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Identification of parasites in haddock (*Melanogrammus aeglefinus*) for use in stock discrimination

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FACULTY OF LIFE- AND ENVIRONMENTAL SCIENCES

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Thesis submitted in partial fulfillment of a
Baccalaureus Scientiarum degree in biology

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Faculty of Life- and Environmental Sciences
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Útdráttur

Frá því að fyrsta árángursríka tilraunin til þess að nota sníkjudýr sem líffræðilegar merkingar fiska var framkvæmd árið 1939 hefur faginu oxið fiskur um hrygg svo nú eru slíkar merkingar fiska reglulega nýttar til þess að greina á milli stofna. Rannsóknir í evrópskri lögsögu Norður-Atlantshafsins hafa sýnt fram á árangur þess að greina stofna síldar, kolmunna, þorsks, hrossamakríls, skeggþorsks, lýsu, ýsu og makríls með notkun ólíkra sníkjudýra sem henta tegundum þeirra og slóðum. Áður fyrr leiddi skortur á aðgegni að sameindabúnaði til falskra jákvæðra niðurstaðna við aðgreiningu torræðra tegunda í birtum rannsóknum en með auknu aðgengi að slíkum búnaði er vandamálið nú á undanhaldi. Rannsóknir á sníkjudýrum á Íslandsmiðum eru takmarkaðar og skráning þeirra nánast engin. Margar tegundir sem teljast mikilvægar í viðskiptalegu tilliti hafa verið vanræktar í þessum efnum, þar á meðal ýsa (Melanogrammus aeglefinus) sem hefur þriðja stærsta leyfilegan heildarafla á Íslandsmiðum samkvæmt ráðleggingum Hafrannsóknarstofnunar 2022/2023. Þrátt fyrir viðskiptalegt mikilvægi ýsu eru rannsóknir á stofngerðum hennar ekki nægilegar. Í opinberum gögnum Hafrannsóknarstofnunar er þannig tekið fram að „upplýsingar um stofngerð ýsu við landið eru af skornum skammti“. Í þessari rannsókn var sjónum beint að líffélögum sníkjudýra á tveimur slóðum við Íslandsmið í von um að varpa nýju ljósi á fyrirliggjandi stofngerðir og leggja grunn að heildstæðari skráningu sníkjudýra sem finnast í ýsustofnum á Íslandsmiðum. Ein tegund flatorma sem ekki hefur áður verið skrásett í ýsu var staðfest.

Abstract

Since the first successful experiment with using parasites as biological tags in 1939 the field has advanced to a level where it is now a common practice in stock discrimination. Studies on the European side of the North Atlantic have already had successes in the identification of stocks of herring, blue whiting, cod, horse mackerel, pout, whiting, haddock, horse mackerel and mackerel using a variety of parasites suitable for each locality and species. In the past the lack of access to molecular tools to distinguish between cryptic species has led to false positives in published research but with these tools at our fingertips this is a diminishing problem. The research on parasites in Icelandic waters has been minimal and inventories on them practically non-existent. Many commercially important species have been neglected in these matters including haddock (*Melanogrammus aeglefinus*), that ranks third highest in the list of total allowed catch in Icelandic waters according to the 2022/2023 MFRI recommendations. Despite haddocks commercial important the research on their stock structure is lackluster with the official MFRI documents stating that “information about stock structure (metapopulation) of haddock in Icelandic waters is limited”. This research examined the parasite communities of the North- and South of Iceland in attempts to shed a new light on the existing stock structure and lay the groundwork for a more comprehensive list of parasites of haddock in Icelandic waters. One species of trematodes not yet documented in haddock was confirmed.

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Abbreviations

ITS : Internal transcribed spacer

PCR: Polymerase chain reaction

MFRI: Marine and Freshwater Research Institute of Iceland

Acknowledgements

Firstly, I’d like to thank my professor Haseeb Randhawa for introducing me to the world of parasitology, his endless amount of patience for my questions and his incredible ability to inspire and motivate students. Secondly, I’d like to thank my friend Alex Rafn Elfarsson for being my partner in crime through these last three years, for all the laughs and for always having my back. Lastly, I’d like to thank Hlynur Pétursson from the Marine and Freshwater Institute of Iceland for organizing logistics in getting samples from the north to Reykjavík.

1 Introduction

1.1 Parasites and stock discrimination

A crucial factor in fisheries ecology and the prerequisite for the assessment and management of marine fisheries is the ability to understand the population structures of the fish species in question (Cadrin et al., 2014). Life history traits such as rate of growth- and mortality, recruitment, time of maturity and reproduction are widely monitored by agencies invested in fisheries and are useful to identify fish stocks along with tool like morphometric-, biochemical-, merisic-, physiological-, behavioural- and genetic characteristics (Ihssen et al., 1981). Solid understanding of stock structures allows fisheries to monitor populations of fish and through doing so, responsibly manage them as resources. This makes it possible for fisheries to optimize their harvest while still protecting nurseries and spawning grounds important to the stock in question (Begg & Cadrin, 2016). Despite our growing understanding of population structures, over exploitation of marine resources is still a persistent problem and it is believed that 90% of the global marine fish stock is currently over exploited (Gebremedhin et al., 2021). Other tools that can be used in stock discrimination is the application of parasites as biological tags, which can be deployed in combination with other tools such as otolith microchemical analysis (Brickle et al., 2021) to understand where the specimen spends their adult lives as well as where spawning and development occurs (Begg & Cadrin, 2016). The use of parasites as biological tag has some advantages over other practices (MacKenzie & Abaunza, 2014). They are ubiquitous in nature and are believed to inhabit almost every free-living organism on the planet (Raga et al., 2009). Despite their great dispersal throughout the biomes many species of parasites are endemic to their geographical locations. These endemic species of parasites, due to their limited range, can be utilized as biological tags as they can provide solid evidence of whether the hosts, they inhabit have spent any time within their habitat (Poulin & Kamiya, 2015). The first use of parasites as biological tags goes back as early as 1939 when Dogiel and Bychovsky used monogeneans to differentiate between two stocks of sturgeons in the Caspian Sea (in Williams et al., 1992). Their pioneer research inspired scientists and has brought the field to where it is today, where parasites have become widely recognized for this purpose and are commonly implemented for stock discrimination by commercial fisheries (Poulin & Kamiya, 2015). However, the use of parasites has its limitations and not all parasites are equal when it comes to their usefulness in stock assessments. MacKenzie and Abaunza published a paper in 1998 named “Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods” where they outlined the criteria needed for the selecting of parasites as biological tags:

- The level of infection in the host species ought to be significantly different between the areas of study.
- The parasite needs to have a lifespan longer than the time it takes to perform the research. Parasites with a lifespan of one or more years are suitable for recruitment studies and stock identification while studies on the seasonal migrations can have a shorter life time than one year.

- Parasites with a simple life cycle (one host life cycle) are easiest to use. That includes trematodes, crustaceans and protozoans. Parasites with complex life cycles are only deemed acceptable if information on factors, abiotic and biotic, that affect their transmission between hosts is well known.
- The level of infection should stay fairly stable between years.
- Ease of detection and identification of the parasite is important to avoid time constraints. The examination should not require labour intensive dissection.
- Parasites that manipulate host behaviour or cause selective mortality of hosts should be avoided (MacKenzie & Abaunza, 1998).

1.2 Parasites as biological tags in haddock

Two studies have been conducted on the use of parasites as biological tags in haddock in the North Atlantic. The first one, conducted by a polish parasitologist, Kabata (1963), focused on myxosporean parasites in the gall bladders of haddock. By comparing the prevalence of the infection of two species; *Myxidium* and *Leptotheca*, between haddock caught of the plateau outside the Faeroe Islands and haddock caught further off shore, Kabata successfully concluded that those were two separate stocks of haddock (in Mackenzie & Hemmingsen, 2015). In 1977 Lubieniecki published a paper on his research where he used plerocercoids (the last larval form) of the cestode species *Grillotia erinaceus* as a biological tag for haddock in the North Sea, around the Faeroe Islands and outside the west of Scotland. His results indicated that based on the prevalence of the cestode he could identify a few separate groups in those areas. Firstly, the results using the cestode larvae supported the aforementioned research of Kabata of two stocks outside the Faeroe Islands. Secondly the use of the cestode he could conclude that there were three different stocks of haddock outside the coast of Scotland, two of which had a high infestation of the cestode and one separating the two that had very low occurrence of the parasite (Lubieniecki, 1977). Stock structure analysis on other gadoid species using parasites as biological tags has been successful at using a plethora of parasites in their work but as many parasites have complex life cycles the abundance of intermediate hosts in a particular area can affect the composition of the parasite fauna there. It is important to find a species of parasites that can function as such within the parameters of the area of research (Mackenzie, 2002). When research into the stock structure of haddock in the West Atlantic was performed, they found that using parasites such as *Grillotia erinaceus* was useless due to them not being a major parasite in the haddock there (Mackenzie, 2002). However, preliminary research in the West Atlantic did show potential in using *Lepidapedon rachion*, a species of trematodes and *Myxidium bergense*, a myxosporidian as biological tags for haddock due to their host specificity and abundance. Due to economic reasons more focus has been placed on parasite research of cod than there has been on haddock (Marcogliese & Jacobson, 2015), demonstrated clearly by the number of published papers on the matter. Studies already show the usefulness of parasites in discriminating complex stock structures of gadoids in the Atlantic Ocean (Mackenzie & Hemmingsen, 2015). The research presented in this thesis aims to lay some groundwork for the field in Icelandic waters.

1.3 Haddock (*Melanogrammus aeglefinus*)

Haddock (*Melanogrammus aeglefinus*) is a demersal teleost species belonging to the Gadidae family. They have a wide distributional range, inhabiting areas from the Northeast- to the Northwest of the Atlantic Ocean. As a demersal species they spend their adult lives close to the ocean's floors at a depth of 50 – 200 meters (Tam et al., 2016). They mainly feed on a variety of benthic invertebrates in their young years (Klimpel & Rückert, 2005) but become more opportunistic in their adult years, incorporating other teleost species into their diet (Link et al., 2005). Depending on temperature and other abiotic factors they can grow to be over a meter in size but mature at a length of 23–73 cm or between the ages of 2 -7 years with males reaching sexual maturity earlier and at smaller size than females (Rogers et al., 2016; Taylor & Stefánsson, 1999). Like cod the Icelandic haddock broadcast spawn off the west coast of Iceland in the spring (Butler et al., 2020; Marcogliese & Jacobson, 2015) (Taylor & Stefánsson, 1999) leaving their eggs and larvae to flow with the West Iceland Irminger Current that flows clockwise around the country (Casanova-Masjoan et al., 2020). The larval drift has occasionally led to juveniles from the Icelandic stock to be recorded outside the East coast of Greenland but no other links of larval drift outside of Iceland have been confirmed (Marine and Freshwater Research Institute, 2022). The spawning of both the haddock and cod coincides with the spring bloom of algae in the area (Jonasson et al., 2009) which in return brings *Calanus finmarchius*, a copepod species that feeds on the algae (Jónasdóttir et al., 2002) but is also known both for being a staple in the diet of larval gadids (Rowlands et al., 2008) as well as being a host for larval nematodes (Svendsen, 1990). Timings of these algae blooms and the spawning of haddock is believed to be a key element in survival of the larvae and when the timing of either falters it can lead to weak recruitment to the stock in the following years (Platt et al., 2003).

Haddock plays a crucial role both in the food webs of the Atlantic Ocean (Tam et al., 2016) and in the economy of Iceland, ranking as the second most important species of fish for commercial fisheries (Þórðarson & Viðarson, 2014). They are known to inhabit the areas south and west of Iceland where the waters are warmer as well as off the north coast but historically have only been known to spawn in the waters south of the island (Marine and Freshwater Research Institute, 2022). The annual catches of Iceland's haddock have wavered greatly between years reaching records hights around 1960 and again between 2005 and 2008 accounting for catches over 100.000 tonnes. However, the landings of Icelandic haddock in the year of 2021 are estimated to be about half the amount of those records or 57.599 tonnes (Marine and Freshwater Research Institute, 2022). Total allowable catches in 2022/2023 are the highest that they have been in the last ten years at 62.219 tonnes.

The Icelandic haddock has historically been treated as a single stock and according the MFRI the information available on its stock structure and meta-populations is limited (Marine and Freshwater Research Institute, 2022). The commercial importance of the species however warrants further examination into the matter. The aim of this research is to review this by inspecting whether a thorough inventory and a comparison of the composition of parasite communities found in haddock between the north- and south of Iceland can provide a new insight into our understanding of the haddock's movement and population structures around the island.

1.4 Parasites of haddock

Table 1 summarises the known species of parasites that have been found in haddock in the Atlantic Ocean. The list includes thirteen higher taxa, with a total of 59 different species.

Table 1: An inventory of the parasites recorded in haddock in the Atlantic Ocean

Taxa	Species	Reference
Protozoa	*Eimeria gadi*	Margolis & Arthur, (1979)
	Glugea branchiale	Margolis & Arthur, (1979)
	Haemogregarina aeglefini	Margolis & Arthur, (1979)
	Haemohormidium terraenovae	Margolis & Arthur, (1979)
Myxozoa	*Myxidium bergense*	Margolis & Arthur, (1979)
	Myxidium gadi	Margolis & Arthur, (1979)
	Myxidium sp.	McDonald & Margolis, (1995)
	Myxosporea Gen. sp.	McDonald & Margolis, (1995)
Trematoda	*Bucephaloides gracilescens*	Johnston & Halton, (1981)
	Cryptocotyle lingua (metacercaria)	McDonald & Margolis, (1995)
	Derogenes varicus	McDonald & Margolis, (1995)
	Digenea Gen. sp. (metacercaria)	McDonald & Margolis, (1995)
	Genolinea laticauda	McDonald & Margolis, (1995)
	Hemiurus levinseni	McDonald & Margolis, (1995)
	Lecithaster gibbosus	McDonald & Margolis, (1995)
	Lepidapedon rachion	McDonald & Margolis, (1995)
	Opechona sp.	MacKenzie, (1974)
	Podocotyle reflexa	McDonald & Margolis, (1995)
	Prosorhynchus squamatus	McDonald & Margolis, (1995)

Cestoda	*Abothrium gadi*	McDonald & Margolis, (1995)
	Bothriocephalus scorpii	McDonald & Margolis, (1995)
	Cestoidea Gen. *s*p. (plerocercoid)	McDonald & Margolis, (1995)
	Grillotia erinaceus (plerocercoid)	McDonald & Margolis, (1995)
	Tetraphyllidea Gen. sp. (plerocercoid)	McDonald & Margolis, (1995)
Nematoda	*Anisakis simplex* (larva)	McDonald & Margolis, (1995)
	Anisakis sp. (larva)	Margolis & Arthur, (1979)
	Ascarophis arctica	McDonald & Margolis, (1995)
	"Capillaria" sp.	McDonald & Margolis, (1995)
	Contracaecum osculatum	Levsen et al., (2022)
	Contracaecum sp.	Margolis & Arthur, (1979)
	Cucullanus cirratus	McDonald & Margolis, (1995)
	Hysterothylacium aduncum	McDonald & Margolis, (1995)
	Hysterothylacium sp.	McDonald & Margolis, (1995)
	Nematoda Gen. sp.	McDonald & Margolis, (1995)
	Phocanema sp. (larva)	Margolis & Arthur, (1979)
	Pseudoterranova decipiens larva	McDonald & Margolis, (1995)
	Thynnascaris adunaca	Margolis & Arthur, (1979)
	Thynnascaris melanogrammi	Margolis & Arthur, (1979)
Acanthocephala	*Corynosoma* sp. (juvenile)	McDonald & Margolis, (1995)
	Corynosoma strumosum (juvenile)	McDonald & Margolis, (1995)
	Corynosoma wegeneri (juvenile)	McDonald & Margolis, (1995)
	Echinorhynchus gadi	McDonald & Margolis, (1995)

	Echinorhynchus sp.	McDonald & Margolis, (1995)
Copepoda	*Calicurtus elongatus*	Margolis & Arthur, (1979)
	Caligus curtus	Margolis & Arthur, (1979)
	Clavella adunca	McDonald & Margolis, (1995)
	Lernaeocera branchialis	Margolis & Arthur, (1979)
Apicomplexa	*Eimeriorina* Gen. sp.	McDonald & Margolis, (1995)
	Goussia gadi	McDonald & Margolis, (1995)
	Goussia sp.	McDonald & Margolis, (1995)
	Goussia spraguei	McDonald & Margolis, (1995)
Microspora	*Loma branchialis*	McDonald & Margolis, (1995)
	Microsporida Gen. *s*p.	McDonald & Margolis, (1995)
Mastigophora	*Ichthyobodo* sp.	McDonald & Margolis, (1995)
	Spironucleus torosa	McDonald & Margolis, (1995)
	Trypanosoma sp.	McDonald & Margolis, (1995)
Annelida	*Johanssonia arctica*	McDonald & Margolis, (1995)
Amphipoda	*Lafystius sturionis*	McDonald & Margolis, (1995)
Isopoda	*Aega psora*	McDonald & Margolis, (1995)

1.4.1 Abiotic effects on sample areas

The two sample areas, the southwest of Iceland and the north of Iceland, are very different abiotically. The area SW of Iceland is dominated by a warm and saline upper current, the Irminger current, that flows towards the Icelandic coast from the south. It flows alongside the west of Iceland where it meets the Greenland Ridge which limits its flow further north, moving its flow towards Greenland and then south again. Some of the current however splits off and carries onwards alongside the northwest of Iceland and to the north of the county where it meets a cold, less saline upper current that flows down from the Arctic (Casanova-Masjoan et al., 2020). The distribution of species is affected by many abiotic factors, but it is widely recognized that temperature is one of the most important of these factors. Research on the benthos around the island found that many of the benthic species have their distributional limits at the Greenland Ridge and Faeroe Ridge, showing preference for either the colder or warmer waters on either side (Brix & Svavarsson, 2010). These distributional limits mean a difference in the benthos between the two localities sampled which could in

turn affect the distribution of parasites with complex life cycles that often rely on benthic invertebrates as one of their intermediate hosts (Klimpel & Rückert, 2005). As the haddock is a benthic species and its diet is reflective of that, the difference in benthos between the localities could prompt a difference in species composition of trophically transmitted parasites in the haddock.

1.5 Research questions

This project seeks to answer two questions:

- **Whether there is a difference in the parasite fauna between the two localities and**
- **whether the parasites found can be used as biological tags to differentiate the existing stock structure into two separate stocks.**

2 Methods

2.1 Sample collection

Catch from fishing vessels was provided by the Marine and Freshwater Institute of Iceland. The specimens were landed in Sandvík in the southwest of Iceland and in Dalvík in the North. Geographical coordinates of the catch were logged by the captains and saved for future reference. To ensure the freshness of the samples the catch was required to be from the day of the vessels return to dock. Samples from the north were shipped to a local fish market where they were collected and brought to the necropsy room at the University of Iceland. Samples from the south were collected directly from the harbour of Sandgerði and likewise brought to the necropsy room. All specimens were stored in a cooler during the processing period that was regularly restocked with crushed ice to preserve them.

Table 2 A summary of catch data

Fish ID	TL (cm)	Sex	Location	Latitude	Longitude	Depth (m)
HAD 01	78	M	North	N66°06´33	W18°33´31	80
HAD 02	46	N/A	North	N66°06´33	W18°33´31	80
HAD 03	55	F	North	N66°06´33	W18°33´31	80
HAD 04	38	N/A	North	N66°06´33	W18°33´31	80
HAD 05	63	N/A	North	N66°06´33	W18°33´31	80
HAD 06	57	N/A	North	N66°06´33	W17°34´41	125
HAD 07	67	F	North	N66°06´33	W17°34´41	125
HAD 08	65	F	North	N66°06´33	W17°34´41	125
HAD 09	55	F	North	N66°06´33	W17°34´41	125
HAD 10	60	F	North	N66°06´33	W17°34´41	125
HAD 11	40	N/A	North	N66°06´33	W17°34´41	125
HAD 12	34	N/A	North	N66°06´33	W17°34´41	125
HAD 13	56	F	North	N66°06´33	W17°34´41	125
HAD 14	76	F	South	N63°57’057	W22°48’20	77
HAD 15	70	M	South	N63°57’057	W22°48’20	77
HAD 16	76	F	South	N63°57’057	W22°48’20	77
HAD 17	76	F	South	N63°57’057	W22°48’20	77
HAD 18	70	M	South	N63°57’057	W22°48’20	77
HAD 19	52	F	South	N63°55’37	W22°46’49	68
HAD 20	63	F	South	N63°55’37	W22°46’49	68
HAD 21	64	F	South	N63°55’37	W22°46’49	68
HAD 22	40	M	South	N63°55’37	W22°46’49	68
HAD 23	52	N/A	South	N63°55’37	W22°46’49	68
HAD 24	72	M	North	N65°42’44	W20°26’03	53
HAD 25	74	F	North	N65°42’44	W20°26’03	53
HAD 26	30	N/A	North	N65°42’44	W20°26’03	53

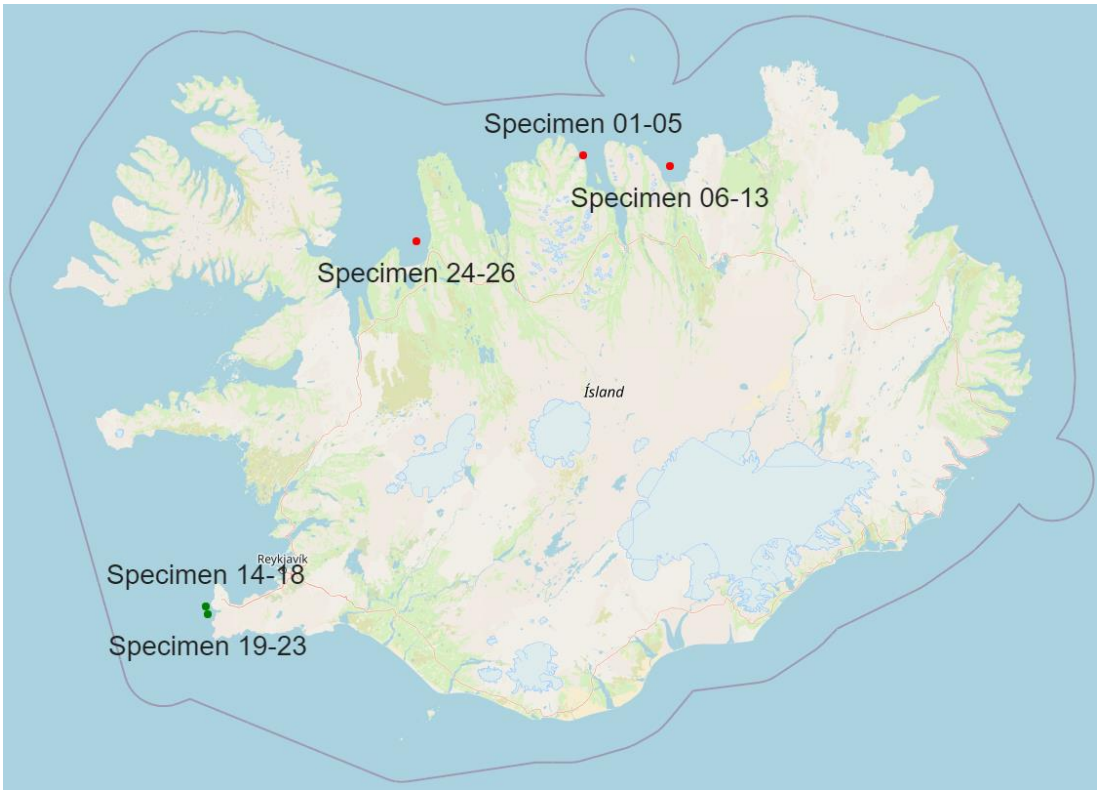

Figure 1 A map of Iceland with the geographical coordinates of sampling sites with specimen numbers included

2.2 Necropsies and examination of the specimen

Specimens of haddock were individually processed with each fish being assigned a serial number (example HAD-01). The specimens were measured using a measuring board and their total length, rounded to the closest complete centimeter, was recorded. External examination was performed in search of ectoparasites, observing in detail the areas behind the fins and around the mouth. Nostril flush was performed by injecting saline solution (8ppt) via pipette into both nostril openings of the fish and the fluid that secreted from them collected in a petri dish for examination under a dissection microscope. The gills and eyes was removed with a scalpel and placed into petri dishes. A large knife was used to open the cranium to gain access to the internal structure of the head. The brain along with the cranial fluid were collected into a petri dish and the otoliths extracted, bagged and labelled for further research. The eyes were examined both externally and internally under a dissection microscope, the gills were separated into individual gill arches with bone cutting pliers and examined thoroughly and finally the brain and cranial fluid were examined. 5-10 gill filaments were removed after examination and preserved in 96% ethanol for further research. An incision was made down the abdomen on the ventral side of the specimen and the body cavity opened up to expose the viscera. The internal organs (heart, spleen, liver, stomach, pyloric caeca, intestines, gonads, mesenteries, gall bladder, urinary bladder) were extracted individually, each of them labelled with the corresponding serial number and placed into

separate zip-lock bags, apart from the urinary- and gall bladder that were placed in vials and preserved in 96% ethanol. Due to the quantities of fish received at a time the organs were frozen for later examination so that each shipment of specimen could be processed fresh, excluding a few times when an organ of particular interest was chosen to be processed immediately.

2.3 Parasite collection

The organs were thawed out in batches varying in sizes depending on availability of time. The individual organs were placed into appropriately sized glass petri dishes and examined for parasites using pincettes and teasing needle and viewed under a dissecting microscope. UV flashlights were utilized to assist with finding nematodes that otherwise alluded the eye under normal lighting. The parasites found were transported into separate petri dishes, cleaned in saline solution (8ppt) and sorted by genus and life stage into appropriate vials depending on their size. The vials were filled with 96% ethanol to preserve the parasites for later molecular confirmation of their identity and labelled with the serial number of the host, site of discovery and the phylum of the parasite. The same details were logged into a workbook along with a count of the parasites found and the details from the finding transcribed into an excel sheet.

2.4 DNA extraction

To procure a complete data set to use for statistical analysis, parasite samples were selected from the excel data sheet; two samples from every parasite type (when possible), from each site of infection from two host specimen from both the north and the south. The larger parasites (cestodes, acanthocephalans, nematodes) were severed around the midsection in two places, carving out an approximately cubic millimeter sized piece of their tissue. Both the posterior and anterior parts of the nematode were left to be stored in vials and given new serial numbers for morphological identification while the parasite samples taken were isolated in a separate vial and given new serial numbers (example: TG-001) that correlated with the previously used host associated serial numbers. The smaller parasites (trematodes, metacercarias) were taken whole and isolated in Eppendorf vials. In both cases when the sample size exceeded one, two samples were isolated separately and given individual serial numbers.

A master mix was prepared comprising of (1) 10 µl of Fish Buffer, (2) 1 µl 20% Tween 20, (3) 2 µl Proteinase K and (4) 2 µl of double distilled water (MQ H_2O) per DNA sample. 15µl of the mix was allocated to each sample via P20 pipette. The samples were then placed into a heat block at 65°C in order to optimize the breakdown of genetic material via the proteinase K. This process lasted for a total of two hours with the samples being removed at 20-minute intervals to be flicked and spun down in a centrifuge. Following this step, the heat blocks were set to 95°C and the samples placed back in for the duration of ten minutes to deactivate the proteinase to prevent further breakdown of the genetic material. After the deactivation was completed, the samples were left to cool down to room temperature before being placed into a freezer to preserve them for further processing.

2.5 PCR protocols

In preparation for every PCR session all worksurfaces and tools were wiped down, first with distilled water followed by 70% ethanol, to prevent contamination of the samples and the thermal-cyclers were activated to allow them time to warm up. 200µl strip tubes were tagged with new serial numbers (from one and up) and each sample assigned a tube. The tubes were labelled on top and on the side to minimize the possibility of loss of tags. For each reaction two tubes were reserved for a positive- and a negative control, apart from the acanthocephalan reaction where no positive control could be obtained. A master mix specific to each group of parasites was prepared (see table 1) in an Eppendorf tube and 24.5µl of the mix allocated into each of the labelled strip tubes. The samples were removed from the freezer and allowed to thaw out before being flicked and spun in a centrifuge. Once thawed, the samples were preserved on ice to avoid deterioration of the genetic material. 0.5 µl of each sample was taken, placed in the tube assigned to it and the strip tubes closed. The samples were then placed into a thermocycler and set to a cycle specific to the reagent used for each parasite group (see table 2). Once the cycle completed the samples were removed from the thermal-cyclers, placed into racks and refrigerated to await further processing.

Table 3: A list of the primers and protocols used for each group of parasites during the PCR process

Parasite groups	Primers and protocol
Monogeneans, Cestodes, Unknowns	➢ Primers used: **T01N** and **T13N**(Harper & Saunders, 2001) Thermal-cycler protocol: • 4 m at 94°C • 38 cycles: o 30 s at 94°C o 30 s at 50°C o 2 m at 72°C • Then 7 m at 72°C
Trematodes	➢ Primers used: **BD3** and **536**(García-Varela & Nadler, 2005) Thermal-cycler protocol: • 1 min at 94°C • 35 cycles: o 60 s at 94°C o 60 s at 50°C o 90 s at 72°C • 10 m at 72°C
Nematodes ITS	➢ Primers used: **93** and **94**(Nadler et al., 2005) Thermal-cycler protocol: • 3 m at 94°C • 35 cycles: o 30 s at 94°C o 30 s at 52°C o 60 s at 72°C • 7 m at 72°C
Acanthocephalans	➢ Primers used: **LCO1490a** and **HCO2189**(García-Varela & Nadler, 2005) Thermal-cycler protocol: • 3 m at 94°C • 35 cycles: o 60 s at 94°C o 60 s at 40°C o 60 s at 72°C ➢ 7 m at 72°C

2.5.1 PCR gel electrophoresis

A gel electrophoresis test was run in order to establish the viability of the PCR products. In preparation for making the gels, all work surfaces were wiped down, first with distilled water followed by 70% ethanol. Clean gel rigs were set out with casting rigs placed upon them and 20 tooth combs placed horizontally in the gel in order to create a total of 40 wells per gel. The rig was sealed on the top and bottom of the casting tray to ensure their function. Agarose gels were made by combining 1.50 grams of agarose powder and a 1x concentration of TAE (tris base, acetic acid and ethylenediaminetetraacetic acid) buffer in an Erlenmeyer flask. The bottle was swirled in order to mix the two ingredients before placing it in a microwave to be heated to boiling point to allow the powder to dissolve in the liquid, upon which the bottle was removed from the microwave with heat resistant gloves. The liquid was cooled down to a temperature which allowed for contact with the flask without burning one's hands yet not so cool that it began to congeal. The liquid was checked for clarity to ensure that no congealing had occurred before 5 µl of ethidium bromide was added using a P10 pipette and the mixture was swirled again. The concoction was gently poured into the casting tray and any unwanted pockets of air that formed were removed using pipette tips before giving the gels 20 minutes to set. The PCR products prepared in the PRC protocols were collected in batches not exceeding 38 at a time. Loading dye along with a vial of 1 kb Quick-Load ladder (from BioLabs) was collected from the freezer and allowed to thaw before being placed on ice for preservation. A strip of parafilm was placed upon a table and samples were prepared on it by allocating and combining (1) 1 µl of loading dye, (2) 3 µl of dH_2O and (3) 2 µl of PCR sample per sample. Ladders were prepared with the same first two steps and 2 µl of the 1 kb ladder was added to the samples. Once the gels congealed the combs were pulled out and they were placed into electrophoresis chambers, containing 1x concentration of TAE, making sure that the wells were submerged in the buffer. The wells in the gel were oriented towards negative node to allow the DNA (which carries a negative charge) to be pulled from the wells and down through the gels towards the positive node. The ladders were added to the first well of either row of the gel and the PCR samples loaded into well 1 – 19 of each row, making sure to load the negative and positive into the last wells on the second row. The nodes of the electrophoreses chamber were connected to a power supply, the volt meter set to 80 volts and the electricity given 40 minutes to flow through the chamber in order to pull the strands of genetic material through the agarose gel. The gels were then placed under a cone to be photographed with an orange filter on a digital camera whilst backlit with blacklight to maximise the visual results of the ethidium bromide. This provided visual verification of whether the genetic material had been successfully processed so that viable samples could be selected to be sent for genetic identification of the species. All results were logged down and a table was made listing the successfully processed PCR products for further processing.

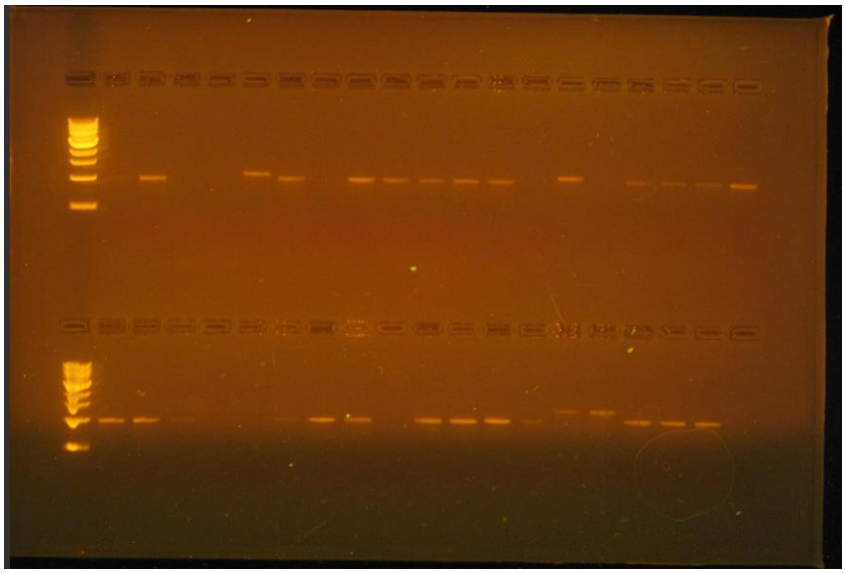

Figure 2 A photograph of one of the agarose gels with bands showing the successfully extracted DNA from three separate species of nematodes. The photo was taken using UV backlighting and an orange filter for optimum results.

2.5.2 PCR purification process

Worksurfaces were cleaned with distilled water and 70% EtOH in preparation for the procedure. The list of successful samples was used to assign numbers to- and label new 200 µl strip tubes for the purification process. Exo-Sap (a 50/50 mixture of Exonuclease I and Shrimp Alkaline Phosphatase) was collected from the freezer and kept on ice as it is sensitive to heat changes. The tubes were placed into empty 10 µl pipette tip trays and the container holding the trays filled with ice in order to preserve the materials going into them. 10 µl of the PCR products containing viable genetic material were transferred to the corresponding vial and 2 µl of the Exo-Sap added to the PCR products in the vials. The vials were flicked and spun before being placed into the thermal-cycler and run on an Exo-Sap protocol. Once the thermo-cycler finished the product was placed into a freezer at -20°C to be stored for further processing. The finished product was shipped to the facilities of Microsynth in Germany where the DNA was sequenced in order to confirm the species found during this research.

Table 4 Exo-Sap protocols

PCR Product Cleaning	Exo-Sap Protocol
10 µl PCR product 2 µl Exo-Sap	15 min at 37°C 15 min at 80°C Freeze

2.6 Data and statistical analysis

The information on parasites, intensity of infection, site of infection, host ID and locality that was gathered during necropsies was compiled into a spreadsheet and processed into datasets. The packages MASS, ggplot2, readxl, klaR, psych, devtools were used to process the datasets with the addition of ggord to make the plots visually appealing. Linear discriminatory analysis of the data gave coefficients of liner discriminants that were used to produce histograms and density plot, and train a predictive algorithm in predicting where a host specimen was from, based on the infections it had and the intensity of those infections. This produced statistical information that provided necessary insight into the statistics of the parasite communities and whether they were of significance for the use as biological tags.

3 Results

The samples used in this research totalled 26 specimens of haddock, 10 from the south (HAD14 - HAD23) and 16 from the north (HAD01 - HAD13 and HAD24 - HAD26). The samples from the north included seven females, two males and seven unassigned specimens (five juviniles,two not recorded). The samples from the south included six females, three males and one unassigned specimen (juvenile).

The procedures for molecular identification were performed on the helminth parasites found in the fish but other parasites such as copepods were not processed. Morphological confirmation of identification was used to identify copepods, myxozoan and xcellidaes. Copepod parasites on the gills were identified as *Lernaeocera branchialis* and *Clavella adunca* with the former found more frequently and in higher numbers. *Clavella adunca* was confirmed from the gills of specimen HAD19 from the southern locality. Myxobolus infections could be confirmed in four fish with equal distribution between the two areas sampled.

HAD 21 from the southern locality had a particularly interesting infection. Upon opening the viscera, the internal organs were fused to inside of the body cavity. Furthermore, the gonads (f) appeared to have been partly appropriated by a parasite and that part had grown in size. It was filled with a liquid and upon closer examination the organ contained a yellow/green hard mass inside it that appeared to be made up of multiple single cells. The specimen had numerous infections throughout its body including a systematic myxozoan infection clearly visible in the cranium, metacercariae on the brain, 18 acanthocephalan-, three cestode larvae, one nematode and five trematodes in the intestines, 40 nematodes in the stomach and a metacercariae-, *Gladixcellia gadi*- and *Loma morhua* infection in the gills. The mass was inspected, logged and photographed before being saved for further analysis.

Dataset of collected parasites was analyzed using Rstudio and an algorithm was trained by running through it a series of datasets based on the parasites recovered during the necropsies. A model was utilized to predict the locality of each individual specimen based on the parasite data accompanying each fish to test the statistical information that the data provided. A total of five different datasets was used to assess this:

1. A dataset containing all the available data but omitting the liver, stomach, pyloric caeca, intestine, gall bladder and gonads.
2. A dataset containing only the liver, omitting the unprocessed livers
3. A dataset containing only the stomach, omitting the unprocessed stomachs
4. A dataset containing only the pyloric caecum, omitting the unprocessed pyloric caecum
5. A dataset containing only the intestine, omitting the unprocessed intestine

Dataset one was run through the algorithm and based on the parasites, their abundance and intensity in the liver it correctly predicted 12 samples from the North and 1 in the south, with 6 samples being placed into the wrong locality.

	Actual	
Predicted	North	South

North	12	6
South	0	1

The algorithm was fed a dataset comprising only of the stomach omitting the unprocessed samples from that grouping. The predictions based on the parasites in the stomach, their abundance and intensity show a misclassification of one sample.

	Actual	
Predicted	North	South
North	10	0
South	1	7

The algorithm was fed a dataset comprising only of the pyloric caecum, omitting the unprocessed samples from that group. The predictions based on the parasites in the pyloric caecum, their abundance and intensity correctly classify 12 specimens to the North but 0 to the south. 6 specimens were classified incorrectly.

	Actual	
Predicted	North	South
North	12	6
South	0	0

The algorithm was fed a dataset comprising only of the intestine, omitting the unprocessed samples from that group. Predictive information about the location of the specimen based on an intestine only dataset, with unprocessed samples omitted. The classification shows correct prediction of 12 specimen to the North and 8 to the South with one misclassification.

	Actual	
Predicted	North	South
North	12	0
South	1	8

The algorithm was fed a dataset comprising of all sites of infections, omitting the liver, stomach, pyloric, intestine, gall bladder and gonads. Predictive information about the

location of the specimen based on that dataset shows the correct classification of specimen from the North was 14 and the correct classification of specimen from the south as 5

	Actual	
Predicted	North	South
North	14	5
South	2	5

3.1 Prevalence, intensity and abundance

Calculations were made for the prevalence, intensity and abundance for every infection found in every organ/site in both localities. The sample size was always 16 from the North and 10 from the South unless expressed otherwise in the "Organ" column. Two samples were taken from the organs for molecular identification, leading to the confirmation of two species within the site of infection.

Table 5 Prevalence, intensity and abundance calculations from compared between the North and South localities with either morphological or molecular identification of parasites listed.

Parasite	**Organ**	**Prevalence**		**Intensity of**		**Abundance**	
		North	South	North	South	North	South
Acanthocephala							
Echinorhynchus	Intestine	64	75	9.22	7.83	5.93	5.88
Cystacanth larva	Body cavity	0	10	0.00	1.00	0.00	0.10
Cystacanth larva	Gonads	11	11	1.00	1.00	0.11	0.11
Apicomplexa							
Loma morhua	Gills	6	30	1.00	1.00	0.06	0.30
Cestoda							
Cestoda Gen. sp.	Intestine	14	0	1.00	0.00	0.14	0.00
Cestoda Gen. sp.	Intestine	7	13	1.00	3.00	0.07	0.38
Cestoda Gen. sp.	Pyloric caecae	31	14	2.25	1.00	0.69	0.14
Cestoda Gen. sp.	Pyloric caecae	8	14	5.00	1.00	0.38	0.14
Copepoda							
Clavella adunca	Gills	0	10	0.00	1.00	0.00	0.10
Lernaeocera	Gills	31	60	2.80	4.50	0.88	2.70
Holobomolochus	Nostrils	6	20	1.00	2.00	0.06	0.40
Myxozoa							
Myxobolus aeglefini	Brain cartilage	13	20	1.00	1.00	0.13	0.20
Myxobolus aeglefini	Sclera	13	10	1.00	1.00	0.13	0.10
Nematoda							
Anisakis simplex,	Body cavity	25	30	2.25	2.00	0.56	0.60
Anisakis simplex	Flesh	13	0	3.00	0.00	0.38	0.00
Anisakis simplex	Gonads	22	11	2.50	1.00	0.56	0.11
Anisakis simplex	Heart	0	10	0.00	1.00	0.00	0.10
Anisakis simplex,	Intestine	50	100	4.00	3.63	2.00	3.63
Anisakis simplex x	Liver	25	0	34.75	0.00	11.58	0.00
	Liver	8	0	2.00	0.00	0.17	0.00
Anisakis simplex	Liver	85	86	20.55	7.17	18.83	6.14
Anisakis simplex and	Mesenteries	19	30	2.67	3.67	0.50	1.10
Anisakis simplex	Pyloric caeca	85	100	16.73	6.71	14.15	6.71
Anisakis simplex	Spleen	6	10	1.00	2.00	0.06	0.20
Anisakis simplex and	Stomach (ext)	30	29	2.00	1.50	0.60	0.43
Adult	Stomach–	0	14	0.00	3.00	0.00	0.43
Anisakis simplex,	Stomach–	20	29	1.00	39.00	0.20	11.14
Perkinsozoa							
Gadixcellia gadi	Gills	50	80	2.63	3.13	1.31	2.50
Trematoda							
Metacercaria	Brain	0	10	0.00	1.00	0.00	0.10
Metacercaria	Brain	0	20	0.00	3.00	0.00	0.60
Metacercaria	Gills	25	60	2.50	24.17	0.63	14.50

Derogenes varicus	Gills	19	80	2.67	1.75	0.50	1.40
Adult	Nostrils	0	10	0.00	2.00	0.00	0.20
Derogenes varicus	Intestine	29	38	9.50	17.00	2.71	6.38
Cyst of unknown							
CUE	Brain	6	10	1.00	1.00	0.06	0.10
CUE	Gills	6	20	1.00	1.00	0.06	0.20
CUE	Heart	19	10	2.00	5.00	0.38	0.50
CUE	Nostrils	13	20	1.50	2.00	0.19	0.40

3.2 Molecular confirmation results

The results from the sequencing data confirmed the identities of four different species of nematodes, one hybrid species and two species that could not be fully identified to a genus level. The sequencing for the two that were not fully identified indicated a <88% similarity to the respective barcode sequence of a spirurid nematode (referred to as species 1) and the other had a 90-91% similarity to a second species of spirurid nematodes. Two species of trematodes were identified, one of which (*Lepidapedon desciersae*), was not previously listed as a known parasite of haddock. One species of acanthocephalans was confirmed.

Table 6 A list of molecularly confirmed identities of species from the North

#	DNA sample	Host ID	Parasite	Site of infection	BLASTn ID
143	TG 074 B	HAD 09	Acanthocephala	Intestine	*Echinorhynchus gadi*
149	TG 077 B	HAD 08	Acanthocephala	Intestine	*Echinorhynchus gadi*
148	TG 077 A	HAD 08	Acanthocephala	Intestine	*Echinorhynchus gadi*
53	TG 050 A	HAD 09	Nematoda	Body cavity	*Anisakis simplex*
54	TG 050 B	HAD 09	Nematoda	Body cavity	*Anisakis simplex*
65	TG 057 A	HAD 01	Nematoda	Body cavity	*Anisakis simplex*
44	TG 044 B	HAD 04	Nematoda	Flesh	*Anisakis simplex*
63	TG 056 A	HAD 01	Nematoda	Flesh	*Anisakis simplex*
64	TG 056 B	HAD 01	Nematoda	Flesh	*Anisakis simplex*
60	TG 054 A	HAD 01	Nematoda	Gonads	*Anisakis simplex*
92	TG 059 A	HAD 08	Nematoda	Gonads	*Anisakis simplex*
93	TG 059 B	HAD 08	Nematoda	Gonads	*Anisakis simplex*
36	TG 038 A	HAD 22	Nematoda	Intestine	*Anisakis simplex*
48	TG 047 A	HAD 11	Nematoda	Liver	*Anisakis simplex*
90	TG 058 A	HAD 01	Nematoda	Liver	*Anisakis simplex*
91	TG 058 B	HAD 01	Nematoda	Liver	*Anisakis simplex*
94	TG 060 A	HAD 01	Nematoda	Liver	*Anisakis simplex*
95	TG 060 B	HAD 01	Nematoda	Liver	*Anisakis simplex*
99	TG 063 A	HAD 09	Nematoda	Liver	*Anisakis simplex*

100	TG 063 B	HAD 09	Nematoda	Liver	*Anisakis simplex*
45	TG 045 A	HAD 01	Nematoda	Mesentery	*Anisakis simplex*
46	TG 046 A	HAD 05	Nematoda	Mesentery	*Anisakis simplex*
47	TG 046 B	HAD 05	Nematoda	Mesentery	*Anisakis simplex*
30	TG 033 A	HAD 16	Nematoda	Pyloric caeca	*Anisakis simplex*
31	TG 033 B	HAD 16	Nematoda	Pyloric caeca	*Anisakis simplex*
49	TG 048 A	HAD 09	Nematoda	Pyloric caeca	*Anisakis simplex*
75	TG 025 A	HAD 12	Nematoda	Pyloric caeca	*Anisakis simplex*
76	TG 025 B	HAD 12	Nematoda	Pyloric caeca	*Anisakis simplex*
38	TG 039 A	HAD 14	Nematoda	Spleen	*Anisakis simplex*
96	TG 061 A	HAD 04	Nematoda	Spleen	*Anisakis simplex*
57	TG 052 B	HAD 01	Nematoda	Stomach	*Anisakis simplex*
67	TG 020 A	HAD 24	Nematoda	Stomach	*Anisakis simplex*
68	TG 020 B	HAD 24	Nematoda	Stomach	*Anisakis simplex*
58	TG 053 A	HAD 01	Nematoda	Stomach	*Anisakis simplex*
97	TG 062 A	HAD 01	Nematoda	Liver	*Contracaecum osculatum*
59	TG 053 B	HAD 01	Nematoda	Stomach (ext)	*Contracaecum osculatum*
78	TG 027 A	HAD 25	Nematoda	Intestine	*Hysterothylacium aduncum*
89	TG 057 B	HAD 01	Nematoda	Body cavity	*Pseudoterranova decipiens*
33	TG 035 A	HAD 16	Nematoda	Intestine	Spirurid sp. 1
61	TG 055 A	HAD 03	Nematoda	Stomach	Spirurid sp.2
62	TG 055 B	HAD 03	Nematoda	Stomach	Spirurid sp.2
52	TG 049 B	HAD 10	Nematoda	Stomach	No results
56	TG 052 A	HAD 01	Nematoda	Stomach	No results
102	TG 094 A	HAD06	Trematoda	Gills	*Derogenes varicus*
103	TG 094 B	HAD06	Trematoda	Gills	*Derogenes varicus*
114	TG 100	HAD13	Trematoda	Stomach	*Derogenes varicus*
115	TG 101	HAD03	Trematoda	Stomach	*Derogenes varicus*

Table 7 A list of molecularly confirmed identities of species from the South

#	DNA sample	Host ID	Parasite	Site of infection	BLASTn ID
146	TG 076 A	HAD 16	Acanthocephala	Intestine	*Echinorhynchus gadi*
144	TG 075 A	HAD 20	Acanthocephala	Intestine	*Echinorhynchus gadi*
145	TG 075 B	HAD 20	Acanthocephala	Intestine	*Echinorhynchus gadi*
34	TG 036 A	HAD 14	Nematoda	Stomach	*Anisakis simplex*
37	TG 038 B	HAD 12	Nematoda	Liver	*Anisakis simplex*
39	TG 041 A	HAD 13	Nematoda	Pyloric caeca	*Anisakis simplex*
40	TG 042 A	HAD 26	Nematoda	Stomach	*Anisakis simplex*
66	TG 019 A	HAD 19	Nematoda	Intestine	*Anisakis simplex*
77	TG 026 A	HAD 23	Nematoda	Heart	*Anisakis simplex*
82	TG 029 A	HAD 21	Nematoda	Mesenteries	*Anisakis simplex*
86	TG 031 A	HAD 19	Nematoda	Liver	*Anisakis simplex*
87	TG 031 B	HAD 19	Nematoda	Liver	*Anisakis simplex*
88	TG 032 A	HAD 14	Nematoda	Gonads	*Anisakis simplex*
69	TG 021 A	HAD 23	Nematoda	Liver	*Anisakis simplex X Anisakis pegreffi* (Hybrid)
71	TG 022 A	HAD 23	Nematoda	Gills	*Contracaecum osculatum*
74	TG 024 A	HAD 18	Nematoda	Body cavity	*Contracaecum osculatum*
83	TG 029 B	HAD 21	Nematoda	Mesenteries	*Contracaecum osculatum*
101	TG 029 B	HAD 21	Nematoda	Mesenteries	*Contracaecum osculatum*
42	TG 043 B	HAD 22	Nematoda	Intestine	*Hysterothylacium aduncum*
73	TG 023 B	HAD 16	Nematoda	Stomach	Spirurid sp.2
81	TG 028 B	HAD 21	Nematoda	Stomach	Spirurid sp.2
104	TG 095 A	HAD14	Trematoda	Gills	*Derogenes varicus*
106	TG 096 A	HAD23	Trematoda	Gills	*Derogenes varicus*
108	TG 097 A	HAD17	Trematoda	Gills	*Derogenes varicus*
109	TG 097 B	HAD17	Trematoda	Gills	*Derogenes varicus*
112	TG 099 A	HAD16	Trematoda	Stomach	*Derogenes varicus*

122	TG 105 A	HAD19	Trematoda	Intestine	*Lepidapedon desciersae*
123	TG105 B	HAD19	Trematoda	Intestine	*Lepidapedon desciersae*
106	TG 096 B	HAD23	Trematoda	Gills	No results

4 Discussion

Eleven of the gill examinations of the specimen revealed *Derogenes varicus* that normally reside in the oesophagus but had likely migrated to the gills post mortem but those infections had an 80% prevalence in the southern location.

The results from the discriminatory analysis showed a varying degree of reclassification success between the two localities. This was due to the dataset collected throughout the project having incidences of a single parasite found in one organ in a single host. This creates what is called the rarity effect, where the statistical analysis favours that one infection, providing results pointing to that single infection being a great difference between the two localities. However, as the guidelines layed out by MacKenzie and Abaunza (1998) point out, a single infection, no matter how heavy, is not viable for use as a biological tag.

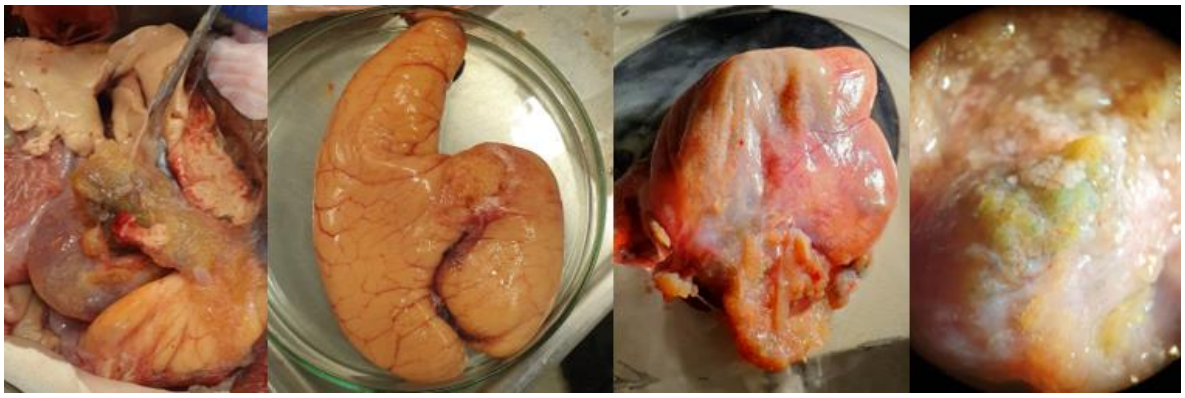

Figure 3 The fused organs, the severed gonads, the ball of liquid and the green mass that was inside it

4.1 Known parasites and their life cycles

4.1.1 Acanthocephala

The acanthocephalan *Echinorhynchus gadi* is a helminth commonly found in gadid fish. Filter feeding crustaceans become their first intermediate host as the crustaceans consume their eggs and there, they grow from acanthors to acanthella (Khan, 2008). Several species of smaller teleosts can become the second intermediate host when they consume the crustaceans including capelin (*Mallotus villosus*) and young cod (Khan, 2008) . They have a range of definitive hosts, including gadids that they reach through tropic transmission as the gadids eat the primary fish host (Khan, 2008). The distribution between the two localities was fairly even indicating the existence of suitable intermediate hosts in both areas as well as similarities in diet of the haddock, both the North and South of Iceland.

The acanthocephalan *Corynosoma* sp. undergoes early development in their first intermediate host, arthropods where they await the host to become prey to fish (Ekbaum, 1938). The fish acts as a second intermediate host, where the acanthocephalan makes its way into the body cavity where it encapsulates and does not go through any development

(Ekbaum, 1938). Their definitive hosts are warm blooded vertebrates, either avian or marine, where they reach sexual maturity (Ekbaum, 1938). The processing acanthocephalan DNA did not yield usable results for molecular confirmations so the presence of *Corynosoma* sp. could not be confirmed in either locality.

4.1.2 Copepoda

The copepod *Lernaeocera branchialis* is a parasite known parasitise gadids as their definitive host (Brooker et al., 2007). They, unlike most copepod parasites have a complex life cycle involving two hosts. Their life cycle starts with two free living naupilus stages, the first one only lasting a few minutes before it moults and sinks down to the benthic environment (Brooker et al., 2007)where it moults into a copepodid in about 48 hours at a water temperature of 10°C (Whitfield et al., 1988). There it seeks out a benthic fish host imbedding itself into the gill tissue where it feeds and goes through four morphs, from chalimus I to chalimus IV and finally into an adult (Whitfield et al., 1988). They can then move freely and swim in search of its definitive host if need be although they can also simply remain in the primary fish host, making it their definitive host (Whitfield et al., 1988). The prevalence, intensity and abundance of the species was greater in the specimen from the southern locality. They difference was however not deemed great enough to warrant further investigation their use as biological tags.

4.1.3 Microspora

Loma morhua is a spore forming microsporidian parasite that is often found on the gills of gadids. The fish gets infected upon ingestion of infective spores that expel their polar tubes when exposed to gastric juices within the hosts stomach leading to the cells getting infected with the sporoplasm released from the spores (Rodriguez-Tovar et al., 2003). The infection is then believed to be carried to the gills via infected blood cells where the infection is most visible (Rodriguez-Tovar et al., 2003). The prevalence, abundance and intensity were calculated on the basis of the presence or absence of *L. morhua* within the two localities.

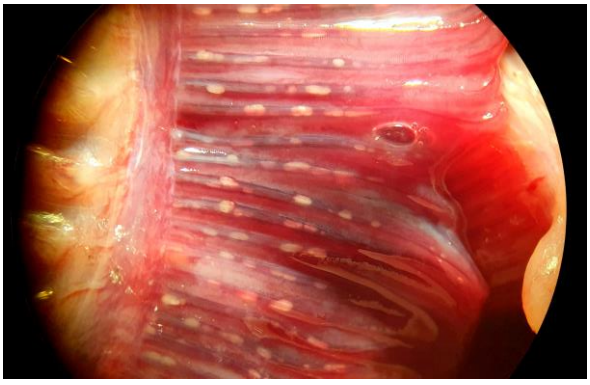

Figure 4 A particularly heavy Loma morhua infection from specimen HAD 21

Visual confirmation from the necropsies indicated greater intensities of infection in the specimen from the southern locality (see Figure 4). A larger sample size would be required to provide further statistical information in order to determine whether *L. morhua* could be viable as a tag species.

4.1.4 Myxozoa

Myxidium bergense and *Myxidium gadi* are two species of myxozoa that both infect the gall bladders of gadoids. However, little is known about their life cycles or distribution. *Myxidium bergense* has shown potential for being used in stock discrimination off the coast of Canada but more time, a deeper understanding of these parasites and better tools would be needed for them to be included into this research.

4.1.5 Nematoda

Anisakis simplex like many other nematodes, they start their lives as their eggs are passed out from their mammalian definitive hosts with feces. In the eggs they molt twice before hatching as free swimming third stage larvae ensheathed in second stage larval cuticle (Køie et al., 1995). Their first intermediate host are usually small crustaceans such as krill or copepods, from which they are trophically transmitted to teleosts. They stay as third stage larvae as they get trophically transmitted, sometimes between a few species of fish until they reach their definitive host, cetaceans, where they moult twice and become adults (Buchmann & Mehrdana, 2016).

The anisakid nematode *Contracaecum osculatum* has a complex life cycle that starts as adults release their eggs in the definitive host, often a seal. They hatch in the water and third stage free-swimming larvae are believed to infect copepods or amphipods as their first intermediate host (Haarder et al., 2014). Small fish such as herring or other species from the Clupidae family then eat the invertebrates and become their primary fish host. Larger fish, such as species from the Gadidae family often serve as their secondary fish host after they eat the infected smaller fish. The infection tends to be in the liver in gadids where they get encapsulated by the hosts. Their definitive hosts are warm blooded vertebrates, usually seals, that get infected with third stage larvae as they feed on the gadids (Zuo et al., 2018). The larvae go through two moults in the stomach of the definitive host before becoming adults (Haarder et al., 2014).

Hysterothylacium aduncum an anasakid nematode has been found to be incredibly abundant in haddock in the North Sea. Their life cycle was described in detail by Køie in her paper published in 1993, where she experimented with and documented the life stages of *H. aducum*. It moults twice inside the egg before emerging as a third stage larvae ensheathed in the cuticle of the second stage larvae and appears only to be capable of infecting crustaceans such as copepods. Køie's experiment showed that when the crustaceans were consumed the larvae inside the crustacean is not infective to all invertebrates, but four species of polychaetes and one species of brittle star were susceptible to the infection. Larvae longer than 2 mm were however able to survive in the primary fish host as third stage larvae and those that penetrated the body cavity could grow. The larvae that were encapsulated in the intestinal wall could survive and grow to about 1 cm if they were not completely encapsulated while those that were did not achieve much growth and died. Much like with other nematodes, *H. aducum* usually has a smaller fish host as an intermediate host but different from nematodes that have their definitive host as warm-blooded mammals, *H. aducum* has larger fish as their definitive host (Køie, 1993).

Pseudoterranova decipiens, commonly known as sealworm, has a similar life cycle as *Contracaecum oculatum*. As the name indicates their definitive hosts are pinnipeds from which their eggs emerge from when the seals defecate. Due to the economic loss, they have

inflicted upon fisheries their life cycle has been researched well and is thoroughly described by McClelland in their review "The trouble with sealworms". The larvae moult inside the egg although there is some uncertainty to what stage it is that emerges from the egg. Once out they can survive in cold water (0-5°C) and be infective to copepods for over 100 days. The ensheathed larvae ingested by copepods or amphipod where they exsheathe and penetrate the haemocoel. At this stage the larvae are not infective to fish but are infectious to macroinvertebrates such as crustaceans, polychaetes, nudibranchs and others if they consume the first intermediate host. The larvae do not moult in the second intermediate host but they do achieve growth. *Pseudoterranova* sp. are known for not being host specific and have been found in at least 75 species of fish in the North Atlantic. They rely on small fish of various species, including young commercially important fish such as gadids, as their primary fish host, where they usually move into the musculature of the fish. Their secondary fish host are larger piscivorous fish such as monkfish and cod where if they are not trophically transmitted to their definitive host they are often killed by the hosts immune responses (McClelland, 2002).

While the statistics showed some degree of difference of prevalence, intensity and abundance of different nematode species between the two regions, I conclude that none of the nematode species would be suitable as biological tags as it proved difficult to separate them into species based on morphological confirmation.

4.1.6 Trematoda

The digenean species *Derogenes varicus* has a complex life cycle. Their vulnerable free living metacercariae are known to infect calanoid and harpactacoid copepods as their first intermediate host. They can then be trophically transmitted from the miniscule copepods to small fish and can infect larger predatory fish such as haddock when those fish become prey (Koie, 1979).

4.2 Data

Due to inconsistencies in collected data from specimen the dataset had to be broken down into several subsets of data. The main reason for this being the incomplete processing of organs either due to them not being located, or not being examined because of time constraints. The urinary- and gall bladders of the specimen were often found empty or not located at all. Not all specimen had reached sexual maturity so despite all observed gonads being processed, they were omitted from the datasets due to insufficient presence in the sampling pool. Mesenteries were isolated when possible but when firmly attached to the organs they were processed with the organs themselves. Due to the labor-intensive work of isolating parasites from heavily infected organs, the processing of the entire load of organs could not be fully completed, with 27 organs still awaiting processing. The organs missing from the datasets are seven livers, eight stomachs, six pyloric caeca and five intestines.

4.3 Analysis of data

The list of prevalence, intensity and abundance did not show any variation between the two localities that could indicate a that target species for the use in stock discrimination of haddock had been found. Incidences of the presence of the copepod *Clavella adunca* in the gills the specimen was infrequent, but the presence, abundance and intensity of the copepod *Lernaeocera branchialis* presented at a much higher frequency and intensity in the southern locality. Nematodes were ubuiquitus in both localities but did not present any statistical importance within the data. The prevalence of one species, the digenean trematode *Derogenes varicus*, showed much higher prevalence in the southern locality. *Derogenes varicus* normally resides in the oesophagus of teleosts but the specimen found during this research had migrated into the gills of a few hosts post mortem. A completed dataset (including all organs of the specimens) as well as a larger sample size would provide a more accurate statistical insight into differences between the two localities.

4.4 Research questions addressed

To address the two questions proposed for this project I conclude that:

1. The statistical analysis showed a higher prevalence, intensity and abundance of two species, the trematode *Derogenes varicus* and the copepod *Lernaeocera branchialis* in the southern region, indicating their possible use as biological tags for stock discrimination purposes.
2. This research could not definitively conclude any of the parasite's viability as a biological tag. *Derogenes varicus* does however fit the criteria laid out by MacKenzie and Abaunza (1998). The life cycle of *D.varicus* is well documented, their life-spans can be up to 15 months (Meskal, 1967), their distribution spans both localities and they are both visible and identifiable. I believe that this warrants further research.

4.5 Conclusion

The results from this research indicated that at least two species, the trematode *Derogenes varicus* and the copepod *Lernaeocera branchialis*, could prove to be viable as biological tags for stock discrimation of haddock in Icelandic waters. Both species are easily recognized based on morphological features and showed a significant statistical difference between the two localities making them viable candidates for further research. A more accurate representation of the parasite fauna would be achieved from performing a study that (1) included a larger sample size, (2) sampled from more locations, including the area between the localities sampled in this study, (3) would be long enough to span annual and seasonal changes to verify the viability of *Derogenes varicus* and *Lernaeocera branchialis* as biological tags for stock discrimination. The two parasites could potentially be used for future stock discrimination of other gadoid species and could prove more convenient than the use of microscopic myxosporid parasites that have been the focus of previous studies, as they are clearly visible with the naked eye.

5 References

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6 Appendix

6.1 R codes

Rstudio was used to generate a map with coordinates on it. The code used in that process is as follows:

```
library(leaflet)
locations <- data.frame(Name = c("Specimen 01-05", "Specimen 06-13", "Specimen 14-18", "Specimen 19-23", "Specimen 24-26"), Lat = c(66 + 6/60 + 33/3600, 66 + 3/60 + 33/3600, 63 + 57/60 + 57/3600, 63 + 55/60 + 37/3600, 65 + 42/60 + 44/3600), Lon = c(-18 - 33/60 - 31/3600, -17 - 34/60 - 41/3600, -22 - 48/60 - 20/3600, -22 - 46/60 - 49/3600, -20 - 26/60 - 3/3600))
map <- leaflet() %>%addTiles(urlTemplate =
"https://{s}.tile.openstreetmap.org/{z}/{x}/{y}.png")
for (i in 1:nrow(locations)) {offset <- c(0, 0)  # Default offset values if (i == 1) { offset <- c(0,-65) } else if (i == 3) { offset <- c(0,-65)}
map <- map %>%
addCircleMarkers(lat = locations$Lat[i], lng = locations$Lon[i],radius = 4,
fill = TRUE, fillOpacity = 1, fillColor = colors[i], color = colors[i], stroke = FALSE,label = locations$Name[i],labelOptions = labelOptions(noHide = TRUE, direction = "bottom", textOnly = TRUE, offset = offset, style = list(fontSize = "28px")), popup = locations$Name[i]
map    offset <- c(0,-65)  }
map <- map %>%
addCircleMarkers(lat = locations$Lat[i],  lng = locations$Lon[i], radius = 4,
fill = TRUE, fillOpacity = 1, fillColor = colors[i], color = colors[i], stroke = FALSE,
label = locations$Name[i], labelOptions = labelOptions(noHide = TRUE, direction = "bottom", textOnly = TRUE, offset = offset, style = list(fontSize = "28px") ), popup = locations$Name[i])}

map
```