Parasites of Redfish (*Sebastes* spp.) in Icelandic Waters

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90 ECTS thesis submitted in partial fulfillment of a
Magister Scientiarum degree in Biology

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Hér með lýsi ég því yfir að ritgerð þessi er samin af mér og að hún hefur hvorki að hluta né í heild verið lögð fram áður til hærri prófgráðu.

I declare that this thesis is supported by my research work, written by myself and has not partly or as a whole been published before to higher educational degree.

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Ásthildur Erlingsdóttir

Abstract

Sphyrion lumpi is a marine ectoparasitic copepod that has a significant negative impact on fisheries of redfish, *Sebastes* spp. in the North Atlantic as it reduces the commercial value of the fillets. Long-term data of infestation by *S. lumpi* and abnormalities in *Sebastes mentella*, in Icelandic waters were analyzed in this study. Five categories of external abnormalities were applied; black spots, red spots, mixed spots, remnants or lesions caused by *S. lumpi* and the parasite itself. Infestation intensity of the copepod was not found to be related to redfish condition (K). During the period, the prevalence of *S. lumpi* infections declined from 25% in 1995 to 9% in 2013. Significant differences in infestations were found between female and male fish and the shallow pelagic and deep pelagic stock. These results show the host response of *S. lumpi* infections and give a good overall view of trends in *S. lumpi* infestation, which could be a contributing factor towards defining *S. mentella* population stock structure.

Microparasites in the field of icthyoparasitology are generally limited to Myxozoa (Kingdom Animalia, Phylum Cnidaria), Protozoa (Kingdom Protista) and Microsporidia (Kingdom Fungi). The microparasitic fauna of *Sebastes* spp. in Icelandic waters was mapped in this study. All major organs were examined thoroughly with a stereoscope and/or a compound microscope. Histopathological examinations were carried out with regards to parasitic infections in order to determine the exact site of the infection. DNA was extracted from myxozoans from the gallbladder and apicomplexans from the intestines and the urinary bladder. The parasites' DNA was amplified with PCR and sequenced. Six different parasite species were identified, two myxosporeans and four apicomplexans. Results identified the myxosporean species to be *Myxidium bergense* and *Ceratomyxa adeli*. The apicomlexan species are not fully identified but are thought to be previously undescribed. Accurate identification awaits further study.

Útdráttur

Krabbadýrið *Sphyrion lumpi*, er ytra sníkjudýr á karfategundum (*Sebastes* spp.) við Ísland og veldur efnahagslegu tjóni þar sem það festir sig í vöðva fisksins og veldur þannig afurðaskemmdum. Í þessu verkefni voru áður ógreind langtímagögn er varða sýkingar á úthafskarfa (*Sebastes mentella*) af völdum *S. lumpi* við Ísland skoðuð. Í rannsókninni voru frávik á ytra borði fisksins sett í fimm flokka: svartir blettir, rauðir blettir, blandaðir blettir, kýli eða leifar eftir *Sphyrion lumpi* sýkingar og sníkjudýrið sjálft. Niðurstöður sýndu að smitmagn hefur ekki áhrif á ástand fisksins (K). Á rannsóknartímabilinu lækkaði tíðni sýkinga úr 25% árið 1995 í 9% árið 2013. Marktækur munur er á smitmagni milli kynja hjá *S. mentella* og einnig á milli stofna úthafskarfa. Niðurstöðurnar gefa góða mynd af þróun *S. lumpi* sýkinga við Ísland, ásamt smittengdum hýsilsvörunum karfans, og gætu þær haft áhrif á sýn vísindasamfélagsins á samsetningu karfastofna hér við land.

Á sviði fisksjúkdóma á hugtakið smásæ sníkjudýr almennt við slímdýr (Myxozoa), frumdýr (Protozoa) og sníkjusveppi (Microsporidia). Í rannsókninni var fána smásærra sníkjudýra tveggja karfategunda (*Sebastes* spp.) við Ísland kortlögð. Öll helstu líffæri voru skoðuð með víðsjá og/eða smásjá. Einnig var vefjameinafræðileg skoðun framkvæmd með tilliti til sýkinga. Erfðaefni þeirra sníkjudýra sem fundust var magnað upp með PCR og raðgreint. Slímdýr er fundust í gallblöðru voru greind til tegundanna *Myxidium bergense* og *Ceratomyxa adeli*. Líkur benda til að fjórar tegundir gródýra (Apicomplexa) er fundust í görnum og þvagblöðru, séu áður óþekktar en fullnaðargreiningu er ólokið.

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Abbreviations

K = Fulton's condition factor

AGD = Amoebic gill disease

PV = Parasitophorous vacuole

MRI = Marine Research Institute

FSMI = Icelandic Fish Market

PBS = Phosphate-buffered saline

SEM = Standard Error of Mean

LM = Linear Regression Model

CI = Confidence Intervals

SSU = Small sub unit

rRNA = Ribosomal ribonucleic acid

PCR = Polymerase chain reaction

fwd = Forward

rev = Reverse

bp = Base pairs

BLAST = Basic Local Alignment Search Tool

MCL = Maximum Composite Likelihood

U = Units

P = Prevalence

W = Whole body wet weight in grams

L = Total fish length in centimeters

µm = Micrometers

µl = Microliters

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1 Introduction

1.1 *Sebastes* spp. Cuvier, 1829

Redfish or rockfish of the genus *Sebastes* contain more than 100 species, which are distributed worldwide with a great diversity of ecological and morphological traits (Land et al. 2001). The vast majority inhabit the North Pacific Ocean but two species are found in the Southern Hemisphere and four in the North Atlantic Ocean, of which three inhabit Icelandic waters (Love et al. 2002). Those species are the Norway redfish (*Sebastes viviparous* Krøyer, 1845), golden redfish (*S. norvegicus* Linnaeus, 1758) (syn. *S. marinus*) and beaked redfish (*S. mentella* Travin, 1951). The group includes many heavily fished species such as the golden and beaked redfish (Hyde and Vetter 2007). Their unique life history characteristics and longevity make them vulnerable to overfishing (Magnusson-Ford et al. 2009).

1.1.1 Life History

Sebastes species have unique life history traits with ovoviviparity, longevity and wide dispersal as their main characteristics (Love et al. 2002). Ovoviviparity involves internal fertilization and development of ova, where the egg, containing the embryo, is retained inside the female until it is ready to hatch (Love et al. 2002). *Sebastes* spp. are highly fertile, a single fish thought to be able to produce as much as 400 thousand larvae (Jónsson and Pálsson 2013), a characteristic quite uncommon among live-bearers, which mostly produce few offspring (Magnússon 1956). Sexual maturity, mating and fertilization of eggs take place at different times of the year. Males mature in September - October and the females in February – March. Mating and copulation occurs in autumn but the eggs are not fertilized until winter. In spring (April – June, but mainly in May) the females extrude larvae soon after they hatch from the eggs (Magnússon 1955; Rocha-Olivares 2004). At that time, the sexes have commonly separated (Magnússon and Magnússon 1995). The length of released larvae differs for *S. norvegicus* and *S. mentella,* as they are 5-7 mm and 7-8 mm, respectively (Moser and Boehlert 1991). Survival of redfish larvae seems to depend largely on the availability of eggs and larval stages of the planktonic copepod *Calanus finmarchicus* (Anderson 1994). Unlike the consistent timing of spawning of redfish, there are considerable annual fluctuations in the timing and the amount of the copepod production, which can affect the survival of the redfish larvae (Runge and de Lafontaine 1996).

Sebastes are slow growing, long living and late maturing fish species (Perlmutter and Clarke 1949; Sandeman 1969). Age at maturity is thought to be around 12-15 years in the North Atlantic (Jónsson and Pálsson 2013) and some *Sebastes* species are frequently aged over 100 years old (Munk 2001). Furthermore, the maturity and longevity differ between

sexes, where the females mature later and live longer than males (Saborido-Rey et al. 2004a; Sandeman 1961; Sandeman 1969).

1.1.2 Geographical Distribution

Globally, the genus *Sebastes* has a wide geographic range where species are habituated to diverse ecological niches (Johns and Avise 1998). Despite their broad distribution and diversity in form and function, warm, oligotrophic waters represent a significant barrier for *Sebastes* species, which are limited to cool-temperate, upwelling driven systems (Hyde and Vetter 2007). The geographic distribution of *S. norvegicus* and *S. mentella* in the North Atlantic is wide (Magnússon and Magnússon 1995). The distribution of *S. norvegicus* extends from Svalbard and by Novaya Zemlya in the Barents Sea along the Norwegian coast, south to Kattegat and the northern part of the North Sea. Furthermore, it is found west and north of Scotland to the Faroe Islands and Iceland along the continental shelf to eastern and western Greenland and along the North American coast southward to Flemish Cape, Grand Banks and Gulf of St Laurence. *S. mentella* is found throughout the North Atlantic, from the Barents Sea, along the Norwegian coast, around the Faroe Islands, Iceland, Greenland, the Irminger Sea and the East-Atlantic waters of Canada and the United States (Jónsson and Pálsson 2013). The main areas of larval release are in the Irminger and Norwegian Sea (Cadrin et al. 2010). Nursery grounds for the stocks found in the Irminger Sea and adjacent waters are thought to be on the continental slope of Greenland (Moser and Boehlert 1991).

These species are generally classified as demersal, occurring along the slopes of fishing banks and deep-water channels (Gascon 2003), but *S. mentella* is also found in pelagic waters (Magnússon and Magnússon 1995).

The depth distributions of the species differ with some overlapping of habitats. Golden redfish around Iceland mainly inhabit depths of 100 - 400 m, while the beaked redfish is found from 200 m to 1,100 m (Jónsson and Pálsson 2013).

1.1.3 Population Stock Structure

The stock structure of *S. mentella* in the Irminger Sea and adjacent waters has been debated for decades (Cadrin et al. 2010), where one to four stocks of the species have been hypothesized. Saborido-Rey et al. (2004b) argued a one stock hypothesis based on the ecology of beaked redfish. Information leading to that conclusion included a single spawning area above the Reykjanes Ridge, a continuous distribution of larvae and common nursery grounds in East Greenland. They also concluded that the previously observed genetic differences could be derived from genetic drift, selection or temporal variation.

Phenotypic diversity has played a large role in this field of study. Magnússon (1972) reported the existence of a second, pelagic stock in the Irminger Sea and, among others, described morphological characteristics distinguishing the shallow pelagic type from the deep pelagic type (Magnússon and Magnússon 1995; Yatsu and Jørgensen 1989). They include a darker complexion, abnormal coloration of the skin, frequent muscular pigmentation and heavy infestation of the parasitic crustacean *Sphyrion lumpi* along with ulcers caused by its post-mortem remnants. Also, pelagic forms are considered more

streamlined, a possible result of habitat adaptation (Cadrin et al. 2010). Behavioural and biological differences of the stocks have been noted as well. The shallow pelagic and deep pelagic stocks differ in size at sexual maturity, spawning depth, size of released larvae and feeding depth (Magnússon 1995). Molecular studies have further strengthened the separation of stocks with differences in frequencies of several genetic characters, such as: allozymes, mitochondrial DNA haplotypes, hemoglobins and microsatellites (Johansen et al. 2000; Johansen 2003; Melnikvov et al. 2007; Ingimarsdóttir 2008; Pampoulie and Daníelsdóttir 2008).

Cadrin et al. (2009) proposed a three stock hypothesis of *S. mentella* in the Irminger Sea and adjacent waters based on recent genetic research and certain information on the life history of the species. Later, Cadrin et al. (2010) suggested the fourth stock to be introduced, a western stock from south and west of Newfoundland, and advised a redefinition of practical management units near the Irminger Sea based on geographic areas rather than depth. Makhrov et al. (2011) challenged this, mainly criticizing sampling methods and eventually concluded that the evidence brought forward by Cadrin et al. (2010) did not disprove Saborido-Ray's hypothesis of a single population. The discussion of the population structure of beaked redfish is an on-going debate where the scientific community is not unanimous regarding the subject. However, in the central and eastern North Atlantic, the fisheries management is currently based on the existence of three stock units: (1) A deep pelagic stock (>500m), (2) a shallow pelagic stock (<500m) and (3) an Icelandic slope stock as suggested by Cadrin et al. (2009). Whether *S. mentella* consists of a single or multiple stocks, it seems evident that intra- and inter stock diversity of *Sebastes* is complex (Anon 2004) and further research would be ideal for sustainable fishery management in the future.

1.1.4 Exploitation

Exploitation of *S. norvegicus* and *S. mentella* has a long history and targeted redfish fisheries are known since the beginning of the 1930s. Initially the fishery was exclusively based on golden redfish, as the existence of beaked redfish was unknown until the late 1930s (Magnússon and Magnússon 1995). Beaked redfish was then first described as a species in 1951 (Travin 1951). Information on species composition of redfish catches is limited prior to 1965 (Björnsson and Sigurðsson 2003). That could even be argued today, as considerable experience is required to separate the two species and to distinguish between *S. mentella* phenotypes, due to substantial morphological similarities.

S. mentella is a commercially valuable species. The Soviet Union started a pelagic redfish fishery in 1982. Currently, it is conducted by multiple nations where Germany, Iceland and Russia have been the main fishing nations (Sigurðsson et al. 2006). The fishery expanded to deeper waters in the early 1990s, also targeting redfish below 500 m. Since the beginning of exploitation, landings have fluctuated with a peak of 180 thousand tonnes in 1996. In recent years catches have drastically declined, especially in the shallower waters of the southwest Irminger Sea. Annual landings have ranged between 30,000 and 70,000 tonnes since 2005 in the northeast Irminger Sea, the main fishery for deep pelagic beaked redfish. In 2010, ICES advised fishery managers to close the directed fishery of the shallow pelagic stock due to its poor state (Cadrin et al. 2010). For the deep pelagic stock a total catch limit of maximum 20,000 tonnes was recommended.

1.2 *Sphyrion lumpi* (Krøyer, 1845)

Sphyrion lumpi was first mentioned by Krøyer (1845) who obtained his specimen from lumpfish (*Cyclopterus lumpus* L.) caught in Icelandic waters. *S. lumpi* is a parasitic marine crustacean that belongs to the subclass Copepoda. In addition to *S. lumpi*, two further species of genus *Sphyrion* Cuvier, 1830, are known, i.e. *S. quadricornis* (Gaevskaya & Kovaleva, 1984) and *S. laevigatum* (Quoy & Gaimard, 1824). All these species are extremely derived marine siphonostomatoid copepods (Dojiri and Deets 1988) and frequently found as ectoparasites on teleost fishes (Boxshall 2001). *S. quadricornis* and *S. laevigatum* are mostly found parasitizing Gadiformes while *S. lumpi* is quite non-specific with regards to hosts, being recorded from 29 fish species belonging to four different orders of fishes: Perciformes, Pleauronectiformes, Gadiformes and Scopaeniformes (Ho and Kim 1989; Ho 1992). However, Kabata (1979) considered *Sebastes* spp. as the main hosts of this parasite.

1.2.1 Life Strategies

The life cycle of *S. lumpi* is poorly known, especially with regards to larval stages (Ho 1989). It is however known that it exhibits a mesoparasitic mode of life, as other members of the Sphyriidae family (Kabata 1979), where they live partially imbedded in their hosts (Boxshall 2004). As described by Wilson (1919), *S. lumpi* is sexually dimorphic in the adult stages and host attachment is exclusive to the post-metamorphic female (Ho 1989). The metamorphosis is so extreme that only the maxillipeds remain unmodified (Moran and Piasecki 1994). The cephalothorax, or the “head”, is used as an anchor process in somatic muscles while the posterior part, the neck and the trunk, remain outside the fish host. The trunk consists of the genital process, posterior process, cement glands and two egg sacs (Najarian 1952) (Figure 1.1). Body length, excluding egg sacs, can vary from 9 to 80 mm (Templeman and Squires 1960). Six stages of maturity have been determined for the adult female depending on ova diameters in ovary and the presence or absence of egg sacs (Squires 1966).

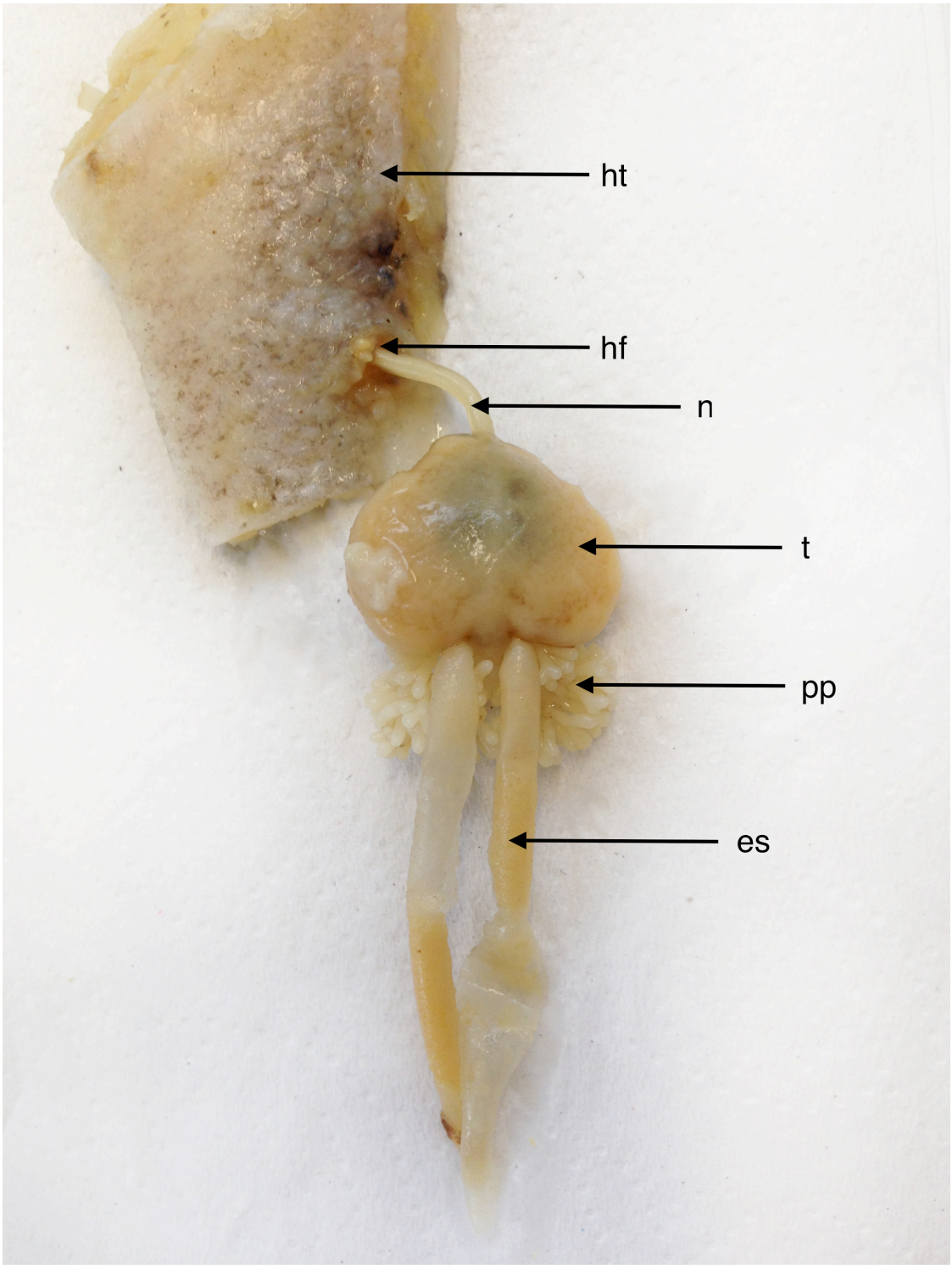

Figure 1.1 *Sphyrion lumpi. ht, host tissue; hf, holdfast of S. lumpi, within lies the cephalothorax; n, neck; t, trunk; pp, posterior process; es, egg sack.*

Few male specimens have been described in details and only three papers have been published describing their morphology (Wilson 1919; Squires 1966; Moran and Piasecki 1994). The body can be divided into two parts, cephalothorax and a thoraco-genito-abdominal trunk, the trunk being smaller in examined specimen (Moran and Piasecki 1994). Squires (1966) defined two stages of maturity, adult or immature. Adult males (1.8 - 2.1 mm in length) had fully developed reproductive organs while testes could not be easily seen in the immature males (0.8 mm in length). Also, no data is available of the larval development except for the early naupilus and metanaupilus stages, removed from egg sacs and described by Jones and Mattews (1968).

The life cycle could be confined to a single host as males were found on the same host as the females (Moran and Piasecki 1994) or even attached to them (Ho 1989). Templeman and Squires (1960) suggested that *S. lumpi* might have an intermediate host, like its relative *Lernaeocera branchialis* (L.).

Whether the female experiences single or multiple spawning, i.e. extrusion of larvae is not clear. However, Squires (1966) found simultaneous development of embryos in egg sacs and of ova in ovaries. After spawning the egg sacs are lost and Squires' findings suggest that new egg sacs could be ready for extrusion and therefore multiple spawning is probable. When spawning is complete the animal withers and eventually dies, leaving the cephalothorax in the muscle where it likely remains for the fish's entire lifespan (Templeman and Squires 1966). High level of infestation of *S. lumpi* among other abnormalities, such as pigmentation of the fillets, decreases the economic value of the catch. Commercial trawlers have been reported to dispose 16-26% of the fillets per trip due to these abnormalities (Magnússon 1992).

1.2.2 Distribution

Although *Sphyrion lumpi* has mostly been reported from the North Atlantic (Boxshall 2001) its geographical distribution is much wider. It has been reported from New Zealand, Japan, the Beaufort Sea and most recently, off the coast of Brazil (Ho 1989; Webber 2010; Miller 2012; Alves 2013).

The family Sphyriidae is primarily found parasitic on deep-water marine fishes. Some of those species, e.g. *Lophoura* spp., exhibit high host specificity whereas *Sphyrion lumpi* is non-specific with regards to its hosts. It has been speculated that the low host specificity of *S. lumpi* could be correlated with the low probability of encountering a suitable host (Boxshall 1998). The relationship of depth and the infestation of *S. lumpi* has been speculated. These studies are however quite controversial; some studies showing an increase with depth while others show the opposite (Templeman and Squires 1960; Sarralde et al. 1997; Gayevskaya 1996). Studies on the distribution of *S. lumpi* on redfish have shown that the greatest abundance of alive parasites and old cephalothoraxes is between the base of the dorsal fin and the lateral line (Perlmutter 1951; Bakay 1988). In addition, females are generally thought to be more heavily infested than males (Templeman and Squires 1960; Magnússon 1977; Yatsu and Jørgensen 1989; Magnússon and Magnússon 1990; Nagel et al. 1991; Magnússon 1992; Shibanov et al. 1994; Gayevskaya 1996; del Río et al. 1996; Sarralde et al. 1997; Melnikov et al. 2003).

1.3 Microparasites of Fishes

According to the Oxford dictionary, a parasite is defined as *"an organism which lives in or on another organism (its host) and benefits by deriving nutrients at the other's expense"*. Commonly, parasites are divided into "microparasites" and "macroparasites". Macroparasites are defined as "*those parasites (e.g., helminths, arthropods) that do not multiply within their definitive host, cycling instead through transmission stages (eggs and larvae), which pass to the outside*" while microparasites are in the broadest sense defined as *"…pathogens, such as viruses, bacteria, protozoans and fungi, that are characterized by small size, short generation time and an extremely high rate of direct reproduction within the host…."* (Anderson and May 1982). However, in the field of ichthyoparasitology, the term "microparasites" generally refers to three major groups of parasites, i.e. Myxozoa (Kingdom Animalia, Phylum Cnidaria), Protozoa (Kingdom Protista) and Microsporidia (Kingdom Fungi).

Most individual fish in wild or cultured populations are infected with parasitic organisms. The number of fish parasites species already described is measured in thousands, and thousands are likely to be discovered (Wooten 2012).

Microparasites are a highly diverse group of organisms that have evolved different strategies for infecting their hosts. Major differences are not only found between the three groups but also between genera and species within each group. Some species are obligate parasites while others are facultative or opportunistic and also have a free-living stage.

Life cycles of parasites are normally defined as direct or indirect. For direct life cycles, no intermediate host is required; only a definitive host: the species in which the parasite reaches sexual maturity and produces progeny. Indirect life cycles involve one or more intermediate hosts, which are required by the parasite for completion of its life cycle. In addition, paratenic hosts or transport hosts are present in some parasite life cycles, in which no parasite development occurs. Instead, it provides both an ecological and temporal bridge for the parasite to move through the environment and infect the definitive host. Parasites are furthermore divided into ectoparasites, i.e. found on the external parts of the host, while others are endoparasites and found inside the host.

Pathogenicity varies greatly between parasite species; some being highly pathogenic, causing mortality to its host, while others seem relatively harmless. Many factors affect that, e.g. type of host: parasite coevolution, mode of parasitism in host tissues (intracellular, extracellular, epicellular) and environmental factors. As for other pathogens, the epidemiological/disease triangle can be attributed to parasites.

In aquaculture, where biomass is commonly high, fish generally live under sub-optimal conditions, which can induce stress that can cause immunosuppression in the fish, making it more susceptible to infections. Furthermore, the high biomass increases the possibility of transmission of pathogens between fish. In wild populations parasitic diseases mostly go unnoticed, unless they lead to rejection of the commercial product or cause an epidemic with high mortality rates. Consequently, the effect of parasitic diseases on wild fish is likely to be underestimated in many cases (Wooten 2012).

1.3.1 Protozoa

Amoebae

Amoebae are a diverse group of protozoans that belong to the phylum Amoebozoa Lühe 1913 (Adl et al. 2012). They have a simple cell structure where the membrane can be bare or with an external test. An internal skeleton is present in some groups. Their locomotive form includes pseudopodia that they are able to extend and retract. Reproduction can be either asexual, by binary or multiple fission, or sexual, by means of flagellated or amoeboid gametes (Lom and Dyková 1992). Amoebas are ubiquitous in nature and found globally in water, soil and air (Martines and Visvesvara 1997), mostly as free-living organisms (Lom and Dyková 1992) but also as commensal, a symbiotic relationship from which the amoebas benefit while the other organisms remain unaffected. However, some amoebas are opportunistic parasites (Lom and Dyková 1992) causing serious diseases in humans and lower vertebrates as well as invertebrates. Infections can either be systematic or affect single organs, which are difficult to identify and hence their prevalence is probably underestimated. Histological examination has been the dominant research method for host response, which can range from hyperplasia in gill infections to acute or chronic inflammation in internal organs (Dyková et al. 2005; Constenla and Patrós 2010; Dyková et al. 2010). Why they become parasitic is not well known but unfavourable environmental factors are thought to play a vital role.

Amoebic gill disease (AGD) is a serious disease of farmed marine fish. It is caused by *Neoparamoeba perurans*, and has caused severe economic losses in aquaculture worldwide, as the absence of treatment results in mortality (Bridle et al. 2010; Nowak et al. 2014).

Apicomplexa Levine, 1970

Apicomplexans are a vast collection of single celled eukaryotic organisms, known to parasitize both vertebrate and invertebrate hosts in terrestrial and aquatic environments. They exhibit a wide variety of morphological forms, depending on their genus and life cycle stage (Šlapeta et al. 2003).

The taxonomy of apicomplexans is a highly dynamic field, which can be attributed to their complex life cycle that in some cases has resulted in various stages of the same apicomplexan species being re-described as new species (Duszynski 2011). Also, the majority of species descriptions are based on the morphological features of the oocysts, which are highly resistant to known fixative techniques; therefore no satisfactory method is known to permanently preserve its structural features. Even so, with over 6000 species currently described, the number of apicomplexan species is still thought to be grossly underestimated (Adl et al. 2007). As the order Coccidia accounts for the majority of apicomplexans that parasitize fish, it will be the main subject of this chapter. Coccidia comprises two suborders, i.e. Adeleorina Léger 1911, including species of the genus *Haemoggregarina*, and Eimeriorina Léger 1911, a particularly speciose group, containing e.g. *Eimeria* and *Goussia* species.

The life history characteristics of coccidians are discussed in detail by Lom and Dyková (1992) and will be briefly summarized below. Apicomplexans can either be homoxenous (only one host required for life cycle) or heteroxenous (more than one host required). They

are obligate intracellular parasites which go through three stages of proliferation, merogony, the asexual stage, and gamogony and sporogony, the two stages of sexual reproduction which end product are the sporozoites, the infective form (Figure 1.2).

A simplified life cycle is as follows: The sporozoite enters a host cell and subsequently transforms into a meront, which initiates merogony. During this process the meront produces numerous cells called merozoites, normally by multiple fission but binary fission and budding is also known. The merozoites then enter neighbouring host cells by induced phagocytosis or active penetration. There they are able to repeat merogony several times and produce several generations of merozoites. When the last generation of merozoites enter a host cell they alter and develop into macrogamonts (corresponding to eggs) or a male microgamonts (sperm sac). Macrogamonts become enlarged macrogametes while the microgamonts divide and produce numerous mature microgametes. Whether they are flagellated or not, can vary between groups. These anisogamous gametes subsequently merge giving rise to zygotes, which initiate the last proliferative stage, the sporogony. During sporogony, division of the zygote and secretion of a protective coat, or the oocyst wall, forms an oocyst harbouring two or more sporocysts, each containing two infective sporozoites. By their outburst the stages of proliferation and development can start anew. There are two known mechanisms for sporozoites release. The former is when there is an apical opening plugged by a stieda body on the sporocyst shell. This is the case for e.g. many *Eimeria* species. The latter is when two equally sized halves of the sporocyst wall separate and the sporozoites are released; this is typical for many *Goussia* species.

The main feature all apicomplexans share is the apical complex (Levine 1961), which is situated in the anterior end of the invasive stages, i.e. the merozoites, sporozoites and ookinetes, which are the fertilized female gamete of some groups. This complex consists of several organelles visible with an electric microscope: the conoid, polar rings, subpellicular microtubules, rhoptries and micronemes. The number and shape of these organelles can vary between groups (Blackman and Bannister 2001; Oborník et al. 2009). The apical complex is absorbed during the transformation of sporozoites into meronts and merozoites into gamonts. Furthermore, this intricate apical complex plays a vital role in host cell invasion. The micronemes are thought to play a role in host cell recognition as well as binding and possibly motility. The club shaped rhoptries are used for the formation of the parasitaphorous vacuole (PV) (Debremetz 1998), which surrounds the parasite inside the host cell (Lom and Dyková 1992; Bannister and Mitchell 1989). Dense granules then remodel the PV into a metabolically active compartment (Debrametz 1998).

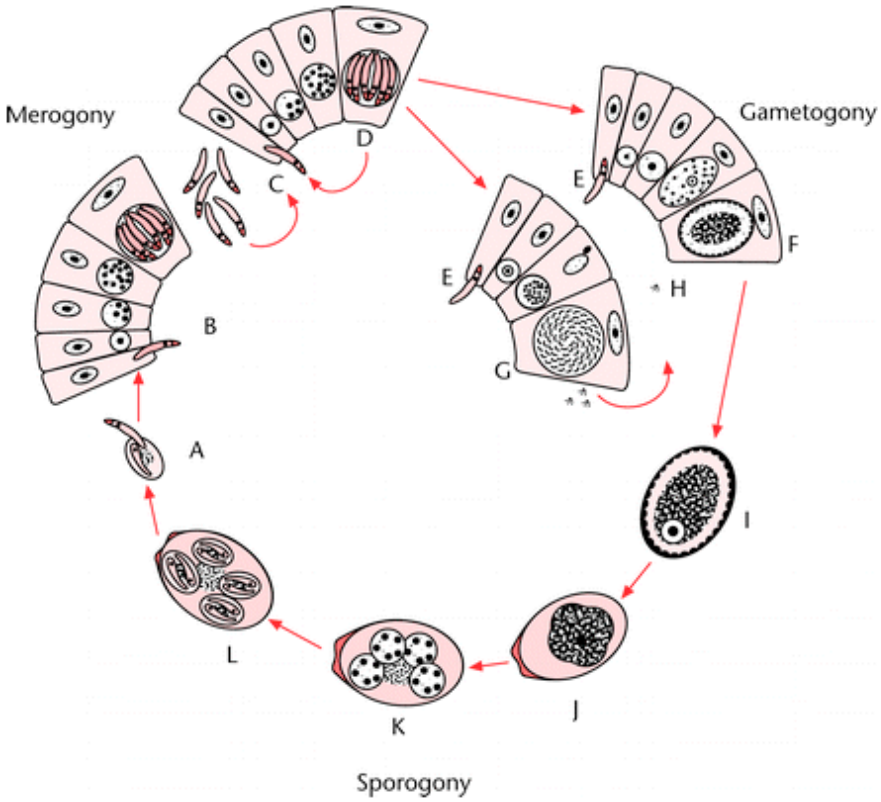

Figure 1.2. *Simplified apicomplexan life cycle, exemplified by a typical eimeriid parasite (from Barta 2001, Figure 1). (A) Sporozoites penetrate host cells and initiate merogony (B-D). After one or more cycles gametogony begins (E) resulting in macrogametes (F) or microgametes (G), which fertilize (H) the macrogamete. The zygote (I) develops into an oocyst during sporogony (J-L)*

The most eminent of the apicomplexan parasites are probably *Plasmodium* spp., which cause malaria, responsible for over 1 million human deaths annually (Manguin et al. 2010). Apicomplexans are common parasites of fish in marine-, brackish- and freshwater (Molnár et al. 2012). Most of the fish coccidians invade the digestive tract, although many infect other organs such as liver, kidney, spleen, swim bladder, urinary bladder and gall bladder. According to Dyková and Lom (1981) the pathology of coccidians have been underestimated, e.g. due to the lack of host response to infections.

Dinoflagellata Bütschli, 1885

As previously mentioned, the phylum Dinoflagellata is included in superpylum Alveolata. The phylum consists of three classes: Noctilucales Haeckel 1894, Dinophyceae Pascher 1914 and Incertae sedis Dinoflagellata (Adl et al. 2012).

The anterior part of the dinoflagellates body is called the episome (also epitheca or epicone) whereas the posterior part is the hyposome (or hypotheca or hypocone). The cell is coated with a three-membrane complex that in some cases has thecal plates as a shield. The trophozoites have two grooves that hold unequal flagella: one is the cingulum that lays

horizontally and within it the transversal flagellum. The other is the vertical sulcus, where the longitudinal flagellum is found. Some groups have two or more monomorphic nuclei but a single nucleus, the unique dinokaryon, is the most common. It is not composed of typical chromatin as it lacks histones and is visible through interphase. In many parasitic species, exceptions can be found as the basic characteristics of dinoflagellates are extremely modified (Lom and Dyková 1992).

This group has been studied both by botanists and zoologists because of their great diversity of nutritional strategies. Many dinoflagellates are highly important primary producers in aquatic biotopes, however, not all are photosynthetic as phototrophic, heterotrophic and mixothrophic dinoflagellates can be found. Phototrophs may be free-living or occur as endosymbionts whereas heterotrophs are apoplastic or, if mixotrophic, contain chloroplasts. They may even use foreign chloroplasts for photosynthesis. Most of the parasitic dinoflagellates are ectoparasites, some intracellular, living on or in aquatic animals such as fish. The life cycle of the ectoparasites involves a feeding and growing stage, called the trophont. It develops an apparatus for attachment and feeding that extends from the sulcus. When it is large enough it disengages from its host and becomes a tomont. The tomont divides several times and the result is a free-swimming, migratory and infectious stage, called a dinospore or gymnospore. This stage is often the only state of the organism that is recognizable as a dinoflagellate because of its parasitizing adaptations. Reproduction in or on the fish is generally by longitudinal binary fission. However, sexual processes are known and are carried out by syngamy (Lom and Dyková 1992).

The most common dinoflagellate parasitizing fish is *Amyloodinium ocellatum,* which is considered among the most important pathogen of marine fish. It causes serious morbidity and outbreaks that can result in 100% mortality within days (Woo et al. 2011). Repeated outbreaks of infestation by the parasite in cultured gilthead bream (*Sparus aurata*) and seabass (*Dicentrarchus labrax)* have been noted since 1977 and caused severe economical loss (Paperna 1980). New hosts are frequently recorded, the latest being in farmed mearge (*Argyrosomus regius*) in Portugal (Soares et al. 2012). *Piscinoodinium* causes a disease similar to *Amyloodinium*, but in tropical freshwater fish. It has the same life cycle and is morphologically alike. *Ichthyodinium chabelardi* parasitizes the eggs of several marine species. High prevalences have been observed in Atlantic sardines (*Sardina pilchardus*), with up to 50% of examined eggs being infected (Woo et al. 2011). Recently, *I. chabelardi* was identified in the eggs and yolk sack larvae of European eel (*Anguilla anguilla)* and blue fin tuna (*Thunnus orientalis*), respectively (Ishimaru et al. 2012; Sørensen et al. 2014).

Ciliophora Doflein, 1901

As the apicomplexans and dinoflagellates, the ciliates (Phylum Ciliophora) belong to the Superphylum Alveolata (Adl et al. 2012). The ciliates split into two sub-phyla: Postciliodesmatophora Gerassimova and Seravin, 1976 and Intramacronucleata Lynn, 1996. Free-living ciliates are ubiquitous in aquatic environments and are found in diverse habitats at both temperature extremes. Symbiotic species are found as commensals of sea urchins or parasitic on or in both invertebrates and vertebrates such as fish (Lynn 2001).

These unicellular organisms are coated by a pellicle that is covered by cilia. In some groups the ciliature is extremely reduced or even not present, however, ciliates typically have cilia at some stage of their life cycle that are used for various purposes such as

locomotion, attachment or feeding. They can be single or in a group that forms a specialized organelle and can be associated with the buccal apparatus, which serves for ingestion of granular food. Not all groups have a well-developed buccal apparatus; therefore some groups feed by pinocystosis or by polystomy (Lom and Dyková 1992).

Ciliates show nuclear dimorphism as they have one or several polyploid macronuclei and at least one diploid micronucleus. The small micronucleus, low in RNA, contains the full ciliate genome, while the large macronucleus is RNA rich and contains an incomplete genome. The periphery of the cell is called the cortex, which consists of the outer cell membrane, pellicular alveoli and the epiplasm. The cortex is supported by microtubules, microfilaments and kinetostomes that form a kinetid, which is localized at the kinetostomal territory. Finally, the entire system of all kinetids of a ciliate is called the kinetome (Lom and Dyková 1992).

Most ciliates have direct life cycles, although complex life cycles occur in the group. Reproduction can be either asexual, by simple division, or sexual through a process known as conjugation.

Some ciliates parasitizing fish can be serious pathogens, e.g. species like *Tetrahymena* spp. and *Ichthyophthirius* spp., which cause destruction gills and skin tissue that can result in death. As for many other parasites, problems related to ciliate species, such as *Trichodina* spp., *Brooklynella* spp. and *Cryptocaryon* spp. are much less common in wild fish populations than in aquaculture where they can seriously impact the health of the fish (Lom and Dyková 1992). Many ciliates, like *Trichodina* spp. and *Uronema marinum* are opportunistic parasites, with extremely low host specificity and a wide geographic distribution (Cheung et al. 1980).

1.3.2 Myxozoa Grassé, 1970

The myxozoans (unranked sub-phylum Myxozoa) were formerly classified as protistans. However, with the emergence of molecular approaches, they are now accepted as members of the phylum Cnidaria Verrill, 1865 (Cairns et al. 2009), and hence relatives of squids and octopuses. To date, the myxozoans are comprised of two classes: Myxosporea Bütschli, 1882 and Malacosporea Canning et al., 2000, the latter including only two genera, i.e. *Tetracapsuloides* Canning et al., 2002 and *Buddenbrockia* Schröder, 1910 and four described species. Conversely, the myxosporeans are an extremely diverse group containing over 2000 nominal species. In older literature Myxosporea and Actinosporea are classified as separate phyla but the discovery of a two-stage myxosporean life cycle by Markiw and Wolf (1983) lead to the recognition that the two groups were alternate life stages of same etiology, i.e. the myxosporean stage, found almost exclusively in fish (occasional findings amphibians and higher vertebrates) and the actinospore stage in oligochaetes or polychaetes (Figure 3) (Lom and Dyková 2006; Yokoyama et al. 2012). Both life stages have the characteristic cnidarian polar capsules, usually 3 in actinospores (can also be only 1) and 2 in myxospores (can be 1-10). When the actinospore encounters a fish after being released from its annelid host, the polar capsules discharge their polar filaments, which attach to the fish and force the infective material, the sporoplasm, from between the valve shells into the host. Entry gateways vary between species but include skin, gills, fins, buccal cavity, stomach and intestine, however, mucosal tissues seem to be the primary invasion route (Lom and Dyková 2006; Goméz 2014). After the invasion, an asexual replicative phase called the presporogonic phase follows. Meanwhile, the parasite

migrates to the final infection site and forms plasmodia or pseudoplasmodia that begin spore development during the sporogonic phase. Plasmodial stages can be histozoic, i.e. found in host tissues (intracellular, intercellular or epicellular) or coelozoic and found in body cavities, frequently in the gall bladder and urinary bladder. Large plasmodia produce numerous spores and are therefore polysporic while the pseudoplasmodia compose one or two spores, making them mono- or disporic. The myxospores mainly consist of the polar capsules and the infective sporoplasms, which are enclosed in two to seven shell valves that are joint at the suture lines. When spores are released from the fish, e.g. by urine, feces or upon its death, they encounter their annelid and definite host where the sexual phase takes place. After ingestion by the annelid, the myxospores open and release the sporoplasm, which then penetrates the intestinal epithelium where some species continue their development, while others do so in the body cavity or -wall. After the fusion of α and β gametic cells, which form zygotes in the pansporocyst, the development of 4 to 8 actinospores is complete (Lom and Dyková 2006; Gómez 2014).

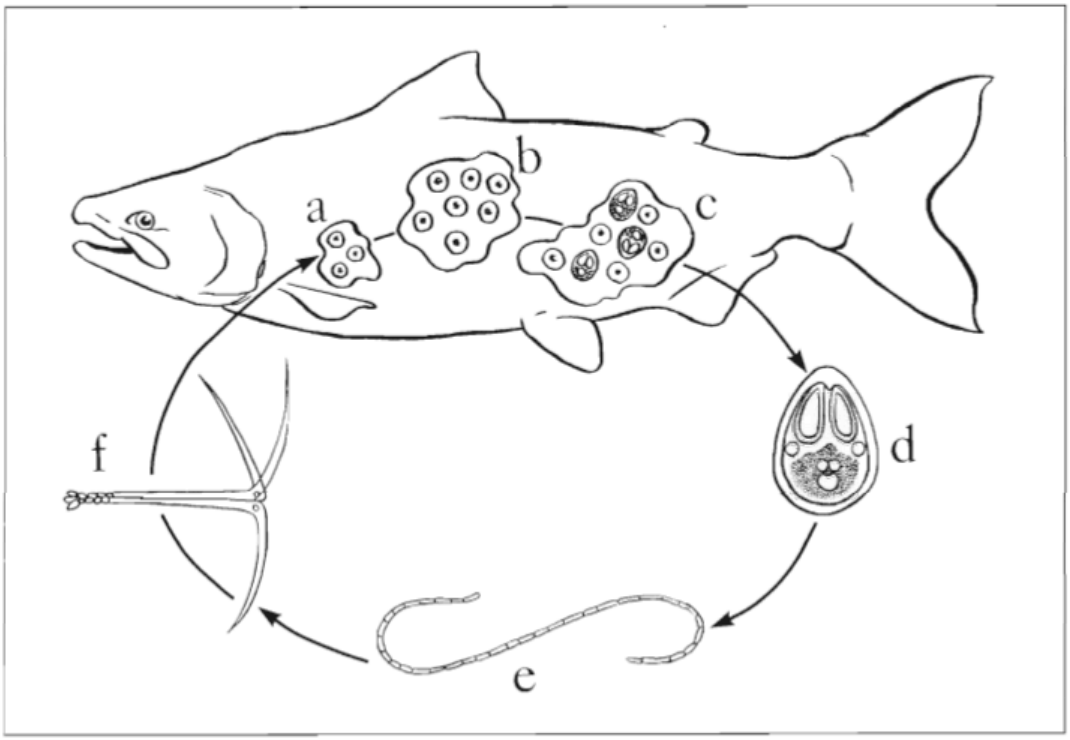

Figure 1.3. *Simplified life cycle and development of the Myxozoa, exemplified by Myxobolus sp. (from Kent 1992, Figure 17). (a-b) Presporogonic phase. (c) Development of myxospores during sporogonic phase. (d) Myxospore. (e) Annelid host. (f) Actinospore.*

Many freshwater myxosporeans life cycles have been fully described, however, such descriptions are mostly missing for marine species (Mackenzie and Kalavati 2014). Nevertheless, marine myxosporeans have been reported as pathogens in wild and farmed fish with evidence of direct fish to fish transmission (Diamant 1997; Moran et al. 1999; Kent et al. 2001; Redondo et al. 2002; MacKenzie et al. 2005). Generally, histozoic species are thought to be more problematic than the coelozoic, e.g. *Ceratomyxa shasta* and *Myxobolus cerebralis* (Nehlsen et al. 1991; Baldwin et al. 2000), both serious pathogens of

salomonids and *Kudoa* species which can cause post-mortem myoliquefaction, causing considerable economical loss (Kristmundsson and Freeman 2014). Compared to freshwater myxosporeans, many marine species have low host specificity, e.g. *Kudoa thyrsites, K. yasunagai* and *Enteromyxum leei* (Yogoyama et al. 2012). The use of myxozoans as biological tags has been implemented in several cases. For example, some gall bladder myxosporeans have been used in population studies and stock identification of gadoid fishes, such as whiting, haddock and cod (MacKenzie et al. 2005). However, the duration of infection in the fish host is unknown and therefore the use could be limited.

1.3.3 Microsporidia

Microsporidia is one of many groups once classified as protistans. However, to date, it is recognized as a phylum belonging to Fungi. Fishes are known to host over a hundred species of microsporidia assigned to 14 genera, including the notorious *Glugea* Thélohan, 1891, *Loma* Morrison and *Sprague*, 1981 (Lom and Nilsen 2003).

The microsporidians are eukaryotic, unicellular organisms. They are obligate intracellular parasites that form spores and invade all major animal groups. The main characteristic feature of the group is the hatching apparatus, which consists mainly of a single polar tube that is coiled around the interior of the spore. It is used to infiltrate the host cell and they do so by discharging the tube from the anterior pole of the spore. Consequently, it can pierce a neighbouring cell and inject the sporoplasm into that cell (Lom and Dyková 1992). Some genera (e.g. *Kabatana*) are found in the cytoplasm with no boundaries. However, the vast majority of microsporidians transform the host cell (normally a macrophage or leucocyte) into a xenoma, a special formation where the development of the parasite takes place (Lom and Nilsen 2003).

Fish microsporidia have simple life cycles that include merogony and sporogony. The proliferative phase of merogony produces a great number of parasites as the meronts generally divide by multiple fission. The following phase, sporogony, leads to the production of spores when meronts differentiate into sporonts, which may have special organelles. The sporont divides into two sporoblasts or grows into a multinucleate sporogonial plasmodium that later divides into sporoblasts (Lom and Dyková 1992). The formation of the hatching apparatus organelles can either begin in the sporogonial plasmodium (only known in *Nucleospora*) or in the already separated sporoblast. Within species spore structure is congruent, except for the genera *Pleistophora*, *Ovipleistophora* and *Heterosporis*, which produce macro- and microspores that vary in size and polar tube turns (Lom and Nilsen 2003). Spores are then released to the environment and transmitted through ingestion of the new host, when the xenoma ruptures or the host dies (Wooten 2012). Many microsporidians cause serious diseases in freshwater and marine fishes. Examples are *Glugea stephani* and *Loma branchialis*, which are found in economically important species, such as winter flounder (*Pleuronectes americanus*) and Atlantic cod (*Gadus morhua*), respectively (Morrison 1983; Khan 2004). Furthermore, *Glugea hertwigi* was found responsible for millions of dead smelt (*Osmerus mordax*) (Nepszy et al. 1978) and *Loma salmonae* remains a threat to freshwater and marine salmonids, causing microsporial gill disease (Kent et al. 1989; Becker and Speare 2007).

2 Materials and Methods

2.1 Sample Collection

2.1.1 Samples for analyses of long term data on *Sphyrion lumpi* infestations

A total of 41,405 *Sebastes mentella* specimens from the commercial fishery were collected during the period of 1995-2013. The data collection was in a collaborative assignment between commercial vessels and the Marine Research Institute of Iceland (MRI). Samples were sent frozen to the MRI laboratory for further examination. Each sample consisted of about 100 fish and 2-4 samples were collected from each commercial trip. Sampling varied spatiotemporally, where most fish (30,864 specimens) were caught in May to July at 200-900 meters depth southwest off Iceland.

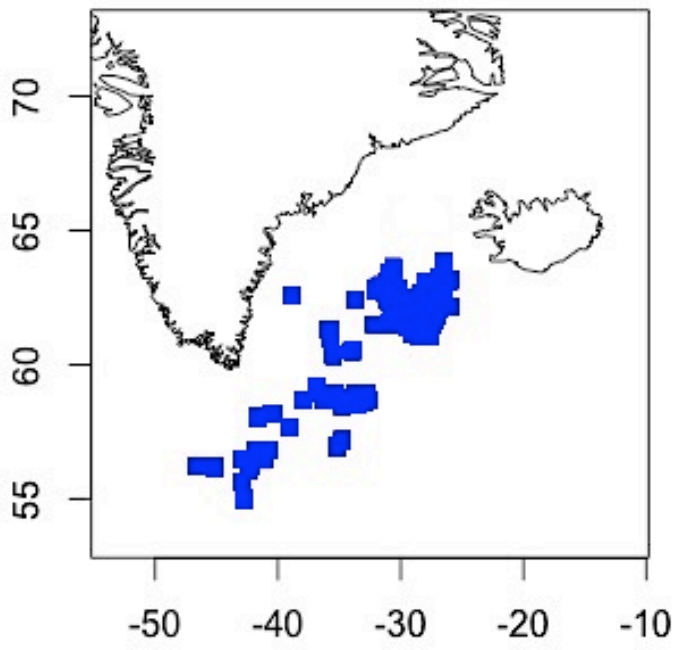

***Figure 2.1**. Sampling sites of Sebastes mentella specimens during the years 1995-2013.*

A routine examination of each specimen of *S. mentella* included; total fish length (cm), weight (g), sex and maturity stage. Furthermore, for fish aging, otoliths were collected from 50 fish per sample. All signs of infestation of the parasite *Sphyrion lumpi*, including lesions, abnormal pigmentation and muscular pigmentation, were systematically recorded in all cases. Five categories of external abnormalities were applied; black spots (B), red spots (R), mixed spots (RB), remnants or lesions caused by *S. lumpi* and the parasite itself. The sites of infections and/or abnormalities were accurately noted according to four defined body sections (Figure 2.2) on each side of the fish. Regarding external pigmentation, section A was excluded. The muscular pigmentation was categorized as light, medium or severe.

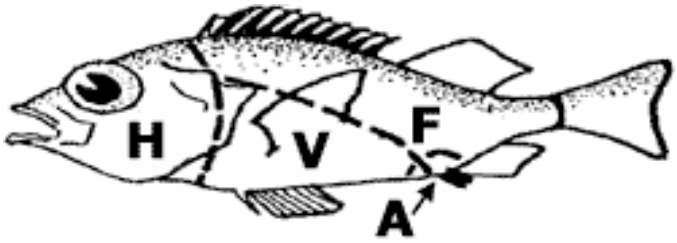

***Figure 2.2**. Defined body sections of Sebastes mentella. H, head; V, ventral; F, fillet; A, anal.*

2.1.2 Samples for Parasitological examination

General screening of parasites

A total of 35 individuals of *Sebastes norvegicus and Sebastes mentella*, from five different sampling times and -sites, were subjected to general parasitological examination.

(i) Four deep pealgic *Sebastes mentella* were collected in October 2013 from three different sites during the autumn survey of the MRI. (ii) Five *S. mentella* and five *S. norvegicus* were collected during the MRI spring survey in April 2014 from one site east off Iceland, (iii) six redfish were obtained from the Icelandic Fish Market (FMSI) in April 2014, one shallow pelagic *S. mentella* and five *S. norvegicus* that were caught by a commercial trawler Klakki, north off Iceland, (vi) five *S. norvegicus*, caught west off Iceland in July 2014, were obtained from the seafood company HB Grandi (v) ten *S. norvegicus*, caught by a commercial trawler south off Iceland were collected from HB Grandi in August 2014.

All fish were frozen and brought to the Fish disease Laboratory at Keldur for further analysis.

Examination of Sphyrion lumpi

Samples from four deep pelagic *S. mentella* were collected in October 2013 from three different sites during the autumn survey of the MRI were specifically examined with regards to *Sphyrion lumpi*. The samples consisted of one attached adult female *S. lumpi* per fish, except for one fish that had two attached parasites. The *S. lumpi* specimens were cut off its host on board, along with host tissue surrounding the attachment site. Four copepods were fixed in 10% buffered formalin for histologic examination and one in 96% ethanol for DNA analysis.

2.2 Statistical Analyses

As none of the data on *S. lumpi* infestations and abnormal pigmentation were available in a digital format, they were manually entered using Excel (version 14.4.7) before the data could be analysed.

Linear regression models (LM) were used to investigate the relationship between sampling time and three variables, i.e. mean infection intensity of *S. lumpi* on *S. mentella,* prevalence of the parasite and fish condition. For that purpose the Fulton's (1902) condition factor (K) calculated as follows:

$$K = 100(W / L^3)$$

Where W is the whole body wet weight in grams and L is total fish length in centimetres.

Prevalence was determined as the proportion of infected fish among all the fish examined and mean infection intensity was expressed as the mean number of parasites and post mortem remnants found in the infected fish excluding uninfected hosts. Pearson product-moment correlation coefficient (r) was calculated to examine the relationship between mean infection intensity and three variables, i.e. depth, age and total length of fish. The shallow pelagic and deep pelagic stocks were compared using t test or, when comparing spatial groups, the non-parametric Wilcoxon test. 95% confidence intervals (CI) were calculated and applied to proportions and the standard error of means (SEM) was applied to mean infection intensity. They were calculated as follows:

$$CI = \rho \pm z \times \sqrt{(\rho(1-\rho) / n)}$$

Where ρ is the sample proportion, n is the sample size and z is the appropriate value from the standard normal distribution for the desired confidence level, in this case 1.96.

$$SEM = s / \sqrt{n}$$

Where s is the estimated standard deviation of the population distribution and n is the sample size.

Mean intensity of *S. lumpi* was compared between body sections using the non-parametric Kruskal-Wallis test. The sectional distribution of *S. lumpi* and abnormal pigmentation on the skin were examined using ANOVA.

All statistical tests and plots were performed using RStudio (version 0.98.1062).

2.3 Parasitological Examination

2.2.1 Dissection and examination of wet mounts

Examination was carried out at the Fish Disease Laboratory at Keldur and were limited to microparasites (Protozoa, Myxozoa, Microsporidia). All fish were kept frozen at -20°C until examined. Prior to examination, the fish were thawed overnight. Initially, gill arches and the fish exterior were thoroughly examined with the naked eye and through a stereoscope for any abnormalities indicative of parasitic infections. Subsequently, tissue smears (diluted in phosphate-buffered saline - PBS) were prepared from gills, eyes, brain, spinal cord and lateral line and microscopically examined for parasites. Following that, the visceral cavity was cut open, and all organs monitored for macroscopic abnormalities and smears and scrapings from all major organs (heart, brain, liver, kidney, spleen, gastrointestinal tract, gall bladder, urinary bladder and swim bladder) prepared and examined thoroughly using a compound microscope. Lastly, slices of the skeletal muscle were homogenized in PBS using a pestle and mortar, filtered with a strainer and the fluid centrifuged at 1500 g for five minutes before the pellet was microscopically examined.

The identification of parasites was based on morphological characters of mature myxozoan spores and apicomplexan oocysts according to Lom and Dyková (1992). These characters include the number and shape of sporocysts and sporozoites, the shape and size of oocysts and the presence of a Stieda body.

2.2.2 Histological Examination

Histological examination can be divided into two categories: (i) Samples for examination of host reaction to *Sphyrion lumpi* infections as well as general histology of the parasite itself. Those included both whole animals and fish tissue at the site of the infection. (ii) Samples for general histopathological examination with regards to parasitic infections. Those included tissue samples from all major organs of redfish, i.e. gills, heart, brain, spinal cord, liver, kidney, spleen, gall bladder, urinary bladder, swim bladder, pyloric caeca, stomach and intestine.

All samples were fixed in 10% buffered formalin and prepared for histological examination according to routine protocols, i.e. embedded in paraffin wax, cut in to 4 µm thick sections, stained with Giemsa and mounted in resin based medium. The stained histological slides were thoroughly screened for parasites and histopathological changes using a compound microscope.

2.2.3 PCR Amplification and Sequencing

Parasite tissue, host tissue or scrapings from host tissue containing parasites were placed in 260 µl of Lyse T and 10 µl protease K and extracted according to directions from GeneMATRIX Tissue DNA purification Kit by EURx.

The target region for PCR amplification was the 18S small subunit (SSU) ribosomal RNA (rRNA) gene. The oligonucleotide primers used to amplify the target regions are listed in table 2.1. The primers SFC-340f, SFC-1260r, SFC-1120f, 18gM, Gou-380r, CAP-250f, 830r were used to amplify apicomplexan species. 606fwd, 1415rev, 830r, 606rev, 1415fwd and 1830r were used to amplify myxosporeans species. 390fwd, 870fwd, 18gM and 870 rev were used to amplify the copepod.

Table 2.1. *List of primers used in this study.*

Primer name	Sequence (5'-3')	Primer name	Sequence (5'-3')
390fwd	AGAGGGAGCCTGAGAAACG	**1830r**	TCTAAGGGCATCACAGACCTG
870rev	GTTGAGTCAAATTAAGCCGCA	**830r**	TTCCGCTTGCTTTGAGCAC
870fwd	TGCGGCTTAATTTGACTCAAC	**CAP-250f**	TCATATAACCGAACGAATCGC
18gM	CTTCCGCTGGTTCACCTACG	**SFC-1120f**	GAACGAAAGTTGGGGTCG
606fwd	TGCGTTTAAAACGCTCGTAG	**gou-380r**	GTTACCCGTCACTGCCACG
606rev	CTACGAGCGTTTTAAACGCA	**SFC-340f**	AGTTTCTGACCTATCAGC
1415fwd	TTAGTTCGTGGAGTGATCTG	**SFC-1260r**	TCAGCCTTGCGACCATACTC
1415rev	CAGATCACTCCACGAACTAA		

All PCRs were performed in 20 µl volumes containing dNTPs 0.2 mM, primers 0.8 µM, iTaq polymerase 0.02 U/µl, 2 µl 10× buffer PCR and purified H_2O to achieve the correct final volume. PCR conditions were as follows: Initial DNA denaturation at 95°C for 4 minutes, followed with 37 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds and elongation at 72°C for 45 seconds, and then final elongation 72°C for 7 minutes.

All PCR amplifications were performed in 2720 Thermal Cycler from Applied Biosystems and the PCR products were subsequently visualized in an ethidium bromide- stained 1% agarose gel with loading dye and a 100bp ladder for amplicon size estimation.

Positive PCR products of expected sizes were purified using a PCR purification kit from EURx and sent to First BASE Laboratories Sdn Bhd in Malaysia for sequencing. Nucleotide Basic Local Alignment Search Tool (BLAST) searches was performed for each sequence to confirm their origin.

2.2.4 Phylogenetic Analysis

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000). Trees with the highest log likelihood were made with available sequences obtained from PubMed and sequences from this study. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analyses involved 67 nucleotide sequences. Phylogenetic analyses were conducted in MEGA6 (Tamura et al. 2013).

3 Results

3.1 Infestation by *Sphyrion lumpi* and abnormal pigmentation

The relationship between weight and length for *Sebastes mentella* is strong and statistically significant (n =41,405, Pearson, r = 0.91, p < 0.001). The intensity of *Sphyrion lumpi* infections seems to have no effect on the condition (K) of the fish (n = 9,199, Pearson, r = 0.07, p > 0.05, Figure 3.1).

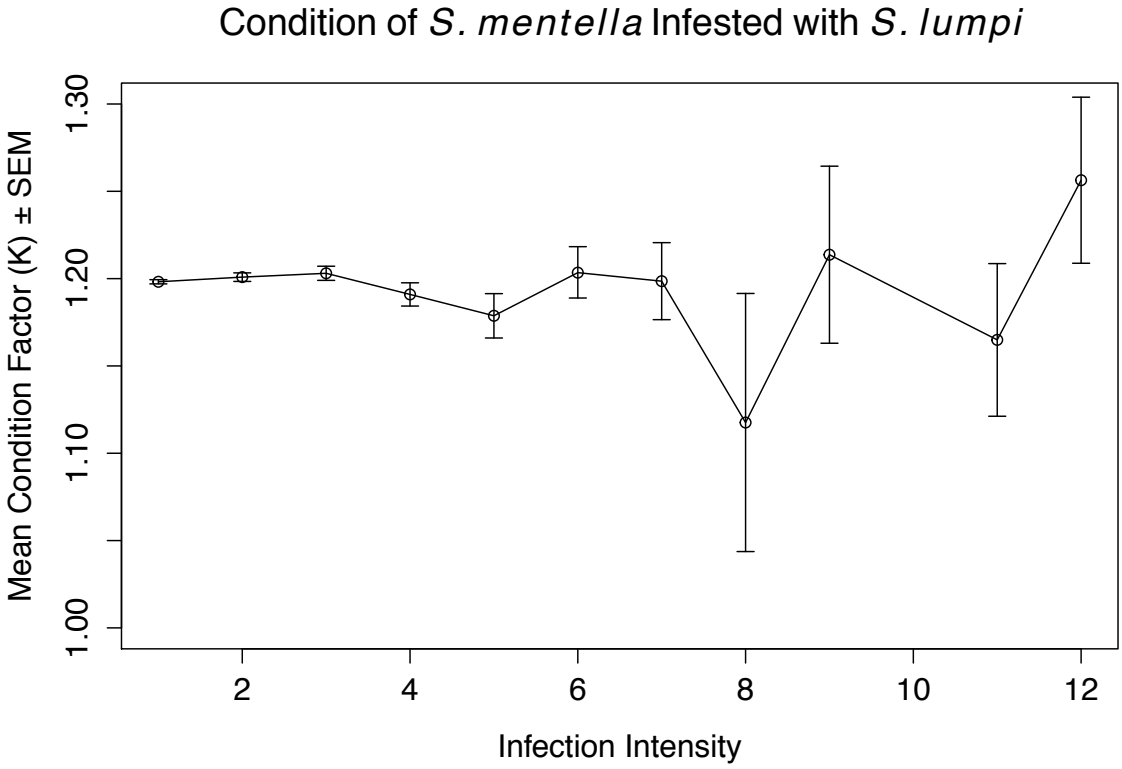

Figure 3.1 *The relationship between the condition (K) of S. mentella and infection intensity of S. lumpi ± SEM.*

The condition of *S. mentella* infested with the copepod (Figure 3.2, green) seems to harmonize with the condition of those without *S. lumpi* (Figure 3.2, orange), as they do not differ significantly (t test, $p > 0.05$). The condition of the fish declines somewhat with time, i.e. from 1.20 for both groups in 1995 to 1.13 for infested fish (LM, n = 9,199, $R^2 = 0.75$, $p < 0.001$) and 1.16 for fish without the copepod (LM, n = 32,206, $R^2 = 0.49$, $p < 0.001$) in 2013.

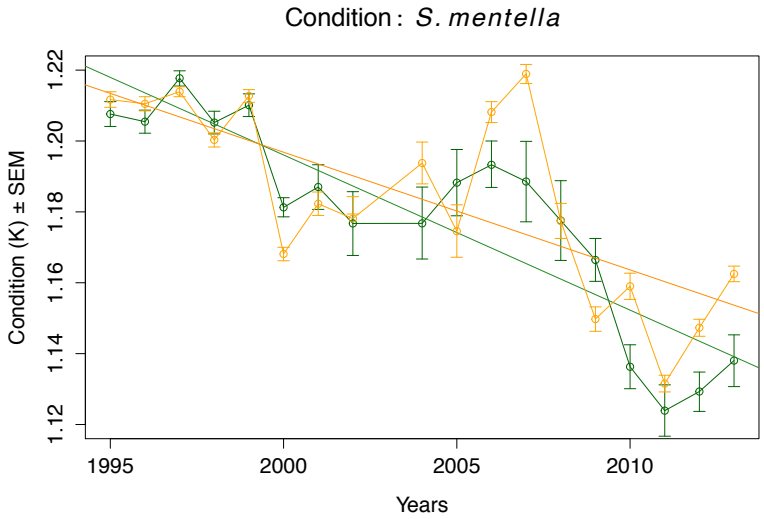

Figure 3.2. *Condition of S. mentella infested with S. lumpi (green) and those not infested (orange) ± SEM from 1995-2013 with regression lines.*

Over the period 1995-2013 the prevalence of visual external infestation and abnormal pigmentation was 31% for the fish examined. Of those, 3.7% were infested with living *S. lumpi* with as many as 15 individuals per fish while 22.2% were infested with living *S. lumpi* and/or it's post mortem remnants. Overall, the prevalence of *S. lumpi* infections declined from 25% in 1995 to 9% in 2013 (LM, n = 41,405, $R^2 = 0.59$, $p < 0.001$) with a peak of 28.6% in 1997 (Figure 3.3).

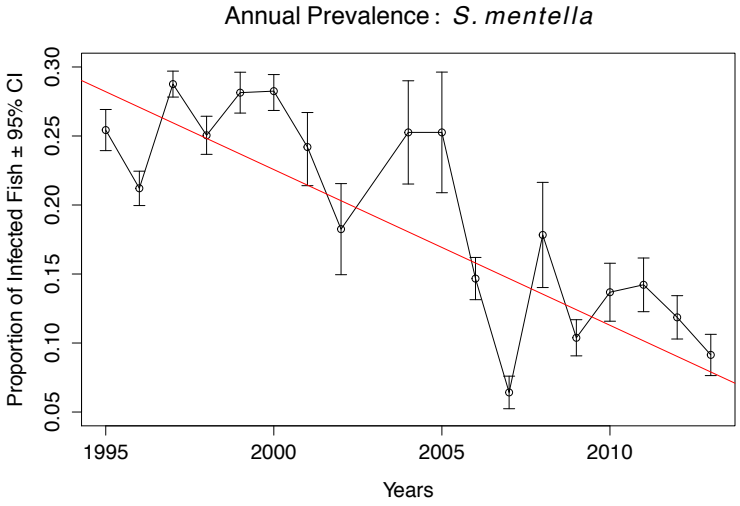

Figure 3.3. *Annual prevalence of S. lumpi infestation on S. mentella from 1995 to 2013 with regression line (red) and 95% confidence intervals.*

When prevalence figures are viewed separately for the two stocks the annual prevalence of infections in the shallow pelagic stock (Figure 3.4, blue) does not decrease like the deep pelagic stock (Figure 3.4, red). *S. lumpi* infections have declined in the deep pelagic stock (n = 29,142) from 24% in 1995 to 6% in 2013 (LM, $R^2 = 0.59$, $p < 0.001$), whereas the prevalence has increased in the shallow pelagic stock (n = 12,263) from 25% to 32% during the same period (LM, $R^2 = -0.04$, $p > 0.05$).

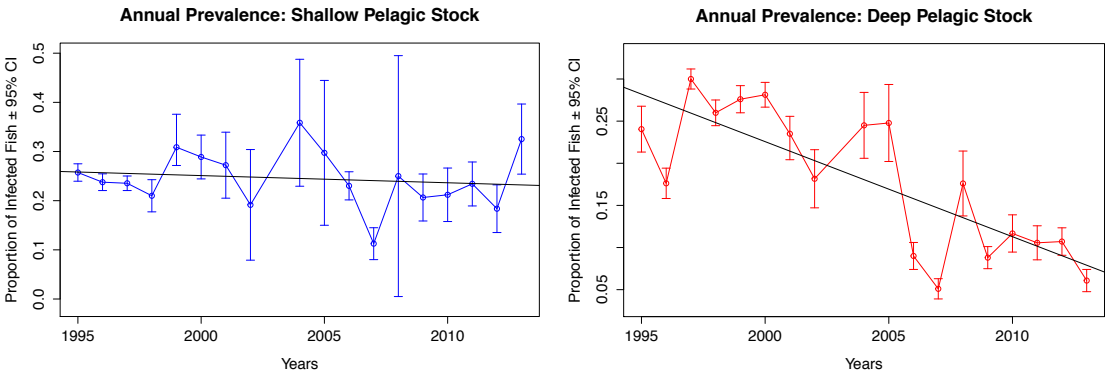

Figure 3.4. *Annual prevalence of S. lumpi infections for the shallow pelagic stock (left) and deep pelagic stock (right) of S. mentella from 1995-2013 with regression lines (black) and 95% confidence intervals.*

The annual mean infection intensity shows that the shallow pelagic stock (n = 3,040) and deep pelagic stock (n = 6,159) differ significantly (t test, $p < 0.0001$, Figure 3.5) during the research period, where the shallow pelagic stock (blue) is generally more heavily infested than the deep pelagic stock (red).

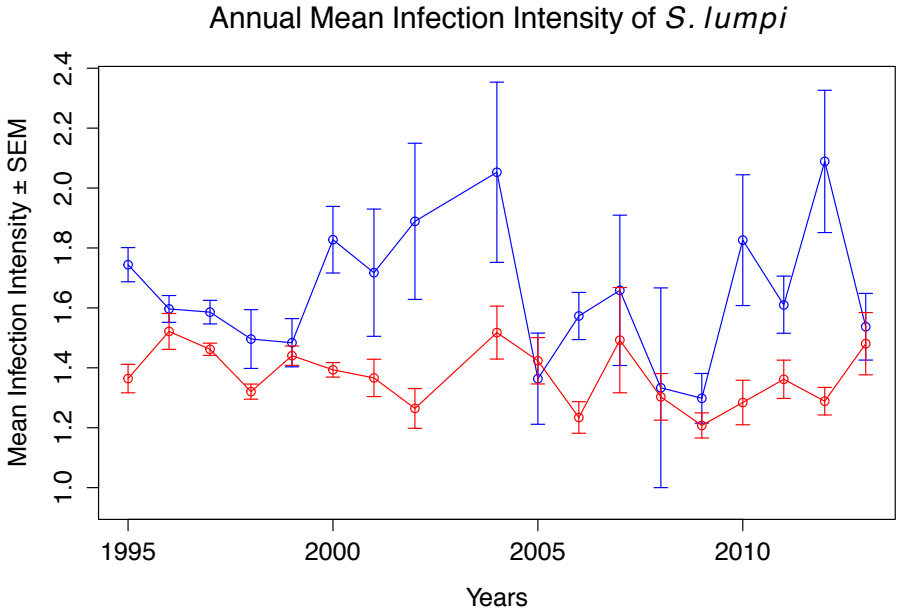

Figure 3.5. *Mean infection intensity by Sphyrion lumpi on shallow pelagic (blue) and deep pelagic (red) stocks from 1995 to 2013 ± SEM.*

The mean intensity of infections significantly differs between the stocks (t test, $p < 0.001$) with regards to depth (Figure 3.6) where the shallow pelagic stock (n = 2,786) has a higher infection intensity than the deep pelagic stock (n = 5,265). Mean infection intensity does not show a significant correlation with depth in the deep pelagic stock (Pearson, $r = -0.02$, $p > 0.05$). The shallow pelagic stock shows a strong negative correlation between mean infection intensity and depth, but it is not significant (Pearson, $r = -0.98$, $p > 0.05$)

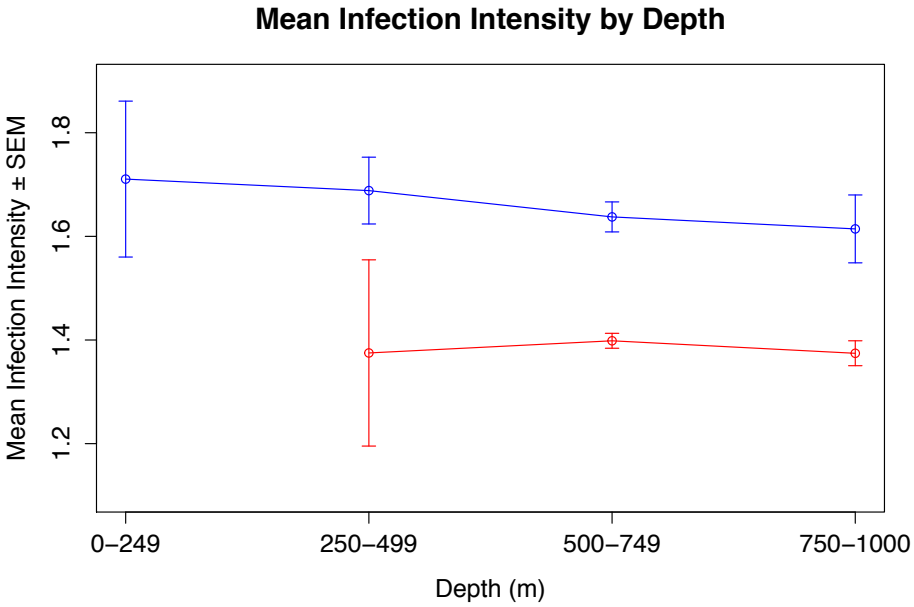

Figure 3.6. *Relationship between mean infection intensity of S. lumpi on shallow pelagic (blue) and deep pelagic (red) stocks and depth ± SEM.*

Mean infection intensity shows a strong positive correlation with length (Figure 3.7) in the shallow pelagic stock (n = 3,040, Pearson, $r = 0.99$, $p < 0.01$). The deep pelagic stock (n = 6,159, Pearson, $r = -0.28$, $p > 0.05$) shows a weak and non-significant negative correlation between the infection intensity and total length. The two stocks do not differ significantly with regards to infection intensity and total length (t test, $p = 0.07$).

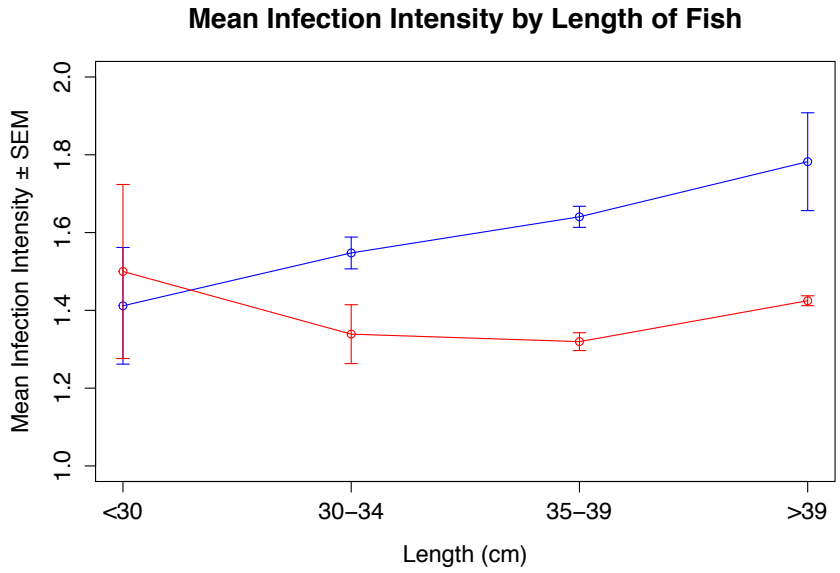

Figure 3.7. *Relationship between mean infestation of S. lumpi on shallow pelagic (blue) and deep pelagic (red) stocks and total fish length in cm ± SEM.*

The mean infections intensity shows a moderate non-significant positive correlation with fish age in the shallow pelagic stock (n = 367, Pearson, r = 0.60, p > 0.05) and the deep pelagic stock (n = 546, Pearson, r = 0.66, p > 0.05) (Figure 3.8).

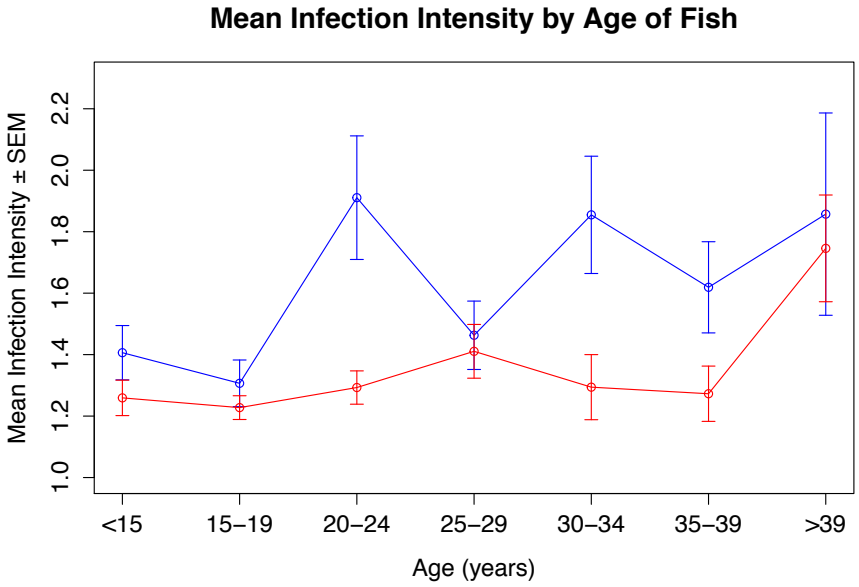

Figure 3.8. *Relationship between mean infection intensity of shallow pelagic (blue) and deep pelagic (red) stocks and age ± SEM.*

When variance in spatial infestation is analysed between two geographical zones split by latitude 60° (Figure 3.9) a significant difference in infection intensity is observed (Wilcoxon, n = 41,405, $p < 0.001$) where the group inhabiting lower latitudes (n = 3,395) is more heavily infested than the group localized at latitudes higher than 60° (n = 38,010). Stock composition also varies between the two groups as the majority of fish examined at latitudes higher than 60° come from the deep pelagic stock (n = 28,409) and the majority at lower latitudes are shallow pelagic redfish (n = 2,662). If stocks are compared between zones there is not a significant difference in infestation intensity in the shallow pelagic stock (Wilcoxon, n = 12,263, $p > 0.05$). However, that is not the case for the deep pelagic stock as a significant difference in infection intensity is observed between the two zones (Wilcoxon, n = 29,142, $p < 0.001$), where infection intensity was higher at lower latitudes. Statistical tests within zones revealed significant differences between stocks in the blue zone (Wilcoxon, n = 38,010, $p < 0.001$) where infection intensity was higher in the shallow pelagic stock, but not the green zone (Wilcoxon, n = 3,395, $p > 0.05$).

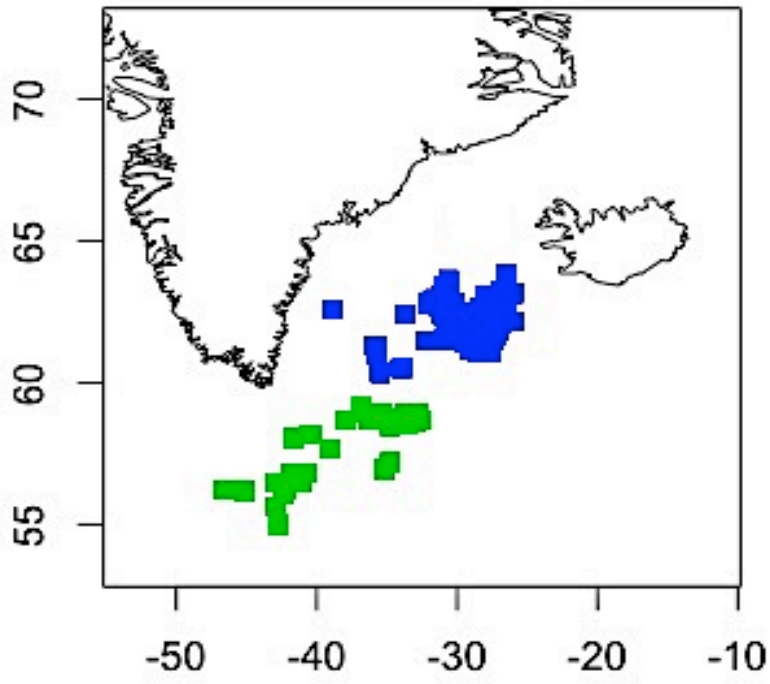

Figure 3.9. *S. mentella spatial groups. Group at higher latitudes than 60° (blue) and group at latidutes 60° lower than 60° (green).*

The four body sections examined showed variability in infection intensity of *S. lumpi* (Figure 3.10). The fillet section (F) had the highest mean infection intensity (Kruskal-Wallis, n = 10,247, $p < 0.001$) and the head (H) the lowest with 1.1 individuals on average per fish. The anal section (A) had a mean infection intensity of 1.4 and the ventral section (V) 1.2.

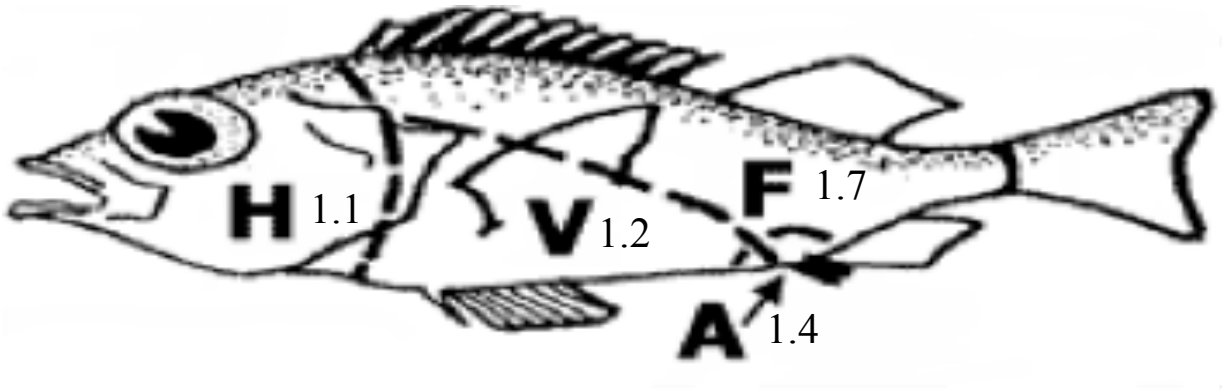

Figure 3.10. *Mean infection intensity of S. lumpi of four sections examined. F, fillet; H, head; A, anal; V, ventral.*

Of all fish examined during the period 1995 – 2013, most fish were infested with *S. lumpi* on section F (ANOVA, n = 9,199, $p < 0.001$, Figure 3.11).

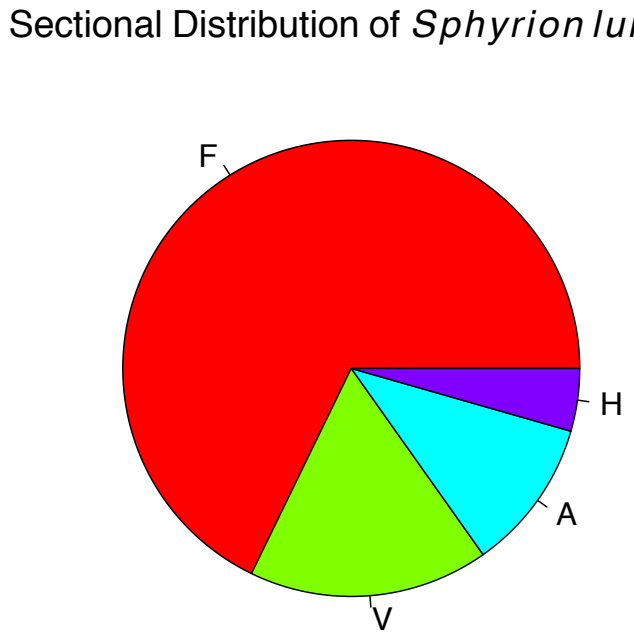

Figure 3.11*. Fish infested with S. lumpi per section from 1995 to 2013. F (red), A (blue), H (purple) and V (green).*

Abnormal external pigmentation had the highest occurrence in section F, or in 73% of all cases (ANOVA, n = 3560, $p < 0.001$ Figure 3.12). Within the three sections, black spots (B) were the most common (F: ANOVA, n = 2601, $p < 0.01$; H: ANOVA, n = 335, $p < 0.05$; V: ANOVA, n = 624, $p < 0.01$) and accounted for 48% of all spots in sections F and H. In section V black spots were evident in 66% of the fish. The main difference between sections is that section H is the only section that has red spots (R) in second highest occurrence or in 43% cases compared to 21% and 15% in sections F and V, respectively. In all sections mixed spots (RB) had the lowest occurrence.

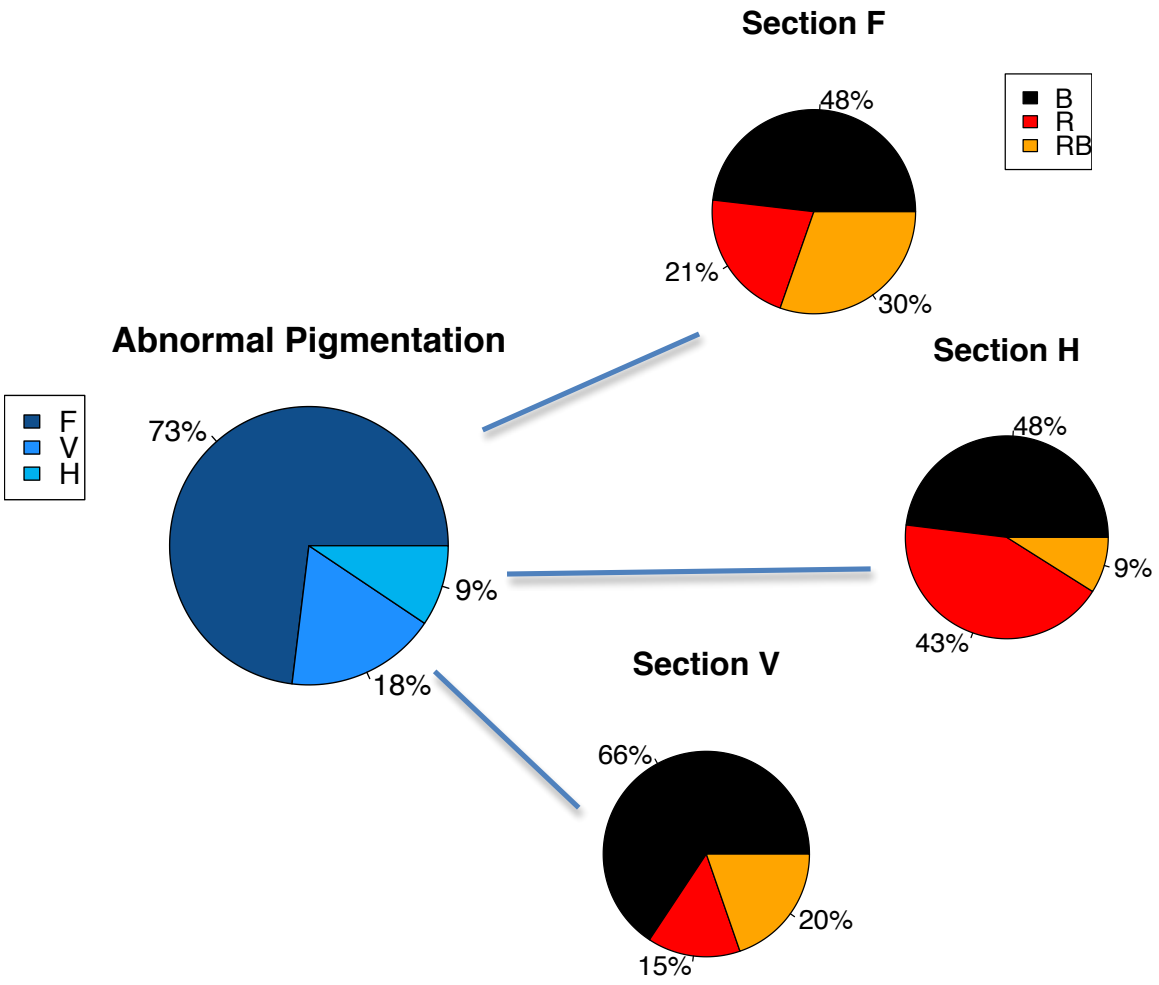

Figure 3.12. *Abnormal pigmentation of S. mentella skin in three sections from 1995 to 2013. F, fillet; V, ventral; H, head; B, black spots; R, red spots; RB, mixed spots.*

In total, 3614 fish were examined for abnormal muscular pigmentation (Table 3.1). Occurrence of these muscular spots is higher in fish infested with *S. lumpi* than in those not infested (t test, $p < 0.05$). The shallow pelagic stock has a much higher occurrence of muscular spots compared to the deep pelagic stock (t test, $p < 0.0001$). In all cases the majority of pigmentations are categorized as light. Severe pigmentation was similar in all groups, except for the shallow pelagic stock, which had the highest occurrence.

Table 3.1. *Abnormal muscular pigmentation in S. mentella from 1995 to 2013.*

Abnormal Muscular Pigmentation	All fish (n=3,614)	Fish with *S.lumpi* (n= 899)	Fish without *S.lumpi* (n=2,715)	Shallow pelagic stock (n= 1,476)	Deep pelagic stock (n= 2,138)
Light	51.5%	53.6%	50.8%	66.2%	41.4%
Medium	7.1%	8.5%	6.6%	14.6%	1.9%
Severe	1.3%	1.3%	1.2%	3.1%	-
Total	**59.9%**	**63.4%**	**58.7%**	**83.9%**	**43.3%**

A significant difference in mean infection intensity was observed between the sexes for both stocks (t test, $p < 0.001$) where females were more heavily infested than males in both cases (Table 3.2).

Table 3.2. *Infestation prevalence of the sexes of the two S. mentella types from 1995 to 2013.*

Sex	Prevalence of *S. lumpi* infestation	
	Shallow pelagic stock (n= 12,262)	Deep pelagic stock (n= 29,136)
Male	20.8%	17.5%
Female	27.2%	24.1%

3.2 Parasitological Examination

3.2.1 Dissection and examination of wet mounts

Microparasites belonging to two separate phyla, i.e. Apicomplexa and Myxozoa, were observed in *S. norvegicus* and *S. mentella* (*Sebastes* spp.) (Table 3.3).

Based on morphological features of mature oocysts, four different apicomplexan species were found (Figure 3.15), two of which were identified as *Goussia* sp. and one of each species of the genera *Crystallospora* and *Eimeria*. The two *Goussia* spp. (referred to as *Goussia* sp-I and II) were most commonly found, in 50% of examined fish, while the prevalence of *Crystallospora* sp. and *Eimeria* sp. was 2.9% and 22.6%, respectively. Both the *Goussia* spp. and *Crystallospora* sp. were found infecting the intestines while *Eimeria* sp. was found in the urinary bladder. The two *Goussia* species had spherical oocysts containing four ellipsoid sporocysts, each with two sporozoites. *Goussia* sp-I was considerably smaller than *Goussia* sp-II with oocysts measuring 8-10 µm in diameter and a sporocyst's length of 4.3 by 4.3 µm, while *Goussia* sp-II had an oocyst diameter measuring 18-20 µm and the length of the sporocysts measured 8.8 by 6.9 µm. The *Crystallospora* sp. had round oocysts measuring 10-12 µm and containing four sporocysts, diamond shaped in longitudinal view and hexagonal in transverse view, measuring 5.9 by 5.6 µm in length. The oocyst of *Eimeria* sp. in the urinary bladder measured 13.5 µm in diameter and had four sporocysts each measuring 4.4 by 4.2 µm, with a visible Stieda body. In addition, developmental stages, presumably of apicomplexan origin, were observed in the intestine of 37% further fish examined. They were also found in 3% and 11.5% of the stomach and pyloric caeca, respectively. In the urinary bladder, presumable apicomplexan developmental stages were observed in 42.9% of examined specimens. The presence of both *Eimeria* sp. oocysts and developmental stages were linked to clinical symptoms, i.e. enlargement of the urinary bladder, thickening of the bladder wall and a presence of milky white goo in the bladder lumen (Figure 3.13).

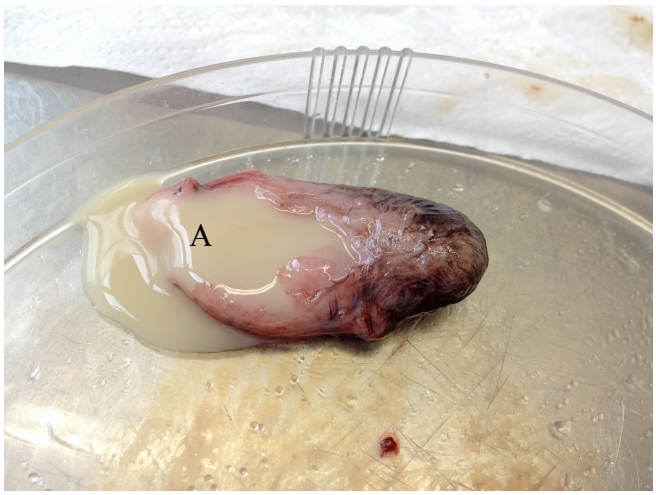

Figure 3.13. *Enlarged and abnormally thick walled urinary bladder of Sebastes sp. with milky whitish goo (A), clinical symptoms due to infection with Eimeria sp.*

Mature coelozoic myxosporean spores were found floating freely in the gallbladder bile of 38.7% of the fish (Figure 3.14). *Myxidium* sp. was present in all cases and co-infection with *Ceratomyxa* sp. in 14.3% of fish. The *Myxidium* sp. spores measured 4.5-7.2 µm in length and 9.5-14.7 µm in width. Polar capsules measured 1.7-3.0 µm in length and 2.0-4.4 µm in width. The spores of *Ceratomyxa* sp. measured 10-14 µm in length and 15.7-24.8 µm in width. Polar capsules measured 3.3-5.0 µm in length and width. Developmental stages of myxosporeans were also observed in the bile of 22.9% further fish, presumably belonging to either *Myxidium* sp. or *Ceratomyxa* sp.

Table 3.3. *Prevalence (P) and location of microparasites found in Sebastes species in Icelandic waters (n = 35).*

Parasite	Location	*P* (%)
Coccidia		
***Goussia* spp.**	int	50.0
***Eimeria* sp.**	ub	22.6
***Crystallospora* sp.**	int	2.9
Myxosporea		
***Myxidium* sp.**	gb	38.7
***Ceratomyxa* sp.**	gb	14.3

Note: int, intestine; ub, urinary bladder; gb. gall bladder.

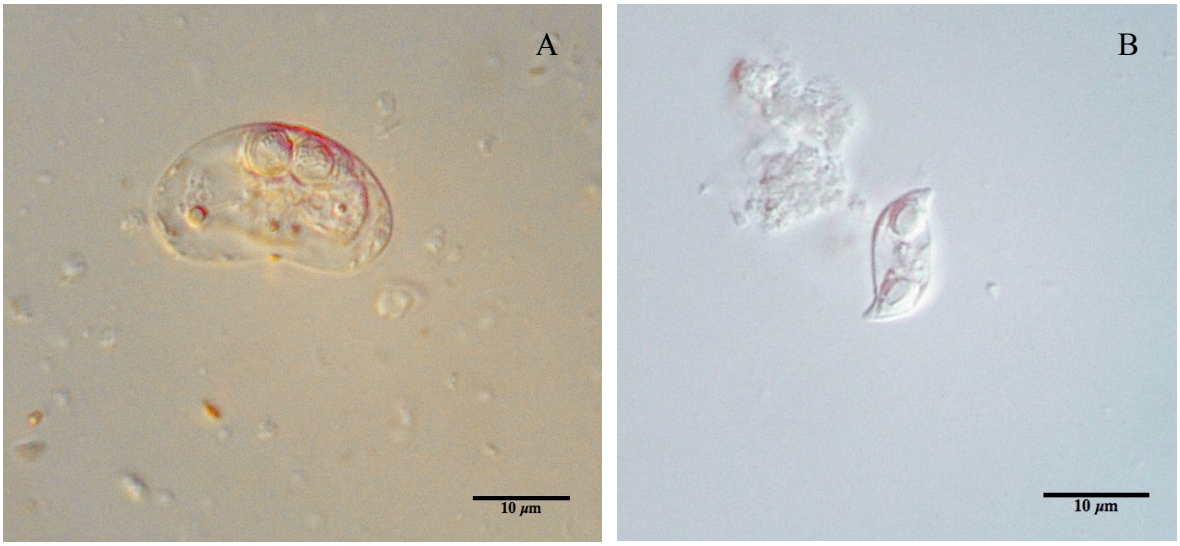

Figure 3.14. *Myxospores found in Sebastes sp. gallbladder. (A) Ceratomyxa sp. (B) Myxidium sp.*

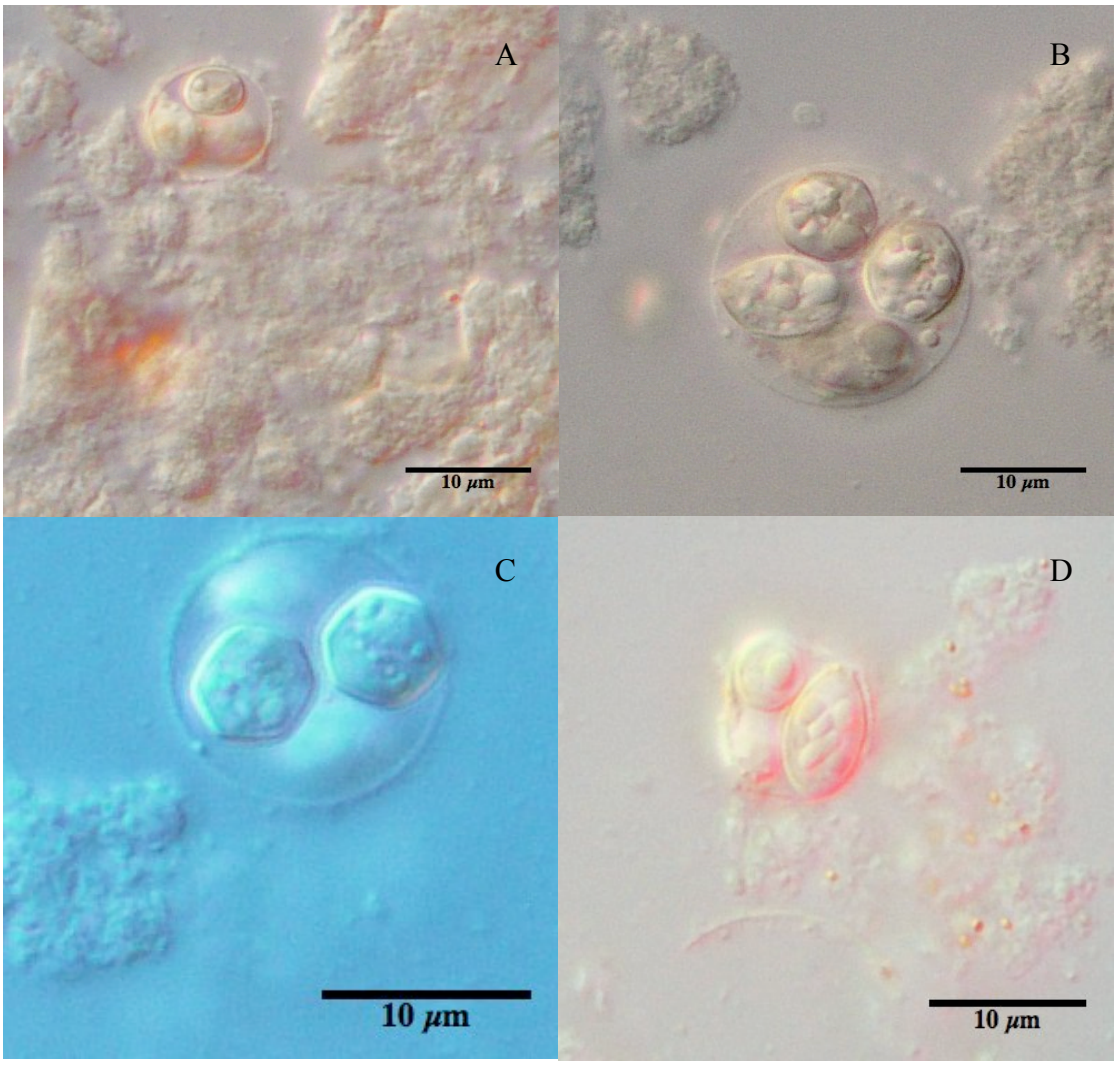

Figure 3.15. *Sporulated apicomplexan oocysts found in the intestines and urinary bladder of Sebastes spp. (A) Goussia sp.- I from the intestine. (B) Goussia sp.- II from the intestine. (C) Crystallospora sp. from the intestine. (D) Eimeria sp. from the urinary bladder.*

3.2.2 Histological Examination

General histological examination

Apicomplexan infections were observed in the intestinal tract and the urinary bladder. Infections were found both intracellular in the epithelium and extracellular in the lumen of both the intestinal tract and the urinary bladder (Figure 3.16). Both immature forms, e.g. trophozoites and gamonts, as well as mature oocysts, containing four sporocysts, each with two infectious sporozoites, were observed. In the intestinal tract, intracellular infections were most commonly found in the basal part of the columnar epithelial cells of the mucosal layer but also, to a lesser extent, in the submucosa layer. Intracellular infections in the urinary bladder were restricted to epithelial cells. As the myxozoan species found in the redfish were coelozoic, these infections were not detected in histological samples. No parasite infections were detected in other organs.

Histopathological changes were frequently seen associated with infections. These consisted of hypertrophy of columnar epithelial cells followed by sloughed off epithelium both in the intestinal tract and the urinary bladder.

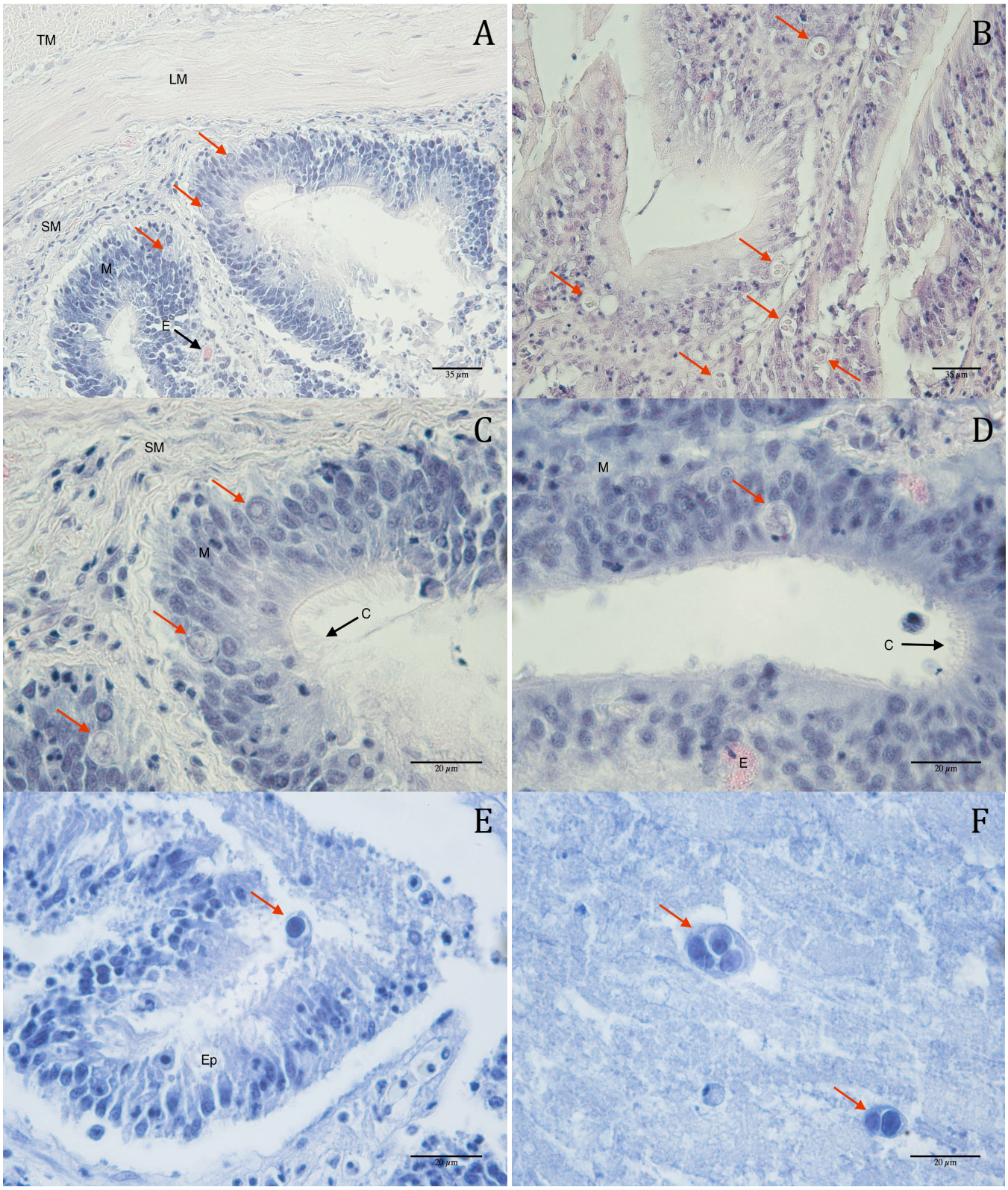

Figure 3.16. *Apicomplexans infecting the intestines and the urinary bladder of Sebastes sp. (A) Transection of Sebastes sp. intestine. TM, transectional muscle; LM, longitudinal muscle; SM, submucosa; M, mucosa; E, eosinophil granulocyte. Apicomplexan macrogamonts (red arrows) are situated in columnar epithelial cells. (B) Sporulated apicomplexan oocysts (red arrows) infecting Sebastes sp. intestine. (C) Apicomplexan macrogamonts seen with greater magnification. C, cilia. (D) Macrogamont (red arrow) infecting Sebastes sp. intestine. (E) Apicomplexan macrogamont (red arrow) infecting Sebastes sp. urinary bladder. Ep, epithelium. (F) Apicomplexan oocyst (red arrow) in urinary bladder.*

Histology of Sphyrion lumpi and host response to infections

A cut through a whole specimen of *Sphyrion lumpi* showing all major tissues is shown in Figure 3.17. A section showing more details of selected organs are shown in Figure 3.18. Severe host reaction to *S. lumpi* infections was observed. These were characterized by massive infiltration of white blood cells and fibroblasts at the site of penetration and a thick layer of fibrotic tissue isolating the parasite from the surrounding musculature.

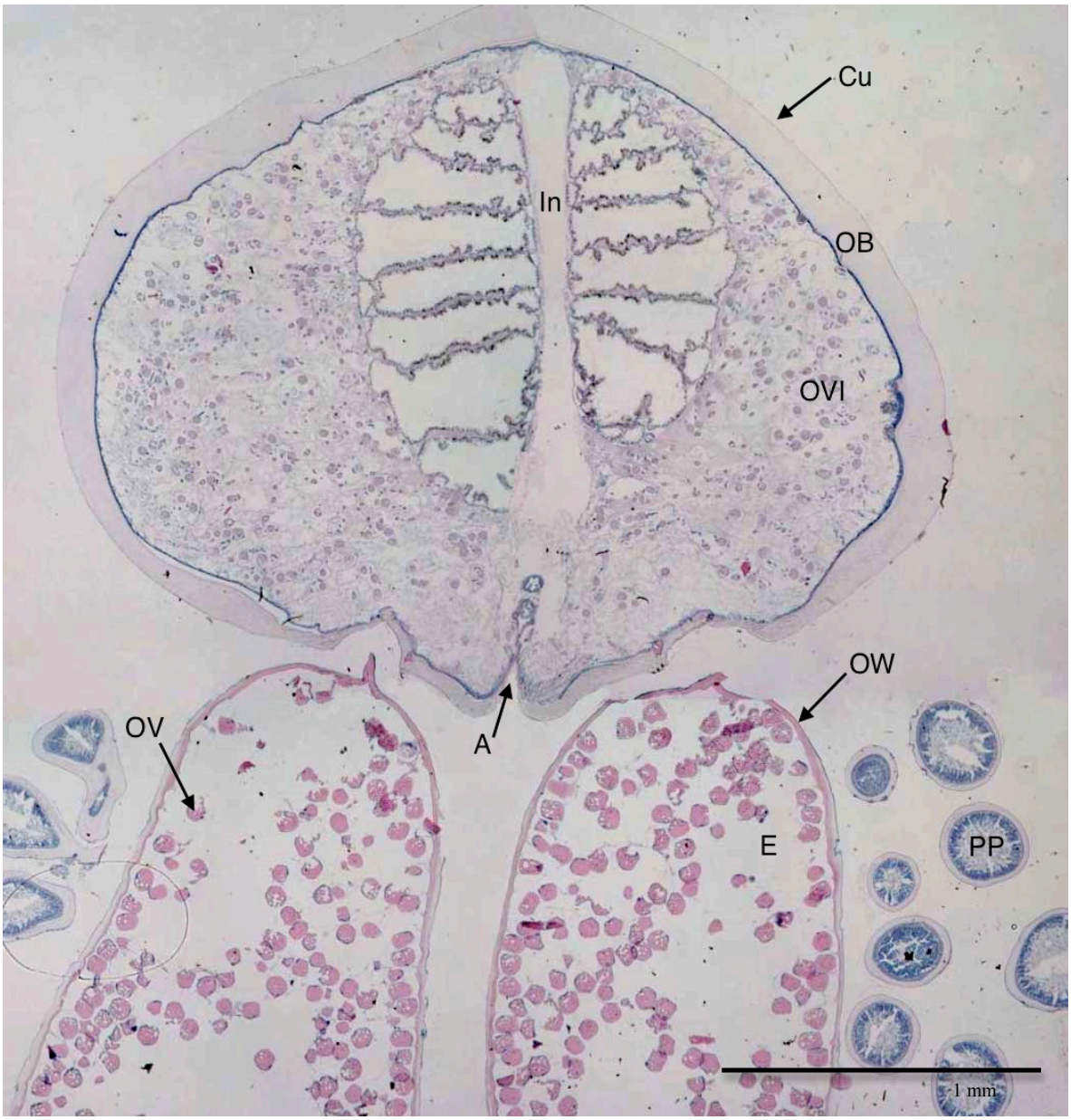

Figure 3.17. *Longitudinal section of S. lumpi trunk, egg sacks and posterior process. Cu, cuticle; In, intestine; OB, outer body wall; OVI, oviduct; A, anus; OV, ovum; OW, outer wall; E, egg sack; PP, posterior process.*

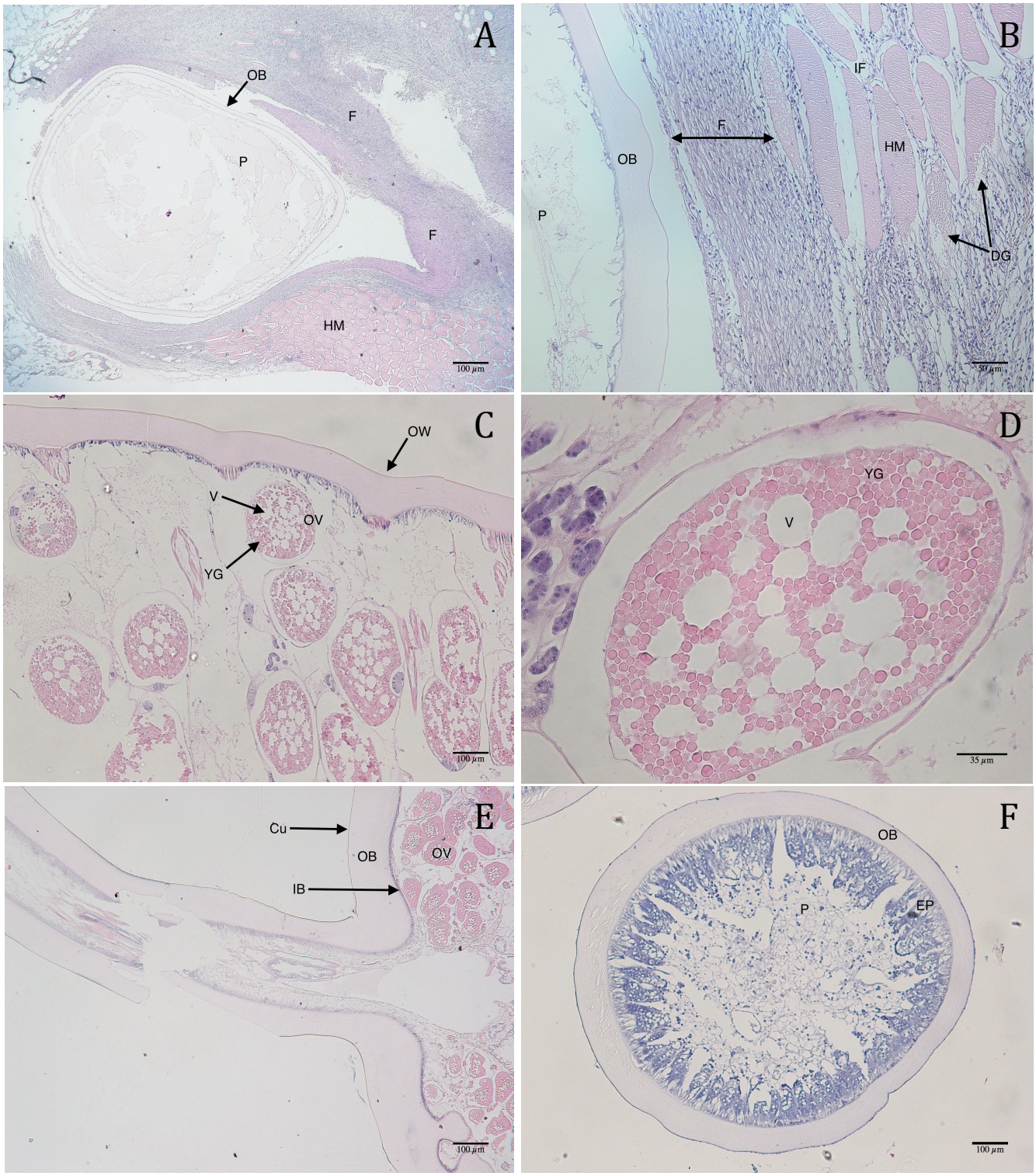

Figure 3.18. *S. lumpi infecting S. mentella. (A) Transection of S. lumpi neck region penetrating S. mentella muscle. OB, outer body wall; P, parenchyma; F, fibrosis; HM, host muscle. (B) Figure A at higher magnification. IF, infiltration of white blood cells and fibroblasts; DG, degeneration of host muscle fibres. (C) Longitudinal section of S. lumpi egg sack. OW, outer wall; OV, ovum; V, vacuole; YG, yolk granules. (D) Longitudinal section of a single S. lumpi egg. (E) Transection of S. lumpi neck and upper trunk. Cu, cuticle; IB, inner body wall. (F) Longitudinal section of S. lumpi posterior process. EP, epithelium.*

3.2.2 PCR Amplification and DNA sequencing

PCR amplification and DNA sequencing was successful in six cases (Appendix A).

A complete sequence of 1776 bp of *Sphyrion lumpi* 18S small subunit (SSU) ribosomal RNA (rRNA) gene was obtained. BLAST searches of 18S SSU rRNA sequence of 1125 bp of *Myxidium* sp. showed a high identity to *Myxidium bergense* (99%) and searches of 1835 bp of *Ceratomyxa* sp. showed a lower identity of 85% to *Ceratomyxa porrecta*. BLAST searches of three distinctive 18S SSU rRNA apicomplexan sequences purified from redfish intestine showed high identities to *Eimeria* species. The sequence from *Goussia* sp.-I of 1781 bp showed 97% identity to *Eimeria variabilis* and *Eimeria anguillae*. The Sequence from *Goussia* sp.-II of 1486 bp showed 98% identity to *Eimeria variabilis* and the sequence from *Crystallospora* sp. of 810 bp showed 89% identity to *Eimeria nementhi*. Amplification of apicomplexans from the urinary bladder remains unsuccessful.

3.2.3 Phylogenetic Analysis

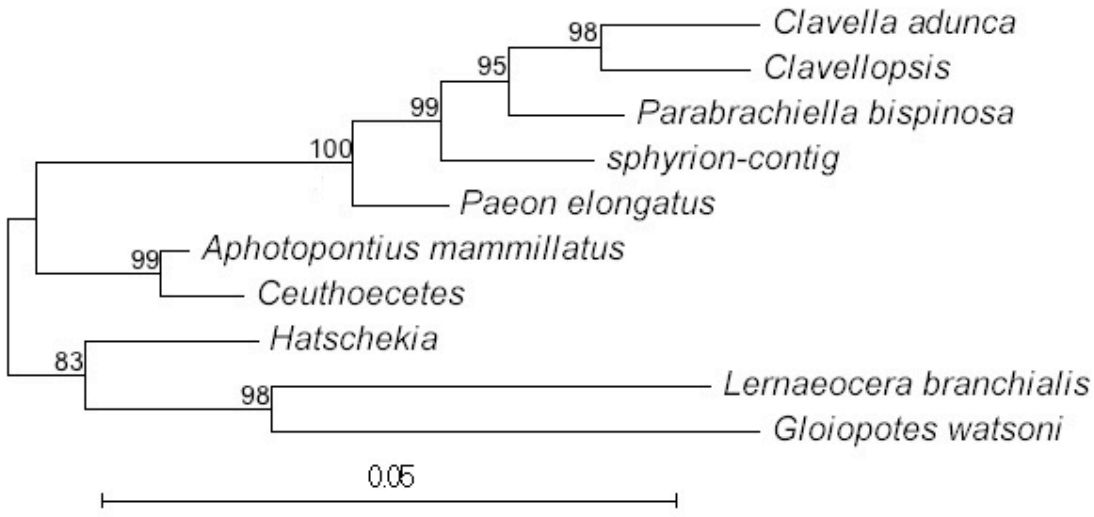

Figure 3.19. *SSU rRNA maximum likelihood tree topology for Sphyrion lumpi. Numbers at nodes = maximum likelihood bootstrap support. Scale = 0.05 substitutions per site.*

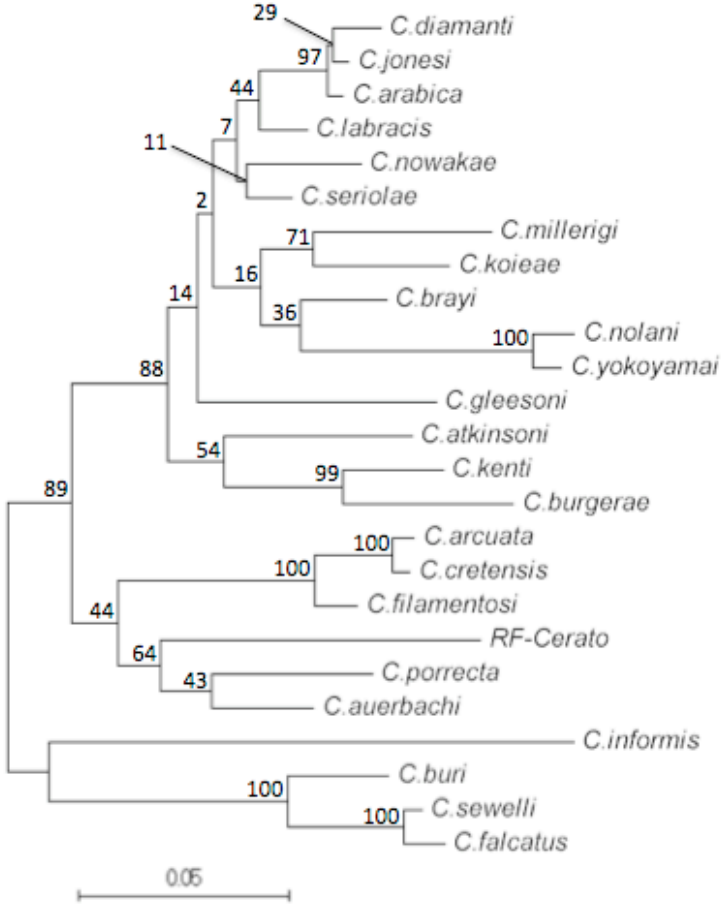

Figure 3.20. *SSU rRNA maximum likelihood tree topology for Ceratomyxa spp. Numbers at nodes = maximum likelihood bootstrap support. Scale = 0.05 substitutions per site.*

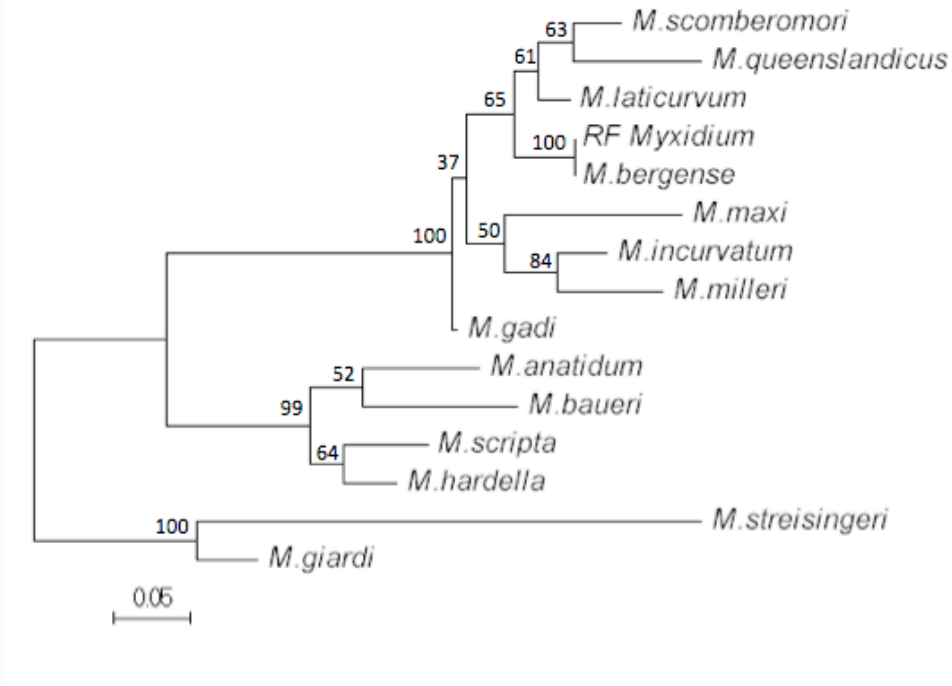

Figure 3.21. *SSU rRNA maximum likelihood tree topology for Myxidium spp. Numbers at nodes = maximum likelihood bootstrap support. Scale = 0.05 substitutions per site.*

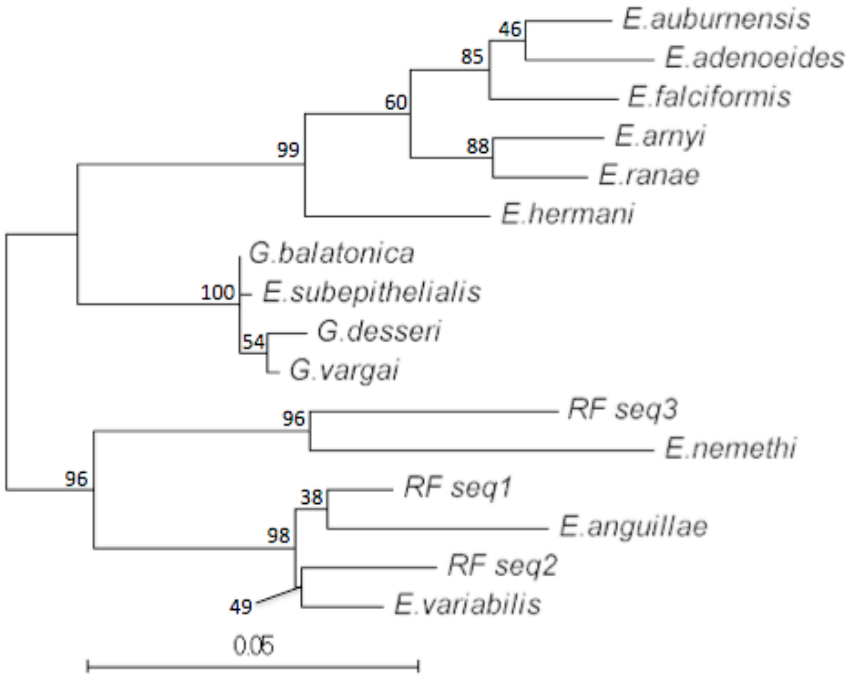

Figure 3.22. *SSU rRNA maximum likelihood tree topology for Apicomplexa. Numbers at nodes = maximum likelihood bootstrap support. Scale = 0.05*

4 Discussion

4.1 *Sphyrion lumpi*

This is the first study that attempts to give a holistic view of *Sphyrion lumpi* infections on *Sebastes mentella* in Icelandic waters. Similar studies have been carried out in other areas, such as the Irminger Sea (Magnússon 1992), the Gulf of St. Lawrence (Templeman and Squires 1960), East Greenland (Yatsu 1989) and the Barents Sea (ICES 2010).

In the present study, the intensity of *S. lumpi* infections did not correlate with the condition factor of *S. mentella* (Figure 3.1) and hence does not seem to have negative impact on its host. This is further supported as fish with and without the copepod are of similar condition. However, in the most severe infections, *S. lumpi* could adversely affect the fish's mobility as invasion of *S. lumpi* results in focal destruction of muscle fibres at the site of infection followed by a fibrosis, where the skeletal muscle is substituted by fibroblasts (Figure 3.20).

During the period this study spanned, the prevalence of *S. lumpi* infections in populations of *S. mentella* decreased considerably, i.e. from 25% in 1995 to 9% in 2013 (Figure 3.3). This trend has been previously reported in the Barents Sea (ICES 2010). However, a comparison of the shallow pelagic and deep pelagic stock (Figure 3.4) shows that this overall decreasing prevalence is largely due to lower infection intensity in the deep pelagic stock, as no significant decrease was observed in the shallow pelagic stock. Why this decrease is restricted to the deep pelagic stock is unclear but must be attributed to some factors, which hamper the parasite's transmission. That could be related to different habitats of the beaked redfish stocks in the water column and the biomass of these two stocks, which has drastically declined since 1995 (MRI 2014). As previously said, the life cycle of *S. lumpi* is still unknown, it could be simple, i.e. direct transmission between fish or it could be complex, where an intermediate host is needed. If the life cycle is simple, the biomass of fish at a certain site is a crucial factor, where high density increases the possibility of one redfish infecting another. In case, *S. lumpi* requires an intermediate host, this decrease could be related to both the density of the redfish as well as the availability of the intermediate host species, its presumable decline or its movement to other areas. The shallow pelagic stock has significantly higher infection intensity than the deep pelagic stock (Figure 3.5), which is in agreement with other studies (see Cadrin et al. 2010). In fact, the shallow pelagic stock exceeds the deep pelagic stock in most aspects regarding *S. lumpi* infestation and other abnormalities (Figures 3.4 – 3.9 and Table 3.1). They also show a geographic variance where most of the deep pelagic stock is located at latitudes higher than 60° and most of the shallow pelagic stock is at lower latitudes (Figure 3.9). However, this could be due to the timing of sampling as the majority of samples were collected in May - July. Parasites have successfully been used as natural biological tags of marine fish populations (Marcogliese and Jacobson 2014) and *S. lumpi* has frequently proved as a

useful indicator of stock structure, distinguishing *Sebastes* populations in the North Atlantic (Nigrelli and Firth 1939; Templeman and Squires 1960; Bakay 1988; Moran et al. 1996; Marcogliese et al. 2003). Based on the results of this study *S. lumpi* could serve as a biological tag for pelagic stocks of *S. mentella* in the Irminger Sea and adjacent waters to separate the shallow pelagic stock and the deep pelagic stock. However, parasitological data alone are not sufficient for stock separation and therefore other factors, such as life history information, need to be considered for that purpose.

Studies on the distribution of *S. lumpi* on redfish have shown that the greatest abundance of living copepods and old cephalothoraxes is between the base of the dorsal fin and the lateral line (Perlmutter 1951; Bakay 1988). That area corresponds to section F in this study, which had the highest intensity of *S. lumpi* infections (Figure 3.10). This is most likely the optimal attachment site for the copepod as it is rich in soft musculature and hence easy to penetrate. Consequently, getting rid of parasites on that area could be more difficult than from the other attachment sites. Abnormal pigmentation on the skin was also most intense in section F. This fact, and that melanism is a well known host reaction to parasite infections (Horth et al. 2013), could indicate that the abnormal pigmentation observed on the *Sebastes mentella* skin are a consequence of *S. lumpi* infections, Moreover, muscular pigmentation was more commonly observed in the shallow pelagic stock than the deep pelagic stock (Table 3.1) and was also greater in fish infested with the copepod than in those not, which further supports this. Bakay and Melnikov (2008) reported the skin and muscular melanosis to be age-dependent changes and ontogenetic movement to deeper pelagic habitats. However, the interpretation of movement from shallow to deep pelagic environments is refuted by recent genetic evidence of reproductively isolated groups (Cadrin et al. 2010). Also, this study did not find any correlation between age and depth, neither within stocks or areas.

Results of the present study show a significant difference in the intensity of *S. lumpi* infections between the sexes of *S. mentella* where females are more heavily infested (Table 3.2), which is in accordance to many previous studies (e.g. Templeman and Squires 1960; Magnússon 1977; Yatsu and Jørgensen 1989; Sarralde et al. 1997; Melnikov et al. 2003). Females migrate to special areas for extrusion of larvae and are separated from males during that period (Magnúson and Magnússon 1995). This spatial separation of the sexes could possibly play a role in this, i.e. the females being, for some reason, more exposed to *S. lumpi* infections than the males.

Studies on patterns of *S. lumpi* infestation are inconsistent. Some researchers found infestation rates to increase with depth (Magnússon et al. 1995; Magnússon and Magnússon 1995) while others have reported decreasing infestation with depth (Del Rio et al. 1996; Sarralde et al 1997). No significant relations were found between depth and mean intensity of *S. lumpi* infections in this study. This is surprising as a decrease with depth was expected because of the significant difference between stocks. However, shallow pelagic *S. mentella* was caught throughout the depth distribution, which could explain the lack of correlation.

It is unknown whether the female *S. lumpi* experiences single or multiple spawning. These results suggest the possibility of multiple spawning, as eggs were observed simultaneously in egg sacs and ovaries in histological samples. This supports Squires' (1966) findings, but further research would be ideal to confirm this. Infection trials would also serve as a key

element in solving the life cycle of *S. lumpi*. Furthermore, it is important to know the environmental limits of the parasite, to strengthen its use as a biological tag.

Here, a novel sequence from the 18S SSU rRNA of *S. lumpi* is published (Appendix A). Unfortunately, PCR amplification was only achievable from one individual as all other specimens were fixed in formalin, which causes damage to the genetic material. Further research could include specimens in greater numbers from different areas and different host species to determine genetic variations and possible sub-populations of *S. lumpi*, which could increase the potential use of the parasite as a biological tag. Currently, the identification of *S. lumpi* is solely based on morphological characters. In many fields, this approach to species identification has been overturn with molecular methods (Lawrence et al. 2002). Priebe (1986) reported significant morphological differences between adult female specimens of *S. lumpi* from golden redfish and roughhead grenadier (*Macrourus berglax*) and concluded them to be separate species. Even though host induced intraspecific morphological variability of parasites have been noted in several studies (Palmieri 1976; Kazubski 1982; de Léon 1995), genetic research could elucidate Priebe's conclusions. A recent study showed genetic evidence suggesting that the amphipod *Eurythenes gryllus* might represent at least nine species-level lineages, therefore questioning the assumption of its cosmopolitan and eurybathic distribution (Havermans et al. 2013). The wide geographical range and extremely low host specificity of *S. lumpi* raises the same questions, and genetics could have the answer.

4.2 Microparasites

4.2.1 Myxosporea

Myxosporeans are one of the most common parasites infecting fishes (Lom and Dyková 2006). At least 13 myxosporean species have been reported from *Sebastes* species in the North Atlantic. The majority of those belong to two genera, i.e. *Myxidium* (four species) and *Ceratomyxa* (Syn. *Leptotheca*) (five species) (Bakay, and Trudnee 2009**;** Eiras et al 2011; Bakay 2012), but one species of each of these genera were found in the present study.

The *Myxidium* species found in this study showed a high similarity to *Myxidium bergense*, both genetically (99% identity) and morphologically. Furthermore, the maximum likelihood phylogenetic tree (Figure 3.22) places the redfish *Myxidium* sp. sequence from this study parallel to *M. bergense* with the bootstrap support of 100. *M. bergense* has been previously reported in *Sebastes* in the North Atlantic, e.g. from fishing grounds SW off Iceland (Bakay 2012). It is also known to be quite non-specific with regards to fish hosts, e.g. commonly found in Atlantic cod and haddock in the North Atlantic (MacKenzie and Kalavati 1995). Consequently, it can be stated with high confidence that this species is *Myxidium bergense*.

Ceratomyxa sp., which was commonly found in this study, showed low identity to known myxozoan sequences available in gene banks. The species showed the highest identity (85%) to *Ceratomyxa porrecta* and hence quite distantly related genetically. Furthermore, *C. porrecta* is considerably different morphologically to the *Ceratomyxa* species observed in this study. Although quite different to other known species genetically, it shows high morphological similarity to *Ceratomyxa adeli*, originally described as *Leptotheca adeli*, which has previously been reported in *Sebastes* spp. in the North Atlantic (Bakay 2001). Unlike *M. bergense*, this species has only been reported from *Sebastes* spp. and could therefore be host specific. No molecular data have been published for this species but most likely the *Ceratomyxa* sp. in the study belongs to this species. Phylogenetic analysis grouped the *Ceratomyxa* sequence of this study with *Ceratomyxa auerbachi* and *C. porrecta*, which are very morphologically distinctive from *C. adeli*. These results lead to the conclusion that morphology alone is not sufficient for true myxosporean taxonomy.

The prevalence of myxosporean infections is likely to be underestimated in Table 3.3 in this study, as the prevalence of the parasites was limited to the presence of mature spores. However, developmental stages that were frequently observed in the gall bladder are without much doubt myxosporean as they were the only species found in this organ.

4.2.2 Apicomplexa

Reports on apicomplexan infection in *Sebastes* species from the North Atlantic are scarce, *Eimeria gadi* apparently the only nominal species. Two further unidentified species, i.e. *Eimerida* gen sp. and *Haemogregarina* sp. have been reported (Moran 1995; Bakay 2010)

Sequences from apicomplexan *Goussia* sp.-I and -II showed 97% and 98% identity to *Eimeria variabilis,* respectively. Sequence from *Goussia* sp.-I also showed 97% identity to *Eimeria anguillae*. These *Eimeria* species are found in freshwater and therefore highly unlikely to be conspecific. *Crystallospora sp.* was quite distantly related to known apicomplexan sequences, the highest identity being to *Eimeria nementhi*, merely 89% similar. However, it bears a great morphological resemblance to *Crystallospora* sp. but to date, no sequences of species from this genus are available in gene banks. The amplicon sequenced consisted only of 810 bp, but for optimal results a longer sequence is required. Due to time restrictions, the accurate identification of these apicomplexan species awaits further study. Despite thorough literature study, no apicomplexans fitting the morphological descriptions were found recorded from *Sebastes* species. Furthermore, no close matches were found in gene banks. It therefore seems likely that the apicomplexan species found in this study are previously undescribed or new host records.

Sporulated apicomplexan oocysts were found in the intestines of *Sebastes* spp. However, developmental stages, most likely of apicomplexan origin, were found in the stomach and pyloric caeca. This leads to the assumption that route of entrance is oral and these species infect the whole gastrointestinal tract where the exit route is with faeces. This is common among apicomplexans (Dyková and Lom 1981). These infections are, like the myxosporeans infections, probably underestimated as the prevalence of the parasites was limited to sporulated oocysts.

Detailed evaluation of histopathological changes due to apicomplexan infections was somewhat problematic as the samples obtained weren't fresh enough for that purpose. Due to the intracellular nature of apicomplexans, some pathology is doomed to be associated with such infections. Despite suboptimum histological samples, pathological changes, such as hypertrophy of infected columnar epithelial cells followed by sloughing off the epithelium was frequently observed, both in the intestinal tract and the urinary bladder mucosa. In severe cases of infection, they would prolifically inhibit the fish's nutritional absorption. As a result the fish would languish due to nutritional deficiency. Furthermore, urinary bladders infected with apicomplexans showed macroscopic clinical signs (Figure 3.15). The clinical symptoms are enlargement of the urinary bladder, thickening of the bladder wall and the presence of milky white goo in the bladder lumen, undoubtedly cause urine disposal problems. The functionality of the urinary bladder is surely impaired and severe cases are likely to result in mortality.

5 Conclusions

This is the first study that gives a holistic view of *Sphyrion lumpi* infections on *Sebastes mentella* in Icelandic waters. The copepod has no impact on the redfish's condition (K) but has a potential to affect it's mobility during severe infections. The parasite is a good candidate as a biological tag in this area, as mean infection intensity and prevalence is significantly different between the shallow pelagic and deep pelagic stock. They also show a geographic variance where most of the deep pelagic stock is located at latitudes higher than 60° and most of the shallow pelagic stock inhabit lower latitudes. However, further research, solving the life cycle of the parasite and studying its environmental limits, is important to strengthen its use as a biological tag.

Microparasites in *Sebastes* spp. in Icelandic waters were found in the gallbladder, intestines and urinary bladder. Two coelozoic species of myxosporeans were confirmed in the gallbladder, *Myxidium bergense* and *Ceratomyxa adeli*. Three species of apicomplexans were found in the intestines, two of which are *Goussia* spp. and one *Crystallospora* sp., causing hypertrophy of infected columnar epithelial cells followed by sloughing off the epithelium. One species belonging to *Eimeria* spp. was found in the urinary bladder, which apparently causes macroscopic clinical signs. These apicomplexan infections are thought to be new host records or previously undescribed species.

Here, a novel sequence from the 18S SSU rRNA from one individual of *Sphyrion lumpi* is published. Also, no sequences from *C. adeli*, *Goussia* spp, *Eimeria* sp. and *Crystallospora* sp. are available in gene banks and unpublished.

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Appendix A

Sphyrion lumpi sequence (1776 bp)

CTCAGTATCATAGCTTGTCTCAAAGATTAAGCCATGCATGTTTAAGTCCAAGCCTTATTAAGGTGAAAC
CGGGAATGGCTCATTAAATCACATCTAATATACTGGATATTAACAGTTACTTGGATAACTGCGGTAATT
CTGGAGCTAATACATGCATTCAAGCCCTGAACTTACGTGAGGGGCGCTTTTATTAGATCAAAACCAAAC
GCTTCACGGCGTACCTTTGGTGACTCTGGATAACCTTTTGCTGATCGCATGGCTTTATGCCGGCGACGT
ATCCTTCTAAGGTGTGCCCTATCAACTGTCGACTGTGGCATAGACGCCCACAGTGGTTTTGACGGGTAA
CGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCTACGGAAGGCAGCAG
GCACGCAAATTACCCACTGGTCGAAGACCGAGGTAGTGACGAAAAATAACGATACCGGACTCATCCGAG
GCCCGGTAATCGGAATGAGTACACTTTAAATCCTTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCC
AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAATTTGTTGCGGTCAAAAAGCTCGTAGTTGG
ATCTCGCAGATTGGGGGTGGTTCATTATTTAATGTTAACTGCTCCACATTTCTGTGTTTTTTGACAGAG
GTTCCAAGGTGCTCTTGATTGAGTGTCGTGGGATGCTGTCAGGTTTACTTTGAAAAAATTAGAGTGCTC
AAAGCAGGCTTTACAAGCTTGAATATTGGTGCATGGAATAATAGAATAGGATGTTGTGTCCTTTTTGTT
GGTTATACGGATATTAACATAATGATTAATAGGGACAGTCGGGGGCATTAGTATTCAGACGACAGAGGT
GAAATTCTTGGACCGTTTGAAGACTAACTACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAG
AACGAAAGTTAGAGGTTCGAAGGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCCAGCTAGC
GATCCGCTTGTGTTTCTTATAAGGCCCTGCGGGAAGCTTCCGGGAAACCAAAGCTTTTGGGTTCCGGGG
GAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGG
CTTAATTTGACTCAACACGGGAAATCTCACCAGGCCCGGACACTGGAAGGATTGACAGATTGAGAGCTC
TTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCC
GATAACGAACGAGACTCTGTCCTGCTAAATAGTTTCAATGTCTTTAATTACATTGGAAATTCTTCTTAG
AGGGACTGGCGGCCTATAGTCGCACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGG
GCTGCACGCGCGCTACACTGAAGAGTTCAACGTGTTTTCCTTTCCTGAGAAGGACGGGTAACCCGCTGA
ACCCTCTTCGTGGTAGGGATCGGGGCTTGCAATTATTCCCCGTGAACCAGGAATTCCCAGTAAGCGCAA
GTCATAAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAACG
TTTTAGTGAGGTATTTGGACTGGATCGCCGAAGTTTTACTTTGGTGTTTTCCGGAAAGACTCCCAAACT
TGAGCGTTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGACCAA

Myxidium sequence (1125 bp)

GCTCGTAGTTGGATTACAAAGGTCTGGCGTCAATTGAGCATTAGTTTGATTGGCGTTGGGCTTTTTTAT
CGCAGGAACCATTTTGTTTACTTAACTGTGAACGTTTGGCCACTTGCGGAGCGTGCCTTGAATAAAGCA
CAGTGCTCAAAGCAGGCGTAACGCTTGAATGTTATAGCATGGAACGAATACCCTGACTCGGTTCAGTTA
GTTGGTTCTCTGAACTGGGTCGTGATTAAAAGGGACATTTGAGGGCGTTAGTACTTGGTGGCGAGAGGT
GAAATTCTTAGACCCACCAAAGACTCACTAATGCGAAAGCATTCGCCAAGAATGTTTTCATTGATCAAG
AACGAAAGTTAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTATACAGTAAACTATGCCACCACG
GGATCAGTCCGGAAAACGATCCAGGTTGGTCCCCCAAGGAAACTTGAAGTGTTCGGGTTCCGGGGGGAG
TACAGTCGCAAGTCCGAAACTTGAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTA
ATTTGACTCAACACGGGGCAACTCACCAGGTCCAGACATTGAAAGGATTGACAGACTGATAGATCTTTC
AAGATCCAATGATTGGTGGTGCATGGCCGTTCTTAGTTGTTGGAGTGATATGTCAGGTCTATTCCGGTC
ACGAGCGAGACCGCGATCTCTATTTGATTGCAGCTGCTTTGTTGTAGTTGGTCATAGAGAGACTACCGG
AATACAAGCCGGGGGAAGCGTGGCAATAACAGGTCTGTGATGCCCTTCGATGTTCTGGGCCGCACGCGC
GCTACAATGGCAGTGACAACAAGTTTCTGCTTTGAGAAGAGTGGATAATCTTGACAATCGCTGTCGTGA
TTGGGATTGAGCCTTGTAATAATTGCTCATGAAAGAGGAATTCCTCGTAAGCGCGAGTCACCAACTCGT
GTTGAATACGTCTCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGCTTTTCGAGAAG
TCGGGATTGGTGATCGCAAGGTCACCGAGAGCTGCTTCAAGGAAAGGTATTTAGAGGAAGTAAAAGTCG
TAACAAGGTTTCCGTAGGTGA

Ceratomyxa sequence (1835 bp)

NTTCTGGGTTGATTTTGCCAGTGGTCATATGCTCGTCTCAAAGACTAAGCCATGCATGTGCTAGTTCAT
AACCTTAGGGTTGAAACTGCGAAGCGCTCAGTAAATCAGTTATAGTCTGTTCGATCGTTAGAATGGCTG
GATAACTATGGCAAATCTATAGCTAATACATGGGAACCCCTGCAACAAGCCTGGGGACATTTATTGACT
AACCGACACCGCCTTGATGGCAGCAGGTGAATCTAGATAACTGTATATACCGCAGAGCATTAGGCTGGC
GGTAGTTTGATCGAATTTCTGCCCTATCAACTAGTTGGTAAGGTAGTGGCTTACCAAGGTAGTAACGGG
TAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAGACGGCTACCACATCCAAGGAAGGCAG
CAGGCGCGCAAATTACCCAATCCAGACATTGGGAGGTAGTGACGAGAAATACTGGGGGTGCCCCTAGTG
GGTGCCATCCGGAATGAATGCAAGATAAAAAGTTCAATGAGGATCTACTGGAGGGCAAGTCTGGTGCCA
GCAGCCGCGGTAATTCCAGCTCCAGTAGTGTATATCAACATTGTTGCGCTTAAAACGCTCGTAGTTGGA
TTACAGGGATGTGAGTGGGTGAATGTGTTGTGCGTTGCTCTAGGTGGCCTTGGTTGTGAGGTAATACTT
ATGGCCGGGGGCACCGCCATATCCCTTTGTTCGCCAGGTTTGTGGCTGCCCTTTGTTGGGTGGACGCAG
TGGCTGGCGGAGTGTGCCTTGAATAAAGCATAGTGCTCAAAGCAGGCGTACGCTCGGTGTGTAATAGCA
TGGAACGAATAAAGGACCCAATGTATCGTAAGGTGCAGTGATCAATGAGTGTTGGTTTTGACCCAGGTC
TAGACGGTTAAGAGGGATGTTTGAGGGCGCTAGTATTCTGTGGCGAGAGGTGAAATTCTTAGACCCACA
GAGGACTGACTACTGCGAAAGCATTCGCCAAGGGTGTTTTCATTAATCAAGAACGAAGGTCAGAGGATC
GAAGACGATCAGATACCGTCCTAGTTTTGACAGCAAACTATGCCAGCATAGGAGCAGGGCAGCCACCCT
TAAGTGGGACGTGCTACCTTGGCCCTTAAGGGAAACCTAGCTTTCGGGCTGCTGGGAGAATATCGCTGC
AAGGCTGAAATTTAAAGAAATTGACGGAGAGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTC
AACACGGGGAAACTCACCAGGTCCAGACATTGGTAGGATTGACAGACTGGTAGATCTTTCATGATTCGG
TGAATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATCTTTGCGGTTTAAACCGGTAGTAGTTGGA
GAACACGGATTTGAGTGGGGGTATGCAGTGTCCTTTACTAGGGGGAGTGTGCAGGGAAATAGAGGGACA
ATTGGTACCAAGCCAGGGGAAGCGTGGCAATAACAGGTCTGTGATGCCCTTCGATGTCCTGGGCTGCAC
GCGCGCTACAATGGCAGCATGAAAGAGTTGCTTCCCCGAGAGGGGCGGCTAACCTGTAAATGGCTGTCG
TGATAGGGATTGAGCCTTGGAATTATTGCTCATGAAAGAGGAATTCCTCGTAAGCACGAGTCATCAGCT
TGTGTTGAATACGTCTCTGCCTCTTGTACACACCGCCCGTCGCTACTACCGATTGAGTTGCCTTCAAAG
CTGGCTGGAGTGTCCTGGCTTTGTGGTTGTGGATGTGAAGGCCAACGATGGAGGTTGTTTAGAGGAAGT
AAAAGTCGTAACAAGGTTTCCGTAGGTGACCNAGCGGAAGA

Apicomplexa sequences:

Sequence 1: *Eimeria* sp.-I (1781 bp)

TCTGGTTGATTCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTATAAGTT
TTTATACGGCGAAACTGCGAATGGCTCATTACAACAGTTATAGTTTATTTGATGGTCTTTTTTACATGG
ATAACCATGGTAATTCTGTGGCTAATACATGCGCACGCACATTGCCTCTGGTAGTGTGGTGTTTATTCG
ATACCAAACCAACACATCTTCGGATGTAGATCTGGTGATTCATAGTAACCGAACGGATCGCACTATGGC
TTCGGCCGGCGATAGATCATTCAAGTTTCTGACCTATCAGCTTTCGACGGTAGGGTATTGGCCTACCGT
GGCAGTGACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATC
TAAGGAAGGCAGCAGGCGCGCAAATTACCCAATGAAAACAGTTTCGAGGTAGTGACGAGAAATAACAAT
ACAGGGCATATAATGTCTTGTAATTGGAATGACTTAAATCCAAACCCCTTTCGGAGTAACAATTGGAGG
GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAGAGTTGTTGCAGTTAAA
AAGCTCGTAGTTGGATTTCTGTTGGGGGCAGCCTGTCCGCCTTCGGGTGTGCATGGGTCATCCCTAGCA
TTTTTCTGGTTCCCCTTTGTACTTTATTGTGCATTGGTTCTCCAGAACATTTACTTTGAGAAAAATAGA
GTGTTTCAAGCAGGCTATCGCCTTGAATACTGCAGCATGGAATAATAAGATAGGACTTTGGCCCTATTT
TGTTGGTTCTAGGACTAAAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGG
TGAAATTCTTAGATTTGTTAAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAA
GAACGACAGTAGGGGGTTTGAAGACGATTAGATACCGTCGTAATCTCTACCATAAACTATGCCGACTAG
AGATAGGGAAACGCCTGTCTTGGCTTCCCCTGCACCTCGTGAGAAATCAAAGTCTCTGGGTTCTGGGGG
GAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGC
TTAATTTGACTCAACACGGGAAATCTCACCAGGTCCAGACATGGGAAGGATTGACAGATTGATAGCTCT
TTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATCTGTCTGGTTAATTTCG

ATAACGAACGAGACCTTAGCCTGCTAAATAGGGTCAGGAATTTTTTTTCCTGTATCGCTTCTTAGAGGG
ACTTTGCACGTCTAGTGCAAGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGC
CGCACGCGCGCTACACTGACGCATCCAACAAGTTCTCCCTTGGCCGATAGGTCTAGGAAATCTTTTGAG
TGTGCGTCGTGATGGGGATAGATTATTGCAATTATTAATCTTCAACGAGGAATGCCTAGTAGGCGCAAG
TCATCAGCTTGCGCCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCAACCGATCGGAGGG
TCCTGTGAATTTATTGGACTGTTCTTTGGTGCTTCCGCACCTTCGGATGGAAAGATATGTAAACAGAGC
CCTCTAAAGGATGCAAAAGTCGTAACACGGTTTCCGTAGGTGACCCAGCCGGAAGA

Sequence 2: *Eimeria* sp.-II (1486 bp)

TTTAGTTTNTTGGACTTCAGCTTTCGACGGTAGGGTATTGGCCTACCGTGGCAGTGACGGGTAACGGGG
AATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCG
CAAATTACCCAATGAAAACAGTTTCGAGGTAGTGACGAGAAATAACAATACAGGGCATTAAATGTCTTG
TAATTGGAATGACTTAAATCTAAACCCCTTTCGGAGTAACAATTGGAGGGCAAGTCTGGTGCCAGCAGC
CGCGGTAATTCCAGCTCCAATAGCGTATATTAGAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCT
GTTGGGGGCAGCTCGTCCGCCTTTTGGTGTGCACAAGTCATCCCTAGCATTTTTCTGGTTCCCCTTTGC
ACTTTATTGTGCTTTGGTTCTCCAGAACGTTTACTTTGAGAAAAATAGAGTGTTTCAAGCAGGCTTTTG
CCTTGAATACTGCAGCATGGAATAATAAGATAGGACTTTGGCCCTATTTTGTTGGTTCTAGGACTAAAG
TAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAGATTTGTTA
AAGACGCACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGACAGTAGGGGGTTTG
AAGACGATTAGATACCGTCGTAATCTCTACCATAAACTATGCCGACTAGAGATAGGGAAATGCCTGTCT
TGGCTTCCCCTGCACCTCGTGAGAAATCAAAGTCTCTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGA
AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGG
AAATCTCACCAGGTCCAGACATGGGAAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATGGGTGGT
GGTGCATGGCCGTTCTTAGTTGGTGGAGTGATCTGTCTGGTTAATTTCGATAACGAACGAGACCTTAGC
CTGCTAAATAGGATCGGGAATTTTATTCCCGTATCACTTCTTAGAGGGACTTTGCACGTCTAGTGCAAG
GAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCTGCACGCGCGCTACACTGACG
CATCCAACAAGTTTCTCCCTTGGCCGATAGGTCTTGGAAATCTTGTGAGTGTGCGTCGTGATGGGGATA
GATTATTGCAATTATTAATCTTCAACGAGGAATGCCTAGTAGGCGCAAGTCAACAGCTTGCGCCGATTA
CGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCAACCGATCGGAGGGTCCTGTGAATTTATTGGACC
GTTTTCAAGTGCTTCTGCATTTTTGAATGGAAAGATACATAAACAGAGCCCTCTAAAGGATGCAAAAGT
CGTAACACGGTTTCCGTAGGTGACCCAACCGAAAGAA

Sequence 3: *Crystallospora* sp. (810 bp)

TCTGGTTGATTCTGCCTCTGGTCGAATGTGCCAGTAGTCATATGCTTCTTTCAAAGATTAAGCCATGCA
TGTCTAAGTGTAAGCTTTTATACAGTGAAACTGACAATGGTTCATTACAACAGTTATAGTTTACATGAT
GGTCCCTTTACAAGGATAACCATGGCAATTCTGTGGCTACTACATGCGTAAAGGCTTCACTTTGGAGCT
GTGTTTATTAGATACCAAAACCAAATAACCTGAAGATTTATGGTGATTCATAGTAACCGAACGAATCGC
ATTATAGCTTCGGCTGGCGATAGATCATTCAAGTTTCTGACCTATCAGCTTTCGACGGTAGGGTATTGG
CCTACCGTGGCAGTGACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCT
ACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATGAAAACAGTTTCGAGGTAGTGACGAGAA
ATAACAATACAGGGCATTAAATGCTTTGTAATTGGAATGACTTAAATTTAAACCCCTTTCGGAGTAACA
ATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAGAGTTGTTG
CAGTTAAAAAGCTCGTAGTTGTATTTCTGCTGGGGCAGACTGTAGGTTCGTCCTACTGGTCTAACCTAG
CTTCTTCTCGGTAGCTCTCCCATCTTTATCGATTGGGTTTGTATTTCCGAGACTTTTACTTTGAGAAAA
ATAGAGTGTTTCAAGCAGGCTAACGCCTTGAATACTGCAGCATGGAATAAT