FINAL REPORT

Improving our knowledge about *Hysterothylacium aduncum* in cod, saithe and haddock from Norwegian waters with special focus on preventive measures for the industry

FHF project nr. 901553

Miguel Bao, Paolo Cipriani, Arne Levsen

Havforskningsinstituttet (IMR)

27.02.2020

1. Summary

During spring and summer of 2018, fish lots of Northeast arctic cod (*skrei*) were sold from a Norwegian fishing company to a Spanish customer. The client realised the presence of big and lively roundworms (*Hysterothylacium aduncum*) over the fish and styrofoam boxes, and as a consequence, the fishing lots were rejected. This event resulted in important monetary losses to the Norwegian fishing company and to some extent also client distrust on cod products from Norway. The present project has been carried out aiming to investigate and solve the problematic caused by the presence of *H. aduncum* in whitefish industry.

The parasitic nematode *H. aduncum* uses a wide range of intermediate invertebrate hosts (e.g. copepods, euphausiids, etc.) and second intermediate/transport or final fish hosts in the marine environment. This generalist strategy broadens immense possibilities of dispersion and successful completion of its life cycle, making *H. aduncum* one of the most abundant and ubiquitous parasite of fishes of NE Atlantic waters. The presence of *H. aduncum* in fish from the Barents Sea waters has been reported in the 50s, however its life cycle and epidemiology in fish is poorly documented or unknown. In here, we have morphologically and molecularly identified *H. aduncum* from Northeast Arctic cod (skrei), haddock and saithe captured in West-Finnmark during winter, early spring and late spring. Results show that the infection values in the digestive tract of fish are highly variable, ranging from 100% prevalence with mean abundance 238 in cod to 27% prevalence with mean abundance 1 in haddock. Results suggest that season is the most important factor explaining the parasite abundance in fish. Infection values were higher during winter and early spring and lower during late spring. It is hypothesized that the three fish species gain the maximum load of *H. aduncum* through predation on spawning capelin during winter and early spring. After that, *H. aduncum* will reproduce and disappear from the fish in a few months. New infections may still occur during late spring and probably throughout the whole year. Due to the rapid heaving speed during line fishing, some fishes have their stomach expelled when landed on deck. This phenomenon facilitates the movement of *H. aduncum* from the fish stomach (where they naturally live) to the head cavities (i.e. mouth, gills and pharynx region). Evisceration would remove most of *H. aduncum* present in the fish, however some (maybe many) can remain hidden within the head cavities. As observed, *H. aduncum* can crawl out from there to the fish body surface or styrofoam boxes during transportation and be observed by the customer at destination point. Thus, *H. aduncum* has the potential to cause significant monetary losses to Norwegian exporters of fresh whitefish. In addition, results show that *H. aduncum* can remain alive and active if kept in humid and cold conditions for at least 14 days. The parasite is not present in fish fillets. Finally, *H. aduncum* can be completely removed by evisceration, beheading and rinsing of fish during handling/cleaning practices at processing plants. Alternatively, apart from evisceration and rinsing, a thorough cleaning or completely removal of the head cavities (i.e. the gill/pharynx region) may be considered.

2. Introduction

Parasitic nematodes commonly known as "*kveis*" in Norwegian, often occur in the viscera and muscle of many North East Atlantic commercially important marine fish species, such as cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aegleginus*) (Gay et al., 2018; Levsen et al., 2018; Pierce et al., 2018; Strømnes and Andersen, 1998). Nematodes of the family Anisakidae (Nematoda: Ascaridoidea), and particularly the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*,

are of medical and socioeconomic concern as they are the causative agents of a fish-borne zoonosis called anisakidosis (EFSA-BIOHAZ, 2010).

Besides the presence of anisakids in fish, the occurrence of other ascaridoid nematodes belonging to the family Raphidascarididae (Nematoda: Ascaridoidea), i.e. *Hysterothylacium* spp., are also very common (Klimpel and Rückert, 2005). *Hysterothylacium* spp. use fish as final host, whilst *Anisakis* spp. and *Pseudoterranova* spp. use marine mammals (cetaceans and seals, respectively) as final hosts and fish serve as intermediate and/or transport host during the life cycle. The species *Hysterothylacium aduncum* inhabits the viscera of fish (forth larval stage (L4) and adults are located and reproduce within the digestive tract, whilst third larval stage (L3) is commonly located on the internal organs such as pylorus) (Berland, 1989). Since *H. aduncum* lives exclusively in cold-blooded organisms (i.e. marine invertebrates and fish), it is per definition not adapted to the conditions that prevail in the alimentary tract of mammals (Karl and Levsen, 2011). Thus, *H. aduncum* is commonly considered non-zoonotic. In addition, *H. aduncum* can indeed heavily infect some fish species (Klimpel and Rückert, 2005), and the massive presence of larval or adult specimens may heavily reduce the aesthetical appeal of fish products, potentially causing socioeconomic problems to the industry.

3. Problematic and objectives

3.1 Initial problematic

Briefly, during spring and summer of 2018, fish lots of Northeast arctic cod (hereinafter cod) were sold from a Norwegian fishing company to a Spanish client. At arrival, the client realised the presence of big and lively roundworms over the fish and styrofoam boxes (Figure 1). In consequence, the client rejected the fishing lots. This event resulted in important monetary losses to the Norwegian fishing company and to some extent also client distrust on cod products from Norway. Initial advice was provided to the Norwegian fishing company and the Spanish client by the Parasitology Group of the IMR (Institute of Marine Research) of Bergen. Eventually, this "*Hysterothylacium*" problematic was communicated from the Norwegian fishing company to the FHF who decided to open a call for a project in order to investigate and solve the problem. The Parasitology Group of the IMR-Bergen submitted a project proposal and eventually got the project.

Figure 1: *Hysterothylacium* sp. nematodes over the fish and styrofoam boxes at destination point.

3.2 Duration of the project

Start date: 18.02.2019

End date: 28.02.2020

3.3 Participants

Havforskningsinstituttet: Miguel Bao-Dominguez (project leader), Paolo Cipriani, Arne Levsen

FHF: Frank Jakobsen (FHF project responsible)

Members of the reference group: Terje Kjølsøy (Aalesundfisk AS), Håvard Henriksen (ToBø Fisk AS), Leif Kvivesen (Andenes Fiskemottak AS), Arne Karlsen (Gunnar Klo AS), Martin Flåten (Lerøy Norway Seafoods AS)

3.4 Objectives

The main objective was to gain a better knowledge in relation to the presence of *H. aduncum* in cod, Northeast Arctic saithe (hereinafter saithe) and Northeast Arctic haddock (hereinafter haddock) caught in Norwegian waters, as well as to study the current handling procedures along the fish value chain in order to help the industry to develop/improve better parasite preventive practices/strategies.

The specific objectives were:

1) To collect and process information on *H. aduncum* available in the literature by performing a literature study. There is a need to gain more knowledge about the parasite life cycle, ecology, its presence in cod, saithe and haddock, and other fish species (e.g. capelin), and its possible zoonotic potential to humans.

2) To perform an epidemiological study on these target fish species caught in different seasons with special focus on infection level and anatomical location of the parasite in the fish, and particularly if it can also be present in the muscle.

3) To assess the survival and motility of the parasite by mimicking the temperature conditions during transport and storage of fresh cod to European markets.

3.5 Impact for the industry

The parasitic nematode *H. aduncum* may be present in high numbers in whitefish, and its presence may have a significantly negative impact due to loss of fish marketability and customer/consumer confidence.

Based on new knowledge about the presence of *H. aduncum* in cod, saithe and haddock, and through revision of handling practices, the industry could implement improved parasite control measures adapted to fish species, fishing ground, season, etc. This again, in principle, could significantly mitigate or even eliminate the socioeconomic impact caused by *H. aduncum*.

4. Methodology and project implementation

4.1 Fish sampling

4.1.1 Fish sampling on-board/factory

The 12th of March 2019, Miguel Bao and Paolo Cipriani joined a regular coastal fishing vessel in West-Finnmark. This gave us the opportunity to understand how the fish was handled from catch at sea to landing at the processing plant.

The following day, we sampled 10 recently landed cod for the presence of *Hysterothylacium* sp. Further details about the sampling methodology are given in section 4.2.

4.1.2 Fish sampling at the IMR facilities

Fishes were caught by professional fishermen (jigging, longline or Danish seine) at catching area 80425, Hjelmsøybanken (Barents Sea) from March to May 2019 (see Table 1; the tables are located in the appendix). Fishes were frozen as soon as possible after capture and sent to the IMR facilities for parasitological inspection (see below, section 4.2). In addition, 18 cod were sampled at the beginning of February as part of the annual IMR winter cruise in the Barents Sea.

4.2 Parasitological sampling

Data on total length (TL), total weight (TW) and sex were recorded for each fish individual (Table 2). Skin, fins, mouth and gills were visually inspected by naked eye for the presence of L4 and/or adult *Hysterothylacium* sp. Later, the body cavity was opened to expose the internal organs. The digestive tract (i.e. stomach, pyloric caeca and intestine) was removed for later inspection. The stomach and intestine were carefully opened to expose their content, and *Hysterothylacium* sp. inspection was carefully performed using forceps and a lamp. After inspection, the whole digestive tract including the pyloric caeca was placed into a transparent plastic bag for further inspection of L3 *Hysterothylacium* sp. by UV-press method (further details below, section 4.3). Fish fillets, liver and gonads were also inspected by the UV-press method. All parasites were counted, and their anatomical location reported (Table 2). Subsamples were morphologically and molecularly identified (see details below, section 4.4 and 4.5).

4.3 UV-press methodology

The UV-press method utilises the fluorescence of dead-by-freezing nematodes when exposed to UV-light (Pippy, 1970). For that, fish fillets or internal organs are placed in transparent plastic bags and flattened to 1-3 mm thin layers using a hydraulic press before UV-light exposure at 366 nm in a darkened room (Karl and Leinemann, 1993; Levsen and Lunestad, 2010). Larval and adult *Hysterothylacium* sp. may be tentatively distinguished from other nematodes present in fish (i.e. *Anisakis* spp., *Pseudoterranova* spp. and *Contracaecum* spp.) by differences in shape as well as intensity and shade of fluorescence. Internal organs (i.e. liver, gonad, stomach, pyloric caeca and intestine) and fish fillets were inspected for L3 *Hysterothylacium* sp. and their anatomical location recorded (Table 2).

4.4 Morphological identification

Hysterothylacium sp. subsamples from all three fish species were identified to species level and to life cycle stage (i.e. L4/adult or L3) by light microscopy using morphological characters such as presence or absence of lips/boring tooth, intestinal caecum, ventricle, ventricular appendix and ‘‘cactus’’ tail (Berland, 1989, 1961).

4.5 Molecular identification

Hysterothylacium sp. subsamples recovered from gills, skin, stomach or intestines of cod (n= 7), haddock (n= 6) and saithe (n= 5) were molecularly identified by sequence analyses of the internal transcribed spacers of nuclear ribosomal DNA (ITS rDNA) (Zhu et al., 2000).

4.6 *Hysterothylacium* sp. viability assessments

Hundreds of *Hysterothylacium* sp. adults were collected from fresh cod (n=10) recently caught and delivered at a fish factory in West-Finnmark the 13th of March 2019. Living nematodes were obtained from cod digestive tracts and placed in falcon tubes with freshwater. The nematodes were transported the following day to IMR facilities in Bergen, and they were transferred to petri dishes with physiological water and placed in a fridge at around 4-5 °C. Parasites showed vigorously movements until the 19th of May when the survival assessment started.

Hysterothylacium aduncum is not a mammal parasite, therefore it will eventually die in a warm mammalian stomach. However, it is not known for how long it could stay alive under such conditions. Parasite viability was assessed in accordance with CODEX standard for salted herring and sprat (Codex Alimentarius, 2004). A nematode is considered viable when it is physically intact and motile, as demonstrated by spontaneous movements when stimulated mechanically with forceps (Codex Alimentarius, 2004; EFSA-BIOHAZ, 2010).

Hysterothylacium sp. viability was challenged by using different media (physiological water and pepsin solution), temperature conditions (approx. 4.5 °C and 36.5°C) and exposure time (Table 3). The parasites were placed in petri dishes (N= 20 parasites per petri dish) containing 100 ml of respective media and challenged under different temperature and timely conditions (Table 3). A second survival experiment was additionally performed only for warm conditions (Table 4).

4.7 Data analyses

Basic infection parameters (prevalence, abundance and intensity) per infection site (gill/mouth/skin, digestive tract, pyloric caeca and overall) were calculated as described in Bush et al. (1997).

The relationship between total nematode numbers in viscera and fish size (TL and TW), sex and season were statistically studied in Statistica 13.4.0.14.

5. Results

5.1 Morphological and molecular results

Hysterothylacium sp. subsamples were morphologically identified as adult/L4 or L3 *H. aduncum*. Based on the ITS rDNA sequences obtained, n= 17 *Hysterothylacium* sp. were molecularly identified as *H. aduncum* (The ITS rDNA sequences (908 to 943 bp) showed 99.8 to 100% similarity with the ITS rDNA sequences of previous works deposited in GenBank).

5.2 Infection levels and sites

Hysterothylacium aduncum was present in cod, haddock and saithe from West-Finnmark in all sampling periods. However, the level of infection varies with fish species, site of infection and season (Table 2). The highest infection levels were found in cod sampled at the factory in the middle of March, with 100% prevalence and 238 ± 337 mean abundance ± standard deviation in the digestive tract.

Hysterothylacium aduncum varies considerably in length in cod, haddock and saithe. The average length ± SD (range) of *H. aduncum* adults (N= 287) from cod fished in February and March was 4.9 ±

1.3 cm (1.8 - 10.5 cm). The parasite can grow quite big, and this clearly shows the potential of the parasite to cause client/consumer rejection if present in the final product (Figure 2).

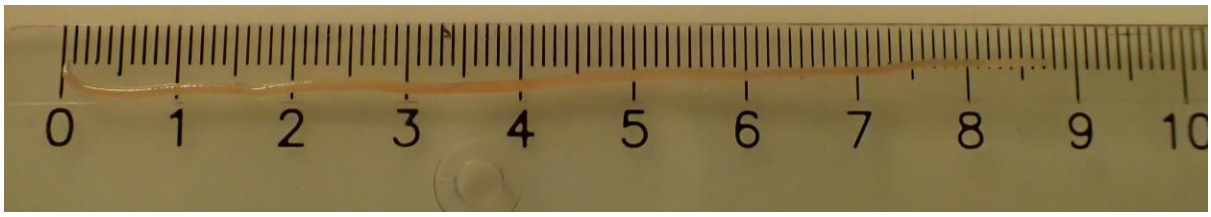

Figure 2: Adult *H. aduncum* recovered from the stomach of saithe.

Adults and L4 larvae were found on the skin, mouth, pharynx, gills and within the stomach and intestine, and L3 larvae in the pyloric caeca of cod (Figure 3), haddock (Figure 4) and saithe (Figure 5). The parasite was frequently found in the head cavities of fish (i.e. pharynx, mouth and gills) and/or on the skin (Table 2), occasionally in large numbers (Figure 6). Numerous *H. aduncum* were also frequently observed in fish stomachs where they wriggled around in newly eaten capelin, apparently aiding in the initial digestive split-up of the prey (Figure 7). *Hysterothylacium aduncum* was absent from fish fillets.

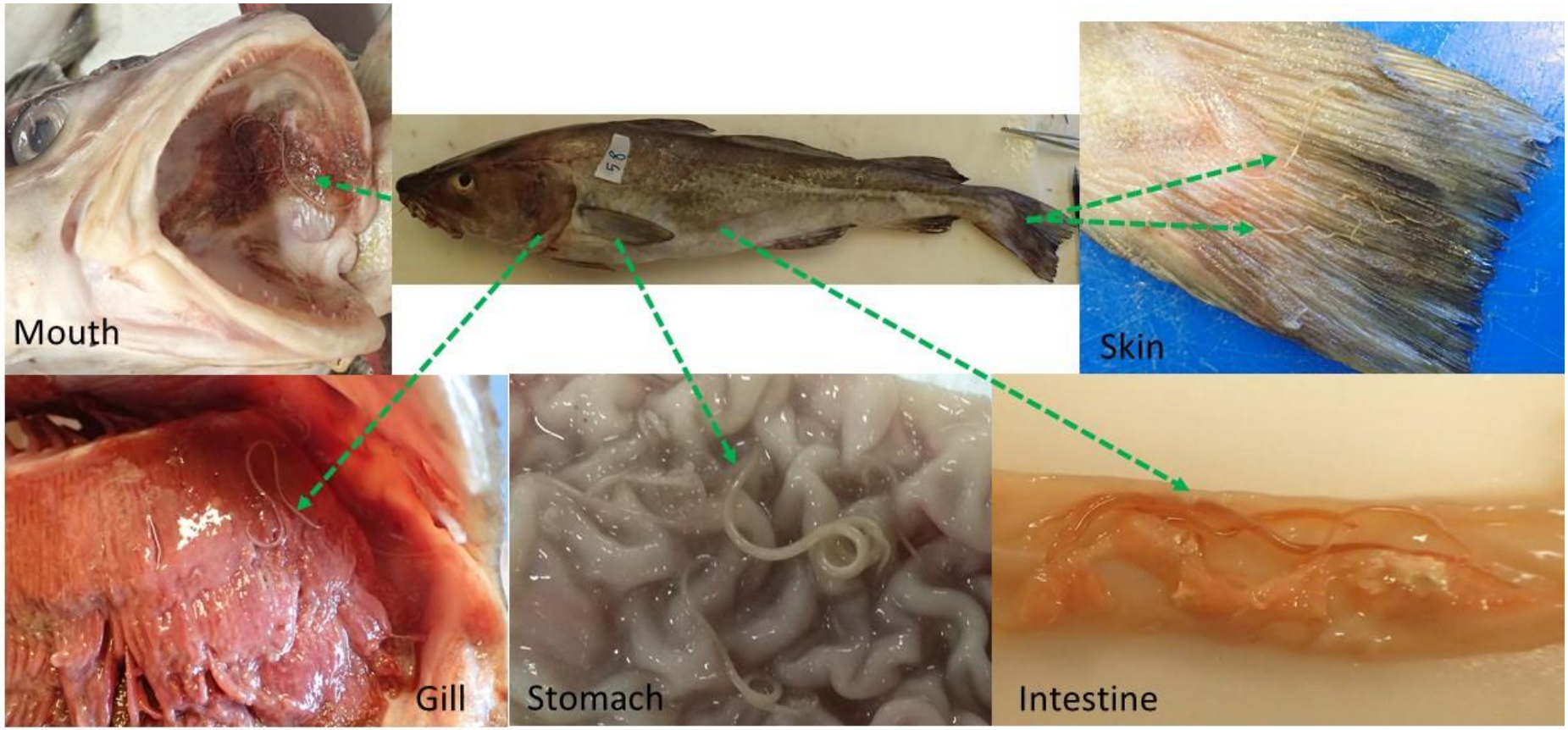

Figure 3: Natural and unnatural finding sites of *H. aduncum* during cod inspection.

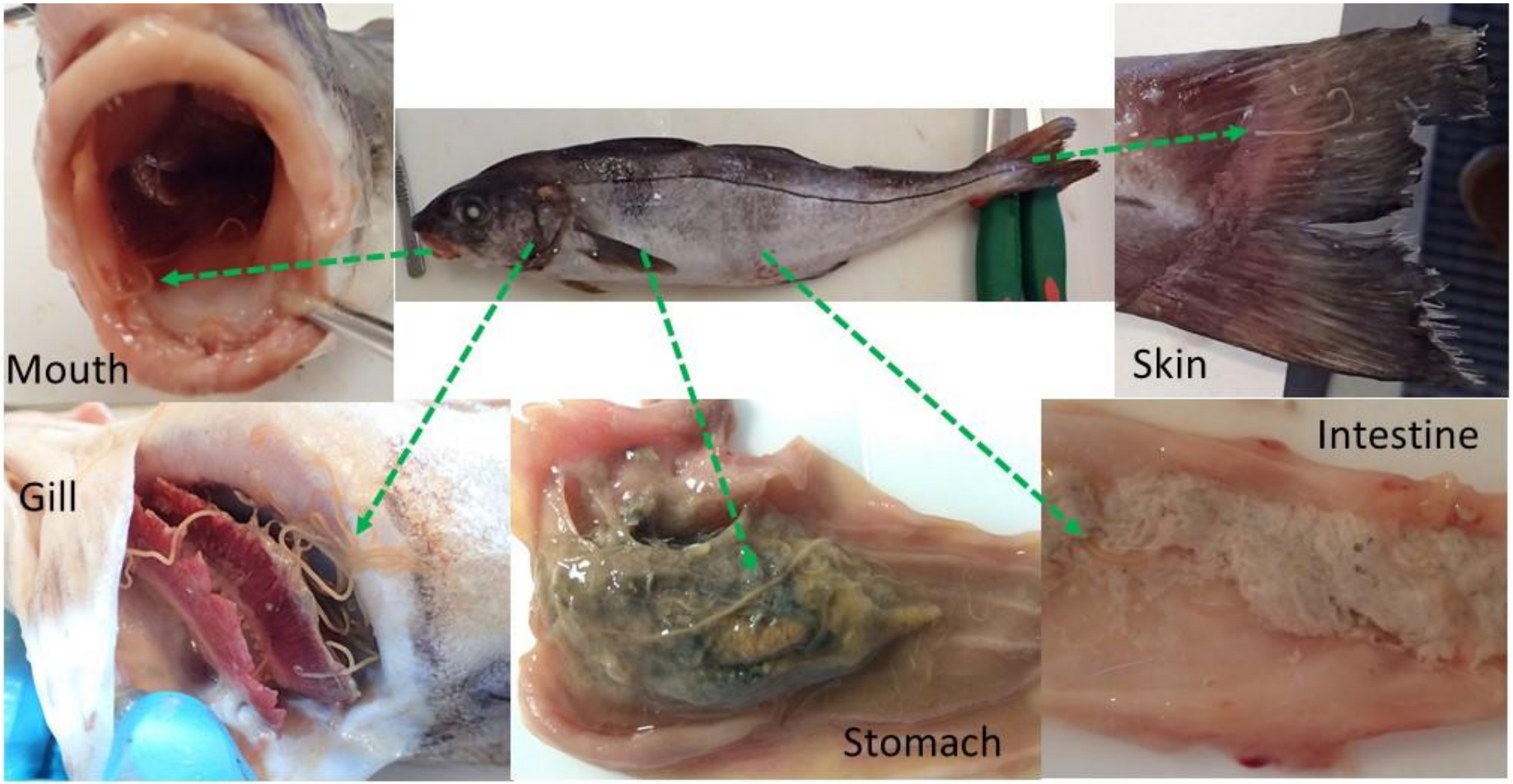

Figure 4: Natural and unnatural finding sites of *H. aduncum* during haddock inspection.

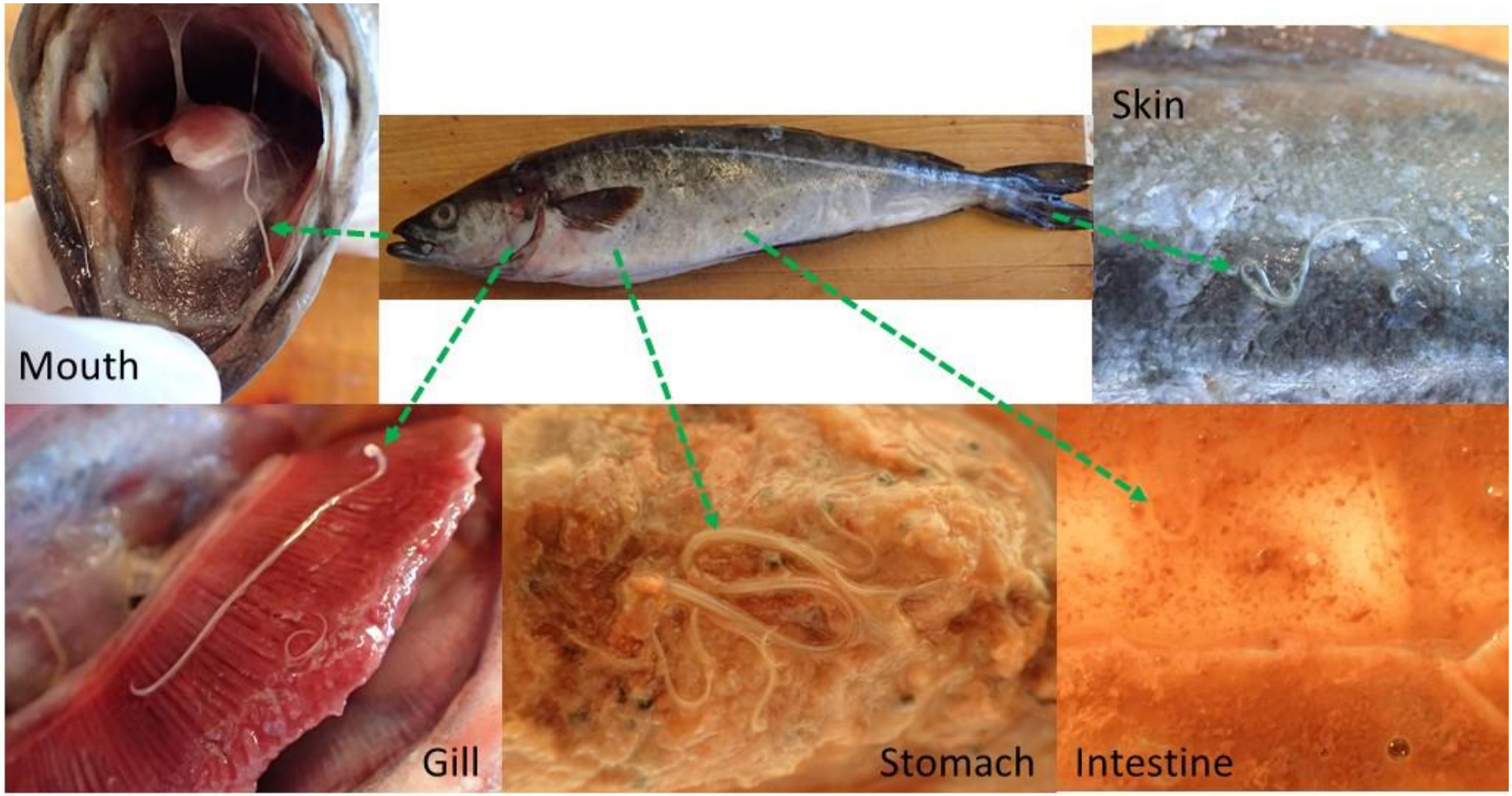

Figure 5: Natural and unnatural finding sites of *H. aduncum* during saithe inspection.

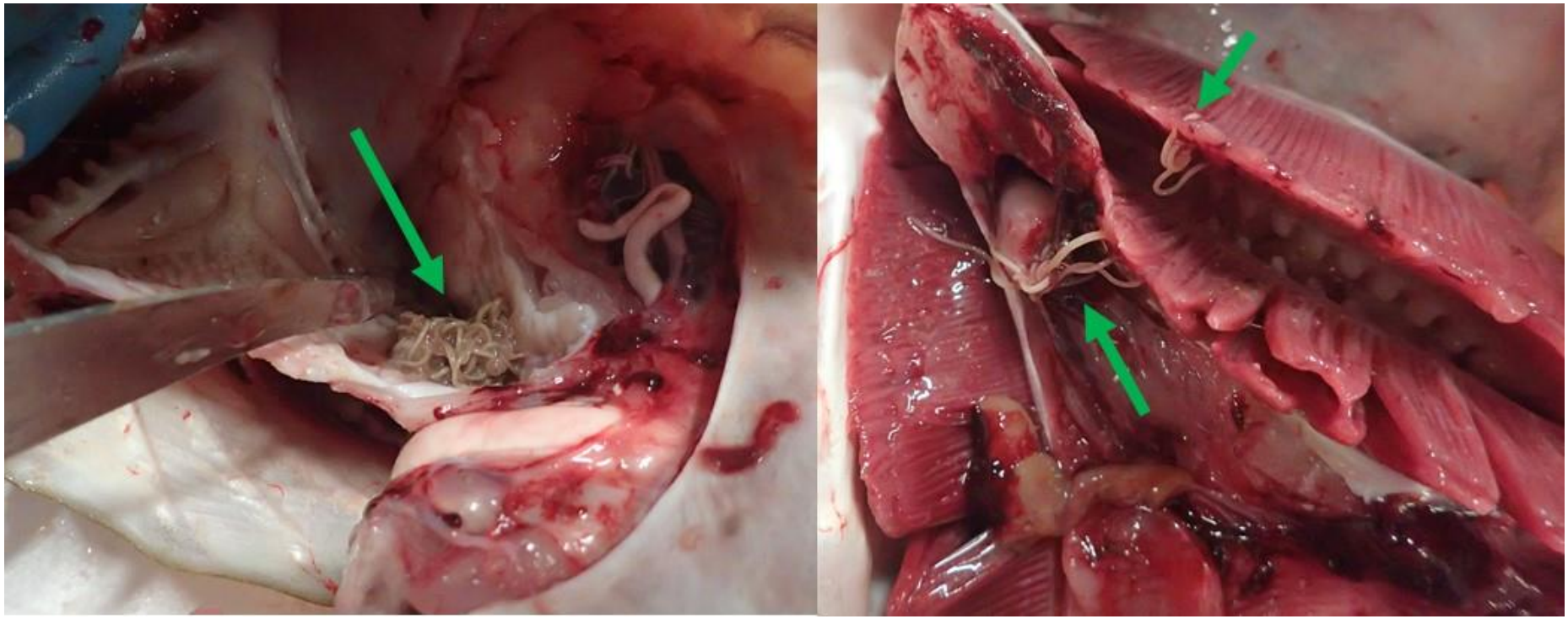

Figure 6: Many *H. aduncum* in the pharynx and on the gills of cod.

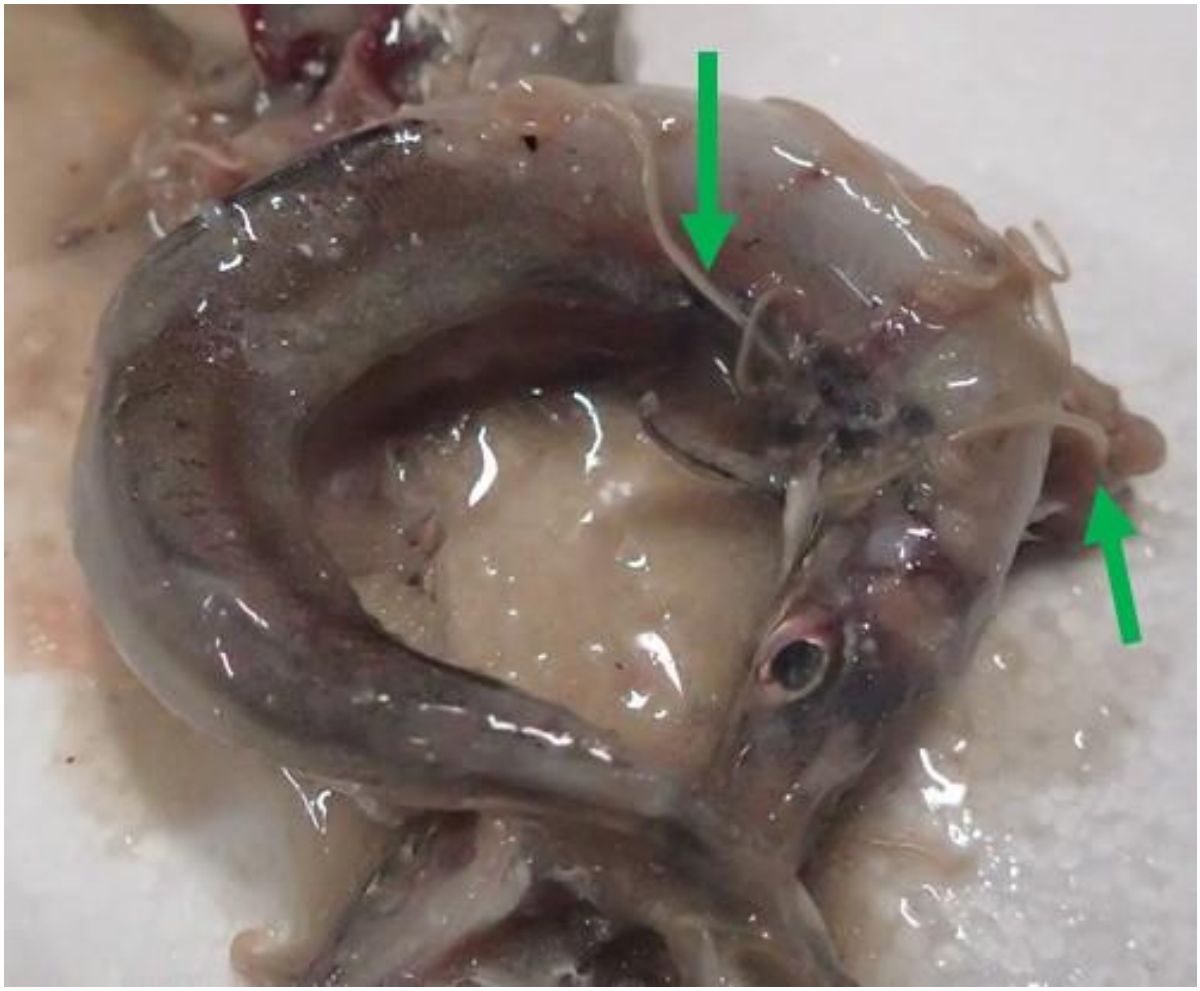

Figure 7: *H. aduncum* in and around newly eaten prey (i.e. capelin) of cod.

5.3 *Hysterothylacium aduncum* viability assessments

Hysterothylacium aduncum (n= 20) that were checked on a daily basis were clearly viable after 14 days in a refrigerator. In fact, many *H. aduncum* left unattended in petri dishes with physiological water were still alive after two months of cool-storage in the fridge (on 13_05_2019).

Table 3 and 4 suggest that the nematodes appear to suffer from thermic shock when transferred from 4.5 ºC physiological water in a fridge to 36.5 ºC pepsin solution or physiological water. Nematodes were apparently dead, as they showed no movements even when stimulated with needles. However, it appears that they can somehow adapt to the high temperature after a while (i.e. 30 min), since some of them started to show very slow movements, particularly after stimulation.

Results show that these parasites are fine when maintained in physiological water in the fridge, and can tolerate to be maintained in a pepsin medium in the fridge (most of them showed motility (19 out of 20 parasites) in pepsin medium after 20 hours) (Table 3).

It appears that *H. aduncum* cannot tolerate being at 36.5 ºC for 20 hours since all of them were found dead (i.e. not moving after stimulation and body seriously damaged) regardless of the media (i.e. pepsin or water) (Table 3). Those kept in pepsin media seemed partially digested nematodes, and those in water appeared cooked nematodes.

Table 4 shows that, even though some nematodes were considered dead after two hours storage at 36.5 ºC in physiological water or in pepsin medium, they can recover and get back to active motility if they are transferred into physiological water and kept in the fridge for the whole night. These nematodes seemed to be dead, since they did not react to manipulation with forceps, however they have shown ability to recover if put back to "good" conditions. Moreover, the appearance of the cuticle and body content seemed to be damaged (i.e. a "rigid" and transparent cuticle as well as lacking internal content (see Figure 8)), however they were still alive (i.e. moving).

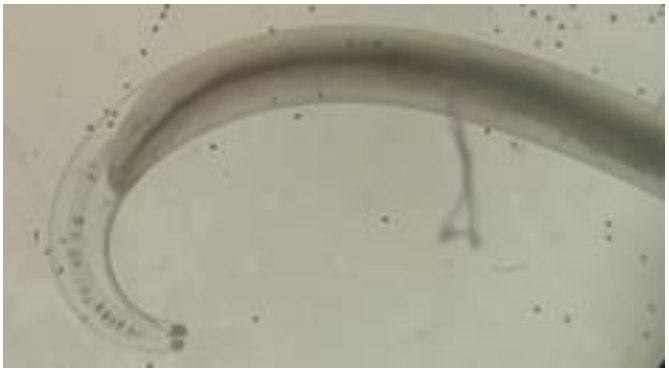

Figure 8: *Hysterothylacium aduncum* adult, with apparently no content in the anterior end of the body, after being in the fridge for two days in a pepsin solution medium. The parasite still showed sporadic movements after manipulation with forceps.

5.4 Statistical analyses

Statistical inference of relationships between number of adult *H. aduncum* present in the digestive tract and fish host data (i.e. season, total length and total weight) was performed in Statistica 13.4.0.14 or R (R-project). Cod (n= 10) that have been inspected for parasites in the factory were removed from the analyses since parasite counts were estimated when fishes were heavily infected.

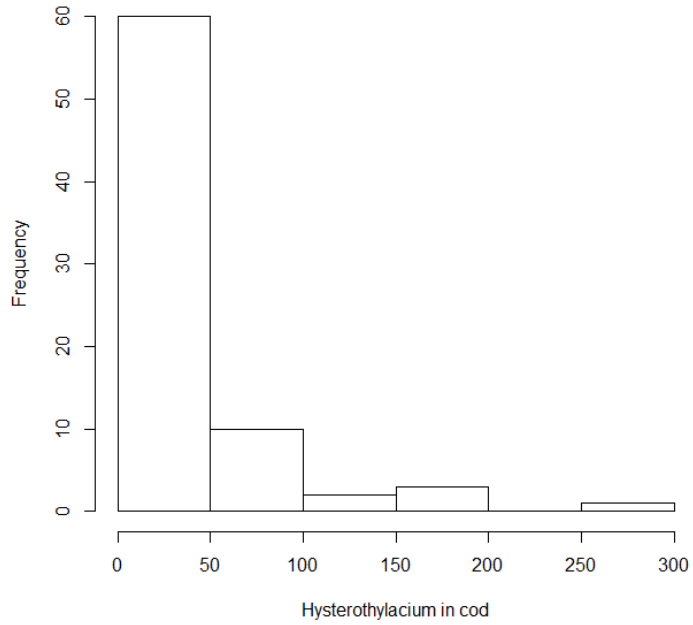

Figure 9: Histogram representing the distribution of adults and L4 larvae *H. aduncum* recovered from the digestive tract of cod (n= 76).

Spearman's correlation coefficients between *H. aduncum* numbers and fish length and weight were positive and highly statistically significant for cod, whilst they were negative and statistically significant for saithe (Table 5). However, correlations were not significant for haddock (Table 5). In other words, bigger cods had higher number of parasites, bigger saithe had less parasites and there was no association between fish size and number of parasites for haddock.

One-way ANOVA tests were conducted to examine the differences on *H. aduncum* abundance in fish (i.e. cod and saithe) according to fishing seasons and sex.

Cod were found to have higher parasite numbers in winter and fewer in late spring ($P<0.001$). Females have higher number of parasites than males ($P<0.05$). The variables season and fish size were correlated (see further explanation in the discussion section). In other words, big cods were sampled in winter and smaller cods in late spring (both variables are correlated), therefore it is not possible to say which one is explaining best the abundance of *H. aduncum* in cod.

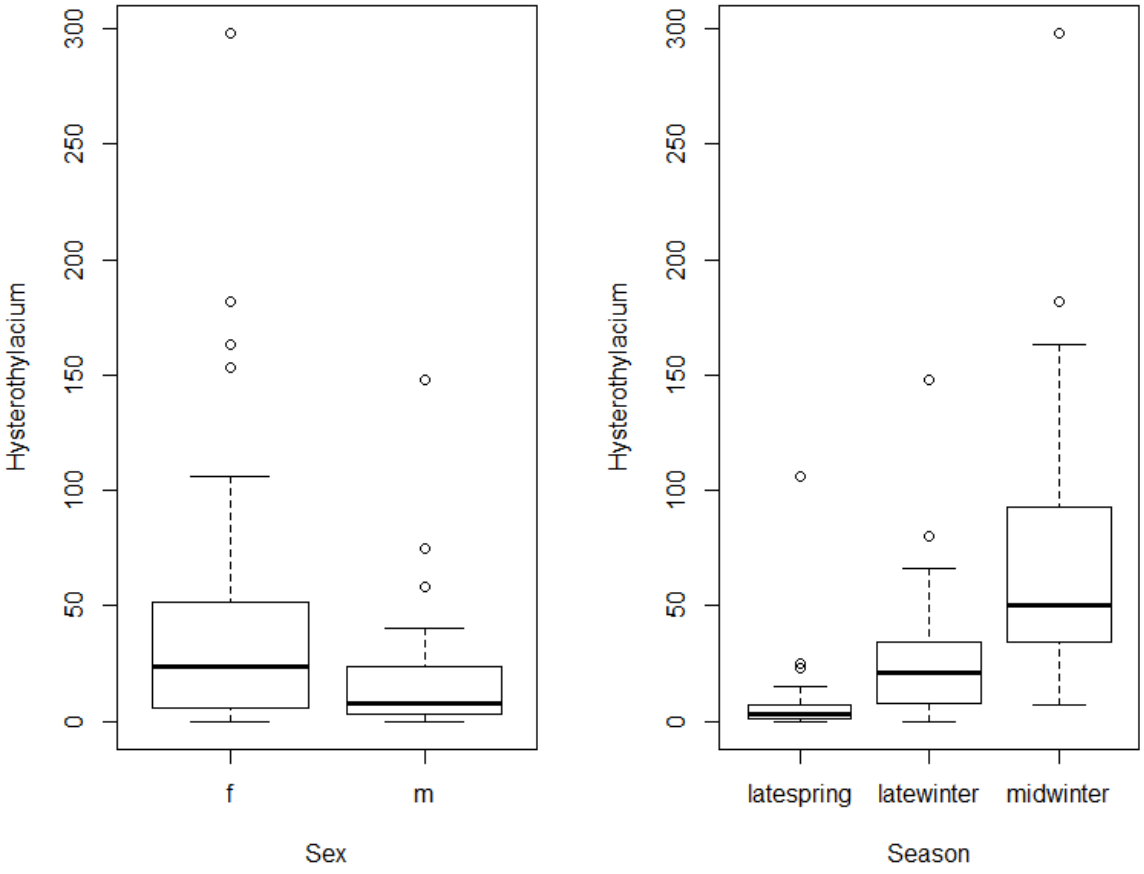

Figure 10: boxplot of *H. aduncum* abundance in cod conditional on sex and season.

Similar results were found for saithe with higher number of parasites in early spring compared to late spring ($P<0.001$), but no statistical difference was found between females and males (see boxplots in Figure 11). Contrary to cod, season and fish size were not correlated (see further explanation in the discussion section).

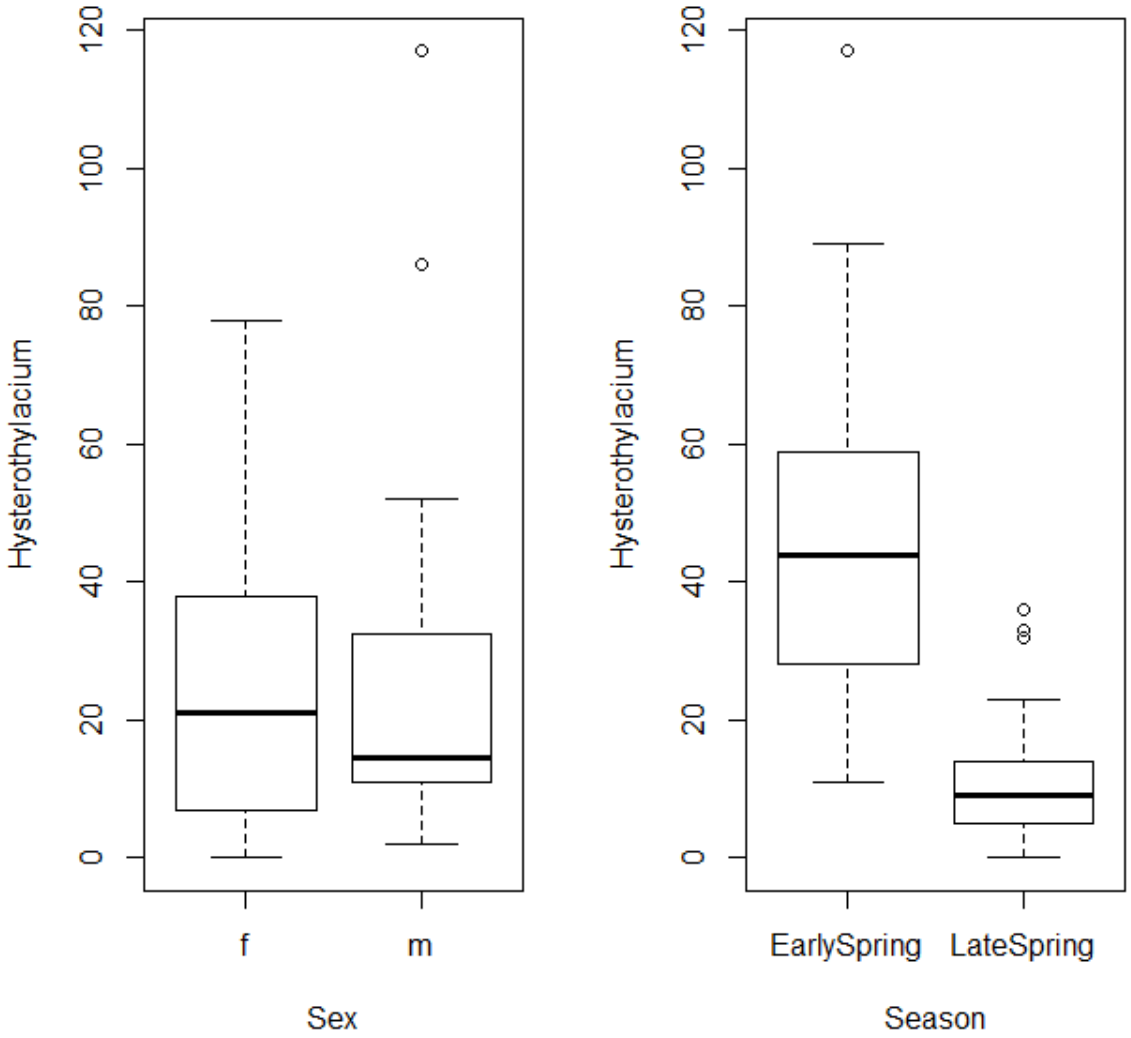

Figure 11: boxplot of *H. aduncum* abundance in saithe conditional on sex and season.

Regression analyses have not been performed for haddock data due to zero inflation (i.e. too many fishes with zero parasites in late spring) and unequal sampling balance (many females sampled in early spring (22 out of 30) whilst more males sampled in late spring (19 out of 30). However, a boxplot (Figure 12) showing the abundance of *H. aduncum* in haddock conditional on season and sex, shows clearly that haddock has more parasites on early spring than in late spring, and suggests that females might have more parasites than males. Like it occurred for saithe and contrary to cod, season and fish size were not correlated (see further explanation in the discussion section).

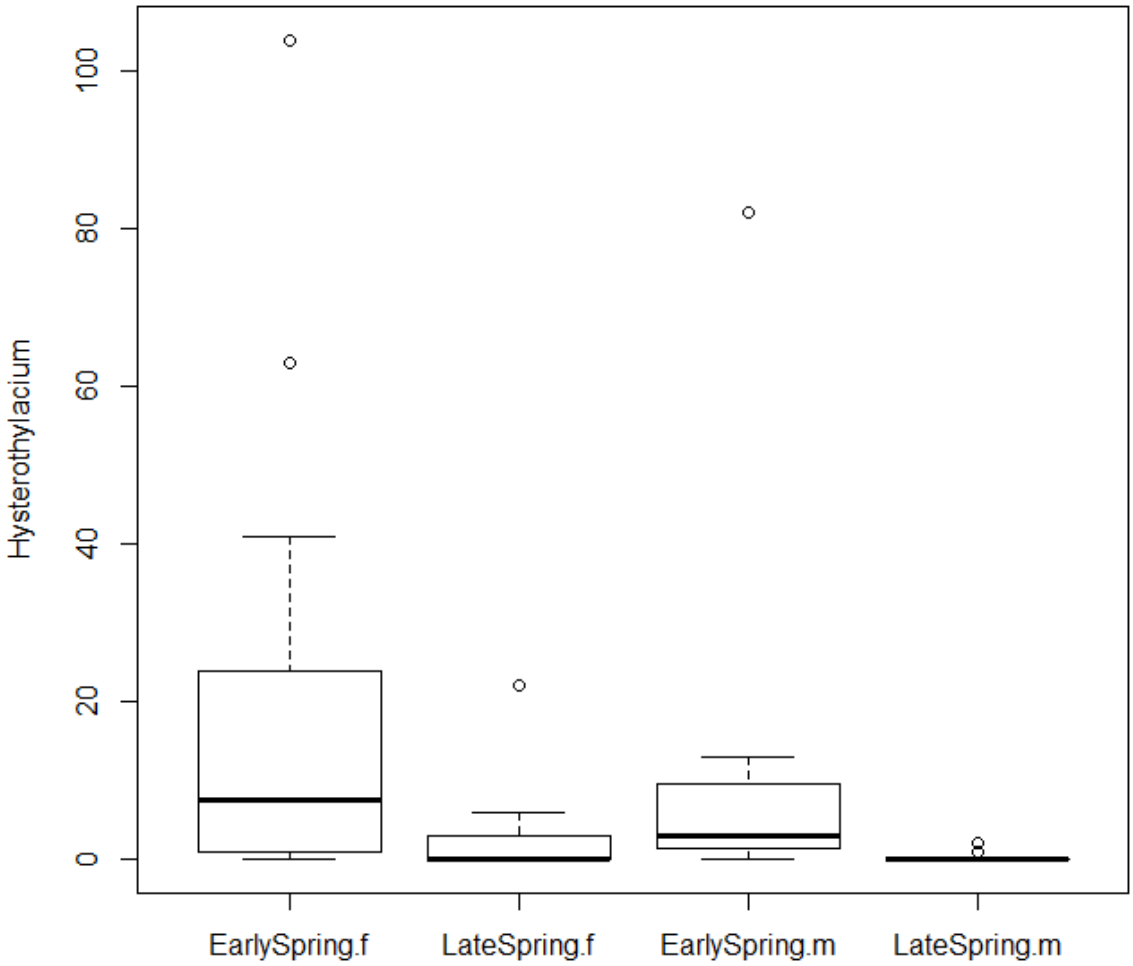

Figure 12: boxplot of *H. aduncum* abundance in haddock conditional on season and sex.

6. Discussion

6.1 *Hysterothylacium aduncum* biologic aspects

The taxonomy of the genus *Hysterothylacium* is unresolved, and this also applies to the species *H. aduncum*. Most authors assume *H. aduncum* to be a single species; one of the most common fish parasitic nematodes of NE Atlantic waters (Balbuena et al., 1998; Klimpel et al., 2007). However, Hartwich (1975) considered three different species; *H. aduncum* from clupeids, *H. gadi* from gadids and *H. auctum* from eelpout (*Zoarces viviparous*) and various flatfishes. Petter and Cabaret (1995) based on biometric data collected from different species proposed only two subspecies; *H. aduncum aduncum* and *H. aduncum gadi*. Klimpel et al. (2007) studied the genetic variability of *H. aduncum* from sprat (*Sprattus sprattus*) caught in four different geographical areas of NE Atlantic by investigating the ITS rDNA region. Klimpel et al. (2007) could not found significant genetic variability in those parasites and concluded that cryptic speciation has not occurred. In here, we assume *H. aduncum* as a single species parasitizing many fish species of NE Atlantic waters. However, it cannot be discarded that future parasitological studies, which may integrate morphological and genetic

methods, may reveal *H. aduncum* as a complex of sibling species, as previously suggested by Balbuena et al. (1998). Morphological and molecular results based on ITS gene identified *H. aduncum* as the parasite species infecting cod, haddock and saithe from West Finnmark.

Generally, the life cycle of *H. aduncum* occurs in the marine ecosystem and includes predatory fishes, such as gadoids, as final hosts where adults reproduce within the digestive tract (L4 larvae may be also present) and die (Berland, 1961; Køie, 1993). Third stage larvae (L3) are known to occur in small crustaceans such as copepods, euphausiids, amphipods, mysids, decapods and other invertebrates such as chaetognaths (intermediate hosts) (reviewed by Busch et al. (2012)) and also in the viscera (especially pylorus) of fishes (reviewed by Berland, 2006, 1961). It appears that small planktivorous pelagic fishes, such as capelin, herring, sprat, anchovy and sardine, foraging in infected invertebrates may serve as second intermediate or transport hosts, even though they can also act as final hosts since small adult specimens may be also present (Dessier et al., 2016; Klimpel et al., 2007; Levsen et al., 2016; Tolonen and Karlsbakk, 2003). The fact that *H. aduncum* may use an enormous diversity of benthic/pelagic invertebrate and fish hosts make this parasite one of the most abundant and ubiquitous ascaridoid parasite of NE Atlantic waters. Based on bibliography and our own findings we postulate the life cycle of *H. aduncum* in west Finnmark (Figure 13).

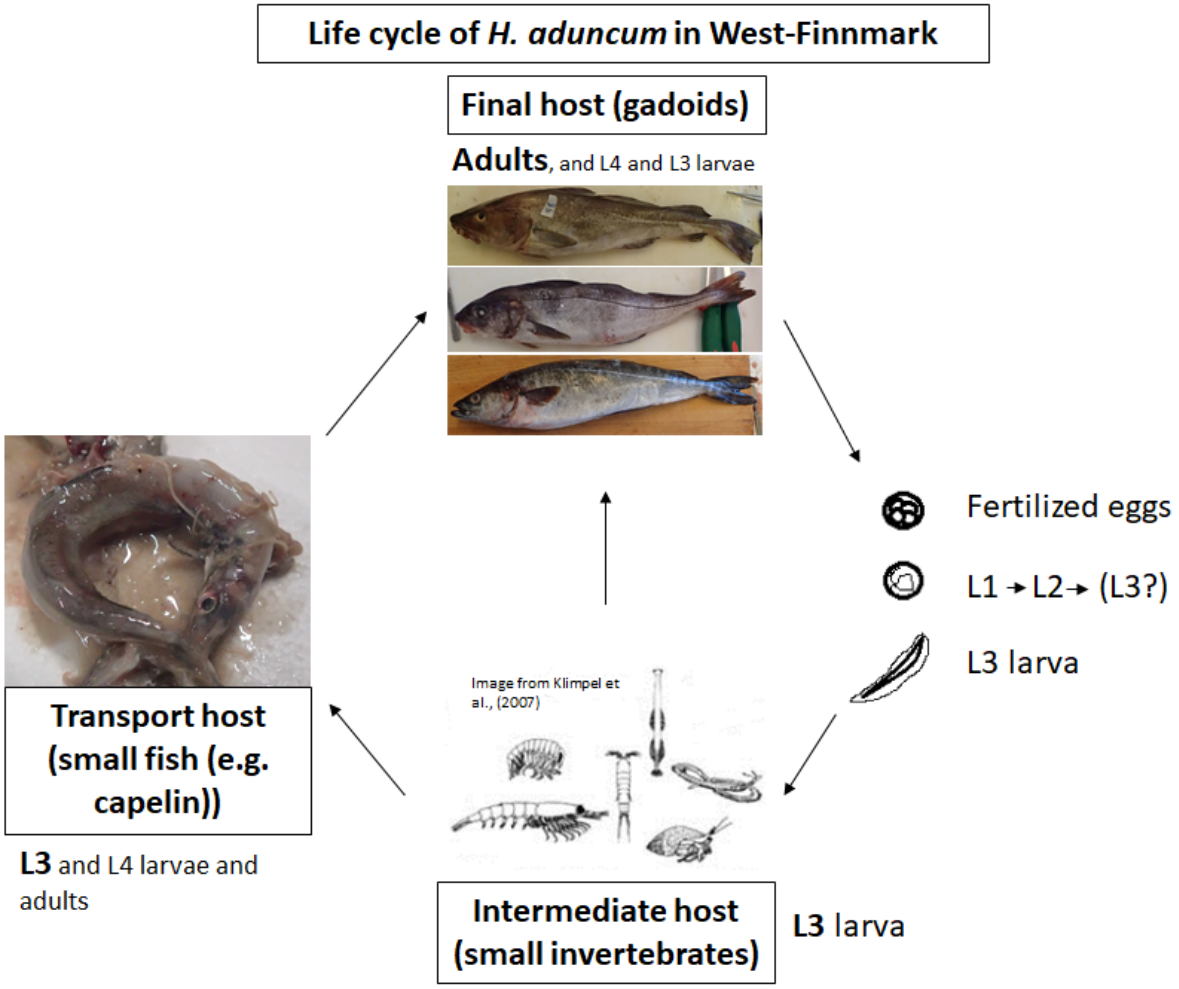

Figure 13: Life cycle of *H. aduncum* in West-Finnmark.

6.2 *Hysterothylacium aduncum* in the Barents Sea

The presence of parasitic nematodes ("*kveis*") in fish has been known for centuries to fishermen and coastal people of Norway (Berland, 2006). In the 50s, Bjørn Berland identified and described the most common ascaridoid nematodes (including *H. aduncum*) of marine fishes from Norway (Berland, 1961), and, in 1974, Soleim and Berland used *H. aduncum* recovered from the digestive tract of cod and haddock from the Barents Sea for morphological studies (Soleim and Berland, 1981). Since then, the occurrence of this parasite in cod fished in Norway has been documented in a number of studies: in cod from the Oslofjord (Andersen, 1993), Balsfjord (Hemmingsen et al., 2000), Trondheimsfjord (Perdiguero-Alonso et al., 2008) and Øksfjord (Heuch et al., 2011). In addition, the presence of *Hysterothylacium* sp. in cod from Altafjord (Hemmingsen et al. (1993)) and Barents Sea (Gay et al., 2018) has been reported.

Recently, Najda et al. (2018) reported 100% prevalence with mean abundance of 51 of *H. aduncum* in cod (n= 7) fished in the Barents Sea (precisely somewhere in between Svalbard and Bear island) in October/November of 2011. Two master theses carried out at the University of Tromsø reported the presence of *H. aduncum* in cod from the Barents Sea captured in February of 2015 (Alvestad, 2017) and May, June and September of 2015 (Løvland, 2017). In particular, Løvland (2017) inspected the whole digestive tract of cod (n= 26, mean body weight of 2 Kg) for *H. aduncum* reporting 85% prevalence with mean abundance 34 of *H. aduncum*. In here, we reported 81% to 100% prevalence with mean abundance ranging from 9 to 238 of *H. aduncum*. It appears that *H. aduncum* prevalence remains high and that mean abundance can be very variable (further discussion below).

6.3 Why has the "*Hysterothylacium* problematic" occurred now?

It can be concluded that *H. aduncum* has been part of the Barents Sea ecosystem for decades (if not "always") and that cod, saithe and haddock are optimal final hosts. But, why has recently the industry encountered with the *Hysterothylacium* problem? It appears that the "*Hysterothylacium*" problem just started when head-on "*skrei*" were sent to Spain in 2018. During fishing, some fishes may vomit or eject the stomach due to stress or differences in body pressure while ascending to the surface from the deep. By this phenomenon, *H. aduncum* that inhabits the digestive tract of the fish, can be transferred immediatly from the stomach to the oral cavities. This implies that, even though the common evisceration practices carried out by the industry will remove most of the *H. aduncum* present in the fish, some (maybe many) can remain hidden in the oral cavities. Eventually, *H. aduncum* can crawl out from the head cavities to the body surface and styrofoam boxes during transportation, hence being alive and visible at destination point.

It cannot be discarded that, in addition, there has been an increase in the infection levels of *H. aduncum* in cod in recent years, especially considering the very high infection values observed in February and March (Table 2). However, due to lack of comparable historical data this cannot be tested.

6.4 Factors explaining *H. aduncum* abundance in whitefish

Results suggest that season is the most important variable explaining the abundance of *H. aduncum* in these three whitefish gadoid species. For cod, season and fish size were correlated, (i.e. bigger cod in winter and smaller in summer) and this makes not possible to determine which variable is the most important explaining the abundance of *H. aduncum* in this fish species. Interestingly, these variables (i.e. fish size and season) were not correlated for saithe and haddock. Moreover, *H. aduncum* abundance was negatively correlated with fish size for saithe and not correlated for haddock. These results suggest that season is the most important variable explaining the parasite abundance in these

three whitefish species, having higher number of parasites soon in the year (winter and early spring) and lower in late spring.

The seasonal variation in infection levels found for this parasite in west Finnamark whitefish may be explained by its biological nature, and by the trophic relationship and migration behaviour of its hosts. The three gadoid species appear to have similar migration behaviour from oceanic regions of the Barents Sea to southern spawning grounds near the northern Norwegian coast (particularly important is the Lofoten/Vesterålen area) in winter/spring (reviewed by Bergstad et al., 1987; Olsen et al., 2010). Similarly, capelin spends its whole life cycle in the Barents Sea, but performs extensive seasonal migration (reviewed by Gjøsæter, 1998). During winter and early spring, capelin starts its spawning migration towards the coast of northern Norway and Russia, while during summer and autumn feeding occur in the central and northern Barents Sea (see reviews by Gjøsæter, 1998; Gjøsæter et al., 2016).

In addition, cod, saithe and haddock show clear seasonality in their prey selection, feeding extensively on spawning concentrations of capelin during winter and spring, and feeding capelin near the polar front in summer (reviewed by Bergstad et al., 1987; Olsen et al., 2010). In particular, the three gadoids predate intensively on spawning capelin along the coast of Finnmark, constituting 97%, 96% and 87% of the stomach biomass in cod, saithe and haddock, respectively (Bogetveit et al., 2008). This trophic link and overlapping distribution is particularly strong between cod and capelin (Fall et al., 2018; Johannesen et al., 2016). In general, saithe and cod are more piscivorous with cod having a more diverse benthic diet, and haddock being mainly a benthic feeder with crustaceans and echinoderms composing the major part of its diet (Bogetveit et al., 2008; Jiang and Jørgensen, 1996). Shrimps (*Pandalus borealis*) can be also an important prey throughout the year, as well as euphausiids that may be highly predated by cod and haddock during summer (reviewed by Bergstad et al., 1987) and can be important food components for saithe (Mironova, 1961).

Thus, it appears then that these gadoids will acquire most of *H. aduncum* through intensive predation on capelin at the beginning of the year, as capelin is known to be a host for this parasite (Levsen et al., 2016). *Hysterothylacium aduncum* behave as a short-lived parasite when living as adults in the fish digestive tract. Hence, we hypothesize that after a peak of *H. aduncum* infection in winter/early spring, the parasite will reproduce and disappear from the fish gut before late spring. It is important to highlight that new infections can continuously occur throughout the whole year, as herein observed in late spring, since the parasite can use multiple ways to be transferred to its gadoids' final hosts (Figure 13).

Future samplings are recommended to study any changes/trend in parasite load in whitefish throughout seasons and in following years.

6.5 Viability assessments

During the context of the present project, the parasite has shown a great potential to cause monetary losses to the Norwegian whitefish industry. Many parasites were found alive and moving over the cod and boxes when the fish arrived at destination point in Spain, and the fish was immediately rejected. In this sense, we have provided further evidence that *H. aduncum* can survive for more than 14 days in a wet and cold media (i.e. in physiological water and in fridge).

The nematode species *H. aduncum* is a parasite of cold-blooded organisms and is generally considered non-zoonotic, however, this aspect is not fully understood and remains controversial (Shamsi et al., 2018). For instance, it is not known for how long it will survive under mammalian digestive conditions. Results showed that *H. aduncum* cannot tolerate being at 36.5 ºC for 20 hours since all of them were

dead regardless of the media (i.e. pepsin or water). However, they have shown some tolerance to elevated temperatures and acidic conditions in short periods of time (i.e. at least two hours), since some were showing movements after manipulation with needles. Thus, in the unlikely event that an adult *H. aduncum* could be ingested by humans, it appears not possible (but cannot be completely discarded with the present results) that it can cause any physical damage to a human stomach/intestine. Further studies (e.g. animal studies) are recommended in order to test *H. aduncum* zoonotic potential.

6.6 Fish handling recommendation to the industry

Since the parasite can remain hidden in the head cavities of the fish, and afterwards crawl out from there to the fish surface and/or transport boxes, we recommend the following handling/cleaning procedures:

1) To eviscerate the fish as soon as possible, cut off the fish head and rinse carefully the fish body would eliminate *H. aduncum* from the fishery product (Figure 14).

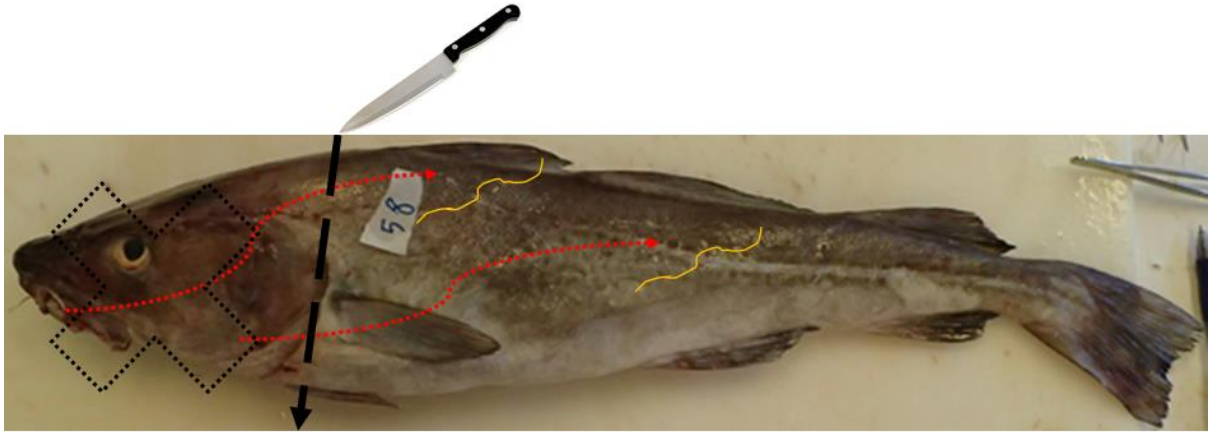

Figure 14: Evisceration, cutting off head and rinse would eliminate the parasite.

2) Apart from evisceration and rinse, a thorough cleaning and completely removal of the head cavities (i.e. the gill/pharynx region) may be considered, particularly during the off-peak *H. aduncum* season (i.e. late spring).

7. Conclusion

The fish parasitic nematode *H. aduncum* can be very abundant within the digestive tract of cod, saithe and haddock, however it is not present in the fillets. Season appears to be the most important factor explaining *H. aduncum* abundance in whitefish, with more parasites during winter and early spring and less parasites during late summer. It is hypothesized that whitefish may gain the maximum load of parasites through predation of spawning capelin during winter and spring in western Finnmark, since the later is known to be a host of *H. aduncum*. The parasite can survive for long periods in cold and humid conditions, and therefore being alive (if present in the fish lots) and actively moving when arriving at destination point. *Hysterothylacium aduncum* can be eliminated if fishes are eviscerated, beheaded and rinsed carefully at plants. Alternatively, apart from evisceration and flushing with water the fish body, a thorough cleaning and completely removal of the head cavities (i.e. the gill/pharynx region) may be considered.

8. Main findings

- *Hysterothylacium aduncum* is very prevalent and can be very abundant in cod, saithe and haddock.
- *Hysterothylacium aduncum* it is not present in fillets.
- The parasite is much more abundant during winter and early spring than in late spring in West-Finnmark, and this appears to be linked to an intense feeding period on spawning capelin.
- The parasite can be removed by evisceration, beheading and flushing carefully the fish at processing plants. As an alternative, apart from evisceration and flushing with water the fish body, a thorough cleaning and completely removal of the head cavities (i.e. the gill/pharynx region) may be considered.

9. Deliverables

- Academic final report.
- Administrative final report.
- Presentation of project results to the industry.
- Summary of the final meeting.
- Informative sheet in Norwegian, English and Spanish.

10. References

Alvestad, A., 2017. Parasites of Northeast Arctic cod (*Gadus morhua*) in the Barents Sea : effects on reproduction. Univeristy of Tromsø.

Andersen, K., 1993. *Hysterothylacium aduncum* (Rudolphi, 1862) infection in cod from the Oslofjord: Seasonal occurrence of third-and fourth-stage larvae as well as adult worms. Parasitol. Res. 79, 67–72. https://doi.org/10.1007/BF00931220

Balbuena, J.A., Karlsbakk, E., Saksvik, M., Kvenseth, A.M., Nylund, A., 1998. New Data on the Early Development of *Hysterothylacium aduncum* (Nematoda, Anisakidae). J. Parasitol. 84, 615. https://doi.org/10.2307/3284732

Bergstad, O.A., Jørgensen, T., Dragesund, O., 1987. Life history and ecology of the gadoid resources of the Barents Sea. Fish. Res. 5, 119–161. https://doi.org/10.1016/0165-7836(87)90037-3

Berland, B., 2006. Musings on nematode parasites.

Berland, B., 1989. Identification of fish larval nematodes from fish, in: Möller, H. (Ed.), Nematode Problems in North Atlantic Fish. Report from a Workshop in Kiel, 3-4 April 1989. pp. 16–22.

Berland, B., 1961. Nematodes from some Norwegian marine fishes. Sarsia 2, 1–50. https://doi.org/10.1080/00364827.1961.10410245

Bogetveit, F.R., Slotte, A., Johannessen, A., 2008. The ability of gadoids to take advantage of a short-term high availability of forage fish: The example of spawning aggregations in Barents Sea capelin. J. Fish Biol. 72, 1427–1449. https://doi.org/10.1111/j.1095-8649.2008.01808.x

Busch, M.W., Kuhn, T., Münster, J., Klimpel, S., 2012. Marine crustaceans as potential hosts and vectors for metazoan parasites, in: Mehlorn, H. (Ed.), Arthropods As Vectors of Emerging Diseases. Berlin Heidelberg: Springer, pp. 329–360. https://doi.org/10.1007/978-3-642-28842-5

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. J. Parasitol. 83, 575. https://doi.org/10.2307/3284227

Codex Alimentarius, 2004. Standard for salted atlantic herring and salted sprat - CODEX STAN 244-2004.

Dessier, A., Dupuy, C., Trancart, T., Audras, A., Bustamante, P., Gérard, C., 2016. Low diversity of helminth parasites in *Sardina pilchardus* and *Engraulis encrasicolus* (Clupeidae) from the Bay of Biscay. Mar. Freshw. Res. 67, 1583. https://doi.org/10.1071/MF15147

EFSA-BIOHAZ, 2010. Scientific Opinion on risk assessment of parasites in fishery products. EFSA J. 8, 1543. https://doi.org/10.2903/j.efsa.2010.1543

Fall, J., Ciannelli, L., Skaret, G., Johannesen, E., 2018. Seasonal dynamics of spatial distributions and overlap between Northeast Arctic cod (*Gadus morhua*) and capelin (*Mallotus villosus*) in the Barents Sea. PLoS One 13, 1–26. https://doi.org/10.1371/journal.pone.0205921

Gay, M., Bao, M., MacKenzie, K., Pascual, S., Buchmann, K., Bourgau, O., Couvreur, C., Mattiucci, S., Paoletti, M., Hastie, L.C., Levsen, A., Pierce, G.J., 2018. Infection levels and species diversity of ascaridoid nematodes in Atlantic cod, *Gadus morhua*, are correlated with geographic area and fish size. Fish. Res. 202, 90–102. https://doi.org/10.1016/J.FISHRES.2017.06.006

Gjøsæter, H., 1998. The population biology and exploitation of capelin (*Mallotus villosus*) in the barents sea. Sarsia 83, 453–496. https://doi.org/10.1080/00364827.1998.10420445

Gjøsæter, H., Hallfredsson, E.H., Mikkelsen, N., Bogstad, B., Pedersen, T., 2016. Predation on early life stages is decisive for year-class strength in the Barents Sea capelin (*Mallotus villosus*) stock. ICES J. Mar. Sci. 73, 182–195. https://doi.org/10.4135/9781412953924.n678

Hartwich, G., 1975. Schlauchwürmer, Nemathelminthes Rund-oder Fadenwürmer, Nematoda Parasitische Rundwürmer von Wirbeltieren. I. Rhabditida und Ascaridida. Die Tierwelt Deutschlands, 62 Tiel.

Hemmingsen, W., Halvorsen, O., MacKenzie, K., 2000. The occurrence of some metazoan parasites of Atlantic cod, *Gadus morhua* L., in relation to age and sex of the host in Balsfjord (70°N), North Norway. Polar Biol. 23, 368–372. https://doi.org/10.1007/s003000050457

Hemmingsen, W., Lysne, D.A., Eidnes, T., Skorping, A., 1993. The occurrence of larval ascaridoid nematodes in wild-caught and in caged and artificially fed Atlantic cod, *Gadus morhua* L., in Norwegian waters. Fish. Res. 15, 379–386. https://doi.org/10.1016/0165-7836(93)90088-O

Heuch, P., Jansen, P., Hansen, H., Sterud, E., MacKenzie, K., Haugen, P., Hemmingsen, W., 2011. Parasite faunas of farmed cod and adjacent wild cod populations in Norway: a comparison. Aquac. Environ. Interact. 2, 1–13. https://doi.org/10.3354/aei00027

Jiang, W., Jørgensen, T., 1996. The diet of haddock (*Melanogrammus aeglefinus* L.) in the Barents Sea during the period 1984-1991. ICES J. Mar. Sci. 53, 11–21. https://doi.org/10.1006/jmsc.1996.0002

Johannesen, E., Johansen, G.O., Korsbrekke, K., 2016. Seasonal variation in cod feeding and growth in a changing sea. Can. J. Fish. Aquat. Sci. 73, 235–245. https://doi.org/10.1139/cjfas-2015-0052

Karl, H., Leinemann, M., 1993. A fast and quantitative detection method for nematodes in fish fillets and fishery products. Arch. Lebensmittelhyg. 44, 105–128.

Karl, H., Levsen, A., 2011. Occurrence and distribution of anisakid nematodes in Grey gurnard (*Eutrigla gurnardus* L.) from the North Sea. Food Control 22, 1634–1638. https://doi.org/10.1016/j.foodcont.2011.03.021

Klimpel, S., Kleinertz, S., Hanel, R., Rückert, S., 2007. Genetic variability in *Hysterothylacium aduncum*, a raphidascarid nematode isolated from sprat (*Sprattus sprattus*) of different geographical areas of the northeastern Atlantic. Parasitol. Res. 101, 1425–1430. https://doi.org/10.1007/s00436-007-0662-0

Klimpel, S., Rückert, S., 2005. Life cycle strategy of *Hysterothylacium aduncum* to become the most abundant anisakid fish nematode in the North Sea. Parasitol. Res. 97, 141–149. https://doi.org/10.1007/s00436-005-1407-6

Køie, M., 1993. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can. J. Zool. 71, 1289–1296. https://doi.org/10.1139/z93-178

Levsen, A., Lunestad, B.T., 2010. *Anisakis simplex* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. Vet. Parasitol. 171, 247–253. https://doi.org/10.1016/j.vetpar.2010.03.039

Levsen, A., Paoletti, M., Cipriani, P., Nascetti, G., Mattiucci, S., 2016. Species composition and infection dynamics of ascaridoid nematodes in Barents Sea capelin (*Mallotus villosus*) reflecting trophic position of fish host. Parasitol. Res. 115, 4281–4291. https://doi.org/10.1007/s00436-016-5209-9

Levsen, A., Svanevik, C.S., Cipriani, P., Mattiucci, S., Gay, M., Hastie, L.C., Bušelić, I., Mladineo, I., Karl,

H., Ostermeyer, U., Buchmann, K., Højgaard, D.P., Pierce, G.J., 2018. A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—Introducing the FP7 PARASITE exposure assessment study. Fish. Res. 202, 4–21. https://doi.org/10.1016/J.FISHRES.2017.09.009

Løvland, A.B., 2017. Parasites from cod in two different localities , the Barents Sea and Øksfjord in the coastal region of northern Norway : a comparison. University of Tromsø.

Mironova, N. V., 1961. Biology of the Barents Sea saithe, *Pollachius virens* (L.). Int. Rev. der gesamten Hydrobiol. und Hydrogr. 46, 447–459. https://doi.org/10.1002/iroh.19610460311

Najda, K., Kijewska, A., Kijewski, T., Plauška, K., Rokicki, J., 2018. Distribution of ascaridoid nematodes (Nematoda: Chromadorea: Ascaridoidea) in fish from the barents sea. Oceanol. Hydrobiol. Stud. 47, 128–139. https://doi.org/10.1515/ohs-2018-0014

Olsen, E., Aanes, S., Mehl, S., Holst, J.C., Aglen, A., Gjøsæter, H., 2010. Cod, haddock, saithe, herring, and capelin in the Barents Sea and adjacent waters: A review of the biological value of the area. ICES J. Mar. Sci. 67, 87–101. https://doi.org/10.1093/icesjms/fsp229

Perdiguero-Alonso, D., Montero, F.E., Raga, J., Kostadinova, A., 2008. Composition and structure of the parasite faunas of cod, *Gadus morhua* L. (Teleostei: Gadidae), in the North East Atlantic. Parasit. Vectors 1, 23. https://doi.org/10.1186/1756-3305-1-23

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

Pierce, G.J., Bao, M., MacKenzie, K., Dunser, A., Giulietti, L., Cipriani, P., Mattiucci, S., Hastie, L.C., 2018. Ascaridoid nematode infection in haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) in Northeast Atlantic waters. Fish. Res. 202, 122–133. https://doi.org/10.1016/J.FISHRES.2017.09.008

Pippy, J.H.C., 1970. Use of Ultraviolet Light to find Parasitic Nematodes in situ. J. Fish. Res. Board Canada 27, 963–965. https://doi.org/10.1139/f70-107

Shamsi, S., Steller, E., Chen, Y., 2018. New and known zoonotic nematode larvae within selected fish species from Queensland waters in Australia. Int. J. Food Microbiol. 272, 73–82. https://doi.org/10.1016/J.IJFOODMICRO.2018.03.007

Soleim, Ø., Berland, B., 1981. The Morphology of *Thynnascaris adunca* (Rudolphi) (Nematoda, Ascaridoidea). Zool. Scr. 10, 167–182. https://doi.org/10.1111/j.1463-6409.1981.tb00494.x

Strømnes, E., Andersen, K., 1998. Distribution of whaleworm (*Anisakis simplex*, Nematoda, Ascaridoidea) L3 larvae in three species of marine fish; saithe (*Pollachius virens* (L.)), cod (*Gadus morhua* L.) and redfish (*Sebastes marinus* (L.)) from Norwegian waters. Parasitol. Res. 84, 281–285. https://doi.org/10.1007/s004360050396

Tolonen, A., Karlsbakk, E., 2003. The parasite fauna of the Norwegian spring spawning herring (*Clupea harengus* L.). ICES J. Mar. Sci. 60, 77–84. https://doi.org/10.1006/jmsc.2002.1307 ER

Zhu, X., Gasser, R.B., Jacobs, D.E., Hung, G.C., Chilton, N.B., 2000. Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. Parasitol. Res. 86, 738–744. https://doi.org/10.1007/PL00008561

11. Appendix

Tables

Table 1: Fish sampling batches from Hjelmsøybanken (West-Finnmark).

Species	Number of fish	Date of capture	Season	Fishing method	State at sampling	Sampling place
Cod	18	1.2.19	Winter	Jigging	Fresh (in ice)	IMR-lab
	10	13.3.19	Late winter	Jigging	Fresh (in ice)	Factory
	32	12.3.19	Late winter	Jigging	Frozen	IMR-lab
	27	31.5.19	Late spring	Longline	Frozen	IMR-lab
Haddock	30	2.4.19	Early spring	Danish seine	Frozen	IMR-lab
	30	31.5.19	Late spring	Danish seine	Frozen	IMR-lab
Saithe	29	2.4.19	Early spring	Danish seine	Frozen	IMR-lab
	29	31.5.19	Late spring	Danish seine	Frozen	IMR-lab

Table 2: Infection levels of *Hysterothylacium aduncum* in cod, haddock and saithe from Hjelmsøybanken (West-Finnmark). NA= not available data.

					Adults and L4 larvae				L3 larvae		All		
					skin/mouth/pharynx/gill		Digestive tract		Pylorus		Total		
Species	**N**	**Date of capture**	**Length ± SD**	**Weight ± SD**	**P (%)**	**mA ± SD**	**P (%)**	**mA ± SD**	**P (%)**	**mA ± SD**	**P (%)**	**mA ± SD**	**Range**
Cod	18	1.2.19	101 ± 6	10.0 ± 1.9	NA	NA	100%	79.7 ± 74.8	50%	3.7 ± 5.0	NA	NA	NA
	10	13.3.19	90 ± 12	6.7 ± 3.6	50%	4.2 ± 11.2	100%	238.2 ± 336.8	NA	NA	NA	NA	NA
	32	12.3.19	77 ± 7	4.4 ± 1.3	41%	2.3 ± 5.9	97%	28.7 ± 29.8	0%	0	97%	31.1 ± 29.4	0-148
	27	31.5.19	67 ± 7	2.4 ± 0.7	19%	0.3 ± 0.8	81%	8.9 ± 20.5	4%	0.0 ± 0.2	81%	9.2 ± 21.2	0-110
Haddock	30	2.4.19	51 ± 5	1.4 ± 0.4	53%	5.0 ± 12.0	80%	16.0 ± 25.8	3%	0.1 ± 0.4	87%	21.1 ± 34.9	0-143
	30	31.5.19	54 ± 6	1.4 ± 0.5	3%	0.1 ± 0.5	27%	1.3 ± 4.2	7%	0.2 ± 0.6	33%	1.6 ± 4.7	0-25
Saithe	29	2.4.19	53 ± 5	1.6 ± 0.4	100%	11.0 ± 7.0	100%	45.9 ± 24.9	28%	1.7 ± 5.1	100%	58.6 ± 27.7	15-129
	29	31.5.19	62 ± 9	2.2 ± 1.1	66%	2.8 ± 4.8	97%	12.1 ± 10.3	10%	0.2 ± 0.5	100%	15.1 ± 12.2	1-43

Table 3: Number of adult *Hysterothylacium aduncum* **motile/non-motile** per time, temperature and liquid medium. At time 0, each petri dish contained 20 adult *H. aduncum* showing active movements.

Time 0 (Day 1)	**Pepsin (36.5 ºC) (replicate 1)**	**Pepsin (36.5 ºC) (replicate 2)**	**Physiological water (36.5 ºC) (replicate 1)**	**Physiological water (36.5 ºC) (replicate 2)**	**Physiological water (4.5 ºC)**	**Pepsin (4.5 ºC)**
15 min	0/20	0/20	11/9	11/9	20/0	20/0
30 min	5/15	4/16	11/9	6/14	20/0	20/0
1.15 h	1/20	2/20	11/9	4/16	20/0	20/0
1.45 h	0/20	0/20	13/7	4/16	20/0	20/0
Day 2 20 h	0/20	11/9 (transferred to the **fridge for all night**)	20/0 (dish transferred to the **fridge for all night**)	0/20	20/0	19/1
Day 3 48 h	End of trial	End of trial	End of trial	End of trial	20/0	13/7
Day 4 70 h	End of trial	End of trial	End of trial	End of trial	20/0	10/10

Table 4: Number of adult *H. aduncum* classified by viability criteria per time and liquid medium at 36.5 ºC. At time 0, each petri dish containing 20 adult *H. aduncum* showing active movements.

Time 0	**Viability criteria**	**Pepsin (36.5 ºC)**	**Phys. water (36.5 ºC)**
15 min	Spontaneous movement	1	1
	Movement stimulating by needle	18	15
	Non-motile*	1	4
30 min	Spontaneous movement	10	4
	Movement stimulating by needle	8	12
	Non-motile*	1	0
45 min	Spontaneous movement	9	10
	Movement stimulating by needle	9	5
	Non-motile*	0	1
1 h	Spontaneous movement	11	8
	Movement stimulating by needle	7	7
	Non-motile*	0	0
2 h	Spontaneous movement	0	0

	Movement stimulating by needle	2	15
	Non-motile*	16	0
* The non-motile nematodes were transferred into a petri dish with physiological water and placed in the fridge to check their ability to recover. The following day, the petri dishes containing the nematodes were moved out from the fridge to room temperature and checked for viability criteria for up to 2 h. All the nematodes were viable at the end of the experiment.			
2 h	Spontaneous movement	18	5
	Movement stimulating by needle	-	-
	Non-motile*	-	-

Table 5: Spearman rank coefficients (r) and *p-values* (P) of the relationships between *H. aduncum* counts and fish host data (TL= total length, TW= total weight).

	TL	TW
Cod (n= 76)	r= 0.68, **P< 0.001**	r= 0.69, **P< 0.001**
Haddock (n= 60)	r= -0.01, P=0.96	r= 0.19, P=0.15
Saithe (n= 58)	r= -0.45, **P<0.001**	r= -0.29, **P=0.03**