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Quality issues related to the presence of the fish parasitic nematode *Hysterothylacium aduncum* in export shipments of fresh Northeast Arctic cod (*Gadus morhua*)

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ABSTRACT

The parasitic nematode *Hysterothylacium aduncum* commonly occurs in many fish species in the Northeast (NE) Atlantic. The parasite is considered non-pathogenic to humans. During spring and summer of 2018, lots of head-on eviscerated fresh NE Arctic cod (*Gadus morhua*) were shipped from Norway to Spain for commercialization. At arrival, the presence of lively roundworms (i.e. *H. aduncum*) in the transporting boxes was noticed. Consequently, fish lots were rejected causing substantial monetary losses to all involved parties. As part of the hazard assessment process, the epidemiology of *H. aduncum* in cod, NE Arctic saithe (*Pollachius virens*) and NE Arctic haddock (*Melanogrammus aeglefinus*) fished off West-Finnmark, Norway, was studied. Parasites were morphologically and molecularly identified to species level. Additionally, the viability of *H. aduncum* was assessed by simulating the conditions that prevail during transport of fresh cod to European market. The infection values of L4 larval stage or adult *H. aduncum* in the digestive tract of fish were highly variable, ranging from 100% prevalence with mean abundance (range) 238 (10–1092) in cod to 27% prevalence with mean abundance (range) 1 (0–22) in haddock. Fishing season was identified as most important explanatory factor for parasite abundance. Infection levels were higher during winter and early spring, and lower during late spring. Infection peaks of *H. aduncum* in the three fish species seemed to coincide with heavy predation on spawning capelin (*Mallotus villosus*) during winter and early spring. Thorough evisceration seems to remove most of the nematodes from the fish. However, parasites may remain hidden within the head cavities where they can stay alive and active under regular transport conditions for at least 14 days. *Hysterothylacium aduncum* can be completely removed from the product by evisceration, beheading and rinsing the fish prior to shipping. Alternatively, thorough cleaning or completely removal of the gills and pharynx may be considered.

1. Introduction

Parasitic nematodes commonly known as "*kveis*" in Norway, often occur in visceral organs and muscle of many Northeast (NE) Atlantic commercially important marine fish species, such as Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*) (Bao et al., 2020; Gay et al., 2018; Levsen et al., 2018; Pierce et al., 2018; Strømnes & Andersen, 1998). Nematodes of the family Anisakidae (Nematoda: Ascaridoidea), and particularly species of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*, are of medical and socioeconomic concern globally as they are the causative agents of a fish-borne zoonosis called anisakidosis (reviewed by Bao et al., 2019; Buchmann & Mehrdana, 2016; Eiras, Pavanelli, Takemoto, & Nawa, 2017; Mattiucci, Cipriani, Paoletti, Levsen, & Nascetti, 2017; Shamsi, 2014).

Besides the presence of anisakids in fish, the occurrence of other ascaridoid nematode belonging to the family Raphidascarididae, i.e. *Hysterothylacium aduncum* is also very common (Klimpel & Rückert, 2005). *Hysterothylacium aduncum* uses fish as final host, whilst *Anisakis* spp., *Pseudoterranova* spp. and *Contracaecum* spp. use cetaceans, seals and seals/fish-eating birds, respectively in their life cycle (Berland, 2006). Small crustaceans serve as first intermediate hosts and fish serve as second intermediate or paratenic host for all the former species (Berland, 2006). *Hysterothylacium aduncum* inhabits the viscera of fish. The fourth larval stage (L4) and adults are located and reproduce within the digestive tract (i.e. stomach and intestine), whilst the third larval stage (L3) is commonly located on the internal organs such as pyloric

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caeca (Berland, 1961, 1989bib_Berland_1961bib_Berland_1989). Since *H. aduncum* lives exclusively in cold-blooded organisms, it is not adapted to the conditions that prevail in the alimentary tract of mammals (Karl & Levsen, 2011). Thus, *H. aduncum* is commonly considered non-zoonotic (Cavallero et al., 2020). Nevertheless, *H. aduncum* can indeed heavily infect many fish species in NE Atlantic waters (Berland, 1961; Klimpel, Kleinertz, Hanel, & Rückert, 2007; Klimpel & Rückert, 2005), and the presence of larval or adult specimens may heavily reduce the aesthetical appeal of fish products, potentially causing socioeconomic problems to the fishing industry.

During spring and summer of 2018, fish lots of NE Arctic cod (*Gadus morhua*) (hereinafter cod), called *skrei* in Norwegian, were sold from Norway to Spain. The distributor realized the presence of big and lively roundworms (which resulted to be *H. aduncum*) over the fish (i.e. head-on eviscerated fresh cod) and styrofoam boxes, as a consequence, the fishing lots were rejected. This special event with big volumes packed resulted in important monetary losses to all parties involved.

This study aimed to investigate the current handling procedures during the cod value chain from sea to processing at land in order to help the industry to improve/develop better parasite preventive practices/strategies. In addition, we aimed to study the epidemiology of *H. aduncum* in cod, NE Arctic saithe (*Pollachius virens*) (hereinafter saithe) and NE Arctic haddock (*Melanogrammus aeglefinus*) (hereinafter haddock) caught in different periods of the year with special focus on the infection levels and anatomical location of the parasite in the fish. The viability of the parasite kept in conditions that resemble those that occur during storage and transportation of fresh cod to European markets was also investigated.

2. Materials and methods

2.1. Fish sampling

Fishes (i.e. cod (n = 76), saithe (n = 58) and haddock (n = 60)) were caught by professional fishermen using jigging, longline or Danish seine at Hjelmsøybanken (Barents Sea) from February to May 2019 (Table 1). Fishes were frozen after capture and sent to the lab facilities for parasitological inspection.

In addition, ten whole freshly landed cod were inspected for the presence of *Hysterothylacium* sp. at the factory.

2.2. Parasitological sampling

2.2.1. Necropsy

Data on total length (TL), total weight (TW) and sex were recorded for each fish (Table 1). Skin, fins, mouth, pharynx and gills were visually inspected by naked eye for the presence of *Hysterothylacium* sp. 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 opened to expose their content, and *Hysterothylacium* sp. inspection was carefully performed using forceps and a lamp by naked eye. 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 (details at section 2.2.2). Fish fillets, liver and gonads were also inspected by the UV-press method. All parasites were counted, and their anatomical location reported (Table 1). Subsamples were morphologically and molecularly identified by using molecular/genetic markers (details at section 2.3 and 2.4, respectively).

2.2.2. UV-press

The UV-press method utilizes the fluorescence of dead-by-freezing nematodes when exposed to UV-light (Pippy, 1970). For that, fish fillets and 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 & Leinemann, 1993;

Table 1
Infection levels of *Hysterothylacium aduncum* in cod, saithe and haddock samples from Hjelmsøybanken (West-Finnmark, Norway).

						Adults and L4 larvae					L3 larvae			All	
						skin/mouth/pharynx/gill			Digestive tract		Pylorus			Total	
Species	N	Date of capture	Season	TL ± SD	TW ± SD	Sex (F/M/U)	PR (%)	A ± SD	Range	P (%)	mA ± SD	Range	P (%)	mA ± SD	Range	P (%)	mA ± SD	Range
Cod	18*	1.2.19	Mid Winter	101 ± 6	10.0 ± 1.9	13/5	NA	NA	NA	100%	79.7 ± 74.8	7–298	50%	3.7 ± 5.0	0–19	NA	NA	NA
	10**	13.3.19	Late winter	90 ± 12	6.7 ± 3.6	6/4	50%	4.2 ± 11.2	0–36	100%	238.2 ± 336.8	10–1092	NA	NA	NA	NA	NA	NA
	32	12.3.19	Late winter	77 ± 7	4.4 ± 1.3	21/11	41%	2.3 ± 5.9	0–30	97%	28.7 ± 29.8	0–148	0%	0	–	97%	31.1 ± 29.4	0–148
	26	31.5.19	Late spring	67 ± 7	2.4 ± 0.7	13/13	19%	0.3 ± 0.8	0–4	81%	9.0 ± 20.8	0–106	4%	0.0 ± 0.2	0–1	81%	9.3 ± 21.6	0–110
Saithe	29	2.4.19	Early spring	53 ± 5	1.6 ± 0.4	17/7/5	100%	11.0 ± 7.0	1–37	100%	45.9 ± 24.9	11–117	28%	1.7 ± 5.1	0–20	100%	58.6 ± 27.7	15–129
	29	31.5.19	Late spring	62 ± 9	2.2 ± 1.1	16/13	66%	2.8 ± 4.8	0–24	97%	12.1 ± 10.3	0–36	10%	0.2 ± 0.5	0–2	100%	15.1 ± 12.2	1–43
Haddock	30	2.4.19	Early spring	51 ± 5	1.4 ± 0.4	22/8	53%	5.0 ± 12.0	0–61	80%	16.0 ± 25.8	0–104	3%	0.1 ± 0.4	0–2	87%	21.1 ± 34.9	0–143
	30	31.5.19	Late spring	54 ± 6	1.4 ± 0.5	11/19	3%	0.1 ± 0.5	0–3	27%	1.3 ± 4.2	0–22	7%	0.2 ± 0.6	0–3	33%	1.6 ± 4.7	0–25

TL = total length (cm; mean ± SD = standard deviation), TW = total weight (Kg; mean ± standard deviation), F = female, M = male, U = unknown, PR (%) = positivity rate (as percentage), A ± SD (average ± standard deviation), P (%) = Prevalence (as a percentage), mA ± SD (mean abundance ± standard deviation), Range = minimum and maximum number of parasites per fish in the sample, NA = not available data. * Fish sample delivered fresh (in ice) to lab facilities. ** Fish sample inspected at the factory. The other fish samples were delivered frozen to lab facilities.

Levsen & Lunestad, 2010). Larval and adult *Hysterothylacium* sp. may be distinguished from other nematodes present in fish (i.e. *Anisakis* spp., *Pseudoterranova* spp. and *Contracaecum* spp.) by differences in shape as well as color, intensity and shade of fluorescence.

2.3. Morphological identification

Hysterothylacium sp. subsamples from all three fish species were identified to species level and to developmental 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, cuticle ornamentation and "cactus" tail, as well as the position of the excretory pore relative to the boring tooth (Berland, 1961, 1989bib_Berland_1961bib_Berland_1989). The total length and sex (presence of caudal papillae and spicules in males) of adult *Hysterothylacium* sp. from cod were recorded.

2.4. Molecular identification

Genomic DNA from *Hysterothylacium* sp. subsamples recovered from gills, skin, stomach and intestines of cod (n = 7), saithe (n = 6) and haddock (n = 4) was extracted using DNeasy Blood & Tissue kits (Qiagen) according to the manufacturer's instructions with few modifications (i.e. DNA was eluted by adding 30 or 50 μl of buffer AE) and stored at −20 °C prior to amplification. The DNA concentration and purity were measured using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

The internal transcribed spacers of nuclear ribosomal DNA (ITS rDNA) of the nematodes was amplified following procedures by Zhu, Gasser, Jacobs, Hung, and Chilton (2000).

In addition, for amplifying the mitochondrial cytochrome *c* oxidase subunit II (*cox2*) gene, polymerase chain reaction (PCR) was performed using the primers 211F (5′-TTTTCTAGTTATATAGATTGRTTTYAT-3′) and 210R (5′-CACCAACTCTTAAAATTATC-3′) (Nadler & Hudspeth, 2000). PCR was carried out according to the procedures provided by Mattiucci et al. (2014) with modifications. PCR reactions were subjected to the following cycle conditions: 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 60 s, 72 °C for 90 s, followed by post-amplification at 72 °C for 10 min.

Purification and sequencing of PCR products were carried out by Eurofins (Cologne, Germany) using the same primers as the ones for the amplification.

The sequences obtained at the ITS rDNA and mtDNA *cox2* for the adult nematodes were searched for similarity using BLAST (Basic Local Alignment Search Tool) through web servers of the National Center for Biotechnology Information (USA) (Altschul, Gish, Miller, Myers, & Lipman, 1990).

2.5. Infection parameters

Basic infection parameters such as prevalence and mean abundance per infection site (digestive tract, pyloric caeca and overall) were calculated as described in Bush, Lafferty, Lotz, and Shostak (1997) (Table 1). When the nematodes occurred on the gills, mouth, pharynx and/or skin (an unnatural location), the following descriptors were used: positivity rate (percentage of the number of fish with at least 1 parasite/total number of fishes sampled) and average (mean number of parasites in the sample) (Table 1).

2.6. Statistical modelling

Hysterothylacium sp. abundance in the digestive tract of cod, saithe and haddock was investigated for variation related to fish length and weight, sex and fishing season (categorized into mid-winter, late-winter, early-spring and late-spring). Generalized additive models (GAMs), allowing to model non-linear effects of explanatory variables, were fitted by backwards selection based on Akaike information criterion (AIC) values and significance of individual variable effects. Model assumptions were verified by plotting Pearson residuals versus fitted values, explanatory variables in the model and explanatory variables not in the model. Data exploration, model selection and model validation were carried out using R (R Core Team, 2018) following Zuur and Ieno (2016); Zuur, Ieno, and Elphick (2010); Zuur, Ieno, and Smith (2007).

2.6.1. Cod

Data exploration showed high correlation among TL, TW and season (correlation coefficients > 0.8). Variance inflation factors (VIFs) were used to assess which variables were highly related (Zuur et al., 2007). Backwards selection method to remove one variable with the highest value at a time was used. The process was repeated until VIF values were smaller than 3 (Zuur et al., 2010). Total weight (TW) was dropped in first place, followed by season. The first Poisson GAM including TL and sex as explanatory variables was overdispersed (ascaridoid abundance in fish is commonly overdispersed (Gay et al., 2018; Pascual, Rodríguez, Pierce, Hastie, & González, 2018; Pierce et al., 2018)), so negative binomial GAMs with log link function were fitted.

2.6.2. Saithe

Data exploration showed high correlation among TL and TW (correlation coefficients > 0.8). Total length (TL) was dropped from the model (variable with highest VIF value). Firstly, a Poisson GAM including TW, season and sex as explanatory variables was overdispersed. A negative binomial GAM was fitted, but sex effect was non-significant, so it was dropped from the model. Model validation showed a highly influential observation (Cook's distance >1). This observation corresponded to a very big saithe (e.g. four times the average TW) that was behaving as an outlier, so it was dropped from the final model.

2.6.3. Haddock

Like above, TL and TW were highly correlated, so TL was dropped from the model because it was the variable with highest VIF value. A Poisson GAM including TW, season and sex as explanatory variables was overdispersed. A negative binomial GAM was fitted, but TW and sex effect were non-significant, TW having the highest *p*-value, so it was dropped from the model. Model validation shown the presence of a highly influential observation (Cook's distance >1). This observation corresponded to an average size haddock (TL = 51.5 cm, TW = 1.5 Kg) with high number of parasites (n = 82) caught in early spring that was behaving as an outlier, so it was dropped from the final model.

2.7. Viability assessment

Hundreds of *Hysterothylacium* sp. adults were recovered from cod (n = 10) recently caught and delivered at the fish factory. Living nematodes were randomly obtained from the digestive tracts and placed in falcon tubes with freshwater. The nematodes were transported the following day to the lab facilities in Bergen (Norway), 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 viability assessment started (i.e. 6 days). The nematodes (n = 20) were placed on a Petri dish containing 100 ml of physiological water and their viability checked on a daily basis for 8 days.

Nematode 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).

3. Results

3.1. Morphological and molecular results

Hysterothylacium sp. subsamples were morphologically assigned to adult/L4 or L3 of *H. aduncum*. Species identity was confirmed by sequence analysis of ITS rDNA and cox2 mtDNA genes. The ITS rDNA sequences obtained (n = 13) (908–943 bp; GenBank accession numbers MW131974 to MW131976) were identical and showed 100% identity with corresponding *H. aduncum* sequences previously deposited in GenBank. The mtDNA cox2 sequences obtained for 11 of the latter parasites plus 4 new (i.e. n = 15) (571 bp; GenBank accession numbers MW149060 to MW149074) showed 99.1–99.8% identity with sequences of L3 *H. aduncum* from horse mackerel (Trachurus trachurus) caught in the Adriatic Sea (GenBank accession numbers JQ934891-93).

3.2. Infection levels and sites

Hysterothylacium aduncum was present in cod, saithe and haddock in all sampling periods. However, the level of infection varies with fish species, site of infection and season (Table 1). 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 (SD) in the digestive tract.

The parasite varied considerably in length in cod, saithe and haddock. 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). A total of 459 *H. aduncum* from cod were sexed, 323 (70%) females and 136 (30%) males of which 249 were also measured. The average length ± SD (range) was 5.3 ± 1.3 cm (1.8–10.5 cm) for females (n = 169) and 4.1 ± 0.8 cm (25–70 cm) for males (n = 80).

Adults and L4 of *H. aduncum* were frequently found on the skin, mouth, pharynx, gills and within the stomach and intestine (Fig. 1, at supp. mat.), and L3 in the pyloric caeca of cod (L3 were yellowish in color when being exposed to UV light), saithe (Fig. 2, at supp. mat.) and haddock (Figure 3, at supp. mat.), occasionally in large numbers (Figure 4, at supp. mat.) (Table 1). Numerous *H. aduncum* were also frequently observed in fish stomachs where they wriggled around in newly eaten capelin (*Mallotus villosus*) (Figure 5 at supp. mat.). The parasite was absent from fillets.

3.3. Factors associated with H. aduncum abundance in fish

3.3.1. Cod

Hysterothylacium aduncum numbers in cod followed a negative binomial distribution (theta = 0.861, dispersion statistic = 1.439) and the best final model included a significant positive linear effect of TL ($p < 0.0001$) and sex ($p < 0.049$) (Deviance explained (DE) = 35.1%, N = 76). Parasite abundance increased with TL (Fig. 1A) whilst females had higher abundance than males. Additionally, a negative binomial GAM including a) season and sex as covariates, and b) an interaction term between TL and season and sex as covariates were also fitted but did not improve the model (i.e. sex was not significant, data not shown).

The final model was satisfactory in terms of absence of highly influential data points and of serious trends in residuals versus fitted values and explanatory variables (Figure 6, at supp. mat.). The visual representation of the model and numerical output can be observed at Fig. 2A and at Figure 7 at suppl. mat., respectively.

In addition, the individual effect of season variable on *H. aduncum* abundance was analyzed. Higher parasite numbers were found in cod fished in mid-winter, followed by late-winter and the fewest in late-spring seasons ($p < 0.0001$).

3.3.2. Saithe

The final negative binomial model (theta = 3.133, dispersion statistic = 0.952) of *H. aduncum* abundance in saithe included an effect of

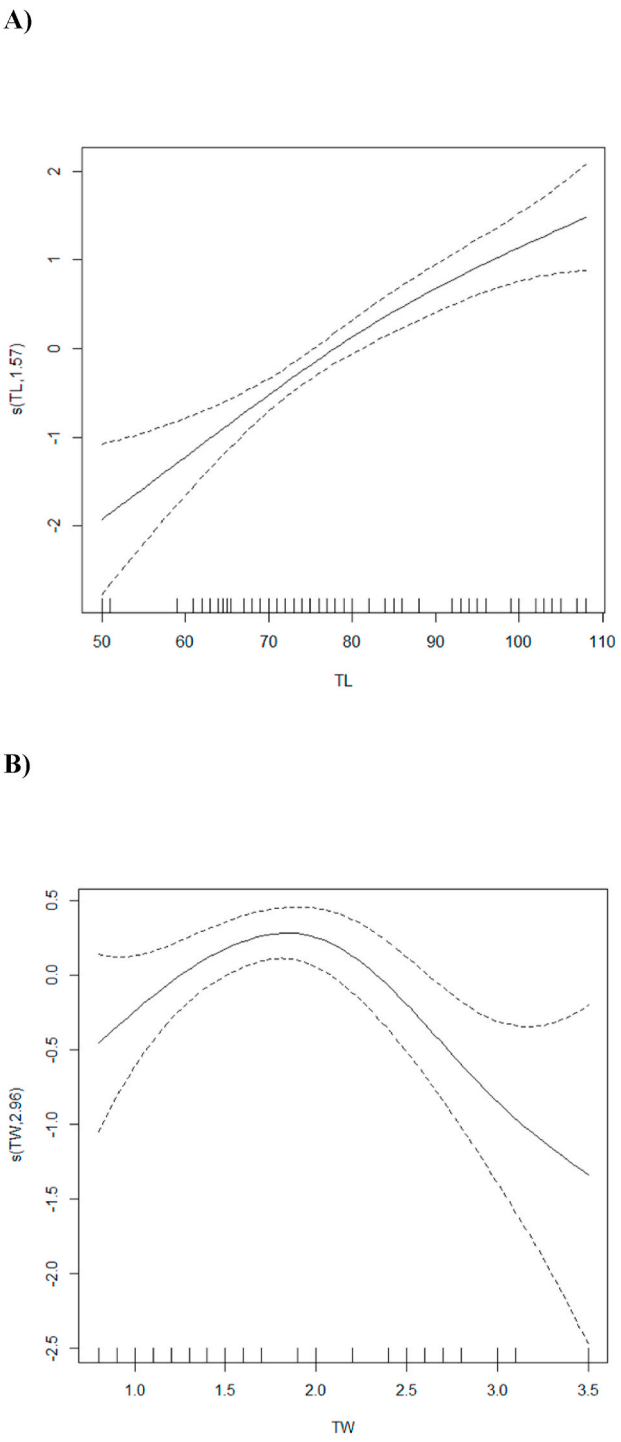

Fig. 1. GAM smoothing curves showing the partial effect of fish total length (TL) and total weight (TW) on *Hysterothylacium aduncum* abundance in the digestive tract of A) cod and B) saithe, respectively. The x-axes show the values of TL or TW (vertical lines), and the y-axes the contribution of to the smoother to the fitted values. The solid line is the smoother and the dashed lines 95% confidence bands. The notations s (TL, 1.57) and s (TW, 2.96) means that the smoothers are applied on TL and TW and cross-validation is used to estimate the optimal amount of smoothing. The little vertical lines along the x-axes indicate the TL or TW values of the observations.

TW ($p < 0.003$) and season ($p < 0.0001$) (DE = 59.9%, N = 57). Parasite abundance increased with TW in fish up to around 1.7 kg, then remains stable up to around 2 kg, and decreased after that (Fig. 1B). There were higher number of parasites in early spring compare to late spring. A

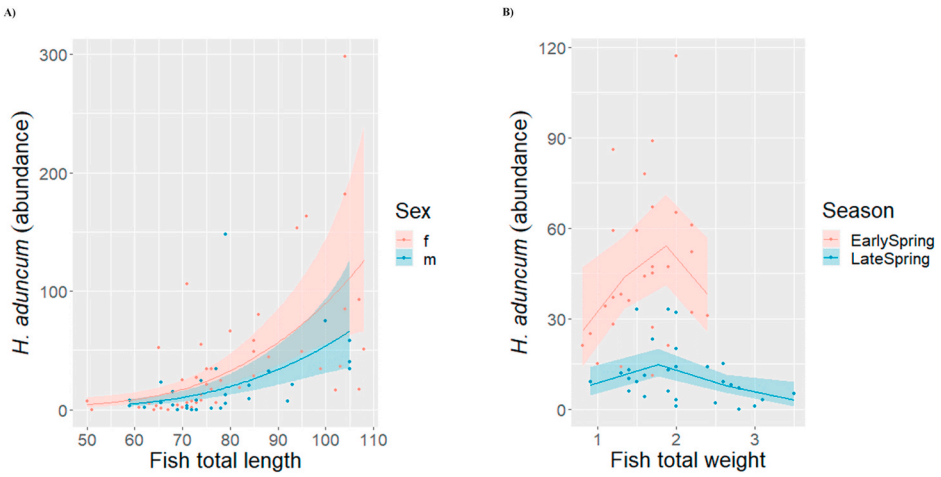

Fig. 2. Model fit of the negative binomial GAM for cod (A) and saithe (B).

negative binomial GAM including the interaction between TW and season did not improve the model (i.e. highest AIC, data not shown).

Model validation indicated no problems (Figure 8, at supp. mat.). The visual representation of the model and numerical output can be observed at Fig. 2B and at Figure 9 at suppl. mat., respectively.

3.3.3. Haddock

The final negative binomial model (theta = 3.365, dispersion statistic = 0.945) of *H. aduncum* abundance in saithe included an effect of season ($p < 0.0001$) and sex ($p = 0.0002$) (DE = 42%, N = 59). Females had a higher abundance, and the abundance was higher in early spring compared to late spring. Model validation indicated no problems (Figure 10 at supp. mat.). The numerical output of the model can be observed at Figure 11 at supp. mat.

3.4. Viability assessments

Hysterothylacium aduncum (n = 20) that were checked on a daily basis were viable after 8 days in a refrigerator (14 days since capture). 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.

4. Discussion

4.1. Hysterothylacium aduncum biological aspects

In the present study, morphological and molecular results based on the ITS rDNA and *cox2* mtDNA genes identified *H. aduncum* as the parasite species infecting cod, saithe and haddock from Northern Norway. The *cox2* mtDNA sequences obtained represent the first sequences of *H. aduncum* adults from NE Atlantic waters available at GenBank so far.

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 and euphausiids (intermediate hosts) (reviewed by Busch, Kuhn, Münster, and Klimpel (2012)) and also in the viscera (especially pylorus) of fishes (reviewed by Berland (1961; 2006)). It appears that small planktivorous pelagic fishes (e.g. capelin, sprat, Atlantic herring (*Clupea harengus*), etc.) foraging on infected invertebrates may serve as second intermediate or transport hosts, even though they can also act as final hosts since small adult specimens may also be present (Dessier et al., 2016; Klimpel et al., 2007; Levsen, Paoletti, Cipriani, Nascetti, & Mattiucci, 2016; Tolonen & Karlsbakk, 2003). The fact that *H. aduncum* may use an enormous diversity of benthic/pelagic invertebrate and fish hosts makes this parasite one of the most abundant and ubiquitous ascaridoid parasite of NE Atlantic waters, including the Barents Sea.

4.2. Hysterothylacium aduncum in cod from the Barents Sea

The presence of parasitic nematodes in fish has been known for centuries to fishermen and coastal people of Norway (Berland, 2006). In the 1950s, Bjørn Berland studied and described the morphology of the most common ascaridoids of marine fishes from Norway, including *H. aduncum* (Berland, 1961). In addition, Soleim and Berland (1981) used *H. aduncum* recovered from the digestive tract of cod and haddock from the Barents Sea for morphological studies. Since then, the occurrence of *H. aduncum* in cod fished in Norway has been documented in a number of studies: in cod from the Oslofjord (Andersen, 1993), Balsfjord (Hemmingsen, Halvorsen, & MacKenzie, 2000), Trondheimsfjord (Perdiguero-Alonso, Montero, Raga, & Kostadinova, 2008) and Øksfjord (Heuch et al., 2011). In addition, the presence of *Hysterothylacium* sp. in cod from Altafjord (Hemmingsen, Lysne, Eidnes, & Skorping, 1993) and Barents Sea (Gay et al., 2018) has been reported.

Recently, Najda, Kijewska, Kijewski, Plauška, and Rokicki (2018) reported 100% prevalence with mean abundance 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 thesis carried out at the University of Tromsø reported the presence of morphologically identified *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. In here, we reported 81%–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).

4.3. Why has the industry faced this parasite-related quality issue precisely now?

Hysterothylacium aduncum can be found parasitizing cod, saithe and haddock in the Barents Sea. But, why has recently the industry encountered with the "*H. aduncum* problem"? It appears that all started when head-on cod 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, the parasite that inhabits the digestive tract of the fish, can be transferred immediately from the stomach to the head 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, a number of nematodes (ranging from 0 to 58) may remain hidden in the head cavities. Eventually, *H. aduncum* can crawl out from there to the body surface and styrofoam boxes during transportation, hence being alive and visible at destination point. *Hysterothylacium aduncum* can grow quite big, and this clearly shows its potential to cause client/consumer rejection if present in the final product (Figure 12 at supp. mat.).

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 1). However, due to lack of comparable historical data this cannot be tested. In relation to this, it has been suggested an increasing abundance of Atlantic copepods (e.g. *Calanus finmarchicus*) and euphausiids (*Thysanoessa inermis* and *Meganyctiphanes norvegica*) in western Barents sea over the last decades probably linked to increased water temperatures (Aarflot, Skjoldal, Dalpadado, & Skern-Mauritzen, 2018; Dalpadado & Mowbray, 2013; Eriksen et al., 2016). These crustaceans are known to be most important prey for capelin (Dalpadado & Mowbray, 2013) and potential intermediate hosts of *H. aduncum* (reviewed by Busch et al. (2012).

4.4. Factors explaining H. aduncum abundance in cod, saithe and haddock

Results suggest that season is the most important variable explaining the abundance of *H. aduncum* in cod, saithe and haddock. 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 (in fact, the best final model included effects of TL and sex). However, when accounting for the individual effect of season on *H. aduncum* abundance, they were found to be highly significantly related. Interestingly, the former variables (i.e. season and fish size) were significantly correlated for saithe but not for haddock (data not shown). The best final models for these species included highly significant effects of season. It appears then, that season is the most important variable explaining the parasite abundance in these three gadoids, having higher number of parasites soon in the year (i.e. "mid and late winter" and "early spring") and lower in "late spring".

The seasonal variation in infection levels may be explained by the biology of *H. aduncum*, and by the trophic relationships and migration patterns of its hosts. The three gadoids appear to have similar migration behavior 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, Jørgensen, & Dragesund, 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, Hallfredsson, Mikkelsen, Bogstad, & Pedersen, 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, Slotte, & Johannessen, 2008). This trophic link and overlapping distribution is particularly strong between cod and capelin (Fall, Ciannelli, Skaret, & Johannesen, 2018; Johannesen, Johansen, & Korsbrekke, 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 & Jørgensen, 1996). Shrimps (i.e. *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 that these gadoids will acquire most of *H. aduncum* through intensive predation on capelin in the first quarter 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 from "mid-winter" to "early spring", the parasite will reproduce and disappear from the fish gut before "late spring".

Interestingly, female cod had higher abundance of *H. aduncum* than males (just weakly significant), and especially in haddock (highly significant). This might be related to temporary differences in feeding habits, habitat distribution or migration routes between sexes of both species (particularly haddock).

4.5. Viability assessments and zoonotic aspects

Hysterothylacium aduncum has shown great potential to cause monetary losses to the Norwegian-Spanish cod 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 lots were immediately rejected. In this sense, we have provided further evidence that *H. aduncum* can survive for at least 14 days in a wet and cold environment (i.e. in physiological water and in fridge).

Hysterothylacium aduncum is a parasite of cold-blooded organisms and is generally considered non-zoonotic, however, this aspect is not fully understood and remains controversial (Cavallero et al., 2020; Shamsi, Steller, & Chen, 2018). The parasite was reported as causative agent of human infection twice to date (González-Amores, Clavijo-Frutos, Salas-Casanova, & Alcain-Martínez, 2015; Yagi et al., 1996). However, these studies lack a confirmatory molecular identification of the nematode, so diagnosis remains open to doubt.

In addition, *H. aduncum* share common antigens (and possibly has species-specific antigens) with anisakids (e.g. *A. simplex* s.l.) responsible of allergic responses in humans, and therefore, it may potentially be involved in the allergic reactions presented by some sensitized patients following fish consumption (Fernández-Caldas et al., 1998; Lozano Maldonado et al., 2004; Valero, Terrados, Díaz, Reguera, & Lozano, 2003).

4.6. Fish handling recommendation to the industry

Head-on eviscerated cod have been sent cooled from Norway to Spain in styrofoam boxes, which resulted in the present parasite-related fish quality issue with important economic losses for all involved parties. 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, it would be recommended to eviscerate the fish as soon as possible, cut off the fish head and rinse carefully the fish body in order to eliminate the parasite from the fishery product. Alternatively, 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").

5. Conclusions

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" (strongly related to fish migration and diet) appears to be the most important factor explaining the abundance of

H. aduncum in these gadoid species, with more parasites during winter and early spring and less during late spring. It is hypothesized that fishes acquire the highest infection levels of *H. aduncum* through predation of spawning capelin during winter/spring in northern Norway. The parasite can survive for long periods in cold and humid conditions, and therefore being lively (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.

CRediT authorship contribution statement

Miguel Bao: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Paolo Cipriani:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Visualization, Funding acquisition. **Lucilla Giulietti:** Methodology, Resources, Writing - review & editing. **Natalia Drivenes:** Methodology, Investigation, Resources, Writing - review & editing. **Arne Levsen:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107724.

References

Aarflot, J. M., Skjoldal, H. R., Dalpadado, P., & Skern-Mauritzen, M. (2018). Contribution of *Calanus* species to the mesozooplankton biomass in the Barents Sea. *ICES Journal of Marine Science, 75*(7), 2342–2354. https://doi.org/10.1093/icesjms/fsx221

Alimentarius, C.odex (2004). *Standard for salted atlantic herring and salted sprat - CODEX STAN 244-2004*. Retrieved from http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

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. *Parasitology Research, 79*(1), 67–72. https://doi.org/10.1007/BF00931220

Bao, M., Cipriani, P., Giulietti, L., Roiha, I. S., Paoletti, M., Palomba, M., et al. (2020). Air-dried stockfish of Northeast Arctic cod do not carry viable anisakid nematodes. *Food Control, 116*, 107322. https://doi.org/10.1016/j.foodcont.2020.107322

Bao, M., Pierce, G. J., Strachan, N. J. C., Pascual, S., González-Muñoz, M., & Levsen, A. (2019). Human health, legislative and socioeconomic issues caused by the fish-borne zoonotic parasite *Anisakis*: Challenges in risk assessment. *Trends in Food Science & Technology, 86*, 298–310. https://doi.org/10.1016/J.TIFS.2019.02.013

Bergstad, O. A., Jørgensen, T., & Dragesund, O. (1987). Life history and ecology of the gadoid resources of the Barents Sea. *Fisheries Research, 5*(2–3), 119–161. https://doi.org/10.1016/0165-7836(87)90037-3

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

Berland, B. (1989). Identification of fish larval nematodes from fish. In H. Möller (Ed.), *Nematode problems in north Atlantic fish. Report from a workshop in Kiel. 3-4 April 1989* (pp. 16–22).

Berland, B. (2006). *Musings on nematode parasites*. Retrieved from http://www.imr.no/filarkiv/2003/12/Nr.11_2006_Musings_on_nematode_parasites.pdf/nb-no.

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. *Journal of Fish Biology, 72*(6), 1427–1449. https://doi.org/10.1111/j.1095-8649.2008.01808.x

Buchmann, K., & Mehrdana, F. (2016). Effects of anisakid nematodes *Anisakis simplex* (s.l.), *Pseudoterranova decipiens* (s.l.) and *Contracaecum osculatum* (s.l.) on fish and consumer health. *Food and Waterborne Parasitology, 4*, 13–22. https://doi.org/10.1016/j.fawpar.2016.07.003

Busch, M. W., Kuhn, T., Münster, J., & Klimpel, S. (2012). Marine crustaceans as potential hosts and vectors for metazoan parasites. In H. Mehlorn (Ed.), *Arthropods as Vectors of Emerging diseases* (pp. 329–360). Berlin Heidelberg: Springer. 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. *The Journal of Parasitology, 83*(4), 575. https://doi.org/10.2307/3284227

Cavallero, S., Lombardo, F., Salvemini, M., Pizzarelli, A., Cantacessi, C., & D'Amelio, S. (2020). Comparative transcriptomics reveals clues for differences in pathogenicity between *Hysterothylacium aduncum, Anisakis simplex sensu stricto* and *Anisakis pegreffii*. *Genes, 11*(3), 321. https://doi.org/10.3390/GENES11030321

Dalpadado, P., & Mowbray, F. (2013). Comparative analysis of feeding ecology of capelin from two shelf ecosystems, off Newfoundland and in the Barents Sea. *Progress in Oceanography, 114*, 97–105. https://doi.org/10.1016/j.pocean.2013.05.007

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. *Marine and Freshwater Research, 67*(10), 1583. https://doi.org/10.1071/MF15147

EFSA-BIOHAZ. (2010). Scientific Opinion on risk assessment of parasites in fishery products. *EFSA Journal, 8*(4), 1543. https://doi.org/10.2903/j.efsa.2010.1543

Eiras, J. C., Pavanelli, G. C., Takemoto, R. M., & Nawa, Y. (2017). Fish-borne nematodiases in South America: Neglected emerging diseases. *Journal of Helminthology*. https://doi.org/10.1017/S0022149X17001006, 1–6.

Eriksen, E., Skjoldal, H. R., Dolgov, A. V., Dalpadado, P., Orlova, E. L., & Prozorkevich, D. V. (2016). The Barents Sea euphausiids: Methodological aspects of monitoring and estimation of abundance and biomass. *ICES Journal of Marine Science, 73*(6), 1533–1544. https://doi.org/10.1093/icesjms/fsw022

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*(10), 1–26. https://doi.org/10.1371/journal.pone.0205921

Fernández-Caldas, E., Quirce, S., Marañó, F., Gómez, M. L. D., Botella, H. G., & Román, R. L. (1998). Allergenic cross-reactivity between third stage larvae of *Hysterothylacium aduncum* and *Anisakis simplex*. *The Journal of Allergy and Clinical Immunology, 101*(4), 554–555. https://doi.org/10.1016/S0091-6749(98)70364-1

Gay, M., Bao, M., MacKenzie, K., Pascual, S., Buchmann, K., Bourgau, O., et al. (2018). Infection levels and species diversity of ascaridoid nematodes in Atlantic cod, *Gadus morhua*, are correlated with geographic area and fish size. *Fisheries Research, 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*(6), 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 Journal of Marine Science, 73*(2), 182–195. https://doi.org/10.4135/9781412953924.n678

González-Amores, Y., Clavijo-Frutos, E., Salas-Casanova, C., & Alcain-Martínez, G. (2015). Direct parasitologial diagnosis of infection with *Hysterothylacium aduncum* in a patient with epigastralgia. *Revista Española de Enfermedades Digestivas, 107*(11), 699–700.

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 Biology, 23*(5), 368–372. https://doi.org/10.1007/s003000050457

Hemmingsen, W., Lysne, D., 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. *Fisheries Research, 15*(4), 379–386. https://doi.org/10.1016/0165-7836(93)90088-O

Heuch, P., Jansen, P., Hansen, H., Sterud, E., MacKenzie, K., Haugen, P., et al. (2011). Parasite faunas of farmed cod and adjacent wild cod populations in Norway: A comparison. *Aquaculture Environment Interactions, 2*(1), 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 Journal of Marine Science, 53*(1), 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. *Canadian Journal of Fisheries and Aquatic Sciences, 73*(2), 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. *Archiv Für Lebensmittelhygiene, 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*(10), 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. *Parasitology Research, 101*(5), 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. *Parasitology Research, 97*(2), 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). *Canadian Journal of Zoology, 71*(7), 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. *Veterinary Parasitology, 171*(3–4), 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. *Parasitology Research, 115*(11), 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., et al. (2018). A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—Introducing the FP7 PARASITE exposure assessment study. *Fisheries Research*, 4–21. https://doi.org/10.1016/J.FISHRES.2017.09.009, 202.

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.

Lozano Maldonado, J., Martín Hita, L., Díaz Sáez, V., Mañas Almendros, I., Valero López, A., & Campos Bueno, M. (2004). Cross-reactivity between antigens of *Anisakis simplex* s.l. and other ascarid nematodes. *Parasite, 11*(2), 219–223. https://doi.org/10.1051/parasite/2004112219

Mattiucci, S., Cipriani, P., Paoletti, M., Levsen, A., & Nascetti, G. (2017). Reviewing biodiversity and epidemiological aspects of anisakid nematodes from the North-east Atlantic Ocean. *Journal of Helminthology, 1–18*. https://doi.org/10.1017/S0022149X1700027X

Mattiucci, S., Cipriani, P., Webb, S. C., Paoletti, M., Marcer, F., Bellisario, B., et al. (2014). Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). *The Journal of Parasitology, 100*(2), 199–214. https://doi.org/10.1645/12-120.1

Mironova, N. V. (1961). Biology of the Barents sea saithe, *Pollachius virens* (L.). *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie, 46*(3), 447–459. https://doi.org/10.1002/iroh.19610460311

Nadler, S. A., & Hudspeth, D. S. S. (2000). Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology hypotheses of structural and sequence evolution. *The Journal of Parasitology, 86*(2), 380–393. https://doi.org/10.1645/0022-3395(2000)086[0380:POTANA]2.0.CO:2

Najda, K., Kijewska, A., Kijewski, T., Plauška, K., & Rokicki, J. (2018). Distribution of ascaridoid nematodes (Nematoda: Chromadorea: Ascaridoidea) in fish from the Barents sea. *Oceanological and Hydrobiological Studies, 47*(2), 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 Journal of Marine Science, 67*(1), 87–101. https://doi.org/10.1093/icesjms/fsp229

Pascual, S., Rodríguez, H., Pierce, G. J., Hastie, L. C., & González, A. F. (2018). The NE Atlantic European hake: A neglected high exposure risk for zoonotic parasites in European fish markets. *Fisheries Research, 202*, 69–78. https://doi.org/10.1016/J.FISHRES.2017.12.008

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. *Parasites & Vectors, 1*(1), 23. https://doi.org/10.1186/1756-3305-1-23

Pierce, G. J., Bao, M., MacKenzie, K., Dunser, A., Giulietti, L., Cipriani, P., et al. (2018). Ascaridoid nematode infection in haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) in Northeast Atlantic waters. *Fisheries Research, 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*. *Journal of the Fisheries Research Board of Canada, 27*(5), 963–965. https://doi.org/10.1139/f70-107

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/.

Shamsi, S. (2014). Recent advances in our knowledge of Australian anisakid nematodes. *International Journal for Parasitology: Parasites and Wildlife, 3*(2), 178–187. https://doi.org/10.1016/j.ijppaw.2014.04.001

Shamsi, S., Steller, E., & Chen, Y. (2018). New and known zoonotic nematode larvae within selected fish species from Queensland waters in Australia. *International Journal of Food Microbiology, 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). *Zoologica Scripta, 10*(3), 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. *Parasitology Research, 84*(4), 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 Journal of Marine Science, 60*, 77–84. https://doi.org/10.1006/jmsc.2002.1307 ER

Valero, A., Terrados, S., Díaz, V., Reguera, V., & Lozano, J. (2003). Determination of IgE in the serum of patients with allergic reactions to four species of fish-parasite anisakids. *Journal of Investigational Allergology & Clinical Immunology, 13*(2), 94–98. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12968392.

Yagi, K., Nagasawa, K., Ihikura, H., Nakagawa, A., Sato, N., & Ishikura, H. (1996). Female worm *Hysterothylacium aduncum* excreted from human: A case report. *Japanese Journal of Infectious Diseases, 45*(1), 12–23. Retrieved from http://www.tmd.ac.jp/med/mzoo/parasites/JJP/45(1)/Contents45(1).html.

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. *Parasitology Research, 86*(9), 738–744. https://doi.org/10.1007/PL00008561

Zuur, A. F., & Ieno, E. N. (2016). A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution, 7*, 636–645. https://doi.org/10.1111/2041-210X.12577

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution, 1*(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Zuur, A. F., Ieno, E. N., & Smith, G. M. (2007). *Analysing ecological data*. New York, NY: Springer New York. https://doi.org/10.1007/978-0-387-45972-1