Parasites Found in Pink Salmon (*Oncorhynchus gorbuscha*) Caught in the Feeding Areas in the Norwegian Sea

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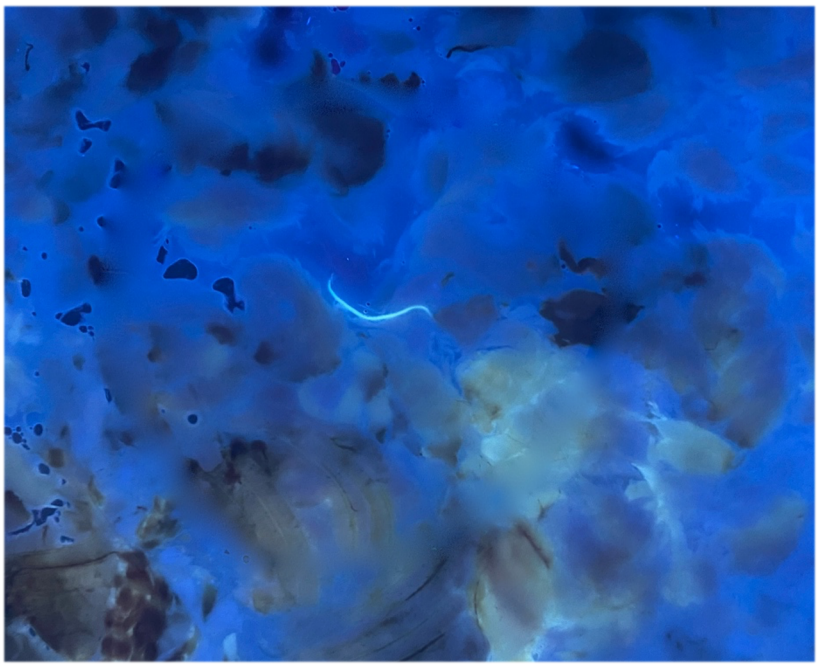

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http://bora.uib.no/

Front cover motive: The nematode *Anisakis simplex* under UV-light.

Abstract

The pink salmon (*Oncorhynchus gorbuscha*) is native to the North Pacific but has repeatedly been introduced to the North Atlantic by Russian researchers since 1956. This has resulted in the establishment of populations in north-western Russia and northern Norway. In 2017 pink salmon invaded at least 272 rivers along the whole Norwegian coast. This species, being alien to Norway, pose a potential risk to the native salmonids.

Parasites have the potential to be used as indicators for feeding ecology, migration patterns and place of origin. However, the pink salmon exhibit anorexia when entering rivers to spawn, causing parasite loss. This reduce the information gainable from the parasites and complicates the interpretation.

The aim of the present study was to investigate the parasite repertoire of pink salmon caught at sea, before parasite loss affects any patterns. Also, the occurrence of viral infections relevant to salmonid aquaculture was examined. A total of 86 pink salmon were caught in the Norwegian Sea by the Institute of Marine Research. The fish were examined for external and internal parasites and molecularly tested for microparasites and viruses.

The pink salmon were found to be infected with 13 marine parasite species and Piscine orthoreovirus-1 (PRV-1). The parasite fauna of ocean-caught pink salmon consisted of four trematode species (*Derogenes varicus*, *Brachyphallus crenatus*, *Hemiurus levinseni* and *Lecithaster gibbosus*), six cestode species (*Clestobothrium* sp., *Diphyllobothrium* sp., *D. schistochilos*, *Tetrabothrius* sp., *Clistobothrium* sp. (*Scolex pleuronectis* type A) and *Solex pleuronectis* type B, two nematode species (*Anisakis simplex* and *Hysterothylacium aduncum*) and one crustacean species (*Caligus elongatus*). This study is the first to report the cestodes *Tetrabothrius* sp., *D. schistochilos, Clestobothrium* sp. and the sea louse *C. elongatus* from pink salmon.

Most of the abundant parasites detected were food derived and could be contracted in feeding areas in the Norwegian Sea (*A. simplex*, *Clistobothrium* sp.), the Barents Sea or coastal shelves (*D. varicus*, *L. gibbosus*, *H. aduncum*) or particularly in Arctic waters (*Diphyllobothrium* spp.). Based on the finding of trematode parasites likely acquired in northern nursery areas (*B. crenatus*), it is suggested that most pink salmon originate in an area from Finnmark in Norway to north-western Russia down to the White Sea. It was also found that this indicator species, *B. crenatus*, was absent in fish over 1000 grams, thus a short parasite life span is likely responsible for its absence in river-caught pink salmon from western Norway.

The pink salmon harbour many of the same parasites as wild Atlantic salmon, suggesting a high degree of diet overlap. The viral agent detected (PRV-1) is of little concern, infections are common in both wild and farmed salmon and usually benign. The zoonotic nematode *A. simplex* was detected in the muscle of 23 % of the pink salmon. Freezing kills the parasite and is therefore recommended before consumption, removing any risk of the disease anisakidosis.

Acknowledgements

The present thesis on the parasite fauna of pink salmon concludes five years of integrated masters in aquamedicine at the Univeristy of Bergen (UiB) and is a collaborative study with the Institute of Marine Research (IMR). The work has been conducted at the Faculty of Mathematics and Natural Sciences (Institute of Biological Science) at the University of Bergen.

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Terms

Abundance	The number of individuals of a parasite species in/on a host, regardless of whether they are infected or not (Bush et al., 1997).
Arctic	The north polar region.
Arctoboreal	In Arctic and boreal regions.
Boreal	Of the north.
Cercariae	A tailed and motile larva of trematodes (Cribb et al., 2003).
Ectoparasite	A parasite living in the surface of a host (Cheng, 1986) e.g., skin, fins and gills.
Endoparasite	A parasite living within a host (Cheng, 1986).
Final host	The host where a parasite attain sexual maturity (Cheng, 1986).
Genital pore	The joint open ending of the male and female reproductive system (Gibson, 1996).
Helminths	Worms. Term used about members of certain invertebrate phyla, such as Platyhelminthes and Nematoda.
Intensity	Intensity of infection, meaning the number of individuals of a parasite species in a single infected host (Bush et al., 1997).
Intermediate host	If the host serves a temporary but essential environment for the completion of the parasite's life cycle (Cheng, 1986).
Juvenile	An immature organism.
Locality	A geographical location where a parasite is found (Bush et al., 1997).
Macroparasite	Parasites that do not multiply in/on their final host but instead produce eggs or larvae which is passed to the environment.
Mean abundance	The total number of individuals of a parasite species in a sample of hosts, divided by the total number of hosts (Bush et al., 1997).

Metacercariae	The stage following cercariae of trematodes, when the larva loses its tail (Cribb et al., 2003)
Microparasite	Small parasites which reproduce in/on its host.
Plerocercoid	The larval stages of cestodes in the second intermediate host.
Preacetabular	Before/anterior to the ventral sucker (acetabulum).
Presomatic pit	An invagination mid-ventrally in the posterior half of the forebody of the trematode *Brachyphallus crenatus.*
Prevalence	Number of hosts infected with one or more individuals of a parasite species (Bush et al., 1997).
Seminal vesicle	Vesicle containing spermatozoa in trematodes, ending in a genital pore (Gibson, 1996).
Site	The topological or spatial location in a host were a particular sample of parasites is found (Bush et al., 1997).
Sucker	Attachment organ of some animals. Digenean trematodes have oral- and ventral suckers. The oral sucker is positioned anterior of the animal at the mouth and the ventral sucker (also called acetabulum) is positioned posterior to the mouth (Gibson, 1996).
Transport/paratenic host	A host not needed for the development of the parasite but serves to maintain the life cycle of the parasite.
Viscera	The internal organs.
Vitellaria	Vitelline glands of trematodes with cells that form yolk and eggshell components. The vitellaria can be lobed or oval to globular, or dispersed in the tissues.
Zoonotic	When a parasite can be passed on from animals to humans and cause disease.

Abbreviations

Ab	Abundance, often used about mean abundance in this thesis
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
DNA	Deoxyribonucleic acid
x g	Relative centrifugal force
IMR	The Institute of Marine Research
ITS	Internal transcribed spacer
KW	Kruskal-Wallis test
L3	Larvae stage III (Nematoda)
LSU	Large Sub-Unit, a part of the ribosome containing protein and RNA (LSU rRNA). Ribosomal LSU RNA is also called 28S RNA in animals.
Lum	Luminal
Min	Minutes
mL	Microliter
MLR	Multiple linear regression
N	Number
NCBI	The National Center for Biotechnology Information
Nt	Nucleotides
p	P-value

Pl	Plerocercoid
PCR	Polymerase chain reaction
rDNA	Ribosomal DNA, DNA sequence coding for ribosomal RNA
rxn	Reaction
Saline	Saltwater diluted to about physiological salinity (10 ‰)
SD	Standard deviation
Sec	Seconds
Sensu	In the sense of
Sensu lato	In the broad (old) sense, used when a species has been split
Sensu stricto	In the strict (new) sense, used when a species has been split into several
SLR	Simple linear regression
SSU	Small Sub-Unit, a part of the ribosome containing protein and RNA (SSU rRNA). Ribosomal SSU RNA is also called 18S RNA in animals.
UiB	University of Bergen
µL	Microliters
µM	Micromolar
µm	Micrometres

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1 Introduction

1.1 Pink salmon

The pink salmon, *Oncorhynchus gorbuscha* (Walbaum, 1792), is the most abundant of the seven species of Pacific salmon (Heard, 1991). The species is naturally widespread in the North Pacific with spawning grounds on the Asian and North American continents (Groot & Margolis, 1991) (Fig. 1). The name pink salmon originates from the morphology changes both males and females undergo during maturation, giving them a pink colour on the flanks (Heard, 1991). Pink salmon is also called hunchback salmon, because of the hump on the back that characterizes a mature male. Immature males and females found in the open sea do however look identical to each other, having streamlined bodies with a silvery colour.

As most other salmonids, pink salmon are anadromous, meaning they spawn in freshwater and migrate out to the sea to live parts of their juvenile and premature life. The pink salmon has a two-year life cycle. The fry migrates quickly to the sea to grow, and the adults return to fresh-water the following year to spawn and die (Heard, 1991). The species is also known to have a rapid growth of body mass, allowing them to reach significant sizes in a short amount of time. This makes the species a good candidate for commercial fishing. There have been established several pink salmon hatcheries in the northern hemisphere to release fingerlings into the wild and increase the populations. This includes Prince William Sound and Kodiak Island in southeast Alaska, southern Alaska Peninsula (Hilborn & Eggers, 2000), Sakhalin, Kamtchatka and the Amur river in east Russia (Dushkina, 1994). There have also been many attempts to establish populations in the North Atlantic.

Russian researchers have introduced fry from pink salmon to its rivers in the northwest since 1956, in order to establish self-sustaining populations. Most of these attempts have been unsuccessful (Dushkina, 1994). Still, since 1960 there have been irregular observations of adult pink salmon along the Norwegian coast. After 2000, the frequency of these observations increased to happening annually, with a higher amount in odd years (Mo et al., 2018). In 2017 there was a big invasion of pink salmon in Norwegian waters, where at least 272 rivers were ascended (Mo et al., 2018). Due to lack of knowledge of pink salmon and its invasive nature it

is of great interest to Norway to map out any potential adverse effects they may have on our native salmonid species.

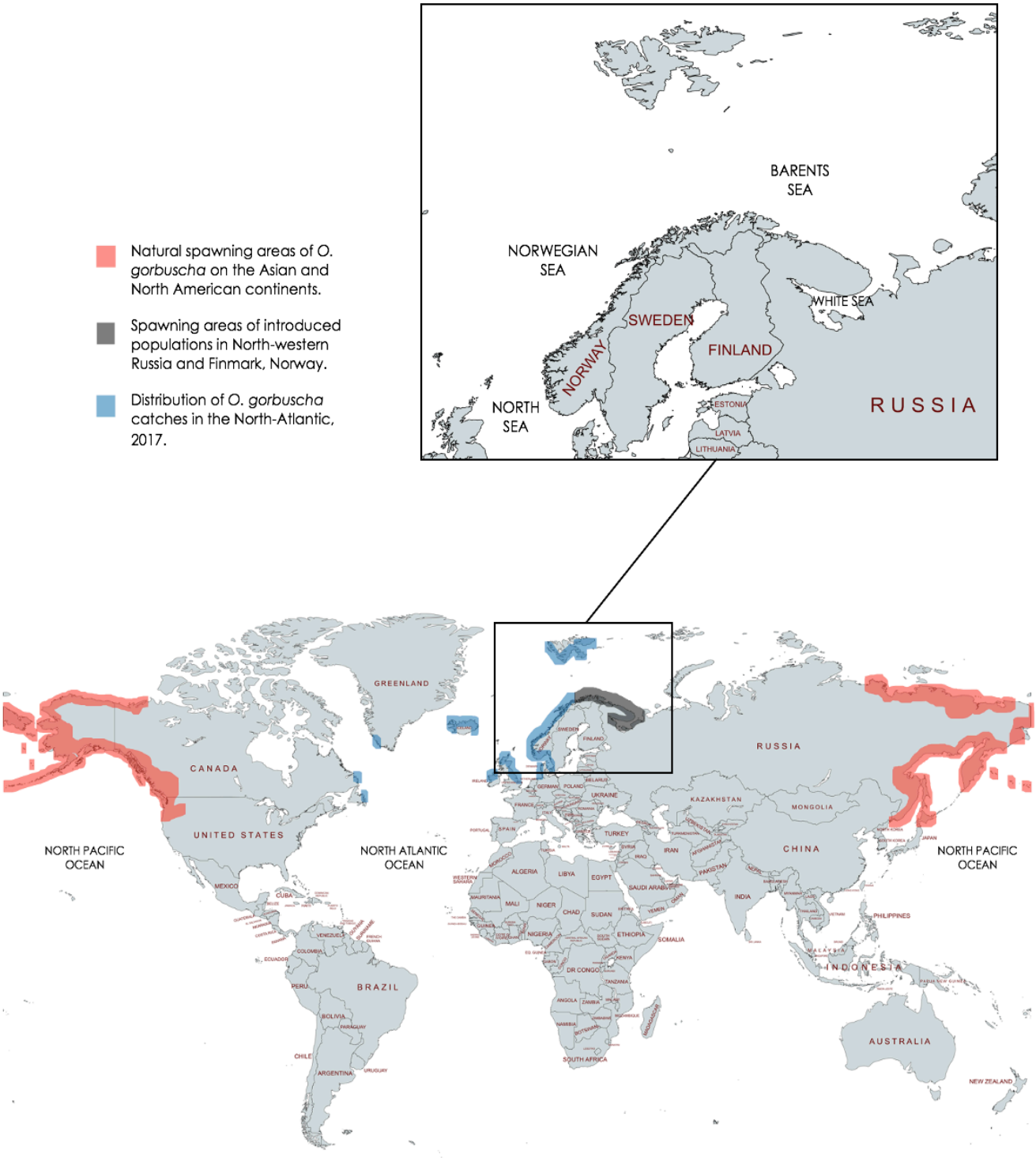

Figure 1 Map of the distribution of *Oncorhynchus gorbuscha* in the North Pacific and North Atlantic Ocean, including the invasion of northern Europe in 2017 (Dushkina, 1994; Heard, 1991; Sandlund, 2019). Template gathered from mapchart.net, colouring added by hand.

1.2 The fixed life cycle of pink salmon

Pink salmon has a fixed life cycle of two years (Fig. 2), meaning they spawn in odd or even years with no intermixing. This biannual life cycle has therefore given rise to two genetically different populations within single rivers (Gordeeva & Salmenkova, 2011; Heard, 1991). The spawning takes place from August to October in rivers on the Asian and North American continents, from Korea (37°N) to the river Lena in Siberia (73°N) and from the Sacramento river in California (38°N) to the northern tip of Alaska (71°N), respectively (Heard, 1991) (Fig. 1). When hatching at the riverbed late December to late February (Bonar et al., 1989), the alevins stay in the gravel for 41 to 64 days until the yolk sack is almost gone (Heard, 1991). When they are about 3 cm long, the now smoltified fry starts to emerge and quickly migrate downstream to the estuary and form schools. This downstream migration usually occurs during the spring months (Bonar et al., 1989; Heard, 1991).

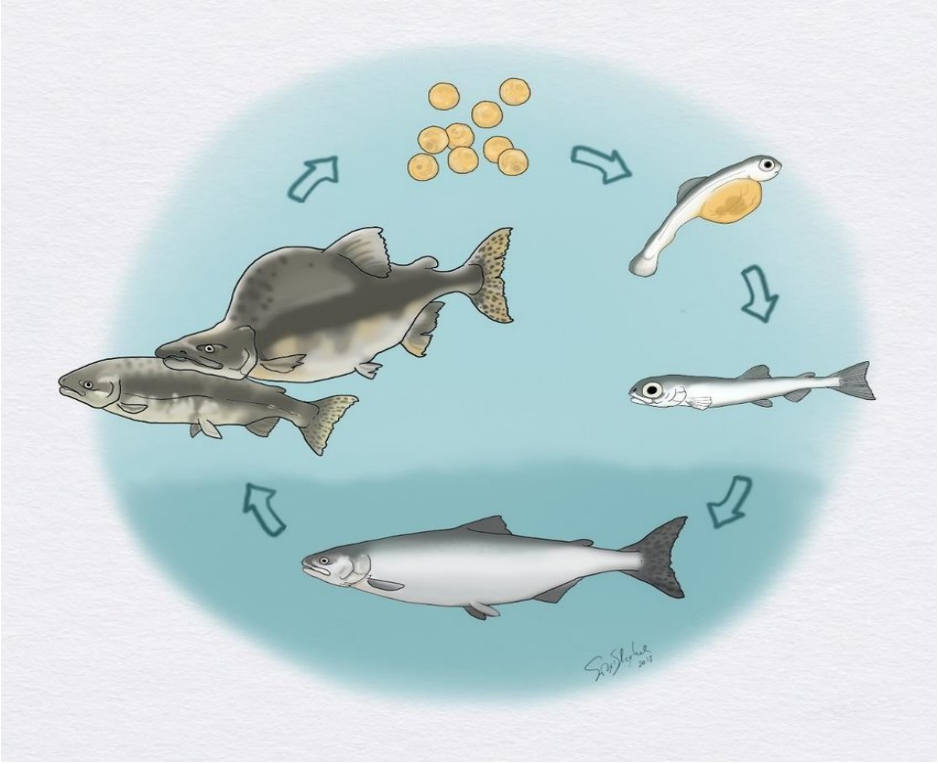

Figure 2 The two-year life cycle of pink salmon. Spawning takes place from August to October and the following year the eggs hatch into alevins. After using its yolk sack, the now smoltified fry migrates downstream to the estuaries and out to sea. They live their juvenile life in the ocean where they grow rapidly and return to the rivers to spawn after 18 months at sea. Illustration: Sigrid Skoglund (NINA).

Pink salmon spend on average less time in freshwater than other pacific salmon and show a preference for salt water already when emerging from the riverbed. When reaching the

estuaries, the pink salmon fry forms schools and usually stays (weeks, even months) to feed along the shoreline ("nursery areas"). They migrate out to sea when some 6-7 cm long (Heard, 1991). In the ocean-phase, schooling seems to be less structured. Catches and echogram images suggest that pink salmon schools consist of a few tens of fish and often only pairs (Heard, 1991).

The native feeding areas of pink salmon are found in the North Pacific, however, after the Russian insertion they are now also found in the North Atlantic (see below). During the ocean-phase the pink salmon grows rapidly and a study from the northern Bering and Chukchi Seas showed that their diet consisted of high-energy prey, like fish, squids, krill, larvaceans, crab megalopa and amphipods (Moss et al., 2009). The diet of pink salmon in the Norwegian Sea is unknown.

In the Pacific, pink salmon usually start to migrate back to the rivers from May to July, still being active feeders (Heard, 1991). In northern Norway, most pink salmon have reached the shore by late June or early July (Bjerknes & Vaag, 1980). Salmonids often return to their native river governed by biological mechanisms like memory imprint, a behaviour called homing. Pink salmon does not seem to have the same affiliation to its river as other salmonids and their homing behaviour is less extensive (Quinn, 1993). This likely reflects the short riverine residence of the pink salmon juveniles and may account for their rapid spread when being introduced to new habitats. Some pink salmon spawn closer to the sea, others far inland, which can affect homing degree by the time spent migrating downstream.

The arriving maturing pink salmon may stay at bays, estuaries and near streams for some time before migrating into the stream when water level rise or they reach sexual maturity. In the North Pacific, they have at this stage reached a weight of 2-4 kg and are about 45-55 cm in length (Bonar et al., 1989; Heard, 1991; Niemelä, 2016). In the North Atlantic, they typically weigh less, from 1.3 to 2.3 kg (Niemelä, 2016). The pink salmon undergo major morphological changes as a part of their sexual maturation (Fig. 3). Males develop marked secondary sexual characteristics, such as a hump on the back, a kype at the tip of the lower jaw and enlarged head and teeth. Females on the other hand undergo minor changes. Both genders undergo a thickening of the skin, absorption of scales and changes in colour. The colour darkens from a silvery appearance to a brown or greenish colour on the back and sides, often with a trace of pink colour on the flanks. Small, irregular black spots occur on the back, sides, dorsal and caudal fins (Heard, 1991). There is also atrophy of the alimentary canal and digestive organs, as a result of the energy reserves being used for maturation and that they stop feeding.

Figure 3 Sexually mature pink salmon caught in Etne river in July 2017. The male has prominent secondary sexual characters, as a hump on the back and a kype at the lower jaw. Its´ green colour with traces of pink and the black spots on the back is evident. The females' colour is not fully changed, and the silvery appearance is still dominant. Photo: Ingrid Otnes.

Males usually arrive to the spawning site first, but the females select redd site and prepares the nests. The spawning takes one to eight days, and the male dies shortly thereafter. The female, however, stays to defend the redd for almost two weeks after the egg deposition, before dying (Heard, 1991). Gordeeva & Salmenkova (2011) found that a female pink salmon in northwest Russia may lay 1677 to 1936 eggs.

1.3 Invasion of Norwegian rivers

There have been attempts to establish pink salmon populations in Russian rivers draining to the Barents and White seas in Kola Peninsula in northwest Russia since 1956. The motivation was to increase fish production (Dushkina, 1994). Eggs from wild pink salmon were collected mainly from the rivers in Sakhalin in eastern-Russia, then reared in hatcheries to be released into the rivers in north-western Russia. In the beginning, alevins were released soon after hatching, resulting in no adults returning. From 1959 hatcheries in Murmansk began to release fry between 0.2 and 0.7 grams, proving to be successful. The first year, more than 15 million fry were released and returning pink salmon were reported from the White, Barents, Kara and Norwegian seas, in addition to the coasts of Scotland, Greenland and Spitzbergen (Dushkina, 1994; Niemelä, 2016).

Pink salmon has been recorded along the Norwegian coast and in Norwegian rivers since 1960. In 1960 the species invaded about 40 rivers, primarily in northern Norway (Berg, 1961; Niemelä, 2016). In 1976 downstream migrant fry were observed in rivers in Finnmark, which proves that pink salmon had successfully spawned in Norwegian rivers (Bjerknes, 1977). Although pink salmon fry is not released anymore, populations have been established in northwest Russia and northern Norway. These can however not be considered stable populations. Historically the numbers of pink salmon in Norway have fluctuated, however lately there has been an appreciable trend upwards. There seems to be peaks in odd numbered years, with exceptionally large numbers of pink salmon recorded in 1965, 1973 (Bjerknes, 1977) and 2017 (Mo et al., 2018).

In 2017, many observations were made of adult pink salmon in rivers in northwestern Europe, including Norway. At least 272 Norwegian rivers were invaded, from the Russian border in the northeast to the Swedish border in the southeast, and 3400 pink salmon were caught as bycatch (Mo et al., 2018). In addition, numerous pink salmon were caught at sea. The reason for the mass invasion is uncertain but there may have been favorable conditions in the rivers and at sea for the salmon spawning in 2015. Warmer temperatures seem to increase the likelihood of natural breeding (Dushkina, 1994).

Since pink salmon is a non-native species to Norway, there is uncertainty of how it will affect the native salmonid species Atlantic salmon (*Salmo salar* L.), brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* (L.)). Atlantic salmon is of great economic importance to Norway, both as wild and farmed. The wild salmon is widespread throughout the country and is particularly vulnerable due to recent decline in abundance (Hindar et al., 2011).

The Atlantic salmon has a longer and less fixed life cycle than pink salmon. I Norway, it spawns in the autumn and the eggs hatch in the spring the following year (Mo et al., 2018). The Atlantic salmon often spends two to five years in the river before emigrating out to sea in the spring. Before this, the salmon undergo a smolting process that preadapt them for a life in saltwater. This process already starts in late summer or autumn the year before the emigration (Aas et al., 2011). When smoltified, the Atlantic salmon spends one to four years in the North Atlantic Ocean (Forseth et al., 2017; Mo et al., 2018). Catches of pink salmon in the Norwegian Sea indicates that the two species share feeding grounds and may compete for food resources.

A possible consequence of the pink salmon invasion of Norwegian rivers is the disturbance of spawning Atlantic salmon. Spawning pink salmon were observed in Norway from July to mid-September, often in the lower parts of the river, but some tens of kilometres upstream (Mo et al., 2018; Niemelä, 2016). Wild Atlantic salmon spawn in September to November far upstream and remain in the river they were spawned in for several years. Even though the spawning of pink salmon seems to occur earlier than that of Atlantic salmon, the Atlantic salmon starts the migration upstream many months ahead (Aas et al., 2011), which means the two species likely interact when in the river. The presence of pink salmon may cause negative consequences by occupying spawning sites and the males are known to behave aggressively when protecting the nests (Heard, 1991). Another theorized consequence is increased competition for food when in the rivers, though this is unlikely due to anorexia of mature pink salmon and the rapid emigration of fry, during which they not usually feed (Bonar et al., 1989; Heard, 1991).

Although pink salmon is not considered a major threat to Atlantic salmon (Forseth et al., 2017), there is a potential risk that they carry unwanted pathogens. Their migration to Norwegian rivers is potentially problematic, since the numerous fish farms in the Norwegian fjords provides conditions for proliferation of diseases (Forseth et al., 2017).

1.4 Parasites in pink salmon

Pink salmon are potential carriers of parasites, that can pose a threat to Atlantic salmon and other native salmonids. A parasite is often defined as an organism living in or on another organism, called the host, where it derive nutrients and cause some harm (Poulin, 1998) and hence in the broad sense includes viruses, parasitic bacteria, fungi and protists, and metazoan parasites. Some parasites cause little harm to the host, while others affect the host greatly some even leading to death. Parasites which live within the body of the host is called endoparasites, while parasites attached to the outer surfaces of the host is called ectoparasites (Cheng, 1986).

The parasite fauna of the introduced pink salmon is of interest for several reasons. As previously mentioned, the species could carry and spread disease agents, not least through rapid migration and poor homing. This could be a threat to both wild salmon stocks and to salmonid farms. Also, if irreversibly established in the North-East Atlantic, pink salmon may be subjected to commercial fishing as another introduced species, the red king crab *Paralithodes camtschaticus* (Tilesius, 1815) (Jørgensen & Nilssen, 2011). If a fishery develops, then food safety aspects needs to be examined. Zoonotic parasites occur in other fish in the area, including the related

salmonids (Bristow & Berland, 1991; Bristow et al., 1996; Holst et al., 1993; Mo et al., 2021). There have been conducted several studies on parasite fauna of pink salmon populations from the North Pacific at various stages of its life cycle (Boyce, 1969; Margolis, 1956). However, when introduced to the Atlantic very few (if any) parasites followed the eyed eggs from the Pacific. Therefore, the parasite fauna developed by the pink salmon is interesting, regarding which types of parasites can exploit the novel host, and what these parasites tell us about the poorly known oceanic phase.

Some studies have been conducted in rivers draining to the White and Barents seas (Barskaya et al., 2005; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963), as well as to the North Sea (Fjær, 2019), with findings of marine and freshwater parasites (Table 1). The most common metazoan parasites found were the trematodes *Derogenes varicus* (Müller, 1784), *Brachyphallus crenatus* (Rudolphi, 1802) and *Lecithaster gibbosus* (Rudolphi, 1802), cestode larvae such as *Scolex pleuronectis* (Müller, 1788), and the nematodes *Anisakis simplex* (Rudolphi, 1809) and *Hysterothylacium aduncum* (Rudolphi, 1802).

However, there were notable and interesting differences between the parasite fauna of pink salmon in the west-Norwegian rivers compared to those from northwestern Russian rivers. Few parasites of freshwater origin have ever been found (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963), most are marine. This likely reflects the brief freshwater residence of the juveniles. In the sea, the pink salmon may become infected with coastal parasites in the estuarine nursery areas, before emigrating to the high oceans where parasite types with oceanic life cycles are acquired. The coastal life cycles involve alternate hosts such as snails and polychaetes, limiting the spread from the coast to shallow or shelf-areas. Oceanic parasites may mature in fish, marine mammals and birds, and involve zooplankton and fish as intermediate hosts. Such parasites, e.g. *Anisakis simplex*, may infect fish feeding over large depths in the mid of the Norwegian Sea (Tolonen & Karlsbakk, 2003).

Additionally, when migrating to the coast while maturing, pink salmon may again be exposed to coastal parasites. Fjær (2019) discovered that pink salmon caught in west-Norwegian rivers had become infected with the myxosporean *Parvicapsula pseudobranchicola* Karlsbakk, Sæther, Høstlund, Fjellsøy & Nylund, 2002 and with metacercaria larvae of the trematode *Cryptocotyle lingua* (Creplin 1825). The former is likely acquired through the skin or gills from water borne spores released from some annelid (Nylund et al., 2018), while the latter parasite has free-swimming cercaria-larvae released from littoral snails acting as first intermediate host.

The cercariae actively penetrate the skin of fish (Sindermann & Farrin, 1962; Stunkard, 1930). It is not known if these parasites were acquired as juveniles, or when migrating towards the rivers as adults.

Fjær (2019) also found several gastrointestinal trematodes, including *Hemiurus communis* Odhner 1905, *H. luehei* Odhner 1905 and *Brachyphallus crenatus*. The trematodes *H. communis* and *H. luehei* are common at the Atlantic coast of Europe, including Norway, but are not present or common in the White and Barents Sea (Køie, 1990, 1995). *H. communis* is particularly abundant at the coast of Norway but not north of Lofoten (Hemmingsen & Mackenzie, 2013), while *H. luehei* can penetrate as far north as Finnmark in juvenile herring (Karlsbakk et al., 2000). The trematode *Brachyphallus crenatus*, however, has an Arctoboreal distribution, and has been reported from northern Norway, the White and Barents Sea (Køie, 1992), being rare in western Norway. Another species, *Hemiurus levinseni* Odhner, 1905, which was found in the Keret River (Barskaya et al., 2005) but not at all by Fjær (2019), is a northern species (Krupenko et al., 2020). These trematodes can therefore be used as biological indicators, reflecting past as well as recent feeding areas (MacKenzie, 2002; Margolis, 1982a, 1982b).

It is now well established that parasites can be used as biological indicators of, among others, populations, migration, habitat and diet of fish (Williams et al., 1992). The presence and abundance of a parasite species can tell us something about the pink salmon's origin and migration, based on the known distribution of the parasite. When examining river-caught pink salmon in western Norway, Fjær (2019) found a lack of a relationship of parasite abundance with size or sex of the fish. However, she found a negative correlation between time spent in the river and parasite abundance, interpreted as evidence of parasite loss. The underlying mechanism for this is thought to be anorexia and the following atrophy of the digestive system. Therefore, Fjær (2019) could not fully utilize the parasites as biological indicators.

Key phases in the life history of pink salmon in the North Atlantic can be reflected in the parasite profile of juveniles caught in the feeding areas. Acquiring and studying this parasite profile is the focal point of this study. A selection of pink salmon caught in the Norwegian Sea was examined to achieve this. Besides from a few young sea-caught pink salmon examined by Grozdilova (1974) (*n=35*), no such studies of the parasite fauna of pink salmon from the oceanic feeding areas have previously been conducted.

Table 1 Overview of previous studies on the parasite fauna of pink salmon (North-Atlantic populations), with prevalence and abundance. With the exception of 35 fish (Grozdilova, 1974), all were river-caught returnees.

Locality	**Murmansk**		**White Sea**[a]		**Keret river**		**Ekso, Etne and Guddal rivers**	
Year (n)	1961 (25)		1964, 1965 (68)		1993, 2003 (30)		2017 (80)	
Reference	Ninburg, 1963		Grozdilova, 1974		Barskaya et al., 2005; Ieshko et al., 2016		Fjær, 2019	
Parasite species	P (%)	Ab[b]	P (%)	Ab[b]	P (%)[c]	Ab[c]	P (%)	Ab
Microsporidia								
Microsporidia gen. sp.	0	-	47	-	-	-	-	-
Acantocephala								
Echinorhynchus gadi	0	-	9	0.3	3	-	0	-
Monogenea								
Discocotyle sagittata	0	-	3	<0.1	-	-	0	-
Gyrodactyloides bychowskii	-	-	12	0.8	-	-	-	-
Trematoda								
Cryptocotyle lingua	0	-	0	-	0	-	63	130.8
Digenea gen. sp.	88	74.69	-	-	-	-	-	-
Derogenes varicus	68	17.0	74	39.4	70	5.3	89	32.2
Brachyphallus crenatus	52	7.6	71	24.7	67	6.0	1	0.03
Hemiurus levinseni	0	-	0	-	13	0.2	0	-
Hemiurus luehei	0	-	0	-	0	-	3	0.03
Hemiurus communis	0	-	0	-	0	-	4	1.1
Lecithaster gibbosus	67	141.2	82	42.9	70	45.3	70	20.7
Apatemon gracilis	0	-	0	-	0	-	1	0.01
Podocotyle reflexa	20	0.4	26	22.5	20	0.25	-	-
Ichthyocotylurus erraticus	4[d]	1.2[d]	0	-	10	0.15	-	-
Diplostomum spp.	4	<0.1	12	0.9	17	0.5	0	-
Cestoda								
Eubothrium crassum	24	2.4	28	1.3	53	4.4	34	3.2
Diphyllobothium sp.	20[e]	0.3	68	22.8	67[f]	3.4[f]	15	0.2
Scolex bothriosimplex	0	-	0	-	0	-	6	0.1
Scolex pleuronectis	>80	>32.9	93	552.8	80	45.9	78	16.7
Nematoda								
Anisakis simplex	0	-	99	11.2	33	0.9	25	0.8
Pseudoterranova sp.	0	-	0	-	17	0.67	0	-
Hysterothylacium aduncum	>84	21.1	43	1.1	63	3.7	83	6.2
Crustacea								
Ergasilus sieboldi	0	-	-	-	3	0.07	-	-
Lepeophtheirus salmonis	+	+	2,9[g]	-	0	-	3	0.03
Salmincola salmoneus	92	20.2	22	1.7	0	-	3	0.6
Unionidae glochidiae	0	-	9	0.1	0	-	0	-

P – prevalence, Ab – abundance, pl – plerocercoid.

[a] Sea- and river-caught pink salmon, off the coast of Karelia and Tersky district and in the rivers Umba and Kereti, Russia.
[b] Average abundance calculated from prevalence- and intensity data. [c] Prevalence and abundance merged for the years 1993 and 2003. [d] Reported as *Tetracotyle* sp. [e] Reported as Plerocercoid B. [f] Reported as Cestoda l. gen sp. in 2003. [g] Reported as Caligidae larvae, assumed being salmon lice.

1.6 Aim

This study aims to characterize the parasite communities in open ocean caught pink salmon, specifically:

I. Does the pink salmon carry viral agents representing a threat to native salmonids?
II. Is there evidence for spatial differences in parasite load, based on capture locations?
III. Which parasites increase in abundance with fish mass? (i.e., oceanic transmission)
IV. Which parasites show stable or declining abundance during the oceanic phase? (i.e., of nursery area origin)
V. Can the parasites indicate where the fish originate? (e.g., Arctic vs. boreal or southern parasite species)
VI. What do these parasites tell about past and present diet, and diet overlap with Atlantic salmon?

2 Materials and Methods

2.1 Sampling

A total of 89 pink salmon, *Oncorhynchus gorbuscha*, were provided by the Institute of Marine Research (IMR). The pink salmon were sampled by trawling in the Norwegian Sea and adjoining parts of the Barents Sea by the vessels "G.O. Sars" and "Johan Hjort", from 63.04°N to 74.32°N and 4.16°W to 23.32°E, from the year 2013 to the year 2019 (Fig. 4). The majority of the salmon were caught in May and June (*n=77*) and the rest in December (*n=12*, all these 2018). The number of fish caught at the same location (single catch) ranged from 1 to 10 individuals. Individual fish data are listed in Appendix table 1.

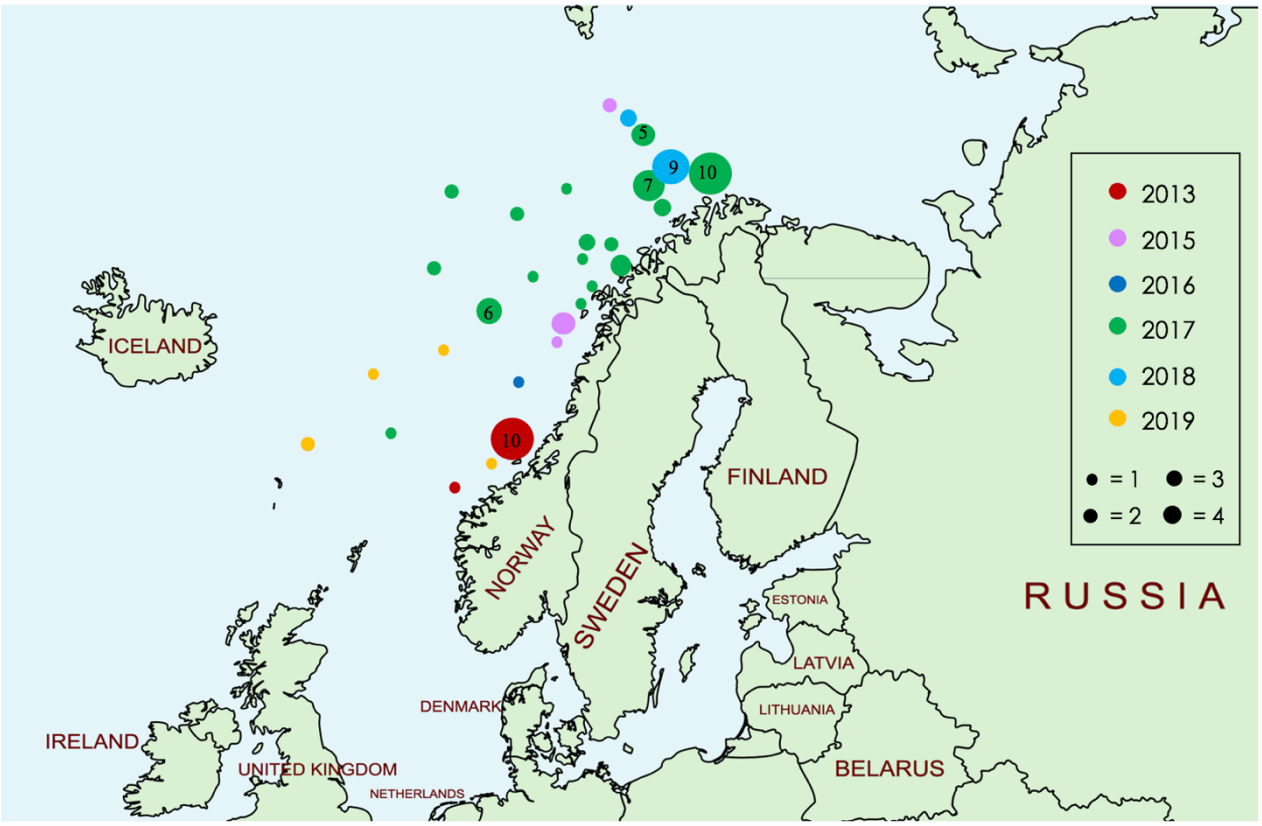

Figure 4 Map of sampling sites of pink salmon in the Norwegian Sea and adjoining parts of the Barents Sea. Increasing size of circles indicates a larger number of fish sampled (1-10 individuals). Each year in colour. Template reproduced from mapchart.net.

The sampling was conducted during ecosystem surveys in the Norwegian Sea, using the Multpelt 832 pelagic trawl. The usual towing speed was 3.3 to 4.7 knot with a tow duration of roughly 30 minutes. The trawl opening was minimum 35 meters (often 45 to 50 meters) and the trawling depth was 0 to 50 meters. At sea, the fish were deep frozen whole in plastic bags with haul and station information. Due to the trawling, the pink salmon had extensive scale loss as seen in figure 5. An overall view of the number of fish, gender and size sampled each year is given in Table 2.

Table 2 Overview of the pink salmon samples collected in the Norwegian Sea 2013-2019, by gender, total length and weight of the fish examined each year. SD = standard deviation, min = minimum and max = maximum.

Year	Sex	n	Length (mm)				Weight (g)			
			Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
2013	♀	6	392	21	373	429	659	142	448	860
	♂	5	407	22	386	448	732	125	598	914
2015	♀	3	426	11	418	441	764	73	662	826
	♂	5	443	32	385	480	918	245	549	1313
2017	♀	28	430	3	341	491	963	291	443	1532
	♂	22	460	38	406	527	1247	460	705	2290
2018	♀	3	256	21	232	284	113	27	83	148
	♂	9	245	36	187	307	114	53	37	210
2019	♀	3	411	9	404	423	690	34	645	726
	♂	2	412	9	403	420	689	121	568	810

Individuals determined to be Atlantic salmon not included (see below).

Figure 5 Pink salmon sampled in June 2017, showing extensive scale loss and haemorrhagic bleeding around the abdomen. Photo: Erling Kåre Stenevik (IMR).

2.2 Study area

Most pink salmon were caught in the Norwegian Sea, but some were from the adjoining western parts of the Barents Sea. The Norwegian Sea is situated right off the coast of Norway and borders to the North Sea in the south and Barents Sea in the north. The Norwegian Sea is a deep sea with an average depth of 1800 meters (Skjoldal et al., 2004). The Barents Sea, on the other hand, is a shallow shelf sea with an average depth of 220 meters (Jakobsen & Ozhigin, 2011).

The Norwegian Sea ecosystem is dominated by marine animals such as macrozooplankton (e.g., copepods and krill) and mesopelagic fish, and is an important feeding area of herring, mackerel, Atlantic salmon, whales and seals (Skjoldal et al., 2004). The new habitant pink salmon spends its oceanic phase in feeding areas in the North Atlantic Ocean, including the Barents- and Norwegian Sea, much like the Atlantic salmon.

2.3 Fish identification

After thawing and inspection, the presumed pink salmon (*n=89*) were morphologically identified by body shape, pigment spots, jaw length, colour of tongue, anal fin rays, scale size, gonad size and otolith shape. The morphological attributes are explained in Table 3. Three individuals were determined to be Atlantic salmon, the one caught in 2016 and two caught in 2017 (see Appendix table 1).

Table 3 Morphological attributes used in fish identification, to separate pink salmon from Atlantic salmon.

Trait	Pink salmon	Atlantic salmon	Comment
Body shape	High weight and more rounded body, in relations to the length	Longer and thinner	
Pigment spots	Large, black spots on the back, dorsal fin and tail	None	Rarely of use*
Jaw length	Upper jaw usually extends past eye	Upper jaw does not extend past eye	
Colour of tongue	Black	Pink	
Anal fin rays	> 12 anal fin rays, often 14-16	< 12 anal fin rays	
Scale size	Very small	Big	
Gonad size	Large gonads relative to size	Small gonads relative to size	
Otolith shape	Oval, oblong	Round to oval	

* More apparent towards sexual maturation.

2.4 Dissection

All 86 pink salmon, as well as the three determined to be Atlantic salmon, were dissected and examined for parasites at the parasitology laboratory at BIO, University of Bergen (UiB). Being a unique material, the fish were subject to extensive sampling besides the parasite analyses. These additional samples taken for researches at IMR are described below (section 2.7). Furthermore, five mature pink salmon caught in Etne river in 2017 were dissected for training purposes and some of the rarer parasites found were kept and used in DNA studies.

The total length and weight of the fish were measured while the fish was still frozen. This was because not all individuals had been measured and weighed at sea, due to ambiguous species identification. For the parasite examination the fish had to thaw for up to two hours, depending on its size. During this process the fish were examined externally for abnormalities, external parasites such as sea lice and other externally detectable parasites such as the metacercaria larvae of the trematode *Cryptocotyle lingua* that causes black spots on the skin. For this purpose,

all fins were removed and carefully examined in water under a dissection microscope (*Wild*) lit from both above and below (candling) at 6.4, 16 and 40x magnification.

For the small fish the digestive tract was prioritized for parasite examination, due to the enzymatic degradation of parasite DNA that can occur after thawing. For larger fish the head thawed before the rest of the body and could be examined first. The mouth, tongue and gill cavity of the fish were examined for macroparasites. The operculum was removed to access the left pseudobranch and the gills. A piece was cut out of the pseudobranch and the middle part of the first gill using sterile equipment and placed in microcentrifuge tubes with 96 % ethanol (for subsequent PCR testing of the myxozoan parasite *Parvicapsula pseudobranchicola* and euglenozoan parasite *Ichthyobodo* spp.). The gills were then removed and placed in a glass Petri dish with water to be examined in a dissecting microscope. Each gill was examined thoroughly by stroking through the filaments with a pair of tweezers.

The eyes were then removed and placed in a glass Petri dish with physiological saline (diluted seawater, ~10‰, hereafter referred to as saline). The cornea, lens, corpus vitreum and the muscle of the eye were separated, and a squash preparation was made by squeezing the components with some saline with glass Petri dishes as illustrated in Fig. 6. The squash was then examined in a dissecting microscope with light from below. This Petri-dish glass plate method was also used for other soft tissues (below).

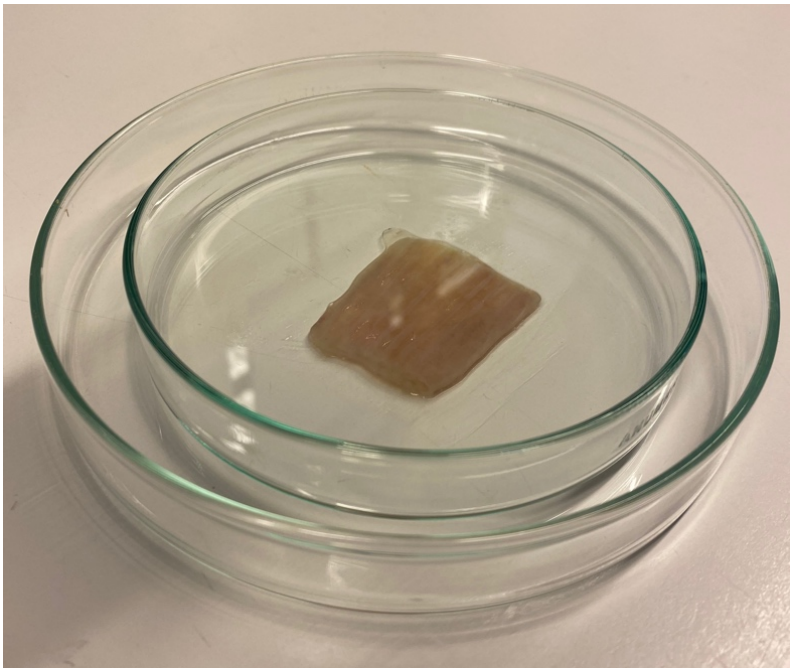

Figure 6 A squeeze preparation of the oesophageal part of the stomach wall of pink salmon, made by placing a Petri dish on top of the tissue and pressing down.

When the fish was sufficiently thawed the abdomen was cut open from the pericardial cavity to just before the anus. The organs were then removed and placed in separate Petri dishes with saline and stored cold (0-4°C). The gender was recorded, and the gonads weighed. The gonads were examined externally for visible parasites and then squashed in saline using glass Petri dishes and candled in a dissection microscope for internal parasites. The heart, liver, spleen and swim bladder were examined following same procedure.

The alimentary canal was divided into the pharynx, oesophagus, stomach (subdivided into an oesophageal part, middle 'posterior' part and a pyloric part), the anteriorintestine with pyloric caeca, mid gut (the rest of the thin limb) and hind gut including the short anal part with the sphincter muscle (Fig. 7). Each part was examined externally for encapsulated parasites and then cut open, and any visible parasites was removed and stored in saline. The mucosa was then scraped off using a scalpel, and both the alimentary wall and the mucosa was pressed with glass Petri dishes and examined in the dissection microscope, to detect any further parasites. Since the stomach contents were to be used for diet analyses at IMR (see below), the stomach was handled first. The prey was quickly separated from any parasites and immediately refrozen in zipped plastic bags.

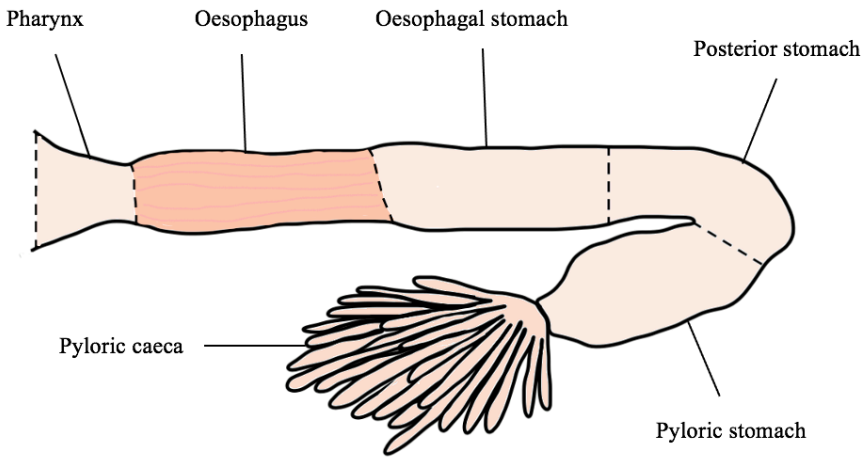

Figure 7 The division of the alimentary canal, from the pharynx to the anteriorintestine with pyloric caeca.

Finally, the plastic bag containing the fish was rinsed and the contents examined for detached parasites or any escaped worms from pharynx or anus. The remaining fish body was placed back into its plastic bag and stored at -18°C, to preserve the filet for later parasite analyses using the UV-press method.

The parasites found were washed, identified and sorted. They were studied in light microscopes (*Olympus cx 41* or *ZEISS Axio Scope A1*) at 40, 50, 100 and 400x magnification when necessary, and digital images were taken of all individuals with any ambiguity in identification or staging, using digital cameras fitted to these microscopes (*Olympus SC 30* and *ZEISS Axiocam*, respectively). Images were also taken of specimens kept for DNA analyses or for the potential deposition in museum collections as voucher specimens. Gender and maturity of the parasites were determined if possible. The trematodes were scored as oviferous if containing eggs. The gastrointestinal nematodes were staged to larvae (stage III) and preadult/adult (stages IV and V) based on morphological traits. The parasites were stored in CryoTubes™ with 96 % ethanol for further testing to determine the species.

2.5 UV-press method

The frozen fish filet was examined for nematodes (e.g. *Anisakis simplex* and *Pseudoterranova* sp.) using the UV-press method (Karl & Leinemann, 1993) at IMR. This method involves pressing fish filets to about 2 mm thickness using a hydraulic press and examining it under UV-light. Frozen anisakidae nematodes will fluoresce when illuminated with UV-light, making them observable to the naked eye.

The fish were thawed overnight and handled the following day. The body was divided vertically on each side of the spine, resulting in two filets (left and right side). The filets were placed in separate vacuum bags of an appropriate size (400 x 900 mm), placed in the hydraulic press (Fig. 8) and pressed flat at 7-8 bar.

Figure 8 Image of the hydraulic press used to press fish filets in vacuum bags.

To achieve proper fluorescence the anisakidae larvae need to be sufficiently frozen. The freezing process can be done before or after flat pressing. In this study, the fish were stored frozen, meaning they were ready to be immediately observed under UV-light using appropriate eye protection due to the 366 nm of UV-light used for this process. To expose any hidden nematodes that might be difficult to detect due to them being in crevices of the flesh, a pen was used on top of the vacuum bag in a stroking motion. Each filet was divided in four sections: dorsal anterior, dorsal posterior, ventral anterior (belly flap) and ventral posterior (Fig. 9). Nematodes found in the flat pressed filet were named after the section they were found in. Finally, the nematodes were dissected/removed from the flat filet using a scalpel and studied in a light microscope to determine the species.

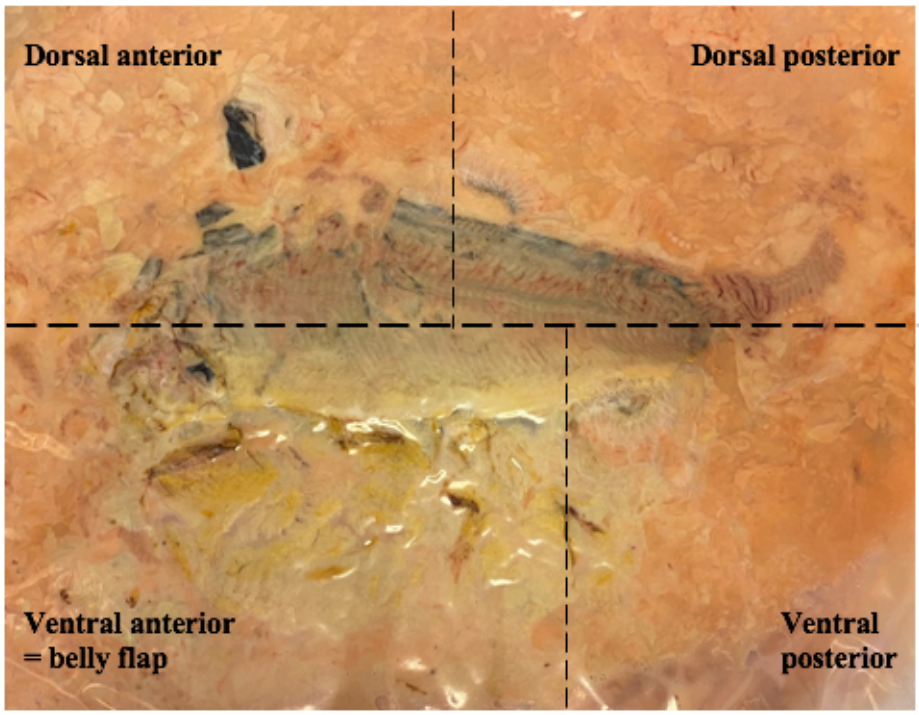

Figure 9 Flat pressed pink salmon filet in vacuum bag, divided in the sections dorsal anterior, dorsal posterior, ventral anterior and ventral posterior.

2.6 Virus analysis

While the fish was still frozen, the head was separated from the body using a hacksaw. The cut to sever the head was placed between the operculum and pectoral fins (Fig. 10). The transverse section reveals the anterior kidney and the pericardial cavity with the heart. With a sterile scalpel, the frozen cut-surface of these organs were cut away, revealing a surface that had not been in contact with the saw. Samples of the deep-frozen anterior kidney and heart was then

collected in a microtube. A sample from the first gill arch on the left gill was also collected. These frozen (-18°C) samples were taken for qPCR analyses at IMR (i.e., virus testing). The equipment used was wiped clean, dipped in ethanol and flame sterilised.

RNA was extracted from kidney, gill and heart tissue and analyzed by Pharmaq Analytic Bergen (https://pharmaq.com/en/analytiq) with their in-house RT-qPCR assays. The samples were tested for the viral agents Infectious hematopoietic necrosis virus (IHNV), Infectious salmon anaemia virus (ISAV), Infectious pancreatic necrosis virus (IPNV), Salmonid alphavirus (SAV), Piscine orthoreovirus-1 (PRV-1) and Piscine myocarditis virus (PMCV).

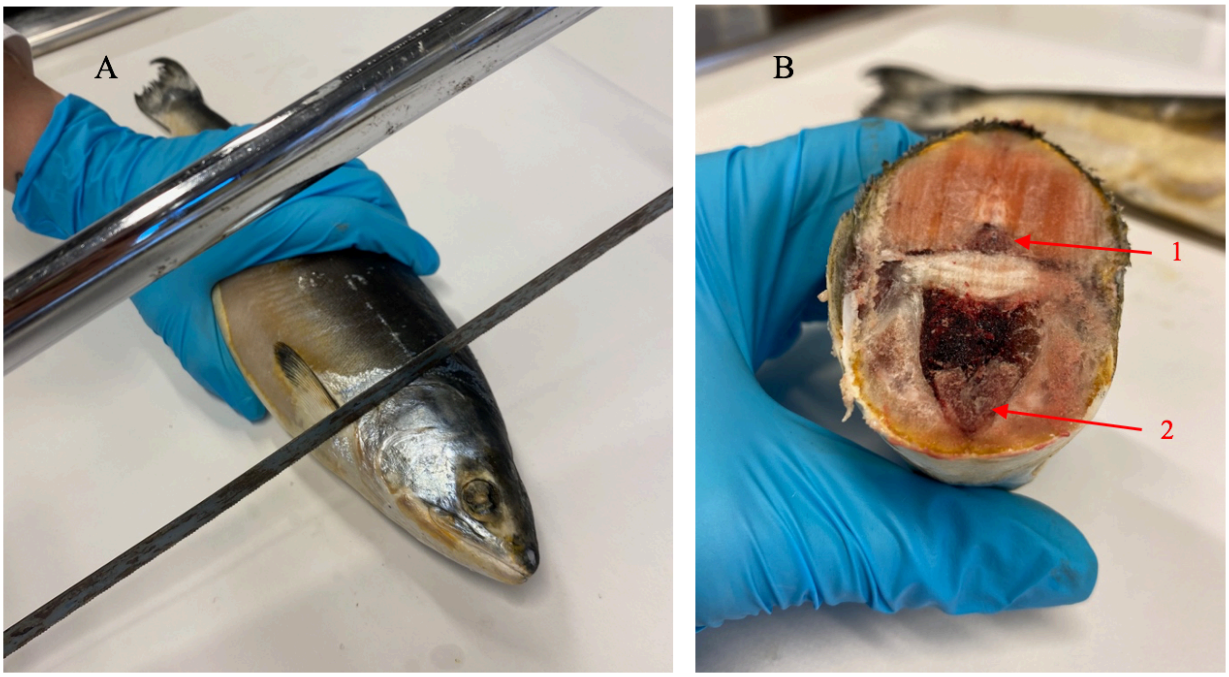

Figure 10 A Demonstration of saw-placement to sever the head, **B** sites were samples of the kidney[1] and heart[2] were obtained, shown by arrows.

2.7 Additional samples taken for IMR researchers

The otoliths were collected for microchemistry analyses. The top of the skull of the fish was removed with a knife to expose the brain and the otoliths laying beneath. The otoliths were washed with distilled water and placed in a CryoTube™ to dry. The brain itself was not examined. A sample from the dorsal fin was taken to a CryoTube™ with 96 % ethanol for later DNA extraction (genetics sample). Muscle tissue was taken from the dorsal part of the fish right in front of the dorsal fin, one cm deep and two to three cm long, and placed in a 6 mL tube and stored at -18°C (Meier et al., 2006; Skilbrei et al., 2015). Fish scales were also removed and

placed in an envelope provided by the IMR for further testing. Scale samples were taken above the lateral line between the position of the dorsal and anal fin if possible.

2.8 Extraction and purification of nucleic acids

DNA was extracted from parasites and tissue samples using the E.Z.N.A.® Tissue DNA kit (*Omega Bio-Tek*). The tissue samples were cut into an appropriate size of 20-30 mg and the parasites were cut in half to speed up lysis, using sterile equipment. The samples were dried (removing ethanol) in tubes by placing them in a heating cabinet at 60°C. Thereafter, the protocol of the tissue DNA kit was followed. A volume of 200 µL TL-buffer and 25 µL of the enzyme solution Proteinase K was added to the dried samples and mixed thoroughly by a vortex mixer and spun down. They were then placed in a shaking heating block at 55°C, for two hours or overnight until lysis was completed.

The lysates were then centrifuged (5 min; 13.000 x g) and the supernatant was transferred to a new, sterile microcentrifuge tube and 220 µL BL-buffer added. The solution was vortexed and spun down, before it was incubated at 70°C in a heating block for 10 minutes. Thereafter, 220 µL absolute ethanol was added to the sample and mixed by pipetting manually. The whole sample was then transferred to a HiBind DNA Mini Column, including any precipitates, and placed in a 2 mL collection tube. The sample was centrifuged (1 min; 10.000 x g) to bind DNA. The collection tube with the solution was discarded and the column was placed in a new collection tube. Then, 500 µL of the wash buffer HBC-buffer, diluted with isopropanol, was added to the column before it was centrifuged (30 sec; 10.000 x g). The washing step was then repeated but now with 700 µL DNA Wash Buffer diluted with absolute ethanol. The solution was discarded but the same collection tube was used when this last step was repeated once more.

The flow-through liquid was again discarded, and the HiBind DNA Mini Column was placed in the same collection tube. The sample was centrifuged (2 min; 15.000 x g) to dry the column. The column was then placed in a sterile 1.5 mL microcentrifuge tube and eluted with preheated (70°C) Elution buffer. A volume of 200 µL Elution buffer was added to the tissue samples and 30-50 µL was added to the parasite samples. After two minutes, the sample was centrifuged (1 min; 13.000 x g) to elute DNA from the HiBind DNA Mini Column.

By using a UV spectrophotometer (*Nanodrop® ND-100*), the concentration (ng/µL) of the DNA was measured. The instrument baseline was set by applying the Elution buffer. The extracted DNA was stored at -18°C.

2.9 Polymerase Chain Reaction (PCR)

In this study, Polymerase Chain Reaction (PCR) was used to amplify DNA from the parasites, with two purposes: i) detecting their presence and ii) obtain DNA sequences for species identification/confirmation. PCR is a method to amplify a part of a DNA string (template), through a cycling reaction with repeated temperature changes. The cycling reaction involves a denaturation step to separate the DNA strands, an annealing-step to bind the primers and an elongation-step to synthetize new complementary strands. The target DNA in this study was the gene for the eukaryotic ribosomal RNA: 18S rDNA of the ribosomal small subunit (SSU) and 28S rDNA of the ribosomal large subunit (LSU).

To run the cycling reaction some specific ingredients are needed: Forward and reverse primers, thermostable DNA polymerase, dNTPs and a suitable buffer in 20 µL. The ratio of reagents to volume used in this study is listed in Table 4. A mixture of GoTaq® G2 Hot Start Colorless Master Mix (*Promega*), forward primer, reverse primer, DNA-template and nuclease-free water was transferred to 0.2 mL PCR-tubes. GoTaq® G2 Hot Start Colorless Master Mix contains GoTaq® G2 DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers necessary to complete the reaction. Usually 1 µL of DNA-template was used but, for the small parasites with low DNA concentration, 2 µL DNA-template and 6 µL nuclease-free water was used to increase the possibility of amplification.

Table 4 Reagents to volume ratio and final concentration of the ingredients used in Polymerase Chain Reaction.

Reagent	Volume (µL) for 1 rxn	[Final]
GoTaq® G2 Hot Start Colorless Master Mix	10	x1
Forward primer (10 µM)	1	0.5 µM
Reverse primer (10 µM)	1	0.5 µM
DNA-template	1	Variable
Nuclease-free water	7	
Final volume	**20**	

The PCR-tubes were placed in a thermocycling machine (*Veriti™ 96-Well Thermal Cycler-Applied Biosystems*) to carry out the reaction. The thermoprofile used in this study is shown in Fig. 11. The initial denaturation of 95°C for 2 minutes was followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 55-60°C for 15 seconds and elongation at 72°C for 30 seconds, before the final extension at 72°C for 7 minutes. The various primers used to amplify the nucleotide sequence are listed in Table 5, along with the annealing temperature depending on the melting temperature of the primers.

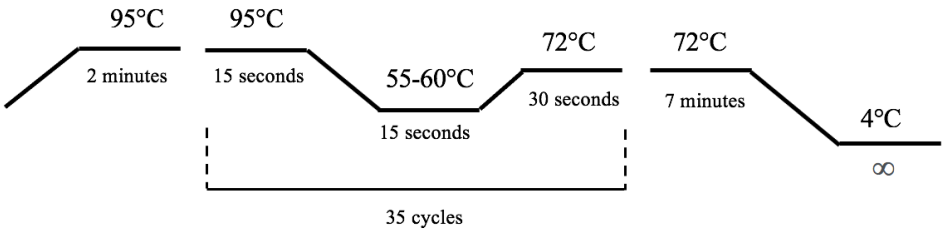

Figure 11 The thermoprofile used during Polymerase Chain Reaction in a thermocycling machine. Stage 1: 2 minutes of denaturation at 95°C. Stage 2: 35 cycles of denaturation at 95°C for 15 seconds, annealing at 55-60°C for 15 seconds and elongation at 72°C fir 30 seconds. Stage 3: 7 minutes of final extension at 72°C.

Table 5 Primers used in the PCR to amplify DNA, along with annealing temperature, target DNA and target parasite.

Primer name	Primer sequence (5´-3´)	Target gene	Annealing temperature (ºC)	Target parasite	Ref.
CoNecF1	AGACCTTCGGGTATGGGATCG	18S	60	*Ichthyobodo necator*	Isaksen et al. (2010)
CoNecR1	TCGGAATCGGAGTCCACC	18S	60	*Ichthyobodo necator*	Isaksen et al. (2010)
CoEurF1	CTCGCCTTCGGGTGAGG	18S	60	*Ichthyobodo salmonis*	Isaksen et al. (2010)
CoEurR1	GCCCGTAGCGTGTGATGAC	18S	60	*Ichthyobodo salmonis*	Isaksen et al. (2010)
PcPcF3	CAACACGGAATCAGTCCGA	18S	58	*Parvicapsula* spp.	E.K.[a]
PcPcR1	CGGACCTGGTGAGTTGC	18S	58	*Parvicapsula* spp.	E.K.[a]
L300F	CAAGTACCGTGAGGGAAAGTTG	28S	55	Platyhelminthes	Littlewood et al. (2000)
28SR3	TCTGGCTTCAACCTACGCAAG	28S	55	Platyhelminthes	E.K.[a]
Ces28F1	CGGGTGGCGTCAAGCTGC	28S	55	Platyhelminthes	E.K.[b]
Ces28R2	ACTTTGCGCAGGCAACACG	28S	55	Platyhelminthes	E.K.[b]
NC5-F	GTAGGTGAACCTGCGGAAGGATCATT	ITS	57	Nematoda	Zhu et al. (1998)
Ani-R1	CAGTGRYCGATGGATTCA	ITS	57	Nematoda	E.K.[b]
CeCOxF	AGWGGATTTTGATCYGGGCT	COI	50	*Caligus elongatus*	E.K.[b,c]
CeCOxR	GGATCAAAAAAYCTGGTRTTTA	COI	50	*Caligus elongatus*	E.K.[b,c]

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).

[a] Primers designed by Egil Karlsbakk (2018), published in Fjær, 2019. [b] Primers designed by Egil Karlsbakk (2020-2021).
[c] Modified WOBCOI primers of Øines & Heuch (2005).

All the DNA samples were from frozen tissues, and many of the parasites were thawed before they were conserved in ethanol for DNA. Enzymatic degradation could therefore affect DNA quality and the results of the PCRs. To account for this, positive control DNA was included from related parasites from optimally collected samples. Hence, positive controls were used to verify that the reaction was working properly and that the primers were binding to the template. When it was difficult to obtain sequences, new primers were also designed for smaller amplicons, to increase the chance of a positive result. The positive control for *Ichthyobodo* sp.

was a sample from a pink salmon previously found infected by Fjær (2019). The positive controls used are listed in Table 6. Negative controls were also used to ensure that no contamination had occurred. For this, the template was substituted with nuclease-free water.

Table 6 Positive controls used in the PCR. The samples were provided by E.K., unless otherwise indicated.

Agents	Host	Organ	Isolate name	Locality	Date
Euglenozoa					
Ichthyobodo sp.[a]	*Oncorhynchus gorbuscha*	Gills	P-90	Etne, Hordaland	25.08.17
Myxozoa					
Parvicapsula pseudobranchicola	*Salmo salar*	Pseudobranch	Parvi1	Solheim, Troms	06.11.07
Trematoda					
Derogenes varicus	*Anarhichas lupus*	Intestine	Dig-EK-1	Barents Sea	27.05.15
Cestoda					
Scolex bothriosimplex	*Krefftichthys anderssoni*	Intestine	ScB-EK-1	Southern Ocean	13.03.08
Scolex bothriosimplex	*Mallotus villosus*	Intestine	ScB-EK-2	Barents Sea	27.05.15
Scolex pleuronectis	*Maurolicus muelleri*	Intestine	ScP-EK-1	Barents Sea	28.05.15
Eubothrium sp.[b]	*Labrus mixtus*	Intestine	Both-pos-1	Espegrend, Hordaland	08.10.20
Diphyllobothrium sp.	*Arctozenus risso*	Intestine	Diph-EK-1	Barents Sea	2015
Nematoda					
Contracaecum sp.[b]	*Cyclopterus lumpus*	Intestine	Nem-pos-2	Espegrend, Hordaland	08.10.20

[a] DNA extracted from P90, a heavily infected individual, material of Marte Fjær (Fjær, 2019). [b] Specimens collected freshly by the author.

2.10 Gel electrophoresis

Gel electrophoresis is used to separate nucleic acids by size using electrical voltage and to visualize the results in a gel made visible by the fluorescence of Gelred™ bound to double stranded DNA. The gel was made of 1 % Agarose dissolved in 1xTAE-buffer, mixed with 1 µL Gelred™. The agarose-gel was submerged in 1xTAE-buffer, covering the wells made in the gel. A volume of 5 µL PCR-product was mixed with 1 µL loading dye, and 5 µL of the mixture was added to the wells. Additionally, 3 µL DNA-standard (*O´RangeRuler 100 bp DNA Ladder, ready-to-use*) was added to one or two empty wells to estimate the molecular weight of nucleic acids. The gel was connected to electrical voltage (80V) for 40 minutes.

Positive samples, meaning primers binding to a DNA-sequence, produce a band of a specific molecular weight on the gel. The bands were visualized in a UV-transilluminator (*Gel Logic 212 Pro, Fisher Scientific*) and digital images were acquired using the software Carestream MI.

2.11 Sequencing

The positive samples were sequenced to identify the species. Before sequencing the PCR-products were treated to inactivate primers and nucleotides from the amplification reactions. This clean-up was achieved by adding 1 µL illustra™ ExoProStar™ 1-step to 2.5 µL of the positive PCR-product. The samples were then incubated in a thermocycling machine at 37°C for 15 minutes and then 80°C for an additional 15 minutes.

The finished product was then added to two sequencing-reactions: one with forward primer and one with reverse primer. The template was mixed with nuclease-free water, Big Dye version 3.1, sequencing buffer and forward or reverse primer, and transferred to a 0.2 mL PCR-tube. The volume of each reagent for one reaction is listed in Table 7.

The sequencing-reaction was placed in a thermocycling machine to amplify the DNA, involving denaturation, annealing and elongation. The thermoprofile used in this study is illustrated in Fig. 12 and contains 5 minutes of initial denaturation at 96°C, 25 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 10 seconds and elongation at 60°C for 4 minutes, and the final step of cooling down to 4°C.

Table 7 Reagents to volume ratio in a sequencing-reaction.

Reagent	Volume (µL) for 1 rxn
Nuclease-free water	6.5
Big Dye v.3.1	1
Sequencing buffer	1
Template (treated with ExoProStar™)	1
Forward or reverse primer (10 µM)	0.5
Final volume	**10**

After the reaction was finished, 10 µL nuclease-free water was added to the samples. The samples were then delivered to the in-house sequencing service (Sanger sequencing). The

finished sequences were assembled in the programs Vector NTI® Software (v.9.0) and SnapGene and analysed using the search tool BLAST (Basic Local Alignment Search Tool) by NCBI (the National Center for Biotechnology Information) (Altschul et al., 1990).

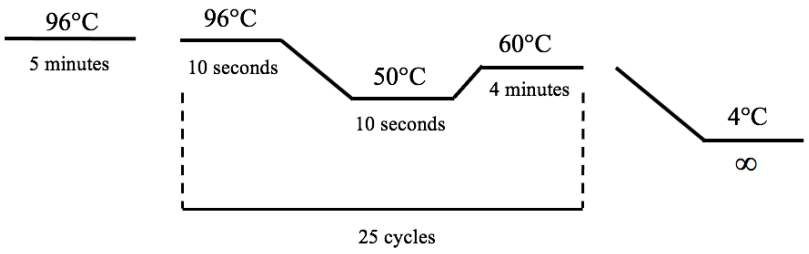

Figure 12 The thermoprofile used during the sequencing-reaction. Stage 1: 5 minutes of denaturation at 95°C. Stage 2: 25 cycles of denaturation at 95°C for 10 seconds, annealing at 50°C for 10 seconds and elongation at 60°C for 4 minutes.

2.12 Statistics

The software GraphPad Prism (v. 9.0.0) was used to carry out statistical analyses, specifically to find which variables that affect parasite load in pink salmon. The parasite abundance was aggregated and not normally distributed, hence nonparametric tests were used when comparing groups. The Mann-Whitney test was used to determine differences between two groups (e.g., gender) and the Kruskal-Wallis test (KW) was conducted to find out if three or more groups differed significantly (e.g., year class) followed by the post hoc Dunn´s Multiple Comparison test. To examine the relationship between one or multiple independent variables and one dependent variable the statistical models Simple Linear Regression and Multiple Linear Regression were used, respectively. For these tests, the parasite abundance (x) of each species was log transformed (log10(x+1)) to meet the requirements of variance homogeneity.

Relationships are considered significant if the P-value < 0.05. The results are presented as * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$ and **** = $P \leq 0.0001$. Only results from meaningful analyses (prevalence > 10) are reported. Microsoft® Excel (v. 16.43) and GraphPad Prism were used to construct graphs.

3 Results

3.1 Parasites

The 86 pink salmon caught in the Norwegian Sea (N female = 43, N male = 43) were infected with a total of 12 morphologically identifiable metazoan parasite species, one of which (*Diphyllobothrium* spp.) proved to represent two species (see below). In addition, Piscine orthoreovirus infections were revealed by molecular screening (Table 8). Each individual fish was infected with two to eight metazoan parasite species (averaging four species for each gender). The macroparasites found were four species trematodes, five (molecularly six) species cestodes, two species nematodes and one species copepod.

Table 8 Overview of the parasite species found or molecularly tested for in ocean-caught pink salmon, including number examined, number infected, prevalence, abundance and intensity.

Parasite species	N examined	N infected	Prevalence (%)	Abundance		Intensity (max.)
				Mean	SD	
Viruses						
Infectious hematopoietic necrosis virus (IHNV)	86	0	0	-	-	-
Infectious salmon anaemia virus (ISAV)	86	0	0	-	-	-
Infectious pancreatic necrosis virus (IPNV)	86	0	0	-	-	-
Salmonid alphavirus (SAV)	86	0	0	-	-	-
Piscine orthoreovirus (PRV)	86	2	2	-	-	-
Piscine myocarditis virus (PMCV)	86	0	0	-	-	-
Euglenozoa						
Ichthyobodo spp.	30	0	0	-	-	-
Myxozoa						
Parvicapsula pseudobranchicola	55	0	0	-	-	-
Platyhelminthes						
Trematoda						
Derogenes varicus	86	82	95	43.5	74.8	487
Brachyphallus crenatus	86	30	35	3.6	10.3	69
Hemiurus levinseni	86	9	10	0.4	1.8	13
Lecithaster gibbosus	86	19	22	2.8	11.6	101
Cestoda						
Bothriocephalidea gen. sp.	86	5	6	0.1	0.50	3
Diphyllobothrium sp. (Pl)*	86	10	12	0.2	0.51	3
Scolex bothriosimplex (Pl)	86	6	7	0.09	0.36	2
Scolex pleuronectis A (Pl)	86	38	44	1.5	2.5	13
Scolex pleuronectis B (Pl)	86	14	16	1.8	7.5	54
Nematoda						
Anisakis simplex (L3)	86	56	65	2.5	3.4	18
Hysterothylacium aduncum (L3)	86	30	35	0.9	1.7	10
Hysterothylacium aduncum (lum)	86	86	100	19.4	38.0	292
Crustacea						
Caligus elongatus	86	2	2	0.02	0.15	1

Pl – plerocercoid, L3 – the infective larval stage, Lum – luminal parasites of different stages (III, IV, V), SD – standard deviation.

* Sequences revealed these to represent two species, see below.

3.1.1 Platyhelminthes

Trematoda

Derogenes varicus (Derogenidae)

The trematode *Derogenes varicus* (Fig. 13), morphologically identified in accordance with the descriptions by Gibson (1996), was present in 95.3 % of the examined pink salmon. The trematode was primarily found in the pharynx, oesophagus and stomach, some even at the gills and in the swimbladder. The specimens measured 0.6 to 2.7 mm in length, the smallest immature with no eggs present.

Twelve of the 86 examined pink salmon had *D. varicus* present in the swim bladder (N = 28) (Fig. 14). Those measured 1.5 to 2.2 mm in length and the intensity varied from 1 to 9 trematodes (average 2.3). All were mature (oviferous). In these twelve fishes, the proportion of the *D. varicus* population found in the swimbladder was 12.7 % (0.74-50 %). Overall, they constituted 0.75 % of all *D. varicus* found.

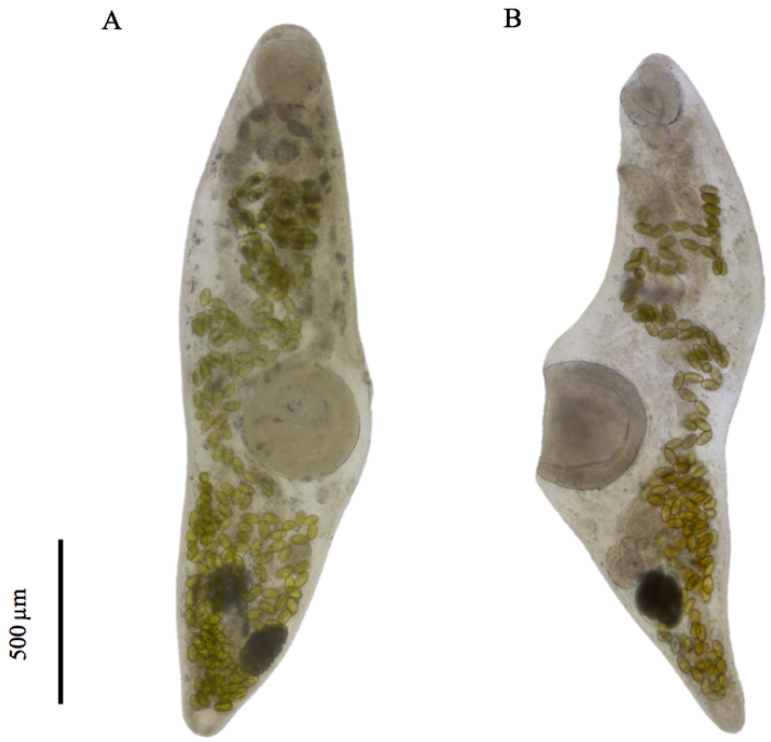

Figure 13 The trematode *Derogenes varicus* found in the oesophagus. **A** Ventral view of the trematode found in pink salmon, **B** lateral view of the trematode found in Atlantic salmon. The identity of both specimens was molecularly verified by LSU rDNA sequencing.

Partial LSU rDNA sequences from three specimens found in the oesophagus and stomach were identical (734-735 nt compared). However, identity was only 98.0 % with *Derogenes varicus* in GenBank, from American plaice (*Hippoglossoides platessoides* (Fabricius, 1780)) from the North Sea (AY222189).

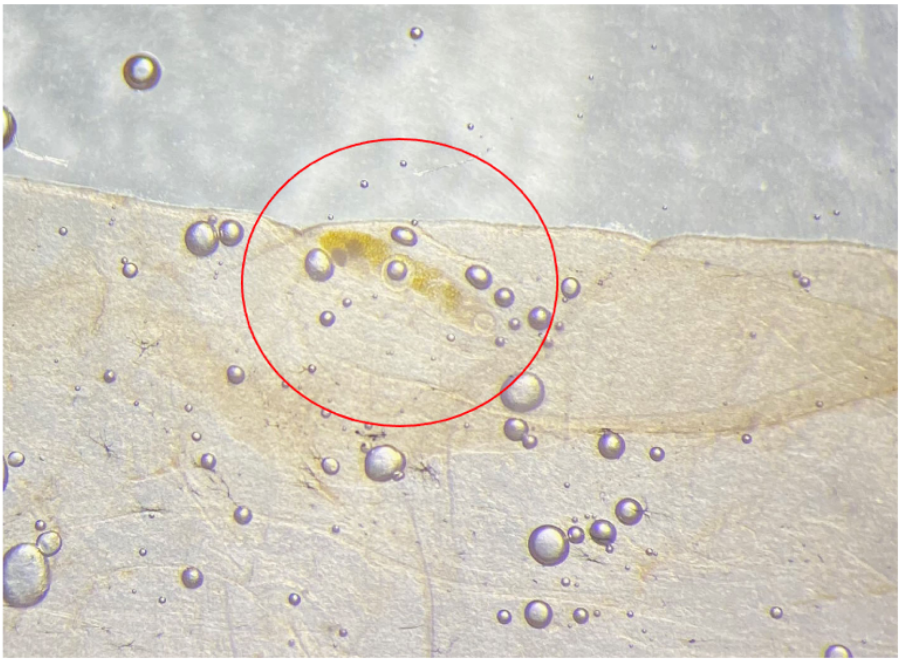

Figure 14 *Derogenes varicus* (marked with a red circle) situated inside the swim bladder of pink salmon.

Bracyphallus crenatus (Hemiuridae)

The trematode *Brachyphallus crenatus* (Fig. 15) was morphologically identified in accordance with the descriptions by Gibson and Bray (1986). The principal characters used were the sucker index (Table 9), occurrence of a presomatic pit, an often prominent seminal vesicle dorsoanterior to the ventral sucker, lobed vitellaria and the position of the genital pore. The overall prevalence in the examined pink salmon was 34.9 % and the trematodes were primarily found in the oesophagal- and posterior stomach (66 %), additionally in the pharynx, oesophagus and pyloric stomach. The length of the trematodes ranged from 1.0 to 4.0 mm. Most were oviferous but the smallest had no or few eggs present.

Partial LSU rDNA sequences were obtained from five specimens, showing 100 % identity (805-831 nt compared) with *Brachyphallus crenatus* from whitespotted char (*Salvelinus leucomaenis* (Pallas, 1814)) from the Sea of Okhotsk, Pacific Ocean (MH628299).

Table 9 Measurements of the trematode species *Brachyphallus crenatus* and *Hemiurus levinseni* from the family Hemiuridae, including length of the trematodes and diameter of the.oral- and ventral sucker.

	Brachyphallus crenatus	***Hemiurus levinseni***
Oral sucker (diameter, µm)	197 (114-261)	195 (170-216)
Ventral sucker (diameter, µm)	212 (126-291)	166 (142-185)
Ratio oral sucker : ventral sucker	1:1.07 (0.96-1.18)	1:0.85 (0.79-0.90)
Length (mm)	1.0-4.0	1.2-2.7

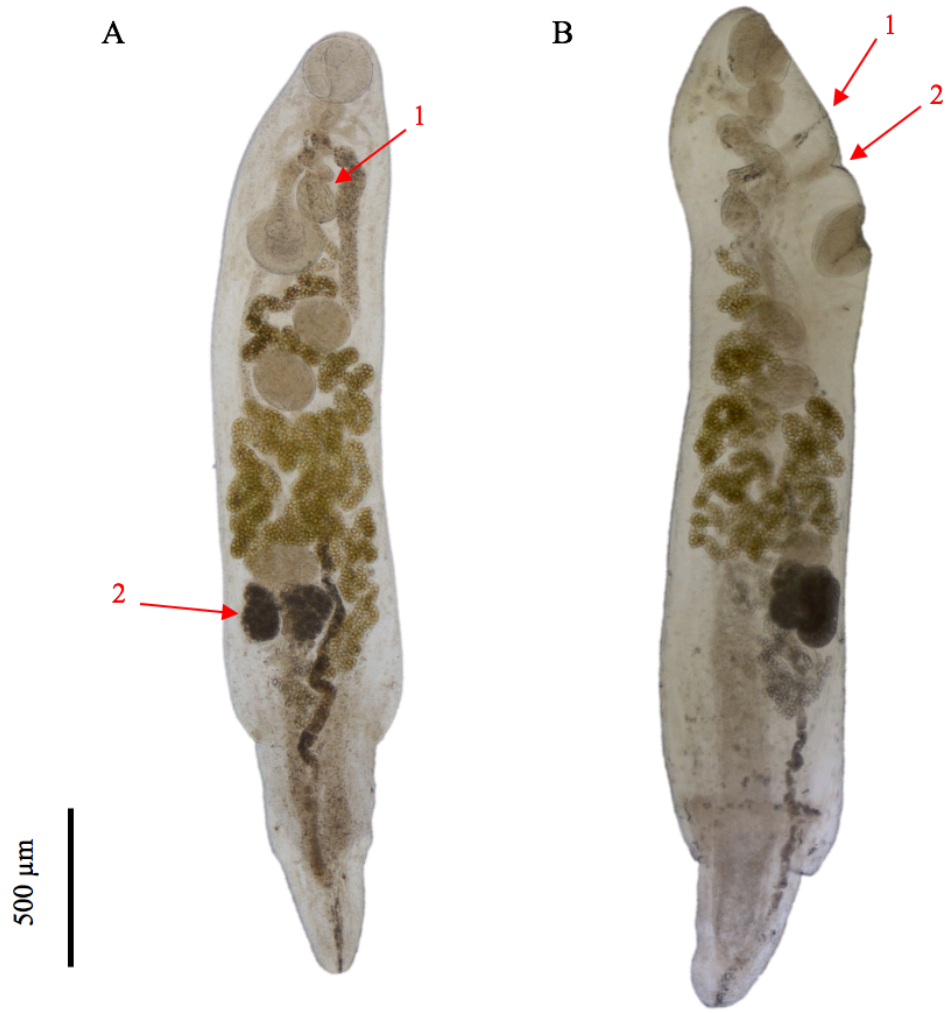

Figure 15 *Brachyphallus crenatus* found in the stomach. **A** Ventral view of the trematode found in pink salmon - the characteristic pre-acetabular seminal vesicle is marked with arrow N° 1 and the lobed vitellaria is marked with arrow N° 2, **B** lateral view of the trematode found in Atlantic salmon – the genital pore is marked with arrow N° 1 and the characteristic presomatic pit is marked with arrow N° 2. The identity of both specimens was molecularly verified by LSU rDNA sequencing.

Hemiurus levinseni (Hemiuridae)

The trematode *Hemiurus levinseni* (Fig. 16) was identified according to the description by Gibson and Bray (1986). The principal characters used were the sucker index, oval to globular vitellaria and the position of the genital pore. A small number of the species was found, with a prevalence of 10.5 % and a maximum intensity of 13. The trematodes, 1.2 to 2.7 mm long, were found in the stomach, except for one in the oesophagus. Partial LSU rDNA sequences were obtained from two specimens, showing 100 % identity (792-794 nt compared) with *Hemiurus levinseni* from Arctic flounder (*Liopsetta glacialis* (Pallas, 1776)) from the White Sea (MN962993).

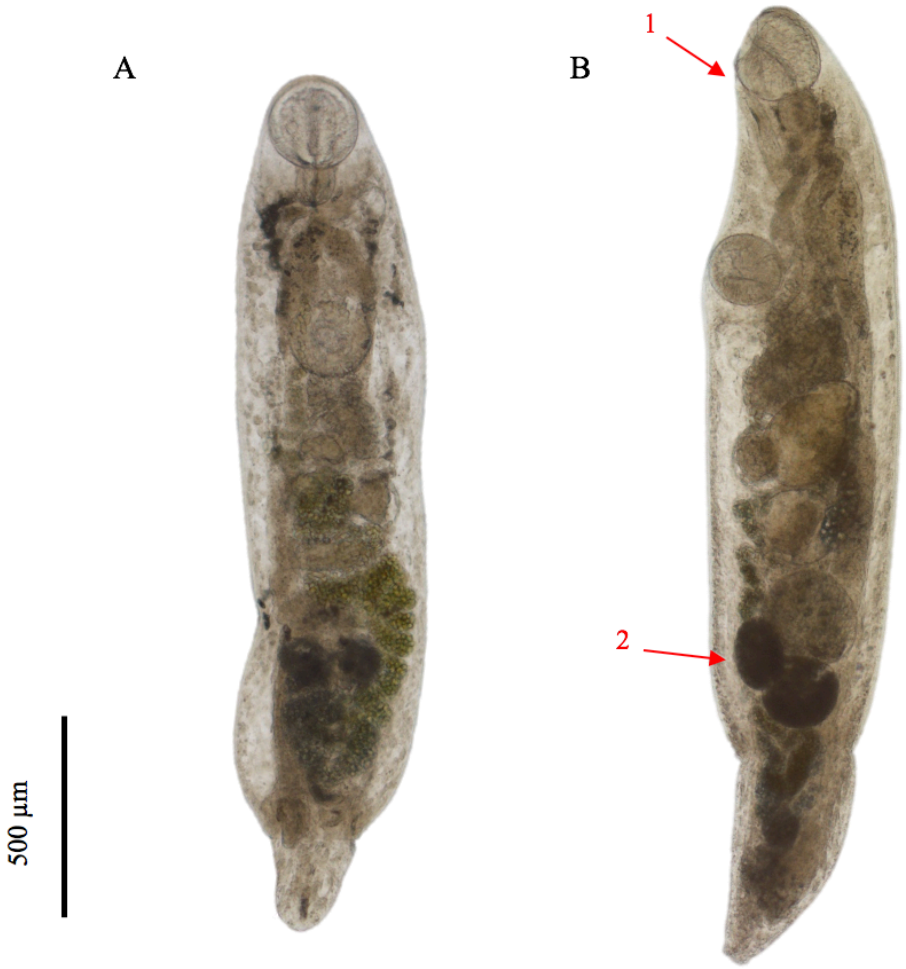

Figure 16 *Hemiurus levinseni* found in the abdomen of pink salmon. **A** Ventral view of the trematode, **B** lateral view of the trematode – the genital pore, situated at the ventral margin of the oral sucker, is marked with arrow N° 1 and the oval to globular vitellaria is marked with arrow N° 2. The identity of the latter specimen was molecularly verified by LSU rDNA sequencing.

Lecithaster gibbosus (Lecithasteridae)

The trematode *Lecithaster gibbosus* (Fig. 17) was identified in accordance with Gibson (1996). The trematode, 1.2 to 2.0 mm long, had a prevalence of 22.1 %. The specimens were mainly found in the hindgut (78.7 %) but were also present in the foregut (11.9 %) and pyloric caeca (9.4 %). A partial LSU rDNA sequence from one specimen showed 99.4 % identity (704 nt compared) with *Lecithaster gibbosus* from whiting (*Merlangius merlangus* (L.)) from the North Sea (AY222199).

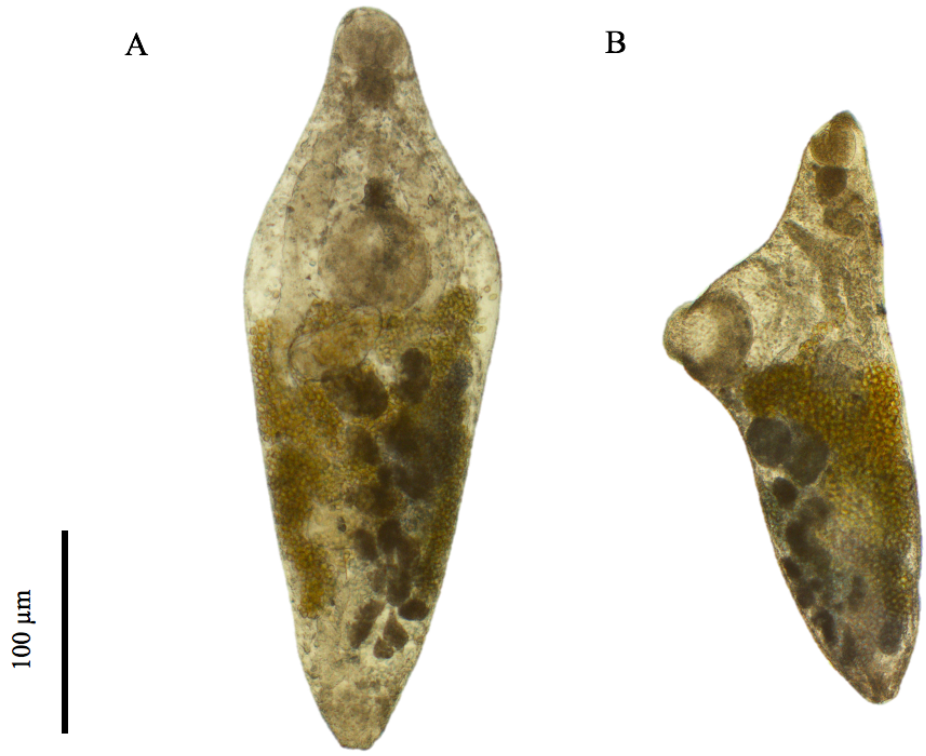

Figure 17 The trematode *Lecithaster gibbosus* found in the hindgut of pink salmon. **A** Ventral view of the trematode, **B** lateral view. No LSU rDNA obtained.

Cestoda

Bothriocephalidea juveniles

A total of ten juvenile cestodes from the order Bothriocephalidea where found in five pink salmon. All were found free in the hindgut lumen, except for one in the pyloric caeca. They had no apparent scolex but a structure resembling a furrow at the anterior part of the body and five to ten proglottids. They measured 1.2 to 3.5 mm in length.

Partial LSU rDNA sequences obtained from two specimens (Fig. 18) showed 99.6 and 99.8 % identity (537 nt compared) with three *Clestobothrium* spp. from hakes (*Merluccius* spp.), including one species, *C. crassiceps* (Rudolphi, 1819), occurring in European hake (*M. merluccius* L.) in the North Atlantic (KR780884).

Figure 18 *Clestobothrium* sp. found in the hindgut lumen of pink salmon. Arrow pointing at the furrow-like structure at the anterior part of the body.

***Diphyllobothrium* spp.** (Diphyllobothriidae)

Cestode plerocercoids (N=14) (Fig. 19) were found encapsulated in the stomach wall of ten pink salmon. The capsules ranged from 500 to 900 µm in diameter, and the freed plerocercoids were 700-1900 µm long (average 1034 µm). DNA was extracted from 11 of these but PCR amplification of partial LSU rDNA was successful from only four specimens. Three of the obtained sequences were identical and showed 98.4 to 99.2 % identity (552-982 nt compared) with *Diphyllobothrium* sp. from California sea lion (*Zalophus californianus* (Lesson, 1828)) from the Pacific coast of USA (KY552829). The fourth specimen showed 100 % identity to *Diphyllobothrium schistochilos* (Germanos, 1895) from ringed seal (*Pusa hispida* (Schreber, 1775)) from Svalbard (KY552821). A sequence was also obtained from an additional plerocercoid from river-caught pink salmon in Etne, with 100 % identity to the other *Diphyllobothrium* sp. sequences obtained.

Figure 19 Encapsulated plerocercoid cestodes found in the abdominal wall of pink salmon. **A** Plerocercoid identified as *Diphyllobothrium schistoschilos*, **B-C** Plerocercoids identified as D*iphyllobothrium* sp., **C** Encapsulated plerocercoid to the right.

Scolex bothriosimplex (Reimer, 1970) (Tetrabothriidae)

Eight specimens of the cestode larval type *Scolex bothriosimplex* (Fig. 20) were found, identified morphologically according to Reimer (1970). They measured 436 to 746 µm in length (average 539 µm) and had a structure resembling a sucker at the end of the body. The single apical sucker ranged from 101 to 119 µm in diameter. Most plerocercoids were found in the hindgut (*N*=7), one in the foregut.

LSU rDNA sequencing was attempted from five specimens from Norwegian Sea pink salmon, and in addition one from river-caught pink salmon (Etne). DNA yield was very low, and only one PCR was successful. A partial LSU rDNA sequence was obtained from the Etne specimen. This *Scolex bothriosimplex* (Fig. 20) showed 95.0 % identity (581 nt compared) with an adult *Tetrabothrius* sp. (AF286952.2) from short-tailed shearwater (*Puffinus tenuirostris* (Temminck, 1835), a seabird from Australia. Two positive controls, *S. bothriosimplex* collected fresh from other marine fish, had better DNA yield. Sequences were obtained both from a specimen from the Antarctic fish *Krefftichthys anderssoni* (Lönnberg, 1905) and one from a Barents Sea capelin, *Mallotus villosus* (Müller, 1776). These partial LSU sequences had 89.8 % identity with *Tetrabothrius forsteri* (Krefft, 1871) and 96.5 % identity with *Tetrabothrius* sp. (AF286952.2) respectively, the latter then identical to *S. bothriosimplex* from pink salmon.

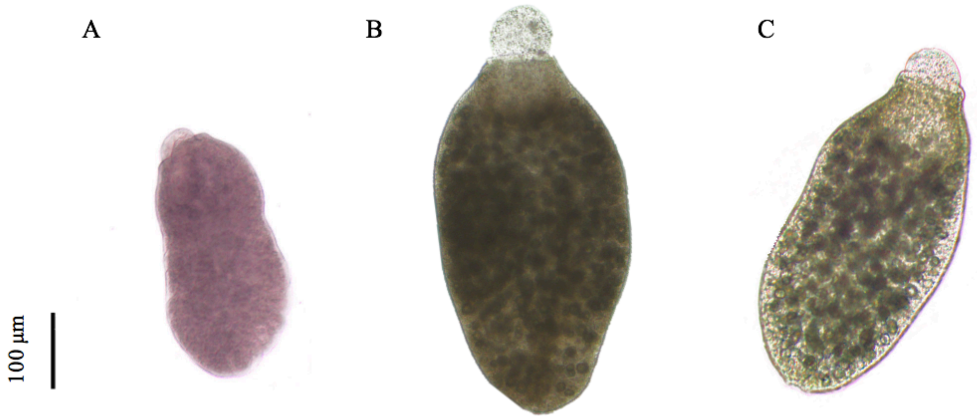

Figure 20 *Scolex bothriosimplex* found in hindgut of pink salmon. **A** Plerocercoid from river-caught pink salmon identified as *Tetrabothrius* sp., **B-C** *Scolex bothriosimplex* from ocean-caught pink salmon.

Scolex pleuronectis

Scolex pleuronectis is a collective term of plerocercoids with locular bothridia and are in this thesis categorized in two types ("A" and "B"), based on the size of the plerocercoid and the diameter of the apical sucker following Fjær (2019) (Fig. 21). Many had scolex and bothridia retracted and hence difficult or impossible to measure, adding an element of uncertainty when categorizing the plerocercoids. The overall prevalence was 48 %, with a mean abundance of 3.3.

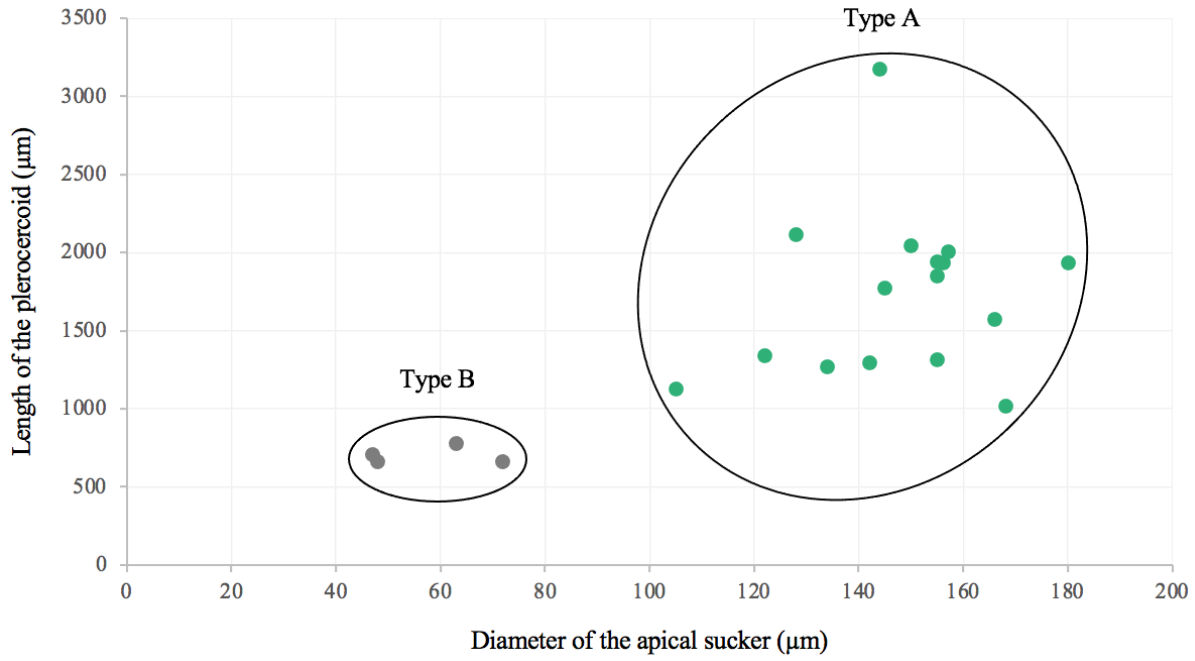

Figure 21 Diamater of the apical sucker relative to the length of the plerocercoids identified as *Scolex pleuronectis*. Grey = type B, green = type A.

The biggest type ("A") (Fig. 22) had a prevalence of 44 % and was typically found in the hindgut (90 %). A few were found in the pyloric caeca and in the anal part of the intestine. These plerocercoids were 0.8 to 3.2 mm long with an apical sucker ranging from 105 to 166 µm in diameter. Partial LSU rDNA was obtained from five type A plerocercoids, all sequences showing 99.4 to 100 % identity to *Clistobothrium* cf. *montaukensis* from porbeagle shark (*Lamna nasus* (Bonnaterre, 1788)).

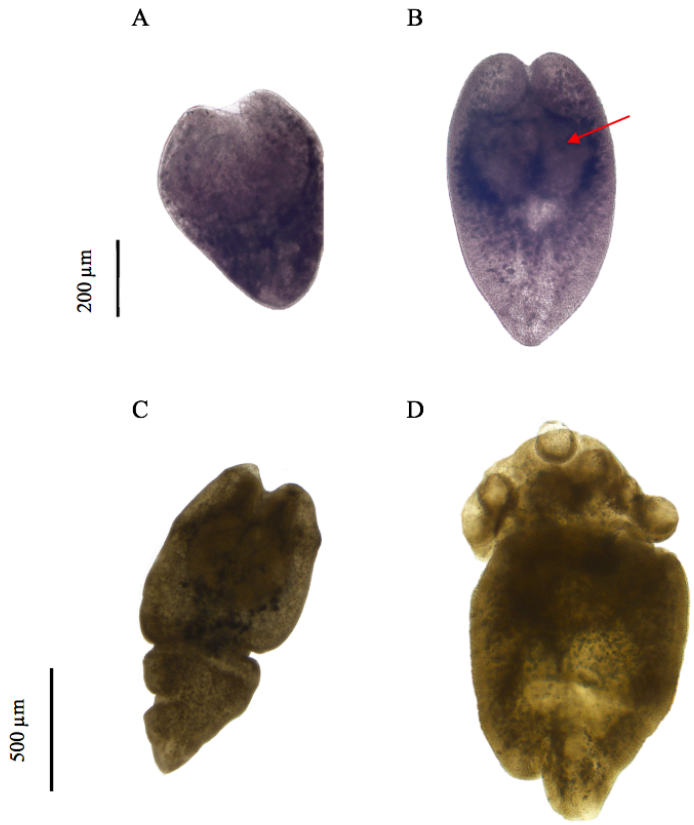

Figure 22 *Scolex pleuronectis* A found in the hindgut of pink salmon. **A** Plerocercoid with retracted bothridia identified as *Clistobothrium montaukensis*, **B-D** plerocercoids identified as *Clistobothrium* sp., **B** plerocercoid with retracted bothridia, marked with a red arrow, **D** plerocercoid with visible bothridia.

The smaller type ("B") was less frequently found, with a prevalence of 16 %. Most of these plerocercoids were found in the hindgut (80 %) and the rest in the pyloric caeca, foregut and anal part. The length of the plerocercoids ranged from 0.44 to 0.78 mm and the apical sucker measured 48 to 72 µm in diameter when it could be measured. Most of the small types had retracted bothridia and morphology like an egg (Fig. 23). DNA yield and quality from the small *Scolex pleuronectis* type B was poor, and PCR amplification of LSU rDNA did not succeed.

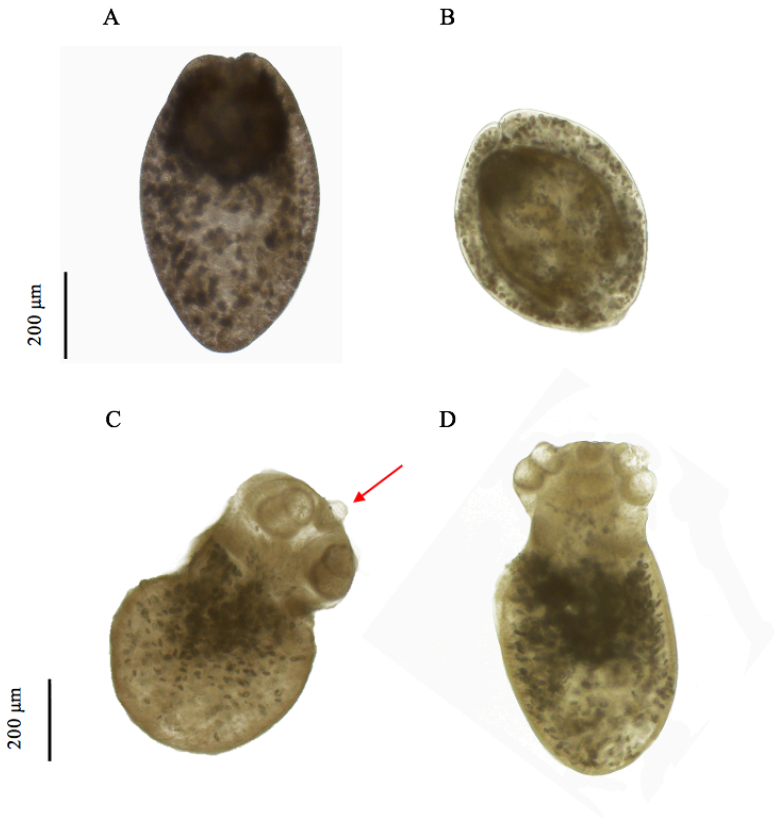

Figure 23 *Scolex pleuronectis* B found in the intestine of pink salmon. **A-B** Plerocercoids with retracted bothridia found in the hindgut and foregut respectively, **C** Plerocercoid with visible apical sucker (arrow) found in the hindgut, **D** Plerocercoid with extended bothridia found in the hindgut. Note the clearly bilocular bothridia in C and D.

3.1.2 Nematoda

Hysterothylacium aduncum (Raphidascarididae)

All fish were infected with the nematode *Hysterothylacium aduncum* (Fig. 24), identified according to Berland (1961), as either the larval stage (III) or preadult/adult stage (IV/V). The larvae, 3.5 to 27 mm long, had a prevalence of 73 % and were found free in the lumen of the digestive tract (82.9 %), free in the abdominal cavity (4.7 %) (Fig. 25) or encapsulated on internal organs (12.4 %). The preadult and adult nematodes, 5 to 36 mm long, were found in the lumen of the digestive tract, with a prevalence of 92 %. The ratio of luminal nematodes stage IV/V and III was 2:1.

The luminal nematodes were most frequently found in the pyloric caeca (91.5 %). Other sites were the stomach, foregut, hindgut and anal part. The encapsulated larvae (L3) were found on

the liver and in the mesenteries surrounding the caeca, foregut, hindgut, gonads, spleen and swim bladder.

All nematodes with ambiguous morphology were molecularly tested, because the occurrence of *Phocascaris* spp. or *Contracaecum* spp. larvae was anticipated. ITS1-5.8s-ITS2 sequences were obtained from eleven specimens, showing 99.4 to 99.5 % identity (942-1013 nt compared) to *Hysterothylacium aduncum* from European anchovy (*Engraulis encrasicolus* L.) from the North Adriatic Sea (KP670310).

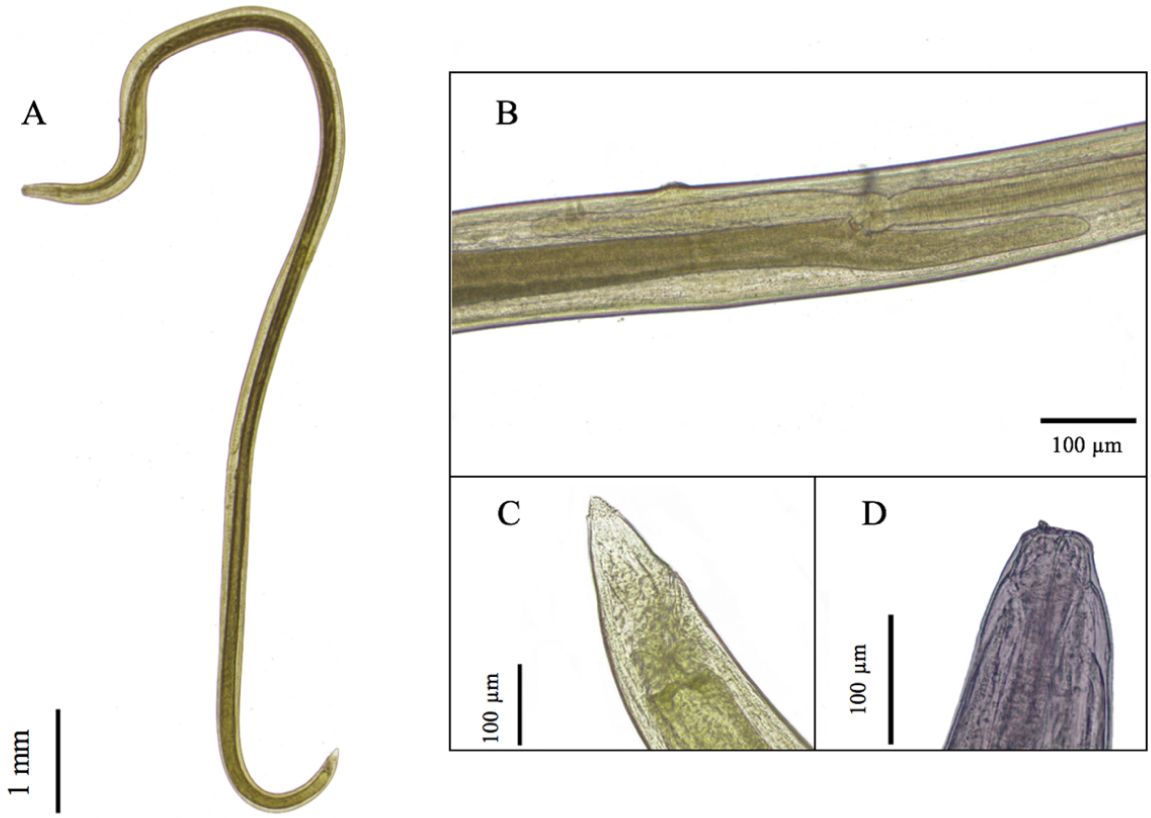

Figure 24 The nematode *Hysterothylacium aduncum* found in the pyloric caeca of pink salmon. **A** Preadult/adult nematode, **B** ventricle with appendix and caecum, **C** characteristic cactus-tail of a preadult/adult nematode, **D** boring tooth of a larvae. All molecularly verified.

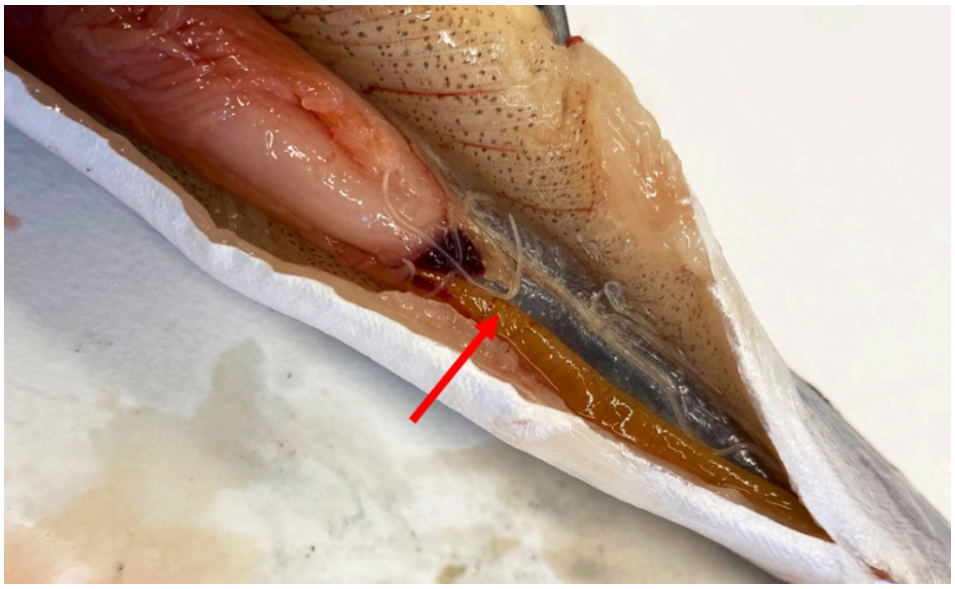

Figure 25 A total of 15 larva *Hysterothylacium aduncum* free in the rear part of the abdominal cavity of young pink salmon, pointed out by the arrow.

Anisakis simplex (Anisakidae)

The larval nematode *Anisakis simplex* (Fig. 26) was identified morphologically according to Berland (1989). Total prevalence was 65 %. The larvae ranged from 10 to 30 mm in length, and most were found free in the alimentary canal (61 %), mainly in the luminal area of the pyloric region. The encapsulated nematodes (26 %) were most frequently found in the serosal covering in the pyloric region, in addition to on the liver, stomach, hindgut and swim bladder. The sites and frequency of the larvae is presented in Fig. 27. Using the UV-press method, 20 pink salmon were found to harbour *A. simplex* larvae in the filets. These constitutes 13 % of the larvae found but when disregarding the luminal larvae, they represented the third (33 %). Most of the nematodes found in the filet were situated in the bellyflap (Fig. 28) and had a maxiumum intensity of four. Also, five fish had *Anisakis* present only in the filet. An overview of the larvae distribution in the filet is presented in Fig. 29.

ITS1-5.8s-ITS2 sequences from five nematodes showed 100 % identity (932-965 nt compared) with *Anisakis simplex* sensu stricto.

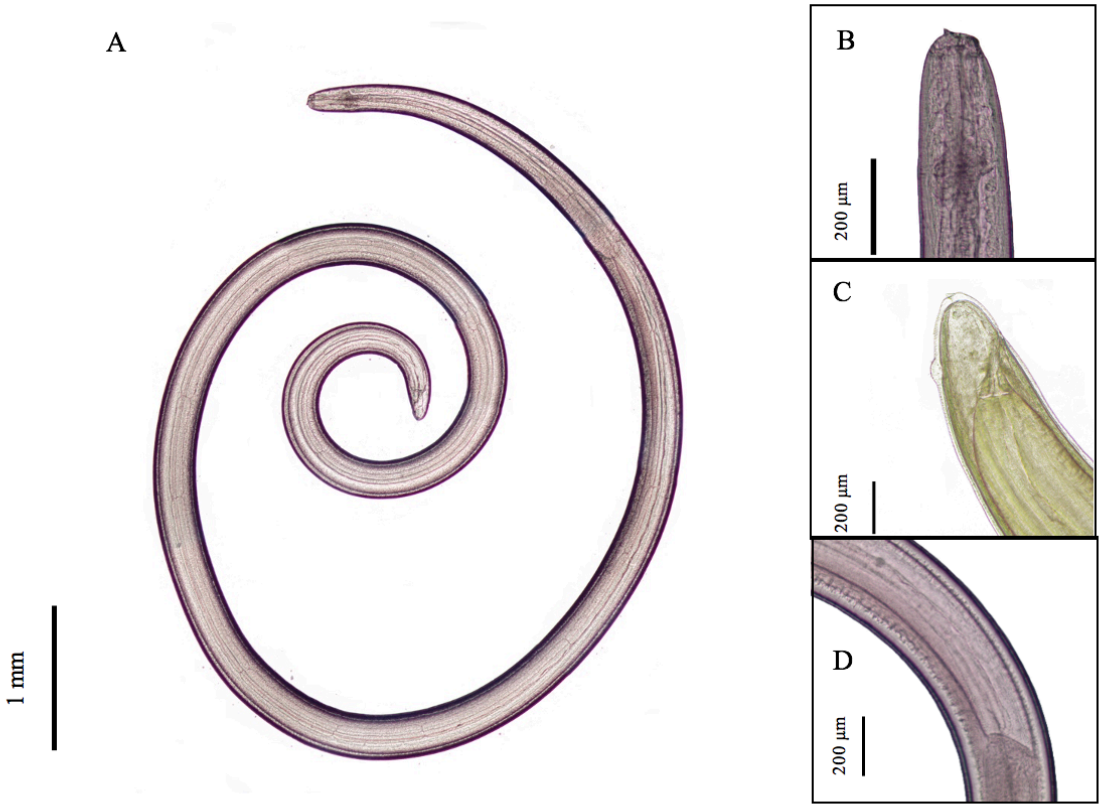

Figure 26 The nematode *Anisakis simplex* (L3) found in pink salmon. **A** Larva found encapsulated on the liver, **B** close up of the boring tooth from the same nematode, **C** tail from larva found free in pyloric region, **D** the oblique transition between the ventricle and intestine.

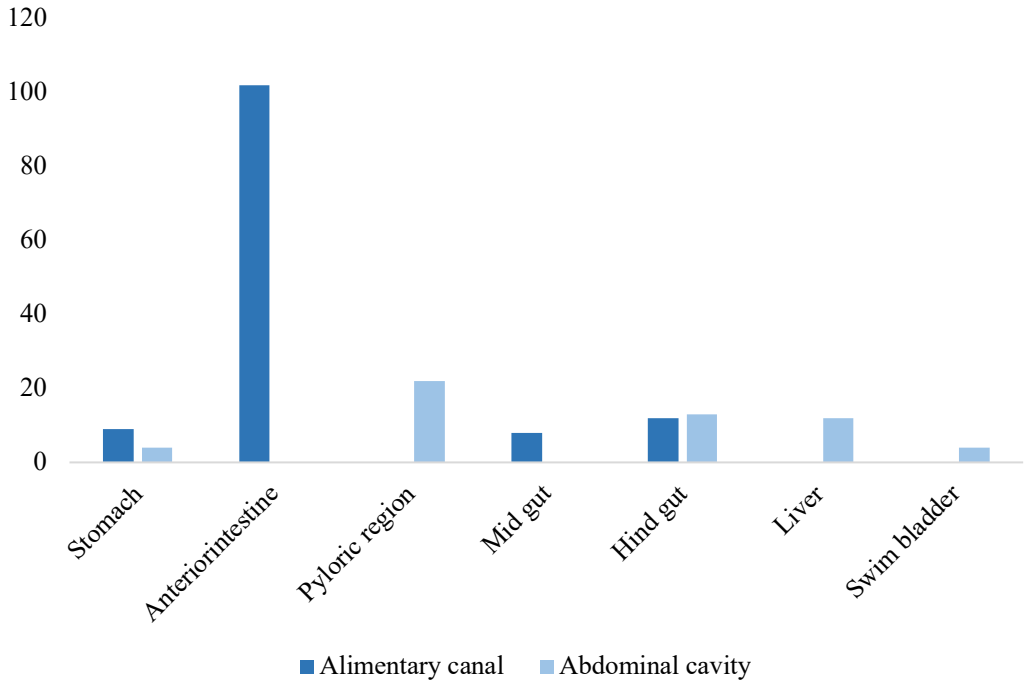

Figure 27 The sites where *Anisakis simplex* was found free in the alimentary canal and encapsulated in the serosal covering on organs in the abdominal cavity.

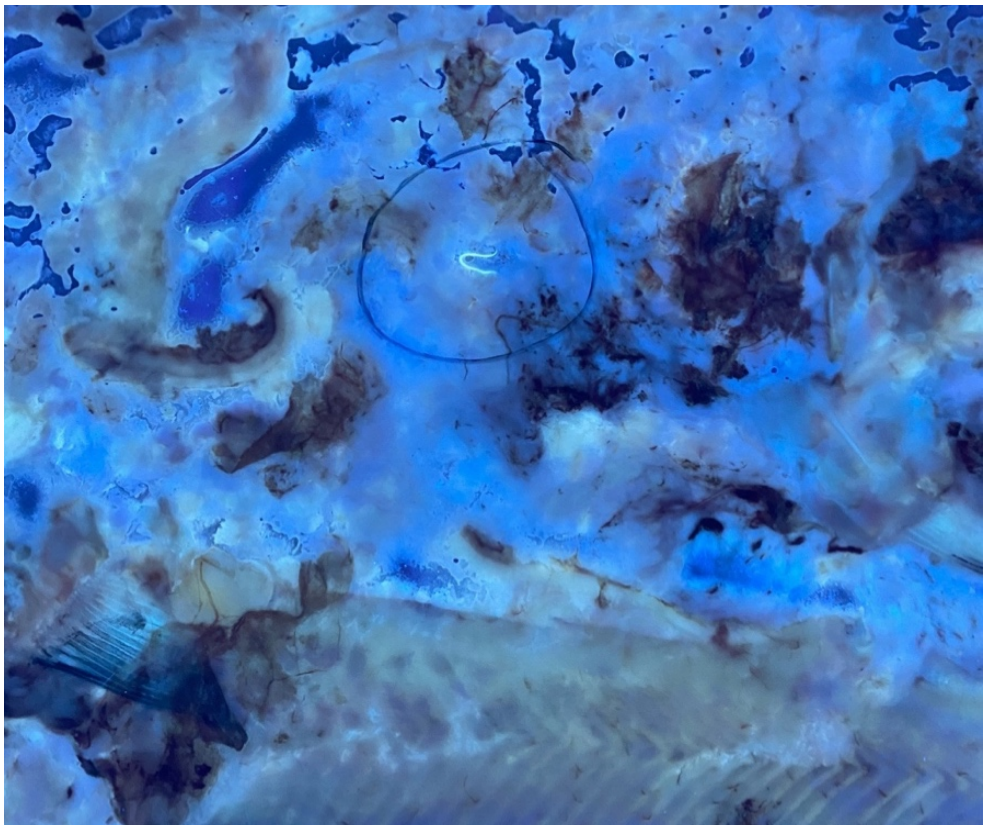

Figure 28 The fluorescent nematode *Anisakis simplex* under UV-light, found in the belly flap of pink salmon after flat pressing.

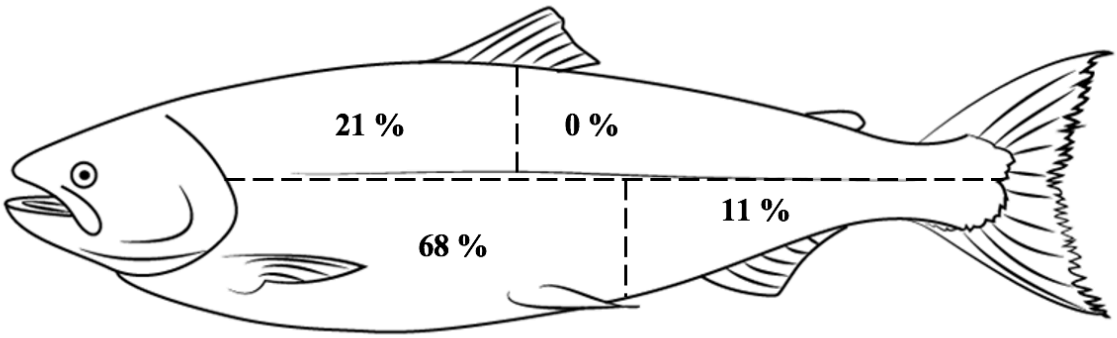

Figure 29 The distribution of *Anisakis simplex* in the filet of pink salmon, shown as the proportion of larvae recovered from the musculature (N = 28).

3.1.3 Crustacea

Caligus elongatus Nordmann, 1832 (Caligidae)

Despite extensive scale loss (60-99 %, mean 92 %), two sea lice were found on two separate fish, caught in June 2017 (69°25'51.6"N, 17°16'51.6"E and 70°25'40.8"N, 14°28'33.6"E). The lice were adult females (6.8 to 7.0 mm long) with egg strings and both fitted the description of *Caligus elongatus* according to Kabata (1992). The lice were collected from the tail fin (Fig. 30) and the skin right below the dorsal fin.

The two specimens were genotyped by MSc student Hanne Log Persson, using an assay targeting the mitochondrial Cytochrome C Oxidase 1 (COI) gene. The sequences confirmed them as *Caligus elongatus* and showed that they belonged to genotype 1 (99.6-99.7 % identity with KT209134).

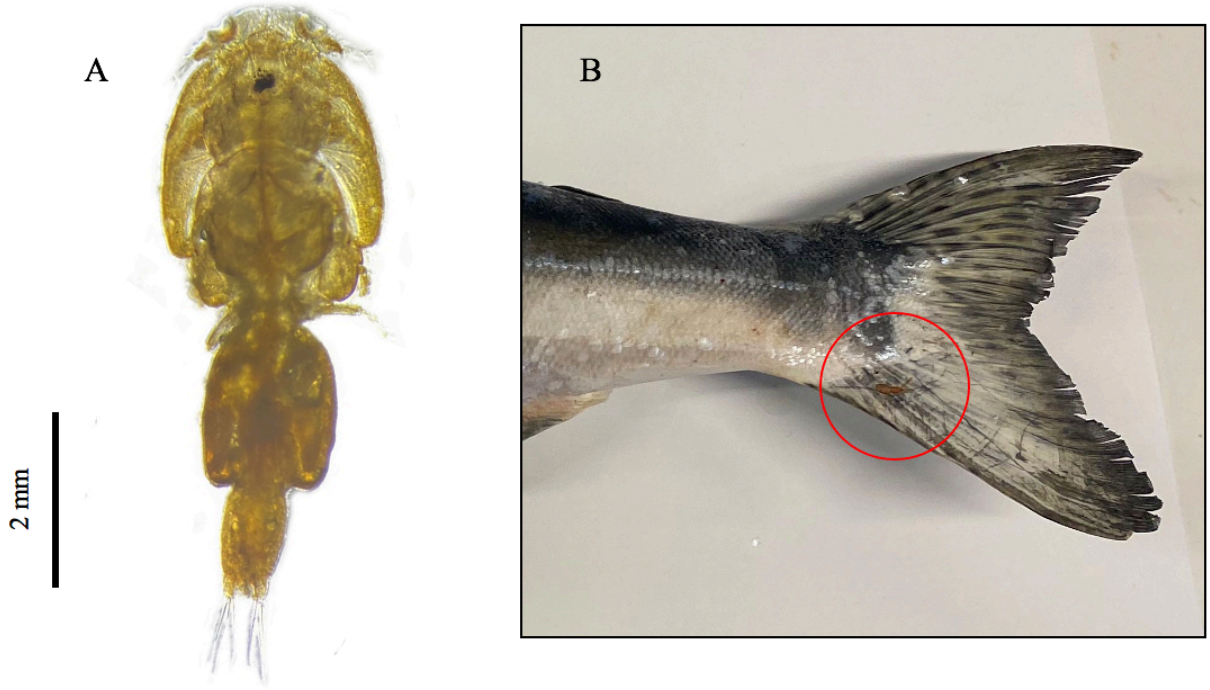

Figure 30 Female adult *Caligus elongatus* found on the tail fin of pink salmon. **A** Dorsal view of the louse (egg strings detached), **B** site on the tail fin marked with a red circle.

3.1.4 Microparasites

Parvicapsula pseudobranchicola

Psuedobranch samples from 55 fish (N 2013 = 1, N 2015 = 8, N 2017 = 32, N 2018 = 9, N 2019 = 5) were molecularly tested for the presence of the myxosporean parasite *Parvicapsula pseudobranchicola*. All samples were negative (Table 8).

***Ichthyobodo* spp.**

The gills from 30 fish (N 2013 = 1, N =2015 = 8, N 2017 = 7, N 2018 = 9, N 2019 = 5) were molecularly tested for infections with kinetoplastid flagellats from the genus *Ichthyobodo*. Alle samples were negative (Table 8). The positive control obtained from river-caught pink salmon (Etne) was positive for both *I. necator* and *I. salmonis*.

3.1.5 Viruses

Heart, gills and kidney samples from the 86 pink salmon were tested for the viral agents IHNV, ISAV, IPNV, SAV, PRV-1 and PMCV. Two pink salmon tested positive for piscine orthoreovirus-1 (PRV-1), with Ct-values of 20.9 and 35.4. No other viral agents were detected.

3.2 Importance of year class

To get a clearer picture of the parasite fauna throughout the life cycle of pink salmon, the year of capture was changed to year class i.e., the year the pink salmon should have spawned. Thus, the fish caught in December 2018 belongs to the 2019-year class.

The importance of year class on parasite abundance was difficult to establish, due to insufficient data and the influence of multiple variables. However, there were indications that year class was significant for the species *Derogenes varicus* (MLR, $P \leq 0.001$), *Lecithaster gibbosus* (MLR, $P \leq 0.05$), *Scolex pleuronectis* A (MLR, $P \leq 0.001$) and *Scolex pleuronectis* B (MLR, $P \leq 0.01$). *D. varicus* was very abundant in 2013, while *L. gibbosus, S. pleuronectis* A and *S. pleuronectis* B were more abundant in 2017.

3.3 Importance of fish mass

The most abundant parasite species *Derogenes varicus*, *Hysterothylacium aduncum* and *Anisakis simplex* showed stable abundance throughout the oceanic phase. The trematodes *Brachyphallus crenatus* and *Lecithaster gibbosus*, however, had a significant relationship to fish mass (both SLR, $P \leq 0.001$). The abundance of *B. crenatus* showed a negative correlation with increasing fish mass (Fig. 31), where the parasite was absent in fish > 1000 gram. The abundance of *L. gibbosus*, however, showed a positive correlation with increasing fish mass (Fig. 32).

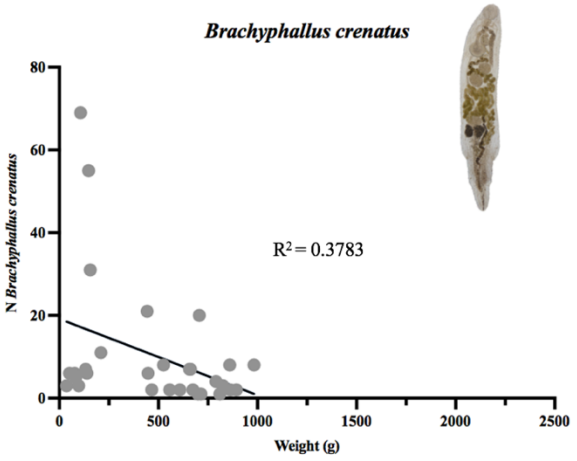

Figure 31 Number of the trematode *Brachyphallus crenatus* found in pink salmon relative to the weight of the fish.

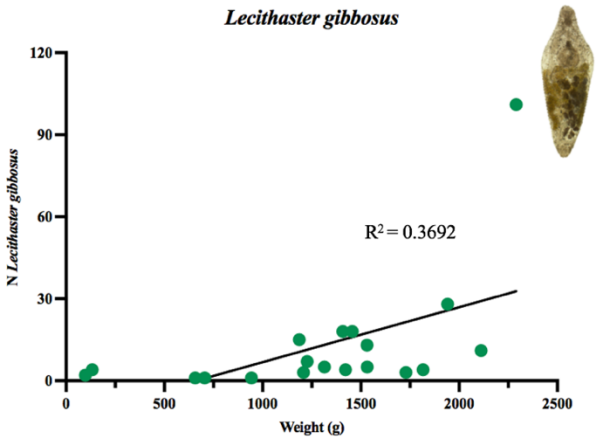

Figure 32 Number of the trematode *Lecithaster gibbosus* found in pink salmon relative to the weight of the fish.

3.4 Importance of sex

The abundance of the most common parasites was compared for males and females, showing no significant differences.

3.5 Importance of area of capture

The geographical distribution of the parasite species *Scolex pleuronectis* A (Fig. 33), *Scolex pleuronectis* B (Fig. 34) and *Lecithaster gibbosus* (Fig. 35) showed significant trends (MLR, $P \leq 0.001$, MLR, $P \leq 0.05$ and MLR, $P \leq 0.001$, respectively). Also, the species *Hemiurus levinseni*, *Scolex bothriosimplex* and the Bothriocephalidea juveniles were more abundant at more northern latitudes, but low numbers did not allow sensible analyses.

Scolex pleuronectis A

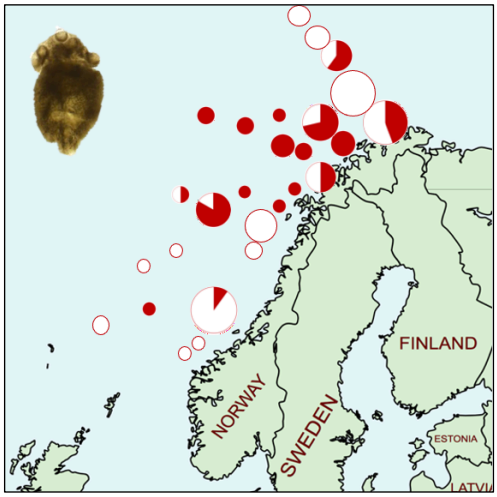

Figure 33 The geographical distribution of *Scolex pleuronectis* A found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of infected fish. Template reproduced from mapchart.net.

Scolex pleuronectis B

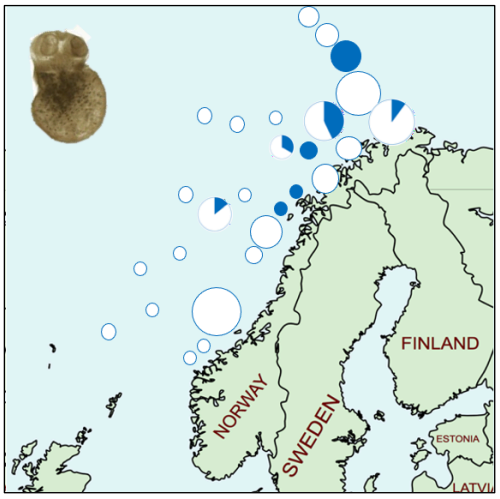

Figure 34 The geographical distribution of *Scolex pleuronectis* B found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of infected fish. Template reproduced from mapchart.net.

Lecithaster gibbosus

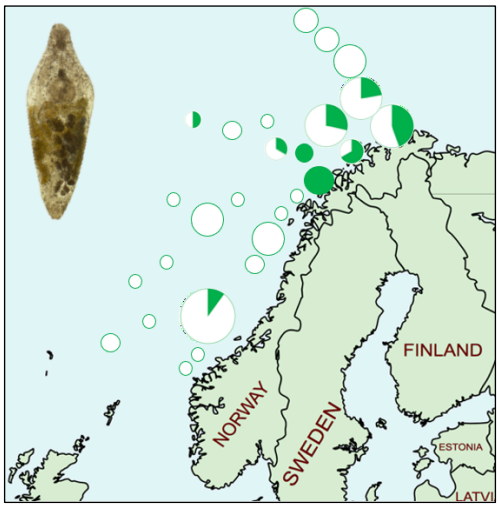

Figure 35 The geographical distribution of *Lecithaster gibbosus* found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of infected fish. Template reproduced from mapchart.net.

3.6 Pink salmon diet affecting parasite load

When examining the stomachs of the salmons for parasites the identifiable prey present was registered, since this may relate to the helminth parasite occurrence. Of the stomachs, 6 % were empty, 33 % scored as full, while the rest contained some prey. *Calanus* spp., krill, *Themisto* spp. and herring larvae (*Clupea harengus* L.) were the four prey species most frequently found.

The prey categories found are listed in table 10**.** Additionally, small pieces of plastic were found in two pink salmon. The three Atlantic salmon had relatively empty stomachs, only containing small amounts of *Calanus* spp. and *Themisto* spp.

Table 10 Prey found in the stomachs of the pink salmon with prevalence.

Prey category	**Common name**	**Proportion of all stomachs (%)**	**Proportion of stomachs with prey (%)**
Calanus spp.	Copepod	50	53
Euphausiacea	Krill	50	53
Themisto spp.	Amphipod	36	38
Clupea harengus larva	Herring larva	33	34
Thecosomata	Sea butterfly	20	21
Eurydice sp.*	Isopod	23	24
Gonatus spp.	Squid	7	7
Natantia	Shrimp	5	5
Myctophidae	Lanternfish	1	1

* Most similar to *Eurydice elegantula*.

When analysing the preys' relationship to parasite load, only the relationship between *Anisakis simplex* and krill was significant (MLR, $P \leq 0.01$) (Fig. 36). When investigating this further, the luminal larvae was the significant factor (MLR, $P \leq 0.0001$) and the encapsulated larvae was not. The relationship between krill and fish weight was not significant.

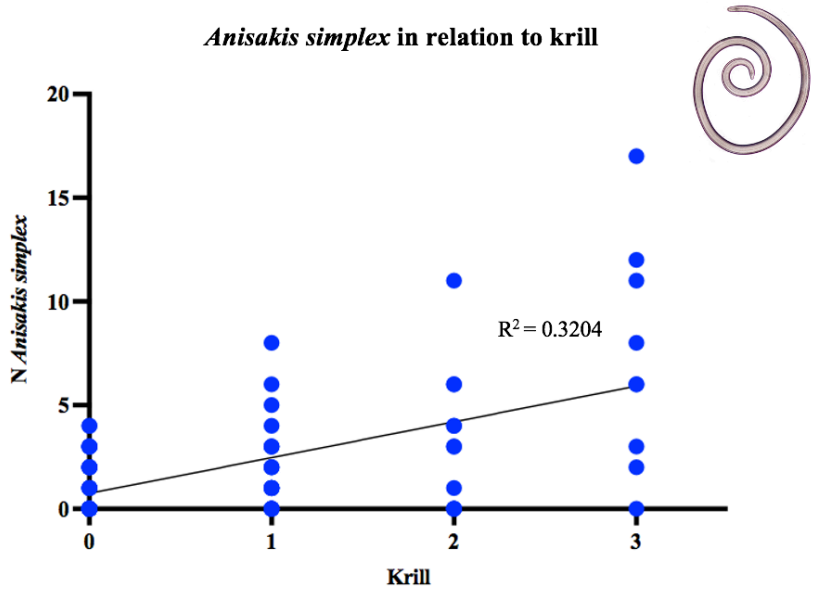

Figure 36 The relationship between the larval nematode *Anisakis simplex* and the prey krill found in pink salmon. Krill is presented as: 0 = not present, 1 = small amount, 2 = medium amount and 3 = large amount.

Statistically significant relationships between prey and area were found, specifically for *Gonatus* spp. (KW, $P \leq 0.0001$) (Fig. 37), krill (KW, $P \leq 0.0001$) (Fig. 38), herring larvae (KW, $P \leq 0.001$) (Fig. 39), isopods (KW, $P \leq 0.0001$) (Fig. 40), *Themisto* spp. (KW, $P \leq 0.0001$) (Fig. 41) and sea butterflies (KW, $P \leq 0.0001$) (Fig. 42).

Gonatus spp.

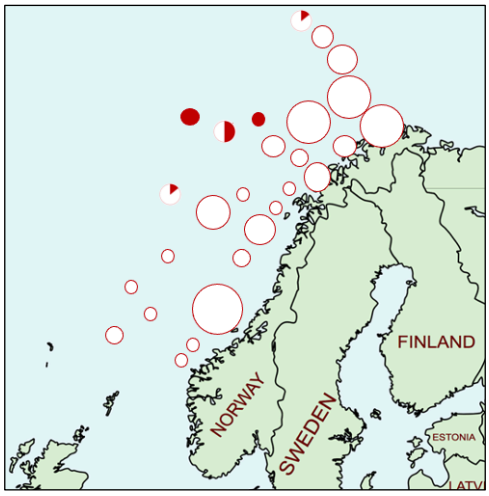

Figure 37 Geographical distribution of the prey *Gonatus* spp. found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of prey found. Template reproduced from mapchart.net.

Krill

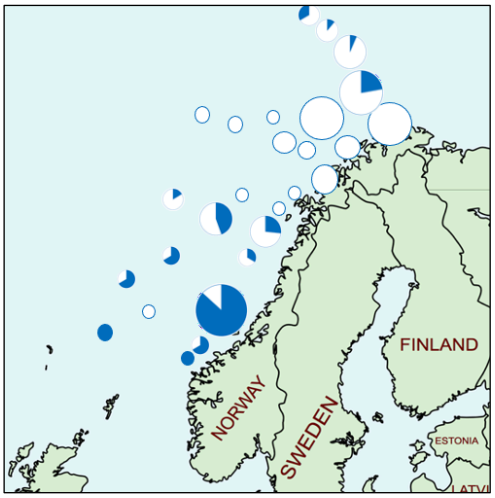

Figure 38 Geographical distribution of the prey krill found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of prey found. Template reproduced from mapchart.net.

Herring larva

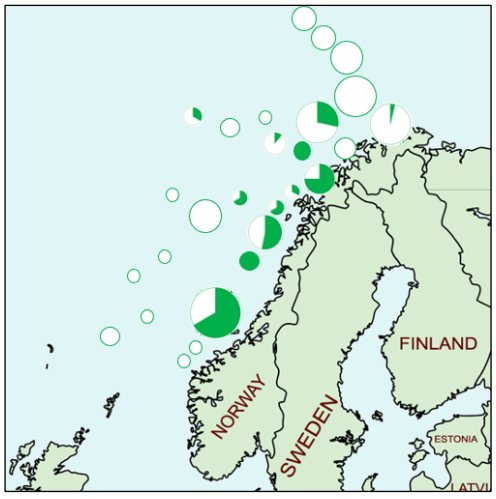

Figure 39 Geographical distribution of the prey herring larvae found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of prey found. Template reproduced from mapchart.net.

Isopod

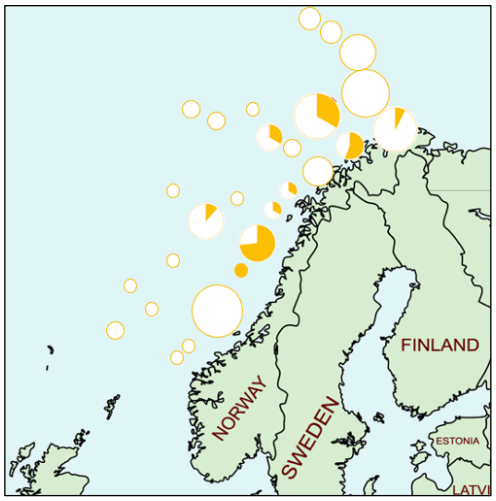

Figure 40 Geographical distribution of the prey isopod found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of prey found. Template reproduced from mapchart.net.

Sea butterfly

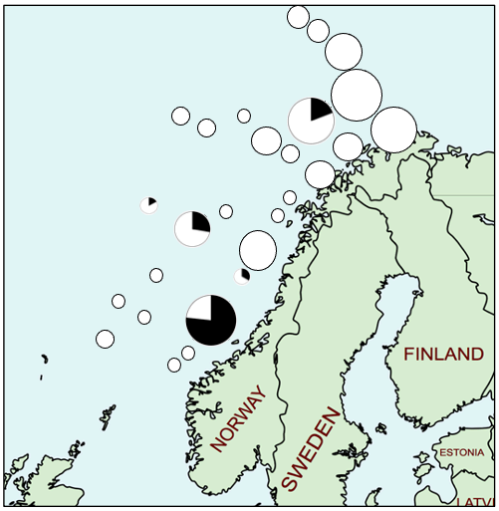

Figure 41 Geographical distribution of the prey sea butterfly found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of prey found. Template reproduced from mapchart.net.

Themisto spp.

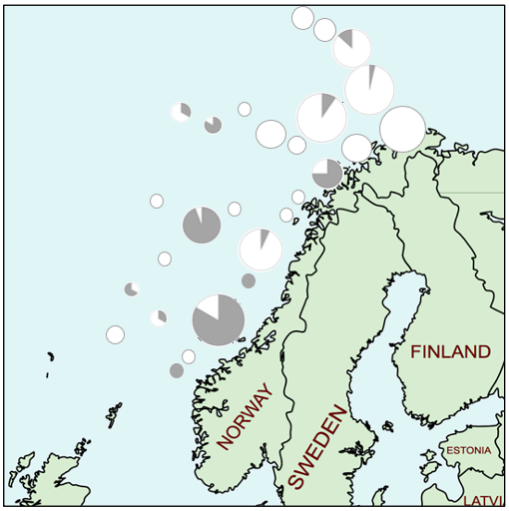

Figure 42 Geographical distribution of the prey *Themisto* spp. found in pink salmon. The size of the circles signifying amount of salmon caught and the colour inside representing ration of prey found. Template reproduced from mapchart.net.

3.7 Parasites in Atlantic salmon

The three Atlantic salmon received together with the pink salmon were also examined for parasites. They were infected with six parasite species (four to five each): the trematodes *Derogenes varicus* and *Brachyphallus crenatus*, the nematodes *Hysterothylacium aduncum* and *Anisakis simplex*, the cestode *Eubothrium crassum* (Bloch, 1779) and the crustacean *Lepeophtheirus salmonis* (Krøyer, 1837). All three fish were infected with immature *Eubothrium*, ranging 1.5 to 60 cm in length. One fish was infected with six salmon lice (*L. salmonis*) (one adult male, one preadult female and four chalimus II), despite extensive scale loss. The parasite data of the Atlantic salmon is listed in Appendix table 2.

4 Discussion

4.1 Discussion of the methods

The pink salmon was sampled by trawl in the Norwegian Sea, frozen and then thawed, before the parasite examination was conducted. The experimental design was not tailored for this study, since the pink salmon mainly was caught as bycatch and handled as such, making the samples suboptimal for parasite examination.

Firstly, the majority of the pink salmon were harvested the same year (2017) in the months of May and June. Furthermore, each year pink salmon was caught, they were concentrated in a geographical area, making relationships based on catch year and/or catch location challenging to ascertain. For example, of the 11 fish caught in 2013, 10 were caught at the same location (64°22'15.7"N, 7°02'51.4"E). These fish had high amounts of the trematode *Derogenes varicus* and because of the intermixing of the mentioned variables, a significant relationship was hard to determine.

Secondly, the trawling caused extensive scale loss on the pink salmon. Holst et al. (1993) found that scale loss was negatively correlated with the prevalence of the salmon louse *Lepeophtheirus salmonis.* Therefore, the prevalence of ectoparasites from pink salmon is not a reliable number and must be an underestimate.

Lastly, since the pink salmon were received frozen and had to be examined after thawing, a reactivation of DNAases in the parasite cells likely led to poor DNA-quality. The ability to obtain sequences varied, although this was always possible with freshly conserved parasites. Several measures were implemented to reduce this problem, well known from previous studies (Fjær, 2019; Haugsland, 2020). The amount of DNA was doubled in the reactions when DNA yield was low (see section 2.9). The parasites were also divided into pieces to ensure lysis, since many gastrointestinal parasites have a cuticula that resists digestion. When long amplicons could not be obtained, shorter ones were tried. These PCRs were sometimes successful, providing products that could be sequenced. However, the RNA samples from the heart, kidney and gills were of good quality, due to the samples being taken from the fish while still in a frozen state.

4.2 Parasites in pink salmon from the Norwegian Sea

A total of 13 marine macroparasite species were found in the 86 pink salmon examined. Previous studies of the parasite fauna of pink salmon in the North Atlantic have discovered 30 species (see table 1), including microparasites. Two pink salmon were also infected with Piscine orthoreovirus-1 (PRV-1).

Of the 13 parasite species, seven were found exclusively as larvae, five exclusively as adults and the last (*Hysterothylacium aduncum*) as both larvae and adults. The total amount of parasites found was 6617, 1170 of them being larvae. None of these species are specific to pink salmon and most have low host specificity, therefore termed generalists. The number of larvae demonstrate that pink salmon may act as an intermediate- or transport host, though not vital for the parasite species to complete their life cycles.

4.2.1 Platyhelminthes

The phylum platyhelminthes, or flatworms, includes three classes with obligatory parasites: Monogenea, Trematoda and Cestoda. A total of four trematode species and six cestode species were found, but no monogeneans. The parasites found have complex life cycles with several hosts involved.

Trematoda

Trematoda has two subclasses, Aspidogastrea and Digenea. All of the four species found to infect pink salmon were digeneans, and they all also belong to the superfamily Hemiuroidea. Hemiuroideans are parasitic in the gut, and especially stomach, primarily of marine teleosts (Gibson, 1996). As most other digenean trematodes these have complex life cycles, involving three obligatory hosts (Cribb et al., 2003). The first intermediate host is a gastropod, the second a crustacean such as zooplankton copepods, and the final host is fish (Gibson & Bray, 1986).

Embryonated eggs from hemiuroidean species are released with the fish feces and swallowed by gastropods, where it hatches to a larva called miracidium. The miracidium transforms to a mother-sporocyst which normally gives rise to a generation of rediae. Still inside the gastropod, the rediae produce a generation of cercariae. The cercariae is a tailed larva which leaves the gastropod and swims in the water. Cercariae grasped by copepods may inject the cercaral body into the copepod hemocoel, becomin a metacercaria (Gibson & Bray, 1986; Krupenko et al.,

2020; Køie, 1979, 1989; Køie, 1992). Small fish must acquire the hemiuroideans directly from feeding on zooplanktonic crustaceans, however, larger fish may acquire the parasite by preying upon plankton-feeders. The gastropod hosts are benthic, confining most life cycles to coastal waters and the continental shelves (Gibson & Bray, 1986).

Derogenes varicus

Derogenes varicus is parasitic in the stomach and oesophagus of marine teleosts (Gibson, 1996). The species is widely distributed and occurs in temperate and subartctic areas of both the Atlantic and Pacific Ocean. In both oceans it infects pink salmon (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Margolis, 1982b; Ninburg, 1963) but *D. varicus* has low host specificity and is recorded from more than 100 teleost species (Gibson, 1996; Køie, 1979, 2000).

The first intermediate host of *D. varicus* is certain snails, *Lunatia* spp., and the second intermediate hosts are copepepods, e.g., *Calanus* spp. Transport hosts can occur, such as chaetognaths (Rolbiecki & Walkusz, 2005). When reaching its final host the trematode normally lives free in the stomach (Køie, 1979). *D. varicus* from pink salmon caught at sea showed both high prevalence and abundance, reaching 95 %. The river-caught pink salmon also had high prevalence of the parasite (68-89 %) (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963).

The parasite is less prevalent in pink salmon from the North-Pacific, but Margolis (1956) found clear onshore-offshore differences, where the prevalence of *D. varicus* was higher in coastal waters. This suggests that the pink salmon primarily gets infected in the nursery areas as fry or as adults when feeding along the coast en route to the spawning grounds. If the trematode has a life span of more than a year, river-caught pink salmon is expected to have a higher prevalence and abundance of the parasite because of reinfection. This however is not the case, thus suggesting that *D. varicus* has a shorter life span.

A notable finding was the presence of the *D. varicus* in the swim bladder of twelve fish. This is an unusual site for the trematode, however, *D. varicus* is very active and can exit through the mouth and gills after host death, possibly because the stomach environment then becomes hostile (Karlsbakk, 1993; Køie, 2000). This phenomenon could cause some individuals to enter

the pneumatic duct. This strange site has rarely been observed but has been reported from Atlantic salmon (Pippy, 1969).

It has been suggested that *D. varicus* in fact is two species (Krupenko et al., 2021). The morpho- and genotype found in the present study is the same found in pink salmon by Fjær (2019) and in European eel found by Haugsland (2020).

Brachyphallus crenatus

Brachyphallus crenatus usually occurs in the stomach of marine teleosts (Gibson, 1996). Many teleost species serve as final hosts and the trematode may survive in anadromous salmonids when these migrate to freshwater. *B. crenatus* is most frequently found in salmonids, clupeids, gasterosteids and pleuronectids (Gibson & Bray, 1986; Køie, 1992). The trematode has an Arctoboreal distribution and is found in both the North Atlantic and the North Pacific Ocean (Gibson & Bray, 1986; Køie, 1992). In both oceans it is a common parasite of pink salmon (Barskaya et al., 2005; Grozdilova, 1974; Ieshko et al., 2016; Margolis, 1982b; Ninburg, 1963)

The life cycle of *B. crenatus* involves at least three hosts. The mollusc *Retusa obtusa* (Montagu, 1803) has been reported as the first intermediate host and the known second intermediate hosts are certain calanoid copepods (*Acartia* spp.), occurring in coastal waters. Copepod-feeding predators in the plankton (e.g., *Sagitta* spp., *Pleurobrachia pileus* (Müller, 1776)) may act as transport intermediate hosts (Køie, 1992).

The prevalence of *B. crenatus* in pink salmon caught in the Norwegian Sea was 35 % and the trematode had a mean abundance of 3.6. After sea entry, the juvenile pink salmon may become infected with this trematode in inshore nursery areas, but as they grow and leave the coast recruitment likely ceases. There was a decrease in abundance with increasing fish mass and the parasite was completely absent in fish over 1000 grams. This is strong circumstantial evidence for a short lifespan of this trematode, likely being less than a year. Fjær (2019) only found two specimens of *B. crenatus* and believed that the parasite could have been lost during the sea-phase. Margolis (1982) observed that pink salmon fry rapidly acquired marine species during their passage through coastal waters, including *B. crenatus.* This species increases in abundance as the adult pink salmon returns back to the shore. The few specimens seen by Fjær (2019) could have been acquired by the adults in coastal waters.

Along the European coast, this trematode occurs from Øresund to Skagerrak, but is rare along the coast of western Norway to Finnmark. In Finnmark and eastwards to Novaja Semlja, the Pechora Sea and the White Sea this trematode occurs in inshore waters, but abundance seems highest in the White Sea (Fjær, 2019). Hence, the parasitological evidence from this parasite suggests that more than a third of the pink salmon could originate from nursery areas in Finnmark and eastwards.

Hemiurus levinseni

Hemiurus levinseni is a stomach parasite of marine teleosts (Gibson, 1996; Gibson & Bray, 1986). The common final hosts are gadoids but the trematode is also found in salmonids and other plankton-feeding fish (Gibson & Bray, 1986). This Arctoboreal species is present in both the Atlantic and Pacific oceans and is the only species from the genus *Hemiurus* reported from the White Sea (Gibson & Bray, 1986; Krupenko et al., 2020).

The gastropod *Cylichna alba* (Brown, 1827) has been found to be the first intermediate host of *H. levinseni* and copepods serve as second intermediate hosts (Krupenko et al., 2020). The chaetognath *Sagitta elegans* Verrill, 1873 is a transport host that can also harbour progenetic worms (Gibson & Bray, 1986; Krupenko et al., 2020). Fish gets infected by preying on copepods, chatognaths or other fish.

H. levinseni is prevalent and abundant in Atlantic cod, *Gadus morhua* L. from Troms og Finnmark and northwards in the Barents Sea (Hemmingsen et al., 2000; Løvland, 2017; Sobecka et al., 2011). The parasite is less commonly found along the Norwegian coast south of Troms og Finnmark (Hemmingsen & Mackenzie, 2013). Løvland (2017) found a negative correlation between the abundance of *H. levinseni* and fish mass, suggesting that the parasite is short-lived.

The trematode has been recorded from several *Oncorhynchus* species including pink salmon (Gibson, 1996). *H. levinseni* is common in pink salmon from the North Pacific and the fish acquire the parasite upon entry into estuaries or passage through coastal waters (Margolis, 1982b). The parasite was present in the pink salmon caught in the Norwegian Sea, where they could not become infected. This suggests that they likely acquired the trematode in the nursery areas in northern Norway or northwestern Russia as juveniles, or while feeding in the Barents Sea.

Lecithaster gibbosus

Lecithaster gibbosus is parasitic in the intestine of marine teleosts (Gibson, 1996). The trematode has a wide distribution and is common in the North Atlantic and North Pacific Ocean (Gibson, 1996). Also, *L. gibbosus* has a low host specificity and parasitize most teleost families, including Clupeidae, Salmonidae, Gadidae and Pleuronectidae (Køie, 1989). The trematode is common in pink salmon from both the North Pacific and the North Atlantic Ocean (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Margolis, 1982b; Margolis & Boyce, 1969; Ninburg, 1963).

The first intermediate host of *L. gibbosus* is the pyramidellid opisthobranch *Brachystomia eulimoides* (Hanley, 1844) and the second hosts are calanoid copepods, like *Acartia* spp. (Køie, 1989). The final host can be planktophagous, benthophagous and piscivorous fish. The latter indicate that the trematode can be transferred from one fish to another (prey-predator) and accumulate (Køie, 1989). The fish gets infected in the coastal areas (Margolis & Boyce, 1969), herring feeding in the Norwegian Sea are never infected (Tolonen & Karlsbakk, 2003). The trematode is common in returning Atlantic- and pink salmon (Bristow et al., 1996; Margolis, 1956; Margolis & Boyce, 1969; Pippy, 1969), which could have acquired the trematodes in the Arctic or boreal coastal areas or in the Barents Sea (Gibson, 1996; Polyanski, 1955).

L. gibbosus has been found to be short lived, only five to eight months in pink and chum salmon, *Oncorhynchus keta* (Walbaum, 1792) (Margolis & Boyce, 1969). In the present study, the abundance of *L. gibbosus* increased with fish mass, which suggests a continuous accumulation of the short-lived parasite, only possible in coastal areas or shallow seas such as the Barents Sea. The prevalence in this study was 22 % but the river-caught pink salmon in western Norway and northwest Russia had prevalences from 67 to 82 % (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963).

Cestoda

The cestodes are obligate, internal parasites that mature and sexually reproduce in the digestive tract of vertebrates. The cestodes have a wide range of body forms but are in general made up of a body called the strobila, consisting of several segments, or proglottids, each containing one or two sets of hermaphroditic genetalia, and a scolex at the anterior end. The mature proglottids can be egg-bearing and are then called gravid (Kuchta et al., 2015; Scholz & Kuchta, 2017).

The life cycles of many cestodes are well known, however, there is still some uncertainty regarding the development and transmission of some orders (Scholz & Kuchta, 2017). In general, fish can be the final-, transport- or intermediate hosts of cestodes. When being the intermediate- or transport host the plerocercoid larvae can occur as free in the intestine or encapsulated in the tissue, depending on the cestode-group and life cycles. A total of five cestode species were found in the pink salmon, all being plerocercoids.

Bothriocephalidea juveniles

Bothriocephalidea is a species-rich order which mature in freshwater and marine teleosts (Caira & Jensen, 2017; Caira et al., 2017; Kuchta et al., 2008; Scholz & Kuchta, 2017). Most species have strict host specificity as adults, being known from only one fish host (Caira & Jensen, 2017; Scholz & Kuchta, 2017).

The juvenile specimens found in pink salmon had clear segmented strobila and resembled juveniles of *Eubothrium crassum* previously found in river-caught pink salmon and in eels (Fjær, 2019; Haugsland, 2020). However, the present juveniles had no apparent scolex. The specimens were molecularly verified as a species of *Clestobothrium*. The genus *Clestobothrium* consists of five species (Świderski et al., 2013). The specimens found in pink salmon had identities closest to *C. crassiceps*, *C. splendidum* Gil de Pertierra, Incorvaia & Arredondo, 2011 and *C. cristinae* Gil de Pertierra, Incorvaia & Arredondo, 2011, parasites of hakes (*Merluccius* spp.). Since the latter two species infects hakes occurring in the southern hemisphere only, the juveniles in pink salmon from the Norwegian Sea should be those of *C. crassiceps* from European hake, *M. merluccius*.

The life cycle is not known in detail but a typical bothriocephalidean cycle is likely (Draoui & Maamouri, 1997; Scholz & Kuchta, 2017), with prey fish containing free plerocercoids in the intestine. This is the first time a fish has been recorded as an intermediate- or transport host for *C. crassiceps*, and this finding in pink salmon suggests it can act as such.

***Diphyllobothrium* spp.**

The main vertebrate host groups of species in the order Diphyllobothridea are homeotherms, mammals and birds (Caira et al., 2017). The diphyllobothridean species have a copepod as the first intermediate host and a vertebrate as the second intermediate host, in aquatic environments

being fish. Most species from the genus *Diphyllobothrium* parasitize pinnipeds but some infect whales (Caira & Jensen, 2017; Kuchta et al., 2015).

The specimens found in the pink salmon from the Norwegian Sea were plerocercoids encapsulated in the stomach wall, belonging to two genotypes. Sequence identity suggests that most of them belong to a species utilizing Arctic seals as the final host, but the species cannot be identified. Eight *Diphyllobothrium* species parasitize Arctic seals, including *D. schistochilos* (Schaeffner et al., 2018). One specimen from pink salmon showed 100 % identity to *D. schistochilos*, previously found as plerocercoids in the stomach wall of *Leptoclinus maculatus* (Fries, 1838) from the Barents Sea (Fjær, 2019; Karlsbakk, unpublished). This species infects the bearded seal (*Erignathus barbatus* (Erxleben,1777) and the ringed seal (*Pusa hispida*) in Arctic waters (Schaeffner et al., 2018).

Diphyllobothrium plerocercoids have previously been found in North Atlantic pink salmon (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963). The findings were reported as plerocercoid B by Ninburg (1963), Cestoda l. gen sp. from Barskaya (2005) and Ieshko (2016) and Diphyllobothriidae gen sp. by Grozdilova (1974). Ninburg believed that the plerocercoids were of freshwater *Diphyllobothrium* spp. (now *Dibothriocephalus* spp.), while Grozdilova argued that they were marine species with pinnipeds as the final host. The present observations support the latter view, since the plerocercoids found were related to pinniped tapeworms. The exact identity is still unknown, pending sequence information from the mature adult worm.

Scolex bothriosimplex

Scolex bothriosimplex is a collective term for a morphotype of cestode larvae, found free in the intestine of marine fishes. The larva is characterized by a large and muscular apical sucker, often 70 to 119 µm in diameter (Hoberg, 1987). This morphology is consistent with the larvae found, with apical suckers ranging from 101 to 119 µm in diameter.

The larva has previously been described from herring (Reimer, 1970), capelin (Palsson & Beverley-Burton, 1984) and pink salmon (Fjær, 2019). A similar cestode larva was also found in seabirds by Hoberg (1987) and was thought to belong to the genus *Tetrabothrius.* Fjær (2019) confirmed that *S. bothriosimplex* from Antarctic fishes indeed was of a *Tetrabothrius* sp., and

the specimens collected from pink salmon in the present study showed closest identity to *Tetrabothrius* sp. from short-tailed shearwater (*Puffinus tenuirostris*).

Hence, fish could indeed be involved in the life cycle of *Tetrabothrius* spp. assumed to involve three hosts, where copepods likely serves as the first intermediate host, teleosts as the second intermediate host or transport host, and an avian as the final host (Caira & Jensen, 2017; Hoberg, 1987). However, the adult *Tetrabothrius* sp. involved in the life cycle of the pink salmon parasite could not be identified, as the GenBank has no closely matching sequences.

Scolex pleuronectis

Scolex pleuronectis is a collective term used for plerocercoids in the past order Tetraphyllidea, which is now split into several orders (Caira & Jensen, 2017). The morphology of the plerocercoids varies greatly, however they all share a characteristic scolex with five distinct structures: four bothridia and one apical sucker. The bothridia each have one to four chambers (loculi) (Caira & Ruhnke, 1991).

The life cycle of these tapeworms involves at least three hosts. The first intermediate host is a crustacean, the second intermediate hosts are usually teleosts or cephalopods, and the final hosts are elasmobranchs (Caira & Jensen, 2017). Because of the morphological differences of the plerocercoids and adult cestodes, knowledge about the full life cycle of "tetraphyllidean" species is insufficient (Caira & Ruhnke, 1991). However, through molecular identification species are now being identified.

The species found in pink salmon had clear bilocular bothridia and resembled the plerocercoids found in river-caught pink salmon by Fjær (2019), called type A and B. As found by Fjær (2019), the large type A was here identified as the phyllobothriidean *Clistobothrium* sp., a likely parasite of the shark *Lamna nasus.* This species is likely the same referred to as *Phyllobothrium caudatum* by Margolis (1982), common in pacific salmon and often occurring in oceanic fish and squids. Fjær (2019) found that type B-specimens represented *Phyllobothrium piriei* Williams, 1968, a phyllobothriidean infecting cuckoo ray (*Leucoraja naevus* (Müller & Henle, 1841) (Ruhnke, 2011) in the North Sea. This interesting finding connects it to the British isles or southward, since the ray is known there but is not known from Norway or northern regions (Maia et al., 2012; Walker & Hislop, 1998). However, sequence identification of the present

specimens did not succeed, hence the occurrence of this parasite in ocean-caught pink salmon cannot be confirmed.

4.2.2 Nematoda

Most parasitic nematodes are endoparasites, that have complicated life cycles. In aquatic environments, an invertebrate act as the first intermediate host harbouring larval stages. Often the food chain is used, so larvae can reestablish in new hosts (second intermediate or transport hosts). Most final hosts are vertebrates, becoming infected by eating invertebrates or other vertebrates harbouring larvae (Berland, 2006). The nematodes have five stages in their life cycle, where the first three are larval stages (L1-3), the fourth is a preadult stage and the fifth is the adult stage. These stages are separated by moulting of the cuticula (Køie et al., 1995). In the ascaridoid nematodes, a particularly common group, there are two moults in the egg, so third stage larvae hatch (Køie, 1993; Køie et al., 1995).

Fish can serve as intermediate or final host, or both. In an intermediate host, the larva usually bore through the gut wall and become encapsulated in the wall, in the body cavity, on the viscera or in the musculature. In a final host, the nematodes live free in the gut (Berland, 2006; Køie, 1993). Two nematode species were found in the examined pink salmon, from the ascaridoid families Raphidascarididae and Anisakidae.

Hysterothylacium aduncum

Hysterothylacium aduncum is a raphidascaridid that lives as sexually mature adults in the digestive tract of marine teleosts, particularly in gadoids, and are very abundant in marine fishes in temperate and cold waters in the North Atlantic, including the coast of Norway (Berland, 1961; Køie, 1993; Setyawan et al., 2019).

The life cycle of *H. adundum* involves a crustacean (normally a copepod) obligate first intermediate host, and may involve non-crustacean invertebrates or fish as obligate intermediate or transport hosts (Berland, 2006; Køie, 1993). The number of intermediate hosts depends on the size of the larva when ingested. Køie (1993) found that larvae less than 2.0 mm long did not survive in fish and larvae between 2.0 to 3.0 mm re-established in the tissues as encapsulated third-stage larvae in the fish. Larger larvae (> 3.0 mm) moult into fourth-stage larvae in the intestinal lumen of the fish. Hence, when fish ingest infected crustaceans, they could become either intermediate or final hosts, depending on larva size.

All ocean-caught pink salmon were infected with *H. aduncum*. Of the larvae, 83 % were luminal. Large larvae develop in *Calanus* spp. and krill (Svendsen, 1990), important prey items of pink salmon, that can explain the dominance of luminal larvae. The encapsulated larvae could represent worms acquired when juveniles are in the nursery areas, where small sized prey harbouring small *H. aduncum* are much more likely. The river-caught pink salmon from western Norway also had high prevalence of the nematode (83 %) (Fjær, 2019), though less than the pink salmon caught at sea.

Fish are infected in the coastal areas and salmonids are known to harbour the parasite, both as intermediate and final hosts (Berland, 1961; Setyawan et al., 2019). Farmed Atlantic salmon in Norway may become infected after sea transfer while feeding on zooplankton, but these infections have disappeared after 5 to 6 months (Ruud, 2019), suggesting a quite short life span in the final host. The pink salmon likely start to lose nematodes shortly after they stop feeding.

Anisakis simplex

Anisakis simplex is an anisakid, all of which mature in homeotherms. The final hosts to *A. simplex* are cetaceans where the nematodes live free in the digestive tract. Large planktonic crustaceans are the first intermediate host, mostly krill. Fish may serve as transport or intermediate hosts (Kent et al., 2020; Klimpel et al., 2004), where *A. simplex* encapsulates as a characteristic coil in the body cavity or in the muscle (Berland, 1961). The nematode can accumulate in piscivorous fish before reaching its final host. *A. simplex* has a low host specificity and have an Arctoboreal distribution. The nematode is reported from a wide spectrum of fish from most oceans (Kent et al., 2020; Klimpel et al., 2004)

Krill is an important prey to salmonids in the oceanic feeding areas, where also whales are present. Hence this parasite can fulfill its life cycle in the ocean (Tolonen & Karlsbakk, 2003), unlike many of the other parasites found in pink salmon. Therefore, an increasing number of *A. simplex* is expected with time spent in the ocean. The prevalence in river-caught pink salmon from western Norway, however, was lower than that of the ocean-caught pink salmon (Fjær, 2019).

The nematode is common in marine fishes off the coast of Norway and is almost always found encapsulated (Berland, 1961; Fjær, 2019; Klimpel et al., 2004). However, in the studied pink salmon an uncommonly high ratio of the *A. simplex* larvae were found in the intestine. This

could be due to recent ingestion of infected prey. Indeed, a correlation with the amount of krill present in the stomachs supports this (see also section 4.5.3).

4.2.3 Crustacea

The subphylum Crustacea harbour several several groups with fish parasites. Parasitic copepods are common on farmed and wild marine fish, and feed on their mucous, tissues and blood (Johnson et al., 2004). The most known and damaging copepods in salmon acuaqulture is the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus.* In Norway, sea lice are one of the major challenges in Atlantic salmon aquaculture with huge economic impact, given the injuries they cause and the costs of treatments (Liu & vanhauwaer Bjelland, 2014; Sommerset et al., 2021).

Pink salmon in the northern Pacific harbour marine copepods such as *L. salmonis* and *C. clemensi* Parker & Margolis, 1964 (Margolis, 1982b). In the North Atlantic, pink salmon can be infected with *L. salmonis* (Fjær, 2019; Grozdilova, 1974; Ninburg, 1963), but *C. elongatus* has never previously been reported from this species.

Caligus elongatus

The caligids *Caligus elongatus* and *Lepeophtheirus salmonis* differ in their host specificity, the latter is salmonid specific while the former has been reported from more than 80 fish species. The geographical distribution of *C. elongatus* is restricted to the North Atlantic (Kabata, 1992; Øines & Heuch, 2007; Øines & Heuch, 2005). The sea louse is common on wild fish in Norway, including salmonids (Bristow et al., 1996; Heuch et al., 2007; Schram et al., 1998).

Pink salmon may get infected by copepodids, which complete their life cycle and become adults on its host, like on Atlantic salmon and sea trout. The motile adult *C. elongatus* is very active and can jump over to another host if preferable (Paulsen, 2018). Therefore, pink salmon could acquire adult *C. elongatus* when in contact with infected fish.

There are two genotypes of *C. elongatus*, type I and II. Both genotypes have a broad host spectrum but the species *Cyclopterus lumpus* L. seem to only harbour genotype I. When investigating *C. elongatus* found on farmed salmon in northern Norway, all was of genotype I (Øines & Heuch, 2007).

This study is the first to report the sea louse *Caligus elongatus* from pink salmon. Two lice on two separate fish despite extensive scale loss due to trawling suggest that the true prevalence and abundance is higher. The same could apply to *Lepeophtheirus salmonis*, which was not observed here.

4.2.4 Viruses

Viral infections are a threat to wild salmonids in Norway, especially in connection with disease outbreaks in Atlantic salmon farms in the fjords. The three most frequent viral diseases in farmed Atlantic salmon are Cardiomyopathy syndrome (CMS) caused by Piscine myocarditis virus (PMCV), Heart and skeletal muscle inflammation (HSMI) caused by Piscine orthoreovirus (PRV) and Pancreas disease (PD) caused by Salmonid alphavirus (SAV) (Sommerset et al., 2021). There has also been an increase in cases of the serious disease Infectious salmon anemia (ISA) caused by Infectious salmon anemia virus (ISAV) (Sommerset et al., 2021).

There have previously been conducted virus analyses on mature river-caught pink salmon in Norway (Fjær, 2019; Garseth et al., 2020; Skjåvik, 2008) (Table 11). Only PRV-1 has ever been detected in North Atlantic pink salmon (Garseth et al., 2020). This virus was also detected in the present study, with a prevalence of 2 %. In the study by Garseth et al. (2020), 171 Atlantic salmon and 16 sea trout caught along the Norwegian coast were tested for viruses. The Atlantic salmon were positive of PMCV (0.6 %), PRV-1 (3 %) and PRV-3 (0.6 %), while the sea trout were infected with PRV-3 (38 %).

Table 11 Overview of previous virus analyses from North Atlantic pink salmon. Except for the present study, all pink salmon were river-caught.

Locality	**Tana river and Neiden river, Troms og Finnmark**	**Etne river, Vestland**	**Karpelva river, Troms og Finnmark**	**Norwegian Sea**
Year (n)	2007 (74)	2017 (40)	2019 (60)	2013-2019 (86)
Reference	Sjåvik, 2008	Fjær, 2019	Garseth et al., 2020	The present study
Viral agent	P (%)	P (%)	P (%)	P (%)
Infectious pancreatic necrosis virus (IPNV)	0	0	-	0
Infectious salmon anaemia virus (ISAV)	0	0	-	0
Hematopoietic necrosis virus (IHNV)	0	0	-	0
Piscine orthoreovirus (PRV)	-	0	7	2
Salmonid alphavirus (SAV)	-	0	0	0
Piscine myocarditis virus (PMCV)	-	0	0	0
Atlantic salmon calicivirus (ASCV)	-	-	0	-

P – prevalence.

Piscine orthoreovirus-1 (PRV-1) attack salmonid erythrocytes and cause infection of myocytes and inflammation of the heart and red skeletal muscle. PRV-1 is the causative agent of Heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (Dhamotharan et al., 2019). The virus is ubiquitous and often non-pathogenic (Garseth et al., 2013).

Madhun et al. (2018) found that the prevalence of PRV in sea-caught returning adult Atlantic salmon increased with sea-age, suggesting that the virus is transmitted in the ocean. This was also supported by Teffer et al. (2021). Hence, the pink salmon could have become infected independent of any aquaculture activities, in the ocean. Since PRV infections are common in wild salmonids in Norway, pink salmon is not viewed as a threat in regard to transmission of this virus.

4.3 Comparison with river-caught pink salmon

In the Atlantic, the parasite fauna of river-caught pink salmon has previously been studied in Norway (Fjær, 2019) and northwestern Russia (Barskaya et al., 2005; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963) (Table 1). This present study is the first to examine pink salmon caught in the oceanic feeding grounds in the North Atlantic Ocean.

The dominating parasite species of this study were also prevalent in river-caught pink salmon. Overall, the most abundant species were *Derogenes varicus*, *Lecithaster gibbosus*, *Brachyphallus crenatus*, *Scolex pleuronectis* and *Hysterothylacium aduncum*. The trematode *B. crenatus* was very prevalent in the studies from northwest Russia (52–71 %) and also present in pink salmon from the Norwegian Sea, with a prevalence of 35 %. It was however rare in river-caught fish from western-Norway. The anorexia and atrophy of the digestive system of mature pink salmon is likely to cause parasite loss among luminal species deriving nutrients from the gut fluid. However, high intensities with trematodes *Derogenes varicus* and *Lecithaster gibbosus*, in addition to other helminths such as *Scolex pleuronectis*, do not support this assumption. A short life span of *B. crenatus* in the fish seems to explain this difference, and that trematodes obtained as juveniles in the nursery areas are lost prior to river entry. There is no acquisition of the parasite in the oceanic waters and hardly any in western Norway where the parasite is rare. However, in the White Sea and Barents Sea coastal areas this trematode may be common, allowing high intensities in the returning pink salmon (Grozdilova, 1974; Ieshko et al., 2016). The fish acquire further infections or get reinfected before entering the rivers, as also seen in Atlantic salmon (Muladal, 2001)

The trematode *Hemiurus levinseni* was detected in pink salmon from Keret river, emptying to the White Sea (Barskaya et al., 2005; Ieshko et al., 2016). It also occurred in pink salmon from the Norwegian Sea, though with low prevalence. The species was not found in pink salmon ascending western Norwegian rivers (Fjær, 2019). *H. levinseni* is reported from gadids with high prevalence and intensity in Greenland (Køie et al., 2008), Svalbard (Sobecka et al., 2011), northern Norway (Hemmingsen et al., 1991; Hemmingsen & Mackenzie, 2013) and northwest Russia (Krupenko et al., 2020), suggesting a northern preference like *B. crenatus*. Hemmingsen & Mackenzie (2013) found a significant relationship between the prevalence of *H. levinseni* and latitude along the Norwegian coast, with higher prevalence in the north. These patterns therefore could reflect that at least 10 % of the pink salmon from the Norwegian Sea have a northern origin. The trematode species *B. crenatus* and *H. levinseni* have a combined prevalence of 43 %, suggesting that almost half of the pink salmon can be traced back to likely nursery areas in Finnmark and northwestern Russia. The absence or rarity of these trematodes in river-caught pink salmon from western Norway is probably due to parasite loss in the oceanic phase, and no acquisition in the coastal areas of southern Norway.

On the other hand, two other species, *H. luehei* and *H. communis*, were only found in pink salmon from rivers in Norway. *H. luehei* is a parasite of clupeids, especially herring, occurring along the Scandinavian coasts north to the Baltic Sea (Køie, 1984; Setyawan et al., 2019; Tolonen & Karlsbakk, 2003), that may survive in predators eating herring. The species is known in herring from the coast of Finmark but does not occur in the Barents and White seas (Muladal, 2001; Polyanski, 1955; Polyanski & Kulemina, 1963). *H. communis* is common in teleost species in the boreal region of the North-East Atlantic, from the British Isles and the western Baltic Sea to about Lofoten in Norway (Køie, 1995). Hemmingsen & Mackenzie (2013) also found a significant relationship between the prevalence of *H. communis* and latitude along the Norwegian coast, with lower prevalence in the north. The few *H. luehei* and *H. communis* found by Fjær (2019) may originate from prey by the adult pink salmon in coastal waters en route to the rivers in western Norway.

Pink salmon from the rivers in western Norway showed infection with the trematode *Cryptocotyle lingua* encysted as metacercaria in the skin. This species was not detected in the fish from the Russian rivers. The trematode is a common parasite in the coastal waters of Europe and North America, where the first intermediate host *Littorina* spp. occurs. Cercariae released from the snails penetrate the skin of fish (Lysne et al., 1998). The pink salmon migrating to rivers in western Norway may have been exposed to cercariae there, causing the infections with characteristic black spots (Fjær, 2019). The present study supports this, since none of the fish were infected, rendering it unlikely that they acquire infections in the nursery areas. This is further supported by the tendency of the cercaria to leave the mollusc more frequently when the water temperature reaches 10 to 12 °C (Sindermann & Farrin, 1962), when the pink salmon juveniles already have emigrated out to sea. In addition, *C. lingua* is not as common in the Barents and White sea areas (Galaktionov & Bustnes, 1999).

The river-caught pink salmon harboured the cestode *Eubothrium crassum,* though only juveniles were found (Barskaya et al., 2005; Fjær, 2019; Grozdilova, 1974; Ieshko et al., 2016; Ninburg, 1963). The species is prevalent (both juveniles and adults) in returning Atlantic salmon in Norway and northwest Russia (36-100 %) (Bristow & Berland, 1991; Bristow et al., 1996; Muladal, 2001), which suggests that pink salmon may only serve as an intermediate-, transport- or accidental host of the cestode. Since the bothroiocephalidean plerocercoids detected in the ocean-caught pink salmon were *Clestobothrium crassiceps* and not *E. crassum*, it seems likely that pink salmon migrating to the coast become infected there while still feeding.

Some of the river-caught pink salmon were infected with salmon lice (*Lepeophtheirus salmonis*) and *Salmincola salmoneus* (L.). The absence of *L. salmonis* in the ocean-caught pink salmon can partly be explained by extensive scale loss and therefore likely parasite detachment. However, the occurrence of the freshwater parasite *S. salmoneus* could be expected since it parasitizes the gills and survives in the marine phase (Kusterle et al., 2012). Ninburg (1963), Grozdilova (1974) and Fjær (2019) all found *S. salmoneus*, with a prevalence of 92, 22 and 3 %, respectively. As in Atlantic salmon, there may be large differences with rivers of origin (Kusterle et al., 2012), so occurrence may be very unpredictable. Of the fish Fjær (2019) examined, three of them carried freshwater parasites, specifically two infected with *S. salmoneus* and one with *Apatemon gracilis* (Rudolphi, 1819). Given the low frequency of freshwater parasite findings by Fjær (2019), no significant difference to the present study is suggested.

Kinetoplastid flagellates *Ichthyobodo* spp. had a prevalence of 88 % in gill samples from pink salmon from Etne river, western Norway (Fjær, 2019). They were later determined to be *Ichthyobodo salmonis* and *I. necator* (Karlsbakk et al., 2020). *I. necator* is a freshwater species so infections must have been acquired after river entry. However, *I. salmonis* is euryhaline and can infect Atlantic salmon also in the sea. Hence, it is possible that *I. salmonis* infections are acquired by juvenile pink salmon or in the oceanic phase. However, this parasite was not detected in pink salmon from the ocean, so the infections detected in pink salmon by Fjær (2019) were likely acquired during migration in the fjords, where farmed Atlantic salmon is also a possible source (Gunnarsson et al., 2017).

A similar case is posed by the myxosporean parasite *Parvicapsula pseudobranchicola*, found at a prevalence of 7 % by Fjær (2019) through PCR testing of the pseudobranchs. Again, this parasite was not detected in the ocean-caught pink salmon, supporting the hypothesis of Fjær that she detected recent infections acquired during the migration in the fjords. In northern Norway and likely in Russian regions along the Barents Sea, farmed salmonids may become infected by *P. pseudobranchicola* starting in July but particularly during the autumn (Nylund et al., 2018). This seems to allow juvenile pink salmon to leave the nursery areas before the infection pressure develops.

4.4 Comparison with Atlantic salmon

Pink salmon harbour many of the same parasite species as Atlantic salmon at sea. Table 12 shows an overview of species found in postsmolts from the Norwegian Sea (Holst et al., 1993) and in returning salmon from the coast of Vestland and Troms og Finnmark in Norway (Bristow & Berland, 1991; Bristow et al., 1996). The most prevalent species were *Anisakis simplex*, *Hysterothylacium aduncum* and *Lepeophtheirus salmonis*, found in all studies. Also, *Eubothrium* sp. (most likely *E. crassum*) and *Derogenes varicus* was present in all three studies, though more prevalent in the coastal areas.

The species *Derogenes varicus*, *Lecithaster gibbosus* and *Scolex pleuronectis* were far more prevalent and abundant in pink salmon than Atlantic salmon. Surprisingly, no salmon from Sotra, Vestland was infected with *L. gibbosus* and *S. pleuronectis.* The same trend of putative northern and southern parasites shown in pink salmon is also evident in the Atlantic salmon. The fish from northern Norway had higher prevalence of *Hemiurus levinseni* and *Brachyphallus crenatus*. The species *H. luehei* and *H. communis* were not present in the same fish. The Atlantic salmon seem to have more freshwater parasites than pink salmon, likely reflecting on a longer residence in the river (parr stage) before migrating out to sea.

Table 12 Overview of previous studies on the parasite fauna of Atlantic salmon at sea and in the coastal areas of Norway, with prevalence and abundance.

Locality	**Norwegian Sea**		**Sotra, Vestland**		**Tanafjorden, Troms og Finnmark**	
Year (n)	1991 (36)		1988 (62)		1995 (21)	
Reference	Holst et al., 1993		Bristow & Berland, 1991		Bristow et al., 1996	
Parasite species	P (%)	Ab[a]	P (%)	Ab[a]	P (%)	Ab[a]
Myxozoa						
Myxobolus sp.	8	-	-	-	19	-
Chloromyxum sp.	53	-	-	-	5	-
Monogenea						
Discocotyle sagittata	3	0.03	0	-	5	0.05
Gyrodactyloides bychowskii	0	-	0	-	62	-
Trematoda						
Cryptocotyle lingua	3	0.06	0	-	0	-
Derogenes varicus	8	0.3	24	1.6	52	39
Brachyphallus crenatus	3	0.08	0	-	19	4.1
Hemiurus levinseni	0	-	8	0.6	29	9.1
Hemiurus luehei	3	0.08	7	1.5	0	-
Hemiurus communis	28	0.6	0	-	0	-
Lecithaster gibbosus	17	1.25	0	-	38	3
Apatemon sp.	31	1.3	0	-	0	-
Diplostomum spp.	0	-	-	-	19	4.1
Crepidostomum farionis	0	-	0	-	19	1.2
Crepidostomum metoecus	0	-	0	-	10	0.2
Cestoda						
Eubothrium crassum	8	0.2	-	-	-	-
Eubothrium sp.	8	0.3	36	2.9	38	0.8
Scolex pleuronectis	39	-	0	-	24	4.6
Proteocephalus sp.	0	-	0	-	5	0.1
Nematoda						
Anisakis simplex	83	4.0	≥ 65[b]	4.7[b]	38	0.8
Contracaecum sp.	14	0.1	0	-	0	-
Hysterothylacium aduncum	78	2.9	≥ 66[b]	56.7[b]	95	38.3
Pseudocapillaria salvelini	8	0.08	0	-	0	-
Cucullanus truttae	3	0.5	0	-	0	-
Acanthocephala						
Acanthocephala (prob.)	8	0.3	-	-	-	-
Echinorhynchus gadi	-	-	0	-	14	0.4
Crustacea						
Lepeophtheirus salmonis	58	3.2	94	6.9	90	6.2
Caligus elongatus	0	-	16	0.2	5	0.1
Salmincola salmoneus	0	-	0.6	-	0	-

[a] Mean abundance calculated from prevalence and intensity data, [b] Three separate values from the stomach, intestine and abdominal cavity – the largest number is included.

4.5 Parasites as biological indicators

Parasites can be used as biological indicators of the population biology, migration pattern, habitat, diet and phylogenetics of fish (Williams et al., 1992). For a parasite to be suitable as an indicator of migrations or origin, the level of infection should differ among areas and the infections must be sufficiently long-lasting (Williams et al., 1992).

The pink salmon were mostly caught at the same time of their life cycle (~1.5 years) and the majority were sampled the same year (2017). Factors affecting the parasite abundance were difficult to disentangle, due to the data being grouped. However, some infections can still act as weak indicators.

4.5.1 Origin

Parasites can indicate the area of origin of fish, if these move between different environments with different parasites. Marine parasites mainly acquired in the nursery areas could be suitable indicators, if they vary in spatial distribution, e.g., along a North-South gradient. When the young pink salmon stay and feed in these estuaries to feed before migrating out to sea, they may be infected by parasites spreading through water (e.g., myxosporeans) or through small sized prey such as copepods. The suitability of these parasites as indicators also depends on their life span, since most information can only be derived from the occurrence of long-lived species. Also, the host should not be exposed and potentially reinfected after leaving the nursery area.

Digeneans are widely used as biological indicators (Boje et al., 1997; Pippy, 1969; Williams et al., 1992). The putative northern parasites *Hemiurus levinseni* and *Brachyphallus crenatus* were found in the ocean-caught pink salmon. The more southern parasites, *H. luehei* and *H. communis*, were however not present. This indicates that the pink salmon caught in the Norwegian Sea have a northern origin, likely from Finnmark or northwest Russia. The few southern parasites found by Fjær (2019) in river-caught fish from western Norway may have been acquired by feeding in the coastal areas before maturation and anorexia. The life span of the trematodes and the longevity of the infections is poorly known, weakening their indicator value.

4.5.2 Migration

Most of the parasites found in the pink salmon caught at sea are widespread generalists. The *Diphyllobothrium* spp. seems connected to Arctic seal species but our poor knowledge on the host range of these cestodes does not allow any finer interpretation. However, the cestode larva *Scolex pleuronectis* B, found to represent *Phyllobothrium piriei* (Fjær, 2019) indicate feeding incursions into the North Sea or British waters where the cuckoo ray final host live. This cestode is then a good indicator, because the *Phyllobothrium* spp. of rays are highly host specific (Caira & Jensen, 2017) and the cuckoo ray does not occur in Norway or in northern regions. While Fjær (2019) found 40 % of the pink salmon ascending western Norwegian rivers infected with this cestode larva type, she was only able to obtain sequence information from three species. Hence, it is still possible that more than one cestode species occur in the type B group. Further studies are therefore needed.

4.5.3 Diet

Fish can get infected by endoparasites through preying on intermediate hosts, and most of the parasites found in pink salmon are acquired through prey. Stomach content tells us the recent food intake, but parasites can indicate the diet over a longer time period. However, this requires a certain degree of prey host-association by the parasite. Many fish species have individual feeding specialization, i.e. they feed on a selection of prey (Knudsen et al., 1996). Salmonids are known for this behavior and Knudsen et al. (1996) showed a clear association between infection with a certain parasite type and the occurrence of its intermediate host in the stomach content of individual Arctic charr.

In the pink salmon a strong relationship was observed between euphausiids (krill) as prey and the abundance of the nematode larva *Anisakis simplex*, regardless of fish size. Euphuasiids are the main first intermediate host of *A. simplex* (Pravettoni et al., 2012; Smith, 1983). This could represent the same phenomenon. However, there was a surprisingly high number of free *A. simplex* larvae in the anterior intestine, suggesting that the fish had just recently been infected.

A digression is the finding of plastic (3–7 mm) in the stomachs of two pink salmon. This is noteworthy, due to the problem of plastic pollution in the oceans, including the Norwegian and Barents Sea (Bråte et al., 2016; Grøsvik et al., 2018), and the properties of plastic being nonbiodegradable (Hopewell et al., 2009). There has been found plastic in several species

inhabiting the Norwegian Sea, including Atlantic cod (Bråte et al., 2016), and ingestion of these substances can cause decreased mobility, feeding and growth (Markic et al., 2020). The prevalence of plastic in certain fish species that do devour it could become an indicator of this pollution problem, revealing increasing or decreasing trends.

4.6 Potentially zoonotic parasites - a food safety perspective

Pink salmon was introduced to northwest Russia mainly because of their promising potential as a food source (Dushkina, 1994). Parasites can be an esthetical problem in warm treated dishes, but they pose no danger to the consumer. However, when eating raw- or undercooked fish or squid there is a risk of disease, such as anisakidosis due to ingestion of anisakid nematode larvae. This can easily be prevented if the food is sufficiently heated or frozen before consumption (Hochberg et al., 2010).

The recent trend of Japanese cuisines such as sushi has led to more people eating raw food. It is therefore important to address food safety, in relation to zoonotic parasites, of pink salmon. There was found one zoonotic parasite, *Anisakis simplex*, and one potential zoonotic parasite, *Diphyllobothrium* sp.

4.6.1 *Diphyllobothrium* spp.

Diphyllobothridean cestodes from the genera *Dibothriocephalus* and *Diphyllobothrium* can parasitize humans, the most known species being the human broad tapeworm *Dibothriocephalus latus* (L.). These zoonotic parasites can cause diphyllobothridiosis in humans that ingest raw and undercooked fish harbouring plerocercoids (Durrani et al., 2020). Patients experience fatigue, gastrointestinal problems and weight loss (Choi et al., 2012).

Of the eight *Diphyllobothrium* species parasitizing arctic seals, two have been recorded from dogs and humans in Greenland and Alaska, namely *D. cordatum* (Leuckart, 1863) and *D. lanceolatum* (Krabbe, 1865) (Schaeffner et al., 2018). Human infections with *D. cordatum* and *D. lanceolatum* are rare, with only a few cases reported, but infections of domestic dogs are more common (Scholz & Kuchta, 2016). There is however a lack of knowledge about the species in *Diphyllobothrium* and their ability to cause zoonosis, and previous reports may be inaccurate and unreliable.

Since the presently found plerocercoids occurred encapsulated in the stomach wall, an ingestion of raw viscera must occur to become infected. This is an unlikely route of transmission for humans, so the zoonotic potential of these cestode larvae seems very low. On the other hand, animals like dogs can be expected to ingest them, such as dead pink salmon encountered along rivers after spawning (Karlsbakk et al., 2020).

4.6.2 *Anisakis simplex*

The final host of *Anisakis simplex* are cetaceans but humans can get accidentally infected when eating raw and undercooked fish or squid harbouring larvae. Ingestion can cause gastric, intestinal, ectopic and allergic disease. Pain, nausea, vomiting and fever can occur a few hours after ingesting the larva (Hochberg et al., 2010). The disease is called anisakiosis (due to *Anisakis*).

The pink salmon caught at sea were infected with *A. simplex*. The species is reported from many teleost species in most oceans (Berland, 1961; Kent et al., 2020) where it most often occurs as encapsulated in the viscera and muscle. The most important site from a food safety perspective is the muscle or filet, that can be eaten raw.

The UV-press method is frequently used to detect anisakid nematodes in the muscle of marine fish in Norway (Klapper et al., 2015; Levsen & Karl, 2014; Levsen & Lunestad, 2010; Levsen & Maage, 2016). By using this method, 23 % of the pink salmon was found to be infected with *A. simplex* in the muscle. There were mostly one or two individuals found in each fish and the majority was situated in the bellyflap.

There have previously been conducted several studies on anisakid nematodes in the muscle of pink salmon, in both the North Atlantic and North Pacific Ocean (Bilska-Zając et al., 2016; Grozdilova, 1974; Nomokonova, 2009). The studies found high prevalence of species in the genera *Anisakis* and *Pseudoterranova*, with high intensities in the muscles of the back and in the bellyflap. The high prevalence suggests filets from pink salmon should not be consumed raw, at risk of anisakidosis.

5. Conclusion

Pink salmon caught in the Norwegian Sea were examined for parasites, discovering a total of 13 marine microparasite species. All fish were infected, with two to eight species, averaging four species. A low prevalence of Piscine orthoreovirus (PRV) was also detected. This is the first study on the parasite fauna of pink salmon caught in the Atlantic Ocean, where previous studies focused on fish caught near or in rivers.

Several of the parasites (*Parvicapsula* sp., *Ichthyobodo* spp., *Cryptocotyle lingua*) detected in pink salmon ascending rivers in western Norway were not detected in the salmon from the ocean. Therefore, these infections were likely contracted by the fish during their migration into the fjords.

An interpretation of the parasite repertoire of pink salmon, used as biological indicators, suggests that the fish originate from Finnmark in Norway or north-western Russia. The pink salmon migrate large distances to the feeding grounds in the Atlantic Ocean and may return to a river far from their original spawning sites. The parasites found have low teleost specificity and many species are shared with Atlantic salmon. There is however a huge lack of knowledge concerning the cestode plerocercoids, where sequence information does not allow species identification.

The zoonotic parasite *Anisakis simplex* was found in the muscle of pink salmon with a prevalence of 23 %. Therefore, fresh raw pink salmon should not be consumed by humans. Heat treatment or freezing is necessary, to remove the risk of anisakidosis.

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Appendix

Table 1 Individual fish data of the 89 salmon caught in the Norwegian Sea from 2013 to 2019 by the Institute of Marine Research (IMR).

No	Serial no	IMR no	Ship	Latitude	Longitude	Bottom depth (m)	Day	Month	Year	TL (cm)	W (g)	Sex	Gonad W (g)
IP-01	23163	IMR_6294	G. O. Sars	74.321133	16.273559	245	26	5	2015	48,0	1313	Male	9
IP-02	23163	IMR_6293	G. O. Sars	74.321133	16.273559	245	26	5	2015	38,5	549	Male	2
IP-03	23137		G. O. Sars	67.422100	10.265800	201	16	5	2015	41,8	826	Female	10
IP-04	24001	IMR_1311	Johan Hjort	63.040000	3.780000	956	3	5	2013	37,5	448	Female	3
IP-05	23009		G. O. Sars	64.113743	-4.161266	-	6	5	2019	42,0	568	Male	1
IP-06	23005		G. O. Sars	63.557177	5.164205	-	4	5	2019	40,5	645	Female	6
IP-07	23024		G. O. Sars	67.114095	3.548475	-	11	5	2019	40,3	810	Male	2
IP-08	23009		G. O. Sars	64.113743	-4.161266	-	6	5	2019	42,3	700	Female	5
IP-09	23020		G. O. Sars	66.484111	-0.4715269	-	10	5	2019	40,4	726	Female	8
IP-10	23137		G. O. Sars	67.703000	10.443000	201	16	5	2015	41,8	662	Female	8
IP-11	23137		G. O. Sars	67.703000	10.443000	201	16	5	2015	44,1	893	Male	1
IP-12	23137		G. O. Sars	67.703000	10.443000	201	16	5	2015	44,7	863	Male	4
IP-13	23137		G. O. Sars	67.703000	10.443000	201	16	5	2015	46,2	974	Male	4
IP-14	23137		G. O. Sars	67.703000	10.443000	201	16	5	2015	44,1	803	Female	9
IP-15	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	27,3	139	Male	0
IP-16	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	23,2	83	Female	1
IP-17	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	28,4	148	Female	1
IP-18	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	22,4	77	Male	0
IP-19	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	25,3	107	Female	1
IP-20	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	27,7	156	Male	0
IP-21	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	24,4	98	Male	0
IP-22	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	26,0	133	Male	0
IP-23	72008		G. O. Sars	71.690000	20.040000	262	2	12	2018	30,7	210	Male	0
IP-24	24071		Johan Hjort	69.431000	17.281000	-	19	6	2017	51,9	1816	Male	64
IP-25	24063		Johan Hjort	70.428000	14.476000	-	15	6	2017	46,7	1531	Male	56
IP-26	24063		Johan Hjort	70.428000	14.476000	-	15	6	2017	42,5	982	Female	34
IP-27	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	42,6	828	Male	15
IP-28	24059		Johan Hjort	71.422861	23.327240	314	14	6	2017	43,6	1226	Male	29
IP-29	24061		Johan Hjort	71.130000	20.140000	-	14	6	2017	46,7	1408	Female	43
IP-30	24063		Johan Hjort	70.428000	14.476000	-	15	6	2017	43,5	1056	Female	42
IP-31	24084		Johan Hjort	68.434000	12.416000	-	23	6	2017	44,4	1003	Female	50
IP-32	72004		G. O. Sars	73.840000	17.800000	250	2	12	2018	22,5	64	Male	0
IP-33	72004		G. O. Sars	73.840000	17.800000	250	2	12	2018	18,7	37	Male	0
IP-34	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	42,2	1101	Male	24
IP-35	72004		G. O. Sars	73.840000	17.800000	250	2	12	2018	20,5	51	Male	0
IP-36*	24066		Johan Hjort	70.129000	14.384000	-	17	6	2017	55,9	1139	Female	3

IP-37	24061		Johan Hjort	71.130000	20.140000	-	14	6	2017	52,7	2112	Male	74
IP-38	24071		Johan Hjort	69.431000	17.281000	-	19	6	2017	50,7	1729	Male	62
IP-39	23148		G. O. Sars	71.010000	09.186600	-	28	5	2017	40,7	754	Female	13
IP-40	23156		G. O. Sars	71.329092	04.052234	-	31	5	2017	40,6	705	Male	4
IP-41	23156		G. O. Sars	71.329092	04.052234	-	31	5	2017	40,1	715	Female	12
IP-42	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	46,8	1059	Female	30
IP-43	23161		G. O. Sars	73.250984	19.077304	478	3	6	2017	44,1	860	Female	12
IP-44	23161		G. O. Sars	73.250984	19.077304	478	3	6	2017	40,4	688	Female	11
IP-45	23161		G. O. Sars	73.250984	19.077304	478	3	6	2017	44,4	1059	Female	20
IP-46	23161		G. O. Sars	73.250984	19.077304	478	3	6	2017	42,2	908	Female	25
IP-47	23161		G. O. Sars	73.250984	19.077304	478	3	6	2017	42,3	895	Male	4
IP-48	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	43,3	1160	Male	32
IP-49	24071		Johan Hjort	69.431000	17.281000	-	19	6	2017	50,6	1941	Male	60
IP-50	24013	IMR_1839	Johan Hjort	64.371026	07.047600	230	6	5	2013	40,9	608	Male	0
IP-51	24013	IMR_1833	Johan Hjort	64.371026	07.047600	230	6	5	2013	37,6	526	Female	4
IP-52	24013	IMR_1831	Johan Hjort	64.371026	07.047600	230	6	5	2013	38,9	706	Male	0
IP-53	24013	IMR_1837	Johan Hjort	64.371026	07.047600	230	6	5	2013	42,9	791	Female	5
IP-54	24013	IMR_1834	Johan Hjort	64.371026	07.047600	230	6	5	2013	44,8	914	Male	1
IP-55	24013	IMR_1835	Johan Hjort	64.371026	07.047600	230	6	5	2013	37,3	657	Female	3
IP-56	24061		Johan Hjort	71.130000	20.140000	-	14	6	2017	49,4	1565	Male	23
IP-57	24013	IMR_1836	Johan Hjort	64.371026	07.047600	230	6	5	2013	41,2	860	Female	7
IP-58	24013	IMR_1838	Johan Hjort	64.371026	07.047600	230	6	5	2013	38,9	674	Female	6
IP-59	24013	IMR_1830	Johan Hjort	64.371026	07.047600	230	6	5	2013	40,5	835	Male	0
IP-60	24013	IMR_1832	Johan Hjort	64.371026	07.047600	230	6	5	2013	38,6	598	Male	0
IP-61	24071		Johan Hjort	69.431000	17.281000	-	19	6	2017	52,4	2290	Male	95
IP-62	23112		G. O. Sars	64.561850	00.559823	-	10	5	2017	38,6	466	Female	7
IP-63	23167		G. O. Sars	70.335976	16.594306	952	5	6	2017	44,8	943	Male	13
IP-64	23167		G. O. Sars	70.335976	16.594306	952	5	6	2017	47,4	1208	Male	20
IP-65	23130		G. O. Sars	68.297205	06.371538	-	19	5	2017	39,6	608	Female	7
IP-66	23130		G. O. Sars	68.297205	06.371538	-	19	5	2017	45,9	734	Male	2
IP-67	23130		G. O. Sars	68.297205	06.371538	-	19	5	2017	45,4	906	Male	3
IP-68	23130		G. O. Sars	68.297205	06.371538	-	19	5	2017	41,0	742	Female	7
IP-69	23130		G. O. Sars	68.297205	06.371538	-	19	5	2017	40,6	793	Male	5
IP-70	23130		G. O. Sars	68.297205	06.371538	-	19	5	2017	44,1	863	Female	14
IP-71	23135		G. O. Sars	69.124400	03.215100	-	20	5	2017	34,1	443	Female	4
IP-72	23135		G. O. Sars	69.124400	03.215100	-	20	5	2017	43,9	978	Male	2
IP-73	23138		G. O. Sars	69.124400	03.215100	-	21	5	2017	39,3	557	Female	8
IP-74	23148		G. O. Sars	71.010000	09.186600	-	28	5	2017	41,4	781	Female	14
IP-75	24076		Johan Hjort	69.131141	14.571824	88.2	22	6	2017	40,3	915	Female	40
IP-76	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	43,2	1036	Male	17
IP-77	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	43,6	855	Male	18
IP-78	23153		G. O. Sars	71.477800	13.342800	-	30	5	2017	42,2	826	Female	16

IP-79	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	43,1	1041	Female	41
IP-80	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	43,5	1177	Female	45
IP-81	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	46,8	1187	Female	40
IP-82	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	44,2	1130	Female	41
IP-83	24058		Johan Hjort	71.426000	19.172000	-	13	6	2017	49,1	1532	Female	60
IP-84*	24029		Johan Hjort	66.086600	07.170600	396	16	5	2016	44,5	738	Female	0
IP-85	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	47,4	1084	Male	30
IP-86*	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	53,7	1295	Female	2
IP-87	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	45,6	1314	Female	49
IP-88	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	47,8	1422	Female	54
IP-89	24059		Johan Hjort	71.422861	23.327240	315	14	6	2017	46,3	1456	Female	55

* Determined to be Atlantic salmon.

TL – total length, W – weight.

Table 2 Parasite data of the three Atlantic salmon caught in the Norwegian Sea in 2016 and 2017 by the Institute of Marine Research (IMR).

Species	**IP-36**			**IP-84**			**IP-86**		
	N	**Length**	**Site**	**N**	**Length**	**Site**	**N**	**Length**	**Site**
Derogenes varicus	3	2.0-3.0 mm, mean 2.5 mm	Pharynx, stomach	33	1.5-2.5 mm, mean 2.0 mm	Pharynx, oesophagus, stomach	43	1.5-2.5 mm, mean 2.0 mm	Pharynx, oesophagus, stomach, swim bladder
Brachyphallus crenatus	0	-	-	10	2.0-2.5 mm, mean 2.0 mm	Stomach	396	2.5-3.5 mm, mean 2.8 mm	Pharynx, oesophagus, stomach
Anisakis simplex	2	22.0 mm	Encapsulated on pyloric caeca and in the filet	3	16.5-21.0 mm, mean 18.5 mm	Encapsulated on pyloric caeca and liver	0	-	-
Hysterothylacium aduncum	19 (18 III and 1 IV/V)	6.0-20.0 mm, mean 9.0 mm	Encapsulated on pyloric caeca, inside pyloric caeca and midgut	1 (III)	16.0 mm	Inside pyloric caeca	33 (6 III and 27 IV/V)	5.0-24.0 mm, mean 17.0 mm	Inside pyloric caeca and hindgut
Eubothrium crassum	3	15.0 mm	Pyloric caeca	6	60-260 mm, mean 190 mm	Pyloric caeca	27	15-600 mm, mean 200 mm	Pyloric cacea
Lepeophtheirus salmonis	6	Adult male: 7.0 mm Preadult female: 3.7 mm Chalimus II: 2.2 mm	Adult male: Left flank Preadult female: Tail fin Chalimus II: Dorsal fin and anal fin	0	-	-	0	-	-