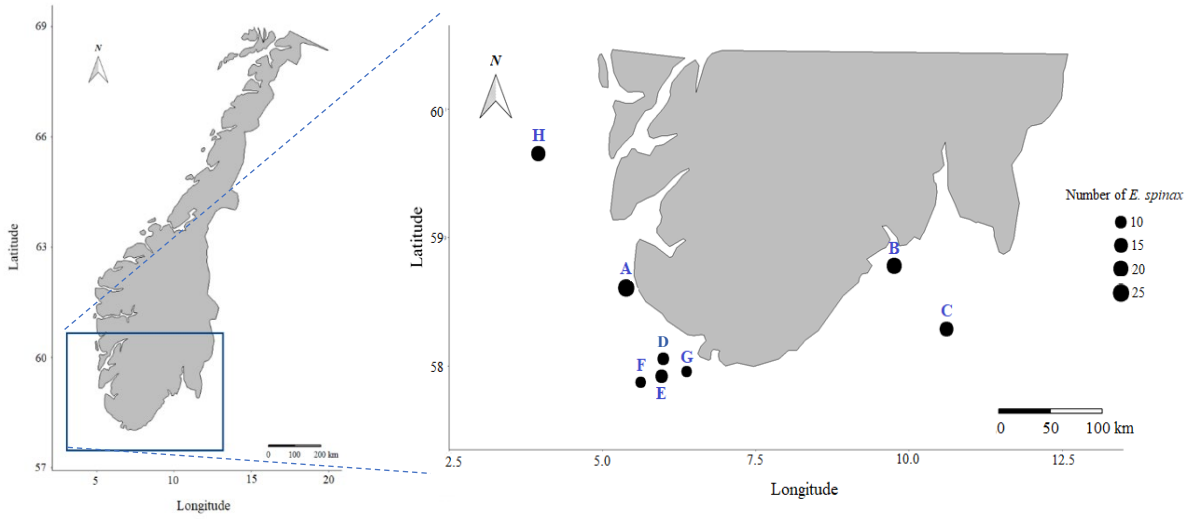

Figure 1 – Sampling stations circles in southern Norwegian waters. Map showing the eight sampling stations (coded A-H; see Table 1) with black circles in southern Norwegian waters. The size of the circle is proportional to the sampling size at a given station.

In the laboratory, the sharks were thawed and prepared for dissection. For each station all the sharks were defrosted, and all specimens processed individually. For each station all the sharks were processed. Prior to dissection, total length (length, in cm) and body weight (weight, in g) were measured and the sex determined (based on presence or absence of claspers on the pelvic fins, see Figure **2**).

Figure 2 – Length measurement of *E. spinax*. Measurement of overall length (in centimetres) from the tip of the snout to the tip of the upper lobe of the caudal fin. Red circle shows the location of claspers on the male specimen.

Dissections

Sharks were dissected following the protocol of Jobling (2015). Two small incisions of surrounded skin to each nostril were made (dorsally and ventrally to the axis of the shark) to access the snout content. An incision in the mid-ventral line was made from just anterior to the cloaca to the level of the pectoral fins. Then lateral incisions were made from the extreme regions of the initial incision. Lateral displacement of the skin flaps was then made to expose the internal organs. Sex was confirmed by examination of the gonads, and the reproductive stage was assessed based on Myrlund (2018) (see Appendix Table **1** and **2**; Figure **3**). Then the three-lobed liver was freed by dissection of mesenteric tissue close to the pectoral girdle, removed from the body cavity, and its weight recorded in order to calculate the hepatosomatic index (HSI). Then, with the help of Mosquito pins the distal part of the intestine and the oesophageal connection to the pharynx were occluded (avoiding the spread of gastrointestinal content). Incisions were made adjacent to the cloaca and the pharynx to free the gastro-intestinal tract. Then the gastro-intestinal tract was gently removed from the body cavity by cutting the supporting mesenteries. An incision was made at the level of the pyloric sphincter, dividing the gastro-intestinal tract into two portions. The two portions were transferred to separate Petri dishes, and the contents of the portions were gently squeezed onto the Petri dishes. A longitudinal incision was then made to each portion of the gastrointestinal tract, and the preparation allowed lying flat.

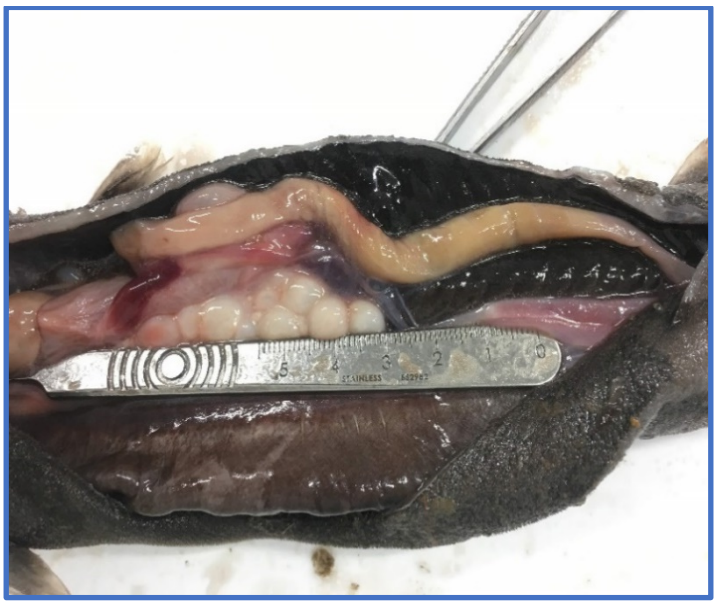

Figure 3- Female specimen of *E. spinax* classified as "Mature - Stage 3", according to Myrlund (2018).

Parasite identification

- Before host dissection

External surfaces (i.e. skin) and buccal cavity were examined macroscopically and under the stereomicroscope looking for ectoparasites (e.g. *Anelasma squalicola*) (see Figure **4**).

A. squalicola is the only ectoparasite found on *E. spinax* (see Introduction and Results from literature review) and therefore easy to identify. It is a monophyletic species of stalked barnacle which parasitizes certain deep-sea sharks of the family Etmopteridae (Yano & Musick, 2000; Rees *et al.*, 2014). Usually, it is attached to the dorsal spine or the area of the pectoral and pelvic fins of their hosts (Baer, 1951; Yano & Musick, 2000).

Figure 4 - *E. spinax* parasitized by two *A. squalicola* specimens.

- **After host dissection**

The contents retrieved from the dissections were examined using a Leica dissecting microscope at 20 and 200× magnification for counting any parasites. All metazoan parasites were collected (with thin brushes and forceps) and preserved in 70% ethanol (for future genetic studies) or fixed in 4% borax-buffered formaldehyde for subsequent taxonomic confirmation. All retrieved parasites were identified to the lowest possible taxonomic level and counted. The taxonomic identification had the supervision of Dr Kenneth Mackenzie, who has a solid and long experience on the identification of marine fish parasites.

Nematodes

To retrieve potential nematodes, the internal surfaces of the two portions of the host's gastrointestinal tract were immersed in saline solution (2,5% NaCl) and scraped with a microscopic slide. Thereafter, a dissecting microscope at 20 to 200× magnification was used for the species identification. The identification of nematodes was based on published morphological characteristics such as body shape, cuticle shape, mouth and lip shape, shape and dimensions of the buccal capsule, spicules and esophagus, tail shape, the position of the vulva and excretory pore and number of caudal papillae in males (Petter *et al.*, 1995; Moravec, 1998; Coomans, 2000).

Cestodes

Cestodes were collected from the gastrointestinal samples. The taxonomic identification of these parasites was done based on general morphologic characteristics (long, flat, ribbon-like organisms) and anatomic featuring (scolex with suckers, proglottids, absence of digestive system, presence of a male and female reproductive system in each proglottid).

Monogeneans

The *E. spinax* snots were extracted from each nostril, with the help of dissection forceps, and they were placed on a separate Petri dishes. Then each nasal cavity was subjected to a sequence of consecutive washes with flush of saline solution (2,5% NaCl) until the remaining snout inside of the nostril was dissolved. The monogenoid specimens collected were fixed and stored in 5% formaldehyde (Varella & Malta, 1995; Varella & Malta, 2001; Jobling, 2015).

The taxonomical identification of Monogeneans was based on qualitative analysis of characteristics of morphological and anatomical features: the shape of the body, the form, and structure of haptor (anterior haptor, prohaptor, and posterior haptor, opisthaptor), hooks, anchors, and clamps, the reproductive system (male copulatory organ, female reproductive organ) and the arrangement of the organs (Brinkmann, 1952; Boeger *et al.*, 2006).

Statistical analysis

Host life history data

As the number of sharks per gender on each sampling location was not normally distributed, a few additional statistical considerations had to be made and the reasoning for using the different statistical tests are outlined in each case.

To investigate the relationship between *E. spinax* gender and locations/stations a non-parametric Fisher's exact test was used. This test is used to determine if there are non-random associations between two categorical variables. To test if there is a significant correlation between length or weight among different locations/stations the non-parametric Kruskal-Wallis test was used. If a significant difference was detected between these associations, a post hoc Dunn's non-parametric comparison (pairwise multiple comparisons) was performed (Whitlock & Schluter, 2015).

The *Hepatosomatic index* (HSI) is an index describing the relationship of the liver weight relative to the overall body weight of an individual and is often used to infer the health status of an individual. It was calculated according to Coelho & Erzini (2008) for all sampled individuals as:

HSI= liver weight (g) / shark weight (g) × 100

Statistical terminology and measurements of infection rates

To investigate differences in parasite species load on the eight sampled stations, two quantitative parameters (prevalence and mean abundance) were analysed for each station.

Prevalence and mean abundance are suitable statistical descriptors to quantify parasites in whole host populations (including the uninfected hosts (Gallagher *et al.*, 1994; Bush *et al.*, 1997). The statistical parameters used in this master dissertation are standardized and based on the terminology suggested by Bush *et al.* (1997):

Prevalence (P) is the number of hosts (sharks) in a sample which are infected with one or more individuals of a particular parasite species (a), divided by the number of the host sample (station) (N). It is frequently expressed in percentage.

$$P = (a / N) * 100$$

Mean abundance (A) is the arithmetic mean of the total number of individuals of a parasite taxon in a sample (b) per host examined on that sample (N) - regardless of infection-status (infected/non-infected).

$$A = b / N$$

In addition, the epidemiological term *Intensity* will be also used in the following parts of this study, so a definition should be revealed.

Intensity, or *intensity of infection*, corresponds to the number of individuals of a parasite taxon in a single infected host.

Influence of host length/age and maturity stage on parasite infection

Since only few sharks were infected by parasites in this study and no apparent pattern in the infection was evident, the use of a generalized linear model (GLM) was taken into consideration. A logistic regression was used to analyse for effects of length and location on the presence/absence of parasites. Separate logistic regressions were performed for each parasite species, using length and location as a predictor variables and presence/absence of parasite as the response variable. Subsequently, a Kruskal-Wallis test (regarded as a non-parametric alternative to ANOVA type I) was performed to summarize the main effects. If any effect on parasite prevalence could be detected for any of the detected parasite species, a graph with a fitted negative binomial was used to visualize the pattern of parasite infections. To fit the model, parasite intensity (i.e. number of a parasite of a certain species (or other taxon) found in a single infected host) was used (Bush *et al.*, 1997).

In order to statistically assess if the prevalence of parasites differed between females and males of *E. spinax,* Fisher's exact test was applied. This test examines the relationship in a 2x2 contingency table and tests the independence of categorical small values (Whitlock & Schluter, 2015).

Software used

Mathematical calculations and descriptive analyses were performed in Microsoft ® Excel ® for Office 365 MSO (16.0.11328.20420) 64-bit. Statistical analysis, graphs and maps relied on the open source software R (version 3.5.1, R Core Team) and its visual representation in Rstudio (version Version 1.1.456 – 2009-2018 RStudio, Inc.). The packages for statistical analysis used in R were "dplyr", "car", "factoextra", "factoMineR", "FSA", "ggplot2", "ggpubr", "gridExtra", "MASS", "muStat", "rcompanion", "readr", "reshape2" (Seefeld, 2007; Meur, 2012).

Results

Part I. A Review of the Parasite Fauna of sharks of the genus *Etmopterus* - Literature Review

The summary data presenting the parasitic fauna of squaliform sharks of the *Etmopterus* genus presented in Table 2 were gathered from the systematic literature review. The binomial nomenclature of the host of each parasite species (when possible) or genus, the parasite's location in the host, and references ranked by year of publication are shown. In addition, the most important parasites are being discussed below, giving important morphological and life history background information.

The results indicated 21 identified parasite species on 9 host species belonging to the *Etmopterus* genus. The parasites were identified from 13 different sites of the host's body. The intestines were the host's sites which had the highest diversity of parasite species. The parasite that had the largest geographic distribution was *A. squalicola* and it was also the most studied parasite among the *Etmopterus* genus hosts. The Atlantic Ocean was the most studied area and showed the highest diversity of found parasites.

E. spinax was the host species within the genus *Etmopterus* with the highest number of found metazoan parasites species (n=12). The parasite species were *S. spinacis, A. menezesi, A. norvegicus, A. pickeringae, A. tasmaniensis, A. tenuis, P. squali*, *L. longibrachia*, *L. spinacis, A. simplex, H. aduncum, A. squalicola*. The cestode *A. norvegicus* was the most studied parasite for the *E. spinax* host. Only four articles investigated metazoan parasites in *E. spinax* in Norwegian waters, two of them on *A. squalicola* (Rees *et al.*, 2014; Ommundsen *et al.* 2015, Rees *et al.*, 2019) and one of them describing parasites of only immature specimen (Klimpel *et al.*, 2003). For all details see Table 2.

Table 2 - Parasitic fauna of squaliform sharks of the *Etmopterus* genus.

Parasite	**Site of Infection**	**Host species**	**Location**	**Reference**
Digenea				
Otodistomum plunketi (Fyfe, 1953)	Body cavity	*Etmopterus princeps* (Collett, 1904)	Rockall, NE Atlantic	Gibson & Bray, 1977
Otodistomum sp.	Intestine	*Etmopterus granulosus* (Günther, 1880)	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018
	Stomach	*Etmopterus spinax* (Linnaeus, 1984)	Off NE Spain, NE Atlantic	Isbert *et al.*, 2014
Monogenea				
Asthenocotyle azorensis (Kearn, Whittington & Thomas, 2012)	Dermal denticles	*E. princeps*	North Atlantic, Azores	Kearn *et al.*, 2012
Squalonchocotyle spinacis (Gotto, 1894)	Gills	*E. spinax* *E. granulosus*	Norwegian Deeps, NE Atlantic Off NE Spain, NE Atlantic Off coast of Chile, SE Pacific	Klimpel *et al*., 2003 Isbert *et al.*, 2014 Espínola-Novelo *et al.*, 2018
Monocotylidae indet.	Nasal cavities	*E. spinax*	Norwegian Deeps, NE Atlantic	Klimpel *et al*., 2003
Asthenocotyle sp.	Skin	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018

Calicotyle sp.	Skin	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018
Monocotylidae gen. sp.	Skin	*E. spinax* *E. granulosus*	Norwegian Deeps, NE Atlantic Off coast of Chile, SE Pacific	Klimpel *et al*., 2003 Espínola-Novelo *et al.*, 2018
Cestoda				
Aporhynchus cf. menezesi (Noever et al, 2010)	Intestine	*E. spinax*	North Atlantic Off NE Spain, NE Atlantic	Noever *et al.*, 2010 Isbert *et al.*, 2014
Aporhynchus norvegicus (Olsson, 1868) Nybelin, 1918	Stomach, Intestine	*E. spinax*	North Atlantic, Azores Norwegian Deeps, NE Atlantic Off NE Spain, NE Atlantic NW Mediterranean deep-sea	Beveridge, 1990 Klimpel *et al*., 2003 Świderski *et al.*, 2012 Isbert et al., 2015 Dallarés *et al.*, 2017
Aporhynchus pickeringae (Noever, Caira, Kuchta & Desjardins, 2010)	Intestine	*Etmopterus pusillus* (Lowe, 1839) *E. spinax*	North Atlantic, Azores	Noever *et al.*, 2010 Caira & Pickering, 2013

Aporhynchus tasmaniensis (Beveridge, 1990)	Intestine	*Etmopterus baxteri* (Garrick, 1957) *E. granulosus* *E. spinax*	SW Pacific SE Indian, Tasmania North Atlantic, Azores	Beveridge, 1990 Palm, 2004
Ditrachybothridium cf. macrocephalum (Rees, 1959)	Intestine	*E. spinax*	Off NE Spain, NE Atlantic	Isbert *et al.*, 2014
Gilquinia squali (Fabricius, 1794)	Intestine	*E. granulosus*	Off coast of Chile, SE Pacific North Atlantic	Carvajal, 1974 Alves *et al.*, 2017
Lacistorhynchus tenuis (van Beneden, 1858)	Body cavity	*E. spinax*	Norwegian Deeps, NE Atlantic	Klimpel *et al*., 2003 Palm, 2004
Plesiorhynchus brayi (Palm, 2004)	Intestine	*E. princeps*	North Atlantic	Palm, 2004 Caira & Pickering, 2013
Plesiorhynchus etmopterid (Beveridge, 1990)	Intestine	*E. baxteri* *E. granulosus* *Etmopterus lucifer* (Jordan & Snyder, 1902)	East Pacific	Beveridge, 1990 Palm, 2004
Phyllobothrium squali (Yamagutti, 1952)	Intestine	*E. spinax*	Mediterranean Sea	Williams, 1968

Aporhynchus sp.	Stomach	*E. granulosus* *E. princeps*	Off coast of Chile, SE Pacific North Atlantic, Azores	Espínola-Novelo *et al.*, 2018 Caira & Pickering, 2013
Gilquinia sp	Intestine	*E. princeps*	North Atlantic, Azores	Caira & Pickering, 2013
Plesiorhynchus sp.	Intestine	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018
Pseudophyllidea indet	Intestine	*E. spinax*	Norwegian Deeps, NE Atlantic	Klimpel *et al*., 2003
Hepatoxylon sp.	Mesenteries	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018
Sphyriocephalus sp.	Stomach	*E. spinax*	Off NE Spain, NE Atlantic	Isbert *et al.*, 2014
Tetraphyllidea fam. gen. sp.	Liver, Intestine, Stomach	*E. spinax*	Off NE Spain, NE Atlantic NW Mediterranean deep-sea	Isbert *et al.*, 2014 Dallarés *et al.*, 2017
Trypanorhyncha fam. gen. sp.	Muscle and Stomach	*E. princeps* *E. spinax* *E. granulosus*	North Atlantic, Azores Off NE Spain, NE Atlantic Off coast of Chile, SE Pacific	Caira & Pickering, 2013 Isbert *et al.*, 2014 Espínola-Novelo *et al.*, 2018
Copepoda				
Lernaeopoda longibrachia (Brian, 1912)	Gills	*E. spinax*	Mediterranean Sea	Raibaut *et al*., 1998
Lernaeopodina spinacis (Brian, 1908)	Gills	*E. spinax*	Mediterranean Sea	Raibaut *et al*., 1998

Albionella etmopterid (Yamaguti, 1939) Kabata, 1979	Skin, Fins	*E. lucifer*	North Pacific	Benz, 1991
Neoalbionella sp.	Fins	*E. granulosus*	Off coast of Chile, SE Pacific	Rodríguez *et al,*. 2010 Espínola-Novelo *et al.*, 2018
Ommatokoita sp	Skin	*E. princeps*	NW Atlantic	Hogans & Brattey, 1986
Lernaeopodidae gen sp.	Gills	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018
Nematoda				
Anisakis simplex (Rudolphi, 1809)	Stomach, Intestine, Liver, Muscle and Gonads	*E. spinax* *E. granulosus*	Norwegian Deeps, NE Atlantic Off NE Spain, NE Atlantic Off coast of Chile, SE Pacific Baltic Sea	Klimpel *et al*., 2003 Isbert *et al.*, 2014 Espínola-Novelo *et al.*, 2018 Więcaszek *et al.*, 2018
Hysterothylacium aduncum (Rudolphi, 1802)	Gonads, Intestine	*E. spinax*	Norwegian Deeps, NE Atlantic	Klimpel *et al.*, 2003
Mooleptus rabuka (Machida, Ogawa & Okiyama, 1982) Özdikmen, 2010	Intestine	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018

Contracaecum sp.	Stomach, Intestine	*E. spinax*	Off NE Spain, NE Atlantic	Isbert *et al.,* 2014
Hysterothylacium sp.	Oral cavity	*E. spinax*	Off NE Spain, NE Atlantic	Isbert *et al.*, 2014
Isopoda				
Elthusa raynaudii (H. Milne Edwards, 1840)	Oral cavity	*Etmopterus joungi* (Knuckey, Ebert & Burgess, 2011)	NW Pacific, Taiwan	Williams *et al.*, 2010
Thoracica				
Anelasma squalicola (*Lovén, 1844*) Darwin, 1851	Skin, Eyes, Fins, Oral cavity	*E. spinax* *E. granulosus*	Norwegian Deeps, NE Atlantic Southern Atlantic North Atlantic Southern Pacific SE Pacific	Causey, 1957 Hickling, 1963 Fernandez-Ovies, 1993 Yano & Musick, 2000 Rees *et al.*, 2014 Ommundsen *et al.*, 2016 Espínola-Novelo *et al.*, 2018 Rees *et al.*, 2019

Anelasma sp.	Skin, Eyes, Fins, Oral cavity	*Etmopterus schultzi* (Bigelow *et al.*, 1953) *Etmopterus unicolor* (Engelhardt, 1912) *E. granulosus* *E. princeps* *E. spinax*	Southern Pacific SE Pacific NW Atlantic	Yano & Musick, 2000
Hirudinea				
Piscicolidae gen sp.	Skin	*E. granulosus*	Off coast of Chile, SE Pacific	Espínola-Novelo *et al.*, 2018

The most prevalent endoparasite species of *E. spinax* in southern Norwegian waters were previously reported as: *Aporhynchus norvegicus*, *Anisakis simplex, Hysterothylacium aduncum* and an underdetermined species of Monocotylidae family (Monogenea) (Klimpel *et al.*, 2003). These are discussed in more detail below.

From those four, the most predominant parasites recorded were an unidentified species of the monogenean family Monocotylidae and *A. norvegicus*, with 83.3% and 81.1% prevalence respectively (Klimpel *et al.*, 2003). The two cestodes *H. aduncum* and *A. simplex,* had a prevalence of 40.5% and 18.9% respectively (Klimpel *et al.*, 2003). *A. simplex* was isolated from the host's stomach and body cavity. Another study found a 20% prevalence of infection of the ectoparasite *Anelasma squalicola* on *E. spinax* sampled in southern Norwegian waters (Rees *et al.*, 2019). This parasite presents the greatest world distribution among the major oceans, on *E. spinax* and on other hosts within the Etmopteridae family (see Table **2**, Yano & Musick, 2000).

Crustacea (Thecostraca)

Anelasma squalicola (Lovén, 1844)

Anelasma squalicola is a crustacean parasite with the most common host being *E. spinax* (see Table **2**) (e.g. Rees *et al.*, 2014; Rees *et al.*, 2019). Morphologically, it is divided into two main regions: penduncular (fleshy stalk-like structure) and capitular (where it erects the rest of the body structure) region (Darwin, 1851; Johnstone, & Frost, 1927). The capitular region is enclosed by a dark purple-brown mantle which also covers the body and feeding appendages (Rees *et al.*, 2014; Ommundsen *et al.*, 2016). This anatomical region comprises a probosciform mouth, cirri (thoracic appendages), a male reproductive tract and egg mass (Rees *et al.*, 2014; Ommundsen *et al.*, 2016) (see Figure **5**). The peduncule is attached to the tissues of the host (Darwin, 1851; Johnstone, & Frost, 1927; Ommundsen *et al.*, 2016). On the peduncule structure there are branching rootlets, which have penetration and anchor functions into the shark's tissues (Darwin, 1851; Ommundsen *et al.*, 2016).

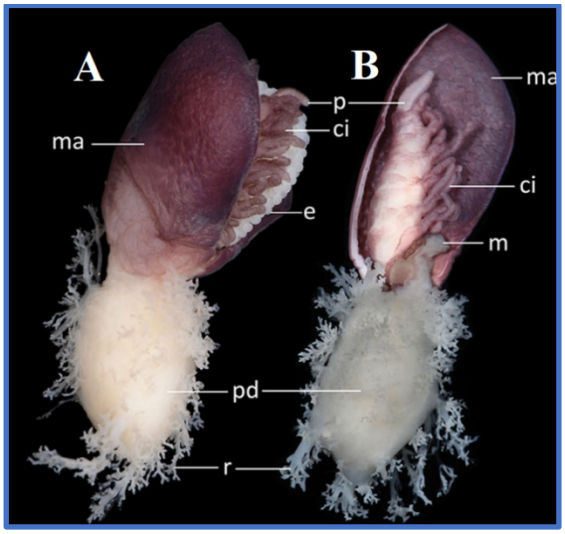

Figure 5 – Morphology of *A. squalicola.* **a.** *A. squalicola* showing the two main morphological regions: the capitulum and the exposed peduncle (the whitish lower half). **b.** *A. squalicola* with the part of the half of the mantle removed. ci=cirri, e=egg mass, m=mouth, ma=mantle, p=penis, r=rootlets; Adapted from Ommundsen *et al.* (2016).

Nematodes

Anisakis simplex (Rudolphi, 1809)

The genus *Anisakis* does not have a ventricular appendix or an intestinal cecum at any stage of its life. This trait anatomically differentiates this nematode genus from *Contracaecum* genus (which is provided of both structures) and *Pseudoterranova* (which has intestinal cecum) (Dallarés *et al.*, 2017). This nematode species has a nerve ring in the anterior region and its tail is conical (Carvalho-Varela, 2005). *A. simplex* stage L3, has two protrusions (i.e. derids), diametrically opposed, which follows the nerve ring (Ventura, 2006; Gomes, 2014) (see Figure **6**). In the cranial extremity of the *A. simplex* stage L3, a slim oesophagus is followed posteriorly by a thicker ventriculus (opaque in transmitted light). The ventriculus creates an oblique intersection at the posterior end with the intestine (Ishii *et al.*, 1989).

The life cycle of this nematode is indirect. Fish is are paratenic (intermediate) host of the stage L3 of this nematode (Ishii *et al.*, 1989; Ventura, 2006; Gomes, 2014). Free-living larvae of *A. simplex* (Second-Stage Larvae) are ingested by intermediate invertebrate hosts, principally planktonic malacostracan crustaceans (Petrie *et al.*, 2005; Smith & Wootten, 1975).

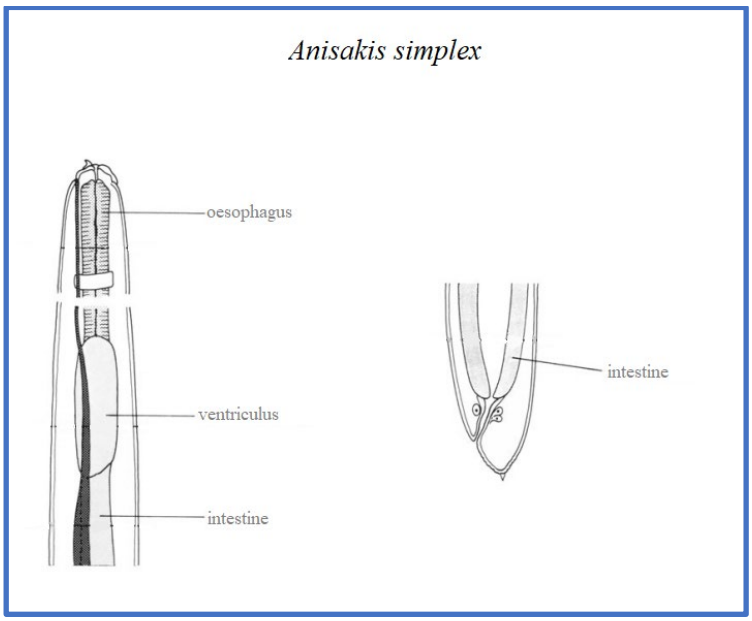

Figure 6 - Schematic representation of the third-stage larvae of *A. simplex*. Adapted from Ishii *et al.* (1989).

Hysterothylacium aduncum (Rudolphi, 1802)

The adult form of this nematode species frequently parasitizes fish's gastrointestinal tract (Coomans, 2000; Navone *et al.*, 1998). Morphologically its third-stage larvae (L3) have a thinner body anteriorly, a cuticle transversally striated, lateral alae with support v-shaped in cross-section, extending immediately behind the anterior extremity up to the caudal end (Navone *et al.*, 1998). L3 and adult stage of this species have an identical disposition of their digestive organs, where the position of the excretory pore it is just behind the nerve ring. (Coomans, 2000; Navone *et al.*, 1998). The first of the two first moults of its life cycle occur in the egg stage (Navone *et al.*, 1998). Reaching L3 there is a need for least one intermediate crustacean host for transmission of this parasite (Coomans, 2000; Navone *et al.*, 1998). During L3, this nematode is only infectious to the host (fish) after a certain development in at least one intermediate host (Coomans, 2000). The last two moults occur in the gastrointestinal tract of the definitive host (fish) (Coomans, 2000; Navone *et al.*, 1998).

Cestodes

Aporhynchus norvegicus (Olsson, 1868) Nybelin, 1918

A. norvegicus, like other species of the Aporhynchinae subfamily, does not have a rhyncheal apparatus (Rees, 1941; Beveridge, 1990) This anatomic characteristic differentiates this genus

among the others trypanorhynch cestodes (Beveridge, 1990). This cestode is relatively small (up to fifteen millimetres long) and presents with ten not overlapping proglottides (acraspedote) in gravid strobilae (segmented part of the cestodes, consisted of proglottids) (Beveridge, 1990). This endoparasite has a scolex which fuses practically imperceptibly into strobila. Its frontal glands are quite prominent (Rees, 1941; Beveridge, 1990).

Monogeneans (Platyhelminthes)

It was not possible to identify the exact species, and it is therefore referred to “Monogenean unident.” throughout the rest of the thesis.

Part II. Metazoan gastrointestinal parasites of *Etmopterus spinax* (L., 1758) from southern Norwegian waters – Empirical Study

Host life history data

A total of 115 individuals of *E. spinax* were examined from 8 stations (see Table **3**) ranging from 12 cm to 45 cm total length, with a mean of 28.7 cm (±7.7 SD), shown in Table 3 and 4. The body weight of these sampled sharks ranged from 7 g to 454 g with a mean of 114.8 g (±81.2 SD). The station with the highest average length and average weight was station B (see Appendix Table **3**, Table **1**).

Table 3 - Number and gender *E. spinax* sampled per station.

Stations Code	Number of individuals	Females	Males
A	27	16	11
B	19	11	8
C	14	7	7
D	10	6	4
E	11	7	4
F	9	4	5
G	9	4	5
H	16	8	8

In total, the selected stations contained 63 females and 52 males. Station E presented the higher sex ratio among the sampled station (7 females:4 males). There was no significant difference between *E. spinax* gender composition and location (stations) based on Fisher's Exact Test ($p>0.05$) (see Appendix Table **4**). However, there was a significant difference between length and location and between weight

and location (Kruskal-Wallis test, $p <0.05$) (see Appendix table **5**). The station combinations which showed significant differences in length distributions were: station A vs. stations B and C, and station B vs. stations F and H (Dunn's test, $p<0.05$). The station combinations which showed significant differences in weight were: station A vs. station B and C, and station B vs. stations E, F and H (Dunn's test, $p<0.05$). The average length was higher in males ($\overline{x}$= 28.8 ±

7.4 SD), although females had higher average body weights with 120.3 g (± 94.0 SD) (see Table **4**). The average length and average weight of the sampled sharks was highest in the station with highest maximum depth (station B) (see Figure **7**).

Most sampled specimens were immature (n=77, out of 115) (see Appendix Table **7**). Of this approximately 60% (n=46) were females and 40% (n=31) males. There were no sampled immature males in the station D and no mature females in the station F. Mature females had the highest length and weight overall.

Table 4 - Life history data by gender and maturity status. Average length (in centimetres) ±SD, average weight (in grams) ±SD, maximum and minimum of length and weight of sampled shark per gender and sexual maturity and the total of sharks sampled on this study.

			Average Length (cm)	Average Weight (g)	Length (cm)		Weight (g)	
					Min	Max	Min	Max
	Female		28.7 ± 7.9	120.3 ± 94.0	12	45	7	454
	Male		28.8 ± 7.4	108.2 ± 62.8	13	40	9	232
Sample size								
46	**Female**	Immature	25.2 ± 5.8	75.7 ± 48.3	12	35	7	194
17		Mature	38.2 ± 3.8	241.1 ± 80.2	32	45	122	454
31	**Male**	Immature	24.6 ± 5.9	68.9 ± 36.4	13	34	9	136
21		Mature	34.7 ± 4.6	138.8 ± 87.4	25	40	62	232

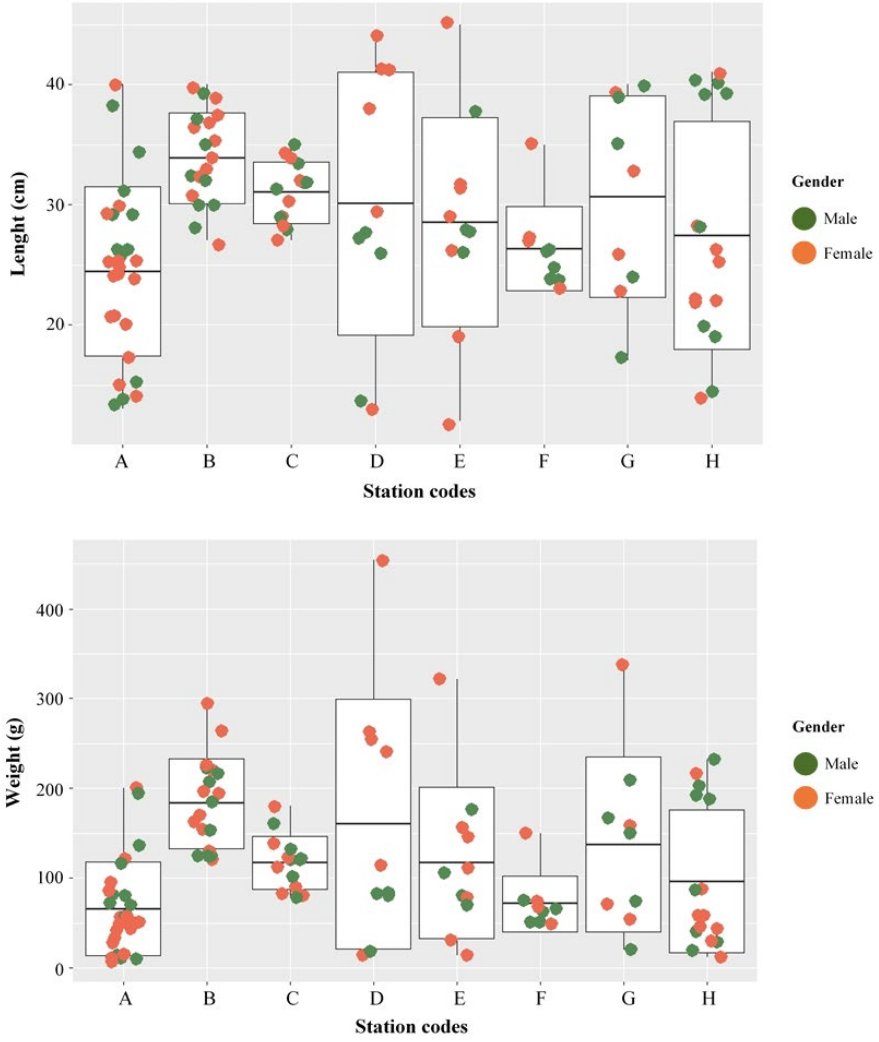

Figure 7 - Average length and average mean of males and females of *E. spinax*, captured in each station. Vertical bars show standard deviation from the mean. Circles show the values of length and weight of each shark sampled. Green circles correspond to male sharks and orange to female sharks.

The individuals of *E. spinax* which were analysed had relatively small livers: about 15.5% (±6.1 SD) of body weight for females and 16.3% (±5.5 SD) for males. Males presented a higher average HSI both in mature ($\overline{x}$= 18.7 ± 5.0) and immature ($\overline{x}$= 14.6 ± 5.4) sexual stages, compared to the females with 17.7 (± 5.5 SD) and 14.2 (± 6.1 SD) respectively. The average HSI is higher in both sexes in the mature stages (see Appendix Table **8**).

Parasite composition

Out of 115 *E. spinax* examined from eight sampling stations only 18% (n=21) were infected with at least one parasite. Four parasites species from four taxonomic groups were found in the following organs: skin, snout (nasal cavity), stomach wall and intestine. Station B had the highest parasite diversity, where all four parasite species were detected, and station D the lowest with no parasite found at all (see Table **5**, Appendix Table **9**).

Table 5 - Number of infected hosts per station infected by given parasite species. Shown are the site within the host, the parasite development stage, and the number of infected hosts by stations (A-H). Dashes indicate absence of parasite. A = adult, I = intestine, L3 = Larval stage 3, S = Skin, Sn = Snout, Sw= Stomach wall

Species	Site	Stage	Number of infected hosts							
			A	B	C	D	E	F	G	H
Monogenean										
Monogenea indet.	Sn	A	-	1	-	-	-	-	-	-
Cestoda										
Aporhynchus norvegicus	I	A	1	1	1	-	1	-	-	-
Nematoda										
Anisakis simplex	Sw	L3	2	2	-	-	1	2	3	2
Crustacea										
Anelasma squalicola	S	A	-	2	-	-	-	-	1	3

Four specimens (n=4) in the adult stage of the endoparasite *A. norvegicus* were found within four host intestines. Eight (n=8) *A. squalicola* specimens were found in six *E. spinax* individuals. Three of those were found embedded near to the spiracle region, three anterior to the first dorsal spine and two on the orbit area (see Figure **8**). Four (n=4) specimens in the adult stage of the endoparasite *A. norvegicus* were found within four host intestines. Sixteen (n=16) specimens of the nematode *A. simplex* were found on the wall of twelve analysed stomachs. All the specimens were on Third-Stage Larvae (L3). Some of the specimens were encapsulated within the stomach walls. Only one monogenean was found in the nasal cavity of one *E. spinax*. This monogenean was severely damaged which only made it possible to taxonomically identify

the class of the parasite. As it was possible to visualize its haptor attached posteriorly, it allowed the distinction from the other classes of Neodermata (e.g. Cestodes and Trematodes).

Figure 8 - *E. spinax* caught in one of the analysed stations that was parasitized by *A. squalicola* on the orbital area.

Host-parasite interaction

Of the twenty-one (n=21) *E. spinax* specimens infected with at least one parasite, twelve (n=12) were females and nine (n=9) males. Around 67% (n=14) of those infected *E. spinax* were sexually mature (see Table **6**), five of them females and nine males. There was no immature males among the infected specimens.

Table 6 – Infection Status and sexual maturity of sampled *E. spinax*.

Infected Status	**Female**		**Male**	
	Immature	**Mature**	**Immature**	**Mature**
Infected	7	5	0	9
Non-infected	39	12	31	12

The most common parasite found was *Anisakis simplex* (Nematoda, Ascaridida) in its larval form, which was present in twelve of the stomachs analysed and was also the most numerous overall of the parasites found (n=16). Only four hosts were infected with two specimens of *A. simplex*. 75% (n=3) of these infected hosts were mature. The second most prevalent parasite (n=8) was *Anelasma squalicola* (Maxillopoda, Pedunculata) which was found in six sharks. Monogeneans and *A. norveggicus* were instead the least common parasites. Females and males of *E. spinax* showed no significant differences in parasite prevalence of each found species (Fisher's exact test; for each parasite species $p > 0.05$) (see Appendix Table **10, 11)**.

Among the eight sampling stations, *A. simplex* was found to be more common at location G whereas *A. squalicola* in location H (see Figure **9**). The Monogenean was only recorded from station B, whereas *A. norvegicus* was generally present in most stations at low intensities. Due to the low parasite load on the sharks (only five hosts were infected with more than one parasite individual of a particular species), mean abundance followed the same pattern. The stations that had the highest prevalence of a specific species also had the highest mean abundance of that species (see Figure **9**; Appendix Table **10**).

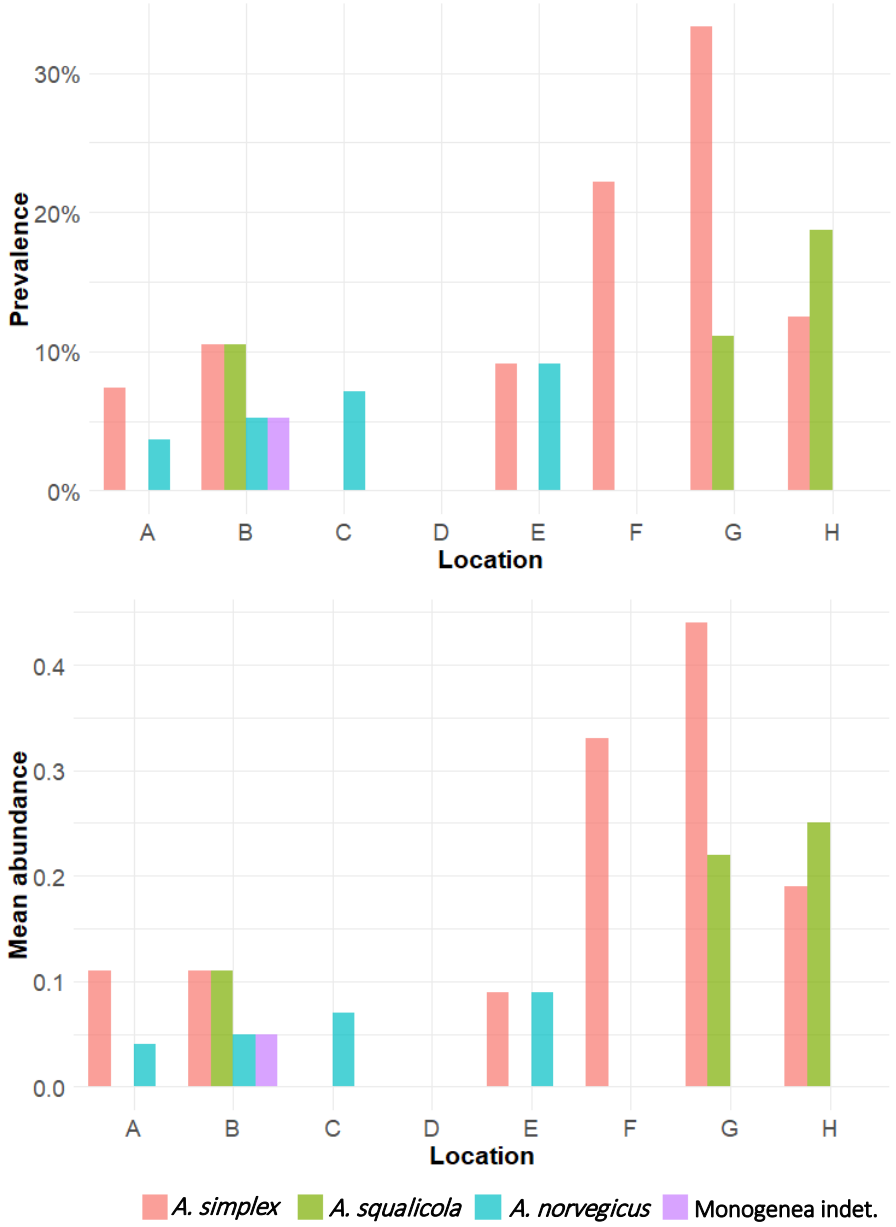

Figure 9 - Prevalence and mean abundance of the four found parasite species by location

Location had no significant effect on parasite infections (see Appendix table **12**). Parasite infection in fact, seems to be driven mainly by host size (length), i.e. larger sharks had more parasites than the smaller hosts. This was particularly evident for *A. simplex* and *A. squalicola* (Kruskal-Wallis, $p < 0.05$), shown in Figure **10**, where it was possible to visualize the pattern of parasite infections in relation to shark length (i.e. increasing host size did result in an increase of infections). Although no effect of length or location was detected on *Aporhynchus norvegicus* and on the Monogenean indet. (Kruskal-Wallis, $p > 0.05$).

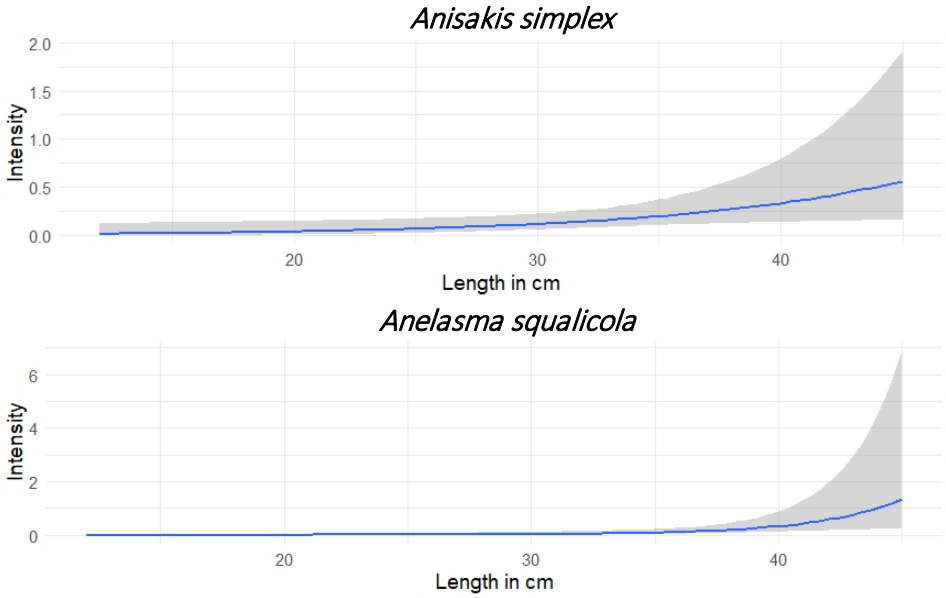

Figure 10 - Relationship between infection intensity and host length. Fitted GLM negative binomial regression for intensity of parasites infecting *E. spinax* with 95% confident interval.

Discussion

The literature review indicated 21 existing parasite species on 9 host species belonging to the *Etmopterus* genus, on 13 different sites of the host's body. The empirical data from eight stations in southern Norwegian waters revealed fairly low parasite prevalence and diversity on *E. spinax*. From a total of 115 studied shark specimen in this study only a total of four different parasite species (from four taxonomic groups) were recorded and only 18 host specimens were infected with at least one parasite species. The comparison of *E. spinax* individuals showed that larger sharks had a significantly higher prevalence of the parasite species *A. simplex* and *A. squalicola*, although the same could not be found for the other two parasite species.

Parasite diversity

During the present study only 4 parasite species were observed and the parasite community therefore differs from the only other previous study in Norwegian waters by Klimpel *et al.* (2003). Klimpel *et al.*'s (2003) study based on samples from May 2001 from the Norwegian Deep waters identified seven endo- and ectoparasite species in total (Klimpel *et al.*, 2003). Another study from two areas located in the NE Atlantic off Spain during the period July and August 2010, found seven and nine parasite species per area (Isbert et al., 2015). The parasitic data for different geographic locations is still too scarce for *E. spinax* for complex and comparative epidemiologic studies (Isbert *et al.*, 2015; Dallarés *et al.*, 2017). Differences between the Atlantic and the Mediterranean are however not unexpected as those have been observed in several studies which could show a widespread dichotomy in teleost parasite diversity and abundance between the two water bodies (Klimpel *et al.*, 2010; Dallarés *et al.*, 2017).

As it can be expected that the parasite composition might change based on the diet of the host organism as this is often the primary route of entry for parasites, an investigation into *E. spinax* diet preferences is important. Unfortunately, it was not able to investigate the stomach content of the here analysed individuals, which is described in more detail under the section methodological considerations below. The diet of *E. spinax* seems to vary with location due to a variation of abiotic factors and with its morphological development (Isbert et al., 2015). Isbert *et al.* (2015) showed that *E. spinax* exhibits opportunistic feeding habits, and that they capture the available benthopelagic prey of suitable size and exploite aggregations of organisms. *E. spinax* from the Skagerrak Deep-water, somewhat east of the current sampling locations, were

found to mostly feed on micronektonic crustaceans such as euphausids (krill) but may also have scavenged on fish carcasses (Bergstad et al. 2003). In the study by Klimpel et al. (2010) only two species were found in the analysed stomachs: krill *Meganyctiphanes norvegica* (in 91.9% of the stomachs) and hatchetfish *Maurolicus muelleri* (in 40.5% of the stomachs). The same study showed a transition of feeding composition from krill to fish with the increase of length of *E. spinax*. Interestingly, the individuals with stomachs only containing the krill, i.e. the small individuals, were not infected by *A. simplex*, which the authors explained by the hatchetfish functioning as a vector, even if it has been described that this parasite use euphausiids as intermediate host (Klimpel *et al*., 2003; Nagasawa, 1990). Klimpel *et al*. (2010) have shown a higher *A. norvegicus* prevalence (81.1%) which might be explained by the krill being a second intermediate host of this parasite species and by the juvenile *E. spinax* investigated in Klimpel *et al*. (2003) feeding on mainly krill as they have not transitioned to fish yet.

The highest parasite diversity, with all four parasite species detected, was found in the deepest location (station B) with 402 m. However, there is no indication that there are significant temperature differences (suggested in the literature to affect diversity) (ICES, 1983; Huthnance, 1991; Ottesen, 2009) between this station and the next deepest stations between 300 and 400 m which could explain such differences in diversity patterns. Interestingly, this deepest station was also the station with the highest average length and average weight. This station had a prevalence between 5 and 11 % for all four parasite species and was the only station where in fact all four species were detected. At this point, given the small infection intensities and methodological limitations (see below), it is not clear what could explain this pattern.

Parasite prevalence

Only 18 out of 115 host specimens were infected with at least one parasite species. This was lower than expected based on previous studies (e.g. Klimpel *et al*. 2003, Rees *et al*. 2014, Isbert *et al*. 2015). No correlation was found between catch depth of the host and its parasite prevalence; however, the deepest station contained host specimen with the highest parasite diversity (see section above). The station depths ranged from 173 m to 402 m, and only one of the eight stations was shallower than the sampled stations by Klimpel *et al*., (2003). It does not seem that depth is the most deterministic and defining abiotic variable for this species as *E.*

spinax can be found in a large depth range from 70 to 2000 m including reported frequent vertical migrations (Isbert *et al*., 2015).

When addressing the parasite species individually, the prevalence of *A. simplex* was lower in the majority of sampling stations compared to Klimpel *et al*. (2003), however, the highest prevalence was found in stations F (station with lowest depth) and G (station with third highest depth). Fish feeding ecology play a role for a specific parasite species distribution *(Klimpel et al*., 2010). This diet shift might be due to a relatively high availability of benthic infected invertebrates and crustaceans (Hemmingsen & Halvorsen, 1995; Hemmingsen & MacKenzie, 2001; Klimpel *et al*., 2010). Although, in most stations here *A. simplex* prevalence was lower than values found in Isbert *et al*. (2015) (location 1: 26.7 % and 2: 48.3 %), the results in Isbert *et al.* (2015) could be explained with high fishing and disposal rate of infected discharge (i.e. liver and stomach from evisceration on board of caught fish) and the opportunist scavenging behaviour of *E. spinax*. Here, station G has a prevalence of 33.3% which is 10% higher than the next highest prevalence station (F), and much higher than all the other stations with prevalence between 0 and 13%.

The second highest prevalence among the parasite species found in this study was *A. squalicola* which has a wide distribution among the major oceans, and on various hosts within the genus *Etmopterus* (Yano & Musick, 2000). Although its prevalence varies also among the world, and may therefore suggest that this parasite is dependent on the specific abiotic and host conditions (Rees et al., 2014; Rees et al., 2019). The mean abundance of each parasite per each station followed the pattern of the prevalence's results because the majority of infected host are infected with a single parasite of a specific species. This can be explained by ecological variations and the availability of potential intermediate hosts (Rohde, 1993; Marcogliese, 2004; Klimpel *et al*., 2006). Mean abundance followed the same pattern due to the fact that there were only very few *E. spinax* that were infected with more than one parasite of a specific species. From the eight stations, F was the station that had a depth most similar to the sampling location from Klimpel *et al*. (2003) and apparently also had the most similar prevalence *of A. simplex*.

Host-parasite relationship

The length-ranges observed in the collected *E. spinax* specimen ranged from 12-45 cm TL for females and 13-40 cm for males, which are similar to the values from other studies comprising immature and mature individuals (e.g. Isbert *et al*., 2015). According to Poulin (2000), as body size increases over time, consequently there will also be an increase in energetic demands, so larger individuals ingest a greater amount of food and have had more time to accumulate parasites than smaller hosts. It was therefore hypothesized that the hosts with larger length should be more parasitized, i.e. show a higher prevalence and mean abundance. The results from this study revealed such apparent positive correlation between length (and indirectly therefore age) and the parasitic prevalence. This correlation was however only significant for two parasite species, *A. simplex* and *A. squalicola*, the two most common among the detected species. This might be explained by the long-term life cycle which these two parasites have (Nagasawa, 1990, Rees *et al*., 2014; Ommundsen *el al*., 2016). A similar correlation between *A. simplex* and the length of the host was found in the Klimpel *et al.* (2003) study which was also on Norwegian *E. spinax* specimen. The apparent non-correlation for the other two detected parasites *A. norvegicus* and Monogenea indet. can most likely be explained by very low, or even null, prevalence of these specimen in some stations. This lack of correlation between *A. norvegicus* with the shark length contradicts the results from Klimpel *et al.* (2003) where all the shark individuals with length up to 17.1 cm were not infected, but specimens with lengths above 19.4 cm had a prevalence of infection of 100%. However, it needs to be pointed out that the study from Klimpel *et al*. (2003) only comprised juvenile individuals of *E. spinax* whereas this study contained also adult individuals of both sexes.

The average hepatosomatic index (HSI), that is the ratio between the liver weight and the total body weight, was highest in mature individuals (males and females), but in direct comparison the HSI was higher for males than for females (i.e. mature males *vs*. mature females, and immature males *vs*. immature females). The higher HSI in males was contrary to literature expectations from populations studied in the North East Atlantic (e.g. Aranha *et al*. 2009). It is not clear why and would need further investigations with larger sample sizes from a variety of maturity stages from both, the NE Atlantic and the North Sea. Generally, the HSI is higher in sharks compared to bony fishes, as expected given the generally very large livers. In dusky sharks for example, Hussey *et al*. (2009) reported values between 6 and 11, with differences in HSI values based on size class, sex and reproductive state. In other species similar values are

published, e.g. in salmon shark (*Lamna ditropis*) with 6.8-8.1 (Jayasinghe *et al*. 2003), and in species within the genus *Carcharinus* (5.7–10%) (Jayasinghe, 1999). Deep-sea sharks on the other hand, have been reported with much higher values, e.g. for *Centrophorus granulosus* (21.4–26.2%), *Dalatias licha* (23.9–26.5%) or *Centroscymmus coelolepis* (25.4–33.3%) (Batista and Nunes, 1992). The values from this study with an HSI of 18-19 are therefore situated somewhere in between those estimates but are more closely resembling estimates of other deep-sea sharks.

Hussey *et al*. (2009) also reported that HSI estimates are further complicated by season and that they reflect short-term energetic and reproductive states, combined with metabolic demands regulated by temperature and other environmental factors, making it necessary to consider a more complex range of indices which could also include reproductive measures and environmental parameters to fully understand HSI data trends. When investigating the effect of various levels of pollution and infection stress, Al-Ghais (2013) found that the HSI in *Tilapia* sp. was over 60% higher in individuals raised in sewage water as compared to the control fish, and Lenhardt *et al*. (2009) reported the highest HSI value in the month with greatest parasite infection numbers for the sterlet (*Acipenser ruthenus* L.) by a factor of 6. These studies all show the importance of sampling different life history stages, seasons and additional areas with different exposure properties where possible.

Seasonality

The opportunity of a holistic comparison between the present study and the one from Klimpel *et al*. (2003), both conducted within a relatively close area but on different seasons, allows for an observation of temporal variation in the intestinal parasite communities of *E. spinax*. Potential explanations can however only be speculated on with the amount of available data.

For parasites which present a short-term life cycle, such as monogeneans, in some cases it is possible to observe a variation with season which follows an annual fixed pattern (parasite seasonality) (Willy Hemmingsen *et al*., 1995). In Klimpel *et al*. (2003), Monogenean indet. presented a prevalence of 84% from spring/summer (May 2001) which is in strong contrast to the null prevalence for the majority of the stations sampled in winter (January 2016) in this study, with the exception of one station which had a 5% prevalence. Although seasonality apparently cannot be applied to *A. simplex,* due to its several year encompassing life cycle in the intermediate fish host, and can therefore not explain the lower values in some stations

(Smith, 1983; Hemmingsen et al., 1995). The seasonality is depended on surrounding abiotic factors, and water temperature might be one of the most important environmental factors influencing the seasonal abundance of aquatic marine parasites (Willy Hemmingsen *et al*., 1995; Hemmingsen & MacKenzie, 2001; Klimpel *et al*., 2010). This abiotic variable can affect either the parasite transmission directly by effecting the free-living transmission stages (larvae) or indirectly by its effects on paratenic hosts (Hemmingsen *et al*., 1995). Temperature may cause changes on host feeding behaviour (because of variations in food availability) and on immune response of the host (i.e. producing seasonal variations in resistance to infection (Hemmingsen et al., 1995; Marcogliese, 2004).

Potential use of the parasites as tags/bio-indicators

From the several studies on marine parasite tags, very few have focused on cartilaginous fishes as hosts (Irigoitia *et al.*, 2017). This could probably be associated with the fact that many sharks are considered apex predators (i.e. predators which are on the top of food chain), which decreases the probability that they are hosts for long-lived larval parasites (Timi & MacKenzie, 2015; Irigoitia *et al*., 2017). They are thus not the most obvious candidates for using parasites as natural markers for stock assessment (Irigoitia *et al.*, 2017). Following the arguments of MacKenzie & Abaunza (1998) and Catalano *et al*. (2014), none of the parasites species found in this study fulfils all suggested guidelines. Although some can be useful as potential biological tags based on their life cycle and geographic distribution, they present strong and weak points (depending on the purpose of use). However, the ability of the parasites to measure impacts in these *E. spinax* populations will dependent on: 1) improving knowledge on the life history patterns of local populations of *E. spinax*, 2) obtaining information on the parasites affecting individuals at various stages in their growth, and 3) establishing trend information on prevalence of infection for each parasite species individually.

A pilot study to investigate potential seasonality of the parasite species would be a pre-requisite before consideration as tags. If they exhibit seasonality, they might be not the best candidates because it would increase the complexity of interactions between the parasite and the ecosystem. The availability of intermediate hosts impacts the parasite species composition in host population and varies between geographically among different habitats (Rohde, 1993; Marcogliese, 2004).

The presence of *A. simplex* in the shark intestines gives an important diet information. Sharks infected by *A. simplex* reveal what they have been feeding on some species (e.g. crustacean) (Smith, 1984; Lamps & Lamps, 2009). Observing Table 1, *A. simplex* presents a large geographic distribution for two *Etmopterus* species inhabiting the northern hemisphere, including *E. spinax*. The combinations of studying feeding ecology, parasite taxonomy and parasite genetics (e.g. frequencies of different acid phosphatase allozymes in the L3 *A simplex* it might be easier) might make it easier to distinguish between host populations (Catalano *et al.*, 2014). Although *A. simplex* accumulates during the host life due to their long-term life cycle (Nagasawa, 1990). Also, sharks are not often the final host of this parasite (Smith, 1983; Lamps & Lamps, 2009; Isbert *et al.*, 2015).

The unidentified species of Monogeneans might be a potential parasite tag species, because monogeneans only have single definitive host (direct life cycle) and are often considered highly host-specific (Whittington et al., 2000; Cribb *et al*., 2002). Biotic factors such as host schooling behaviours (which *E. spinax* can present) and density may affect variations in parasites of this class between locations (Grutter, 1998; Sikkel *et al.*, 2009; Isbert *et al*., 2015). However, the exact species could not be identified, because the specimen was damaged, and only one monogenean was collected from a single host. Species-level identification is however necessary to provide replicable tagging results and this specimen is therefore not suited for the application as a parasite tag.

A. norvegicus presents a typical trypanorhynch life cycle, with elasmobranchs as final hosts (Beveridge, 1990; Klimpel *et al*., 2003). Some of the species of invertebrates and teleosts which are a part of the diet of *E. spinax* from southern Norwegian waters (e.g. *Meganyctiphanes norbegica*) are second intermediate hosts (Klimpel *et al*., 2003). The presence or absence of *A. norvegicus* which is typically found in that area can be important to explain ontogenetic shifts in the host diet and its trophic interactions (Hemmingsen, 1995; Münster *et al*., 2015; Dallarés *et al*., 2017). However, like the undetermined species of Monogenean, the number of *A. norvegicus* specimens found in this study was extremely low, which makes tag applicability evaluations very difficult.

It is suggested that *A. squalicola* is transmitted by close contacts between host and infected host. This might give an indication about the location of breeding grounds (i.e. *A. squalicola* found in mature hosts) and feeding grounds (i.e. *A. squalicola* found in immature hosts) (Magnhagen, 2008). In addition, this parasite is found to have a high geographic distribution,

together with its host and could therefore be very useful as parasite genetic tag. Also, the parasite species could potentially be used as a bio-indicator for pollution (toxicologic studies should be developed for potential calibration of the parasite as a bio-indicator). As for *A. simplex*, this crustacean parasite can accumulate during the host life (Rees *et al.*, 2014; Ommundsen *et al*., 2016).

Methodological considerations and recommendations

The lower prevalence and also diversity could have been facilitated through a few methodological shortcomings with respect to the sampling and the storage of the host specimen. The sharks were collected through an annual research survey as part of an ongoing research effort studying their life history and population structure and have not been intended to be used for parasite study purposes. Hence, all individuals from a station were bulk frozen together by cryopreservation (slow freezing). The ice crystals are hereby relatively bigger than the ones formed with nitrogen preservation. These ice crystals mechanically destroy the cells of the host, as well as the parasite structure. For example, nematodes do not have a cuticula as cestodes, which decreases the probability that the morphological structure of nematodes survives intact during the cryopreservation process. Also, the cryopreservation increased the difficulty to identify the preys on the gastrointestinal content of the sharks. For processing, initially all the sharks of the same station which were frozen in bulk needed be defrosted together and then afterwards individuals needed to be frozen again in smaller batches for individual processing. So, the mechanical damage associated with the ice crystal was induced two times.

In addition, many samples from the range of the survey had already been processed at the point of the project start, leaving a smaller selection of sampling depths to choose form. For consecutive studies additional depths down to 600 m should be chosen for parasite investigation, as well as other areas of the host species' distribution with deeper depth profiles, taking advantage of the topography of the area and depth distribution of the *E.spinax* (Huthnance, 1991; Coelho & Erzini, 2010; Coelho et al,. 2010). It would be a great value for future studies to sample the same areas in two distinct seasons of the year (e.g. one between December-January and another July-August), but this is more difficult to implement given that the sampling in those areas is part of an annual survey to monitor shrimps at a specific time of the year. During the sampling a maximum of abiotic parameters should be registered per station

(e.g. depth, salinity, temperature, pressure, light). Considering a balanced sampling design, per station a minimum of 12 specimens should be collected, with equal composition of males and females and immature and mature individuals. When finer-scale maturity stages should be considered, the same sizes need to be increased per group accordingly. When the sharks are sampled freshly, the total weight needs to be recorded and the gastrointestinal tract should be removed from the body cavity and frozen in separate portions (pharynx-pyloric sphincter; pyloric sphincter-cloaca) in two distinct bags per shark. The remaining body should either be processed directly or frozen for later maturity determination and measurements. Additional stomach content analysis would add value by allowing for direct comparisons with the host's diet. Combining the examination of the gastrointestinal content, which provides a short-term view of the most recent trophic utilization and analysing the structure of shark parasite fauna, which gives an overview of a long-term feeding niche, the study would gain more detailed information on the role of *E. spinax* in the food web in the different locations. Ultimately, this could provide information for the assessment of geographical variation of parasites and their hosts and their combined potential impact on the ecosystem they inhabit (Williams et al., 1992; Marcogliese, 2004; Catalano *et al*., 2014; Isbert *et al*., 2015).

Conclusion

Parasites are fundamental pieces in the biological knowledge puzzle of marine ecosystems. Parasites can contribute to fill numerous knowledge gaps through for example their application as populations tags in order to inform management plans about population structure and potential host migrations (Techera & Klein, 2011; Shiffman & Hammerschlag, 2016). In that way, using parasites as biological tags presents a great potential for future use in ecology studies but also as bio-indicator to advise on the implementation of management strategies for protection or restoration of complex marine ecosystems which are constantly being threatened by anthropogenic activities. Therefore, the comprehensive study of parasites together with their hosts cannot be emphasised enough. I hope that this thesis will contribute to those and hopefully future efforts to design effective management plans for our natural resources.

References

Al-Ghais SM. Acetylcholinesterase, glutathione and hepatosomatic index as potential biomarkers of sewage pollution and depuration in fish. *Marine pollution bulletin*. 2013 Sep 15;74(1):183-6.

Alioshkina, l.D., Gaevskaya, A.V., & Kovaliava, A.A., 1985. Parasitofauna of fishes of the Whale Ridge. NOAA Technical Reports. 25, 29-29.

Alves, P. V., de Chambrier, A., Scholz, T., & Luque, J. L. (2017). Annotated checklist of fish cestodes from South America. *ZooKeys*, *650*(650Special Issue), 1–205. https://doi.org/10.3897/zookeys.650.10982

Aranha, A., Menezes, G., & Pinho, M. R. (2009). Biological aspects of the velvet belly lantern shark, *Etmopterus spinax* (Linnaeus, 1758) off the Azores, North East Atlantic. *Marine Biology Research*, *5*(3), 257–267. https://doi.org/10.1080/17451000802433175

Batista I, Nunes ML. Characterisation of shark liver oils. Fisheries research. 1992 Sep 1;14(4):329-34.

Benz, G.W. (1991). Description of some larval stages and augmented description of adult stages of *Albionella etmopteri* (Copepoda: Lernaeopodidae), a parasite of deep-water laternsharks (Etmopterus: Squalidae). *Journal of Parasitology*, *77*, 666–674.

Benz, George W., & Bullard, S. A. (2004). Metazoan Parasites and Associates of Chondrichthyans with Emphasis on Taxa Harmful to Captive Hosts. In M. Smith, D. Warmolts, D. Thoney, & R. Hueter (Eds.), *The elasmobranch Husbndry manual: Captive care of Sharks, Ray and relatives* (2nd ed., pp. 325–416). Ohio, USA: Ohio Biological Survey, Inc.

Bergstad OA, Wik AD, Hildre O. Predator-prey relationships and food sources of the Skagerrak deep-water fish assemblage. Journal of Northwest Atlantic Fishery Science. 2003; 31:165.

Beveridge, I. (1990). Revision of the Family Gilquiniidae Dollfus (Cestoda, Trypanorhyncha) From Elasmobranch Fishes. *Australian Journal of Zoology*, *37*(5), 481. https://doi.org/10.1071/ZO9890481

Blaylock, R. B., Margolis, L., & Holmes, J. C. (2003). The use of parasites in discriminating stocks of Pacific halibut (*Hippoglossus stenolepis*) in the northeast Pacific. *Fishery Bulletin*, *101*(1), 1.

Boeger, W. A., Vianna, R. T., & Thatcher, V. E. (2006). Monogenoidea. *Amazon fish parasites. Sofia: Pensoft Publishers*, 42-116.

Boxshall, G. (2005). Copepoda (copepods). In J. Rodhe (Ed.), *Marine parasitology* (1st ed., pp. 123–138). Oxford, UK: CABI Publishing.

Boxshall, G., & Lützen, J. (2005). Crustacean parasites. In K. Rohde (Ed.), *Marine parasitology* (1st ed., pp. 123–165). Oxon: CABI Publishing.

Brander, K. (2013). Climate and current anthropogenic impacts on fisheries. *Climatic Change*, *119*(1), 9–21. https://doi.org/10.1007/s10584-012-0541-2

Brinkmann, A. (1952). Fish Trematodes from Norwegian Waters, I: The History of Fish Trematode Investigations in Norway and the Norwegian Species of the Order Monogenea. John Grieg.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. *The Journal of Parasitology*, *83*(4), 575. https://doi.org/10.2307/3284227

Caira, J., & Healy, C. (2004). Elasmobranchs as Hosts of Metazoan Parasites (pp. 523–551). https://doi.org/10.1201/9780203491317.ch18

Caira, J. N., Healy, C. J., & Jensen, K. (2012). An Updated Look at Elasmobranchs as Hosts of Metazoan Parasites. In J. C. Carrier, J. A. Musick, & M. R. Heithaus (Eds.), *Biology of Sharks and Their Relatives* (pp. 547–572).

Caira, J. N., & Pickering, M. (2013). Cestodes from deep-water squaliform sharks in the Azores. *Deep Sea Research Part II: Topical Studies in Oceanography*, *98*(PA), 170–177. https://doi.org/10.1016/j.dsr2.2013.08.008

Caira, J., & Reyda, F. (2005). Eucestoda (true tapeworms). In J. Rodhe (Ed.), *Marine parasitology* (1st ed., pp. 92–103). Oxford, UK: CABI Publishing.

Callaway, R. (2002). Diversity and community structure of epibenthic invertebrates and fish in the North Sea. *ICES Journal of Marine Science*, *59*(6), 1199–1214. https://doi.org/10.1006/jmsc.2002.1288

Carvajal, J. (1974). Records of Cestodes from Chilean Sharks. *The Journal of Parasitology*, *60*(1), 29–34.

Carvalho-Varela, M. (2005). *Parasitas e Parasitoses em Piscicultura*. Lisboa, Portugal: Ordem dos Médicos Veterinários, pp. 487-497

Catalano, S. R., Whittington, I. D., Donnellan, S. C., & Gillanders, B. M. (2014). Parasites as biological tags to assess host population structure: Guidelines, recent genetic advances and comments on a holistic approach. *International Journal for Parasitology: Parasites and Wildlife*, *3*(2), 220–226. https://doi.org/10.1016/j.ijppaw.2013.11.001

Causey, D. (1957). Another barnacle. *The Educational Focus*, *28*(2), 18–20.

Coelho, R., & Erzini, K. (2008). Life history of a wide-ranging deepwater lantern shark in the north-east Atlantic, *Etmopterus spinax* (Chondrichthyes: Etmopteridae), with implications for conservation. *Journal of Fish Biology*, *73*(6), 1419–1443. https://doi.org/10.1111/j.1095-8649.2008.02021.x

Coelho, R., & Erzini, K. (2010). Depth distribution of the velvet belly, *Etmopterus spinax* , in relation to growth and reproductive cycle: The case study of a deep-water lantern shark with a wide-ranging critical habitat. *Marine Biology Research*, *6*(4), 381–389. https://doi.org/10.1080/17451000802644706

Coelho, R., Rey, J., Gil de Sola, L., Fernandez de Carvalho, J., & Erzini, K. (2010). Comparing Atlantic and Mediterranean populations of the velvet belly lanternshark, *Etmopterus spinax* , with comments on the efficiency of density-dependent compensatory mechanisms. *Marine Biology Research*, *6*(4), 373–380. https://doi.org/10.1080/17451000903300885

Compagno, L. J. V. (1984). *Sharks of the world - An annotated and illustrated catalogue of shark species known to date Vol.4 Part I Hexanchiformes to Lamniformes*. (FAO, Ed.), *FAO Species Catalogue* (Vol. 4). rk species known to date. Part I. Hexanchiformes to Lamniformes. FAO Species Catalogue 125.: FAO. https://doi.org/10.1016/0025-

326X(92)90600-B

Compagno L. J. V., Dando M.,& Fowler S. (2005). Sharks of the World. London: Collins. 368 pages.

Coomans, A. (2000). Nematode systematics: past, present and future. *Nematology*, *2*(1), 3–7. https://doi.org/10.1163/156854100508845

Cribb, T. (2005). Digenea (endoparasitic flukes). In K. Rohde (Ed.), *Marine parasitology* (1st ed., pp. 47–116). Oxford, UK: CABI Publishing. https://doi.org/10.5860/CHOICE.43-5289

Cribb, T., Bray, R., Olson, P., & Littlewood, T. (2003). Life Cycle Evolution in the Digenea: a New Perspective from Phylogeny. *Advances in Parasitology Volume 54*, 197–254. https://doi.org/10.1016/S0065-308X(03)54004-0

Cribb, T., Chisholma, L., & Bray, R. (2002). Diversity in the Monogenea and Digenea: does lifestyle matter? *International Journal for Parasitology*, *32*(3), 321–328. https://doi.org/10.1016/S0020-7519(01)00333-2

Dallarés, S., Padrós, F., Cartes, J. E., Solé, M., & Carrassón, M. (2017). The parasite community of the sharks *Galeus melastomus*, *Etmopterus spinax* and *Centroscymnus coelolepis* from the NW Mediterranean deep-sea in relation to feeding ecology and health condition of the host and environmental gradients and variables. *Deep Sea Research Part I: Oceanographic Research Papers*, *129*(June), 41–58. https://doi.org/10.1016/j.dsr.2017.09.007

Darwin, C. R. (1851). Living Cirripedia, A monograph on the sub-class Cirripedia, with figures of all the species. *The Lepadidæ or pedunculated cirripedes*, *1*.Davies, R. W., & Govedich, F. R. (2001). Annelida: Euhirudinea and Acanthobdellidae. *Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, California*, 465-504.

Díaz, S., Settele, J., & Brondízio, E. (2019). Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. In *IPBES - The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*.

Bonn, Germany: IPBES secretariat. https://doi.org/10.5281/zenodo.2671522

Dobson, A. P. (1988). The Population Biology of Parasite-Induced Changes in Host Behavior. *Quarterly Review of Biology, 63*(2), 139-165

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *ELife*, *3*, 1–34. https://doi.org/10.7554/eLife.00590

Eiras, J., & Castro, R. (2016). Crustacea. In Jorge Eiras, A. Velloso, & J. Pereira (Eds.), *Parasitos de peixes marinhos da América do Sul* (1st ed., pp. 287–359). Rio Grande, Brazil: Editora da furg - Universidade Federal do Rio Grande-Furg.

Eliassen, L. (2016). An investigation into the parasitic barnacle, *Anelasma squalicola*; prevalence, infection behaviour and effects on its host, *Etmopterus spinax*, in Lusterfjord, Norway.

Espínola-Novelo, J. F., Escribano, R., & Oliva, M. E. (2018). Metazoan parasite communities of two deep-sea elasmobranchs: the southern lanternshark, *Etmopterus granulosus*, and the largenose catshark, *Apristurus nasutus* , in the Southeastern Pacific Ocean. *Parasite*, *25*, 53. https://doi.org/10.1051/parasite/2018054

FAO. (2011). Fisheries Management - *4. Marine protected areas and fisheries*. (FAO, Ed.), FAO *Technical Guidelines for Responsible Fisheries.* (Vol. 4). Rome.

Fernandez-Ovies, C. L. (1993). Crustáceos parásitos sobre tiburones bentopelágicos del talud continental asturiano. 2. Anelasma squalicola (Loven) (Cirripedia: Thoracica: Anelasmatidae). *Boletín de Ciencias de la Naturaleza*, 43: 7–14

Ferretti, F., Worm, B., Britten, G. L., Heithaus, M. R., & Lotze, H. K. (2010). Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters*, *13*(8), https://doi.org/10.1111/j.1461-0248.2010.01489.x

Gallagher, R., Marx, J., & Hines, P. (1994). Progress in parasitology. *Science*, *264*(5167), 1827–1827. https://doi.org/10.1126/science.8009199

Gibson, D. I., & Bray, R. A. (1977). The Azygiidae, Hirudinellidae, Ptychogonimidae,

Sclerodistomidae and Syncoeliidae of fishes from the north-east Atlantic. *Bulletin of the British Museum (Natural History) (Zoology)*, (32), 167–245.

Gomes, T. A. L. (2014). Anisakis spp. : relevância da sua pesquisa e identificação em peixes. Dissertação de Mestrado. Universidade de Lisboa, Faculdade de Medicina Veterinária, Lisboa

Govedich, F. R. (2001). A reference guide to the ecology and taxonomy of freshwater and terrestrial leeches (Euhirudinea) of Australasia and Oceania. In *Taxonomy Workshop* (pp. 1-67). Cooperative Research Centre for Freshwater Ecology. Govedich, F. R., Bain, B. A., & Davies, R. W. (2005). Hirudinea (leeches). In J. Rodhe (Ed.), *Marine parasitology* (pp. 196–201). Oxford, UK: CABI Publishing.

Grutter, A. (1998). Habitat-related differences in the abundance of parasites from a coral reef fish: an indication of the movement patterns of *Hemigymnus melapterus*. *Journal of Fish Biology*, *53*(1), 49–57. https://doi.org/10.1006/jfbi.1998.0682

Gunn, A., & Pitt, S. (2012). *Parasitology: An Integrated Approach* (1st ed.). Chichester, UK: John Wiley & Sons, Ltd.

Hatcher, M. J., Dick, J. A., & Dunn, A. M. (2012) Diverse effects of parasites in ecosystems: Linking interdependent processes. *Frontiers in Ecology and the Environment*, 10, 186–194.

Hayward, C. (2005). Monogenea Polyopisthocotylea (ectoparasitic flukes). In K. Rohde (Ed.), *Marine parasitology* (1st ed.). Oxford, UK: CABI Publishing.

Heip, C., Basford, D., Craeymeersch, J. A., Dewarumez, J. M., Dorjes, J., De Wilde, P., & Soltwede, T. (1992). Trends in biomass, density and diversity of north sea macrofauna. *ICES Journal of Marine Science*, *49*(1), 13–22. https://doi.org/10.1093/icesjms/49.1.13

Heithaus, M. R., Frid, A., Wirsing, A. J., & Worm, B. (2008). Predicting ecological consequences of marine top predator declines. *Trends in Ecology & Evolution*, *23*(4), 202–210. https://doi.org/10.1016/j.tree.2008.01.003

Hemmingsen, W., & MacKenzie, K. (2001). The parasite fauna of the atlantic cod, *Gadus morhua* L. *Advances in Marine Biology*, *40*, 1–80. https://doi.org/10.1016/S0065-

2881(01)40002-2

Hemmingsen, W., Lile, N., & Halvorsen, O. (1995). Search for seasonality in occurrence of parasites of cod , *Gadus morhua* L . in a fjord at 70 ° N. *Polar Biology*, *15*(7), 517–522. https://doi.org/10.1007/BF00237466

Hermida, M., Cruz, C., & Saraiva, A. (2013). Parasites as biological tags for stock identification of blackspot seabream, *Pagellus bogaraveo*, in Portuguese northeast Atlantic waters. *Scientia Marina*, *77*(4), 607–615. https://doi.org/10.3989/scimar.03859.17A

Heupel, M. R., Knip, D. M., Simpfendorfer, C. A., & Dulvy, N. K. (2014). Sizing up the ecological role of sharks as predators. *Marine Ecology Progress Series*, *495*, 291–298. https://doi.org/10.3354/meps10597

Hickling, C. F. (1963). On the small deep-sea shark *Etmopterus spinax* L., and its cirripede parasite *Anelasma squalicola* (Lovén). *Journal of the Linnean Society of London, Zoology*, *45*(303), 17–24. https://doi.org/10.1111/j.1096-3642.1963.tb00484.x

Hogans, W. E., & Brattey, J. (1986). Ommatokoita sp. (Copepoda: Lernaeopodidae) parasitic on a demersal shark, *Etmopterus princeps* , from the northwest Atlantic Ocean. *Canadian Journal of Zoology*, *64*(4), 833–835. https://doi.org/10.1139/z86-124

Hoggarth, D.D.; Abeyasekera, S.; Arthur, R.I.; Beddington, J.R.; Burn, R.W.; Halls, A.S.; Kirkwood, G.P.; McAllister, M.; Medley, P.; Mees, C.C.; Parkes, G.B.; Pilling, G.M.; Wakeford, R.C.; Welcomme, R. L. (2006). *Stock assessment for fishery management – A framework guide to the stock assessment tools of the Fisheries Management Science Programme (FMSP).* (FAO, Ed.), *FAO Fisheries Technical Paper - T487* (1st ed., Vol. 487). Rome: FAO.

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution*, *21*(7), 381–385. https://doi.org/10.1016/j.tree.2006.04.007

Huthnance, J. (1991). Physical oceanography of the North Sea. *Ocean and Shoreline Management*, *16*(3–4), 199–231. https://doi.org/10.1016/0951-8312(91)90005-M

ICES, (1983). Flushing times of the North Sea. Cooperative Research Report No. 123, 159 pp.ICES, (2018). Greater North Sea Ecoregion – Ecosystem overview. In ICES (Ed.), *Greater North Sea Ecoregion;* (p. 23). Greater North Sea Ecoregion. https://doi.org/doi.org/10.17895/ices.pub.4670

Irigoitia, M. M., Incorvaia, I. S., & Timi, J. T. (2017). Evaluating the usefulness of natural tags for host population structure in chondrichthyans: Parasite assemblages of *Sympterygia bonapartii* (Rajiformes: Arhynchobatidae) in the Southwestern Atlantic. *Fisheries Research*, *195*(April), 80–90. https://doi.org/10.1016/j.fishres.2017.07.006

Isbert, W., Rodríguez-Cabello, C., Frutos, I., Preciado, I., Montero, F. E., & Pérez-del-Olmo, A. (2015). Metazoan parasite communities and diet of the velvet belly lantern shark *Etmopterus spinax* (Squaliformes: Etmopteridae): a comparison of two deep-sea ecosystems. *Journal of Fish Biology*, *86*(2), 687–706. https://doi.org/10.1111/jfb.12591

Ishii, Y., Fujino, T., & Weerasooriya, M. V. (1989). Morphology of Anisakine Larvae. In *Gastric Anisakiasis in Japan* (pp. 19–29). Springer Japan. https://doi.org/10.1007/978-4-431-68290-5_4

Janovy, J., Nadler, S., & Roberts, L. (2013). *Gerald D. Schmidt and Larry S. Roberts' foundations of parasitology* (8th ed.). New York, USA: McGraw Hill.

Jayasinghe C, Gotoh N, Wada S. Variation in lipid classes and fatty acid composition of salmon shark (Lamna ditropis) liver with season and gender. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2003 Feb 1;134(2):287-95.

Jayasinghe, C.V.L., 1999. Composition and stabilization of shark liver oil extracts of selected shark species, M.Phil. Thesis, University of Sri Jayawardanapura, Sri Lanka

Jobling, M. (2015). Laboratory Manual (Cartilaginous Fishes). Bio-2506 Introduction to Fish Biology, UiT. Internal teaching document

Johnstone, J., & Frost, W. E. (1927). *Anelasma squalicola (Lovén): its general morphology*. Liverpool Biological Society.Kearn, G. C., Whittington, I. D., & Thomas, P. (2012). A new species of Asthenocotyle Robinson, 1961 (Monogenea: Microbothriidae), a skin parasite of the great lanternshark Etmopterus princeps Collett from the Azores, with a redescription of A. kaikourensis Robinson, 1961 and observations on A. taranakiensis.

Systematic Parasitology, *83*(2), 145–158. https://doi.org/10.1007/s11230-012-9378-3

Keneedy, C. R. (1965). Taxonomic studies on Archigetes Leuckart, 1878 (Cestoda: Caryophyllaeidae). *Parasitology*, 55(3), 439-451.

Klimpel, S., Busch, M. W., Sutton, T., & Palm, H. W. (2010). Meso- and bathy-pelagic fish parasites at the Mid-Atlantic Ridge (MAR): Low host specificity and restricted parasite diversity. *Deep Sea Research Part I: Oceanographic Research Papers*, *57*(4), 596–603. https://doi.org/10.1016/j.dsr.2010.01.002

Klimpel, S., Palm, H. W., & Seehagen, A. (2003). Metazoan parasites and food composition of juvenile *Etmopterus spinax* (L., 1758) (Dalatiidae, Squaliformes) from the Norwegian Deep. *Parasitology Research*, *89*(4), 245–251. https://doi.org/10.1007/s00436-002-0741-1

Klimpel, S., Wilhelm, H., Wilhelm, M., Kellermanns, E., & Ru, S. (2006). Fish parasites in the Arctic deep-sea : Poor diversity in pelagic fish species vs . heavy parasite load in a demersal fish, *53*, 1167–1181. https://doi.org/10.1016/j.dsr.2006.05.009

Myrlund, M. K. (2018). *Reproduction scales for viviparous and oviparous cartilaginous fishes* (No. 1) (Vol. 1). Tromsø, Norway.

Lamps, L. W., & Lamps, L. W. (2009). Anisakis Simplex and Related Nematodes. In *Surgical Pathology of the Gastrointestinal System: Bacterial, Fungal, Viral, and Parasitic Infections* (pp. 211–213). Springer US. https://doi.org/10.1007/978-1-4419-0861-2_34

Lenhardt M, Jaric I, Cakic P, Cvijanovic G, Gacic Z, Kolarevic J. Seasonal changes in condition, hepatosomatic index and parasitism in sterlet (*Acipenser ruthenus* L.). Turkish Journal of Veterinary and Animal Sciences.

2009 Aug 11;33(3):209-14.

Lent, R., & Squires, D. (2017). Reducing marine mammal bycatch in global fisheries: An economics approach. *Deep Sea Research Part II: Topical Studies in Oceanography*, *140*, 268–277. https://doi.org/10.1016/j.dsr2.2017.03.005

Lester, R. J. G. (2005). Isopoda (isopods). In K. Rohde (Ed.), *Marine Parasitology* (2nd ed.).

Oxford, UK: CSIRO PUBLISHING.

Loker, E. S., & Hofkin, B. V. (2015). *Parasitology: A Conceptual Approach*. (G. Science, Ed.) (1st ed., Vol. 53). Retrieved from http://parasitol.kr/journal/view.php?doi=10.3347/kjp.2015.53.4.507

MacKenzie, K., & Abaunza, P. (1998). Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. *Fisheries Research*, *38*(1), 45–56. https://doi.org/10.1016/S0165-7836(98)00116-7

MacKenzie, K. (1998). Parasites as biological tags in population studies of marine fish. *Parasitology International*, *47*, 43. https://doi.org/10.1016/s1383-5769(98)80066-4

MacKenzie, K. (1999). Parasites as pollution indicators in marine ecosystems: A proposed early warning system. *Marine Pollution Bulletin*, *38*(11), 955–959. https://doi.org/10.1016/S0025-326X(99)00100-9

Marcogliese, D. J. (2004). Parasites: Small Players with Crucial Roles in the Ecological Theater. *EcoHealth*, *1*(2), 151–164. https://doi.org/10.1007/s10393-004-0028-3

Marcogliese, D. J. (2005). Transmission of marine parasites. In K. Rohde (Ed.), *Marine Parasitology* (3rd ed., pp. 280–285). Oxford, UK: CABI Publishing Wallingford.

Martínez, M. L., Intralawan, A., Vázquez, G., Pérez-Maqueo, O., Sutton, P., & Landgrave, R. (2007). The coasts of our world: Ecological, economic and social importance. *Ecological Economics*, *63*(2–3), 254–272. https://doi.org/10.1016/j.ecolecon.2006.10.022

Maunder, M. N. (2008). Maximum Sustainable Yield. In *Encyclopedia of Ecology* (pp. 2292–2296). Elsevier. https://doi.org/10.1016/B978-008045405-4.00522-X

McCallum, H., & Dobson, A. (1995). Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology & Evolution*, *10*(5), 190–194. https://doi.org/10.1016/S0169-5347(00)89050-3

McClelland, G. (2005). Nematoda (roundworms). In J. Rodhe (Ed.), *Marine parasitology* (1st ed., pp. 104–115). Oxford, UK.

McMillan, M. N., Izzo, C., Junge, C., Albert, O. T., Jung, A., & Gillanders, B. M. (2017).

Analysis of vertebral chemistry to assess stock structure in a deep-sea shark, *Etmopterus spinax*. *ICES Journal of Marine Science*, *74*(3), 793–803. https://doi.org/10.1093/icesjms/fsw176

Meur, N. Le. (2012). Analyzing Biological Data Using R: Methods for Graphs and Networks. *Methods in Molecular Biology (Clifton, N.J.)*, *804*, 343–373. https://doi.org/10.1007/978-1-61779-361-5_19

Monteiro, P., Araújo, A., Erzini, K., & Castro, M. (2001). Discards of the algarve (southern Portugal) crustacean trawl fishery. *Hydrobiologia*, *449*, 267–277. https://doi.org/10.1023/A:1017575429808

Moravec, F. (1998). *Nematodes of freshwater fishes of the Neotropical Region*. Academia, Publishing House of the Academy of Sciences of the Czech Republic, 473 p.

Mosquera, J., de Castro, M., & Gómez-Gesteira, M. (2003). Parasites as Biological Tags of Fish Populations: Advantages and Limitations. *Comments on Theoretical Biology*, *8*(1), 69–91. https://doi.org/10.1080/08948550302442

Möller, H., & Anders, K. (1986). *Diseases and parasites of marine fishes*. Kiel: Verlag Möller.

Münster, J., Klimpel, S., Fock, H. O., MacKenzie, K., & Kuhn, T. (2015). Parasites as biological tags to track an ontogenetic shift in the feeding behaviour of *Gadus morhua* off West and East Greenland. *Parasitology Research*, *114*(7), 2723–2733. https://doi.org/10.1007/s00436-015-4479-y

Nagasawa, K. (1990). The Life Cycle of Anisakis simplex: A Review. In *Intestinal Anisakiasis in Japan* (pp. 31–40). Tokyo: Springer Japan. https://doi.org/10.1007/978-4-431-68299-8_4

Navone, G. T., Sardella, N. H., & Timi, J. T. (1998). Larvae and adults of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda: Anisakidae) in fishes and crustaceans in the south west Atlantic. *Parasite*, (1982), 127–136. https://doi.org/http://dx.doi.org/10.1051/parasite/1998052127

Neiva, J., Coelho, R., & Erzini, K. (2006). Feeding habits of the velvet belly lanternshark *Etmopterus spinax* (Chondrichthyes : Etmopteridae) off the Algarve , southern Portugal. *Marine Biological Association of the United Kingdom*, *86*(4), 835–841. https://doi.org/10.1017/S0025315406013762

Nekouei, O., Vanderstichel, R., Thakur, K., Arriagada, G., Patanasatienkul, T., Whittaker, P., … Revie, C. W. (2018). Association between sea lice (*Lepeophtheirus salmonis*) infestation on Atlantic salmon farms and wild Pacific salmon in Muchalat Inlet, Canada. *Scientific Reports*, *8*(1), 4023. https://doi.org/10.1038/s41598-018-22458-8

Noever, C., Caira, J. N., Kuchta, R., & Desjardins, L. (2010). Two New Species of Aporhynchus (Cestoda: Trypanorhyncha) from Deep Water Lanternsharks (Squaliformes: Etmopteridae) in the Azores, Portugal. *Journal of Parasitology*, *96*(6), 1176–1184. https://doi.org/10.1645/GE-2387.1

Ommundsen, A., Noever, C., & Glenner, H. (2016). Caught in the act: phenotypic consequences of a recent shift in feeding strategy of the shark barnacle *Anelasma squalicola* (Lovén, 1844). *Zoomorphology*, *135*(1), 51–65. https://doi.org/10.1007/s00435-015-0296-1

Ottesen, D., Rise, L., Sletten Andersen, E., Bugge, T., & Eidvin, T. (2009). Geological evolution of the Norwegian continental shelf between 61° N and 68° N during the last 3 million years. *Norwegian Journal of Geology/Norsk Geologisk Forening*, *89*(4).

Palm, H. W. (2004). *The trypanorhyncha diesing, 1863*. *PKSPL–IPB Press*.

Palm, Harry W., Waeschenbach, A., Olson, P. D., & Littlewood, D. T. J. (2009). Molecular phylogeny and evolution of the Trypanorhyncha Diesing, 1863 (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution*, *52*(2), 351–367. https://doi.org/10.1016/j.ympev.2009.01.019

Pendleton, L. H., Ahmadia, G. N., Browman, H. I., Thurstan, R. H., Kaplan, D. M., & Bartolino, V. (2018). Debating the effectiveness of marine protected areas. *ICES Journal of Marine Science*, *75*(3), 1156–1159. https://doi.org/10.1093/icesjms/fsx154

Pereira, J., & Velloso, A. (2016). Cestoda. In Jorge Eiras, A. Velloso, & J. Pereira (Eds.), *Parasitos de peixes marinhos da América do Sul* (1st ed., pp. 174–205). Rio Grande,

Brazil: Editora da furg - Universidade Federal do Rio Grande-Furg.

Petrie, A., Wootten, R., Bruno, D., Mackenzie, K., & Bron, J. (2005). *A Survey of Anisakis and Pseudoterranova in Scottish fisheries and the efficacy of current detection methods FSAS Project S14008*.

Petter, A. J., Cabaret, J., & Tcheprakoff, R. (1995). Ascaridoid nematodes of teleostean fishes from the eastern north Atlantic and seas of the north of Europe. *Parasite*, *2*(2S), 217–230. https://doi.org/10.1051/parasite/199502s2217

Porcu, C., Marongiu, M. F., Follesa, M. C., Bellodi, A., Mulas, A., Pesci, P., & Cau, A. (2013). Reproductive aspects of the velvet belly lantern shark *Etmopterus spinax* (Condrichthyes: Etmopteridae), from the central western Mediterranean sea. Notes on gametogenesis and oviducal gland microstructure. *Mediterranean Marine Science*, *15*(2), 313. https://doi.org/10.12681/mms.559

Poulin, R. (2000). Variation in the intraspecific relationship between fish length and intensity of parasitic infection: Biological and statistical causes. *Journal of Fish Biology*, *56*(1), 123–137. https://doi.org/doi.org/10.1111/j.1095-8649.2000.tb02090.x

Poulin, R. (2007). *Evolutionary Ecology of Parasites*. Princeton University Press. ISBN 978-0-691-12085-0.

Poulin, R., & Morand, S. (2000). The diversity of parasites. Quarter. Rev. Biol. 75, 277– 293.

Raibaut, A., Combes, C., & Benoit, F. (1998). Analysis of the parasitic copepod species richness among Mediterranean fish. *Journal of Marine Systems*, *15*(1–4), 185–206. https://doi.org/10.1016/S0924-7963(97)00079-1

Rees, David J., Noever, C., Finucci, B., Schnabel, K., Leslie, R. E., Drewery, J., & Glenner, H. (2019). De novo innovation allows shark parasitism and global expansion of the barnacle Anelasma squalicola. *Current Biology*, *29*(12), R562–R563. https://doi.org/10.1016/j.cub.2019.04.053

Rees, David John, Noever, C., Høeg, J. T., Ommundsen, A., & Glenner, H. (2014). On the origin of a novel parasitic-feeding mode within suspension-feeding barnacles. *Current Biology*, *24*(12), 1429–1434. https://doi.org/10.1016/j.cub.2014.05.030

Rees, G. (1941). The scolex of *Aporhynchus norvegicus* (Olss.). *Parasitology*, *33*(4), 433–438. https://doi.org/10.1017/S0031182000024641

Reiner, F. (1996). Catalogo dos peixes do arquipelago de Cabo Verde. Lisboa: Instituto Portugues de Investigacao Maritima. 339 pages

Renwart, M., Delroisse, J., Claes, J. M., & Mallefet, J. (2014). Ultrastructural organization of lantern shark (Etmopterus spinax Linnaeus, 1758) photophores. *Zoomorphology*, *133*(4), 405-416.

Renwart, M., Delroisse, J., Flammang, P., Claes, J. M., & Mallefet, J. (2015). Cytological changes during luminescence production in lanternshark (Etmopterus spinax Linnaeus, 1758) photophores. *Zoomorphology*, *134*(1), 107–116. https://doi.org/10.1007/s00435-014-0235-6

Roberts, L., & Janovy, J. (2008a). Parasitic Crustaceans. In McGraw-Hill (Ed.), *Gerald D. schmidt & Larry S. Roberts' Foundations of parasitology* (8th ed., pp. 537–559). Boston.

Roberts, L., & Janovy, J. (2008b). Phylum Nematoda: Form, Function, and Classification. In *Gerald D. Schmidt & Larry S. Roberts' Foundations of parasitology* (8th ed., pp. 369–398). Bostom: McGraw-Hill.

Rodhe, J., Tett, P., & Wulff, F. (2004). The Baltic and North Seas: a process-oriented review of the physical oceanography. In A. R. Robinson & K. H. Brink (Eds.), *The Sea* (Vol. 14, pp. 1029–1071). Harvard: President and Fellows of Harvard College.

Rodhe, J. (1998). The Baltic and North Seas: a process-oriented review of the physical oceanography. In: Robinson, A.R. and K. Brink (eds.) The Sea. John Wiley and Sons, New York, p. 699–732.

Rodríguez, S. M., Luque, J. L., & George-Nascimento, M. (2010). A parasitic copepod, Neoalbionella sp. (Lernaeopodidae), on the southern lanternshark *Etmopterus granulosus* (Etmopteridae) off Juan Fernández Archipelago, Chile. *Revista de Biología Marina y Oceanografía*, *45*(2), 359–363. https://doi.org/10.4067/S0718-19572010000200020

Roff, G., Doropoulos, C., Rogers, A., Bozec, Y. M., Krueck, N. C., Aurellado, E., … Mumby, P. J. (2016). The Ecological Role of Sharks on Coral Reefs. *Trends in Ecology and*

Evolution, *31*(5), 395–407. https://doi.org/10.1016/j.tree.2016.02.014

Rohde, K. (1993). *Ecology of Marine Parasites: an Introduction to Marine Parasitology* (2nd ed.). Wallingford, UK: Cab International.

Rohde, K. (2005). The nature of parasitism. In K. Rohde (Ed.), *Marine Parasitology* (3rd ed., pp. 1–10). Oxford, UK: CABI Publishing Wallingford.

Rohde, K. (2015). *Marine Parasitology*. (K. Rohde, Ed.). Victoria, Australia: CSIRO Publishing.

Ruppert, J. L. W., Travers, M. J., Smith, L. L., Fortin, M. J., & Meekan, M. G. (2013). Caught in the Middle: Combined Impacts of Shark Removal and Coral Loss on the Fish Communities of Coral Reefs. *PLoS ONE*, *8*(9), 1–9. https://doi.org/10.1371/journal.pone.0074648

Rynkiewicz, E. C., Pedersen, A. B., & Fenton, A. (2015). An ecosystem approach to understanding and managing within-host parasite community dynamics. *Trends in Parasitology*, *31*(5), 212–221. https://doi.org/10.1016/j.pt.2015.02.005

Sasal, P., & Thomas, F. (2005). Behavioural aspects of parasitism. In K. Rohde (Ed.), *Marine Parasitology* (p. 259-264). Wallingford, UK: CABI Publishing.

Scott, M. (1988). The Impact of Infection and Disease on Animal Populations: Implications for Conservation Biology. *Conservation Biology*, *2*(1), 40–56. https://doi.org/10.1111/j.1523-1739.1988.tb00334.x

Seefeld, K. (2007). *Statistics Using R with Biological Examples*. (K Seefeld, Ed.) (1st ed.).

Serena, F. (2005). Field identification guide to the sharks and rays of the Mediterranean and Black sea. *FAO Species Identification Guide for Fishery Purposes.*, 97.

Shiffman, D. S., & Hammerschlag, N. (2016). Shark conservation and management policy: a review and primer for non-specialists. *Animal Conservation*, *19*(5), 401–412. https://doi.org/10.1111/acv.12265

Shinn, A. P., Pratoomuot, J., Bron, J. E., Paladini, G., Brooker, E. E., & Brooker, A. J. (2015). Economic costs of protistan and metazoan parasites to global mariculture. *Parasitology*,

142(1), 196–270. https://doi.org/10.1017/S0031182014001437

Sikkel, P. C., Nemeth, D., McCammon, A., & Williams, Jr, E. H. (2009). Habitat and Species Differences in Prevalence and Intensity of *Neobenedenia melleni* (Monogenea: Capsalidae) on Sympatric Caribbean Surgeonfishes (Acanthuridae). *Journal of Parasitology*, *95*(1), 63–68. https://doi.org/10.1645/GE-1645.1

Simpfendorfer, C. A., & Kyne, P. M. (2009). Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environmental Conservation*, *36*(2), 97–103. https://doi.org/10.1017/S0376892909990191

Sistiaga, M., Herrmann, B., Larsen, R. B., & Brinkhof, J. (2019). Quantification of bell-shaped size selectivity in shrimp trawl fisheries using square mesh panels and a sorting cone after a Nordmøre grid. *PLOS ONE*, *14*(9), e0222391. https://doi.org/10.1371/journal.pone.0222391

Smith, J. (1983). *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the life-history and ecology. *Journal of Helminthology*, *57*(3), 205–224.

Smith, J. W. (1984). The abundance of Anisakis simplex L3 in the body-cavity and flesh of marine teleosts. *International Journal for Parasitology*, *14*(5), 491–495. https://doi.org/10.1016/0020-7519(84)90030-4

Smith, J. W., & Wootten, R. (1975). Experimental studies on the migration of Anisakis sp. larvae (Nematoda : ascaridida) into the flesh of herring, *Clupea harengus* L. *International Journal for Parasitology*, *5*(2), 133–136. https://doi.org/10.1016/0020-7519(75)90019-3

Sündermann, J., & Pohlmann, T. (2011). A brief analysis of North Sea physics. *Oceanologia*, *53*(3), 663–689. https://doi.org/10.5697/oc.53-3.663

Świderski, Z., Miquel, J., Marigo, A. M., & Gibson, D. I. (2012). Ultrastructure of vitellogenesis and vitellocytes in the trypanorhynch cestode *Aporhynchus menezesi*, a parasite of the velvet belly lanternshark *Etmopterus spinax*. *Comptes Rendus Biologies*, *335*(9), 573–584. https://doi.org/10.1016/j.crvi.2012.07.007

Techera, E. J., & Klein, N. (2011). Fragmented governance: Reconciling legal strategies for

shark conservation and management. *Marine Policy*, *35*(1), 73–78. https://doi.org/10.1016/j.marpol.2010.08.003

Timi, J. T. (2007). Parasites as biological tags for stock discrimination in marine fish from South American Atlantic waters. *Journal of Helminthology* 81, 107–111. doi: 10.1017/S0022149X07726561.

Timi, J. T., & MacKenzie, K. (2015). Parasites in fisheries and mariculture. *Parasitology*, *142*(1), 1–4. https://doi.org/10.1017/S0031182014001188

Varella, A. & Malta, J. (1995). Gamidactylus hoplius sp. n. (COPEPODA, POECILOSTOMATOIDA, VAIGAMIDAE) from Nasal Fossae of *Hoplias maiabaricus* (Bloch, 1794) (Characiformes, Erythrynidae) from the Brazilian Amazon. *Acta Amazônica*, *25*(3-4), 281-288.

Varella, A. & Malta, J. (2001). *Brasergasilus mamorensis* sp. n.(Copepoda: Ergasilidae) from the Nasal cavities of *Hydrolycus pectoralis* (Guenther, 1866)(Characiformes: Cynodontidae) from the Brazilian Amazon, and considerations about Abergasilinae. *Acta Amazonica*, 31: 323-323.

Ventura, C. H. D. (2006). *Parasitas do Peixe-espada preto (Aphanopus carbo, Lowe 1839) de Sesimbra e Madeira*. Dissertação de mestrado em Ecologia Aplicada. Porto, Portugal: Faculdade de Ciências, Universidade do Porto

Whittington, I. D., Cribb, B. W., Hamwood, T. E., & Halliday, J. A. (2000). Host-specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? *International Journal for Parasitology*, *30*(3), 305–320. https://doi.org/10.1016/S0020-7519(00)00006-0

Więcaszek, B., Sobecka, E., Panicz, R., Keszka, S., Górecka, K., & Linowska, A. (2018). First record of the deep-water shark *Etmopterus spinax* (Chondrichthyes: Etmopteridae) from the southern Baltic Sea (Pomeranian Bay). *Oceanologia*, *60*(3), 426–430. https://doi.org/10.1016/j.oceano.2018.02.001

Williams, E., Bunkley-Williams, L., & Ebert, D. (2010). An accidental attachment of *Elthusa raynaudii* (Isopoda, Cymothoidae) in *Etmopterus* sp. (Squaliformes, Etmopteridae). *Acta Parasitologica*, *55*(1). https://doi.org/10.2478/s11686-010-0006-6

Williams, H. H. (1968). The taxonomy, ecology and host-specificity of some Phyllobothriidae (Cestoda:Tetraphyllidea), a critical revision of Phyllobothrium Beneden, 1949 and comments on some allied genera. *Philosophical Transactions of the Royal Society of London*, *253*(786), 231–307.

Williams, H. H., MacKenzie, K., & McCarthy, A. M. (1992). Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. *Reviews in Fish Biology and Fisheries*, *2*(2), 144–176. https://doi.org/10.1007/BF00042882

Willig, M. R. (2001). Latitude, common trends within. *Encyclopedia of biodiversity*, 3(1), 701-714.

Whitlock, M. C., & Schluter, D. (2015). *The Analysis of Biological Data* (2nd ed.). Colorado: Roberts and Company Publishers, Incorporation.

Yano, K., & Musick, J. A. (2000). The effect of the mesoparasitic barnacle *Anelasma* on the development of reproductive organs of deep-sea squaloid sharks, *Centroscyllium* and *Etmopterus*. *Environmental Biology of Fishes*, *59*(3), 329–339. https://doi.org/10.1023/A:1007649227422

Appendix

Appendix Table 7 - Reproduction scales for female specimens of *E. spinax* (Myrlund, 2018)

Maturity	Stage	Description
Immature	1. Immature	**Ovaries**: small dimensions and albicans; without differentiated oocytes. **Oviductal gland:** frequently not visible. **Uterus:** entangled and small diameter (hypoplasic)
	2. Developing (Maturing)	**Ovaries**: increasing dimensions; oocytes on different development stages. Possible visualization of small and medium sized yolked follicles. **Oviductal gland**: possible to distinguish each gland although it is still underdevelopment **Uteri**: Hypoplasic
Mature	3. Capable to Reproduce	**Ovaries**: large size; presence of large yolked follicles ready to be ovulated. **Oviductal glands**: fully developed **Uteri**: fully developed
Maternal	4a. Early Pregnancy	**Ovaries**: different sized follicles are present according to stages of ovulation. **Oviductal glands**: fully developed (possibility of regression) **Uterus:** enlarged rounded shape with yolk content. Embryos cannot be observed.

	4b. Mid Pregnancy	**Ovaries:** small to medium, possibly yolked follicles (active gonads) or small, unyolked and/or atretic follicles (inactive gonads). **Oviductal glands:** fully developed (possibly regressing) **Uterus:** Presence of oedema. Enlarged and round shape. rounded. Embryos are always visible (small and with a relatively large yolk sac)
	4c. Late Pregnancy	**Ovaries:** medium to large yolked follicles (active gonads) or small, unyolked follicles and/or atretic follicles (inactive gonads) **Oviductal glands:** fully developed **Uterus:** embryos fully developed (yolk sacs reduced or absent)
	5**.** Post-Partum	**Ovaries**: Similar to stage Late Pregnancy (4c) **Oviducal glands**: Similar to stage 4c **Uterus:** enlarged and flaccid
	6. Early Regeneration	**Ovaries:** small or medium yolked follicles. **Oviducal glands:** fully developed (may be reduced in size, depending when the partum was). **Uterus:** enlarged (oedema) post-maternal, but not so flaccid
	7. Late Regeneration	**Ovaries:** large yolked follicles of ovulatory size. **Oviducal glands:** fully developed. **Uteris:** enlarged post- maternal, but not flaccid.

Appendix Table 2 - Reproduction scales for male specimens of *E. spinax* (Myrlund,, 2018)

Maturity	Stage	Description
Immature	1. Immature	**Testes:** small dimensions and undeveloped. **Ducts:** narrow and entangled **Claspers**: soft, flexible, noncalcified and usually shorter dimensions than pelvic fins
	2. Developing (Maturing)	**Testes:** maturing with segments visible at this stage - although does not occupy whole surface. **Ducts:** underdeveloped, although the ducts are starting to coil. **Claspers:** flexible, partially calcified and as long as or longer than pelvic fins.
Mature	3a. Capable to Reproduce	**Testes:** fully developed and matured (segments completed). Spermatogenesis **Ducts:** tightly coiled and filled with sperm. Seminal vesicles are developed. **Claspers:** rigid, calcified and longer than the pelvic fins.
	3b. Capable to Reproduce	**Testes:** similar to stage 3a **Ducts:** sperm flowing out of the cloaca on pressure. Seminal vesicles can be full **Claspers:** fully developed (clasper gland dilated, sometimes swollen and/or with erythema). Sperm may be present in clasper groove and glans.
	4. Spent (regressing and regenerating)	**Testes:** anthropic and flaccid **Ducts**: empty and flaccid. Seminal vesicles (when present) empty. **Claspers**: fully formed.

Appendix Table 3– Average total length (in centimetres) ±SD and average weight (in grams) ±SD of sampled shark per station and the total of sharks sampled on this study.

Station codes	Average length (cm)	Average weight (g)
A	24.4 ± 7.0	65.9 ± 51.7
B	33.9 ± 3.8	183.3 ± 50.1
C	31.00 ± 2.5	116.9 ± 29.9
D	30.1 ± 10.9	160.2 ± 139.0
E	28.6 ± 8.7	117.1 ± 84.5
F	26.3 ± 3.5	71.3 ± 31.2
G	33.7 ± 8.4	137.6 ± 97.4
H	27.4 ± 9.5	96.2 ± 79.7
Total of all individuals sampled	28.7 ±7.7	114.8 ±81.2

Appendix Table 4 – Fisher's Exact Test - investigate the relationship between *E. spinax* gender and stations.

Fisher's Exact Test	
p-value	0.9726
Alternative hypothesis	Two sides

Appendix Table 5 – Non-parametric Kruskal-Wallis test for analysing association between length or weight between different stations.

	p-value
Indiscriminate gender Weight per station	4.672e-06
Indiscriminate gender Length per station	5.537e-04

Appendix Table 6 – Stations that have shown significative difference on these associations ($p<0.05$) - Dunn's nonparametric comparison for post hoc (pairwise multiple comparisons)

Stations comparison - Significative difference – length	P.adj
A - B	0.0002323629
A - C	0.0295225090
B - F	0.0296576428
B - H	0.0316867754

Stations comparison - Significative difference – weight	P.adj
A - B	9.144125e-07
A - C	3.402296e-02
B - E	4.919704e-02
B - F	1.620276e-03
B - H	1.879170e-03

Appendix Table 7 – Gender and sexual maturity of sampled *E. spinax* per station.

Station codes	Female		Male	
	Immature	**Mature**	**Immature**	**Mature**
A	15	1	9	2
B	5	6	1	7
C	5	2	4	3
D	2	4	4	0
E	5	2	3	1
F	4	0	4	1
G	3	1	2	3
H	7	1	4	4

Appendix Table 8 - Average of the Hepatosomatic index (HIS) between gender and sexual maturity stage and for each gender.

Sexual Maturity Stage	***Gender***	
	Female	**Male**
Immature	14.2 ± 6.1	14.6 ± 5.4
Mature	17.7 ± 5.5	18.7 ± 5.0
Total of specimens	15.1 ± 6.1	16.3 ± 5.5

Appendix Table 9 – Number of parasites found per station. Parasite development stage, location within the host. Dashes indicate absence of parasite.

A = adult, I = intestine, L3 = larval stage 3, S = skin, Sn = snout, Sw = stomach wall

Species	**Site**	**Stage**	**Number of parasites**							
			A	B	C	D	E	F	G	H
Monogenean										
Monogenea indet.	Sn	A	-	1	-	-	-	-	-	-
Cestoda										
Aporhynchus norvegicus	I	A	1	1	1	-	1	-	-	-
Nematoda										
Anisakis simplex	Sw	L3	3	2	-	-	1	3	4	3
Crustacea										
Anelasma squalicola	S	A	-	2	-	-	-	-	2	4

Appendix Table 10 – Prevalence and Mean abundance of each parasite species per sampled station.

Species	Prevalence %							
	A	B	C	D	E	F	G	H
Monogenea								
Monogenea indet.	0	5.26	0	0	0	0	0	0
Cestoda								
A. norvegicus	3.7	5.25	7.14	0	9.09	0	0	0
Nematoda								
A. simplex	7.41	10.53	0	0	0	22.22	33.33	12.5
Crustacea								
A. squalicola	0	10.53	0	0	0	0	11.11	18.75

Species	Mean Abundance							
	A	B	C	D	E	F	G	H
Monogenea								
Monogenea indet.	0	0.053 ± 0.029	0	0	0	0	0	0
Cestoda								
A. norvegicus	0.037 ± 0.192	0.053 ± 0.229	0.071 ± 0.267	0	0.091 ± 0.302	0	0	0
Nematoda								
A. simplex	0.111 ± 0.424	0.105 ± 0.315	0	0	0.091 ± 0.302	0.333 ± 0.707	0.444 ± 0.726	0.188 ± 0.544
Crustacea								
A. squalicola	0	0.105 ± 0.315	0	0	0	0	0.222 ± 0.667	0.25 ± 0.577

Appendix Table 11 – Number of infected species per gender and Fisher's exact test for each parasite species per host gender.

Prevalence *A. simplex* - Male vs. Female

	Infected	Non-infected
Female	8	55
Male	4	48
fisher's exact test: P=0.5425		

Prevalence *A. squalicola* - Male vs. Female

	Infected	Non-infected
Female	2	61
Male	4	48
fisher's exact test: P=0.4074		

Prevalence *A. norvegicus* - Male vs. Female

	Infected	Non-infected
Female	2	61
Male	2	50
fisher's exact test: P=1		

Prevalence Monogenean - Male vs. Female

	Infected	Non-infected
Female	1	62
Male	0	52
fisher's exact test: P=1		

Appendix Table 12 - Length is important as you can see in the PCA and is confirmed also by the Kruskal-Wallis test results (below) whereas location is not so important as the data are not separated.

Effects	***A. simplex***	***A. squalicola***		***A. norvegicus***	**Monogenea indet.**
Length	0.01573 *	0.002833 **		0.1492	0.7557
Location	0.11688	0.156344		0.6853	0.8851