Fresh- or seawater eels, or mixed life history strategies: what do the parasites tell?

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Front cover motive: The swimbladder nematode, *Anguillicola crassus*.

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Abstract

The European eel (*Anguilla anguilla*) is widely distributed in Europe and has an exceptional tolerance to different salinity environments. Despite of this it is listed as critically endangered on the ICUN Red List of Threatened Species[TM] and vulnerable on the Norwegian Red List of Species. Historically, eels (*Anguilla* spp.) have been considered a catadromous fish species, but research has shown that some eels skip the freshwater phase, and some are habitat-shifters. These alternative life-history strategies may be the dominant at higher latitudes compared to southern Europe. Otolith microchemistry analyses, growth patterns, lipid profile, long-term dietary patterns and parasite fauna can be used to infer the type of residency the eels have had.

The aim of the present study was to get insight into the movement of eels between fresh and seawater using parasites as biological indicators, as well as provide background data on the occurrence of eel parasites in Norway and the geographical range of exotic eel parasites. European eel was caught at seven different localities along the Norwegian coast (58.3°-63.8°N), 93 freshwater and 78 seawater-caught. All 171 eels were dissected and examined for micro- and macro parasites. The eels examined were found to be infected with 34 different parasite species, with 18 species infecting eels caught in freshwater and 22 species in seawater-caught eels. Eleven species were only found in freshwater caught eels, 15 only in seawater caught eels and eight in eels from both habitats.

The three marine parasites found in freshwater eels occurred in 18 % of the fish. However, the studied freshwater locality in Grimstad has seawater influx and is frequented by some marine fishes. In the localities where such influence did not occur, only 3 % had parasites of marine origin, suggestive of inter-habitat shifting (IHS). Yet, myxosporean infections acquired by eels in freshwater were often as prevalent (>30 %) in eels from marine samples as in freshwater localities. This is an indication that most eels at some point had been in freshwater, likely a lake, and been exposed to infectious actinosporeans. These histozoic myxosporean infections are long-lasting, and can reflect only a single periode of freshwater residence, probably as elvers. Other parasites, with a shorter expected longevity in the seawater-caught eels and indicative of recent inter-habitat shifting were rare. These were occasional *Proteocephalus macrocephalus* (cestode) and *Anguillicola crassus* (swimbladder nematode) infections, registered in 4 % of the seawater-caught yellow eels.

In conclusion, some parasites can reveal if an eel has been in freshwater during its lifetime, while others indicate more recent movement between freshwater and marine habitats. Their usefulness depends on the level of knowledge on the life cycles, and knowledge in parasite longevity in the eels.

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Glossary

Abundance   The number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected (Bush et al. 1997).

Cercariae   Free swimming larval stage of trematodes

Coelozoic   Lives in a cavity of an animal's body.

Component community   Refers to all infrapopulations of parasites associated with some subset of a host species or a collection of free-living phases associated with some subset of the abiotic environment (Bush et al. 1997).

Final host   The host in which the parasite attains sexual maturity.

Habitat   The locality or external environment in which the eel lives.

Histozoic   Living within tissues but outside of the cell.

Infracommunity   A community of parasite infrapopulations in a single host (Bush et al. 1997).

Inter-habitat shifter   Eel that move once or twice between freshwater and seawater through their growth face.

Intermediate host   A host in which a parasite passes one or more of its asexual stages.

Intensity   The number of individuals of a particular parasite species in a single infected host (Bush et al. 1997).

Locality	A geographic location of the external environment where the parasite is found (Bush et al. 1997).
Metacercariae	Encapsulated larval stage of trematodes, in the second intermediate host, normally the infective stadium.
Plerocercoid	Larval stage of cestodes.
Prevalence	The number of hosts infected with one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species (%) (Bush et al. 1997).
Site/location	The topological or spatial location on a host where a particular sample of parasites is collected (Bush et al. 1997).
Transport/paratenic host	A host not needed for the development of the parasite, but serves to maintain the parasite's life cycle.

Abbreviations

BLAST	Basic Local Alignment Search Tool
bp	Basepairs
DNA	Deoxyribonucleic acid
FA	Fatty acid
FET	Fisher's exact test
FW	Freshwater
FWR	Freshwater resident
x g	Relative centrifugal force
g	Gram
HES	Hematoxylin-Eosin-Safran
IHS	Inter habitat shifter
IMR	Institute of Marine Research
in vitro	In site, within the sample
ITS	Internal transcribed spacer
IUCN	International Union for Conservation of Nature
km	Kilometer
km^2	Square kilometer
l	Liter
LSU	Large Sub-Unit, about the rRNA gene (LSU rDNA), also called 28S in animals
M/F	Marine to freshwater ratio
Min	Minutes
ml	Milliliter
mm	Millimeter

mm^2	Square millimeter
MWR	Marine water resident
N	Number of specimens
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nt	Nucleotides
p	p-value
PCR	Polymerase chain reaction
rDNA	Ribosomal DNA, DNA sequence coding for ribosomal RNA
r$_s$	Spearman rank correlation-coefficient
rxn	Reaction
Saline	Saltwater diluted to about physiological salinity (10 ‰)
Sec/s	Second
Sensu	In the sense of
Sensu lato	In the old i.e. wide sense, normally used when a species has been split.
Sensu stricto	In the strict i.e. recent sense, normally used when a species have been split into several
SSU	Small Sub-Unit, about the rRNA gene (SSU rDNA), also called 18S in animals
SW	Seawater
UiB	University of Bergen
V	Volt
Vide	"see" (L.), used here to connect citations that must be considered together to make sense.
μl	Microliter

µM	MicroMolar
µm	Micrometer

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1   Introduction

1.1   European eel

The European eel (*Anguilla Anguilla*) is widely distributed, from northern Norway and Iceland in the north, all over Europe including around the Mediterranean, down to the northwest coast of Africa (Tesch 2003). In Norway, the European eel is registered in 1788 waters and lakes representing 361 precipitation areas. However, many lakes and watercourses have not been investigated. In the coastal areas it is seen that the eel abundance decrease northwards (IMR 2017).

The European eel's tolerance to different salinity environments is exceptional. The fish can be found in all kinds of habitats: rivers and lakes, marshes, brackish water, fjord systems and marine coastal waters (Tesch 2003, Daverat et al. 2006). The European eel have historically been considered a catadromous fish species that spawn in seawater and grow in freshwater. In later years the species has been reclassified as a facultative catadromous species due to research done on the strontium and calcium ratio in the otoliths (Tsukamoto et al. 1998). This together with other analyses suggested that some eels never migrate into freshwater, and that some move once or twice between both environments throughout their growth phase. Some research shows that inter-habitat shifting may be the dominant strategy at higher latitudes (Daverat et al. 2006, Durif et al. 2008). The type of residency the eels have had can be inferred from the microchemistry analysis of the otoliths, with the help of growth patterns, lipid profile, long-term dietary patterns and parasite faunas.

1.2   Life cycle

The European eel has a complex life cycle (Fig. 1). The larvae hatch from eggs spawned in the Sargasso Sea area, and develop into characteristic leptocephalus larvae which are transparent with a leaf-like structure (Schmidt 1923).
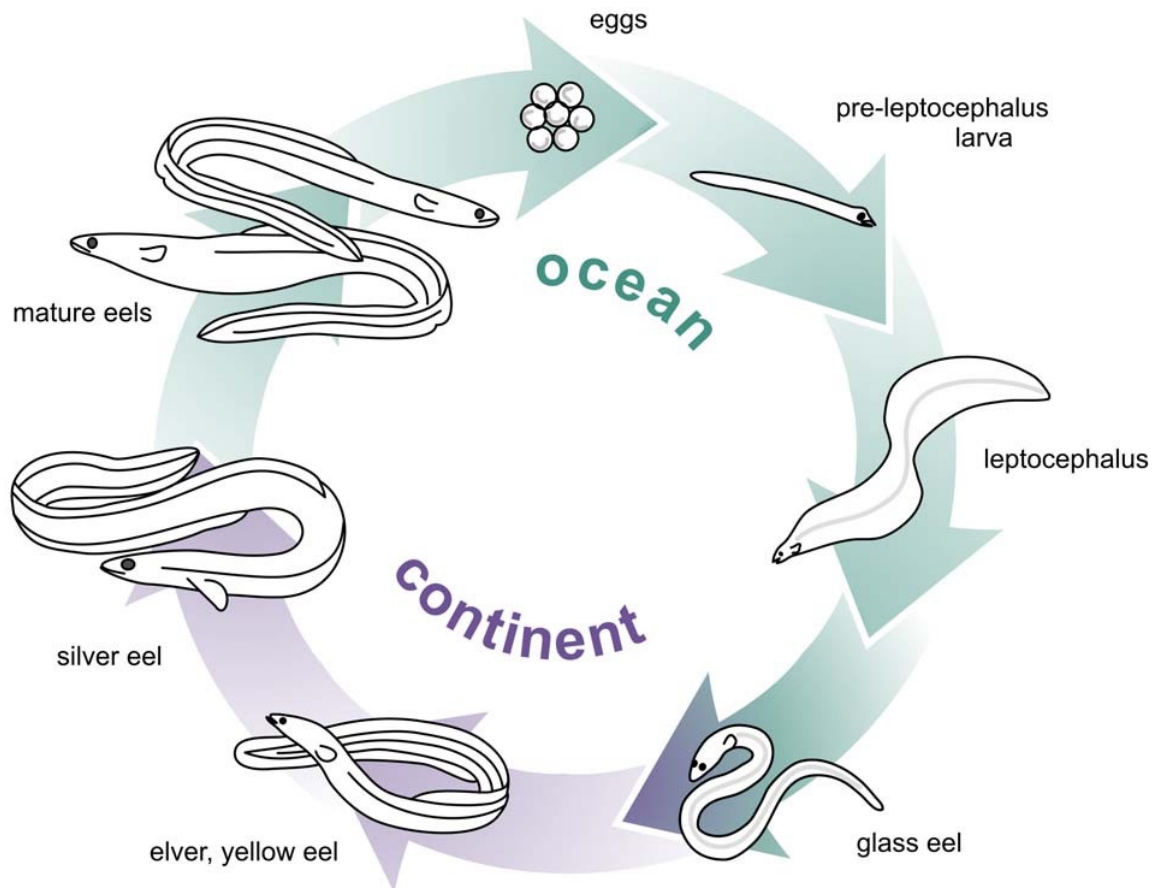
Figure 1 The life cycle of the European eel starting with eggs hatching in the Sargasso Sea area and the leptocephalus larvae drifting with the Gulf stream and enter the European coast as glass eels. Then becoming yellow eels which feed and grow before metamorphosing into silver eels and swim back to the Sargasso sea to spawn (Henkel et al. 2012).

The larvae float in the water column and are transported by the Gulf Stream to Europe where they are dispersed along the European coast (Tesch 2003). The drifting from the western Atlantic to Europe takes approximately one to two years, and the eels arrive at the coast of Europe in the spring (Tesch 2003, Bonhommeau et al. 2009). Before they reach the coast of Europe, they metamorphose into glass eels. Glass eels have the shape of an adult eel but they have no pigmentation. This stage lasts a few months (Schmidt 1923, van den Thillart et al. 2009). Some of the eels will now remain in marine and brackish water in lagoons or estuaries, while others will move upstream and end up in rivers and lakes. The eels feed and grow during their time in freshwater (Tesch 2003). This is the longest period in the life cycle and can last from four to sometimes 30 years or more (Poole & Reynolds 1996, Durif et al. 2020). During this time the eels reach full pigmentation and become yellow eels. There are growth and size differences between males and females. Most large eel, over 45 cm in length, are females (Tesch 2003). After many years in freshwater, the eels undergo a second

metamorphosis known as silvering and transform into silver eels (van den Thillart et al. 2009). They stop growing and start migration towards the ocean. Silvering preadapts the eel to deep-sea conditions and for sexual maturation. This metamorphosis is largely unpredictable, unlike smoltification in salmonids and occurs at various ages and sizes. In females, the age can vary between four and 30 years and in males between 2 and 15 years. Mean total lengths of male European silver eels range between 35 and 46 cm, and females have a range of means from 50 cm to over 1 m (Tesch 2003, Durif et al. 2009b). Several changes occur during silvering. All sensory organs become more developed, including enlarged eyes. The pectoral fin length increases significantly and changes in colour to black. The enlarged fins will contribute with stabilization in the open water during migration. The skin also adapts to the pelagic environment by changing colour. In the silver stage, an eel display a white silver belly separated from a black dorsal region by the lateral line. The skin thickens and an accumulation of fat occurs. Some of the fat is used as an energy source for gonad development, but the majority will be used for swimming. During this period and until the end of their life cycle the eels stop feeding and the alimentary tract degenerates (Schmidt 1923, Durif et al. 2005, van den Thillart et al. 2009). After the eels have gone through silvering and reach the ocean, the long-distance migration to the spawning location in the Sargasso Sea starts. They swim approximately 6000 km, and during this period they undergo sexual maturation. The downstream migration together with the long-distance migration lasts about 6 months. When they reach the spawning grounds they spawn and most likely die afterward (Durif et al. 2008, van den Thillart et al. 2009). Spawning takes place in early spring and can last until late summer (Schmidt 1923).

1.3   Eel fishing and aquaculture in Norway

The eel is a highly valued fish in many countries. But in Norway, there has historically been a low interest for eels (Durif et al. 2011). It has been illegal to fish eel since 2010, due to the European eel population crash. Up until then, the annual recorded eel catch varied between 200-400 tons (Statistics Norway 2019).

The Japanese have farmed eel in ponds since around 1880. Development of eel farming in Europe started in the mid-1900s with the use of the Japanese strategy with eel farming in ponds. This was most feasible in the southern parts of Europe, with France and Italy that had a favourable climate for farming in ponds (Herland et al. 1997, Skiftesvik et al. 2003). The

demand for eel was bigger than the production, therefor new localities and farming models were established (Herland et al. 1997). Eel farming spread northwards to more temperate areas, such as Denmark and The Netherlands. Today European eel farming is mainly associated with recirculation systems, but flow-through systems have also been used (Skiftesvik et al. 2003). The freshwater used in the systems has to be heated for good growth throughout the year, with eels having the best growth at 25°C (Herland et al. 1997). Because the industry has not been successful in rearing larvae and producing glass eel, all eel farming in the world is based on wild caught eel that is placed in tanks and fed until they reach a certain weight. Scientists have however been able to get the eel to spawn in the laboratory (van Ginneken & Maes 2005). Eel farming in Norway was based on imported glass eels in the beginning, but since the import ban on glass eel, the production had to change and be based on capture of yellow eel (Herland et al. 1997, Skiftesvik et al. 2003). The interest for eel farming in Norway has varied, but several eel farms, based on different farming models, have been established (Skiftesvik et al. 2003). Today there are 14 concessions for eel in Norway (Directorate of Fisheries 2018), but no operating eel farms. This is due to the ban on the eel fishery. The last big eel farm in Norway, Farsund Aqua, closed down in 1998 due to problems with different pathogens (Engø 1997, Aasen 1999).

1.4   Eel decline

There has been a steady decline in the European eel stock for the past 40 years and the species is therefore listed as critically endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species[TM] (Freyhof & Kottelat 2010). The adult stock started to decrease in the 1940s and recruitment has collapsed since the early 1980s. The stock is considered outside safe biological limits and the decline seems to occur over most of the natural range of the European eel (ICES 2019). The decline in recruitment has been more pronounced in the North than in the rest of Europe. So with Norway representing the limit of the distribution range, changes in density are more likely to be detected here (Durif et al. 2020). In Norway, as in the rest of Europe, analyses has shown a decline in the eel stock, both in fresh and marine water subpopulations (Durif et al. 2008). The European eel is listed on the Norwegian Red List of Species. It was listed as critically endangered from 2006 to 2015, when the status got improved to vulnerable. This change came as a result of an improvement in the freshwater environment which had a positive effect on the eel abundance (IMR 2017). A new study by Durif et al. (2020) on the age of European silver eels in Norway reported a

mean age of 19 years for female silver eels, which is more than twice the mean age (eight years) used in the previous Norwegian assessment. With the generation length being used to classify endangered species into different IUCN categories, the results of the study will likely have an impact on the next revision of the Norwegian red listing, with the European eel being reassigned to critically endangered or at least an endangered status (Durif et al. 2020).

There are several possible causes for the decline. Two obvious reasons are habitat reduction and overfishing. The eel may have reduced access to the upper reaches of the watershed due to dams and other obstructions and downstream migration can be difficult for the silver eels which get entrained in the turbines of hydroelectric power plants. Other reasons may be pollution and diseases or parasites (Durif et al. 2008, Castonguay & Durif 2016, Aschonitis et al. 2017, Drouineau et al. 2018). Many of these reasons are only present in freshwater habitats, but some marine causes have also been hypothesized, one being a global change in oceanic currents, which could affect the larval drift to Europe. Also changes in ocean productivity and a decrease in food for leptocephalus larvae could cause variation in the recruitment (Durif et al. 2006). Higher temperatures at the spawning ground in the Sargasso Sea may also have a negative effect on the newly hatched larvae (IMR 2017).

European eel parasites, in particular invasive species, are suspected to play an important role in the decline in the population of their host (Fazio et al. 2008). The parasites can be considered as biological stressors. Not all cause disease in fish, but they may be present in a subclinical or carrier state as a potential causative factor in the decline (Mayo-Hernandez et al. 2015). The parasitic infections which leads to severe symptoms and eventually death of eels are caused primarily by highly pathogenic species (van den Thillart et al. 2009).

1.5   Parasites in the European eel

Parasite species that can be considered as serious pathogens for the European eel are *Pseudodactylogyrus anguillae* (Yin et Sproston, 1948), *P. bini* (Kikuchi, 1929) and *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 (Kennedy 2007). Neither of them are pathogenic to their preferred natural host species in the wild, but are found to cause great damage to European eel.

Anguillicola crassus, the swimbladder parasite, is an introduced parasite in Europe. The natural range of *A. crassus* is in tropical and subtropical Asia where it is widespread in the Japanese eel (*Anguilla japonica*) in natural waters and eel farms. It causes little or no damage

to the Japanese eel but can be highly pathogenic to the European eel. The parasite came to Europe in the early 1980s with live eels from Taiwan when eels were imported for stocking and farming purposes (Mo & Steien 1994, Mo 2009). The first reports of the parasite came from Italy and the south of Germany. Later the parasite spread to other countries and was reported from Denmark in 1986 and from Sweden in 1987. The first observation from Norway came in 1993 when two dark-brown nematodes were observed in the swimbladders of eels from an eel farm in Østfold (Fig. 2). The production was based on wild caught eel from the area between Hvaler and Fredrikstad, together with imported eels from Denmark (Mo & Steien 1994). It was apparently observed for the first time in wild-caught eels, from the south of Norway, delivered to an eel farm in Farsund in 1997 (Engø 1997). After the late 1990s, when most eel farms were closed down, the parasite got little attention until it was observed in wild eels caught in several rivers in 2008. It was identified in eel from the river Imsa, the outlet area to the the river Drammenselva and the river Enningdalselva (Fig.2). The hypothesis is that the parasite has spread between Norwegian waterways by migratory eels (Mo 2009), or with the help of different mobile transport hosts (Lindholm 2012). *A. crassus* has not been reported further north than Stavanger, but it is believed that it has spread to Hordaland and Sogn og Fjordane (NBIC 2018a).

Pseudodactylogyrus anguillae and *P. bini* have also been introduced in Europe. They were first reported from an eel farm in the European part of the former Soviet Union where they had been introduced with imported Japanese eel. The introduction to Central Europe most likely happened at the same time as the introduction of *A. crassus* with the import of eel from Taiwan (Buchmann et al. 1987, Køie 1991). The ectoparasitic monogeneans are located on the gills, where they feed on blood, mucus and skin cells. *P. anguillae* was observed for the first time in Norway in 1987 in farmed eel (Mo et al. 1988) and became a big problem for eel farmers (Mo et al. 1988). The first observation of *P. anguille,* together with *P. bini,* in wild eel was made in 1998 in eel from Årungen lake and the river Glomma (Mo & Sterud 1998). How they were introduced to Norway is unknown. One possibility is that there were eggs present in Danish wellboats visiting the farms to collect eel. Another is by the natural migration of infected eel along the coast from Sweden (Mo et al. 1988, Mo & Sterud 1998). Today the known geographical range of *P. anguille* is Østfold, Oslo and Akershus (NBIC 2018b).

Little is known about the parasites of the European eel, especially by comparison with the extensive information available on the parasites of salmonids (Kennedy 2007). Jakob et al. (2016) summarized all published data on the parasite fauna of the European eel and found a total of 161 parasitic species/taxa recorded for European eel. Compared to other fish species in Europe, eels tend to have species poor parasite infracommunities with low parasite abundance.

Most studies of the parasite communities of European eel have been carried out in freshwater localities, and therefore even less is known about the parasite fauna of eels from salt and brackish waters (Filippi et al. 2013). With the suggestion that eels may not necessarily migrate into freshwater (Tsukamoto et al. 1998, Daverat et al. 2006, Durif et al. 2008), and that populations of eels from marine localities contribute primarily to future recruitment of eel populations (Tsukamoto et al. 1998, Mayo-Hernandez et al. 2015), studying the parasite fauna of eels from salt waters can give a better picture of the health status of eel populations.

An eel's parasite infracommunity can provide information on the eel's life strategy. If the parasite life cycles and modes of transmission to fish are known, they can be used as biological tags to provide information on hosts movements and habitats (Kennedy et al. 1992). Parasite communities of European eels strongly relate to the habitat preferences of their hosts and reflect the life history of individual eels. The parasites show very specific salinity-dependence that makes it possible to cluster the them into freshwater, brackish, and marine groups (Jakob et al. 2009, Mayo-Hernandez et al. 2015).

In Norway, knowledge on the parasite communities in European eel is very scant. In a study from 1998, with thirteen specimens of eel from south-eastern Norway, twelve different parasite species were found (Mo & Sterud 1998). Additional species have also been recorded in general parasite studies (Table 1). None of the previous studies on the parasite fauna of Norwegian eel have been carried out in marine localities, but there are scattered parasite record from eels caught in the sea (e.g. Olsson (1868)).

Table 1 Parasite species recorded in European eel (*Anguille anguille*) in Norwegian waters before August 2019.

Parasite species	Locality, year
Trypanosoma granulosum Leveran et Mesnil, 1909	Årungen 1996[1] Glomma 1997[1,] Jæren[7]
Trichodina spp.	Vegsund[7], Bergen[7], Sunnmøre[3]
Paramyxidium giardi* (Cépède, 1906)	Årungen 1996[1], Glomma 1997[1]
Pseudodactylogyrus anguillae (Yin et Sproston, 1948)	Årungen 1996[1], Glomma, 1997[1]
Pseudodactylogyrus bini (Kikuchi, 1929)	Glomma 1997[1]
Gyrodactylus sp.	Mauseidvatn 1990[7]
Diplostomum sp.	Årungen, 1996[1]
Azygia lucii (Müller, 1776)	Årungen, 1996[1]
Deropristis inflata (Molin, 1859)	Bergen 1868[2], Glomma 1997[1]
Helicometra sp.	Bergen 1868[2]
Hemiurus communis Odhner, 1905	Bergen 1868[2]
Lecithichirium rufoviride (Rudolphi, 1819)	Bergen 1868[2], Langesund[5], Sotra[6]
Derogenes varicus (Müller, 1784)	Bergen 1868[2]
Lecithaster gibbosus (Rudolphi, 1802)	Bergen 1868[2]
Triaenophorus nodulosus (Pallas, 1781)	Årungen 1997[1]
Bothriocephalus sp.	Årungen 1996[1], Glomma 1997[1]
Proteocephalus macrocephalus (Creplin, 1825)	Oslofjord 1979[4]
Paraquimperia tenerrima (Linstow, 1878)	Kalandsvatn[8], Årungen 1996[1], Glomma 1997[1] Oslofjoden 1993, Farsund 1997[11]
Anguillicola crassus Kuwahara, Niimi et Itagaki, 1974	Imsa 2008[9], River Drammenselva 2008[9] River Enningdalelva 2008[9], River Storelva2010[10]
Camallanus lactustris (Zoega, 1776)	Årungen 1996[1], Glomma, 1997[1]
Ergasilus sieboldi Nordmann, 1832	Glomma 1997[1]

* Most likely *Paramyxidium branchialis* since recorded from the gills. [1] Mo and Sterud (1998), [2] Olsson 1868, [3] Karlsbakk in Sterud (1999), [4] Andersen (1979), [5] Gibson and Bray (1986), [6] Lönnberg (1890), [7] Karlsbakk (pers.comm), [8] B. Berland (pers. comm. Karlsbakk), [9] Mo (2009), [10] Lindholm (2012), [11] Engø (1997).

1.6   Aim

The main aim of the present study is to get insight into the movement of eels between fresh and seawater using parasites as biological indicators. There are several sub goals:

i) Provide background data on the occurrence of eel parasites in Norway

ii) Examine the spread of the exotic eel parasites *A. crassus* and *Pseudodactylogyrus* spp.

iii) Revealing suitable indicator parasites of habitat shifting.

iv) Compare parasite evidence for habitat-shifting with that from stable isotope and fatty acid data.

2 Material and method

2.1 Sampling

The European eels (Table 2), *Anguilla anguilla*, were caught at seven different localities in Norway (58.3°-63.8°N) (Fig. 2); Landvikvannet (August 2018, N=30) and Inner Grosfjord (August 2018, N=17) in Grimstad, Littledalsvatnet (July 2019, N=45) and Etnefjord (July 2019, N=31) in Etne, the river Botngårdelva in Bjugn (August 2019, N=30), the Edøyfjord in the vicinity of the island Smøla (November 2019, N=30) and a couple of lakes in the Orkla river drainage system (September 2018, N=3). Hereafter the localities will be referred to by place name and habitat (Grimstad FW, Grimstad SW, Etne FW, Etne SW, Bjugn FW, Smøla SW, and Orkla FW).

The captured eels were killed using an overdose of anaesthetics (Eugenol/MS222) or with electricity. All fish from Etne FW and Etne SW were

Figure 2 European eel sampling localities along the Norwegian coast (58.3°-63.8°N). Yellow marker=seawater localities, red marker= freshwater localities

measured (mm), weighed (g) and taken samples from at the locality before they were put into individual plastic bags and frozen (-20 °C). Just after terminal anaesthesia, a gill (1st left) was removed from all the Etne eels. A squash preparation was examined in a compound microscope (100-400x) for protists, myxosporeans and monogeneans. Gill samples from all Etne eels were preserved in ethanol (suitable for DNA extraction) or fixed in a modified Karnovsky fixative (phosphate buffered; Steigen et al. (2015)) for histology. Muscle and liver tissue samples together with both eyes were taken from the Etne eels for fatty acid analysis which were carried out by Camilla Parzanini (Ryerson University, Canada), for another part

of the MAREEL project (funded by the Norwegian Research Council NFR project# 280658 and the Institute of Marine Research). Fish from Bjugn FW and Smøla SW were put straight into individual plastic bags before they were frozen (-18 °C). The eels from Grimstad FW and Grimstad SW were received deep-frozen in blocks and were separated into individual plastic bags after minimal defrosting in the lab, before being re-frozen until examination. Sediments from the water used for defrosting were examined for any detached ectoparasites (e.g. *Argulus* spp., leeches).

Table 2 Overview of total length and weight of the examined eels. Min=Minimum, Max=Maximum, SD=Standard deviation

Locality	n	Length(mm)				Weight (g)			
		Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Grimstad FW	30	531	67	400	647	255	90	124	423
Grimstad SW	17	527	65	420	647	237	110	117	520
Etne FW	45	518	102	370	840	257	201	78	1150
Etne SW	31	497	107	380	512	239	114	80	512
Bjugn FW	30	334	59	204	452	68	30	14	137
Smøla SW	30	527	72	405	704	326	153	140	712
Orkla FW	3	726	323	357	960	1093	913	98	1891

2.2   Localities

2.2.1   Landvikvannet ('Grimstad FW')

Landvikvannet (58.332997°N, 8.518651°E) is located on the Norwegian Skagerrak coast (Fig. 3). It is a 1.85 km^2 lake that is artificially connected via a 3 km long, 1-4 m deep canal to the adjacent fjord, the Strand fjord. Apart from a small 25 m deep basin located at the entrance of the lake, the depth is between 7-10 m. Streams around Landvikvannet add fresh water into the

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lake, and there is an inflow of saltwater during the tidal cycle, resulting in a stratified water column with a transition depth at 4 m. The upper layer has low salinity, and oxygen content above 5 ml/l as opposed to the lower layer that has moderate salinity (> 20 ‰), low and constant temperature (~8°C), and is anoxic and toxic due to hydrogensulphide (Eggers et al. 2014, Berg 2018).

Landvikvannet hosts several different fish species, both marine, and freshwater. The dominant species is rudd (*Scardinius erythrophthalmus*). Other species are brown/sea trout (*Salmo trutta*), herring (*Clupea harengus*), sprat (*Sprattus sprattus*) and eel (*Anguilla anguilla*) (Berg 2018). There are also sticklebacks (*Gasterosteus aculeatus*, *Pungitius pungitius*) inhabiting the water (Haraldstad et al. 2013).

The eels were caught using fyke nets at 3-8 m depth.
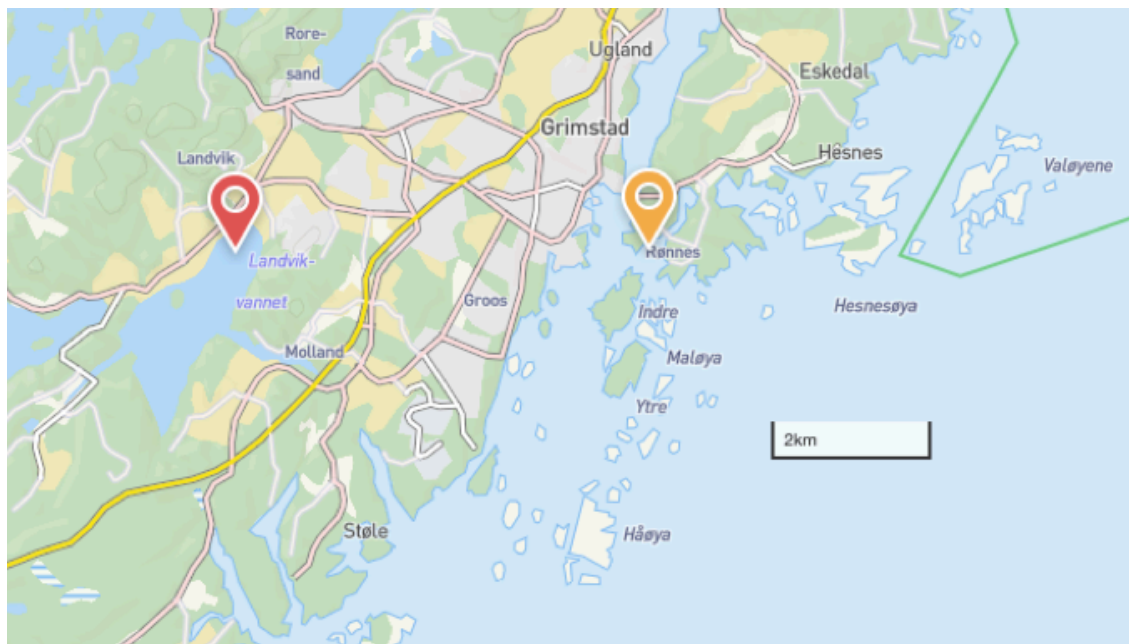
Figure 3 Sampling localities in the Grimstad area, Landvikvannet (red marker) and the Inner Grosfjord (yellow marker). (Obtained from Kommunekart.no)

2.2.2   The Inner Grosfjord ('Grimstad SW')

Inner Grosfjord (58.3334°N, 8.6074°E) is located on the Norwegian Skagerrak coast, in Agder county (Fig. 3). The water in the fjord is polyhaline (16-30 ‰) and low on oxygen (Vann-nett.no). The eels were caught using fyke nets at 3-8 m depth.

2.2.3 Litledalsvatnet ('Etne FW')

Litledalsvatnet, a 0.949 km² freshwater lake, is located on the Norwegian west-coast in the municipality Etne (Fig. 4). The lake hosts several different species, brown trout, Arctic charr (*Salvelinus alpinus*), threespine sticlebacks (*Gasterosteus aculeatus*) and European eel. The lake is connected with the Etnefjord by the river Etneelva. The eels were caught using fyke nets placed at 0.4-1.5 m depth that had been in the water for two-three days. To kill the fish, they were put in a barrel and given an overdose of anaesthesia (Eugenol).
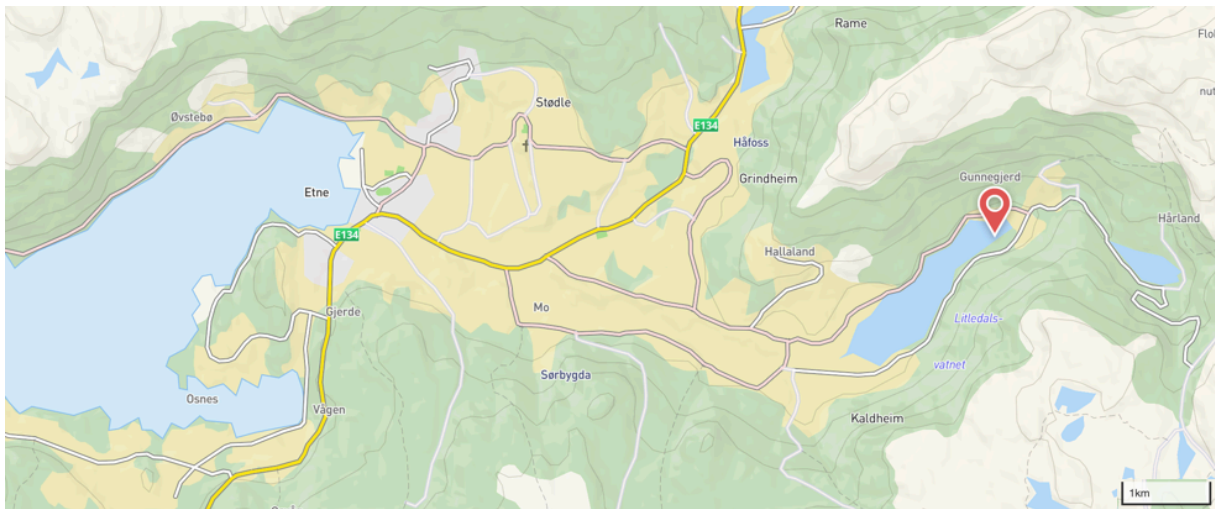
Figure 4 Freshwater sampling locality in Etne, Litledalsvatnet (red). (Obtained from Kommunekart.no)

2.2.4 The Etnefjord ('Etne SW')

The Etnefjord (8.3 km long), an arm of the fjords Ølsfjord and Hardangerfjord, is located on the border between Vestland and Rogaland counties in Norway. The fjord is 8.3 km long and divides into two branches, with Osvågen that branches off into the southeast and the Etnefjord continuing northeast to Etne. The Etnefjord is categorised as euhalin (> 30 ‰) (Vann-nett.no). The eels representing Etne SW were caught in two areas of the fjord, A and B (Fig. 5). Area A is between the river mouth of the river Etneelva and Fjørsnaneset (59.67446°N, 5.92127°E). The fyke nets and eel pots were placed at 1-2 m depth with sandy bottom, seaweed and eelgrass. Area B contains the bay within Melandsholmen (59.64372°N, 5.88671°E). The fyke nets and here eel pots were here placed on a sandy bottom with eelgrass. The fish traps were in the water for two-three days.
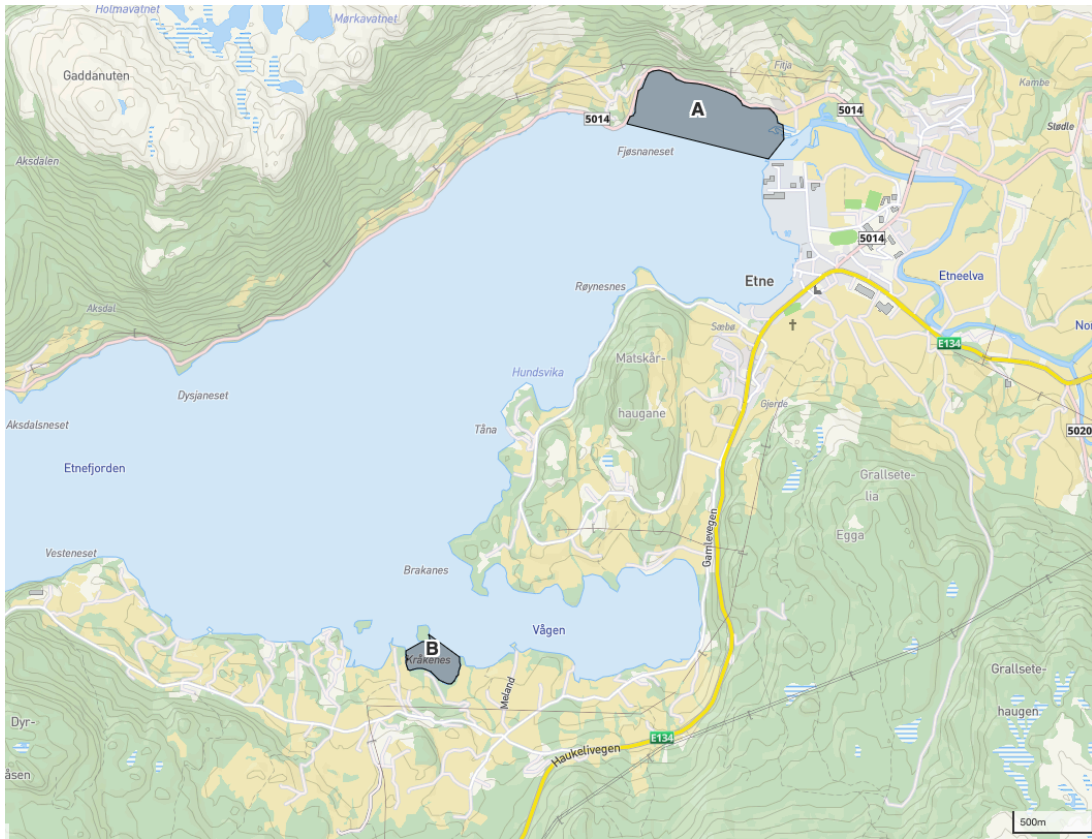
Figure 5 Marine sampling localities in Etnefjorden, Areas A and B (black shaded). (Obtained from Kommunekart.no)

2.2.5 The river Botngårdelva ('Bjugn')

The river Botngårdselva is located in Bjugn municipality in Trøndelag county (Fig. 6). The river is a part of the Botngård drainage system and connects it with the Bjugn fjord. The Fish fauna in The Botngård drainage area is dominated by salmonid fish species, but eel and introduced pike are also present (Bergan 2016). The fish were caught with electrofishing 100-200 m from the river mouth.
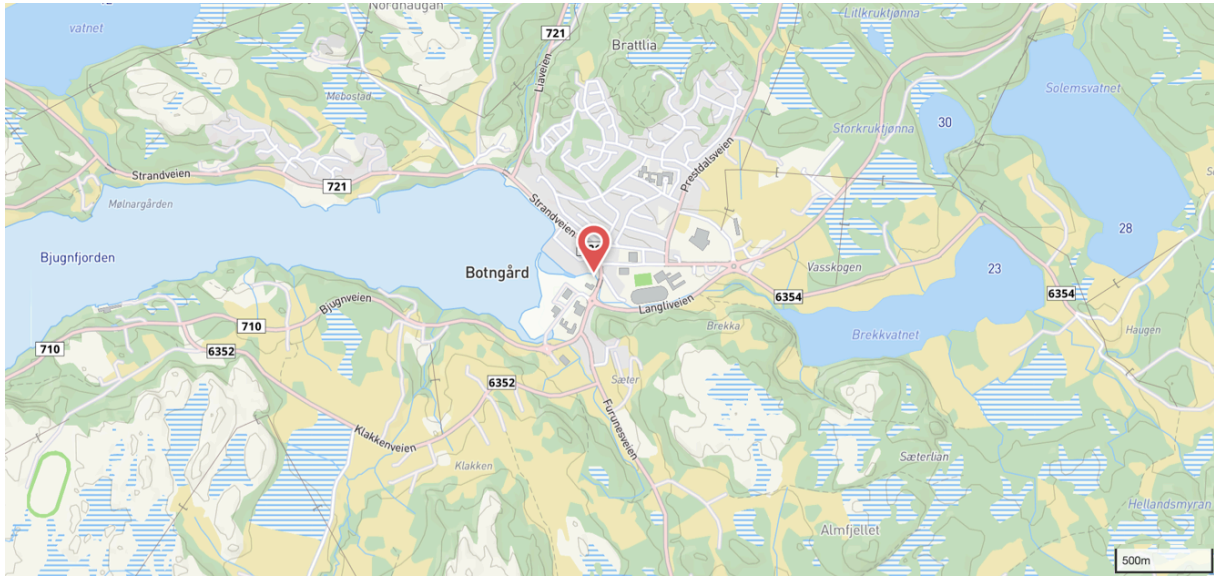
Figure 6 Sampling locality in the river Botngårdelva (red) in Bjugn municipality. (Obtained from Kommunekart.no)

2.2.6 Smøla ('Smøla')

The eels from the 'Smøla' sample were caught in three areas in Edøyfjoden near Smøla in Trøndelag county. Sampling was done during a survey for Atlantic cod juveniles at Smøla and Aure (by Terje van der Meeren, IMR). The localities were in the area around Lauvøysvaet (63.31244°N, 8.16862°E), Åkvika (63.33444°N, 8.43911°E) and Araneset (63.26796°N, 8.32712°E) (Fig. 7). All these areas are categorised as Euhaline (> 30 ‰) (Vann-nett.no). The eels were caught using fyke nets at 2-8 m depth over a time period of three weeks. The fyke nets were checked every second day and the captured eels were kept in nets in the sea until they were killed with an overdose of anaesthesia (MS222), put in individual plastic bags and deep-frozen.
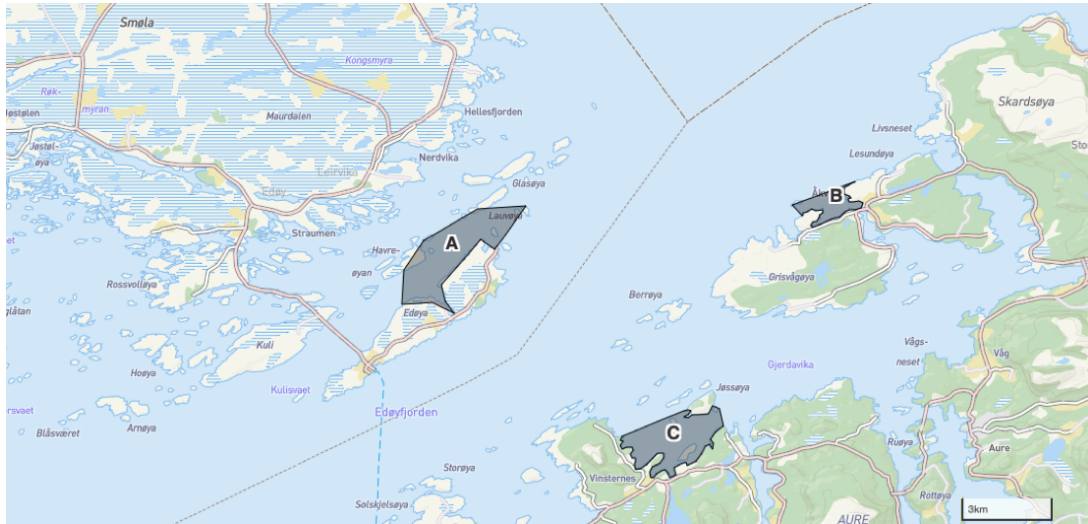
Figure 7 Sampling localities in the area near the island Smøla, Lauvøysvaet (A), Åkvika (B) and Araneset (C). (Obtained from Kommunekart.no)

2.2.7 Orkla River drainage area ('Orkla')

The three eels from two small lakes (Ålvatnet (63.29940°N, 9.78992°E), Brandåstjønna (63.24553°N, 9.65813°E)) connected to the river Sika and the river Skjenaldselva in the Orkla River drainage system (Fig. 8), were killed during a rotenone treatment performed there in September 2018, in order to eradicate the introduced pike (*Esox lucius*) (Bardal et al. 2019). These eels were included in the study only to extend the knowledge on the geographic range of the eel parasites in Norway, and did not constitute a sample equal to the others.

Figure 8 Sampling localities in the Orkla River drainage system, Brandåstjønna (A) and Ålvatnet (B).

2.3   Dissection and parasite examination

A total of 171 eels caught at the seven different localities were dissected and examined for parasites. The general examination included the external surface of the fish, fins, gills, eyes and all internal organs. However, since the eyes of all eels from Etne were used in fatty acid analysis, 15 additional freshwater-caught eels from Etne were examined solely for eye parasites. The dissections took place at the parasitology laboratory at The University of Bergen (UiB).

The eels were thawed in cold water, usually for c. 30 minutes. The total length and weight were then recorded. In accordance with Durif et al. (2009a), eye diameter, and pectoral fin length were also registered, providing a staging for the eels. During the dissection, the sex of the fish was determined by gonad inspection (Tesch 2003). Any prey present in the stomach was recorded, and during the examination of the head (below) the otoliths were taken out. These were sent to The Institute of Marine research (IMR) for microchemical analyses.

The external surfaces were then examined for abnormalities such as lesions. The plastic bag was also examined for potentially detached ectoparasites. During the external examination, the eels were examined for skin and fin cysts or black spots due to endoparasites such as myxosporea or trematode metacercariae. The head was then separated from the body and placed in a glass Petri dish with seawater diluted to about physiological salinity (~10 ‰, henceforth referred to as 'saline') and stored cold (see below), because the digestive tract was necessarily examined first.

The abdomen was cut open from the pericardial cavity to the anus with the use of scissors. All internal organs: heart, liver, gallbladder, spleen, alimentary tract and swim bladder, were removed and placed in separate glass Petri dishes with saline. A urine sample was taken from the urinary bladder and examined microscopically for spores. Gall from the gallbladder was transferred to a 1.5 ml plastic microtube for spore sedimentation of any spores present and later examined microscopically. A squash preparation was made with a piece from the posterior part of the kidney and examined in the microscope for plasmodia and spores (e.g. myxosporea). Occasionally white spots were observed in the kidney, that were also examined further. All these wet and squash preparations (urine, gall, and kidney) were examined in a compound microscope (Olympus cx 41) at 100x, 400x and 1000x magnification for spore producing protists or myxosporea. The other organs were examined macroscopically externally, and any parasites seen were removed, before the organs were flattened between to

glass Petri dishes and candled using a dissecting microscope (Wild Herrbrugg) with under lights.

The alimentary tract was divided into the esophagus/stomach and intestine, with the intestine being further split into an anterior, a posterior and an anal part. The parts were split longitudinally and any prey or big parasites were removed and placed in separate containers. The mucosa was then scraped out with a scalpel and candled, before the wall of the gut was examined in the same way. A small selection of fish from each freshwater locality were examined for the coccidian *Epieimeria anguillae* (Léger et Hollande, 1922), by microscopy on squash preparations from the epithelium from the anterior intestine for oocysts and sporocysts. The swimbladder was examined macroscopically for the presence of *Anguillicola crassus* in the lumen. The heart, liver, spleen, gonad and the rest of the swimbladder was then squashed between two Petri dishes and candled for parasites. Finally, the visceral cavity was inspected.

From the head-part of the body, the eyes were cut out and placed in saline. They were examined in the dissecting microscope by opening the eye and examining the lens, vitreous humour and retina. The gills were taken out and examined separately for helminth parasites such as monogeneans and nematodes (e.g. *Pseudodactylogyrus* spp. and *Daniconema anguillae* Moravec et Køie 1987) using a dissecting microscope. To examine the gills for myxosporeans, about 20 filaments were cut off from the first gill on the left side and examined in the microscope (100-400x). As a measurement of intensity, the proportion of infected filaments was estimated. When the occurrence of the tissue nematode *Daniconema anguillae* was discovered in the gills, and large numbers of larvae subsequently seen in the pectoral fin, a density estimate was taken. In the pectoral fin, the typical number of visible nematodes in a field of view with 40x magnification (18.85 mm^2) was registered.

All the parasites found were, as a standard, counted, roughly measured, washed and examined more closely in the microscope, and identified if possible. Digital photos were taken for documentation, and as an aid in identification. The gender and maturity were determined and registered on certain parasites. Maturity of platyhelminths was determined on the basis of whether or not the specimen contained eggs (were oviferous), with egg containing specimens registered as mature. Pictures were taken of all parasite types and most individuals, but not of all individual of common types. The myxosporean parasites were registered as present and with the number of plasmodia containing spores if possible. Helminth parasites and tissue

samples containing microparasites (e.g. urinary bladder and intestinal wall) were preserved in ethanol (96-100 %).

Also, small pieces of the stomach wall, posterior kidney, and lamellae from the first left gill were taken and stored in ethanol as a standard, allowing for PCR analyses later.

2.4   Extraction and purification of nucleic acids

To extract and purify nucleic acids from tissue samples and parasites the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek) was used. The sample was removed from ethanol and dried using a heating block (55°C). Then 200 μl of digestion buffer (TL) and 25μl of proteinase K solution were added. This was mixed, spun down and incubated at 55°C overnight, except for the platyhelminth parasites that were incubated for only two hours. After the proteinase K treatment, the sample was centrifuged (13,000 x g; 5 min). The supernatant was then transferred to a sterile 1.5 ml micro-tube and 220 μl of BL Buffer was added. The sample was mixed and incubated at 70°C for 10 minutes. Then, the sample was spun down and 220 μl of absolute ethanol was added to the sample and mixed by pipetting up and down. The entire sample was then transferred into a HiBind DNA Mini Column with a collection tube. To bind the DNA, the sample was centrifuged (10,000 x g; 1 min). The HiBind DNA Mini Column was then placed in a new collection tube and 500 μl of the first wash buffer (HB/HBC) (diluted with isopropanol) was added to the column. The sample was centrifuged (10,000 x g; 30 sec). The HiBind DNA Mini Column was then placed in a new collection tube and 700 μl of DNA Wash Buffer (diluted with absolute ethanol) was added and the sample was centrifuged (10,000 x g; 30 sec). This step was then repeated for a second DNA Wash Buffer step. The empty HiBind DNA Mini Column was then centrifuged at maximum speed for two minutes to remove traces of ethanol. To elute the DNA from the column the HiBind DNA Mini Column was placed in a sterile microtube and 50-200 μl of preheated (70°C) Elution Buffer was added. After about two minutes, the sample was centrifuged (13,000 x g; 1 min). The isolated DNA was eluted from the column with the Elution Buffer.

The concentration (ng/μl) of DNA in the samples was measured using a UV spectrophotometer (*Nanodrop® ND-1000*). The instrument baseline was set using the Elution Buffer. The DNA was stored at -30°C.

2.5   Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a method used to amplify a specific DNA region *in vitro*. In this study, PCR was performed on DNA from a selection of parasites, in order to identify them, verify identity or provide reference sequences. The target gene amplified varied with parasite groups, Small Sub-Unit RNA gene (SSU rDNA or 18S), Large Sub-Unit RNA gene (LSU rDNA or 28S) and the internal transcribed spacer DNA region (ITS1-5.8s-ITS2) were used.

PCR requires a thermostable DNA polymerase enzyme, template DNA, primers and nucleotides in a suitable buffer. A mastermix containing 10 µl GoTaq®G2 Colorless Master Mix (GoTaq®G2 DNA Polymerase, dNTPs, MgCl$_2$, reaction buffers), 500 µM forward and 500 µM reverse primer and 7 µl nuclease-free water per sample was prepared (Table 3). 19 µl of the mastermix together with 1µl of the isolated DNA was added to sterile 0.2 ml PCR-tubes. Primers used are listed in Table 4.

Table 3 PCR set up - mastermix

Reagent	Volume (µl) for 1 rxn	Final concentration
GoTaq®G2 Colorless Master Mix	10	X 1
Forward primer	1	500 µM
Reverse primer	1	500 µM
DNA template	1	Variable
Nuclease-free water	7	-
Total reaction volume	20	

The samples were put in a thermocycler (*Veriti™ 96-Well Thermal Cycler-Applied Biosystems*) for the PCR. The following thermo profile was used: two minutes of denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55 or 56°C for 30 seconds and one minute of extension at 72°C. At the end, there was a final extension for five minutes at 72°C (Fig. 9). The temperature used in the annealing step of the PCR depended on the Tm for the primers used.
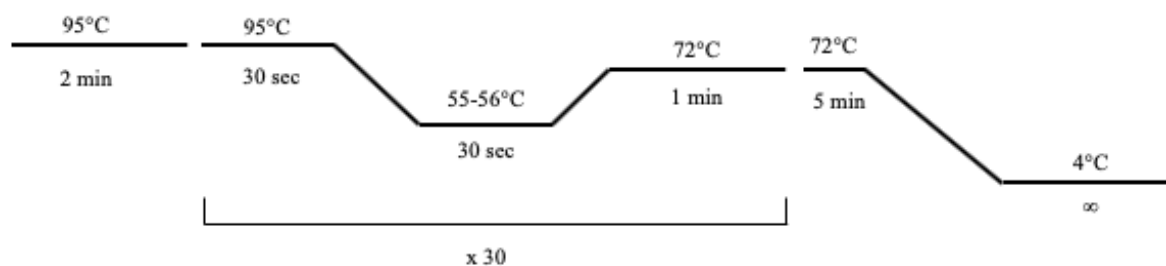
Figure 9 Illustration of the PCR thermoprofile. Stage 1: Two minutes of denaturation at 95°C. Stage 2: 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55 or 56°C for 30 seconds and one minute of extension at 72°C. Stage 3: Final extension for 30 seconds.

Table 4 Primers used in the PCRs in order to amplify various types of rDNA of some parasites.

Primer name	Primer sequence (5'-3')	Target DNA	Target parasite	Ref.
Myxgp2F	WTGGATAACCGTGGGAAA	18S	Myxosporea	Kent et al. (1998)
Ech-R1	CATGCACCACCATACACCG	18S	Myxosporea, Paramyxidium	EK
Ech-R3	CGGGATAAGCCTGACAGATCA	18S	Myxosporea, Paramyxidium	EK
MyEel-R1-RW	AACCGCTCCTCTTAATCATCA	18S	Myxosporea, Myxobolus	EK
MyEel-R2-RW	ACACGATTGTTCGTTCCATG	18S	Myxosporea, Myxobolus	EK
MyxospecF	TTCTGCCCTATCAACTWGTTG	18S	Myxosporea general	Fiala (2006)
ZEel-R3	GCACATTGTATAGCTTGCAC	18S	Myxosporea, Zschokkella	EK
Py28S F1	ATAGCCCAGCACCGAAGC	28S	Cestoda	EK
Ces28S R1	CTCTGGCTTCAACCTACG	28S	Cestoda	EK
L300F	CAAGTACCGTGAGGGAAAGTTG	28S	Trematoda	Littlewood et al. (2000)
28SR3	TCTGGCTTCAACCTACGCAAG	28S	Trematoda	EK
NC5F	GTAGGTGAACCTGCGGAAGGATCATT	ITS	Nematoda	(Zhu et al. 1998)
ANIR1	CAGTGRYCGATGGATTCA	ITS	Nematoda	EK

18S – Small Sub-Unit RNA gene (SSU rDNA), 28S – Large Sub-Unit RNA gene (LSU rDNA), ITS – internal

transcribed spacer DNA region (ITS1-5.8s-ITS2), EK – Egil Karlsbakk (unpublished).

Positive controls were included in the PCR analyses when possible (Table 5). These had been fixed fresh, so DNA quality should be good. This was done to account for potential poor DNA quality in the frozen and thawed parasites.

Table 5 Positive controls used in some of the PCR analyses.

Agens	Host	Locality	Date
Myxobolus species			
Myxobolus neurobius	*Salmo trutta*	Byglandsfjord, Agder	11.12.13
Bothriocephalidean cestode			
Dibothriocephalus ditremus	*Gasterosteus aculeatus*	Lake Gjønavatn, Hordaland	21.08.05
Tetraphyllidean cestode			
Trilocularia gracilis	*Phrynorhombus norvegicus*	Raunefjord, Hordaland	20.10.03
Trematoda			
Steringophorus furciger	*Platichthys flesus*	Lyngen, Troms	20.05.03
Nematoda			
Contracaecum sp.	*Phalacrocorax carbo*	Masfjord	07.05.98

All controls collected and provided by Egil Karlsbakk.

2.6   Gel electrophoresis

Gel electrophoresis was used visualize that the DNA template in the PCR was detected by the primers. A 1 % agarose gel dissolved in 1X TAE-Buffer with 1 µl Gelred™ was used. The gel was submerged in 1X TAE-buffer and the wells were loaded with 5 µl PCR product mixed with 1 µl loading dye. 3 µl Quick-load® purple 2-log DNA ladder (New England Biolabs) was applied to at least one well to estimate the molecular weight of the PCR products. The gel was connected to a power source (80V) for 60 minutes. The bands on the gel were visualized using UV-light (Gel Logic 212PRO, Fisher Scientific) and the program Carestream MI.

2.7   Sequencing and Analyses

PCR-products with proven content of DNA, seen as bands on the gel, were purified using ExoSAP-IT® before sequencing. A mix of 1 μl of ExoSAP-IT® and 2.5 μl of PCR-product was placed in the thermocycler and the standard "Exosap" program was run, 37°C for 15 minutes, then 80°C for 15 minutes. Primers and nucleotides left from the PCR were broken down in this process. The treated samples were used as the template in the sequencing reaction. Template (1 μl) was added to two PCR-tubes together with 1 μl BigDye® (version 3.1), 1 μl sequencing buffer and 6.5 μl nuclease-free water. Then 0.5 μl of forward primer was added to one of the PCR-tubes and 0.5 μl reverse primer to the other. The cycle sequencing was performed using the following thermoprofile: denaturation at 96°C for 5 minutes, then 25 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds and extension at 60°C for 4 minutes. Afterwards, 10μl of nuclease-free water was added to the samples and they were delivered to the sequencing laboratory at UiB for processing. Contigs were assembled using Contig Express, part of the Vector NTI (v 9.0) suite. Sequence searches were done with BLAST (Basic Local Alignment Search Tool) in NCBI (National Center for Biotechnology Information) to find similar sequences that could aid identifying the parasites.

2.8   Histology

Histology was used to clarify the exact location of infection foci with the myxosporean *Myxobolus* sp. within the intestinal wall. Tissue samples containing the parasite were taken from the defrosted eels during dissection, and fixed in buffered formalin (10%). The gut pieces were processed at the histology lab at IMR, where they were and embedded in paraffin wax, and sectioned following standard protocol (Bøgwald 2019). The sections were hematoxylin-erythrosin-saffron (HES) stained.

2.9   Fatty acid analysis

Fatty acids (FA) were extracted by Camilla Parzanini (Ryserson University) following a modified Folch method (Folch et al. 1957) in a chloroform:methanol (2:1) solution, and analysed as methyl esters (FAME) using gas chromatography (GC). Finally, following the

formula provided by Parzanini et al. (Accepted), the marine to freshwater ratio (M/F) was calculated for the identification of M *vs* F eel.

$$M/F = (20{:}1n{-}9 + 22{:}1n{-}11 + 20{:}5n{-}3 + 22{:}6n{-}3) / (18{:}2n{-}6 + 18{:}3n{-}3 + 20{:}4n{-}6)$$

Elements of the numerator are FA that are typically found in high proportions in marine fishes, such as eicosenoic (20:1n-9), cetoleic (22:1n-11), eicosapentaenoic (20:5n-3), and docosaexahenoic (22:6n-3) acids. Conversely, the FA included in the denominator, i.e. linoleic (18:2n-6), linolenic (18:3n-3), and arachidonic (20:4n-6) acids, are generally abundant freshwater fishes (Parzanini et al. Accepted).

Based on the M/F ratio, the eels were categorized into groups with either freshwater (FW), brackish water (BW), intermediate (SW/BW) or seawater signatures (SW).

2.10   Otolith microchemistry analyses

The otolith microchemistry analyses were carried out by Mehis Rohtla (IMR). The sagittal otoliths were prepared in transversal plane until the core was exposed. For trace element analysis, continuous core-to-edge transects were traced using Cetac LSX213 laser coupled with Agilent 8,800× ICPMS. The laser was set to 10 Hz, with a 40 μm ablation spot size, and a scan speed of 5 μm/s. Data were handled following the methods of Miller (2007) as described in Rohtla et al. (2014). Briefly, raw counts were converted to element-to-calcium molar ratios using the NIST-612 standard that was analysed before and after every 10 otoliths. The eels were categorised as either freshwater residents (FWR), marine water residents (MWR) or inter-habitat shifters (IHS).

2.11   Statistics and diversity indices

The relationship between eel size (length) and parasite (helminth) abundance was examined using Spearman's Rank correlation coefficients (r_s). Generally, only significant results from meaningful analyses (prevalence >~10 %) are reported.

3   Results

3.1   Parasites detected

The 171 female eels (N (FW)=93, N (SW)=78) from seven different salinity habitats along the Norwegian coast (58.3 °N- 63.8 °N) were in all infected with 34 different parasite species (Table 6), 11 found only in freshwater, 15 only in seawater and eight in eels from both habitats. The parasites represented one coccidian, six myxosporeans, one monogenean, nine trematodes, five cestodes, one acanthocephalan and eleven nematode species.

3.1.1   Protists

Epieimeria anguillae **(Léger et Hollande, 1922)**

Coccidian oocysts and sporocysts were observed during microscopy on mucus from the intestine of two eels from Grimstad FW (Table 6). A few fish from the other localities examined were negative, but coccidia was not targeted in this study.

The coccidian was identified with *Epierimeria anguillae* according to the descriptions provided by Léger and Hollande (1922) and Lacey and Williams (1983). Oocysts (Fig. 10A,B) measured 10.8-12.1 (mean 11.4) µm in diameter (N=14), while the sporocysts (Fig. 10C) were 8.1-8.9 (8.5) µm long and 4.5-5.1 (4.8) µm thick (N=15). The sporocysts were oval according to the side view but hexagonal in transverse section.
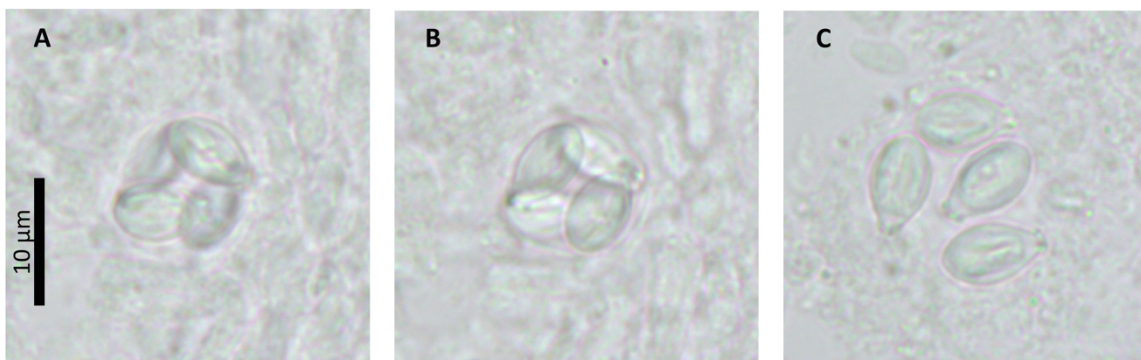

Figure 10 *Epieimeria anguillae* from freshwater-caught eel, Grimstad. All to same scale. **A, B** oocyst with visible sporocysts**, C** four sporocysts**.**

Table 6 Overview over the parasite species recorded in European eels caught in Norwegian waters dissected in the present study.

Locality:	Grimstad FW					Grimstad SW					Etne FW					Etne SW					Bjugn FW					Smøla SW				
No. examined:	30[b]					17[c]					30 (+15)[d]					31					30					30				
Length range (mm):	400-647					420-647					370-840					380-512					204-452					405-704				
Weight range (g):	124-423					117-520					78-1150					80-512					14-137					140-712				
Parasite species:	N inf	P (%)	Mean	SD	Max	N inf	P (%)	Mean	SD	Max	N inf	P (%)	Mean	SD	Max	N inf	P (%)	Mean	SD	Max	N inf	P (%)	Mean	SD	Max	N inf	P (%)	Mean	SD	Max
Protistes																														
Epieimeria anguillae [a]		+										−										−								
Myxosporea																														
Paramyxidium branchialis	11	37				7	41				11	37				11	35				2	7				9	30				
Paramyxidium magi	0	0				0	0				0	0				0	0				0	0				1	3				
Paramyxidium giardi	1	4				0	0				10	33				1	3				11	37				0	0				
Hoferellus gilsoni	1	4				0	0				5	17				0	0				20	67				0	0				
Zschokkella stettinensis	0	0				0	0				10	33				0	0				21	70				0	0				
Myxobolus sp.	9	30				0	0				6	20				3	10				4	13				0	0				
Platyhelminthes																														
Monogenea																														
Pseudodactylogyrus anguillae	24	80	13.8	19.4	90	0	0	0			0	0	0			0	0	0			0	0	0			0	0	0		
Trematoda																														
Deropristis inflata	0	0	0			7	41	2.6	4.6	15	0	0	0			18	58	8.5	13	49	0	0	0			12	40	5.8	17.3	84
Podocotyle atomon	0	0	0			0	0	0			0	0	0			6	19	3.1	11.9	65	2	7	0.3	1.2	5	0	0	0		
Helicometra fasciata	0	0	0			5	29	2.2	4.7	16	0	0	0			0	0	0			0	0	0			0	0	0		
Hemiurus communis	0	0	0			0	0	0			0	0	0			22	71	69.0	77.8	255	0	0	0			29	97	35.6	54.9	248
Brachyphallus crenatus	0	0	0			0	0	0			0	0	0			1	3	0.03	0.2	1	0	0	0			0	0	0		
Lecithochirium rufoviride	0	0	0			13	76	21.2	29.9	83	0	0	0			0	0	0			0	0	0			27	90	31.3	36.1	152
Derogenes varicus	0	0	0			0	0	0			0	0	0			1	3	0.03	0.2	1	0	0	0			7	23	0.5	1.2	5
Lecithaster gibbosus	0	0	0			0	0	0			0	0	0			2	6	0.1	0.4	2	0	0	0			1	3	0.03	0.2	1
Diplostomum sp. (M)	0	0	0			0	0	0			1	7	0.13		2						0	0	0			0	0	0		
Cestoda																														
Scolex pleuronectis (P)	0	0	0			0	0	0			0	0	0			0	0	0			0	0	0			2	7	0.07	0.25	1
Proteocephalus macrocephalus	0	0	0			0	0	0			1	3	0.03	0.2	1	2	6	0.2	0.9	4	0	0	0			0	0	0		
Bothriocephalus claviceps	0	0	0			0	0	0			6	20	0.4	1	4	0	0	0			1	3	0.03	0.2	1	0	0	0		
Dibothriocephalus ditremus (P)	0	0	0			0	0	0			2	7	0.2	0.9	5	0	0	0			0	0	0			0	0	0		
Bothriocephalidea gen. sp. (P)	0	0	0			0	0	0			0	0	0			4	13	2.5	7	27	0	0	0			0	0	0		
Acanthocephala																														
Echinorhynchus gadi	0	0	0			0	0	0			0	0	0			3	10	1.5		40	0	0	0			2	7	0.1	0.4	2
Nematoda																														
Pseudocapillaria tomentosa	1	3	0.03	0.18	1	0	0	0			0	0	0			0	0	0			0	0	0			0	0	0		
Eustrongylides sp. (L)	0	0	0			0	0	0			8	27	0.4	0.8	2	0	0	0			3	10	0.2	0.5	2	0	0	0		
Daniconema anguillae (L)	8	40				0	0				1	3				0	0				0	0				0	0			
Paraquimperia tenerrima	0	0	0			0	0	0			22	73	5.5	7.7	35	0	0	0			5	17	0.3	0.8	3	0	0	0		
Paracuaria adunca (L)	0	0	0			0	0	0			0	0	0			3	10	4.6	18	91	0	0	0			0	0	0		
Cucullanellus minutus	0	0	0			0	0	0			0	0	0			1	3	0.03	0.2	1	0	0	0			0	0	0		
Hysterothylacium aduncum	0	0	0			0	0	0			0	0	0			5	16	0.2	0.4	1	0	0	0			11	37	1.3	2.7	13
Anisakis simplex (L)	2	7	0.07	0.25	1	0	0	0			0	0	0			0	0	0			0	0	0			2	7	0.1	0.4	2
Contracaecum spp. (L)	11	37	2.6	7.51	39	4	24	0.5	1.1	4	2	7	0.07	0.3	1	8	26	2.0	5.2	23	0	0	0			1	3	1.7	9.1	50
Anguillicola crassus	23	77	2.0	2.25	10	1	6	0.1	0.5	2	0	0	0			0	0	0			0	0	0			0	0	0		

M – metacercariae, P – plerocercoids, L – larvae, N inf – number infected, P – prevalence (%), SD – standard deviation, Int – intensity. [a] Not systematically screened for, [b] N=24 examined for *P. giardi*, N=26 examined for *H. gilsoni* and *Z. stettinensis*, N=20 examined for *D. anguillae*, [c] N=7 examined for *D. anguillae*, [d] 15 additional eels were only examined for the presence of *Diplostomum* sp.

3.1.2 Myxosporea

Paramyxidium branchialis **Freeman et Kristmundsson, 2018**

Infections with this myxosporean were detected microscopically in eels from all localities except those from the Orkla area (Table 6). Plasmodia with spores were round to oval, and measured 38-173 x 34-115 µm (N=20) (Fig. 11A,B). Spores (Fig. 11D,E) (N=20) measured 10.7 (9.7-12.0) µm in length and 6.5 (5.7-7.1) µm in width, with polar capsules 3.7 (3.4-4.3) µm long and 3.4 (3.1-4.0) µm in diameter and polar filaments in 5-6 coils.

Across samples, the prevalence of *P. branchialis* in freshwater-caught eels was 26 %, while 35 % of the saltwater-caught eels were infected. Overall prevalence was 30 %. There were up to 0.87 plasmodia per primary lamella, but average density was 0.15 lamella[-1]. Two partial SSU rDNA sequences (Orkla and Etne SW) had >99 % similarity (1242 nt compared) with the *P. branchiale* sequence in the GenBank (MH414926).
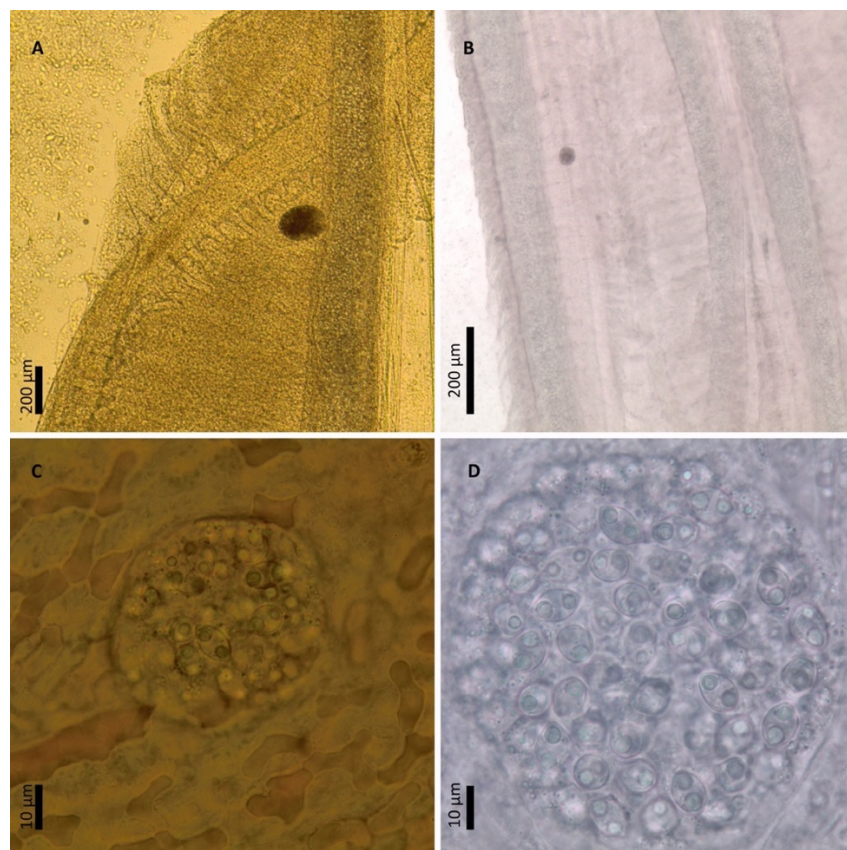

Figure 11 *Paramyxidium branchialis* infections. **A** Plasmodium observed as dark spots in gill lamella from freshwater-caught eel, Grimstad, **B** Plasmodium in gill lamella from freshwater-caught eel, Bjugn, **C** Plasmodium with visible spores from a fresh squash preparation from freshwater-caught eel, Etne, **D** Plasmodium with visible spores from a seawater-caught eel, Smøla.

Paramyxidium magi Freeman et Kristmundsson, 2018

Only one seawater-caught eel from the Smøla area had a *P. magi* infection (Table 6). The parasite formed polysporous plasmodia (reaching 200-300 µm in diameter) in the submucosa of the stomach wall. Mature spores (N=21) measured 9.0 (8.4-9.5) µm in length and 5.8 (5.6-6.1) µm in width (Fig. 12). The polar capsule diameter was 2.9 (2.5-3.2) µm. *P. magi* was also detected on the basis of sequences (490-560 nt) obtained from kidney samples, in an eel from Grimstad and one from the Orkla area. Identification was based on SSU rDNA sequence identity (99-100 %), in comparison with *P. magi* in GenBank (MH414922).
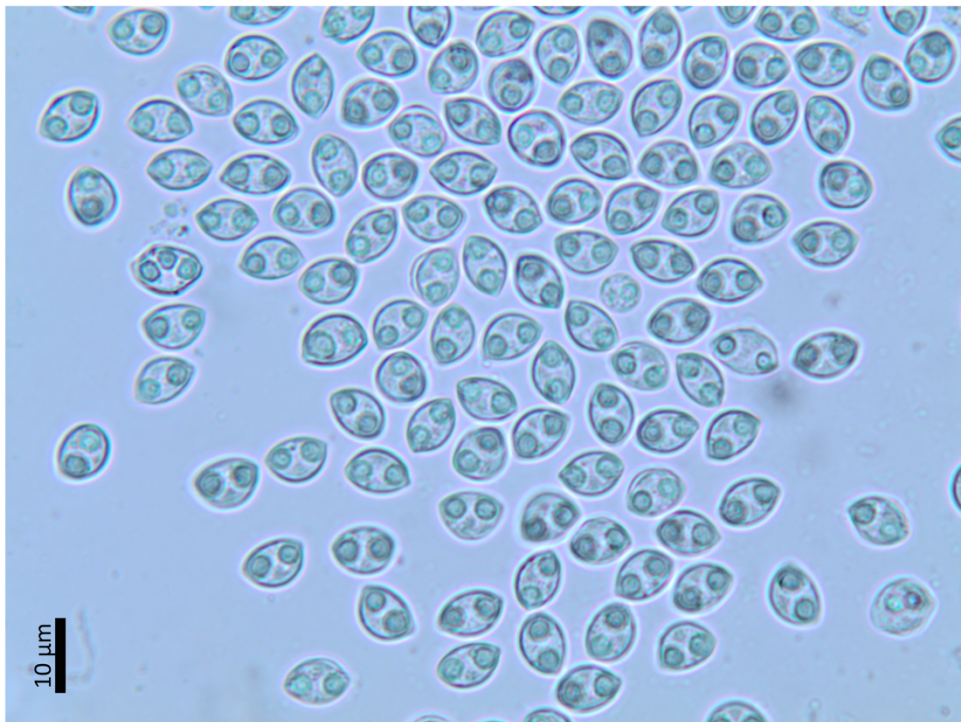

Figure 12 *Paramyxidium* cf. *magi* spores from a plasmodium in the stomach wall of a seawater-caught eel, Smøla.

Paramyxidium giardi (Cépède, 1906)

Infections with this myxosporean were seen in the kidneys of freshwater eels from Grimstad, Etne and Bjugn, and in a single seawater-caught eel from Etne (Table 6). The intact large plasmodia were occasionally seen macroscopically as white spots (0.9-1.4 mm) on the kidneys (Fig. 13A), throughout the posterior half. Spores (Fig. 13B) were observed as aggregates (destroyed plasmodia) in squash preparations of kidney tissue or free. They measured (N=26) 10.7 (9.9-12.2) µm in length and 5.8 (5.3-6.7) µm in width, with polar

capsules 3.7 (2.9-4.4) µm long and 3.3 (2.8-3.6) µm in diameter and polar filaments in 5-7 coils.

Overall, the prevalence was 25 % in freshwater-caught eels and 1 % in the saltwater caught, 13 % overall. The parasite was molecularly identified from two infected eels (Grimstad SW, Etne SW), the SSU rDNA sequence obtained showed 100 % identity (>1240 bp compared) with *P. giardi* in GenBank (MH414925).
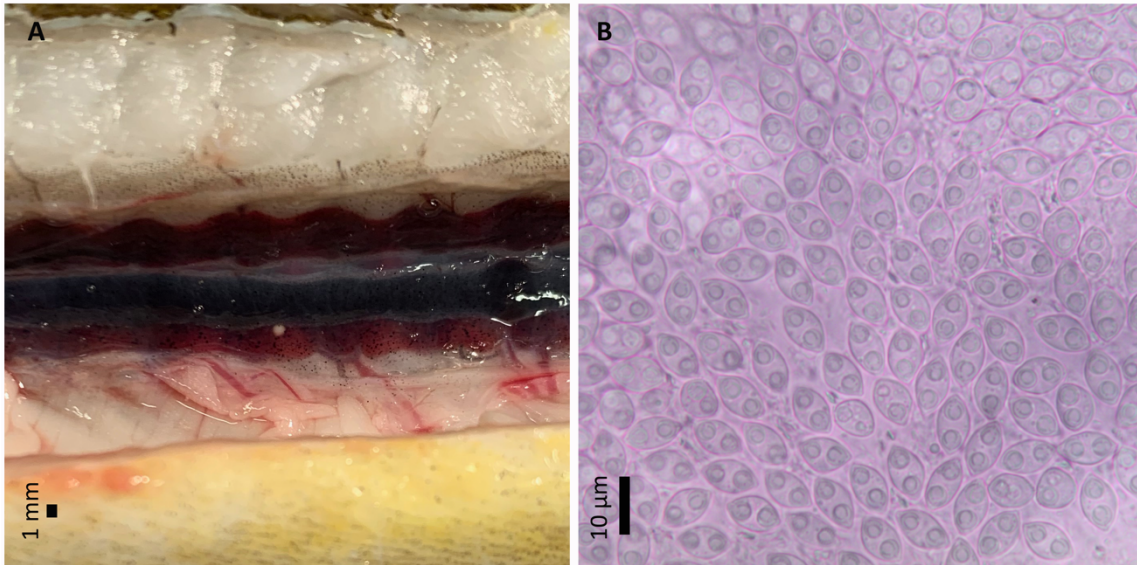

Figure 13 *Paramyxidium giardi* in the kidneys of a freshwater-caught eel, Etne. **A** Intact large plasmodium seen as a white spot on the kidneys, **B** *P. giardi* spores from the plasmodium on picture A.

Hoferellus gilsoni (Debaiseux, 1925)

This parasite was found infecting urinary bladders in fish from Grimstad FW, Etne FW and Bjugn FW. Both free spores and plasmodia were observed. About 29 % of all the examined freshwater eels were infected, with an infection rate of 4 % in Grimstad FW as the lowest and 67 % as the highest in Bjugn FW (Table 6). None of the saltwater caught eels were infected. The myxosporean was identified with *Hoferellus gilsoni* (syn. *Sphaerospora anguillae*) following the description by Wierzbicka (1986) and Lom et al. (1986). Spores developed in diasporic pansporoblasts, in round to elongated polysporic plasmodia (Fig.14A,B) measuring from 29 µm in diameter to 81 µm in length and 45 µm in width. The spores (Fig.14C,D) measured 6.5 (6.1-7.1) µm in length and x 6.2 (5.8-7.1) µm in width, with polar capsules measuring 3.7 (3.0-4.4) µm in length and 2.2 (1.7-2.7) µm in diameter (N=25 spores; 5 eels).

A reference *H. gilsoni* sequence (1210 nt) was obtained from a kidney sample from a Bjugn eel. Identity with the *H. gilsoni* sequence in GenBank (AJ582062; Scottish eel) was 99.8%.
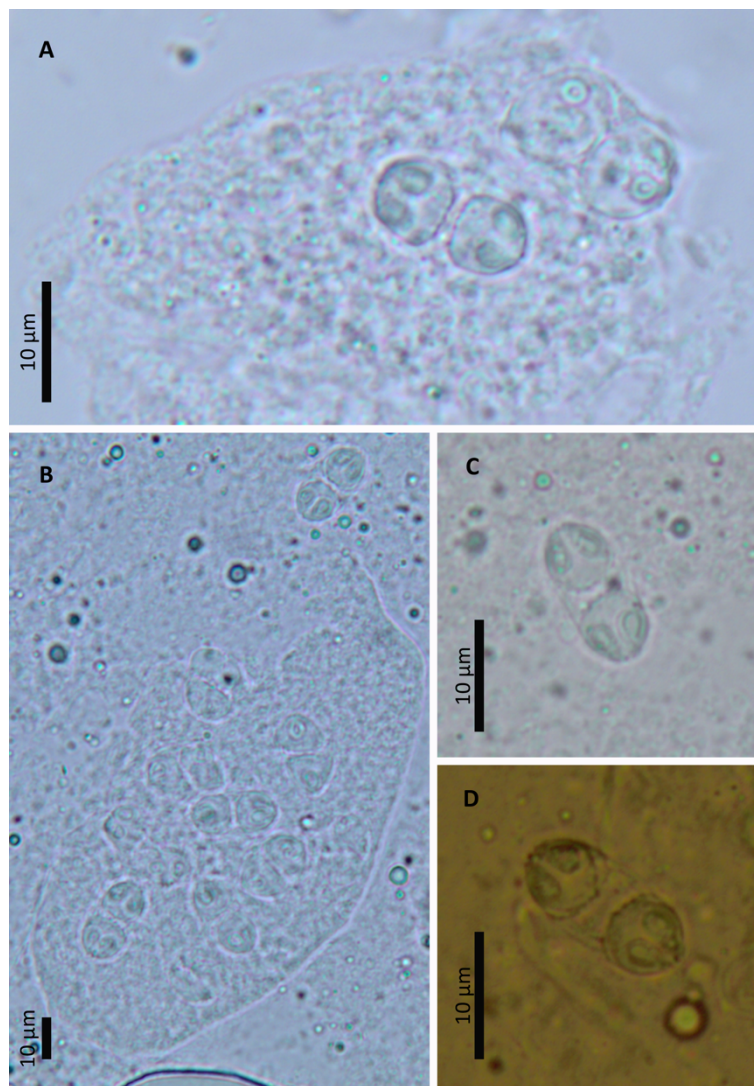

Figure 14 *Hoferellus gilsoni* observed in the urine from freshwater-caught eel. **A** Plasmodium with four *H. gilsoni* spores, **B** Plasmodium with several spores, **C** Two spores sticking together with their posterior parts, **D** Two spores sticking together with their posterior parts

Zschokkella stettinensis **Wierzbicka, 1987**

A myxosporean morphologically similar to *Zschokkella stettinensis* described from eel in Poland (Wierzbicka 1987), were observed in freshwater eels from Etne and Bjugn. The spores developed in polysporic plasmodia (Fig. 15D) in the urinary bladder. The plasmodia measured from 29 μm in diameter to 149 x 74 μm, and were observed to contain from 2-15 spores. The spores (Fig. 15A,B,C) measured 9.8 (8.4-11.3) x 6.6 (5.3-8.4) μm, and the polar capsules 3.3 (2.6-4.3) in length and 2.8 (2.3-3.6) μm in diameter (N=22 spores; 8 eels). Partial

SSU rDNA sequences of the myxosporean (829-1001 nt) were obtained from eels from both localities. These were similar (99.2 % identity) to a *Zschokkella* sp. sequence (AJ581918) from Scottish eel, and to the sequence of the actinosporean *Neoactinomyxon eiseniellae* (AJ582007) (100 %) infecting an aquatic oligochaete.

About 35 % of all examined freshwater-caught eels were infected. The prevalence was highest in Bjugn FW with 70 %, with a prevalence of 33 % in Etne FW (Table 6).
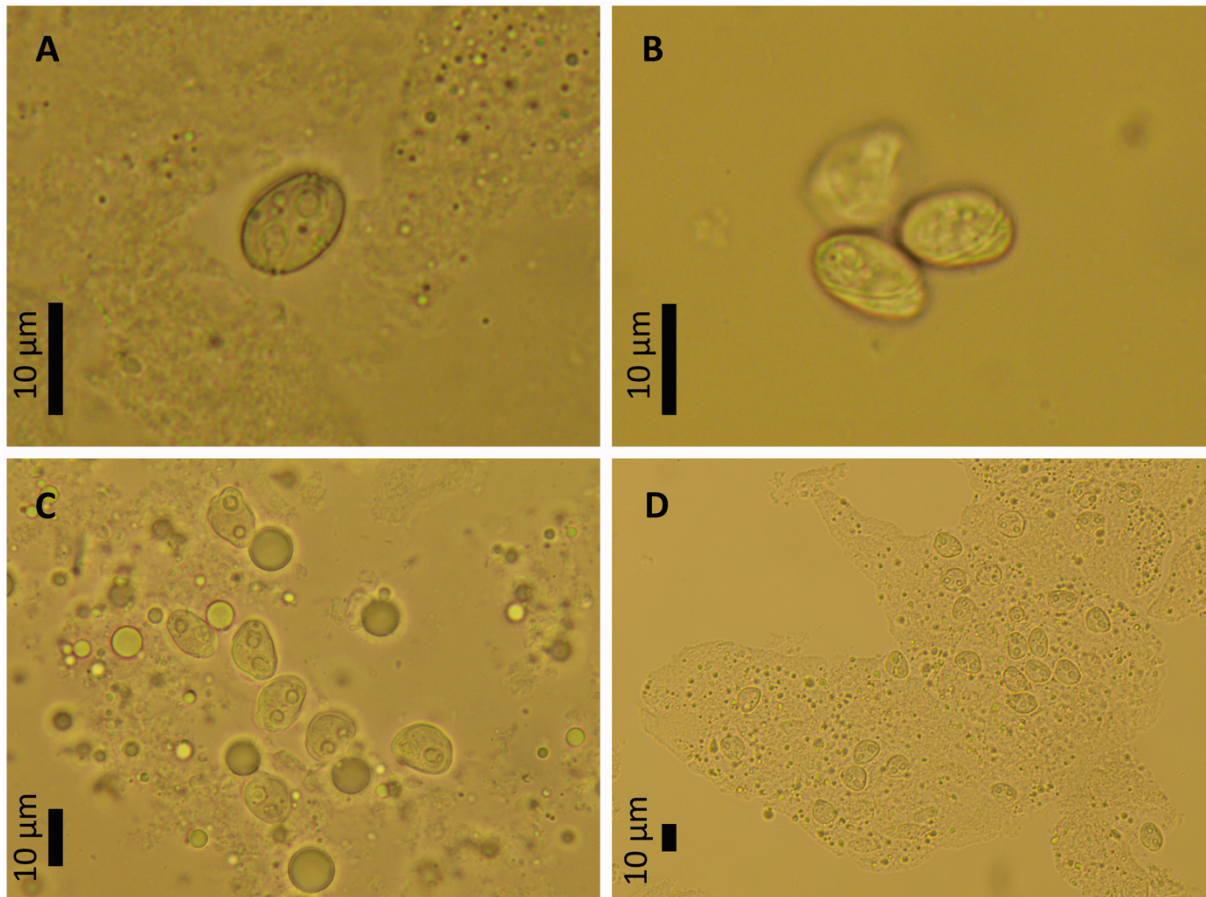

Figure 15 *Zschokkella stettinensis* recorded in urinary samples from freshwater-caught eels, Etne. **A** mature spore, **B** spores with visible striae running along the spore, **C** nearly mature spores, **D** Plasmodium with several spores.

Myxobolus sp.

Brown spots observed in the wall of the posterior and anal part of the intestine was found to represent *Myxobolus* sp. plasmodia packed with mature spores, or spore aggregates among brown-pigmented cells. However, yellowish brown spots in the stomach wall rarely contained *Myxobolus* sp. spores. This Myxosporean was observed in freshwater eels from Grimstad, Etne and Bjugn, with an overall prevalence of 20 %. The parasite was rare in seawater-caught

eels, occurring in 3 fish from the Etne area only (prevalence 4 % in the saltwater caught eels) (Table 6) .

Histology revealed that the spore patches were located to the submucosal connective tissue (Fig. 16).
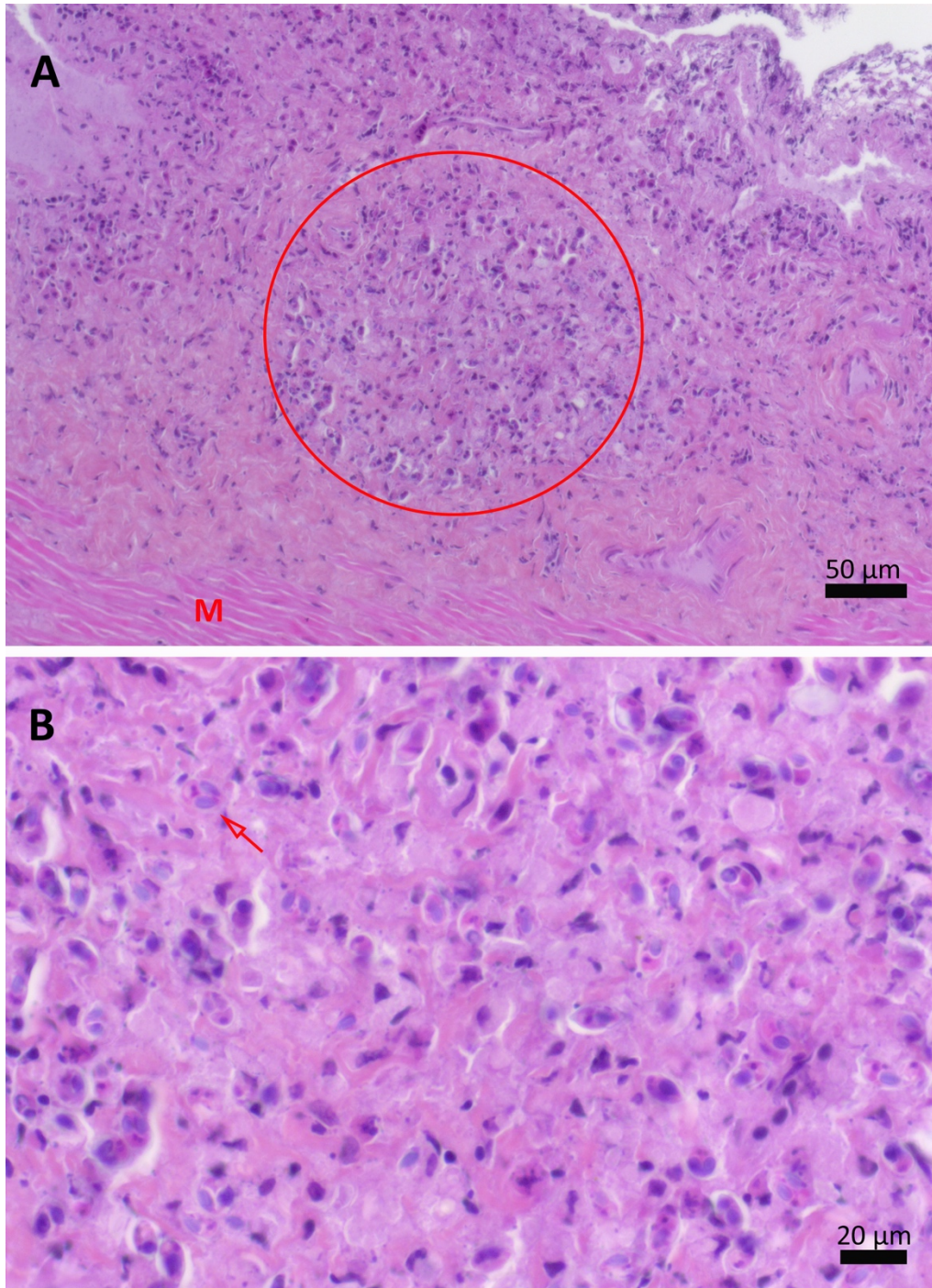

Figure 16 *Myxobolus* sp. aggregate in the submucosa of the intestinal wall from a seawater-caught eel, Etne. **A** Aggregate (red circle) seen in the submucosa, M - smooth muscle, **B** Spores (as indicated with red arrow) seen within the aggregate from picture A.

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The round to oval plasmodia (Fig. 17D,E) measured 190-970 x 120-930 µm, with a mean diameter of 356 µm (N=20). Spore measurements were taken from crushed plasmodia from seven eels representing all three geographic areas. The spores (Fig. 17A,B,C) measured (mean (range)) 10.9 (10.3-11.6) µm in length, 8.6 (7.7-9.1) µm in width and 6.0 (5.5-6.5) µm in thickness. The polar capsules were 5.7 (4.6-6.7) µm long, had diameter 2.9 (2.5-3.4) µm and contained seven (6-8) coils of polar filament (N>30; except spore thickness N=12).
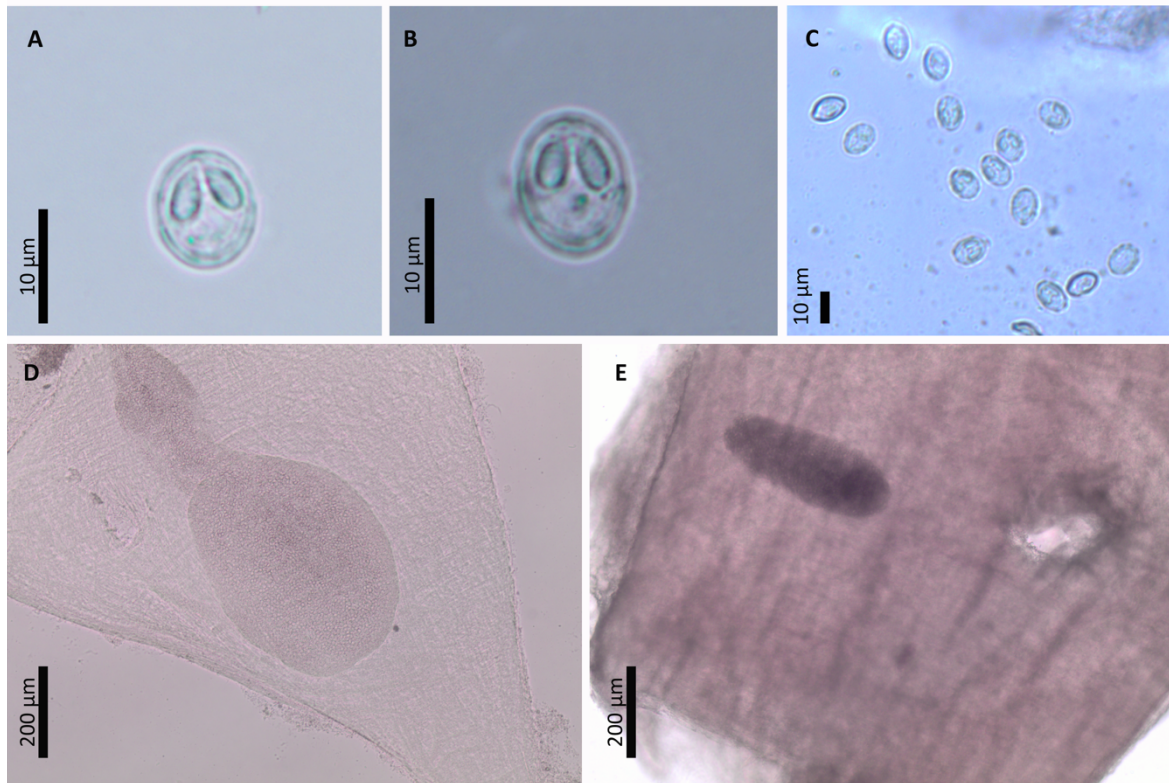
Figure 17 *Myxobolus* sp. spores and plasmodia. **A** Spore from a freshwater-caught eel, **B** spore from a freshwater-caught eel, **C** spores from a seawater-caught eel, **D** Plasmodium from a freshwater-caught eel, **E** Plasmodium from a freshwater-caught eel.

Partial SSU rDNA sequences (880-900 bp) were obtained from five samples (one from stomach, four from intestine) from four eels, representing freshwater eels from Grimstad, Etne and Bjugn. These sequences were identical. In GenBank, they showed highest identity (96.7 %) with *Myxobolus portucalensis* Saraiva et Molnár, 1990 (AF085182), a myxosporean from the fins of European eels.

3.1.3 Plathyhelminthes

Monogeneans

***Pseudodactylogyrus anguillae* (Yin et Sproston, 1948)**

Infections with this monogenean (Fig. 18), as described by Ogawa and Egusa (1976), were only recorded in Grimstad FW eels, where 80 % of the fish were infected (Table 6). The parasites were unevenly distributed on the gills, with a trend that the first gill arch pair harboured fewest worms, and that the number of parasites increase with the gill arch number (Fig. 19). The parasite differs from its congener *P. bini* in the hamuli morphology, and while all worms were carefully examined, no *P. bini* was seen. There was no relationship between *P. anguillae* abundance and eel size (r_s<0.01, p=0.96).
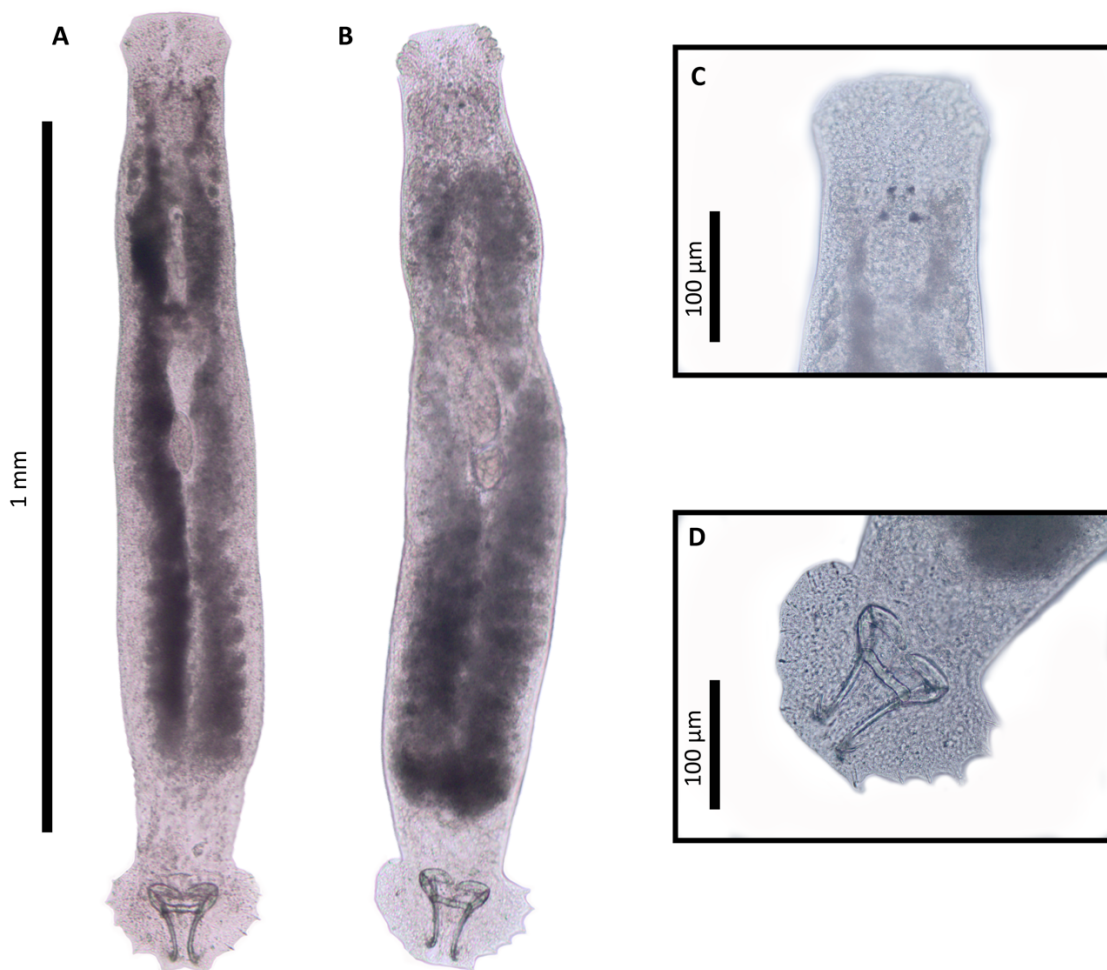

Figure 18 *Pseudodactylogyrus anguillae* observed on the gills of freshwater-caught eels, Grimstad. **A, B** ventral view, **C** anterior end of *P. anguillae* in picture B with visible eyespots, **D** close up of the posterior haptor of worm in picture A showing the hamuli.
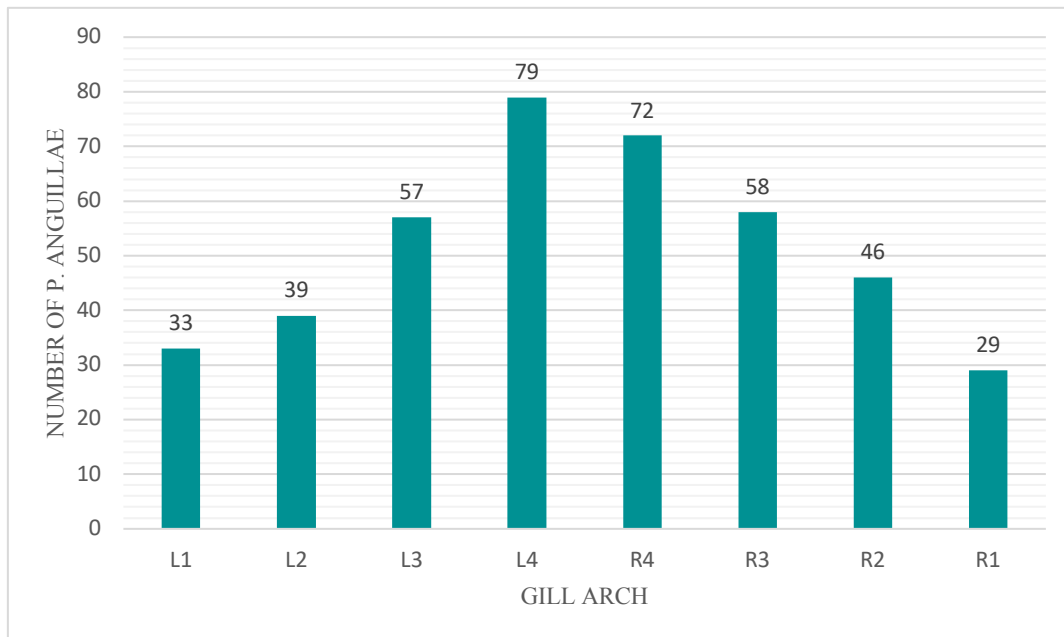
F**igure 19** Number of *P. anguillae* per gill arch, total counts from Grimstad FW. L=Left side, R=Right side.

Trematoda

Deropristis inflata **(Molin, 1859)**

Deropristis inflata (Fig. 20) was identified according to the description in (Dawes 1968). Infections with this trematode were observed at all the saltwater localities (Grimstad, Etne and Smøla), but never in freshwater-caught eels. The trematode primarily occurred in the posterior and anal part of the intestine, with a maximum intensity of 84 (Table 6). Both mature and immature specimens, measuring between 1.7 and 6.3 mm in length, occurred with a distribution of 90 % mature and 10 % immature in Etne and Smøla, and 65 % mature/35 % immature in Grimstad. The overall prevalence in saltwater caught eels was 47 %. Partial LSU rDNA reference sequences were obtained from three selected worms (one per locality). The sequences were invariant (900 bp compared). This represent the first time the *D. infalta* LSU gene is sequenced (partial).
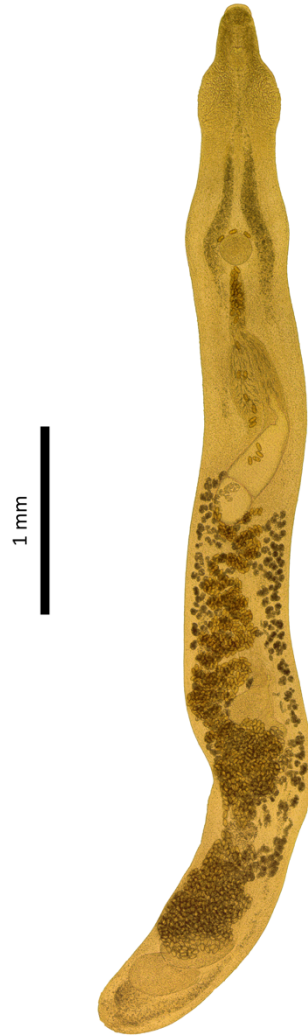
Figure 20 *Deropristin inflata*, ventral view, from the intestine of a seawater-caught eel, Etne.

Podocotyle atomon (Rudolphi, 1802)

This opecoelid trematode, identified using Dawes (1947), was detected in eels from Etne SW, and in two eels caught in Bjugn (Table 6). All the specimens (n=104), were mature and between 2 and 3 mm in length (Fig. 21). They were observed in the posterior and anal part of the intestine. Partial LSU rDNA reference sequences were obtained from three selected worms (Etne, Bjugn). The sequences showed 99.9% identity with *P. atomon* from the White Sea (Russia) (MH161437). No sequence divergence was seen in the Norwegian *P. atomon* sequences from eel (859 bp compared).

Figure 21 *Podocotyle atomon* observed in the intestine. **A** ventral view, from a freshwater-caught eel, Bjugn, **B** dorsal view**,** from a seawater-caught eel, Etne.

Helicometra fasciata (Rudolphi, 1819)

A total of 38 specimens of this opecoelid trematode (Fig. 22) was found in 5 eels from Grimstad SW (prevalence 29 %) (Table 6). They occurred in the hind intestine. Some were immature (c. 1 mm), the oviferous worms reached 2.1 mm in length. Oral sucker width was 208 (195-220) µm, ventral sucker diameter 298 (295-300) µm, sucker index 1:1.4-1.5. Eggs (without filament measured 75 (72-78) µm in length. The vitellarium reached pharynx, but was interrupted medially in the post-testicular region. Testes tandem, lobed, ovary 3-4 lobed. Based on these characteristics, the present specimens are identified with *Helicometra fasciata* Rudolphi following Odhner (1901) and Reversat et al. (1991).

The partial LSU rDNA sequence obtained from the specimen in Fig. 22 (880 nt) showed highest identity with *Helicometra* spp., however there were only 95.1 % identity with a

sequence of *H. fasciata* from *Epinephelus fasciatus* (Forsskål, 1775) from New Caledonia (KJ701238).
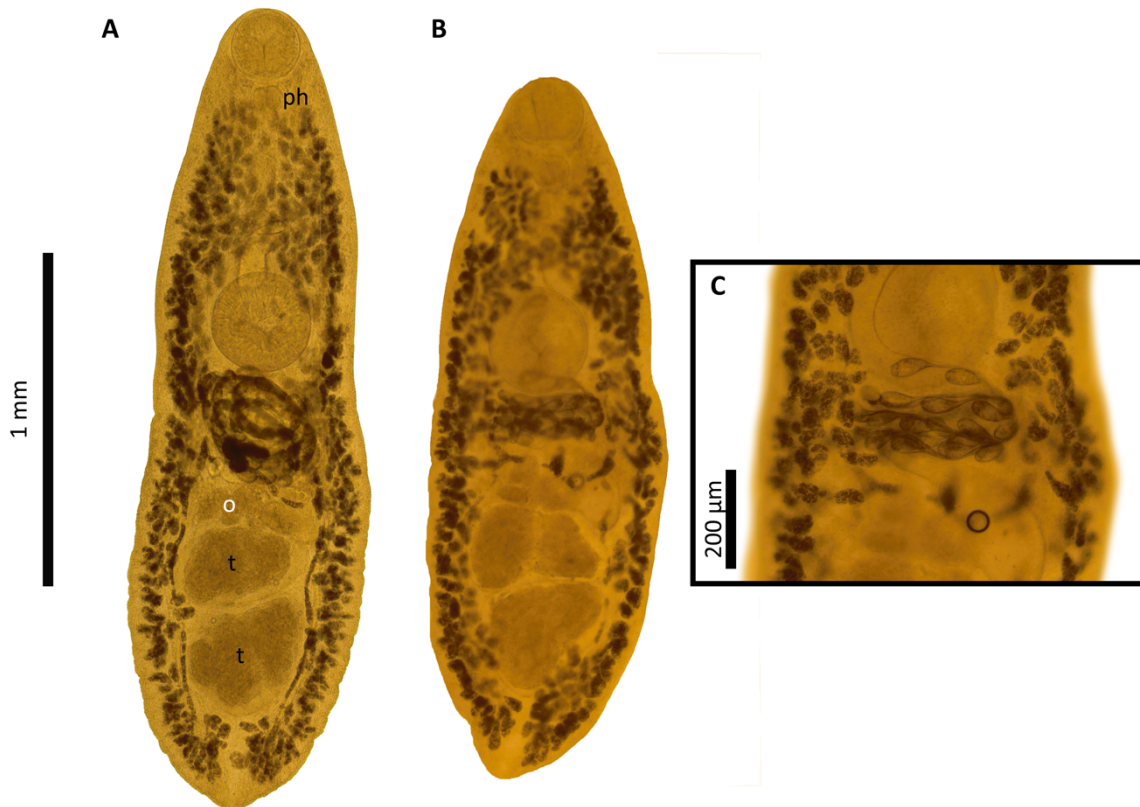
Figure 22 Helicometra fasciata from seawater-caught eel, Grimstad. **A** ventral view, vitellarium almost to pharynx (ph), and interrupted in the post-testicular area. Ovary (o) and testes (t) lobed; **B**, worm in dorsal view, **C**, uterine area, detail, filamented eggs.

Hemiurus communis Odhner, 1905

This trematode (Fig. 23) (family Hemiuridae), identified as *Hemiurus communis* according to Gibson and Bray (1986), was present in fish caught in Etne SW and Smøla SW. Most specimens, 0.8-2.2 mm in length, were mature (76-83 %, N=3206) and were primarily found in the lumen of the stomach with a maximum intensity of 255. A few were observed in the intestine. The prevalence was highest in Smøla with 97 % infected and 71 % in Etne SW (Table 6). Of all the saltwater caught eels, 65 % were infected. LSU rDNA reference sequences were obtained from two selected worms (Etne, Smøla). The sequences showed >93 % identity to hemiurids in GenBank, but no sequences of *H. communis* were

available for comparison. The two *H. communis* sequences differed by three substitutions (810 bp compared).
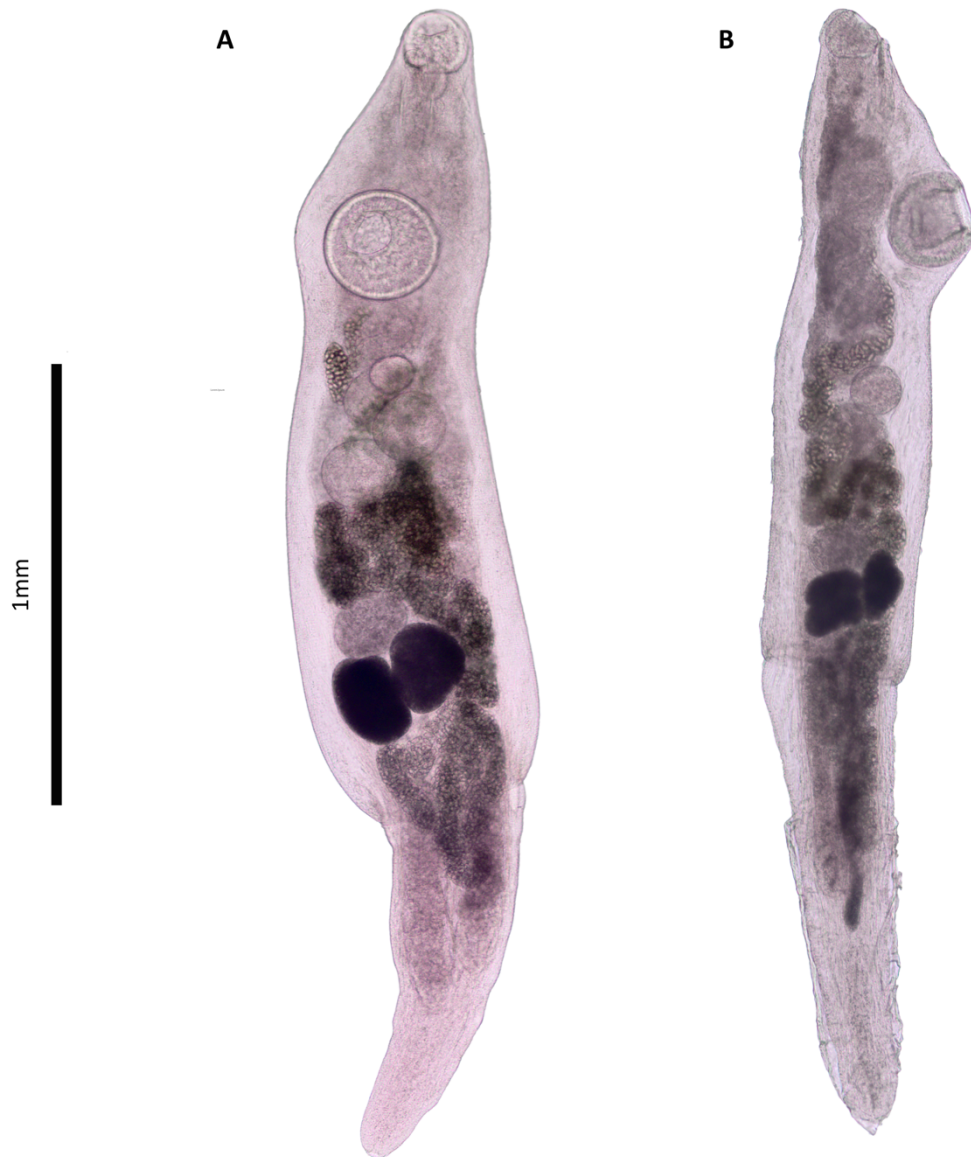
Figure 23 *Hemiurus communis* observed in the intestine of seawater-caught eels, Smøla. **A** ventral view, **B** lateral view.

Brachyphallus crenatus **(Rudolphi, 1802)**

One specimens of this trematode (family Hemiuridae), as described by Gibson and Bray (1986), was observed in an eel from Etne SW (area B) (Table 6). The parasite (Fig. 24) was located in the lumen of the stomach. A partial LSU rDNA reference sequence was obtained

from *B. crenatus* from a seawater-caught eel from Etne. Identity with sequences in GenBank (North Sea (KT767168), North Pacific (MH628299)) was 100 % (378-790 bp compared).
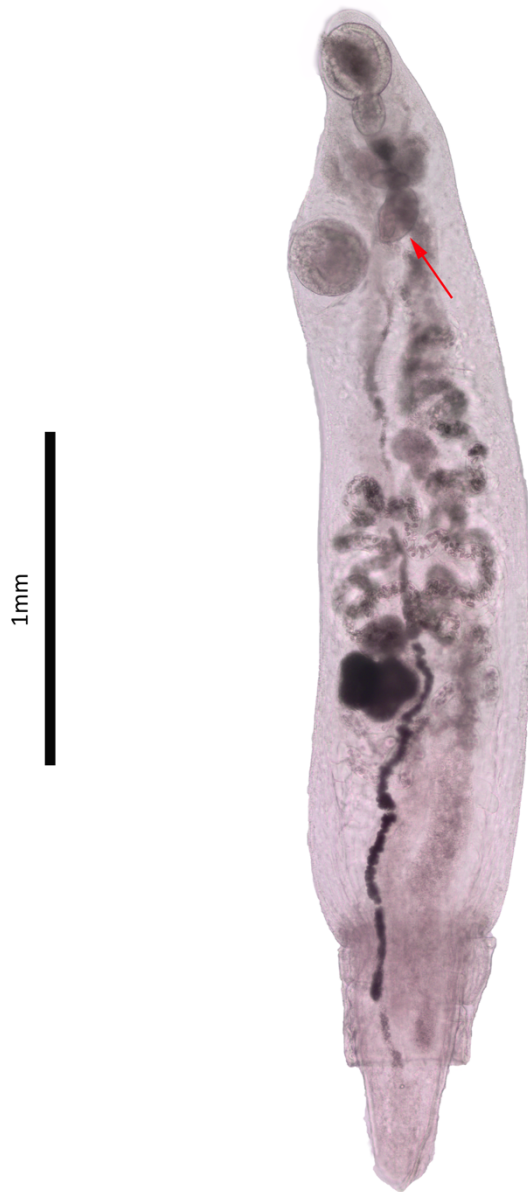
Figure 24 *Brachyphallus crenatus*, lateral view, from an eel caught in the Etnefjord, with visible seminal vesicle (arrow) dorsal-anterior to the ventral sucker.

Lecithichirium rufoviride (Rudolphi, 1819)

Lecithichirium rufoviride (Fig. 25) was identified using the description by Gibson and Bray (1986). Infections with this big trematode (family Hemiuridae), 1-7 mm in length, were observed in eels caught in Grimstad SW and Smøla (Table 6). Most specimens were mature (93-96%, N=1089) and were primarily found in the lumen of the stomach with a maximum

intensity of 152. A few were observed in the intestine. The prevalence was highest in Smøla with 90 % infected and 76 % in Grimstad SW. The parasite abundance correlated with eel size (Grimstad; r_s=0.80, p<0.001, Smøla; r_s=0.61, p<0.001). Partial LSU rDNA reference sequences was obtained from two *L. rufoviride* obtained from seawater-caught eels from Grimstad and Smøla. The sequence (819 nt) showed >92 % identity to related hemiurids in GenBank, but no sequences of *L. rufoviride* were available for comparison.
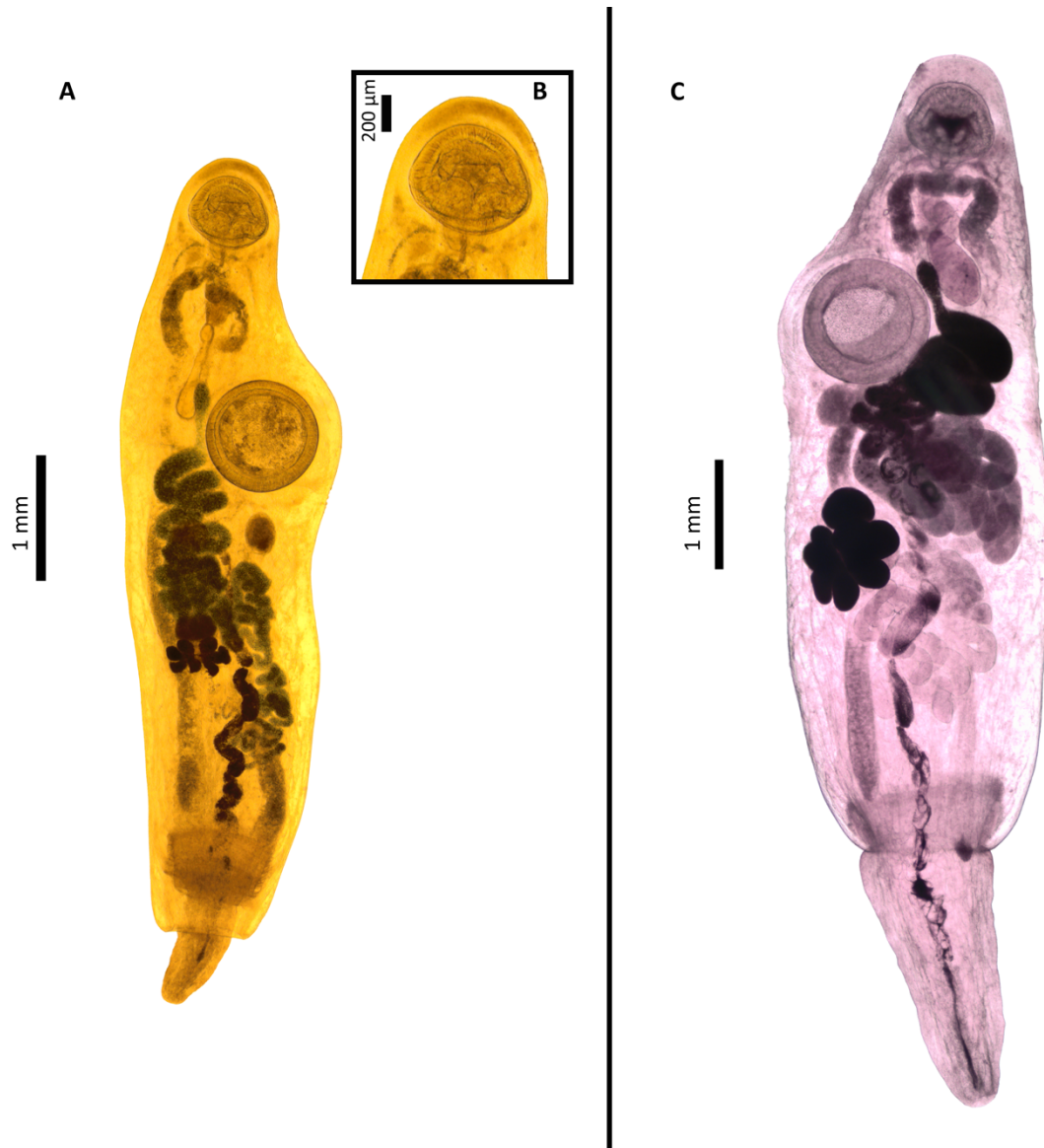
Figure 25 *Lecithochirium rufoviride* found in the lumen of the stomach in seawater-caught eels. **A** ventral view, **B** closeup of the oral sucker with postero-lateral thickening of wall, **C** lateral view.

Derogenes varicus (Müller, 1784)

This derogenid trematode (Fig. 26), identified as *Derogenes varicus* according to Gibson (1996), was observed in seawater-caught eels from Etne and Smøla, with a total prevalence of 10 % among all the seawater-caught eels examined (Table 6). All the specimens (N=16) were mature, 1.5-2 mm in length. Most were found in the oesophagus, some were found in the stomach and one in the anal part of the intestine. Partial LSU rDNA reference sequences were obtained from two selected worms (Etne, Smøla). These sequences were identical (757 bp), and in BLAST search they showed highest identity (97.7 %) with *D. varicus* from Scottish waters.
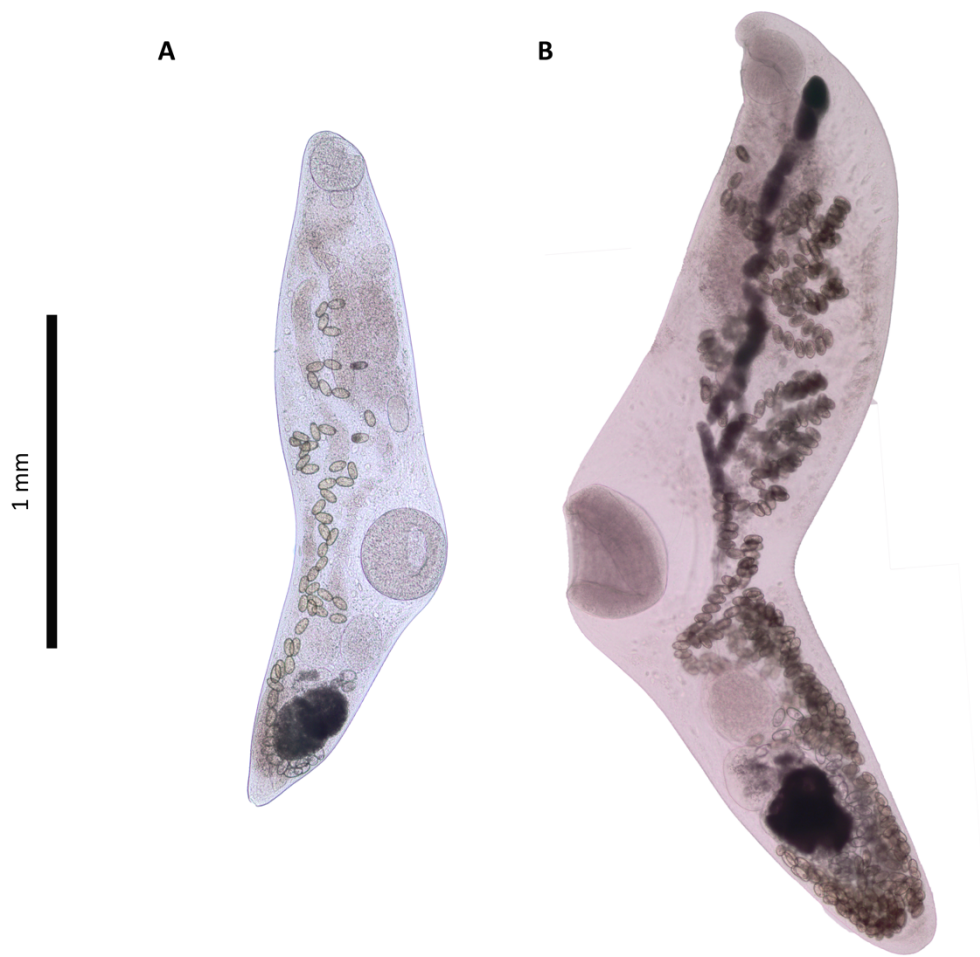
Figure 26 *Derogenes varicus* from seawater-caught eels. **A, B** lateral view.

Lecithaster gibbosus (Rudolphi, 1802)

Specimens of this lecithasterid trematode (Fig. 27), as described by Gibson (1996), were observed in fish caught in Etne SW and Smøla SW. Three fish in total were infected with a

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maximum intensity of two (Table 6). The parasite was found in the lumen of the posterior part of the intestine. Partial LSU rDNA were not obtained from the DNA extraction of two selected worms (Etne, Smøla).

Figure 27 *Lecithaster gibbosus* from the intestine of a seawater-caught eel, lateral view.

Diplostomum sp.

Infection with metacercaria larvae of this trematode (family Diplostomidae), as described by Dawes (1968), Gibson (1996) and Bykhovskaya-Pavlovskaya (1962), was only found in one freshwater-caught eel from Etne (Table 6). One metacercaria (Fig. 28) was observed in the vitreous humour near the lens in both eyes. A partial LSU rDNA sequence was obtained from one of these metacercariae. The sequence (872 nt) showed 99.8 % identity with a sequence (KR269765) of *Diplostomum spathaceum* (Rudolphi, 1819) from a final host, the black headed gull (*Larus ridibundus*).

Figure 28 *Diplostomum spathaceum* found in the eyes vitreous humor in the eye of a freshwater-caught eel from Etne.

Cestoda

'Scolex pleuronectis'

Infection with cestode larvae of this morphotype, with bilocular bothridia (Fig. 29), was observed in two eels caught near Smøla (Table 6). The worms were 1.6-1.8 mm long and 0.6-0.7 mm wide, with the minor anterior bothridial loculus measuring 157-185 µm in diameter, the posterior 189-232 µm and the apical sucker 105-112 µm in diameter. The parasites were located in the lumen of the anal part of the intestine. A partial LSU rDNA sequence (405 nt) was obtained from one of the two worms. It showed highest identity (99.8 %) with the tetraphyllidean *Phyllobothrium squali* Yamaguti, 1952, a parasite of the shark *Squalus acanthias*.
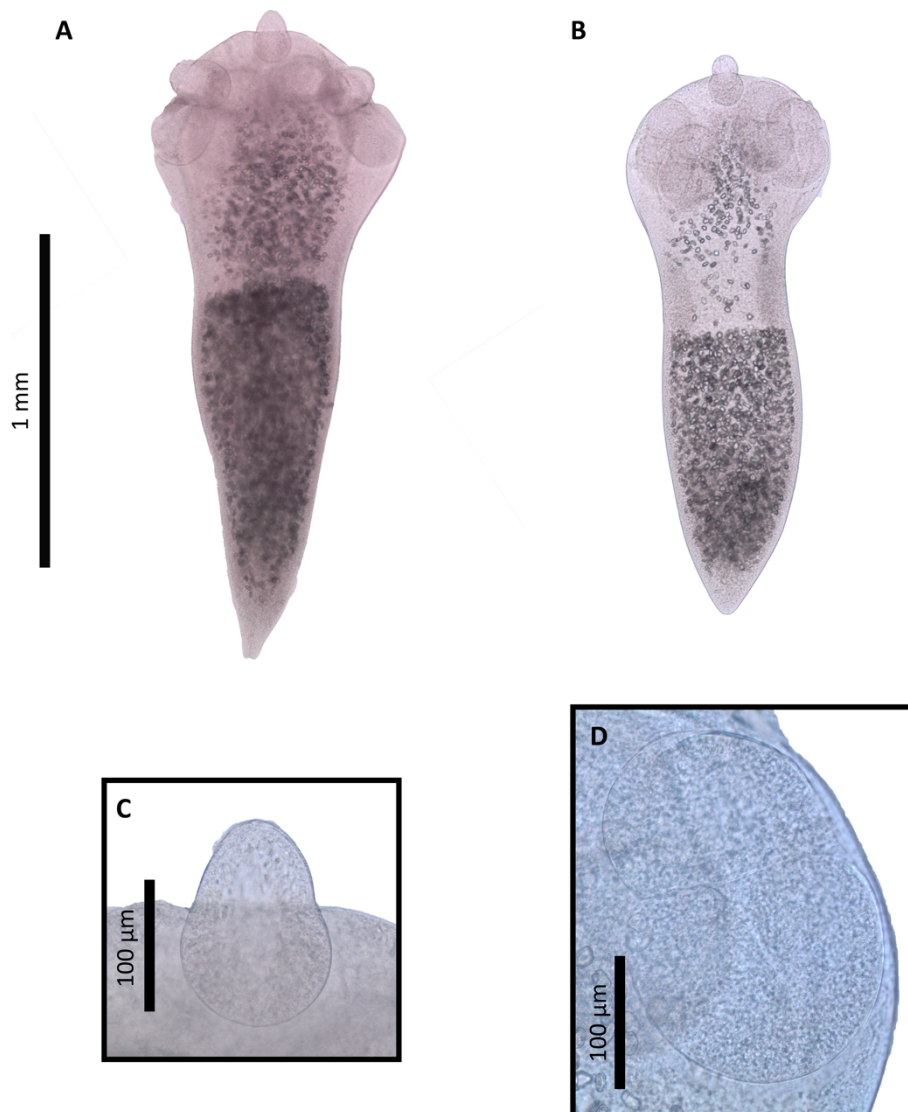
Figure 29 *Phyllobothrium squali* observed in the anal part of the intestine of seawater-caught eels, Smøla. **A** worm measuring 1.8 mm**, B** worm measuring 1.6 mm**, C** close up of the apical sucker of the worm in picture A **D** close up of the bilocular bothridia of the worm in picture B.

Proteocephalus macrocephalus (**Creplin, 1825**)

This cestode (Fig. 30), identified according to Scholz and Hanzelova (1998), was observed in both freshwater and saltwater caught fish from Etne (Table 6). The adult mature parasites, 180-250 mm long, were located in the anterior part of the intestine. A reference LSU rDNA sequence (1243 nt) of *P. microcephalus* from Etne showed 98.7 % identity to an available sequence in GenBank (EF095261) from a River Thames eel (U.K.).
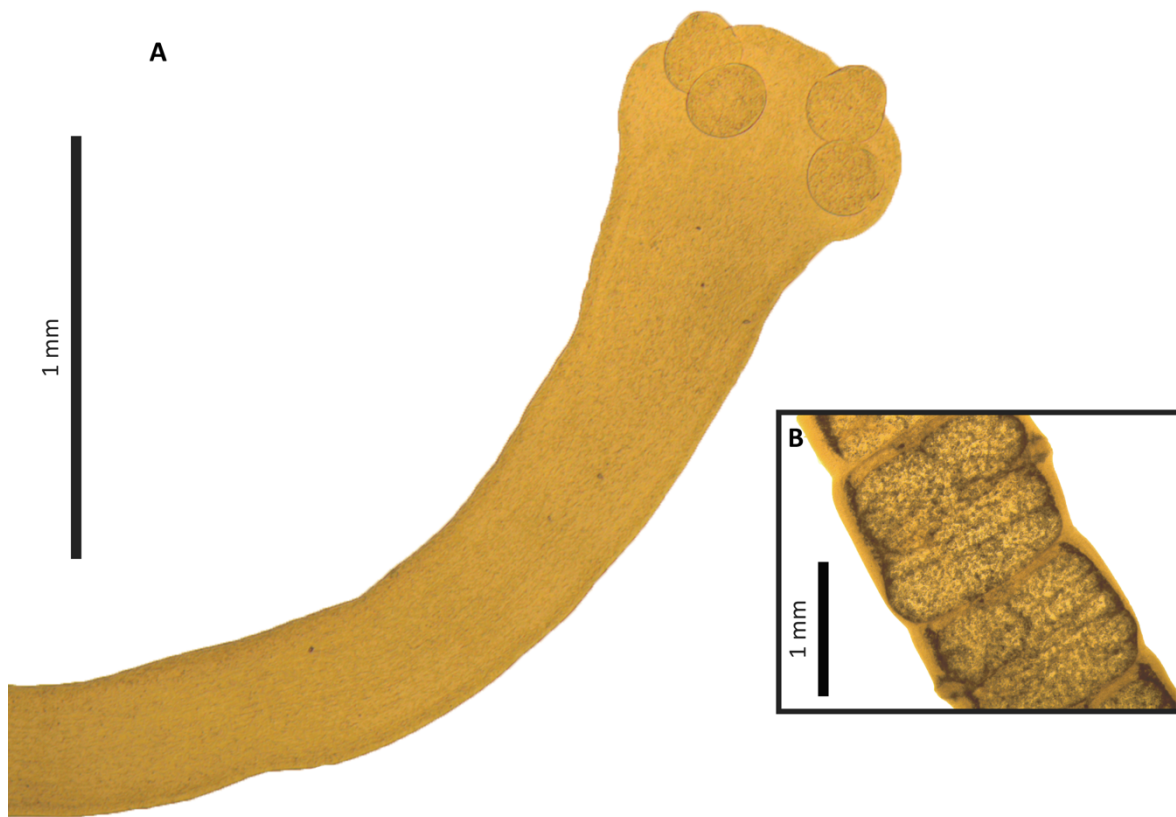
Figure 30 *Proteocephalus macrocephalus* obsrved in the intestine of a seawater-caught eel, Etne. **A** scolex with four suckers, **B** mature progleottids.

Bothriocephalus claviceps (Goeze, 1782)

Specimens belonging to *B. claviceps* (Fig. 31), as described by Scholz (1997), were found in the intestine of fish caught in Etne FW, Bjugn and Smøla (Table 6). The cestodes measured 33-100 mm in length. Both mature and immature specimens were observed. A reference LSU rDNA sequence (1213 nt) of a mature *B. claviceps* from Etne showed 98.8 % identity to an available sequence in GenBank (DQ925323) from a Czech eel.
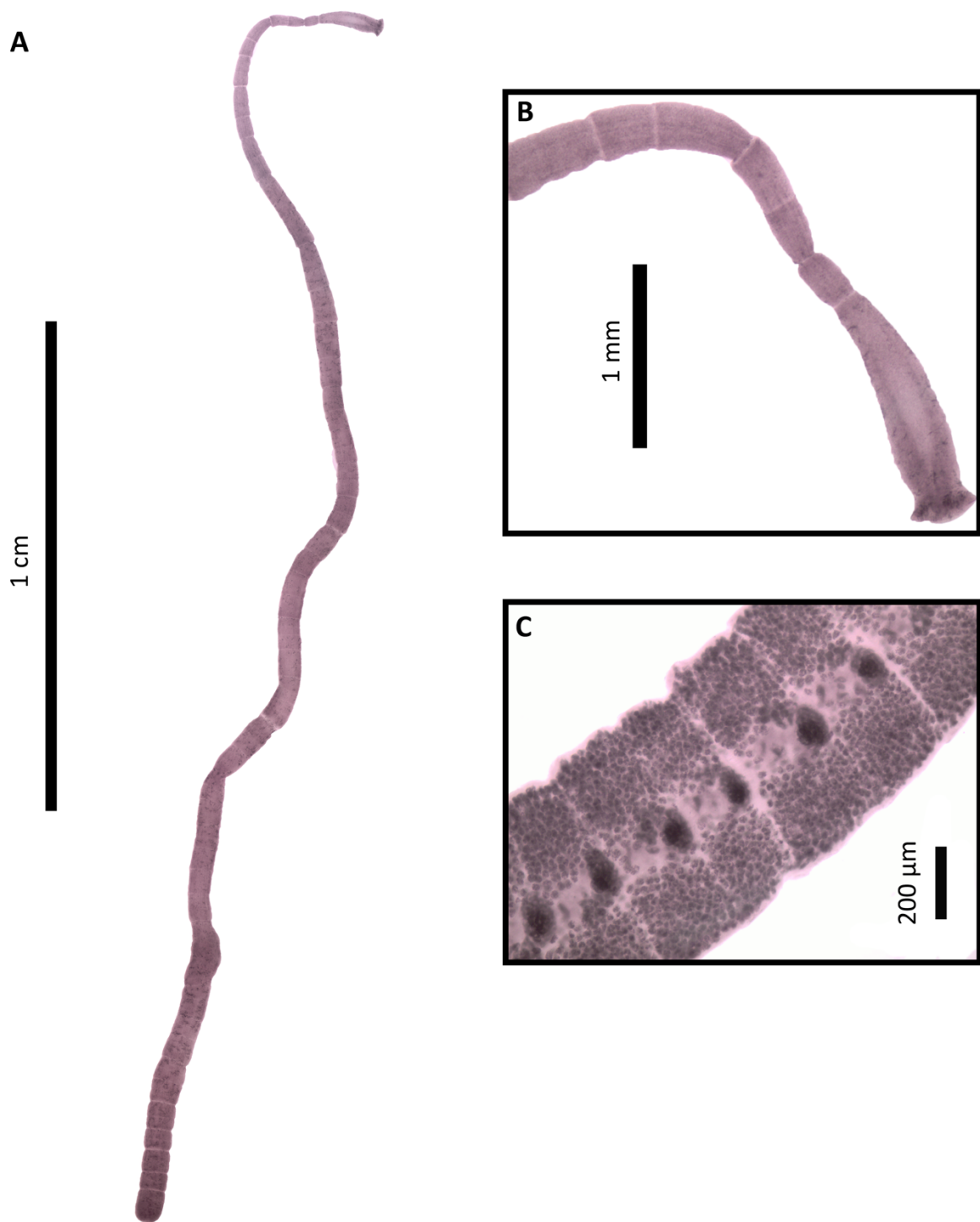
Figure 31 *Bothriocephalus clavicpes* observed in freshwater-caught eels, Etne. **A** immature worm, **B** close up of the scolex, **C** mature progleottids from another specimen.

Dibothriocephalus ditremus (Creplin, 1825)

In eels caught in Etne FW (Table 6), brownish capsules occurred (Fig. 32B,C,D) in the stomach wall. Some of these contained small smooth cestode larvae (Fig. 32A) morphologically identifiable with *D. ditremus* according to Andersen and Gibson (1989). Partial LSU rDNA sequences were obtained from four worms. The consensus sequence (1227 nt) showed 100 % identity with sequences from *D. ditremus* plerocercoids from U.K. (KY552813) salmonids and a plerocercoid from a threespine stickleback (*Gasterosteus aculeatus* L., 1758) from lake Gjønavatn, Fusa.
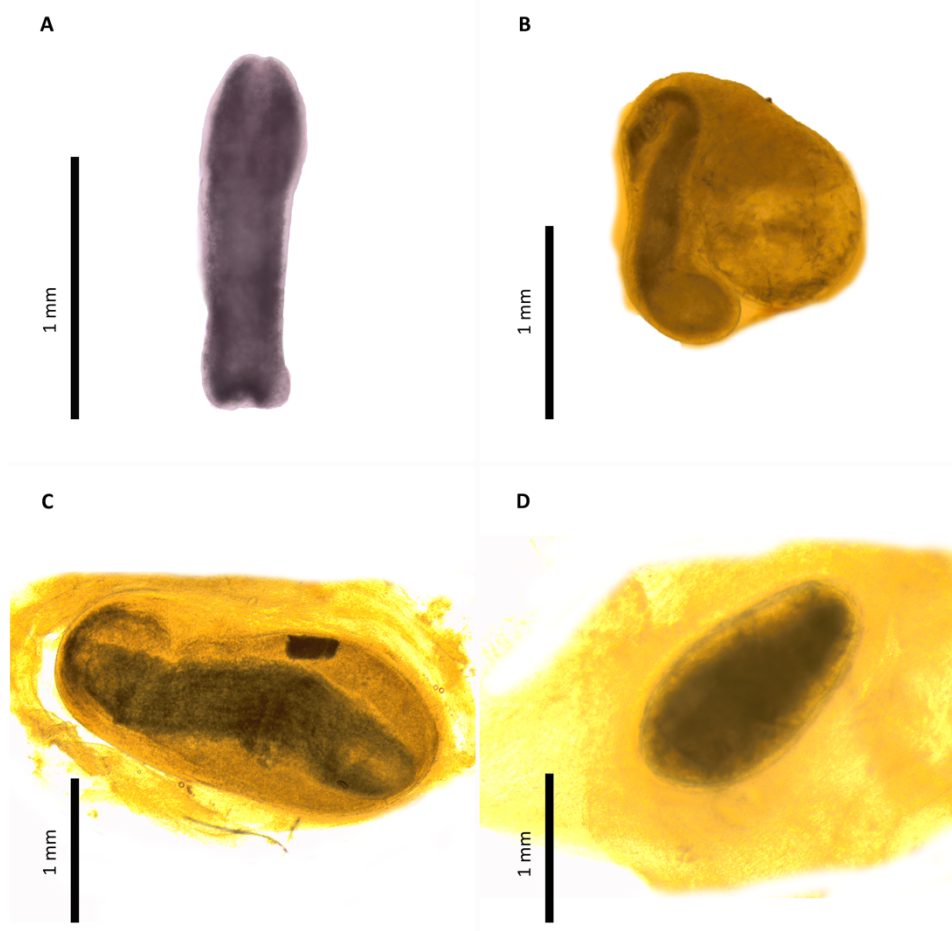
Figure 32 *Dibothriocephalus ditremus* plerocercoids from the stomach wall of freshwater-caught eels, Etne. **A** intact worm dissected out of a capsule, **B, C** worm in their capsules, **D** degraded encapsulated worm.

Bothriocephalidean plerocercoids

Bothriocephalidean plerocercoids were observed in the lumen of the posterior part of the intestine in seawater-caught eels from Etne (Table 6). The worms measured 1.4-3.8 mm in length and had 3-16 proglottids. The biggest worms had craspedote strobila (Fig. 33A,C) and the scolex showed two bothria and often a distinct apical disc (Fig. 33B). Partial LSU rDNA sequences were obtained from five worms. All showed 100% identity (1180 nt compared) with sequences from *Eubothrium crassum* (Bloch, 1779) from *Salmo* spp. in freshwater, as well as from *Eubothrium* sp. from seawater farmed Atlantic salmon (*Salmo salar* L., 1758) in Norway.
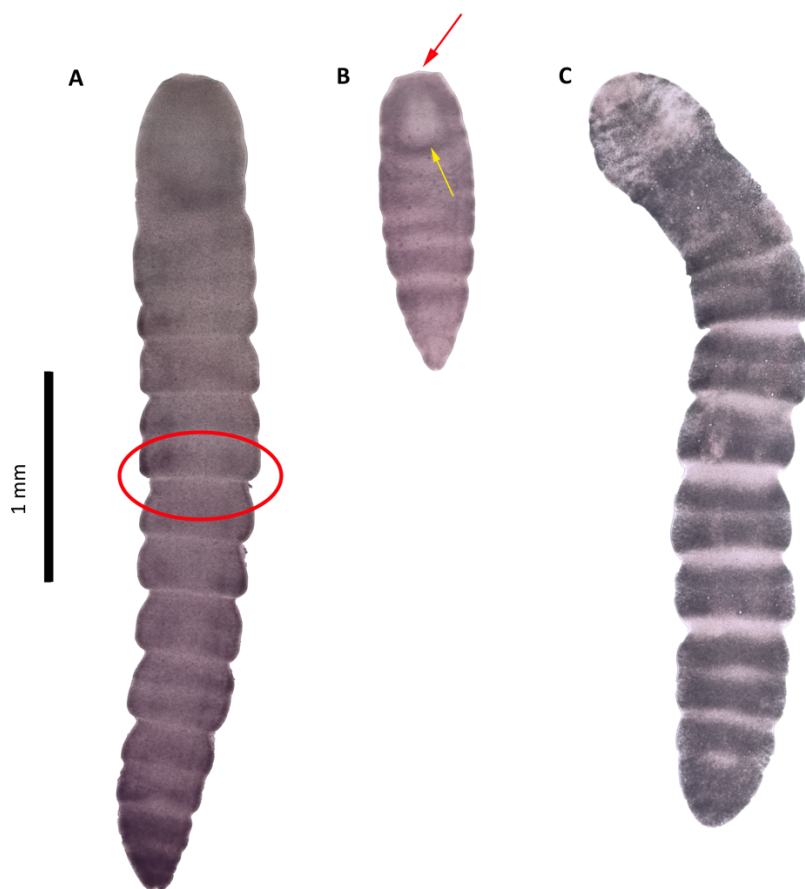
Figure 33 *Eubothrium crassum* from the intestinal lumen of Etne eels (seawater). These larvae were molecularly identified with *Eubothrium crassum,* a cestode maturing in salmonids (*Salmo* spp.). **A** big worm with craspedote stobila (red circle), **B** small worm with visible apical disk (red arrow) and bothria (yellow arrow), **C** worm also showing craspedote strobila. All to same scale.

3.1.4 Acanthocephala

***Echinorhynchus gadi* Zoega, 1776**

Infections with *Echinorhynchus gadi* (Fig. 34), as described by Arai (1989), were observed in seawater-caught eels from Etne and Smøla (Table 6). The 6.5-23 mm long and apparently immature acanthocephalans were located in the lumen of the intestine.
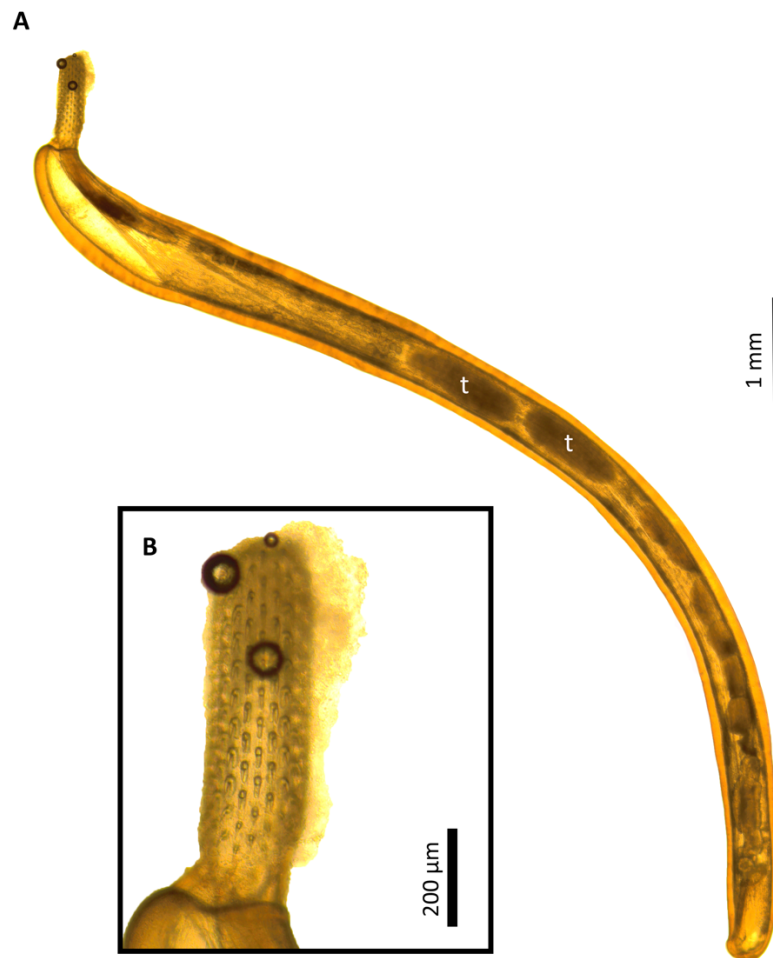
Figure 34 *Echinorhynchys gadi* recorded in the lumen of the intestine of a seawater-caught eel, Etne. **A** Male specimen with two visible testis (t) **B** Close up of the proboscis.

3.1.5 Nematoda

***Pseudocapillaria tomentosa* (Dujardin, 1843)**

Only one freshwater-caught eel from Grimstad had an infection with this capillariid nematode (Table 6), identified as *Pseudocapillaria tomentosa* according to Moravec (1987). The specimen observed (Fig. 35) was male, measured c. 140 mm, and was embedded in the anal part of the intestinal wall.
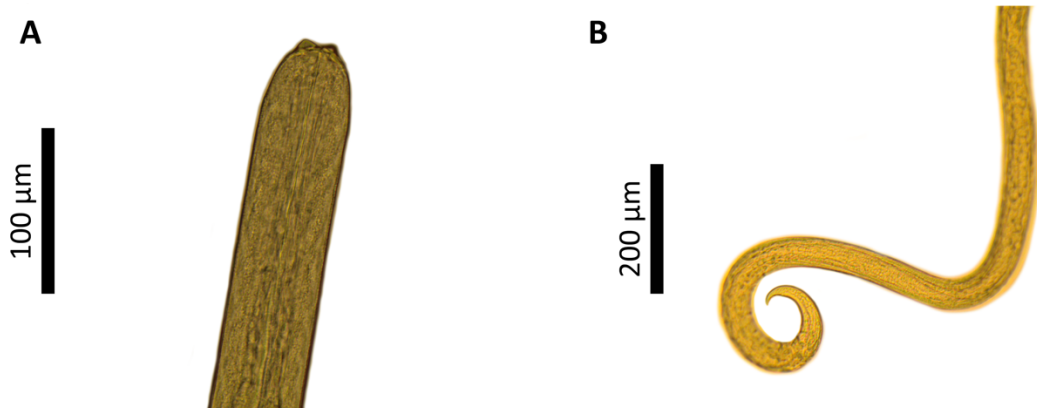
Figure 35 *Pseudocapillaria tomentosa* recorded encapsulated in the intestinal wall of a freshwater-caught eel, Grimstad. **A** posterior part, **B** anterior part

Eustrongylides **sp.**

Infections with small specimens (7.5-8.3 mm) of this dioctophymid nematode, identified according to Berland (1961), were detected in freshwater-caught eels from Etne and Bjugn (Table 6). The reddish coloured parasite (Fig. 36) was encapsulated in the stomach wall, with some of the capsules containing degraded nematodes (not counted).
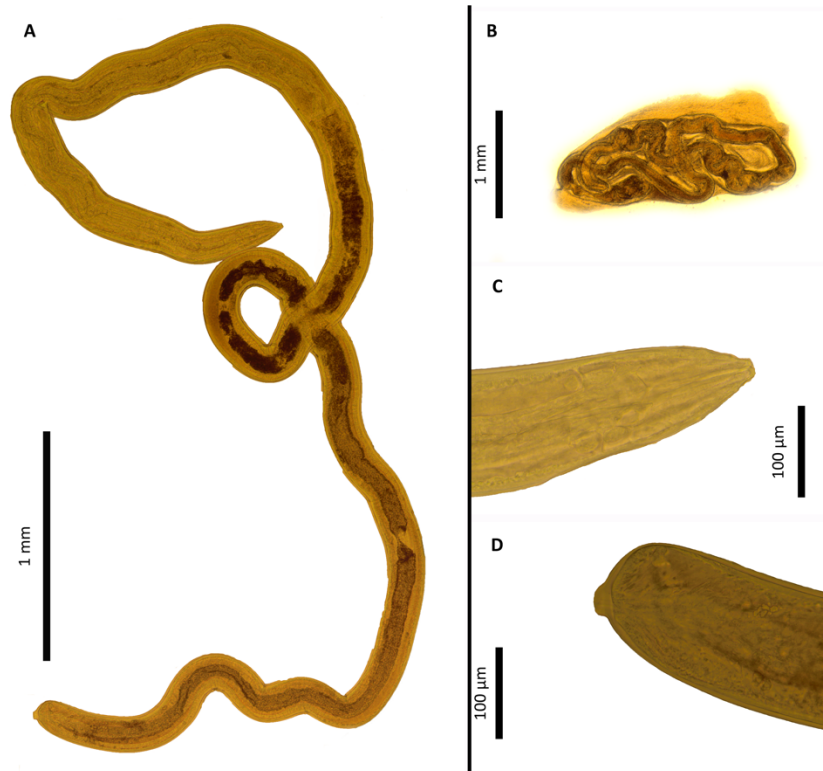
Figure 36 *Eustrongylides* sp. from capsules in the stomach wall of freshwater-caught eels, Etne **A** worm dissected out of the capsule, **B** encapsulated apparently partly degrded worm, **C** anterior part of the worm in A, **D** posterior part of the worm in A.

Daniconema anguillae **Moravec et Køie, 1987**

Nematode larvae of *D. anguillae* (Fig. 37A,B,C), identified according to Moravec and Køie (1987), were first detected and identified from the gills of an eel (Fig. 37E). This discovery then led to examination of the dorsal and pectoral fin where the parasite was also found (Fig. 37D). The larvae were small, 1.4-1.5 mm. The infected fish were freshwater-caught eels from Grimstad, Etne and Orkla (Table 6). A total of ten eels were found infected, where the nematode most often were recorded in the gills, and in greatest amount in the pectoral fin. The intensities were very high, densities in the pectoral fin reached 1.6 larvae per mm^2 (Grimstad eel). No adult specimens were found in the typical site, the swimbladder wall. Abundance showed a positive correlation with eel length (r_s=0.61, p<0.01).
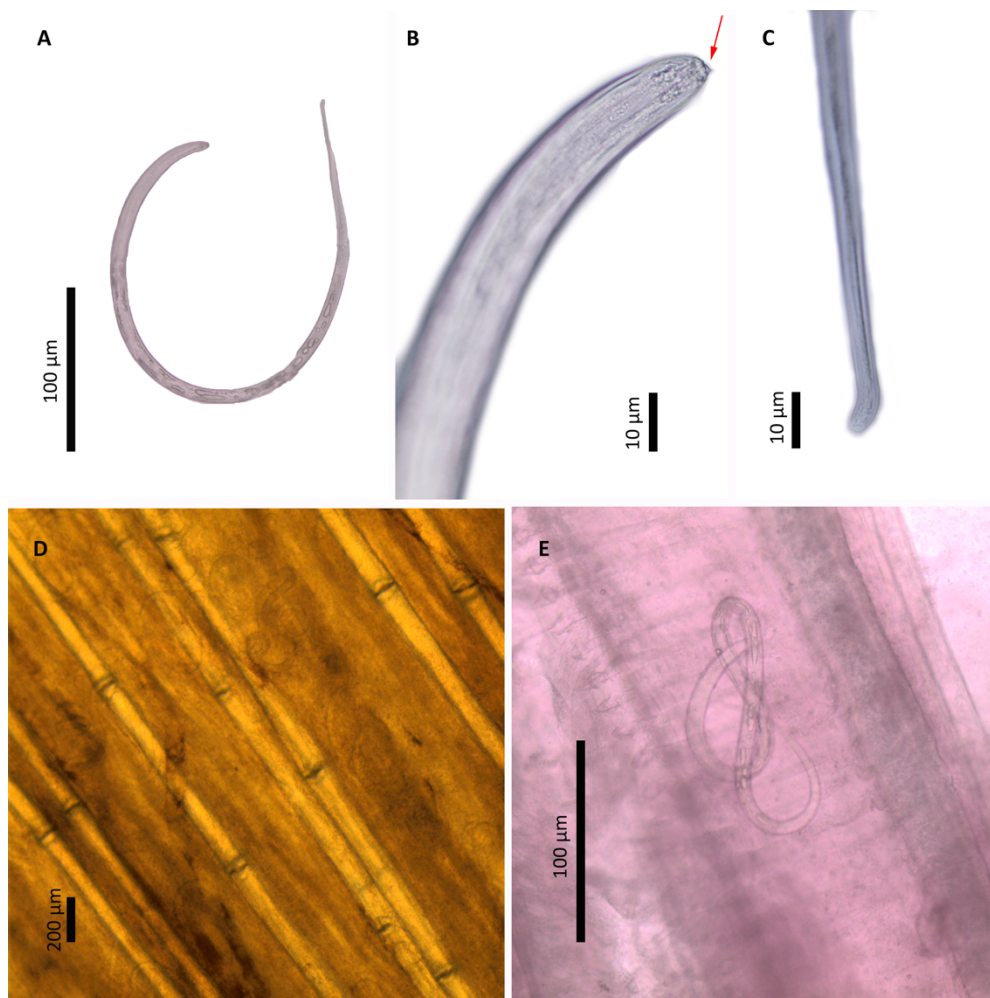
Figure 37 *Daniconema anguillae* larvae, found infecting the gills and the pectoral fin of freshwater-caught eels. **A** dissected out from the gill, **B** close-up of anterior part of the nematode with visible boring tooth (red arrow), **C** close-up of tail end, **D** *in situ* in pectoral fin, **E** *in situ* in gill filament.

Paraquimperia tenerrima (**Linstow, 1878**)

Freshwater-caught eels from Etne, Bjugn and Orkla were registered as infected with this nematode (Fig. 38), identified according to Arai and Smith (2016). The parasites, 1-10.5 mm in length, were located in the lumen of the intestine with a maximum intensity of 37. The prevalence was highest in Etne (73 %) (Table 6) and the overall prevalence in freshwater-caught eels was 37 %.
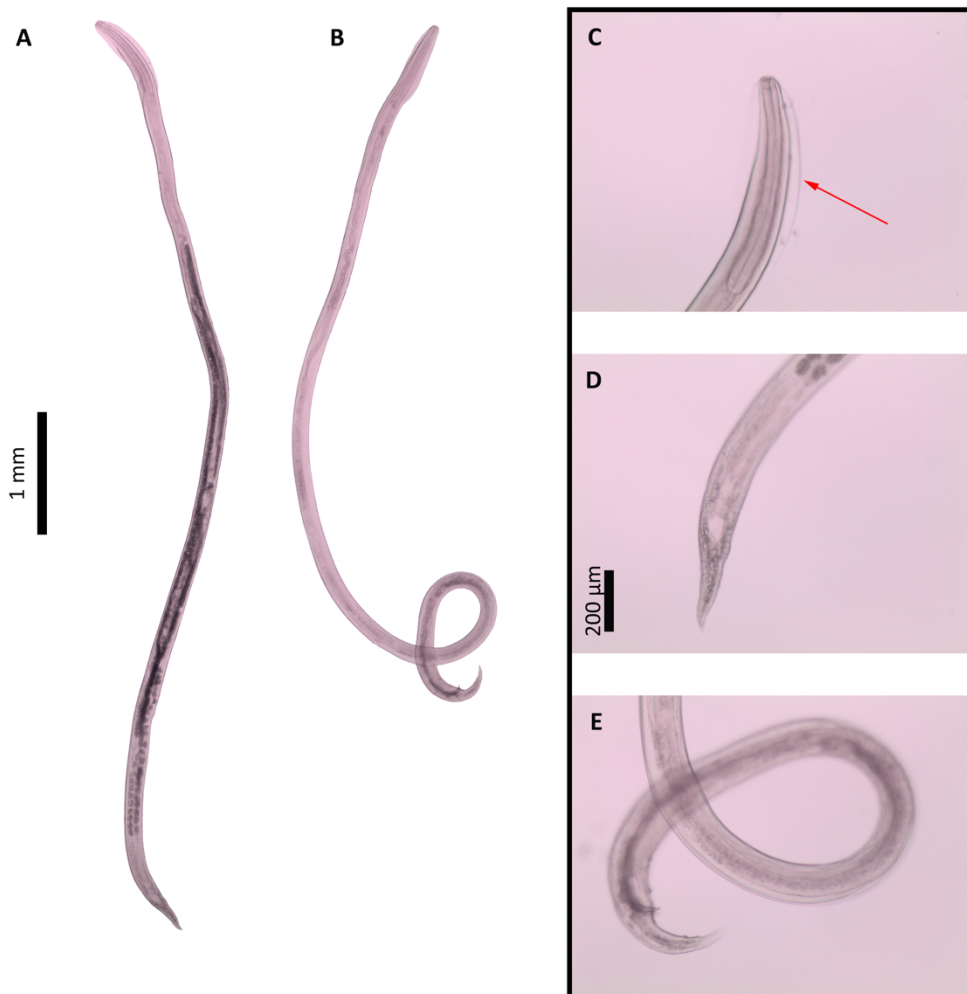
Figure 38 *Paraquimperia tenerrima* from the lumen of the intestine of a freshwater-caught eel, Etne. **A** female specimen, **B** male specimen, **C** anterior part of the female specimen with visible lateral alae (arrow), **D** close up of the straight tail of the female specimen, **E** close up of the ventrally bent tail of the male specimen with visible spicules

Paracuaria adunca (Creplin, 1846)

Infections with this acuariid nematode were only observed in three seawater-caught eels from Etne (Table 6). The larvae were coiled up in spirals and encapsulated (Fig. 39C) in the stomach wall. Out of the 151 specimens registered, 30 were dissected out, examined more closely in the microscope and were identified as *P. adunca* larvae (Fig. 39A,B,D) according to the description by Anderson and Wong (1982).
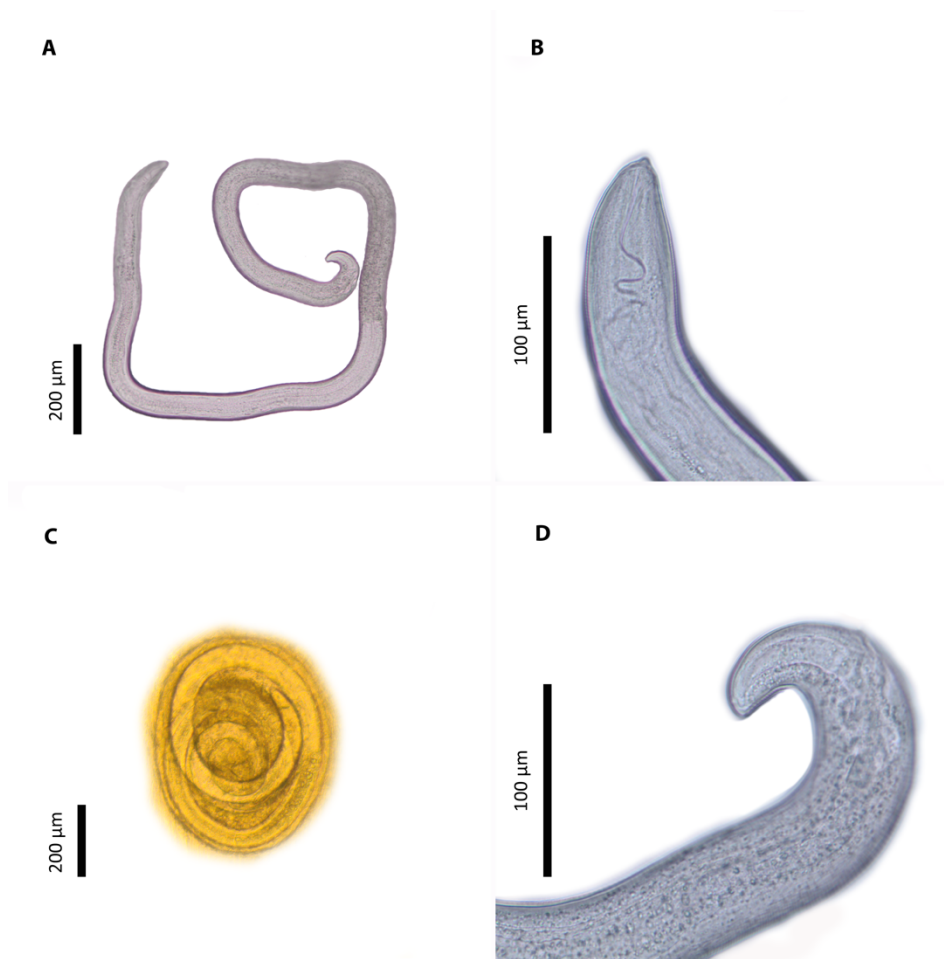
Figure 39 *Paracuaria adunca* recorded encapsulated in the stomach wall of seawater-caught eel, Etne. **A** worm dissected out of capsule, **B** anterior part of the worm, **C** encapsulated worm , **D** posterior end of the worm.

Cucullanellus minutus (Rudolphi, 1819)

Only one seawater-caught eel from Etne was infected with *C. minutus* (Table 6), as described by Arai and Smith (2016). The 3 mm long adult female worm (Fig. 40)was located in the posterior part of the intestine.
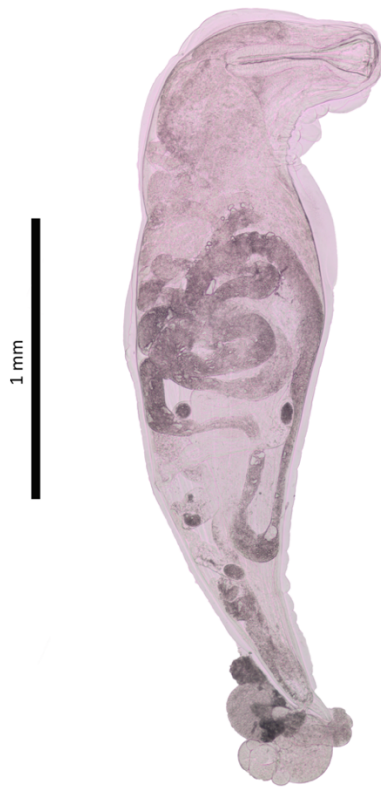
Figure 40 *Cucullanellus minutus* recorded in the intestine of seawater-caught eel, damaged form flattening.

Hysterothylacium aduncum (Rudolphi, 1802)

Infections with this raphidascarid nematode (Fig. 41), identified according to Berland (1961), were detected in seawater-caught eels from Etne and Smøla. The preadult or adult specimens (stage IV-V), 5-45 mm long, were located in the lumen of the intestine with a maximum intensity of 13. The prevalence was highest in Smøla with 37 % (Table 6). ITS1-5.8s-ITS2 sequences were obtained from two worms from Etne and Smøla. Both showed 100 % identity (1052 nt compared) with *Hysterothylacium aduncum* from sprat in Denmark (KU306720).
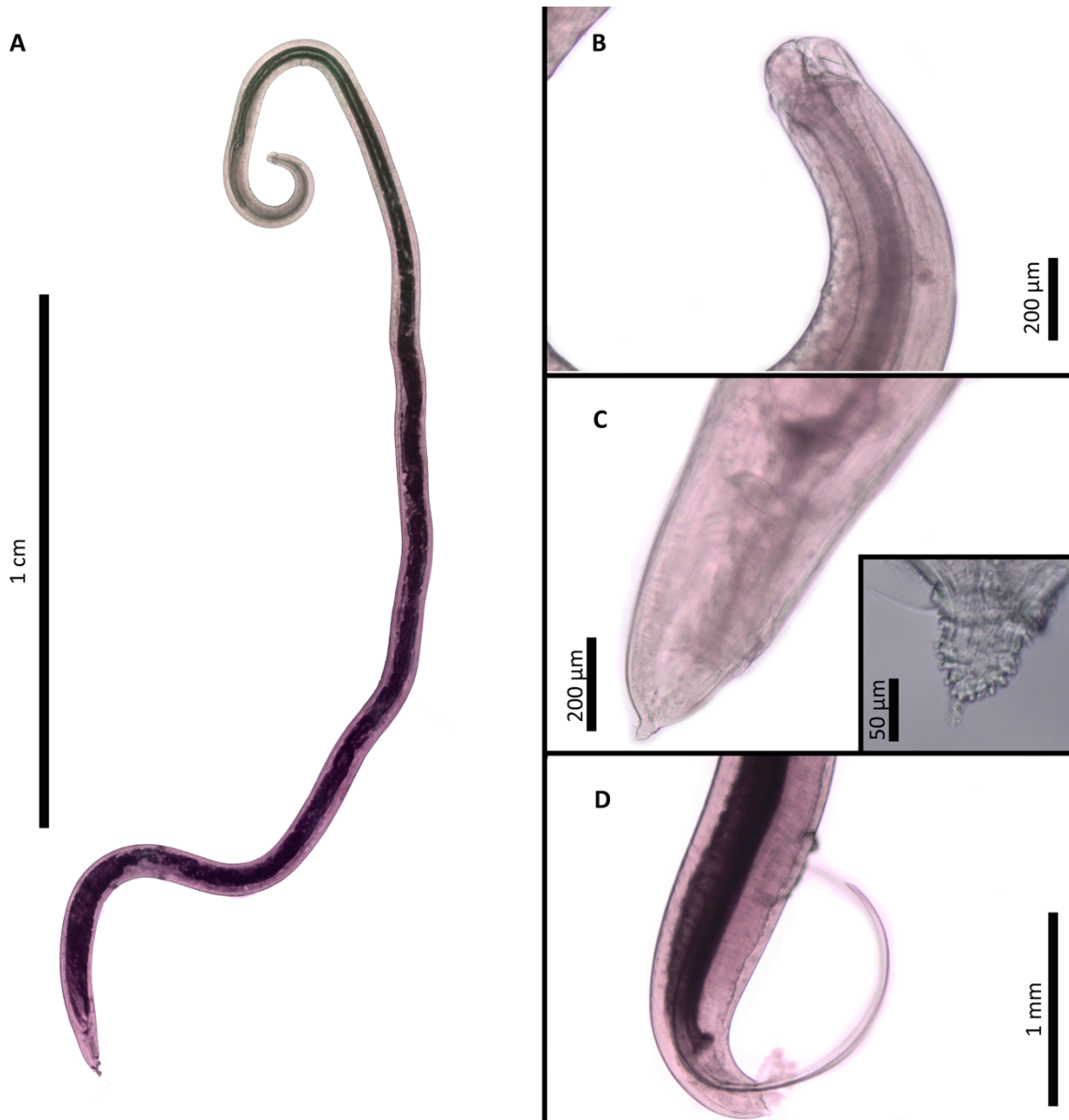
Figure 41 *Hysterothylacium aduncum* from the lumen of the intestine of seawater-caught eels, Smøla. **A** female specimen, **B** close up of the anterior end, **C** close up of the tail of the female specimen and detail of the characteristic "cactus-tail" , **D** close up of a male tail with visible everted spicules.

Anisakis simplex (Rudolphi, 1809)

This nematode (Anisakidae), identified according to Berland (1961), was detected in both freshwater-caught eels from Grimstad and seawater-caught eels from Smøla, each locality represented by two infected fish (Table 6). The parasites (Fig. 42), 18-27 mm long, were encapsulated in the mesenteries around the stomach and intestine. ITS1-5.8s-ITS2 sequences

were obtained from two worms from Grimstad and Smøla. Both showed 99.9 % identity (974-985 nt compared) with sequences from *Anisakis simplex sensu stricto*[1].
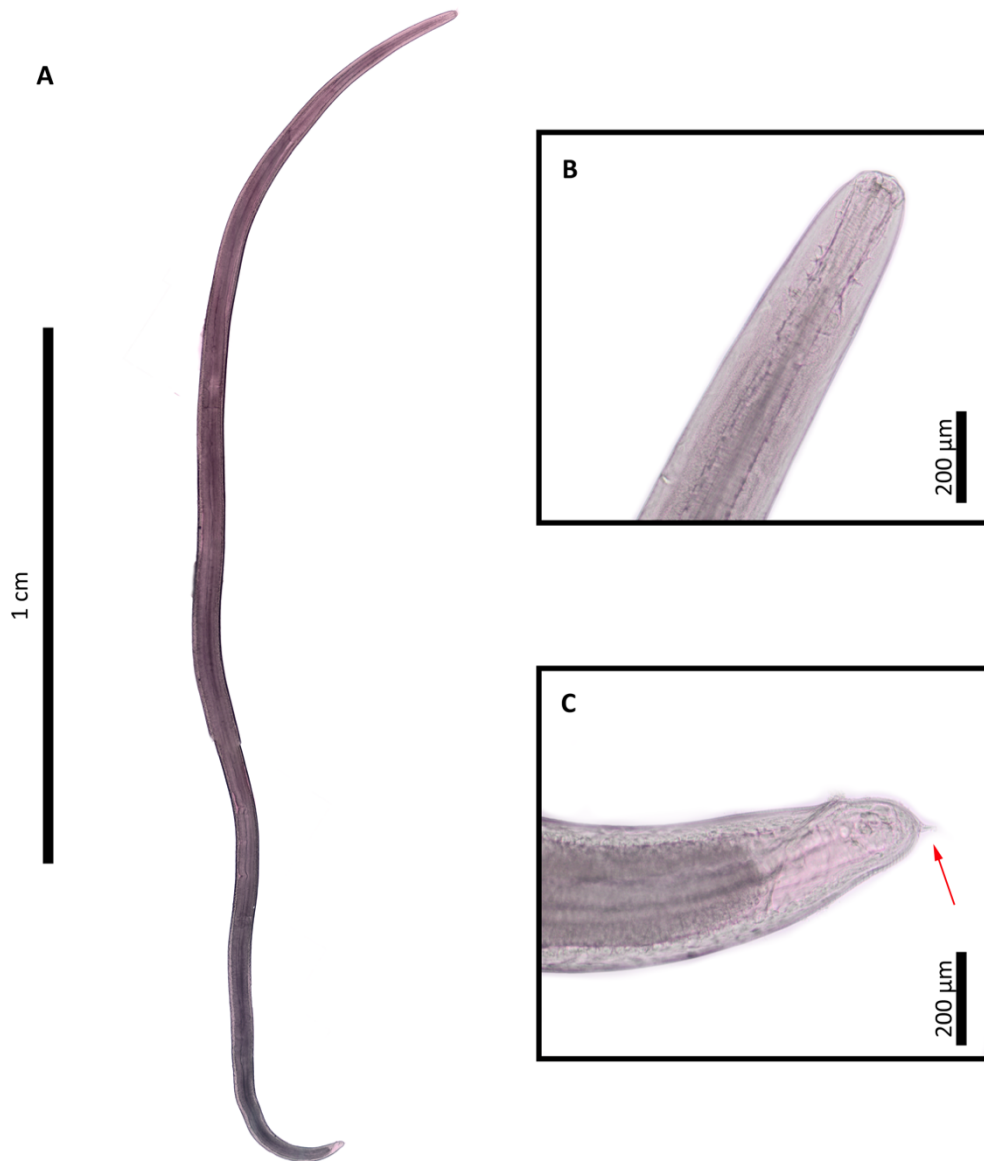
Figure 42 *Anisakis simplex* recorded encapsulated in the mesenteries around the intestine in a seawater-caught eel, Smøla. **A** worm dissected out of the capsule, **B** anterior end, **C** anterior end with the characteristic spike (arrow) at the rounded posterior end.

Contracaecum spp.

Both freshwater-caught eels from Grimstad and Etne, and seawater-caught eels from Grimstad, Etne and Smøla were infected with *Contracaecum* sp (Anisakidae) larvae (Table

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[1] Recent, more restricted sence

6), as described by Arai and Smith (2016). The small (1.8-3.8 mm) nematode larvae were encapsulated in the wall of the stomach and intestine, and in the mesenteries surrounding these organs. ITS1-5.8s-ITS2 sequences were obtained from 15 worms representing all the localities with infected fish. All sequences of larvae obtained from Grimstad (FW and SW) and Etne SW eels showed >99 % identity with *Contracaecum rudolphii* Hartwich, 1964 from cormorants in Sardinia, genotype A (Fig. 43B,C,E). The sequence of a larva from Smøla was identical to that of an adult worm from a cormorant (*Phalacrocorax carbo*) from the Masfjord, W Norway included. In GenBank, these showed >98 % identity with a *Contracaecum* sp. larvae from a marine fish from the Atlantic coast of USA, and >99 % identity with *Contracaecum chubutensis* Garbin, Diaz, Cremonte et Navone, 2008 (Fig. 43D). A specimen from Etne FW (Fig. 43A) revealed an ambiguous *C. rudolphii* sequence, representing a mixed *C. rudolphii* A and B signal in the ITS sequences.
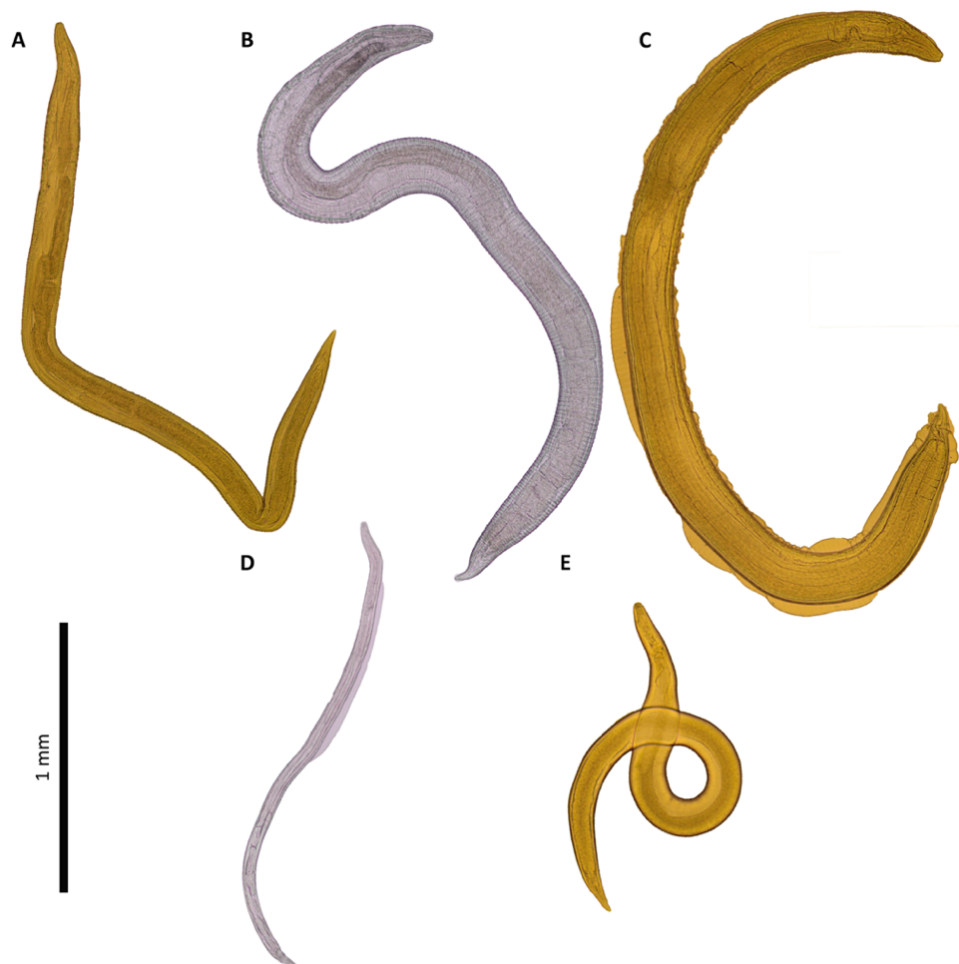
Figure 43 *Contracaecum* sp. worms. **A** *Contracaecum* sp. recorded in freshwater-caught eel, Etne, **B** *C. rudolphii* A recorded in freshwater-caught eel, Grimstad, **C** *C. rudolphii* A recorded in seawater-caught eel, Grimstad, **D** *C. chubutensis* recorded in seawater-caught eel, Smøla, **E** *C. rudolphii* A recorded in seawater-caught eel, Etne.

Aguillicola crassus Kuwahara, Niimi et Itagaki, 1974

Anguillicola crassus (Fig. 44), identified according to Arai and Smith (2016), was only observed infecting the swimbladder of eels caught in Grimstad, in 23 freshwater and a single fish caught in seawater (Table 6). The prevalence in Grimstad FW was 77 % with a maximum intensity of 10. The nematodes measured 2.3-32 mm in length and >99 % of the 63 worms registered were collected from the lumen of the swimbladder. Only one specimen was recorded in the wall of the swimbladder (3.7 mm in length).

Figure 44 *Anguillicola crassus* recorded from the swimbladder of a freshwater-caught eel, Grimstad.

3.2   Fresh gills examination

Most eels examined had been frozen, so protist parasites that do not produce spores could not be revealed. However, squash preparations of the gills of all eels from Etne FW and Etne SW were examined fresh. The only parasites observed during this screening was *Paramyxidium branchialis*. Hence the prevalence of the protists *Ichhtyobodo* sp.*, Trichodina* sp. and monogeneans from the genus *Gyrodactylus* (all previously found in north-European eels) were 0 on the basis of gill examination.

3.3   Parasite diversity

In freshwater-caught eels, 18 species were found. These represented one coccidian, five myxosporeans, one ectoparasite and 12 endohelminths, the latter of six larval and six adult stage forms. Twelve species were eel specialists, the coccidian *Epieimeria anguillae*, four myxosporeans and five endohelmints (*Bothriocephalus claviceps, Proteocephalus macrocephalus, Paraquimperia tenerrima, Anguillicola crassus* and *Daniconema anguillae*) and the ectoparasite *Pseudodactylogyrus anguillae*. Of the endohelminth individuals (N=513), 54 % were adult stage, and 46 % larval forms, the latter underestimated since the *D. anguillae* infrapopulations were not fully censused. For the same reason, > 77 % of the endohelminth individuals were eel specialists while the rest were generalists.

In the seawater caught eels, 22 species were found. These represented four myxosporeans and 18 endohelminths, the latter of five larval and 13 adult stage forms. Eel specialists were represented by eight species, four myxosporeans and four endohelminths (*Lecithochirium rufoviride, Deropristis inflata, P. macrocephalus, A crassus*). Of the endohelminth individuals collected (N=5588), 68 % were of generalists, while 32 % were eel specialists. A total of 94 % were adults, only 6 % larval forms.

The seawater caught eels were less likely to harbour myxosporean infections than eels from freshwater. The endohelminth and gastrointestinal helminth communities in seawater caught eels were richer in number of species and individuals than in the freshwater eels (Table 7).

Table 7 Some diversity measures for the parasite communities in the studied eel samples. N=number.

	Freshwater eels			Seawater eels		
	Grimstad	**Etne**	**Bjugn**	**Grimstad**	**Etne**	**Smøla**
Ectoparasites						
N species	1	0	0	0	0	0
Mean N species per eel	0.8					
Prevalence (%)	80					
Myxosporea						
N species	4	5	5	1	3	2
Mean N species per eel	0.7	1.4	1.9	0.4	0.5	0.3
Prevalence (%)	54	73	90	41	48	33
Endohelminths						
N species	5	8	4	5	13	10
Mean N species per eel	1.5	1.4	0.4	1.8	2.4	3.1
Mean N individuals per eel	8.0	7.7	0.8	26.7	91.5	76.3
Prevalence (%)	90	86	30	94	94	100
Gastrointestinal helminths						
N species	1	3	3	3	11	8
Mean N species per eel	0.03	1.0	0.3	1.5	2.1	3.0
Mean N individuals per eel	0.03	6.0	0.6	26.1	85.0	74.5
Prevalence (%)	3	79	27	88	94	100

3.4 Habitat and parasite origin

According to their life cycles, discussed below (p. 66), the parasites were classified as freshwater or marine (Table 8). They were further subdivided into parasites that are acquired from free infective stages in the water ('penetration'; Myxosporean actinospores, monogenean oncomiracidia, trematode cercariae) or through prey (endoparasitic helminths).

Table 8 Indicator parasites (freshwater, marine) used in the evaluation of eel interhabitat shifting (IHS). The freshwater residence indicators infect the fish through the external surfaces. The freshwater and marine feeding parasites are acquired by feeding on freshwater or marine prey harbouring parasite larvae. The background data for this list is provided in Appendix Table 1 and Table 2, which represents a summary of the information provided on this subject in the Discussion.

Indicator of:	Parasite	Indicator of:	Parasite
Freshwater residence indicators		*Marine feeding indicators*	
	Paramyxidium branchialis (My)		*Hemiurus communis* (T)
	Paramyxidium magi (My)		*Brachyphallus crenatus* (T)
	Myxobolus sp. (My)		*Lecithochirium rufoviride* (T)
	Diplostomum spathaceum (T)		*Derogenes varicus* (T)
	Daniconema anguillae (N)		*Lecithaster gibbosus* (T)
			Podocotyle atomon (T)
Freshwater feeding indicators			*Helicometra faciata* (T)
	Proteocephalus macrocephalus (C)		*Deropristis inflata* (T)
	Bothriocephalus claviceps (C)		*Eubothrium* sp. (C)
	Dibothriocephalus ditremus (C)		*Phyllobothrium squali* (C)
	Pseudocapillaria tomentosa (N)		*Cucullanellus minutus* (N)
	Eustrongylides sp. (N)		*Hysterothylacium aduncum* (N)
	Paraquimperis tenerrima (N)		*Anisakis simplex* (N)
	Anguillicola crassus (N)		*Contracaecum rudolphii* A (N)
Not usable as indicators			*Contracaecum chubutensis* (N)
	Paramyxidium giardi (My)		*Paracuaria adunca* (N)
	Hoferellus gilsoni (My)		*Echinorhynchus gadi* (A)
	Zschokkella stettinensis (My)		
	Pseudodactylogyrus anguillae (Mo)		
	Contracaecum sp. A/B (N)		

My – myxozoa, T – trematode, C – cestode, N – nematoda, Mo – Monogenea, A – Acanthacephalan

Overall, 15 parasite species were clearly of freshwater origin, and 17 were marine. *Paramyxidium giardi* and an ambiguous genotype of *Contracaecum rudolphii* could not be assigned to a habitat, and one species, the coccidian *Epieimeria anguillae* could show transmission in both habitats and was not considered. Hence 32 parasite species were clearly connected to either FW or SW. Three of the 15 freshwater parasite (*H. gilsoni, Z. stettinensis, P. anguillae*) could not be used as indicators, since they do not survive in seawater, The final list therefor contained 12 freshwater parasites and 18 seawater parasites were considered as indicator species and used to examine habitat shift (inter-habitat shifters, IHS).

Marine parasites detected in freshwater eels were all acquired through prey. They occurred in 0-43 % (Table 9) of the freshwater eels. In Grimstad FW, marine parasites constituted 56 % of all the endohelminth individuals (N=141). In Bjugn the only marine parasite occurring was *P. atomon,* while in Grimstad FW 97 % were *Contracaecum rudolphii* A larvae and the rest *A. simplex*.

Table 9 Occurrence of marine parasites (%) in freshwater-caught eel.

FW locality	% infected with marine parasites
Grimstad	43
Etne	0
Bjugn	7

Five parasites of freshwater origin (the myxosporeans *P. branchialis, P. magi* and *Myxobolus* sp., the cestode *P. microcephalus* and nematode *A. crassus*) occurred in seawater-caught eels. Freshwater parasites were detected in 33-47 % (Table 10) of the 78 seawater-caught eels. With the exception of one eel from Grimstad SW with *A. crassus* and two from Etne SW infected with *P. microcephalus,* close to all these individuals harboured histozoic myxosporeans (*Myxobolus* sp., *P. magi* and *P. branchialis*). Of the 32 seawater-caught eels with freshwater parasites, 31 harboured freshwater myxosporeans.

Table 10 Occurrence of freshwater parasites (%) in seawater-caught eel

SW locality	% infected with FW parasites
Grimstad	47
Etne	45
Smøla	33

3.5   Fatty acid analysis

Fatty acid analysis were performed by Camilla Parzanini on the eels from Etne (FW/SW) that were examined for parasites. Based on the M/F fatty acid ratios, the eels were by her categorized as having either freshwater (FW), brackish water (BW), intermediate (SW/BW) or seawater signatures (SW). All the FW eels had clear FW fatty acid profiles. However, the seawater-caught eels had BW, SW/BW and SW signatures, but some harboured parasites of freshwater origin.

The freshwater parasites detected in the Etne SW samples were *Myxobolus* sp., *Paramyxidium branchialis* and *Proteocephalus macrocephalus*. The proportion of freshwater-acquired parasites in eels with BW signature was not significantly higher than in eels with SW or SW/BW signature (Table 11; FET, p=0.15).

However, two of the eels with BW signature carried *P. macrocephalus* infections (3 and 4 large worms), while none of the eels with SW or SW/BW signature were infected. This difference was significant (FET, p<0.03).

Table 11 2 x 2 table. Occurrence of freshwater acquired parasites in seawater-caught eels in Etne SW, according to fatty acid signature categories. BW=brackish water, SW=seawater.

	Freshwater parasites	
	Present	Absent
BW signature	4	1
SW or SW/BW signature	11	15

FET, p=0.15

3.6   Otolith microchemistry analyses

Microchemical analyses were performed on otoliths from the Etne eels by Mehis Rohtla. Based on these analyses, he classified the eels as either freshwater resident (FWR), marine water resident (MWR) or inter-habitat shifters (IHS). All the freshwater-caught eels (Etne FW) were classified from the otoliths as FWR. These eels did not harbour parasites of certain marine origin (but see *Contracaecum* sp. p. 84).

The seawater-caught eels were either IHS, MWR or intermediate (MWR/HIS). All categories of eels harboured parasites of freshwater origin (*Myxobolus* sp., *Paramyxidium branchialis* and *Proteocephalus macrocephalus*).

There were no significant difference between eels that showed a MWS signature and those with an indication of habitat shifting (IHS) in the occurrence of parasites of freshwater origin

(Table 12;FET=0.22). Two eels had intestinal infections by the freshwater cestode *P. macrocephalus*. One was classified as IHS, the other as MWR.

Table 12. 2 x 2 table. Occurrence of freshwater acquired parasites in freshwater-caught eels in Etne SW, according to otolith microchemistry classifications. IHS=inter-habitat shifters, MWR=marine water resident

	Freshwater parasites	
	Present	Absent
IHS or MWR/IHS	6	4
MWR	8	13

FET=0.22

4   Discussion

4.1   Parasites in European eel from Norway

A total of 34 parasite species were observed in the 171 eels examined. Prior to the present study, there were records of 20 species from Norway (Table 1). The list has now increased with 21 additional species.

4.1.1   Protists

Epieimeria anguillae is a coccidian which infect the foregut epithelium of eels. It was first described from the European eel (*Anguilla anguilla*) from Corsica (Léger & Hollande 1922). The parasite has later been reported from most countries in Europe south of Scandinavia and from Iceland Kristmundsson and Helgason (2007), mostly in freshwater eels. This is the first observation of *E. anguillae* in Norway. Like most fish infecting coccidia, *E. anguillae* likely has a direct infection route. *Epieimeria anguillae* occur both in freshwater and seawater-caught eels (Daoudi et al. 1989, Benajiba et al. 1994, Kristmundsson & Helgason 2007). While clearly becoming infected in freshwater (Lacey & Williams 1983), it is unclear if transmission also may occur in the sea. It was therefore dismissed as an indicator species for habitat shift in the present study, and not systematically screened for.

It was surprising that none of the eels from Etne showed any signs of microscopic ectoparasites in the gills. *Trichodina* sp. infections have previously been noted in wild marine eels from Bergen (Karlsbakk, pers. comm.), and the ciliate was registered in a freshwater eel from Sunnmøre (Karlsbakk in Sterud (1999)). These ciliates has also caused problems in freshwater eel culture in Norway (Jensen 1998). In Scottish eels, the prevalence of the flagellate *Ichthyobodo* sp. was high (McGuigan & Sommerville 1985), but these flagellates occurred in highest densities on the skin.

4.1.2   Myxosporea

Myxosporeans have obligate two-host life cycles involving a vertebrate and an annelid (Oligochaeta, Polychaeta). Different types of multicellular spores are produced in these hosts. Myxospores produced in the vertebrate are infective for the annelid host, while the

actinospores produced in the annelid are infective for the vertebrate, which typically is a fish (Kent et al. 2001, Melendy & Cone 2003). Myxosporeans are classified according to the myxospore morphology. Tissue tropism and sites are often characteristic for different phylogenetic groups (Fiala 2006). They are usually either tissue parasites (histozoic) or inhabit cavities such as the gall and urinary bladders (coelozoic).

Paramyxidium spp.

Genus *Paramyxidium* was erected by Freeman & Kristmundsson (2018) for a phylogenetic grouping of *Myxidium*-like histozoic parasites of eel, earlier considered a single species, *Myxidium giardi*. This *M. giardi* in the 'old sense' (*sensu lato*) was originally described from the kidney of the European eel in France. Infections with similar *Myxidium* spp. have since been reported in other *Anguilla* spp. globally. Many of these *Myxidium* spp. were subsequently synonymized with *M. giardi*. Due to this it is unknown if *M. giardi* is a widely distributed parasite which infects numerous species of eels in multiple organs, or if some infections represent other, morphologically similar but different species of myxosporeans. However, Freeman & Kristmundsson (2018) genetically characterised *Myxidium giardi*-like parasites from different infection sites in eels from Iceland and showed there were three different species, which they placed in their new genus *Paramyxidium*. *Paramyxidium giardi* infect the kidney, *P. branchialis* the gills and *P. magi* the gut wall (Freeman & Kristmundsson 2018). The present observations support the validity and tissue tropism of *P. giardi* and *P. branchialis*. However, the *P. magi* sequences from the present study were associated with a *Paramyxidium* sp. with clearly smaller spores than recorded by Freeman and Kristmundsson (2018). This species was in addition to the stomach wall also detected in the kidneys. Further studies are needed, but it now seems possible that the genotype described from Icelandic eels was matched with the wrong phenotype.

In the present study *P. giardi* was found in freshwater-caught eels from Grimstad, Etne and Bjugn, and in seawater-caught eels from Etne. There is a previous record of *Myxidium giardi sensu lato* from Norwegian eels (Mo & Sterud 1998), reporting gill infections in eels from Årungen and Glomma, and also infections in the intestinal wall of eels caught below Sarpsfossen in Glomma. The sites suggest that these records could have represented *P. branchialis* and *P. magi*. Hence the present record represents the first confirmed infections with *P. giardi* in Norway. The present detection of *P. branchialis* in freshwater-caught eels

from Grimstad, Etne and Bjugn and seawater-caught eels from Grimstad, Etne and Smøla and *P. magi* in an eel from Smøla also represent new confirmed records for Norway (for *P. magi* based on the sequence), but these species may have been present in the eels examined by Mo & Sterud (1998) from eastern Norway.

According to Benajiba & Marques (1993), *Myxidium giardi* (very likely *P. giardi*, since kidney cysts) infect freshwater oligochaetes (*Tubifex* sp.) where actinospores of the aurantiactinomyxon type develops. These were infective to glass eels, in which kidney cysts containing spores appeared after 2 months. The whole life cycle was found to take four months at 12°C. However, recently Rocha et al. (2019) showed that a myxosporean developing aurantiactinomyxon spores in the estuarine oligochaete *Tubificoides pseudogaster* was *P. giardi* according to its SSU rDNA sequence. This observation suggest that the life cycle of this species could be completed in estuaries such as river mouths. Freeman and Kristmundsson (2018) showed that numerous freshwater oligochaete actinosporeans belong to the *Paramyxidium* clade, suggesting both a higher diversity in this genus than presently recognized, and that the life cycles are mostly completed in freshwater. The life cycles of *P. branchialis* and *P. magi* have not been demonstrated, but sequences of two actinospore morphs of the Synactimomyxon type from *Tubifex tubifex* in a Scottish river show very high identity with the sequences of these two *Paramyxidium* spp. and they must be conspecific (Holzer et al. (2004) *vide* Freeman and Kristmundsson (2018)). These myxosporeans are therefore regarded as freshwater species, while *P. giardi* is considered as potentially marine.

Hoferellus gilsoni and *Zschokkella stettinensis*

The urinary bladder infecting coelozoic myxosporeans *Hoferellus gilsoni* and *Zschokkella stettinensis* were only found in freshwater eels. These may not survive in eels in seawater, since the eel then should produce hypersaline urine. Therefore, these coelozoic species cannot be used as indicator parasites for freshwater origin. The *H. gilsoni* sequence in GenBank, provided by Holzer et al. (2004) from a Scottish eel was confirmed. The *Zschokkella* sp. and *Myxidium giardi* sequences obtained in that study is here found to represent the same species, identified with *Z. stettinensis*. My *Z. stettinensis* sequences confirm that a sequence from the actinosporean *Neoactinomyxon eiseniellae* (Ormières et Frézil, 1969) is from the same species. That is then evidence for the inclusion of its host, the freshwater oligochaete *Eiseniella tetraedra,* in the *Z. stettinenesis* life cycle (Holzer et al. 2004). Therefore, this

myxosporean may occur throughout the range of the eel in Norway, and also occur in Scotland, Poland, France and Portugal (Debaisieux 1925, Wierzbicka 1987, Wierzbicka & Orecka-Grabda 1994, Saraiva & Eiras 1996, Rodjuk & Shelenkova 2006). The life cycle of *Hoferellus gilsoni* is still unknown.

Myxobolus sp.

The *Myxobolus* sp. detected here in the lamina propria of the hindgut is morphologically identical with *Myxobolus dermatobius* (Ishii, 1915) *sensu* Copland (1982), from the same site in juvenile English eels. However, according to Copland (1982) the site is the muscularis, where the parasite was never found in the present study. Possibly, the much smaller eels in the English study had plasmodia protruding into the muscularis. *M. dermatobius* was originally described from cysts in the skin of Japanese eel (*Anguilla japonica*) from Japan, with spores being clearly smaller (6.3-7.0 µm long) (Ishii 1915). Skin or fin cysts did not occur in the present material, nor in Copland's (1982) eels, and Hoshina (1952) confirmed the small spore size in *M. dermatobius*. Therefore, together with a different site, host species and geographic distribution, *Myxobolus* sp. cannot be identified with *M. dermatobius*. In European eels, *Myxobolus portucalensis* and *Myxobolus kotlani* Molnar, Lom et. Malik, 1986 produce macroscopically visible cysts in the fins and skin respectively (Saraiva & Molnár 1990) (Molnár et al. 1986). The former differs from *Myxobolus* sp. by its much larger spores (averaging 12.6 µm in length) and in the SSU rDNA sequences. *Myxobolus kotlani* has similar but significantly smaller spores, and never occur in the gut. *Myxobolus* sp. is therefore considered a likely new species. Its confinement to freshwater eels, and the fact that related *Myxobolus* spp. also are freshwater parasites indicate that the unknown life cycle is freshwater-restricted.

4.1.3   Platyhelmintes

<u>**Monogenean**</u>

Pseudodactylogyrus anguillae

This gill monogenean is one of two *Anguilla*-specific species of the genus *Pseudodactylogyrus* present in Norway, the other is *P. bini* (Mo 1999). *P. anguillae* has a

direct life cycle, where eggs are released to the environment by adult worms, and oncomiracidia that hatch from the eggs finds and infects an eel (Buchmann et al. 1987).

The gill ectoparasite was only recorded in freshwater-caught eels from Grimstad, whit a prevalence of 80 %. There was, like in the study by Fazio et al. (2009), surprisingly no clear positive relationship between abundance and eel size (400-647 mm), which is the opposite of what has previously been shown in both wild eel (Barker & Cone 1997, Aguilar et al. 2005) and eel farmed in a commercial eel-culture system (Buchmann 1989). Buchmann (1989) explained it with the larger space available in the gills of larger eels, but interactions between the host and the parasites also make such correlations less distinct in older infections (Buchmann 1993). Seawater entering the lake Landvikvannet, Grimstad, allows the eels there to move from freshwater to saline environment. If this occur, then that could cause *P. anguillae* mortality within days. If such habitat-shifting contribute to the observed pattern, then such movements must be most frequent among large eels.

P. anguillae seems to show a preference for the posterior gill arches. The same trend has been noted in other studies (Buchmann 1988, 1993), but then also with *P. bini* present. Hence the present study demonstrates this microhabitat pattern with *P. anguillae* infection alone.

Pseudodactylogyrus anguillae, together with *P. bini*, is geographically widespread worldwide, also in Norway (section 4.2) due to uncontrolled transport of live eels, even intercontinental transfer (Køie 1988c, Matejusová et al. 2003).

Trematoda

Trematodes have complicated life cycles with several hosts involved. The first intermediate host is most often a mollusc, gastropod or bivalve, where cercaria larvae develops. These are usually released and may infect the second intermediate host, where they develop into metacercaria larvae. The final host is most often infected by eating the second intermediate host with metacercariae.

The examined eels hosted nine different trematode species, one species as a metacercaria larvae and eight as adults. All the trematode species, except two, are generalists that are able to infect different teleost species. The specialist trematodes seen were the marine *Deropristis inflata* and *Lecithochirium rufoviride* which only infects eels.

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Deropristis inflata

Deropristis inflata differs from most digeneans by its unusual host-specificity pattern, the trematode is host-specific in regard to the final host, the eel, while the cercariae of *D. inflata* develop in several gastropod groups. The second intermediate hosts are nereid polyachaetes, such as *Hediste diversicolor* that harbour metacercariae (Vaes 1978, Køie 1988b). Most other digeneans are host-specific toward the first intermediate host (Køie, 1988b).

Deropristis inflata appears to be the most common parasite of European eels in saline waters. Kristmundsson and Helgason (2007) compared intestinal helminth component communities between brackish and marine localities in Europe and found that *D. inflata* was the dominant species at 15 of 19 localities (distributed between six countries). In the present study, *D. inflata* was not found to be the dominant species at any of the marine localities, but is the only trematode that is present at all. The infected eels most likely got infected by eating *H. diversicolor*.

The parasite has previously been reported from European eels in Norway from Bergen (Olsson 1868), and from below the Sarpsfossen in Glomma (Mo & Sterud 1998). The latter observation suggest that it may be brought into freshwater by eels, and can be a biological tag for eels that have fed in the sea.

Podocotyle atomon

Podocotyle atomon is a widely distributed parasite of marine fishes (Hunninen & Cable 1943). The life cycle involves cercariae that develop in the littoral snail *Littorina saxatilis*, which is the main snail host and first intermediate host. The second intermediate host is various crustaceans, especially amphipods where infectious metacercariae develop. These then infect the final host, apparently any marine teleost (Hunninen & Cable 1943, Køie 1984).

In the present study *P. atomon* was registered in saltwater caught eels from Etne and freshwater-caught eels from Bjugn. The parasite is most common in sheltered areas, often with reduced salinity (Køie 1984). Estuarine environments are widespread in Norway, including outside the river mouths in Bjugn and Etne. Kennedy et al. (1992) also reported *P. atomon* from freshwater-caught eels in England. They explained these findings by the proximity of the sampling locality to the estuaries. This is most likely also the case in this

study whit the sample locality in Bjugn being a maximum of 200 m from the river mouth. The eels could readily make sojourns downstream to brackish waters and feed on e.g. amphipods.

This is the first registered finding of *Podocotyle atomon* in European eel in Norway, but it has been registered in this host in other European countries like Denmark, Germany, England, Iceland and Portugal (Jakob et al. 2016).

Helicometra fasciata

Stossich (1902) described a separate species, *Helicometra mutabilis* (Stossich, 1902) from marine eels (Adriatic Sea), which is very similar to the present form, except for a relatively larger ventral sucker in the Adriatic species. That species is now considered synonymous with *H. fasciata* (Aken'Ova et al. 2006). *H. fasciata* has been described from many parts of the world, such as Oceania, Australia, the Mediterranean and the northeast North Atlantic. However, as suggested by Aken'Ova et al. (2006), the present LSU rDNA sequences support that *H. fasciata* is a species complex, consisting of several morphologically similar species.

The first intermediate host for *H. fasciata* is certain snails in the genus *Steromphala* (previously *Gibbula*), one of which is common on kelps in the Norwegian fjords. Second intermediate hosts are certain shrimps in shallow water (*Palaemon* spp.), harbouring metacercaria larvae of the trematode in the musculature (*H. fasciata* life cycle reviewed by Blend and Dronen (2015)). Hence, the eels from Grimstad likely acquired their *H. fasciata* infections by feeding on glass shrimps *Palaemon elegans* or Baltic shrimps, *P. adspersus*. Olsson (1868) found *Helicometra faciata* in eels from Bergen.

Hemiurus communis

Hemiurus communis is found in the stomach of fish from several families and is considered unspecific. It has a boreal distribution, in European coastal waters from the Bay of Biscay to about Lofoten in Norway (Køie 1984, Gibson & Bray 1986, Gibson 1996).

The life cycle involves at least three hosts. First intermediate host is a marine snail (*Retusa truncatula*) where the cercaria develops. The free-swimming cercariae infect zooplankton copepods being second intermediate hosts that harbour metacercariae. These are infective for the final host, a teleost fish. Small fish species and young specimens of final hosts acquire the parasite by ingesting infected copepods, while larger fishes most likely acquire the trematode

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by ingesting smaller infected fishes (Køie 1995). Hence, the eels of the sizes studied here likely became infected by eating smaller planktivorous fishes.

Hemiurus communis has previously been reported from European eel from Norway, in Bergen (Olsson (1868) *vide* Odhner (1905)). The species has also been reported from seawater-caught eels in Denmark and Germany (Jakob et al. 2016).

Brachyphallus crenatus

Brachyphallus crenatus has an Arctic-boreal distribution and is found in the stomach of various kinds of marine teleosts (low host specificity). Its life cycle involves at least three hosts, with the first intermediate host being the snail *Retusa obtusa* releasing cercariae. The second intermediate host is a zooplankton copepod where infective metacercariae develops, infecting planktivorous teleost final host. *B. crenatus* survive in anadromous salmonids during migration into freshwater, and thus the species may act as a biological indicator for marine foraging (Køie 1992). The parasite most often infects salmonids, clupeids, gasterosteids and pleuronectids, but is also found infecting European eels in both brackish and marine areas in Denmark and in marine areas in Germany and Poland (Køie 1988b, Køie 1992, Jakob et al. 2016). The parasite has not previously been registered in European eel in Norway.

Lecithochirium rufoviride

Lecithochirium rufoviride is a trematode which is specific to eels, infecting both the European eel and the conger eel, *Conger conger* (Køie 1990).

The life cycle of *L. rufoviride* has been found to involve four hosts (Køie 1990). The cercariae develop in the gastropod *Steromphala cineraria* (Køie 1990), copepods are supposed to act as second intermediate host and small fishes, e.g. goldsinny, as third intermediate host. Eel and conger are final hosts (Køie 1984). Experimental life cycle studies indicate that the species in addition to the four-host life cycle could have a three-host life cycle, depending on the crustacean host (Køie 1990).

In the present study, *L. rufoviride* was found in the stomach of seawater-caught eels from Grimstad and Smøla. The parasite showed a strong correlation between abundance and eel size. In this case the larger eels, eating small fishes with metacercariae, will accumulate a

higher abundance of the trematode compared to the smaller eels that could become infected by feeding on copepods.

Lecithochirium rufoviride has previously been registered in European eels in Norway from Sotra, Bergen and Langesund (Olsson 1868, Lönnberg 1890, Gibson & Bray 1986). It has also been registered in eels from Denmark, Faroe Islands, Germany, Portugal and Spain (Jakob et al. 2016).

Derogenes varicus

Derogenes varicus is a very common trematode in the stomach of marine fish from the North-Atlantic including the entire Norwegian coast. It infects a wide range of unrelated teleosts, and is hence regarded as unspecific (Gibson 1996, Køie 2000).

The life cycle, that takes place in saltwater, involves at least three hosts; the first intermediate host is a snail (*Lunatia* spp.) where the cercariae develop. These infect copepods, the second intermediate host where infective metacercaria develop in the body cavity. The second intermediate host can be a number of common zooplankton copepods, like *Calunus* spp. The final host, normally a teleost, is infected by eating zooplankton. Also, several different transport hosts can be part of the life cycle, like chaetognatha, ctenophores or planktivorous fish (Køie 1979). Predatory fish accumulate *D. varicus* from eating prey and the intensity of infection with the parasite can therefore get high in predators such as cod (Meskal 1967). The seawater-caught eels found infected from Etne and Smøla found infected had likely become infected by feeding on small fish, such as sandeels.

Derogenes varicus has previously been reported from European eel in Norway, from Bergen by Olsson (1868) and the Trondheimsfjord (Land et al. 1966), but has also been registered in other fish species along the entire Norwegian coast (Køie 1984). The parasite has been registered in European eels from Iceland, Denmark and Spain (Jakob et al. 2016).

Lecithaster gibbosus

Lecithater gibbosus is a common trematode that infect the intestine of marine teleosts in the North Atlantic ocean and adjacent seas. The parasite is recorded in the intestine of members from most teleost families, and is therefor not considered host spesific (Køie 1989, Gibson 1996).

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The life cycle is marine with cercariae that develops in the snail *Branchystomia eulimoides*. The released cercariae are eaten by certain zooplankton copepods acting as a second intermediate host. These then contains infective metacercaria in the body cavity. The final host is a plankton feeding teleost or larger predatory fish feeding on them again (Køie 1989).

L. gibbosus (as *Distoma bergense* Olsson, 1868) was reported from European eel from Bergen, Norway (Olsson 1868). The trematode has also been recorded in seawater-caught eels from Denmark and Spain (Jakob et al. 2016).

The European eel of the size studied here probably become infected after eating planktivorous fishes, the trematode likely being transferred from one fish host to another (Køie 1989).

Diplostomum spathaceum

Metacercariae from members of the genus *Diplostomum* inhabit the brain and various regions of the eyes of fish (Field & Irwin 1995). Only one of the eels studied here had metacercariae infecting the vitreous humour of the eye, identifies as *Diplostomum spathaceum.*. Hence European eel may serve as the second intermediate host in the three-host life cycle of this trematode. The first intermediate host is the snail *Lymnaea pereger* were the cercariae develop, and the parasite matures in a fish-eating bird (Køie 1988a, Køie 1988b).

Diplostomum spp. metacercariae have been registered in European eels from Denmark, England, Ireland, Iceland, Latvia and Spain (Jakob et al. 2016). Mo and Sterud (1998) reported *Diplostomum* sp. from European eel in Norway from Årungen lake in Eastern Norway. The present case seems to be the first time that such metacercariae in eel has been identified to species.

Cestoda

Eels can act as both intermediate host for larval stages and final host for adult individuals in the life cycle of cestoda. When fish act as intermediate host the plerocercoids are found either free in the gut or encapsulated in tissues, depending on the cestode group. Encapsulated plerocercoids can accumulate in older fish since they may be long lived. Adult worms are found attached by their scolex to the intestinal mucosa (Bruno et al. 2013). The typical life cycle of cestodes include a free-living egg or larval stage (coracidium), a procercoid stage in the first intermediate host (in general a copepod), and a plerocercoid stage in the second

intermediate host (a large variety of copepod-eating anials), and the adult in the final host (fish-eating fish, birds or marine mammals) (Möller & Anders 1986).

Scolex pleuronectis

Scolex pleuronectis is today used as a designation for tetrabothridial cestode larvae normally found free in the intestine of marine teleosts and in squids. It is now clear that larvae of this type is the alternating stage of several groups of phyllobothriidean and onchobothriidean cestodes (Caira & Jensen 2014). The morphologically very similar plerocercoids of this type found in eels from Etne likely all represented *Phyllobothrium squali* according to the 28S rDNA sequences. This species matures in the spiral valve of dogfish *Squalus acanthias*, a shark very common in the area.

Proteocephalus macrocephalus

Proteocephalus macrocephalus is a specific parasite of eels, found in the intestine (Køie 1988b, Scholz & Hanzelova 1998, Dezfuli et al. 2014). Certain planktonic copepods are first intermediate hosts, containing procercoids developing in their body cavity (Køie 1988b, Scholz 1999). The definitive hosts, eels, can become infected directly when consuming the copepods. However, larger eels rarely feed on copepods, so invertebrates and small prey fishes feeding upon plankton are likely to occur in the life cycle, but their role in the transmission is unclear (Scholz 1999). Free *Proteocephalus* larvae are common in the intestine of small fish such as sticklebacks (Rødland 1980, Zander et al. 2002).

Proteocephalus macrocephalus is a freshwater parasite but tolerant the eel's migration to sea (Dezfuli et al. 2014). In the present study the cestode is recorded from eels in Etne, where it was found both in freshwater and seawater-caught fish. The infected seawater-caught eels were caught near the river mouth of the river Etneelva, which can explain the freshwater parasite infection with habitat shifting eels.

The tapeworm has previously been recorded in European eels from Norway by Andersen (1979). Those eels were caught in brackish waters in the Oslofjord. Besides Norway, the parasite has been registered in countries all over Europe (Jakob et al. 2016).

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Bothriocephalus claviceps

Bothriocephalus claviceps is a common intestinal parasite specific to eels (Køie 1988b, Scholz 1997, Dezfuli et al. 2014). The tapeworm only require one intermediate host, a cyclopid copepod, for completing the development. It is also assumed that small fish can serve as transport hosts, and represent a source of infection for the larger eels (Scholz 1997). The life-cycle is carried out in freshwater (Dezfuli et al. 2014).

In the present study the tapeworm was registered at three of the freshwater localities, Etne, Bjugn and Orkla. It has previously been found eels caught in Årungen and lower River Glomma on the east coast of Norway by Mo and Sterud (1998).

Dibothriocephalus ditremus

Species of the genus *Dibothriocephalus* have complex life cycles. Copepods serve as first intermediate hosts, fish as second intermediate host harbouring encapsulated plerocercoids, and fish-eating birds or mammals as final hosts. *D. ditremus* is mostly found as plerocercoids in salmonids and the final host is mergansers (Henricson 1977, Borgstrøm et al. 2017). In the salmonids, the small plerocercoids are normally found in the stomach wall, the same site they were found in the freshwater-caught eels from Etne. Within the eels, the capsules were brownish, and many contained recognizable but degenerated plerocercoids. In addition there were capsules without identifiable contents, perhaps remains of killed and degenerated worms. This is the first record of this tapeworm species in European eel.

Eubothrium cf. *crassum*

Eubothrium crassum (Bloch, 1779) is a widespread parasite which is found in the alimentary tract of salmonids in freshwater. A similar or conspecific tapeworm is also found to infect salmonids in seawater, and the marine 'race' or species is often referred to as *Eubothrium* sp. (Berland 1997). This is a common cestode in farmed salmon and trout at aquaculture localities on the west coast of Norway. The life cycle of both the freshwater and marine form is relatively simple with only one intermediate host, copepods (Rosen 1919, Saksvik et al. 2001). Small fishes are important transport hosts, but are not essential for the continuation of the life cycle (Vik 1963). In Norway, the natural final hosts are the *Salmo* spp. The importance of *Eubothrium* larvae that end up in the wrong fish host is unknown.

Free bothriocephalidean plerocercoids were found in the lumen of the intestine in seawater-caught eels from Etne. Some of the largest specimens were morphologically similar to *Eubothrium crassum*, whit craspedote strobila. The smaller specimens had a smoother surface and were not possible to identify morphologically. A selection of these, together with specimens morphologically similar to *Eubothrium crassum,* were molecularly identified using LSU rDNA sequencing. All sequences were similar to *Eubothrium crassum* and *Eubothrium* sp. from seawater farmed Atlantic salmon (*Salmo* salar) in Norway. However, marine and freshwater specimens of *E. crassum* cannot be distinguished from each other morphologically or molecularly so they are therefore referred to as *Eubothrium* cf. *crassum*. Since there were no such plerocercoids in freshwater-caught Etne eels, it is highly likely that these plerocercoids represented the marine form, common in farmed Atlantic salmon in the Hardangerfjord.

The tapeworm have been recorded from European eels caught in Lake Neusiedler in Austria (Kritscher 1986, Jakob et al. 2016). However, Scholz et al. (2003) found that the tapeworms from Austria were misidentified, they represented *Bothriocephalus claviceps*. Hence there are no previous valid records of *E. crassum* in eels in Europe.

4.1.4   Acanthocephala

Echinorhynchus gadi

Echinorhynchus gadi is the most common acanthocephalan infecting marine fish in the North Atlantic (Marcogliese 1994) and the only adult acanthocephalan infecting marine fish in Norway. *E. gadi* is a common parasite in codfish, but may be found in almost all marine teleosts in Norway. The life cycle involves two hosts, the intermediate host is certain amphipods and the definitive host is a teleost (Miller 1977, Marcogliese 1994).

The parasite was registered in three eels in total, seawater-caught eels from Etne and Smøla. This is the first record of *E. gadi* in European eels in Norway. It has previously been registered in European eels from Germany and Russia (Jakob et al. 2016).

4.1.5   Nematoda

Nematodes or roundworms are among the most common parasites. As a group they are easy to distinguish from other worms, but species determination is complicated. Roundworms

parasitize fish in both their adult and larvae stage. The life cycle can be direct or involve one or several intermediate hosts, and often transport hosts. Crustaceans are known to play an important role as intermediate hosts for most species (Möller & Anders 1986).

Pseudocapillaria tomentosa

Pseudocapillaria tomentosa is a wide-spread intestinal parasite of freshwater fishes in the Northern Hemisphere. It is mainly a parasite of the intestine of cyprinids in Europe, but is also found in other fishes (Anderson 2000, Leis et al. 2016). The roundworm has a direct life cycle, with oligochaetes which may serve as transport hosts (Leis et al. 2016).

In Europe, the parasite is known form the U.K., Scandinavia and throughout Europe from France to trans-Caucasia (Leis et al. 2016). It is reported from European eels from Denmark, Germany, Italy and Spain (Jakob et al. 2016), it has not previously been reported from European eels in Norway.

Eustrongylides sp.

Eustrongylides spp. are found in both marine and freshwater fish and uses teleosts as an intermediate host in the life cycle. The first intermediate host is aquatic oligochaetes were the nematode develops to a third stage larvae (Measures 1988). These infect fish, which serve as a transport host, and develop into fourth stage larvae. The larvae are encapsulated, mainly in the mesentery and the intestinal serosa. Some of the encapsulated larvae undergo degeneration and die, due to the hosts immune responses. Larvae that survive in the teleost may end up in the final host, a piscivorous bird (Anderson 2000, Urdes et al. 2015).

The *Eustrongylides* sp. specimens observed in the freshwater-caught eels in Etne were encapsulated in the stomach wall. Some were degenerated and presumed dead. This can be an indication that eels are unsuitable hosts. These worms may grow much larger in salmonids which seems to be the dominant hosts in western Norway (Elnan 1995). The identity of the *Eustrongylides* sp. infecting salmonids and eels in western Norway is unknown, but some authorities have identified them with *E. mergorum* (Rudolphi, 1809), a species infecting *Mergus* spp. (Køie 1988a, Elnan 1995). Goosander *Mergus merganser* is a final host in Norway according to Elnan (1995), who found adult mature worms in the proventriculus of this fish-eating duck.

This is the first reported observation of *Eustrongylides* sp. in European eels in Norway, but several *Eustrongylides* spp. have been reported from eels in Europe previously (Urdes et al. 2015, Jakob et al. 2016).

Daniconema anguillae

Daniconema is a tissue parasite, and has been registered in the swim-bladder, intestine and in the subcutaneous connective tissue in the fins of eels in freshwater (Køie 1988b, Molnár & Moravec 1994, Molnár 1997).

The life cycle of the genus *Daniconema* has not been studied, but the life cycles of related deniconematids and skrjabillanids are known. These nematodes develop in the ectoparasitic branchiurans, *Argulus* spp., being the intermediate hosts. It is highly probable that *D. anguillae* larvae also reach the third larval stage, the maturity level necessary for infectivity, in that crustacean (Molnár & Moravec 1994, Molnár 1997). The parasite is transferred to the host's tissue when it penetrates through the crustacean's mouth organs as the crustacean is sucking blood from the host. After entering the connective tissue, the parasite can migrate into internal organs of the fish host for further development and maturation (Molnár & Moravec 1994).

In the present study, *D. anguillae* was found in freshwater-caught eels from Grimstad, Etne and Orkla. Larvae of the hostozoic parasite was detected in the connective tissue of the pectoral fin, dorsal fin and in the gill lamellae, but likely occurred throughout much of the eel's tissues. The abundance of *D. anguillae* larvae, as quantified in the pectoral fins or gills, showed a positive correlation with eel length. If a blood sucking branchiuran of the genus *Argulus* is involved in the life cycle (Molnár 1997), the eels may become infected when the branchiuran feed on eel blood. The older and larger eels may have experienced this several times, a possible explanation for them harbouring more larvae. However, this is uncertain, since this life cycle pattern occurring in other daniconematids has not yet been studied and verified for *D. anguillae*. Curiously, there was a single infected eel in freshwater in Litledalsvatn, Etne. *Arguglus* spp. is not known to occur in western Norway north of Jæren (Økland 1985, Sterud 1999), so this individual is either a migrant or the transmission of the parasite is independent of the branchyuran.

Infections in the gills have not been registered before, and this is also the first reported infection with *Daniconema anguillae* in European eel in Norway. In Europe, the nematode
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has only been detected infrequently and in few countries (Molnár 1997, Jakob et al. 2016). But as the present study evidence, the prevalence might be much higher than what has been registered based on swimbladder examinations, due to the fact that the parasite is dispersed in the tissues and difficult to detect so even heavy infections may be overlooked (Molnár 1997).

Paraquimperia tenerrima

Paraquimperia tenerrima is a specific freshwater parasite of the European eel. Several studies has been done on the life cycle of the nematode and different cycles has been proposed (Kennedy et al. 1992, Barker & Cone 1997, Shears & Kennedy 2005). An indirect life cycle, involving an intermediate host, seems most likely (Shears & Kennedy 2005). The identity of the intermediate host has been considered to be a planktonic invertebrate, or a fish (Kennedy et al. 1992, Barker & Cone 1997, Shears & Kennedy 2005). Shears and Kennedy (2005) studied the capability of stage two larvae to infect invertebrates, eels directly and other fish species. They concluded that *P. tenerrima* require an obligate fish intermediate host and found that in England this was the minnow (*Phoxinus phoxinus*).

Freshwater-caught eels from Etne, Bjugn and Orkla were registered infected in this study. Minnow is not known to inhabit these localities, so other small fish could be involved. Stickleback are widespread in the coastal areas where eels live, is known to be a part of the eels diet (Jakobsen et al. 1988), and seems a candidate.

Paraquimperia tenerrima has been reported from wild eels in Norway previously, Mo and Sterud (1998) reported the parasite from eels caught in Årungen and Glomma. The late prof. B. Berland found it in eel in Lake Kalandsvatn near Bergen (pers. comm. Karlsbakk). The nematode has also been registered in several other countries in Europe (Jakob et al. 2016).

Paracuaria adunca

Paracuaria adunca is an avian parasite that uses fish as a transport host. The fish is infected by third stage larvae which develops in the first intermediate host, an amphipod. The final host is gulls (Laridae) (Anderson & Wong 1982).

Only seawater-caught eels from Etne were infected with this nematode. This is the first registered finding of *P. adunca* in European eels caught in Norway. The parasite has been

recorded in other fish species in Norway (Karlsbakk et al. 1996), and in eels in Denmark and Germany (Jakob et al. 2016).

Cucullanellus minutus

Only a single specimen of this nematode was found. It is a parasite of flatfishes, in particular the flounder *Platichtys flesus*. Intermediate host is the marine polychaete *Hediste diversicolor*. However, immature and even adult specimens occasionally occur in gobies, which also may act as transport hosts (see Køie (2001)).

This is the first record of *C. minutus* in European eel caught in Norway. *Cucullanellus* spp. have previously been reported from eels from the areas around the British Isles, Spain and Russia (Jakob et al. 2016)

Hysterothylacium aduncum

This is a common parasite in the digestive tract of marine teleosts in Norway, Europe and elsewhere. Larvae of the species are known to occur in marine crusteaceans, particularly copepods (benthic or planktonic), which serve as the first intermediate host. Invertebrates or fish ingesting crustaceans with larvae may serve as a second intermediate host or a transport host with third stage larvae (Køie 1993) encapsulated in the viscera. However, when fish ingest prey harbouring larger third-stage larvae (>3 mm), these may moult to preadults and finally adults in the gut (Køie 1988b, 1993). Smaller larvae may re-establish in viscera (Køie 1993). Remarkably, the eels in the present study were only found infected with adults and preadults, suggesting they tend to eat prey with larvae longer than about 3 mm. Kristmundsson and Helgason (2007) found only encapsulated larvae in Icelandic eels, while Køie (1988b) found both larvae and adults in Danish eels. Therefore, eels do seem to act as transport hosts.

This is the first record of *H. aduncum* in eels caught in Norway. The parasite has been registered in eels in other parts of Europe from France in the south to Iceland in the north (Kristmundsson & Helgason 2007, Jakob et al. 2016).

Anisakis simplex

Anisakis simplex is a parasite which is common as a third stage larvae in a variety of marine teleosts. The final host in the lifecycle is certain whales (Mattiucci et al. 2017). Krill (Euphausiacea) represent the most important first intermediate host where third stage larvae develop. The final host can be infected directly by eating krill, but fish or squids who eats krill may act as transport hosts (Højgaard 1998). This parasite may be transmitted from prey to predator fish, accumulating in the food chain (Haarder et al. 2013).

In this study, *A. simplex* were registered in seawater-caught eels from Smøla and in freshwater-caught eels from Grimstad. Being a marine parasite, the finding in freshwater-caught eels is surprising, but there are similar cases. Aguilar et al. (2005) and Saraiva and Eiras (1996) both registered findings of *A. simplex* in Iberian freshwater-caught eels. Saraiva and Eiras (1996) examined eels caught in the rive Este (Portugal), and considered the occurrence of the marine parasite either as due to an infection acquired during their estuarine glass eel phase or an indication that they may migrate down to and feed in the estuarine waters after freshwater entry (inter habitat shifting). Anguilar et al. (2005) examined eels caught in two rivers in Spain and found 89 specimens of the parasite in one large eel from the river Ulla. They also concluded that the parasite most likely had been acquired in the marine environment, probably as a result of ingestion of small marine fish. The freshwater-caught eels from Grimstad were also relatively large and may also have the acquired *A. simplex* infections by feeding on small marine fish. However, the freshwater locality in Grimstad differs from the others studied here with the seawater inflow. Albeit the saline bottom waters tend to be anoxic, certain marine fishes such as herring do enter the lake. Therefore, it is possible that the eels there may have become infected with *A. simplex* larvae when eating a transport host of marine origin in freshwater. A feeding excursion to the Strandfjord is also a possibility, there are no barriers. However, that they should acquire infections in marine waters before they entered the lake as juveniles seems contradicted by the general lack of *A. simplex* in other freshwater eel populations (present study, Jakob et al. 2016)

This is the first registered finding of *A. simplex* in European eels caught in Norway. Other reports of the parasite in European eels are from Germany, Iceland, Spain and Portugal (Saraiva & Eiras 1996, Jakob et al. 2016), mostly from seawater-caught fish.

***Contracaecum* spp.**

Parasites of the genus *Contracaecum* are common anisakids of aquatic organisms in freshwater, brackish and marine ecosystems (Garbin et al. 2011). Besides a phylogenetic clade with *Contracaecum* spp. infecting pinnipeds (Nadler et al. 2005), most *Contracaecum* spp. infects fish-eating birds. The life cycles of the avian species involves several hosts, often with crustaceans as first intermediate host, various fish species as second transport hosts and piscivorous birds as final host (Li et al. 2005, Szostakowska & Fagerholm 2007). Direct infections of fish with *C. rudolphii* larvae is also possible (two-host life cycle), but this may be uncommon in nature (Moravec 2009, Dziekonska-Rynko et al. 2010).

In the present study, *Contracaecum* spp. larvae were in registered in eels caught in both freshwater and seawater. DNA sequences (ITS) showed that worms from Grimstad FW, Grimstad SW and Etne SW belonged to the *Contracaecum rudolphii* complex. Studies on the internal transcribed spacers (ITS) in the rDNA of *C. rudolphii* have shown that this species is genetically heterogenous and there are at least two sibling species in Europe which are reproductively isolated genotypes, referred to as *C. rudolphii* A and B (Li et al. 2005, Szostakowska & Fagerholm 2007). Both are parasites of cormorants (*Phalacrocorax* spp.). The present *C. rudolphii* from Grimstad FW and Grimstad SW and Etne SW was of type A, apparently a species with marine or estuarine life cycle since the larvae are confined to fish on these environments (Szostakowska & Fagerholm 2007). However, the sequences obtained from worms from eels caught in the Smøla area were of another species, these genetically identified with *Contracaecum chubutensis* (Garbin et al. 2011). An adult worm from a cormorant from Masfjord included in the genotyping had the same identity, previously only known from cormorants in Argentina. The worm from Etne FW was not possible to identify, because the genotyping was ambiguous, with similarity to both *C. rudolphii* A and B. Genotype B is considered a freshwater species (Szostakowska & Fagerholm 2007).

This is the first time *Contracaecum rudolphii* and *C. chubutensis* has been reported from European eels caught in Norway. *C. rudolphii sensu lato* has previously been reported from Denmark and *Contracaecum* sp. from eels in several other countries in Europe (Jakob et al. 2016).

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Anguillicola crassus

The blood-sucking swimbladder nematode, *Anguillicola crassus,* is an eel specialist (Køie 1991). The species is ovoiviparous and is trophically transmitted. At least two hosts are involved in the life cycle that takes place in freshwater, an intermediate host and the eel definitive host. The intermediate host is a freshwater cyclopoid copepod that hosts the developing third stage larvae which is infective to eels. Small fishes can serve as transport hosts and be another source of infection for the eels (Køie 1988c, 1991, Kennedy 2007, Lefebvre et al. 2012).

In this study, the parasite was only registered in eels caught in Grimstad, 23 freshwater-caught eels and one seawater-caught eel. The infection found in the seawater-caught eel indicates that this eel has spent time in freshwater. Kennedy and Fitch (1990) showed that the adult parasite could survive for up to four weeks when eels were kept in 100 % seawater. *A. crassus* larvae can also survive in brackish waters, but both the survival and infectivity decreases with increased salinity (Kennedy & Fitch 1990).

A. crassus has not only spread in Europe and Norway (section 4.2), but worldwide and is known to infect six out of 15-20 eel species currently described (Lefebvre et al. 2012).

4.1.6   Parasites not found

Some parasite species previously recorded in European eel from Norway were not registered in the present study. The trematode *Azygia lucii,* vestode *Triaenophorus nodulosus,* nematode *Camallanus lactustris* and copepode *Ergasilus sieboldi* recorded by Mo and Sterud (1998) in eels caught on the east coast of Norway, were not registered at any of the localities examined here because their final hosts are not naturally present there. Pike (*Esox Lucius*) is final host to *A. lucii* and *T. nodulosus,* and pike has been introduced several places in the west but as far as known without these parasites. *Camallanus lacustris* primarily infects perch (*Perca fluviatilis*), also a species that has been spread to the west, but so far this parasite has not been found there. *Ergasilus sieboldin* infects a wide range of freshwater fishes, but the most important hosts are cyprinids (Kabata 1979). Which in Norway is only known from the eastern parts, Østfold and Akershus (Sterud 1999). *Trypansosoma granulosum* is a blood flagellate, transmitted by a freshwater leech, *Hemiclepsis marginata.* This leech occurs in eastern and southern Norway (Økland & Økland 2010). The eels from the present study were mostly examined frozen, so the occurrence of this flagellate was not studied.

Some microparasites such as *Ichthyobodo* spp., *Trichodina* spp. and *Gyrodactylus* spp. cannot or hardly be detected my microscopy in frozen fish, since they are destroyed. Therefore, infections with these may have been present unnoticed, except in the Etne localities where squash preparations of fresh gills were examined without detecting them. *Trichodina* sp. and *Gyrodactylus* sp. has previously been detected in Norwegian eels (see Table 1).

4.2 Parasite communities

The parasite communities in European eel in Norway are less diverse than in more southern parts of Europe. Important crustaceans such as copepods were not detected, or any of the freshwater acanthocephalans occurring in continental Europe (Jakob et al. 2016). Some eel specific parasites such as *Bucephalus anguillae* Spakulova, Macko, Berrilli et Dezfuli, 2002 and *Spinitectus inermis* (Zeder, 1800) also appears to be lacking, likely due to the absence of suitable intermediate hosts (Abdallah & Maamouri 2002, Saraiva et al. 2002).

A similar number of metazoan parasite species can be expected in a random freshwater eel individual (2.3-3.0) and a marine individual (2.2-3.4). A major difference is the number of myxosporeans (0.7-1.4 v.s. 0.3-0.5) will be expected since *Hoferellus gilsoni* and *Zschokkella stettinensis* never occur in seawater eels. However, the number of endohelminth individuals per eel is much higher in the sea, and there are several species. Contrary to in freshwater, most parasites are adult, suggesting that eels have a limited role in transmitting parasites to larger predators. In freshwater however, the situation is different, with perhaps half the parasites being larvae. This could be because glass eels, elvers and small yellow eels can be important prey for e.g. fish-eating birds such as merganers. A higher proportion of the freshwater parasite individuals (>77 %) are eel-specific, that in seawater (32 %). Still there are two marine parasites that exclusively (*Deropristis inflata*) or mainly (*Lecithichirium rufoviride*) infects eels. This remarkable fact is actually a strong indication that a significant proportion of the eels live in the sea. The difference between the freshwater and marine eels is particularly clear when comparing the gastrointestinal parasite communities. In Grimstad, only a single specimen of the capillariid *Pseudocapillaria tomentosa* was found in all the 30 eels examined. In marine eels from Etne and Smøla, 75-85 parasite individuals per eel can be expected. Overall, *Paraquimperia tenerrima* was the dominant parasite in freshwater, and *D. inflata* in the sea; both eel specialists.

4.3   Spread of exotic parasites

Two of the three exotic parasites previously registered in Norway were recorded in the present study. The swimbladder nematode, *A. crassus*, introduced in a Norwegian eel farm in 1993 (Mo & Steien 1994) and later registered in wild-caught eel (Engø 1997, Mo 2009) is believed to have spread further northwards from Imsa in Rogaland to Hordaland and Sogn og Fjordane (NBIC 2018a). The present study documents its presence in Grimstad (Fig. 45) but also suggests, whit the absence of the parasite in Etne, that it has not yet spread to the Hardanger area in western Norway. Still, further spread northwards seems likely.
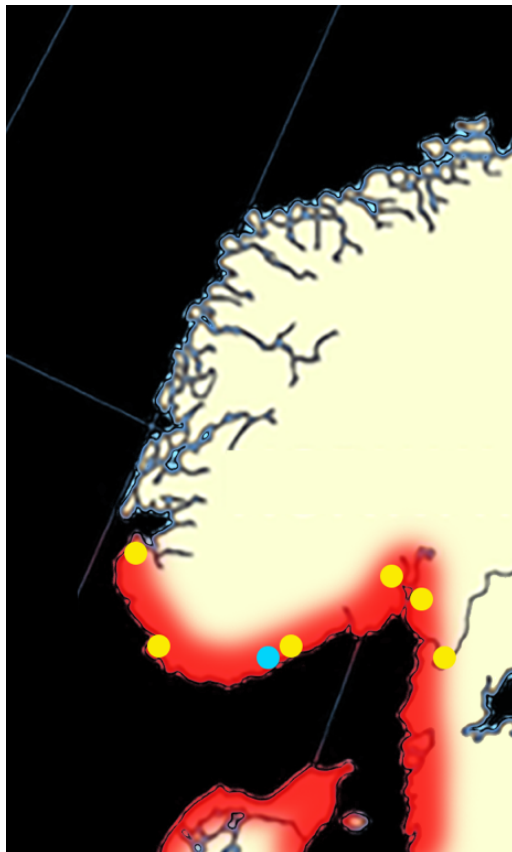

Figure 45 Known geographical range (red colour) of Anguillicola crassus, localities with previous observations (yellow dots) – (left to right) Imsa, Farsund, the river Storelva, Drammenelva , Enningdal selva and the Oslofjord, new observation (blue dot) – Grimstad.

Pseudodactylogyrus anguillae have since it was introduced in a Norwegian eel farm in 1987 (Mo et al. 1988) and later, in 1998 when it was registered in wild eels, spread along the east coast of Norway (Mo & Sterud 1998). The record from Grimstad in this study increases the previously known geographical range of *P. anguillae* further south (Fig. 46). Mo and Sterud (1998) also recorded the other *Pseudodactylogyrus* species, *P. bini*, which was not found in Grimstad. The chances that *P. anguillae* has spread further than *P. bini,* as the present study suggests, is high due to the fact that *P. anguillae* is able to reproduce at elevated salinities (Køie 1988c, Buchmann et al. 1992, Mo & Sterud 1998). *P. anguillae* also has an advantage in spreading when it comes to temperatures required for egg hatching. The eggs of *P. anguillae* needs fewer days to hatch at 10°C then *P. bini'*s eggs which do not develop or develop very slowly at lower temperatures. (Køie 1988c).
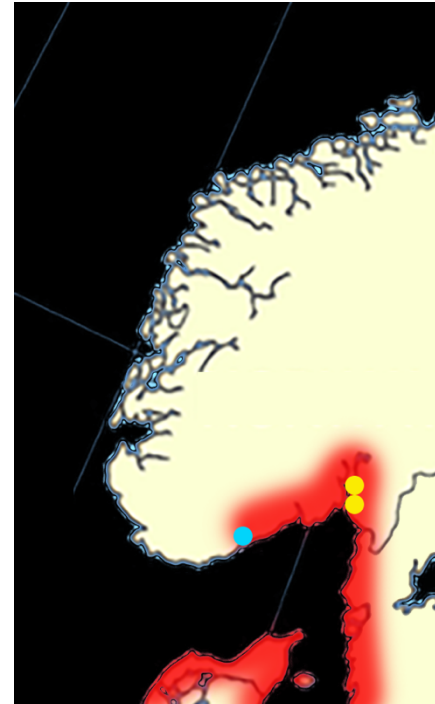
Figure 46 Known geographical range (red colour) of *Pseudodactylogyrus anguillae*, localities with previous observations - Årungen and Glomma (yellow dots), new observation - Grimstad (blue dot).

4.4 Parasites as biological indicators

Parasites can be used as biological indicators of the populations biology, the migration patterns, diet and phylogenetics of fish (Williams et al. 1992). Bailey et al. (1989) looked into freshwater parasites potential as biological indicators in fish caught in seawater, and found several that could suitable due to their long-time survival in marine conditions.

4.4.1 Parasites as inter-habitat shifting indicators

Out of the 34 parasite species recorded, 30 were considered indicator species. Only eight of them were found infecting fish from the opposite habitat of their origin. The freshwater myxosporeans *Paramyxidium branchialis, P. magi, Myxobolus* sp., the cestode *Proteocephalus microcephalus* and the nematode *Anguillicola crassus* were detected in seawater-caught eels. The three seawater species detected in freshwater-caught eels were the

trematode *Podocotyle atomon* and larvae of the nematodes *Anisakis simplex* and *Contracaecum rudolphii* A.

The myxosporean parasites infected 31 of the 32 (41 %) freshwater infected seawater-caught eels. These parasites infect fish through penetration of the body surfaces (skin/gills) by sporoplasms from actinospores. Hence only exposure to freshwater is necessary in order to become infected. Infections with such histozoic myxosporeans are likely long lasting (Margolis 1982), and they are therefore represent good indicators for a freshwater residency in some part of the life history, but does not indicate how recent.

P. branchialis was the most prevalent myxosporean. Based on the molecular data, the following life cycle seems likely: Eels in lakes are exposed to synactinomyxon actinospores released from the oligochaete *Tubifex tubifex*. As in other myxosporeans, the sporoplasm secondary cells eventually give rise to plasmodia at a final site, here the eel gill-filaments. The plasmodia then develop myxospores (Holzer et al. 2004, Kristmundsson & Helgason 2007). The prevalence of *P. branchialis* was high (>30 %) at all the localities, except Bjugn FW, and this could suggest that most eels at some point have spent time in freshwater. In reality the prevalence might be even higher, when considering that only a few lamellas of one gill were examined microscopically. This supports the hypotheses that all eels go into freshwater, even at higher latitudes as in Smøla SW.

The gastrointestinal freshwater parasites which usually live for maximum a year (Chubb 1982, Margolis 1982), could therefore be better indicators of recent inter-habitat shifting. However, the endohelminths found in the present study were limited with only two species registered, *P. macrocephalus* and *Anguillicola crassus*. These parasites should not occur in seawater resident eels unless these have sojourns to freshwater. Such a case may have occurred in Etne SW, where *P. macrocephalus* infections were seen in two eels. Both these eels were among five eels that based on fatty acid analysis were classified as 'brackish water' (BW). They were both caught near the river mouth of the river Etneelva and may have become infected during visits to the river. These parasites were registered in 4 % of the seawater-caught yellow eels and indicate due to their shorter longevity, more recent movement between freshwater and marine habitats.

Among the freshwater-caught eels, 17 (18 %) were infected with parasites of marine origin. Most of these were caught in Grimstad FW, which has seawater influx and is frequented by some marine fishes. This leaves 3 % freshwater-caught eels with parasites suggestive of inter-

habitat shifting at the remaining localities. All the marine species were gastrointestinal and acquired through prey. *Podocotyle atomon* infected eels from Bjugn have most likely recently been feeding in the river mouth due to the short longevity of such trematodes (months) (Margolis & Boyce 1969). The parasites *Anisakis simplex* and *Contracaecum rudolphii* A occurred as encapsulated larvae in the infected eels and the duration of these infections are likely years (Chenoweth et al. 1986, Hemmingsen et al. 1993). Only eels caught in Grimstad FW were infected with these clearly marine nematodes which in this study makes it difficult to use them for indication due to the saltwater input. *A. simplex* has been discovere

The lack of gastrointestinal parasites infecting eels caught in the opposite habitat of their origin and high prevalence of freshwater myxosporeans in both habitas are also seen in studies from Iceland (Kristmundsson & Helgason 2007) and Denmark (Køie 1988b). It seems like most seawater-caught eels have gone into freshwater as elvers, acquired an infection of myxospoeans which they carry their entire life, and very few re-enter freshwater later, given the low prevalence of gastrointestinal parasites.

The fatty acid analysis and otolith microchemistry analyses done on the sample from Etne SW did not always detect a time spent in freshwater as implied by the parasite observations. Freshwater myxosporean infections were seen in eels with a clear marine fatty acid signature, which is not surprising due to the longevity of the myxosporeans. The fatty acid signature has a relatively short temporal resolution, weeks to a year, in comparison to parasites and otolith microchemistry analyses. This makes it possible for an eel who has acquired a myxosporean infection several years previously to also have a clear marine fatty acid signature. The same goes for otolith microchemistry analyses, with freshwater myxosporean infected eels showing marine water resident (MWR) otolith microchemistry.

The challenge with many of the indicator parasites is the lack knowledge about the life cycles and the parasites longevity in the eels. More knowledge about this would increase the amount of information an indicator parasite can provide. The low prevalence of most of the observed parasites makes it hard to use them as indicators at higher than the individual level.

4.4.2   Parasites as diet indicators

Some of the marine eel parasites are good indicators of the hosts diet. These typically occur as larval stages in only one or a few related intermediate host species. *Deropristis inflata* infections are only acquired through feeding on certain nereid polychaetes common in sandy

bottoms in shallow waters. The acanthocephalan *Echinorynchus gadi*, the trematode *Podocotyle atomon* and the nematode *Paracuaria adunca* are all transmitted by amphipods. Indeed some individual eels had very high intensities with *P. adunca* and *E. gadi* while most were uninfected, suggestive of prey specialization (Knudsen et al. 2004).

5 Conclusion and future perspectives

This study provide evidence, through knowledge about the parasite's life cycles, that Norwegian eels migrate between fresh and seawater. Histozoic myxosporeans were the most prevalent indicator species of freshwater residency and could suggest that all eels enter freshwater and stay for a period, after which some re-enter the sea. The gastrointestinal parasites indicates more recent habitat shifting. A low prevalence indicate that the amount of recent inter-habitat shifting is low, <5 % a year.

Marine parasites were detected in freshwater-caught eels, but the prevalence was low when direct marine influence (seawater inflow) could be excluded.

The knowledge on eel parasite diversity in Norway has been significantly increased, with 21 additional species registered in European eel caught in Norwegian waters. A possible new species for science was also found. Exotic parasites were represented by *Pseudodactylogyrus anguillae* and *Anguillicola crassus*, but *Pseudodactylogyrus bini* were not recorded at any localities. The geographic range of *P. anguillae* was expanded to Landvikvannet in Grimstad, while the absence of *A. crassus* in Etne suggests that it has not yet reached Hordaland.

There was no good association between the parasite findings and the results from otolith microchemistry analyses and fatty acid analysis, due to the low prevalence of short lived indicator parasites.

The gill myxosporean *Paramyxidium giardi* seems to be an important indicator for inter-habitat shifting and knowledge about the time span of a *P. branchialis* infection is needed. Future studies should also look into other methods for detection of the parasite, e.g. molecular testing on gill-biopsis.

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Appendix

Table 1 Freshwater indicator parasites used in the evaluation of eel interhabitat shifting (IHS). The background data for this list is provided section 3.4.

Freshwater parasites	Source	Ref.
Paramyxidium branchialis (My)	Penetration	Holzer et al. (2004) *vide* Freeman and Kristmundsson (2018)
Paramyxidium magi (My)	Penetration	Holzer et al. (2004) *vide* Freeman and Kristmundsson (2018)
Myxobolus sp. (My)	Penetration	Present study, El-Mansy (1998)
Hoferellus gilsoni (My)	Penetration	Lom et al. (1986)
Zschokkella stettinensis (My)	Penetration	Present study
Pseudodactylogyrus anguillae (Mo)	Penetration	Buchmann et al. (1987)
Diplostomum spathaceum (T)	Penetration	Køie (1988b)
Proteocephalus macrocephalus (C)	Prey	Scholz (1999)
Bothriocephalus claviceps (C)	Prey	Scholz (1997)
Dibothriocephalus ditremus (C)	Prey	(Vik 1964)
Pseudocapillaria tomentosa (N)	Prey	Leis et al. (2016)
Eustrongylides sp. (N)	Prey	Anderson (2000)
Paraquimperis tenerrima (N)	Prey	Shears and Kennedy (2005)
Daniconema anguillae (N)	Penetration	Molnár and Moravec (1994)
Anguillicola crassus (N)	Prey	Lefebvre et al. (2012)

Table 2 Marine indicator parasites used in the evaluation of eel interhabitat shifting (IHS). The background data for this list is provided section 3.4.

Marine parasites	Source	Ref.
Hemiurus communis (T)	Prey	Køie (1995)
Brachyphallus crenatus (T)	Prey	Køie (1992)
Lecithochirium rufoviride (T)	Prey	Køie (1990)
Derogenes varicus (T)	Prey	Køie (1979)
Lecithaster gibbosus (T)	Prey	Køie (1989)
Podocotyle atomon (T)	Prey	Hunninen and Cable (1943)
Helicometra faciata (T)	Prey	Blend and Dronen (2015)
Deropristis inflata (T)	Prey	Køie (1988b)
Eubothrium sp. (C)	Prey	Saksvik et al. (2001)
Phyllobothrium squali (C)	Prey	Caira and Jensen (2014)
Cucullanellus minutus (N)	Prey	Køie (2001)
Hysterothylacium aduncum (N)	Prey	Køie (1993)
Anisakis simplex (N)	Prey	Højgaard (1998)
Contracaecum rudolphii A (N)	Prey	Szostakowska and Fagerholm (2007)
Contracaecum chubutensis (N)	Prey	Garbin et al. (2011)
Paracuaria adunca (N, L)	Prey	Anderson and Wong (1982)
Echinorhynchus gadi (A)	Prey	Marcogliese (1994)