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ORIGINAL RESEARCH ARTICLE

Distribution of anisakid nematodes in the muscle tissue of cod (*Gadus morhua*) from the Norwegian Sea

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Abstract Atlantic cod (*Gadus morhua*) is an important commercial fish species on the world market. The aim of our studies was to explore the presence, intensity of infection and distribution of the zoonotic nematodes of the different genera of Anisakidae in the muscle tissue of *G. morhua* from the Norwegian Sea. Cod from fishing areas FAO IIa1 (n = 50) and FAO IIa2 (n = 56) were sampled in March 2017. The unskinned flesh of each fish was examined using a white-light transilluminator. Collected parasites were identified to the genus level, and a subsample was identified using molecular methods. We found a higher prevalence of infection with *Anisakis* than with *Pseudoterranova* in the musculature of cod from both fishing areas. In FAO IIa1, a lower prevalence of infection with *Pseudoterranova* was recorded (14%) than in FAO IIa2 (~39%). However, the intensity of infection was higher (53) in FAO IIa2 than in FAO IIa1 (8 parasites per fish). The opposite was found with *Anisakis* (prevalence 88% in FAO IIa1 and ~55% in FAO IIa2, intensity up to 30 and up to 25 parasites per fish respectively). Most *Anisakis* larvae were present in the belly flaps (predominantly the left side), while *Pseudoterranova* spp. were dispersed with descending frequency in belly flaps, dorsal fillet and caudal fillet.

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Molecular identification revealed the presence of *A. simplex* (s.s.), *P. decipiens* (s.s.) and *P. krabbei* in both areas, and a hybrid of *P. decipiens* and *P. krabbei* in FAO IIa2.

1. Introduction

Atlantic cod (*Gadus morhua*) occurs in shelf waters throughout the North Atlantic Ocean and its adjacent seas (Cohen et al., 1990). In Europe, it has been recorded from the northwestern Iberian Peninsula to the Barents Sea, including British and Icelandic waters, as well as some brackish water localities in the Baltic Sea (Bañon et al., 2010; ICES, 2005). Atlantic cod is one of the most important commercial fish species on the world market. According to a report from the Food and Agriculture Organization of the United Nations (FAO) in 2012, it was among the 10 most-fished species in the world in 2010 and 2011, with about 200 000 tonnes landed per year, and was one of the most important species in European commercial fisheries (Cardinale et al., 2013). In 2016, landings of cod in the EU reached more than 91 000 tonnes and had a value of EUR 226 million (EUMOFA, 2018). The top three EU players in the cod fishery were Denmark, the UK and Spain. Most cod landings in Spain are frozen, while those in Denmark and UK are almost entirely fresh products. Fresh cod landed in the UK reached a 10-year peak at more than 15 000 tonnes (EUMOFA, 2018). The reason for such great interest in fisheries of this species is the popularity of cod with consumers, because of its mild flavour and dense, flaky white flesh.

The European Food Safety Authority (EFSA) has stated that all wild-caught fish (particularly if intended to be eaten raw or nearly raw) must be considered at risk of containing viable parasites of concern to human health (EFSA, 2010). The most dangerous parasites for human health that can be found in cod fillets are Anisakidae nematodes: *Anisakis simplex* and *A. pegreffii* (Audicana and Kennedy, 2008; Ishikura et al., 1993; Mattiucci et al., 2013), as well as *Pseudoterranova* spp. (Mattiucci et al., 2013; Mehrdana et al., 2014; Shamsi and Suthar, 2016; Torres et al., 2007). Viable, invasive anisakid larvae accidentally ingested by humans may cause anisakidosis. Symptoms of acute anisakidosis include nausea, diarrhea, vomiting, and intense abdominal pain (Hochberg et al., 2010; Ishikura et al., 1993). There are no published case studies of acute anisakidosis caused by eating infected cod. Nevertheless, two cases of allergy related to anisakidosis following consumption of raw cod have been reported (Alonso-Gómez et al., 2004). Although adequate treatment kills the parasite (Wharton and Aalders, 2002), some of the allergenic proteins of anisakid nematodes are thermostable (Audicana et al., 2002; Moneo et al., 2005). Brunet et al. (2017) demonstrated two cases of infection with *Pseudoterranova decipiens s.s.* in French patients after consumption of a baked cod.

The presence of Anisakidae nematodes in North Atlantic cod muscle tissue has been known for many years: they were recorded in cod fillets from the southern Canadian mainland between 1946 and 1956 (Scott and Martin, 1957), and from different areas adjacent to Newfoundland in the periods 1947–1953 (Templeman et al., 1957) and 1984–1985 (Chandra and Khan, 1988). Parasites, including nematodes, were considered to be biological indicators of stocks of Atlantic cod sampled between 1981 and 1983 off Newfoundland, Canada (Khan and Tuck, 1995). The presence and abundance of *A. simplex* larvae in the flesh of Atlantic cod was examined between 1985 and 1987 in the western Atlantic Ocean around Newfoundland and Labrador: their presence and abundance of parasites varied geographically and increased with cod size (Brattey and Bishop, 1992). Cod from the Gulf of St. Lawrence were examined for the presence of nematodes between 1990 and 1992 (Boily and Marcogliese, 1995), while the occurrence of anisakid nematodes in Atlantic cod was investigated in west Greenland in 2005 (Mouritsen et al., 2010). Cod from Iceland, the Norway coast, the Barents Sea and Arctic waters were examined for the presence of *Pseudoterranova decipiens* (former name *Terranova decipiens*) and *Anisakis* sp. between 1971 and 1973, with different levels of infection being observed in various stocks (Platt, 1975). Fillets of Norwegian Arctic cod from the Barents Sea in 1989 showed a 96% infection rate with *A. simplex*, with a significant difference in mean intensity of infection between oceanic and coastal fish (Aspholm, 1995). Anisakidae larvae were found in 34.7% of cod fillets from fish caught in the northeastern Atlantic ocean (Piccolo et al., 1999). Nematodes were recorded in cod from the Barents Sea in 2002 (Sobecka et al., 2011) and 2011 (Najda et al., 2018). Research conducted during 2013–2014 in the Barents Sea confirmed the high prevalence of cod infection with *A. simplex* (~90% of fillets), although infection with *Pseudoterranova* was lower (less than 10%) (Gay et al., 2018). Most research on cod from the Norwegian Sea was conducted in the coastal waters of Balsfjord and Ullsfjord (Hemmingsen et al., 1991), Balsfjord (Hemmingsen et al., 1992, 1995), Oslofjord (Aspholm et al., 1995; Jensen and Idås, 1992), Altafjord (Hemmingsen et al., 1993), near the island of Vega (Strømnes and Andersen, 1998, 2000) and in Trondheimsfjord (Perdiguero-Alonso et al., 2008). Nematode parasites in cod have also been noted in Icelandic waters (Hauksson, 2011; Perdiguero-Alonso et al., 2008), the Irish and Celtic Seas (Perdiguero-Alonso et al., 2008), and the Central and Northern North Sea (Gay et al., 2018; Perdiguero-Alonso et al., 2008). Nematode parasites were even recorded in cod larvae from the North Sea (Skovgaard et al., 2011). Despite the above research, there is currently a lack of knowledge about the level of cod infection in offshore waters of the Norwegian Sea. Because the level of infection with nematode parasites may differ significantly even between neighboring areas (Molina-Fernandez et al., 2015; Platt, 1975), we decided to focus

on the spatial distribution of cod infection with Anisakidae nematodes in the North East Atlantic.

The Norwegian Sea has been an important and intensively exploited cod fishing ground for many years (Bertheussen and Dreyer, 2019). Norwegian cod products are offered in various forms (whole fresh or frozen fish, fresh fillets, dried, dried salted or wet salted) and are exported to destinations over almost the whole world: Europe, Africa, South America and Asia (Asche et al., 2018).

There are a variety of methods of detecting the presence of nematodes in the fillets of fish. Some of them are not suitable for use during fish processing, because the tissues of the fish are destroyed. For example, both the compression technique (Karl and Leinemann, 1993) and digestion in artificial gastric juice (Llarena-Reino et al., 2013) allow the quantitative determination of nematodes, but lead to the destruction of fish tissue. Moreover, the UV-compression technique (Gomez-Morales et al., 2018), considered today to be the most sensitive among non-destructive methods that assess the risk of human infection by zoonotic nematodes, is limited to frozen products, because it relies on the ability of dead anisakid larvae to show fluorescence under UV light, and is not applicable in the case of fresh fillets. Emerging technologies such as imaging spectroscopy (Heia et al., 2007), multispectral imaging (Stormo et al., 2007; Wold et al., 2001), hyperspectral imaging (Sivertsen et al., 2011), X-ray (Heia et al., 1997) or magnetic resonance imaging (Bao et al., 2017) are used less frequently in the fish processing industry.

Our investigation was conducted using candling, which detects parasites by visual inspection of fish tissues over a light source. Although routine screening of fillets by candling is not 100% effective (Bao et al., 2019; Gomez-Morales et al., 2018; González et al., 2018; Levsen et al., 2005; Mercken et al., 2020a), it is the most widely adopted method in industry, where more accurate laboratory methods of detection are not routinely used. The main advantages of candling are its relatively low cost and simplicity and that it allows detection and immediate removal of visible parasites with minimal damage to the fillet's muscle tissue. In addition, many previous studies have been carried out using candling, allowing better comparison of the results obtained. Thus, the aim of our studies was to explore the level of infection with anisakids and the distribution of the different genera of Anisakidae nematodes in the muscle tissue of *G. morhua* using the detection method routinely applied in fish processing plants.

2. Material and methods

2.1. Sampling and detection of parasites

Cod was caught during a commercial survey in March 2017 in two areas of the North Atlantic, FAO IIa1 (n = 50) and FAO IIa2 (n = 56), as shown on the map (Figure 1). This sampling period was chosen because data (2007—2016) from the Norwegian Directorate of Fisheries showed the monthly average cod catch in this area to be highest in March (Bertheussen and Dreyer, 2019). Randomly chosen whole fish with undercut throats were kept on ice and frozen for further analysis. Subsequently, in the laboratory, after thawing for 24 h, standard ichthyological analysis was performed.

The presence of nematode parasites in different parts of the cod muscle tissue was evaluated. The unskinned flesh from both the right and left side of each animal was divided into three parts: anterior ventral (belly flaps), dorsal fillet and caudal fillet. Each part was examined for the presence of parasites in the muscle tissue using a transilluminator. All parasites detected were collected for further parasitological identification.

2.2. Identification of parasites

All detected parasites were collected and identified to the genus level on the basis of anatomo-morphological features as described by Fagerholm (1982) and Berland (1989). A subsample of parasites (16 *Anisakis* sp. and 34 *Pseudoterranova* sp.) of different origins was molecularly identified. Representative examples of each nematode species were selected from both regions in proportion to the number of nematodes of each species collected for that region. The target of molecular analysis in all cases was internal transcribed spacer 1 of the ribosomal DNA (ITS-1 rDNA). DNA was isolated using a Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the instructions. The analysis of ITS-1: the amplification was performed using NC5 (forward) 5′ GTA GGT GAA CCT GCG GAA GGA TCA TT 3′ and NC13R (reverse) 5′ GCT GCG TTC TTC ATC GAT 3′ primers (Zhu et al., 2000,2002). The reaction mixture consisted of 25 μl PCR Master Mix Plus High GC (ready-to-use PCR mixture containing Taq DNA polymerase, PCR buffer, $MgCl_2$ and dNTPs; A&A Biotechnology), 2 μl each primer (concentration 10 μM) and 5 μl DNA template, supplemented with deionized water up to 50 μl. The PCR conditions were as follows: 3 min at 94°C (initial denaturation) followed by 30 cycles of denaturation at 94°C for 30 s, annealing of primers at 55°C for 30 s, strand elongation at 72°C for 30 s and a final extension step of 5 min at 72°C. If the amplification was weak, the reaction was repeated, increasing the number of cycles to 40. Polymerase chain reaction (PCR) products were sequenced directly using standard procedures and amplification primers. Sequences were analysed using GeneStudio™ Professional (GeneStudio, Inc., USA) and confirmed by a BLAST search of GenBank. The sequences obtained have been deposited in GenBank with the accession numbers given in the Results.

2.3. Parasitological descriptors

The descriptors of parasite distribution used in the present study followed the definitions given by Bush et al. (1997). Prevalence is "the number of hosts infected with one or more individuals of a parasite species (or of a taxonomic group) divided by the number of hosts examined for that parasite species". The abundance is "the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected". The intensity (of infection) is "the number of individuals of a particular parasite species in a single infected host". Both abundance and intensity of infection were calculated, to make it easier to compare the results obtained with the information presented in previous research.

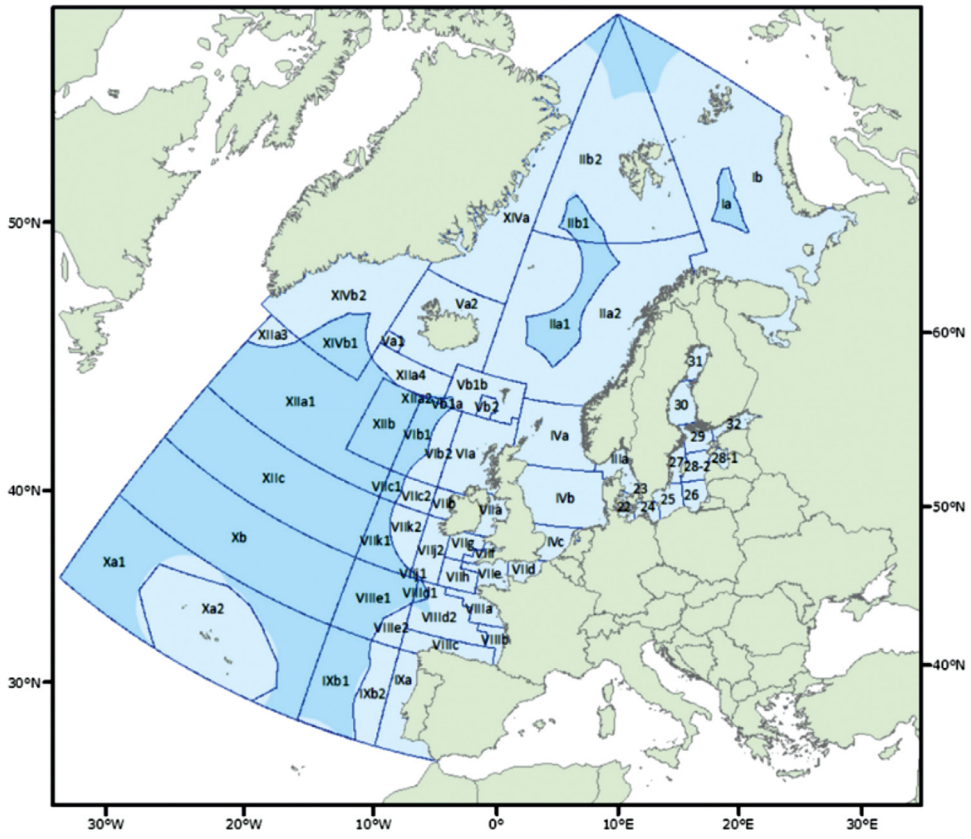


Figure 1 Map of the FAO 27 area (Cardinale et al., 2013).

2.4. Data analysis

Generalized linear models (GLMs) (McCullagh and Nelder, 1989) were applied to analyse the prevalence of cod infection in representatives of the genera *Anisakis* and *Pseudoterranova* in whole cod musculature (gutted fish) with respect to various biological and spatial parameters. The following model was fitted:

$$G(\text{inf}) = \text{area} + \text{sex} + \text{gonad developmental stage} + \text{TL} + \text{error};$$

where G is a link function and inf represents the prevalence of infection with *Anisakis* spp. or *Pseudoterranova* spp. The total body length (TL) was taken as the covariate, whereas the area, sex and gonad developmental stage were treated as factors. The error distribution was assumed to be binomial, and the logit link function was used. Corner point parameterization was imposed, i.e. factor effects for level one were assumed to be zero for all factors. Thus, the factor effects for the other levels may be regarded as the difference between the effect at any given level and the effect at level one. First, the initial model (which included all considered variables and factors) was fitted. The significance of the factors and covariates was then tested, and only significant terms were left in the final model. Tests were performed by deletion and those terms whose deletion did not result in a significant increase in deviance (i.e. the GLM measure of discrepancy between the modelled and observed values) were excluded from the model.

Due to the fact that the presence of parasites in the caudal part of the fillet was very low, for the purposes of the Wilcoxon matched-pairs test, the results obtained from counting the parasites in dorsal and caudal parts of the fillet were combined and compared with results obtained from counting the parasites in the belly flap. The Wilcoxon matched-pairs test was employed to assess the significance of the differences in the distribution of parasites in the various parts of the fish musculature (fillets *vs* belly flaps; left *vs* right fillets; left *vs* right belly flaps).

3. Results

3.1. Biological parameters of the fish

Cod sampled in the FAO IIa1 area were 63—92 cm in length, while fish caught in the FAO IIa2 area were smaller (52—70 cm in length). In both areas, the majority of the catch were males (88% in FAO IIa1 and 78.57% in FAO IIa2) (Table 1). On the basis of anatomo-morphological features, in total in FAO IIa1, 22 specimens of the *Pseudoterranova* genus and 223 individuals *Anisakis* genus larvae were recorded in the muscle tissue of cod, while in FAO IIa2 these numbers were 243 and 130, respectively.

Table 1 Total body length, prevalence (P), intensity (I) and abundance (A) of infection of cod with anisakid larvae. N = number of fish examined.

Area	Sex	N	Length of fish (cm)			*Anisakis*			*Pseudoterranova*			Total		
			Mean	Min	Max	P (%)	I	A	P (%)	I	A	P (%)	I	A
FAO IIa1	males	44	80.43	63	92	86.36	4.74	4.09	13.64	3.33	0.45	86.36	5.26	4.55
	females	6	82.83	68	90	100.00	7.17	7.17	16.67	2.00	0.33	100.00	7.50	7.50
	sum	**50**	**80.72**	**63**	**92**	**88.00**	**5.07**	**4.46**	**14.00**	**3.14**	**0.44**	**88.00**	**5.57**	**4.90**
FAO IIa2	males	44	65.27	46	74	47.73	5.05	2.41	40.91	10.83	4.43	70.45	9.71	6.84
	females	12	66.08	52	70	83.33	2.40	2.00	33.33	12.00	4.00	91.67	6.55	6.00
	sum	**56**	**65.45**	**46**	**74**	**55.36**	**4.19**	**2.32**	**39.29**	**11.05**	**4.34**	**75.00**	**8.88**	**6.66**
Sum		**106**	**72.65**	**46**	**92**	**70.75**	**4.71**	**3.33**	**27.36**	**9.14**	**2.50**	**81.13**	**7.19**	**5.83**

Table 2 Molecular identification of subsample of parasites found in the musculature of the cod (*Gadus morhua*) from the North Atlantic.

Area	*Anisakis simplex* s.s.	*Pseudoterranova krabbei*	*Pseudoterranova decipiens*	*Pseudoterranova decipiens / Pseudoterranova krabbei*
FAO IIa1	10	6	5	
FAO IIa2	6	17	5	1
Sum	**16**	**23**	**10**	**1**

3.2. Molecular identification of Anisakidae parasites

A subsample of parasites was selected for molecular identification, taking into account the number of parasites representing each species found in each fishing area. Accordingly, 21 and 29 Anisakidae larvae were collected from the musculature of cod from FAO IIa1 and FAO IIa2, respectively, and were identified using molecular genetics tools. For FAO IIa1, larvae were identified as *A. simplex* (s.s.), *P. decipiens* (s.s.) and *P. krabbei*. For FAO IIa2, the same species were identified together with one hybrid of *P. decipiens* and *P. krabbei* (Table 2). In the case of hybrid form, a heterozygote pattern was detected in all four nucleotide positions located in ITS-1 rDNA, differentiating *P. krabbei* and *P. decipiens* (Table 3). Examples of DNA sequences (deposited in GenBank) of parasites found in cod caught in FAO IIa1 are *A. simplex* (accession no. MW367082), *P. decipiens* (MW367084) and *P. krabbei* (MW367086); and of those caught in FAO IIa2: *A. simplex* (MW367083), *P. decipiens* (MW367085), *P. krabbei* (MW367087), and a hybrid of *P. decipiens* and *P. krabbei* (MW367088).

3.3. Parasitological descriptor: prevalence of infection

The prevalence of infection with Anisakidae nematodes in both sampling areas was high, at 88% in FAO IIa1 and 75% in FAO IIa2, but the parasite fauna composition was different. In FAO IIa1, a lower prevalence of infection with *Pseudoterranova* of 14% was observed, while in FAO IIa2 this was 39%; the opposite trend was observed for prevalence of infection with *Anisakis*, which was higher in FAO IIa1 (88%) than in FAO IIa2 (55%).

3.4. Data analysis: GLM models of the prevalence of infection

The sampling area had a significant effect in the GLM models of the prevalence of infection with *Anisakis* spp. and *Pseudoterranova* spp. The modeled prevalence of *Anisakis* spp. was higher in FAO IIa1 than in FAO IIa2 ($p<0.001$). In contrast, the prevalence of *Pseudoterranova* spp. was higher in FAO IIa2 than in FAO IIa1 ($p=0.005$). The sex of the host was significant only for *Anisakis* spp., where modeled infection was higher in females than in males ($p=0.026$). The prevalence of cod infection with Anisakidae species and the effect of area on the prevalence model (with standard errors, S.E.) are presented in Figure 2. Parameter estimates are given in Table 4.

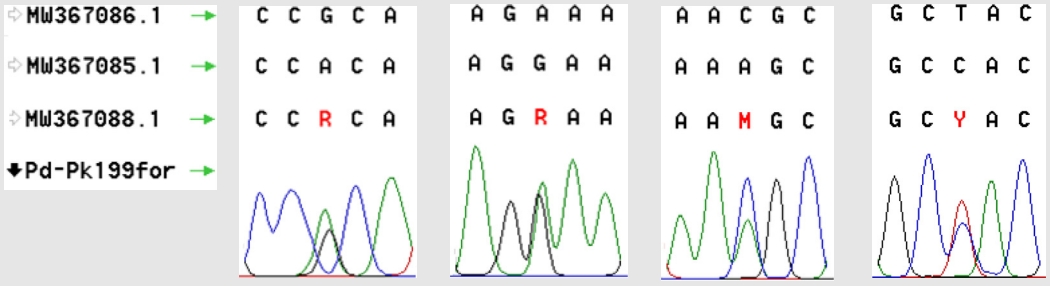
3.5. Parasitological descriptors: intensity and abundance of infection

Intensity of infection with Anisakidae parasites differed in both areas: for *Pseudoterranova* spp. up to 8 parasites per infected fish were found in FAO IIa1 (abundance 0.44) and up to 53 parasites per fish in FAO IIa2 (abundance 4.34); for *Anisakis* spp. there were, respectively, up to 30 parasites per fish (abundance 4.46) and up to 25 parasites per fish (abundance 2.32).

3.6. Distribution of parasites in cod fillets

The distribution of parasites in the various parts of the flesh (right *vs* left, anterior ventral *vs* dorsal *vs* caudal) was recorded for all larvae belonging to the genera *Anisakis* and *Pseudoterranova*. The presence of Anisakidae nematodes in different parts of the cod musculature was analysed. In cod caught in FAO IIa1, *Pseudoterranova* spp. were found in the

Table 3 Comparison of ITS-1 nucleotide sequences with a graphical presentation of diagnostic nucleotide positions differentiating *Pseudoterranova krabbei*, *P. decipiens* and hybrid specimen *P. krabbei* x *P. decipiens* obtained in this study.

		Alignment position according to *P. krabbei* MW367086			
Parasite species	Accession number	45	208	234	260
P. krabbei	MW367086	G	A	C	T
P. decipiens	MW367085	A	G	A	C
Hybrid specimen	MW367088	A/G	A/G	A/C	T/C
Graphical presentation of diagnostic nucleotide positions					

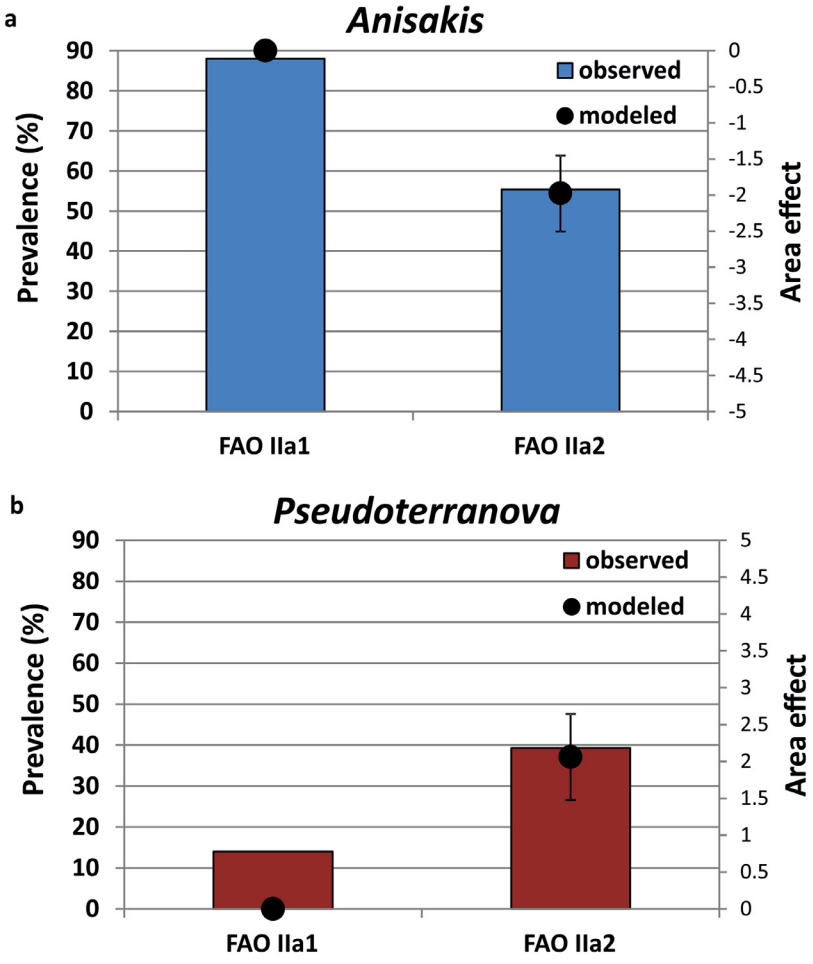


Figure 2 The prevalence of larval infections of *Anisakis* (a) and *Pseudoterranova* (b) genera in cod, relative to the effect of area included in the GLM model (with S.E.).

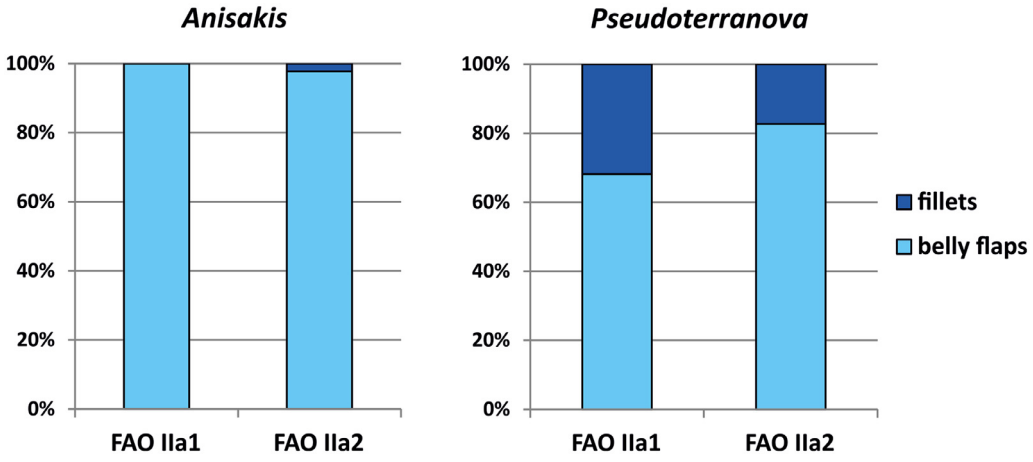


Figure 3 The distribution of *Anisakis* and *Pseudoterranova* in the flesh of cod.

Table 4 Parameter estimates (with s.e.) for models of the prevalence of infection with *Anisakis* and *Pseudoterranova* genera in cod (*Gadus morhua*) from the North Atlantic.

Anisakis				
Parameter		Estimate	S.E.	P
Intercept		1.88	0.44	<.001
Area	FAO IIa1	0.00	Aliased	
	FAO IIa2	−1.98	0.53	<.001
Sex	males	0.00	Aliased	
	females	1.81	0.81	0.026
Pseudoterranova				
Parameter		Estimate	S.E.	P
Intercept		−1.82	0.41	<.001
Area	FAO IIa1	0.00	Aliased	
	FAO IIa2	1.38	0.49	0.005

belly flaps of 5 fish and in 3 of these fish the parasite was also found in the dorsal fillet; we also recorded 2 fish with this parasite only in the dorsal fillet. Caudal fillet musculature was free of *Pseudoterranova* in these fish. In cod caught in FAO IIa2, *Pseudoterranova* spp. were observed in all tissues analysed with the following frequency: belly flaps, 17 fish; dorsal fillets, 11 fish; and caudal fillets, 7 fish. *Anisakis* spp. larvae were observed mainly in the belly flaps (in 44 fish from FAO IIa1 and in 31 fish from FAO IIa2) and only occasionally in caudal fillet (in two fish from FAO IIa2). Detailed information on the distribution of nematodes in each part of the cod musculature is presented in Table 6 and Figure 3.

The presence of nematodes with respect to the side of the musculature was also analysed. *Pseudoterranova* spp. were found in both left and right fillets with similar frequency in both sampling areas, while *Anisakis* spp. larvae were more often observed in the left belly flaps in both areas (Tables 5 and 6).

Wilcoxon matched-pairs tests revealed that the number of larvae representing both *Anisakis* and *Pseudoterranova* genera was significantly higher in belly flaps compared to fillets ($p < 0.001$ and $p=0.025$, respectively). *Anisakis* spp. larvae were found in significantly higher numbers ($p=0.047$) in left than right belly flaps. There were no significant differences in the lateral distribution of *Pseudoterranova* spp.

The intensity of infection differed depending on the location of the fish musculature. *Pseudoterranova* spp. mostly occurred in the belly flaps (15 parasites in fish from FAO IIa1 and 201 in cod from FAO IIa2), with fewer in the dorsal part (7 and 35, respectively) and only occasionally in caudal fillet (only 7 parasites in FAO IIa2 samples). *Anisakis* larvae were present almost exclusively in the belly flaps (223 parasites in FAO IIa1 and 127 in FAO IIa2), but occasionally in the caudal fillet (just 3 parasites in cod from FAO IIa2).

Coinfection with *Pseudoterranova* and *Anisakis* larvae was observed in the case of 18 fish (17%): 7 fish (14%) from FAO IIa1 and 11 fish (almost 20%) from FAO IIa2. Because most *Anisakis* specimens were present in the belly flaps, any coinfection was observed in that part of the fillet.

4. Discussion

Catches of Atlantic cod (*Gadus morhua*) from known spawning grounds are often heavily skewed towards males (Dean et al., 2014), which is also reflected in our sampling results. This might be explained by sex-specific behavior during spawning (Nordeide and Folstad, 2000): mature males aggregate on spawning grounds whereas females seem to be distributed peripherally or above the male aggregations (Morgan and Trippel, 1996; Nordeide, 1998).

Atlantic cod has an exceptionally rich and varied parasite fauna compared with most other species of marine fish (Hemmingsen and MacKenzie, 2001). It is one of the fish species that is most heavily infected with anisakid nematodes in the North Atlantic and its adjacent seas (Nadolna and Podolska, 2014; Mercken et al., 2020b,c).

Molecular identification revealed the presence of *Anisakis simplex* s.s., *Pseudoterranova decipiens* and *Pseudoterranova krabbei* in the muscle tissue of cod from the Norwegian Sea. These species were previously identified in cod by numerous authors (Boily and Marcogliese, 1995; Brattey and Bishop, 1992; Mattiucci and Nascetti, 2008; Mattiucci et al., 1997; McClelland and Marcogliese, 1994;

Table 5 Percentage of total *Anisakis* and *Pseudoterranova* genera detected in the various parts of the fillets of cod in the present study.

Genus/Area	Number of anisakids	Belly flaps		Fillets			
		Left	Right	Left dorsal	Right dorsal	Left caudal	Right caudal
Anisakis							
FAO IIa1	223	61.0	39.0	0.0	0.0	0.0	0.0
FAO IIa2	130	63.8	33.8	0.0	0.0	2.3	0.0
Sum	353	62.0	37.1	0.0	0.0	0.8	0.0
Pseudoterranova							
FAO IIa1	22	18.2	50.0	22.7	9.1	0.0	0.0
FAO IIa2	243	48.6	34.2	6.6	7.8	1.2	1.6
Sum	265	46.0	35.5	7.9	7.9	1.1	1.5

Table 6 Number of anisakids in relation to part of fillets.

Sample	Number of fish	Number of anisakids			Variance	S. D.	S. E.	P**
		Total	Min-Max*	Mean				
Anisakis								
Fillets	106	3	0—2	0.03	0.05	0.22	0.02	< 0.001
Flaps	106	350	0—30	3.30	27.13	5.21	0.51	
Left fillets	106	3	0—2	0.03	0.05	0.22	0.02	0.500
Right fillets	106	0	0—0	0	0	0	0	
Left flaps	106	219	0—22	2.07	14.84	3.85	0.37	0.037
Right flaps	106	131	0—8	1.24	3.36	1.83	0.18	
Pseudoterranova								
Fillets	106	49	0—6	0.46	1.47	1.21	0.12	0.025
Flaps	106	216	0—50	2.04	51.14	7.15	0.69	
Left fillets	106	24	0—3	0.23	0.39	0.62	0.06	1.000
Right fillets	106	25	0—3	0.24	0.51	0.71	0.07	
Left flaps	106	122	0—37	1.15	19.01	4.36	0.42	0.477
Right flaps	106	94	0—32	0.89	12.81	3.58	0.35	

* per fish
** Wilcoxon Matched Pairs Test

Strømnes and Andersen, 1998). Gay et al. (2018) revealed the presence of *A. pegreffii* in fillets of cod from the northern North Sea, but this species occurs rarely and was not detected during our studies. However, we did uncover one example of a hybrid of *P. decipiens* and *P. krabbei* in cod muscle from FAO IIa2. Although such a hybrid has previously been reported from this area, the host was not specified (Paggi et al., 1991).

We found a high prevalence of infection with Anisakidae nematodes in cod fillets in both study areas of the offshore waters of the Norwegian Sea. However, the parasite fauna composition was different in each area. In FAO IIa1, there was a lower prevalence of infection with *Pseudoterranova* spp. than in FAO IIa2, while in contrast the prevalence of infection with *Anisakis* spp. larvae was higher in FAO IIa1. The abundance and intensity of infection with Anisakidae parasites also differed in both areas: for *Pseudoterranova* the number of infected fish caught in FAO IIa2 was higher than in FAO IIa1, while for *Anisakis* the reverse was true.

Previous studies that focused on the presence, intensity and distribution of the different genera of ascaridoid nematodes in *G. morhua* from different regions of the Atlantic Ocean also showed that parasitological descriptors varied depending on the sampling area, for example, for various cod stocks in the North Atlantic in the 1970s (Platt, 1975) or more recently in the Barents Sea *vs* North Sea (Gay et al., 2018). The prevalence of cod infection with *Anisakis* spp. in fillets was only ~10% in the central North Sea (ICES area IVb) and ~85% in the northern North Sea (ICES area IVa), while in the Barents Sea (ICES area I) the prevalence was ~90% (Gay et al., 2018). Our sampling areas were situated between the northern North Sea and the Barents Sea, and therefore it might be expected that the level of infection would be similar, with a high value in the range 85-90%. Nevertheless, our results show differences between the two study areas, FAO IIa1 and FAO IIa2, where the prevalence of *Anisakis* spp. infection in cod fillet was 88% and 55%, respectively. In contrast, the prevalence of infection with *Pseudoterranova* spp. in cod fillets was 20—30% in the northern North Sea and less than 10% in the Barents Sea (Gay et al., 2018) using the UV-compression method, while in our studies the equivalent figures were 14% in FAO IIa1 and 39.29%

in FAO IIa2 using candling. The less-sensitive method used in our studies showed a higher infection than described by Gay et al. (2018) in neighboring areas and indicates that cod from FAO IIa2 generally harbor more *Pseudoterranova* in the musculature than those from other areas studied. Taking into account that candling generally underestimates infection levels compared with UV-compression, which is considered to be more effective (Levsen, 2005), the level of cod fillet infection might be even higher.

According to Gay et al. (2018) the abundance of *Anisakis* in fillet of cod from the northern North Sea was 6.12 ± 6.32 (intensity 7.17 ± 6.27; max 29) and from the Barents Sea 4.84 ± 6.56 (intensity 5.36 ± 6.69, max 65), while for *Pseudoterranova* the abundance values were 0.88 ± 4.41 (intensity 3.29 ± 8.09; max 46) and 0.03 ± 0.18 (intensity 1.00 ± 0.00, max 1), respectively. In our studies, the abundance of *Anisakis* in fillets was lower than recorded previously in the northern North Sea and Barents Sea: 4.46 in FAO IIa1 (maximum intensity 30 parasites per fish) and 2.32 in FAO IIa2 (maximum intensity 25 parasites per fish). In the case of *Pseudoterranova*, the abundance of 0.44 (max intensity 8 parasites per fish) in FAO IIa1 was lower than in the northern North Sea and higher than in the Barents Sea, but in FAO IIa2 the abundance was higher at 4.34 (max intensity 53 parasites per fish) than reported in neighboring regions by Gay et al. (2018). Again, our results might be an underestimated due to the method used (candling *vs* UV-compression).

The differences in the levels of infection between the areas analyzed in our studies and the neighboring regions studied previously (Gay et al., 2018) might be driven by several factors: as mentioned above, different methods were used; sampling was conducted in other areas of North Atlantic, and at a different time (year and season: spring in our study *vs* summer for Gay et al., 2018); the length distribution of the fish might differ between the analyzed samples. Fish length, sampling area, and sampling month or year are all known to influence the distribution of *Anisakis* in fish (Gay et al., 2018). Hemmingsen et al. (1995) reported maximum mean intensity and abundance of *A. simplex* in cod in a subarctic fjord, Balsfjord, in Norway in the autumn, while Strømnes and Andersen (2000) noticed a 'spring rise' of *A. simplex* third-stage larvae in some fish species in Norwegian waters. Natural changes in host and parasite populations should be also taken into account as an explanation of the observed differences.

There are several cod stocks in the eastern North Atlantic, and while all of them exhibit a common life history pattern, considerable regional variations exist in their recruitment, growth rate, age of maturity, migration patterns, food and spawning time (Rätz and Lloret, 2003). Living in different habitats results in different exposure to parasitic infection and each habitat brings unique possibilities for closing the parasite life cycle. The life cycles of nematodes of genera *Anisakis* and *Pseudoterranova* are broadly similar and involve crustaceans and many species of fish that serve as intermediate or paratenic hosts, with marine mammals as the final hosts (McClelland et al., 1990). Important cetacean hosts for *A. simplex* are the harbor porpoise, *Phocoena phocoena* (Herreras et al., 2004), the white-beaked dolphin, *Lagenorhynchus albirostris*, and the common bottlenose dolphin, *Tursiops truncatus* (Smith and Wootten, 1978). The common seal (harbour seal), *Phoca vitulina*, is considered to be the most important final host for *P. decipiens* (Aspholm et al., 1995). The mature parasites produce eggs, which are released with the faeces of the final host and lead to free swimming ensheathed larvae in the marine environment. The larvae undergo one or two moults before being ingested by invertebrates, mostly small crustaceans. Depending on the fishing area, suitable intermediate hosts may occur more or less abundantly and may still be unknown (Klimpel et al., 2004). Wootten and Waddell (1977) revealed that *Phocanema* (current name: *Pseudoterranova*) larvae were common in the musculature of cod from the west coast of Scotland and rare in fish from the central northern North Sea, while *Anisakis* larvae were abundant in cod and whiting from the offshore northern North Sea and less common in other areas. The reason for such variations was explained by differences in the geographical distribution of the invertebrate and vertebrate hosts of the parasites. Studies conducted by Wootten and Bron (2008) revealed that only *Anisakis* was found in fish feeding on planktonic crustaceans, while *Pseudoterranova* has a more benthic and inshore habitat, and is thus more likely to be found in bottom-feeding fish (e.g. cod). This might explain why during our studies more cod infected with *Anisakis* were found in sampling area FAO IIa1, which is located offshore.

However, experimental studies revealed that, in the eggs of *A. simplex* and *P. decipiens*, two moults occur during larval development (Køie et al., 1995); therefore, invasive L3 larvae are likely to be present in seawater and are available for invertebrates, fish and mammals. In this case, an intermediate host is not always needed in the life cycle of the parasite. If they are present, the intermediate hosts are eaten by a wide variety of transport or paratenic hosts, including fish. At this stage, many different life-cycle patterns may be observed (EFSA, 2010), but ultimately infected fish are eaten by the definitive host and the life cycle of the parasite is completed. Fish that live in a habitat where: 1) final hosts are numerous (and consequently there is large-scale transmission of parasite eggs into the environment); 2) the water conditions are favorable to parasite egg dispersion and survival; 3) appropriate intermediate hosts are present and numerous or not needed, are more exposed to parasitic infection. Factors that drive the behavior of the larvae in the paratenic host are not fully known. Most parasites remain in the visceral cavity of the fish or within the visceral organs, whereas in other cases parasites migrate to the musculature of the fish (Cipriani et al., 2016).

Our studies reveal that the distribution of parasites is not uniform in cod muscle tissue. Comparing the distribution of parasites in the flesh of cod using the Wilcoxon matched-pairs test revealed that significantly more *Anisakis* larvae ($p=0.037$) were localized in the left than the right belly flaps. These results are in accordance with the findings of Petrie et al. (2009), but these authors detected significantly more parasites ($p < 0.000001$) of both genera (*Anisakis* and *Pseudoterranova*) in the left than the right side of the body.

The dominance of *A. simplex* larvae (~58%) in the left-sided musculature was demonstrated during research conducted in the 1980s on Atlantic cod from Newfoundland and Labrador (Brattey and Bishop, 1992). An asymmetric distribution of *Anisakis* and *Pseudoterranova* (more larvae

in the left body musculature) was also reported for Atlantic cod by Smith and Hemmingsen (2003). Similarly in all species of fish examined by Petrie et al. (2009), except mackerel, there were significantly greater numbers of larval anisakids in flesh from the left side of the fish. Petrie et al. (2009) speculated that this was because the disposition of organs within the body cavity might to some extent obstruct the path of migrating worms into the right-sided musculature. Smith and Hemmingsen (2003) also proposed that the asymmetrical arrangement of internal organs in the cod (including the digestive tract) might result in asymmetrical distribution of *Anisakis* larvae in the musculature of the fish. However, for fish sampled in the northern North Sea, the right-sided fillets were slightly more infected with Anisakidae parasites than the left, whereas the opposite was observed for fish sampled in the Barents Sea (Gay et al., 2018).

In our studies the majority of larvae were located in the belly flaps (99% of *Anisakis* larvae and 82% of *Pseudoterranova*); indeed, the number of *Anisakis* and *Pseudoterranova* individuals was significantly higher in belly flaps than in fillets ($p < 0.001$ and $p = 0.025$, respectively). Similar results were obtained by Petrie et al. (2009) for *Anisakis*: significantly more larvae ($p < 0.000001$) were detected in the belly flaps compared to the fillets; however, for *Pseudoterranova,* there was no significant difference in the distribution between belly flaps and fillets ($p = 0.396$). In our studies, in the dorsal parts of the cod musculature, only *Pseudoterranova* larvae were present. Similarly the majority of *A. simplex* larvae (∼95%) occurred in the flesh surrounding the body cavity (Brattey and Bishop, 1992). The prevalence and abundance of the parasites in the anterior part of the fillet were always greater than those for the posterior part (Gay et al., 2018; Novotny and Uzmann, 1960). According to Petrie et al. (2009) significantly more *A. simplex* were found in the belly flaps than in the fillets in all fish species. *P. decipiens* was significantly more abundant in the fillets of monkfish, but there was no difference between the numbers of this parasite in belly flaps and fillets in cod. The presence of the majority of larvae in the belly flaps might be explained by the short distance that larvae need to migrate from the digestive tract.

We observed differences in distribution between genera: *Anisakis* were present mainly in the belly flaps, while *Pseudoterranova* were present (with descending frequency) in belly flaps, dorsal fillet and caudal fillet. An important factor influencing the migratory distance and encapsulation site of *A. simplex* L3 larvae might be the special attributes of the particular microhabitats encountered by infecting larvae within their hosts, such as the availability of exploitable nutrients (Strømnes and Andersen, 1998). This suggests that the distribution of larvae might be driven by the diversity of the biochemical environment within the host. One of the most important drivers of parasitic migration seems to be lipids, which are very important for parasite survival (Jordanova et al., 2005). Nematodes must acquire lipids, mainly fatty acids and sterols, because they are not biosynthesized from scratch (Chitwood and Lusby, 1991; Köhler and Voigt, 1988). This hypothesis has been confirmed by the identification of lipids in the cuticle of the parasitic nematode *A. simplex* and the somatic tissues of the Atlantic cod (Mika et al., 2010). *A. simplex* L3 larvae have a preference for host tissue with high lipid content (Strømnes and Andersen, 1998, 2003). In experimental studies, it was shown that in a microhabitat containing few or no lipids, the L3 larvae were apparently stimulated to increase their mobility and actively seek new and possibly better microhabitats (Strømnes, 2014).

Parasitic infections affect almost all fish species and pose a serious problem for the fishing industry in many countries. Therefore, updated knowledge about the level of infection with zoonotic parasites is important, particularly in the case of fish species intended for human consumption. It is helpful to avoid catches in areas where the level of parasitic infection is high, which minimizes the risk to consumers health (Rahmati et al., 2021a,b) and delivers the safest raw material for fish processing. The Norwegian Sea is an important cod fishing ground and Norway is a key player in the European export of cod (as a wide range of products) worldwide; therefore our results may be of commercial as well as scientific value. Recently, the northeast Atlantic has been identified as a high-risk hot spot for the presence of *Anisakis* spp. in Gadidae fish, posing a potential hazard of anisakiosis (Rahmati et al., 2021a). It is worth emphasizing that levels of fish infection vary both temporally and spatially and should be regularly monitored.

Some research suggests that populations of anisakids are shifting over time. Thus, Molina-Fernández et al. (2015) and Rahmati et al. (2021a) demonstrated that the prevalence of *Anisakis* infection in fish may vary even between geographically close locations. Fiorenza et al. (2020) estimated that on average *Anisakis* spp. abundance increased more than 100 times from 1978 to 2015, with the most affected area being the northeastern Atlantic. The elimination of zoonotic nematodes from fish is essential in the context of ensuring the safety and quality of fish products. The phenomenon of accumulation of anisakids in certain parts of the fillet (belly flaps) allows for the elimination of most parasites by cutting off the abdominal parts of the fillet. This simple procedure minimizes the risk of human infection by accidental consumption of nematode larvae and improves the aesthetic value of the product. However, further treatment should be used to at least kill the larvae that may remain in the other parts of the fillet.

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