Distribution of ascaridoid nematodes (Nematoda: Chromadorea: Ascaridoidea) in fish from the Barents Sea

by

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Abstract

The Ascaridoidea are parasites with heteroxenous life cycles. The study shows that fish can be paratenic, intermediate, or final hosts for parasites, and parasitic fauna reflects the feeding behavior of the hosts. Each species of parasites has also different environmental preferences and host specificity. Parasitic nematodes of fish representing Pleuronectidae, Gadidae, Sebastidae, and Macrouridae were studied. Worms were collected separately from different infection sites: stomach, intestine, liver and body cavity. Nematodes were identified using both morphological and molecular methods (PCR-RFLP). Six nematode species were recorded: *Anisakis simplex* s.s., *Contracaecum osculatum* A, B, and *C. osculatum* C (s.s.), *Hysterothylacium aduncum* and *Pseudoterranova bulbosa*. *Anisakis simplex* s.s. was the most numerous nematode species of all catches combined. Differences in parasite species composition were related to the depth and location of sampling areas. In the fish from deep waters, the abundance of *A. simplex* s.s. decreased compared to fish from shallow waters and *P. bulbosa* was the dominant species. Ascaridoid species have specific preferences regarding the impact on various internal organs of fish, which is reflected in their abundance. The presence of Ascaridoidea in the Barents Sea is associated with the distribution of hosts and varying food preferences related to the age of fish. The abundance of parasites varied between different host species.

Key words: Ascaridoidea, Barents Sea, nematodes, parasites, Greenland halibut

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Introduction

The Barents Sea is a marginal shelf sea of the Arctic Ocean with an average depth of 230 m. The physical conditions in the region of the Norwegian Sea and the Barents Sea are governed by the Atlantic water inflow through the Faroe–Shetland Channel. Two main branches of the Atlantic Current create two separate ecosystems, one in the North Sea and one in the region of the Norwegian Sea and the Barents Sea. In the latter area, the interface between the inflow of the warm Atlantic water and the cold Arctic water forms excellent conditions for two highly productive areas: the first one in the Norwegian Sea along the Polar front and the second one in the marginal ice zone of the Barents Sea. The rich production in the latter area is connected with the movement of the ice edge (Volkov et al. 2011).

Approximately 150 fish species occur in the Barents Sea, including some of the world's biggest fish stocks of the Atlantic cod *Gadus morhua* Linnaeus, 1758; haddock *Melanogrammus aeglefinus* (Linnaeus, 1758); saithe *Pollachius virens* (Linnaeus, 1758) and Greenland halibut *Reinhardtius hippoglossoides* (Walbaum, 1792). These economically important fish species are hosts for many parasites, including nematodes of the superfamily of Ascaridoidea. Members of Ascaridoidea (Nematoda: Chromadorea) are widespread around the world (Kuhn et al. 2011), not only in aquatic but also terrestrial environments and include the following families: Anisakidae, Raphidascarididae, Ascarididae, Heterocheilidae, Quimperiidae, and Toxocaridae. The Anisakidae family includes such species as *Anisakis simplex* s.l. (Rudolphi, 1809), *Contracaecum osculatum* s.l. (Rudolphi, 1802) and *Pseudoterranova bulbosa* (Cobb, 1888), which live in the alimentary tract of the vertebrate host (birds, mammals). Whereas *Hysterothylacium aduncum* (Rudolphi, 1802), a member of the Raphidascarididae family, ends its life cycle in a fish. Their life cycles include free-living larval stages and paratenic and intermediate hosts representing both invertebrates and vertebrates such as crustaceans and fish, respectively (Bristow & Berland 1992; McClelland 1995; Marcogliese 1996; Klimpel et al. 2004; Klimpel & Rückert 2005). Nevertheless, each parasite species has its own depth preferences, which determine the spectrum of paratenic and intermediate hosts (Kijewska et al. 2009).

Anisakid nematodes are considered common parasites of a wide range of host species with a worldwide geographical distribution. Molecular analyses have shown that these morphospecies (like *A. simplex* s.l., *P. decipiens* s.l., or *C. osculatum* s.l.) consist of groups of sibling species that show different host preferences and geographical distributions. Genetic methods are necessary for their identification, because different species in a species complex are morphologically very similar or identical. For example, the *C. osculatum* species complex consists of five sibling species: *C. osculatum* A, *C. osculatum* B, and *C. osculatum* C (s.s.) found in the Arctic–Boreal region (Nascetti et al. 1993), and *C. osculatum* D and *C. osculatum* E found in the Antarctic region (Orecchia et al. 1994). These species are morphologically very similar, and the identification based solely on morphological characters is unreliable for both larval and adult stages (Zhu et al. 2000).

The main objective of our fishery research expedition conducted on the Barents Sea was to determine: fish species composition in hauls, fishing efficiency (CPUE) and the biological structure of the Greenland halibut and the main species co-occurring during fishing. These studies were carried out due to the status of the Greenland halibut, which is listed on the Greenpeace seafood red list and its stocks in the Barents Sea are overfished. Scientific data on the biology and ecology of *R. hippoglossoides*, combined with information on the commercial trawling efficiency and ichthyological analysis of caught halibut, are very important during the stock rebuilding and when anticipating the future reopening of the fishery. Data on biological features of the Greenland halibut resource status and trends of its change were collected and evaluated during the research expedition. The prepared report was sent to the Norwegian Directorate of Fisheries and other fishery organizations from Norway and Lithuania to help with planning of sustainable fishery of *R. hippoglossoides* in the Barents Sea.

During the cruise, we carried out parasitological examination as an additional activity and we focused on parasitic nematodes as potentially pathogenic for humans. Parasites from other taxonomic groups (Cestoda, Copepoda, Trematoda, Acanthocephala) were also isolated, and they were used as a reference material for students to learn different preservation and collection techniques. Not all of them were collected systematically as the ascaridoids and the data are not published. Due to the very bad weather and strong waves at the sea, it was not possible to isolate parasites invisible to the naked eye.

The objective of this work was to determine the distribution patterns of parasitic nematodes belonging to the superfamily of Ascaridoidea in relation to the depth and geographical distribution. It continues our previous research in this area (see Karpiej et al. 2013). The presented study helps to explain the distribution of larval ascaridoids in intermediate and paratenic

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hosts and provides further information on the general life cycle strategy of this abundant and economically important group of parasites of sea fish in the pelagic and deep-water environment of the Barents Sea. The estimation of the infection rate and especially the occurrence of parasites pathogenic to humans in the economically important fish as well as their location in the host is useful for fish processing and has a practical aspect.

Materials and methods

The survey was carried out from 22 October to 13 November 2011 and was performed along the slope of the continental shelf from the south of Svalbard and the south of Bear Island, within ICES fishing area IIb2, the Barents Sea (Figure 1). Samples were collected at a depth range of 454–959 m. The mesh size of the net was 145–170 mm.

High selectivity of the net used by the trawler explains the small number (about 3.8% of the total catch) of fish representing the by-catch. The size of the Greenland halibut and of most other fish species exceeded 30 cm due to the big mesh size (145–170 mm). The depth stations were classified as "shallow" or "deep" according to the median depth of all hauls studied. "Shallow" included the continental shelf and shelf break waters up to 660 m, and "deep" slopes were more than 660 m deep. The suggested differentiation was based on the presence of different by-catch species at different depths, e.g. greater argentine *Argentina silus* (Ascanius, 1775), Atlantic cod, beaked redfish *Sebastes mentella* Travin, 1951 and spotted wolfish *Anarhichas minor* Olafsen, 1772 were present at "shallow" stations, while roughhead grenadier *Macrourus berglax* Lacepède, 1801 was present at "deep" stations. Fishing efficiency (CPUE) in the northern area (above the 75th parallel north) was about three times lower than in the southern area (below the 75th parallel north).

The Greenland halibut was the main species in hauls, while beaked redfish, Atlantic cod, roughhead grenadier and American plaice *Hippoglossoides*
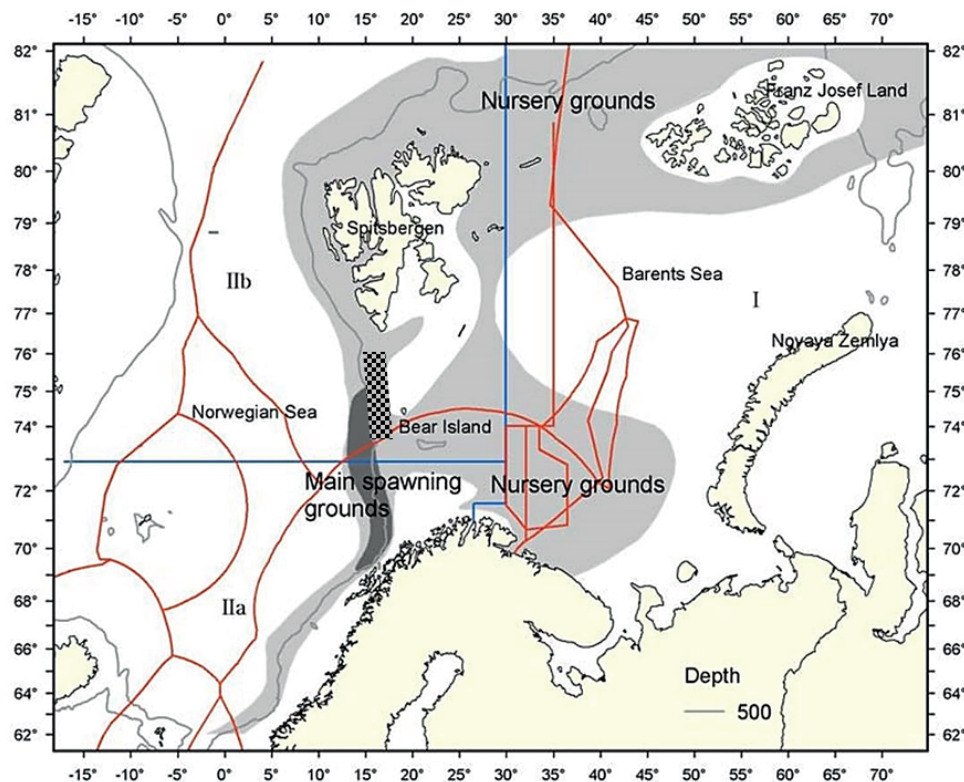

Figure 1

Map of the sampling area on the Barents Sea (checkerboard pattern). Fish were sampled along the shelf from Bear Island to South Svalbard. Greenland halibut nursery and main spawning grounds are marked. National economic zones, the disputed border areas between Norway and Russian (i.e. the Gray Zone), the international Loophole and the ICES areas are shown (Nedreaas & Smirnov, modified).

platessoides (Fabricius, 1780) were the main species in by-catch, representing 3.25% of the total catch and 86.18% of the total by-catch, respectively. Of all the examined fish, *Reinhardtius hippoglossoides* was the most numerous species occurring at all depths and covering almost the full spectrum of the total body length (TL). The total length of the Greenland halibut ranged from 28 to 86 cm and the weight – from 150 to 8310 g. The percentage of halibut in the overall fish catch was 96.79%. From 10 to 102 individuals of the Greenland halibut and all fish from by-catch were collected and measured from hauls represent-ing a given depth and a given geographic location. At least one fish specimen from each fish length class (as defined by Karpiej et al. 2013) was selected for the analysis. As a result, 1–21 fishes from each haul were taken for parasitological analysis. Nematodes were collected separately from the stomach, intestine, liver and the body cavity (including gonads and intestinal mesentery). Three regions of the digestive tract were sampled separately in Gadiformes: stomach, pyloric caeca and intestine. The muscles were randomly checked for the presence of nematodes.

Parasitological analysis

Nematodes were isolated from the host's tissue, washed in deionized water, and preserved in 70% ethanol. Each parasite was divided into 3 parts. The anterior and posterior parts were preserved in 70% ethanol with 5% glycerol. Nematodes were cleared by evaporation of the mixture of ethanol and glycerin, mounted and examined under a light microscope. The identification of worms was based on key morphological characters, i.e. the structure of labiae and ventriculus, the tail shape, the absence or the presence of the mucron, and the position of the excretory pore (Fagerholm 1991). The middle sections of specimens examined were fixed in 70% ethanol and used for molecular identification. Parasites that were excessively damaged for microscopic observations, or difficult to identify using morphological characters, were identified using PCR-RFLP. The same procedure was applied to 25% of nematodes to confirm the morphological identification (Zhu et al. 2000; Kijewska et al. 2002; Dzido 2011).

DNA isolation and amplification

After the morphological identification, the nematode DNA was isolated as described by Hoarau et al. (2002). Due to the small abundance of *Pseudoterranova decipiens* s.l., all specimens of this species were identified using molecular characters.

Because of their large numbers, 27% of *Contracaecum osculatum* s.l., 5% of *Hysterothylacium aduncum*, and 2% of *Anisakis simplex* were identified using PCR-RFLP. The low proportion of *A. simplex* examined based on DNA was supported by the results of our previous study in the Barents Sea (Karpiej et al. 2013), during which only a single species representing the *A. simplex* complex was found in the study area, namely *A. simplex* s.s. In total, parasites from 74 fish individuals (48 Greenland halibut and 26 specimens of by-catch species) were identified using molecular characters.

Amplification of the rDNA marker was carried out according to Zhu et al. (1998). PCR products were separated electrophoretically on 1% agarose gels and visualized by staining with ethidium bromide. The PCR products (ITS1-5.8S-ITS2 fragment of rDNA) were digested with AluI, RsaI, TaqI, HhaI, HinfI, and BsuRI restriction enzymes (Fermentas, Vilnius, Lithuania). The sibling species of the *C. osculatum* complex were determined by digesting the rDNA region with RsaI and XbaI (Fermentas, Vilnius, Lithuania) separately (Table 1). The products were separated electrophore-tically on 4% agarose gels and visualized by staining with ethidium bromide (Sambrook et al. 1989). The pUC Mix Marker (0.5 µg µl⁻¹; Marker 8, Fermentas) was used as a molecular weight marker. The obtained patterns were compared using a molecular key (Zhu et al. 2000; Kijewska et al. 2002; Dzido 2011).

Table 1

Digestion patterns given as approximate lengths of the main DNA fragments (bp), which characterize three sibling species of *C. osculatum* s.l. The missing fragments are represented by short and not diagnostic fragments (total length).

Contracaecum species	RsaI	XbaI
C. osculatum A	390 + 260	960
C. osculatum B	390 + 260	730 + 230
C. osculatum C	390 + 260 + 240	960

Statistical methods and basic parameters

All fish were divided into the following groups: Greenland halibut (overall), Greenland halibut ("deep" water samples), Greenland halibut ("shallow" water samples), and by-catch species. The prevalence and mean abundance (Bush et al. 1997) were calculated for each group separately. All statistical procedures were performed using STATISTICA 12.0 (StatSoft, USA). The non-parametric test (Spearman's rank correlation) was

used because the distribution of parasites deviated from the normal one. The infection abundance–fish length covariance and correlation were examined as well. In addition, the data were tested for differences in the infection intensity and abundance of parasites between the two areas sampled (the northern and the southern one) and two depth categories surveyed ("deep" and "shallow").

Results

A total of 6755 nematodes were collected from 149 specimens of five fish species. The majority of nematodes were *Anisakis simplex* s.l. (4910 individuals). Less numerous were: the *Contracaecum osculatum* species complex (1402), *Hysterothylacium aduncum* (333) and *Pseudoterranova decipiens* s.l. (110). Molecular identification revealed six ascaridoid species: *A. simplex* s.s., *C. osculatum* A, B, and C, *H. aduncum*, and *P. bulbosa*. The majority of nematodes were in the 3rd larval stage except for *H. aduncum,* represented by the 4th larval and adult stages.

Analysis of the distribution of parasites based on the characteristics of their hosts showed that the abundance of nematode species is related to the place of living and preying of their hosts. Demersal species, like American plaice, were characterized by very high abundance of *P. bulbosa,* contrary to benthopelagic fish, like Atlantic cod and Greenland halibut, which contained relatively smaller numbers of this nematode (Table 2). *Anisakis simplex* s.s. was dominant in all the studied fish species (except American plaice) with the abundance higher than the abundance of other nematodes (Table 2). The abundance calculated for all parasites from fish species revealed the following differences between fish collected from different areas (south/north) and depths ("deep"/"shallow"). *Anisakis simplex* s.s. and *H. aduncum* dominated with the higher abundance in the south and "shallow" waters, while *P. bulbosa* was more abundant in the northern "deep" waters. The abundance of *C. osculatum* s.l. did not change between the studied depths and locations (Figure 2).

A total of 48 specimens of Greenland halibut from different locations and depths were checked for the presence of *C. osculatum* s.l. and all parasites were identified using molecular characters. A total of 407 parasites were identified using this approach, including 304 specimens identified as *C. osculatum* A, B, and C (s.s.). One specimen of hybrid origin between two species – *C. osculatum* B and *C. osculatum* C – was found; its identity was confirmed by triple checking of RFLP according to patterns specified in Table 1. One specimen belonged to *C. osculatum* C. Nematodes representing *C. osculatum* A were definitely more abundant (on average 5.2) than *C. osculatum* B (1.2), and their distribution in relation to the depth and location was different. The abundance of *C. osculatum* B was two times higher in southern "shallow" waters

Table 2

Abundance and prevalence of ascaridoids depending on the host and haul depth for *R. hippoglossoides*

	N	All species	*A. simplex* s.s.	*C. osculatum*	*H. aduncum*	*P. bulbosa*
Abundance						
All fish	149	48.7	33.8	9.4	2.2	0.7
R. hippoglossoides all	110	52.0	36.1	11.2	< 0.1	0.5
"shallow"	49		59.2	10.7	< 0.1	0.3
"deep"	61		16.2	11.8	< 0.1	0.6
S. mentella	19	10.2	9.0	0.2	1.0	0.0
M. berglax	7	5.2	9.0	0.3	0.3	0.1
H. platessoides	6	29.0	7.5	12.8	0.0	8.7
G. morhua	7	192.3	126.7	13.0	51.5	1.2
Prevalence (%)						
All fish	149	91	80	82	12	15
R. hippoglossoides all	110	100	81	97	3	14
"shallow"	49		88	96	2	10
"deep"	61		77	100	3	16
S. mentella	19	95	74	11	42	0
M. berglax	7	86	71	14	14	14
H. platessoides	6	100	67	83	0	67
G. morhua	7	100	100	100	100	50

N – the number of analyzed fish
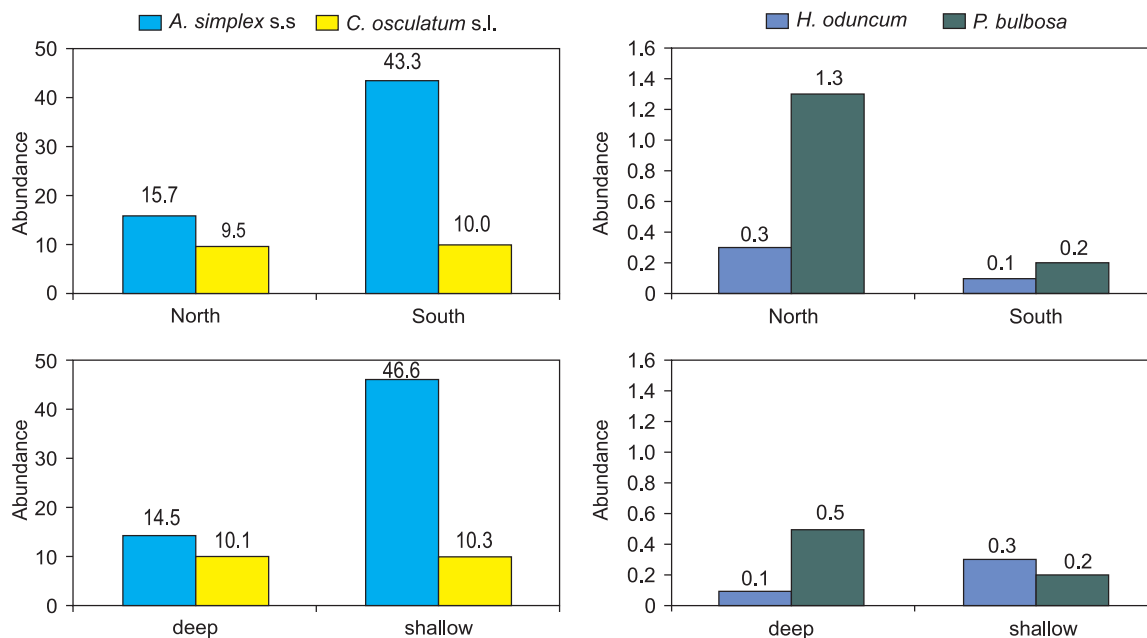
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Figure 2

Abundance calculated for nematode species sampled from fish species identified in both the northern and southern groups (*R. hippoglossoides*, *S. mentella* and *H. platessoides*) and at both depths (*R. hippoglossoides*, *S. mentella* and *M. berglax*). Values for nematode species are shown on separate graphs due to the large difference between the abundance level of the two common species and two much less abundant species.

than in northern "shallow" waters (1.9 vs. 0.6), while the abundance of *C. osculatum* A remained unchanged (5.4 vs. 5.8, respectively) (Figure 3). In other fish species, *C. osculatum* A also dominated compared to *C. osculatum* B (11 individuals vs. 3 individuals).

The number of all parasites found in Greenland halibut increased with the body length of fish (moderate Spearman's rank correlation coefficient $r_s = 0.655$, $p < 0.05$). We also observed positive

covariance between the fish length and the number of *A. simplex* s.l. The host length-class distribution among the nematodes of the *Contracaecum osculatum* complex indicated differences between *C. osculatum* A and B. The abundance of *C. osculatum* B slightly increased with the fish length, while the abundance of *C. osculatum* A rapidly decreased in the middle length class and significantly increased again in fish longer than 60 cm (Figure 4). The abundance of other nematodes was not correlated with the fish total length (TL).

The nematodes found showed their preferences depending on the host species and the infected organs. The most abundant species, *Anisakis simplex* s.s., was found predominantly in liver, stomach and intestine in similar proportions (29.5%, 29.1%, and 28.6%, respectively). *Hysterothylacium aduncum* was the most abundant in the intestine and stomach (45.3% and 38.1%, respectively), whereas *C. osculatum* s.l. clearly preferred the intestine (57.0%). *Pseudoterranova bulbosa* preferred liver, where 45.5% of all individuals representing this species were found (Figure 5).

In Greenland halibut, the largest number of *C. osculatum* B was found in the intestine (72.2%), while only 16.7% and 7.4% of specimens were recorded in the stomach and liver, respectively. Parasites found
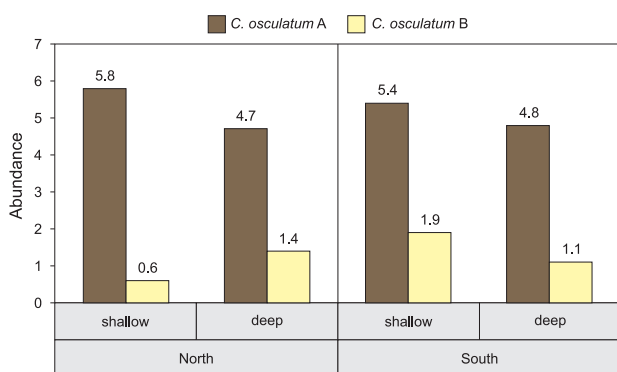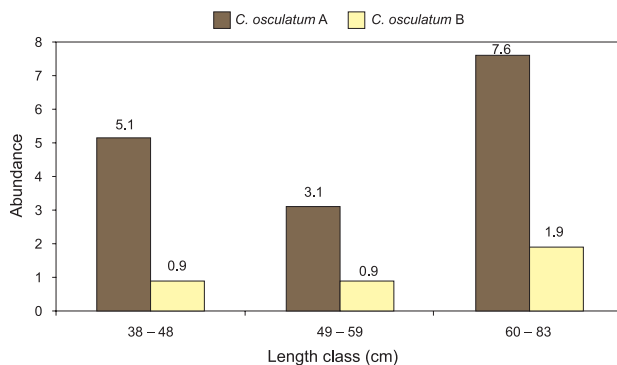

Figure 3

Abundance of *C. osculatum* A and B from Greenland halibut depending on the location and depth

Figure 4

Abundance of *C. osculatum* A and B per length class of *R. hippoglossoides*

in the body cavity represented 3.7% of their total number. This disproportion was less clear in the case of *C. osculatum* A, with 61.1% of nematodes found in the intestine, 28.2% in the liver, 9.4% in the stomach and 1.3% in the body cavity. Moreover, the largest number of *A. simplex* s.s. was observed in the liver of Greenland halibut from "deep" waters, while the ratios between the liver, stomach and intestines were similar in "shallow" waters (Table 3).

Discussion

Assemblages of sea fish harbor a significant number of larval stages of helminth parasite species that use fish as intermediate or paratenic hosts. It is important to study the biodiversity and relative abundance of Ascaridoidea taking also into account the factors that may affect these parameters. We have attempted to analyze the distribution of ascaridoids in teleost fish from the Barents Sea and evaluated the effects of several host traits (body size, depth range, and geographical distribution) on the richness and

abundance of larval helminths, combined with the literature data on fish behavior and feeding habits. Moreover, research of Greenland halibut is all the more important because the species is on the Greenpeace seafood red list.

Anisakis simplex s.s.

The most abundant ascaridoid in the Barents Sea was *A. simplex,* present in all fish species examined. The prevalence of this parasite in Greenland halibut was lower than that observed by Wierzbicka (1992) (81.1% vs. 95.3%), which could be caused by different seasons of sampling performed in the Barents Sea (autumn vs. spring). Michalsen & Nedreaas (1998) observed differences in the Greenland halibut stomach content depending on the sampling season: blue whiting, *Micromesistius poutassou* (Risso, 1827), the squid *Gonatus fabricii* (Lichtenstein, 1818), unidentified fish, herring, *Clupea harengus* Linnaeus, 1758, and Greenland halibut dominated in autumn, while *G. fabricii*, heads of Greenland halibut as offal from fisheries, shrimps, and cod remains (possibly offal) dominated in spring. The comparison of data from the same region with a five-year interval (Karpiej et al. 2013 vs. recently reported results) enables us to assume that the Greenland halibut infection with *A. simplex* s.s. in this part of the Barents Sea has been slightly declining (prevalence 86.2% and abundance 39 according to Karpiej et al. 2013 vs. 81.1% and 36 determined in this work). Dwyer et al. (2010) suggested that many factors such as year, length, and sex may cause a different composition of the halibut diet. Moreover, miscellaneous items in the halibut diet during different seasons may determine the parasite fauna of this species.

It is very likely that the diet of halibut depends on many factors, including the depth and the region, which in turn can affect the anisakid infection level. In the Greenland halibut caught in the "shallow" waters,

Table 3

Percentage of each ascaridoid species in organs of Greenland halibut by catch depth. The number of fish is given in Table 2.

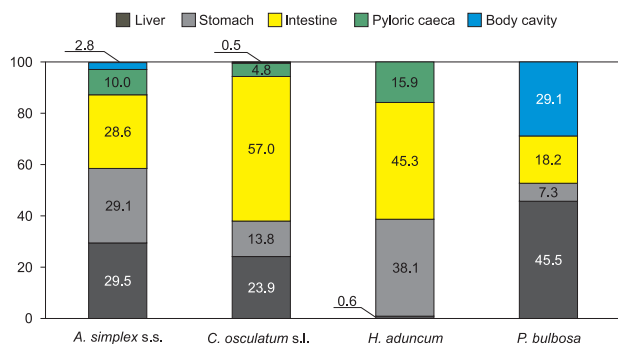| Nematode species		*A. simplex* s.s.	*C. osculatum* s.l.	*H. aduncum*	*P. bulbosa*
Liver	"deep"	41.6	26.0	33.3	60.0
	"shallow"	29.7	27.0	0	86.7
Stomach	"deep"	33.7	17.5	66.7	20.0
	"shallow"	32.5	11.2	100.0	0
Intestine	"deep"	23.7	56.4	0	20.0
	"shallow"	33.9	61.3	0	13.3
Body cavity	"deep"	1.1	0	0	0
	"shallow"	3.9	0.4	0	0

Figure 5

Percentage of nematode species across the main locations in their host organisms. Calculations were carried out for all host species.

the prevalence of *A. simplex* s.s. was only slightly different (10%) compared to the halibut caught in the "deep" waters of the Barents Sea. On the other hand, the abundance of *A. simplex* s.s. was over three times higher in the group of halibut from the "shallow" waters (Table 2) compared to that from the "deep" waters. This observation is consistent with the fact that intermediate hosts of *A. simplex* s.s. are primarily pelagic organisms. According to Dalpadado & Skjoldal (1996), the distribution of dominant species of Arctic krill (*Thysanoessa longicaudata* and *T. inermis*) – the first intermediate hosts for *A. simplex* (see Smith 1971), shows their concentration south of the Svalbard Bank (below 76°N). This pattern of distribution affects the accumulation of parasites in secondary intermediate and paratenic hosts, which are the prey for bigger piscivorous fish. Moreover, a significant increase in the abundance of *A. simplex* in relation to the increased length of Greenland halibut suggests that this parasite is accumulated also in *Reinhardtius hippoglossoides*, known to change its diet with the age and size of the fish (Karpiej et al. 2013). The smaller fish acquire *A. simplex* s.l. by consuming crustaceans and cephalopods, which are the initial paratenic hosts of this nematode. Subsequently, halibut individuals gradually consume more fish as they grow, i.e. paratenic hosts providing anisakid larvae (Karpiej et al. 2013). Moreover, large individuals of Greenland halibut, generally distributed at greater depths than the small ones (Bowering & Chumakov 1989), had different prey items in the diet, with a higher contribution of offal and larger fish.

This anisakid species was dominant in not-benthic species of fish (prevalence of 100% in *G. morhua* and over 70% in other species). This observation is valuable from a practical aspect – *A. simplex* s.s. and other

anisakids pose a consumer health hazard if accidentally eaten alive. People should be aware that the consumption of some fish species is associated with the risk of anisakidosis. Moreover, this anisakid may adversely affect the product quality if abundant in the raw material, which may result in economic losses.

Pseudoterranova bulbosa

In the Northern Hemisphere, adult *P. bulbosa* occurs in the bearded seal in both the North Atlantic and Pacific Oceans, while its larval stages infect the flatfish such as the American plaice, Greenland halibut, Atlantic halibut *Hippoglossus hippoglossus* (Linnaeus, 1758), fourhorn sculpin *Myoxocephalus quadricornis* (Linnaeus, 1758) and rarely the Pacific cod *Gadus macrocephalus* Tilesius, 1810 (see Bristow & Berland 1992; Brattey & Davidson 1996; Mattiucci et al. 1998). The hosts identified so far for *P. bulbosa* are mostly benthic and demersal, with the exception of *G. morhua,* which is benthopelagic (Paggi et al. 1991). This supports the hypothesis that *P. decipiens* s.l. in the Northern Hemisphere has a benthic life cycle with larvae not being able to swim (Bristow & Berland 1992). Moreover, the results of the presented study confirm that, contrary to *P. decipiens* A and B, the intermediate stage of *P. bulbosa* occurs primarily in the liver of the American plaice (*H. platessoides*) and the Greenland halibut (Paggi et al. 1991). The distribution ranges and food preferences of these two pleuronectids overlap (Bristow & Berland 1992). Of all the fish studied from the Barents Sea, the American plaice, which is a strongly demersal species, was characterized by the highest prevalence and abundance of *P. bulbosa* (Table 2). Nevertheless, the observed values were lower than the prevalence and abundance values calculated by Martell & McClelland (1995) for *H. platessoides* sampled on Sable Island Bank. The Atlantic cod was the second species of fish where *P. bulbosa* is very frequently found. In this fish, the prevalence of the above worm reached 50.0%, which is much higher than observed for *P. decipiens* s.l. in *G. morhua* by Sobecka et al. (2011) in the area of Bear Island. Moreover, the Atlantic cod was the most heavily parasitized species among all fish examined during this study, with the infection prevalence equal to 100% for *A. simplex* s.s., the *C. osculatum* complex, and *H. aduncum*. The above-mentioned observation could be an effect of continuous accumulation of parasites in the Atlantic cod and ontogenetic shift in their diet (Link et al. 2009). The mean length of the cod analyzed by Sobecka et al. (2011) was shorter than the length determined for specimens examined in this study (weight: 3478 vs. 4478 g and length: 738 vs. 420 mm), and the study

carried out by Sobecka et al. (2011) was limited to 300 m, while our study – to 660 m. Intensification of parasitic infections may depend on many factors, but most likely it represents a continuous accumulation of parasites in larger cod (Mouritsen et al. 2010).

Hysterothylacium aduncum

A similar situation applies to *H. aduncum* whose larvae were found in many fish species, including capelin *Mallotus villosus* (Müller, 1776) (see Levsen et al. 2016) and a wide spectrum of other fish species (Balbuena et al. 1998; 2000). These species probably transmit *H. aduncum* directly to *Reinhardtius hipopoglossoides* or indirectly to piscivorous fish eaten by Greenland halibut. The list of its prey items contains more than 20 species of fish belonging to Gadiformes, Clupeiformes, Scorpaeniformes, etc. (Froese & Pauly 2017). Infections with *H. aduncum* in the Barents Sea are common and the parasite usually completes its life cycle in fish that feed on crustaceans. This assumption is supported by the observation that *H. aduncum* was recorded only in smaller Greenland halibut that feed on crustaceans. Only 4 specimens (abundance = 0.04) were found in 110 individuals of Greenland halibut, whereas the abundance was higher (2.5) in other fish species. On the other hand, *H. aduncum* was numerous in Atlantic cod, which in the Barents Sea feeds mainly on hyperiids and small fish (Dolgov et al. 1992). Also many *S. mentella* (42%) were infected with *H. aduncum*, which is caused by the diet of this species, containing mainly euphausiids, hyperiids, cephalopods, chaetognaths, and small fish (Froese & Pauly 2017).

Contracaecum osculatum s.l.

McClelland et al. (1985) suggested that *C. osculatum* s.l. competed with the proliferation of *P. decipiens* s.l., because the seasonal and geographical reduction in the number of *P. decipiens* s.l. in gray seals often coincided with increases in the abundance of *C. osculatum* s.l. This negative correlation between the abundance of the two anisakid nematodes was not supported by the data reported by Brattey & Stenson (1993). According to the aforementioned authors, the differences may well result from dissimilarities in the diet among gray seals, as they tend to acquire infection with larval *P. decipiens* s.l. by preying more often on benthic fish (McClelland et al. 1990). Others are more likely to feed on pelagic fish species, such as capelin or cod that harbor larvae of *C. osculatum* s.l. (see Palsson & Beverly-Burton 1984). In our study, the proportions between these two anisakid species in the examined fish are clearly visible and associated with their diet

and biology. We observed that *C. osculatum* s.l. is very numerous in both species, i.e. the Atlantic cod and the Greenland halibut, and also strongly parasitized the American plaice (Table 2), while *P. bulbosa* occurs in the same fish species in smaller numbers. Compared to the results by Karpiej et al. (2013), we also observed the increase in the Greenland halibut infection with these two parasites (abundance for *C. osculatum* s.l. 7.0 vs. 11.2 and for *P. bulbosa* 0.2 vs. 0.5). The said observation appears to contradict the previous assumption by McClelland et al. (1985).

It is interesting to note that fish species infected predominantly with the *C. osculatum* complex are mainly demersal or benthopelagic feeders. Moreover, the highest mean intensity of infection with the *C. osculatum* complex was observed in the longest specimens of the American plaice (39–44 cm). Also in the case of Greenland halibut, the mean intensity of infection was mostly observed in fish with a length of 38–48 cm. According to Rodríguez-Marín et al. (1995), crustaceans, mollusks and small fish accounted for a significant part of the diet of Greenland halibut specimens with a length (TL) ranging from 40 to 49 cm. We cannot exclude the possibility that some of invertebrates, too big for shorter fish, are the main vector for *Contracaecum* sp. Based on the list of food items in the fish diet and the size of invertebrates as a limit for shorter fish, we suspect that representatives of Isopoda and Polychaeta are intermediate or paratenic hosts for the *C. osculatum* complex. Usually they live at the bottom, even below 1000 m, and they are widespread in most oceanic waters around the world, including waters with reduced salinity like the Baltic Sea. An alternative hypothesis assumes that *C. osculatum* is acquired by halibut indirectly, by consuming fish feeding on small invertebrates, like gammarids being a dietary component of many fish, including Greenland halibut from the length class of 39–49 cm (Hovde et al. 2002). These invertebrates are widely distributed and could transmit larvae of *C. osculatum* A to a wide spectrum of secondary hosts. This could explain why the distribution and abundance of *C. osculatum* A does not depend on the location or the depth. The presented hypothesis also explains the fluctuation in the abundance of *C. osculatum* A according to the length classes. The first peak of abundance for the shortest length class may result from feeding on invertebrates (Michalsen & Nedreaas 1998). The second peak observed in the higher length class could be the effect of the accumulation of parasites and feeding on smaller fish species. The prey species included *M. villosus*, which is one of the most preferred species in the diet of the Greenland halibut (Bowering & Lilly 1992). This species, abundant

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in the Barents Sea, is carrying the larval stages of *C. osculatum* B (Levsen et al. 2016). The size of capelin suggests that only halibut representing the middle and upper length classes can feed on capelin. Contrary to the results of Karpiej et al. (2013), the northern halibut population seems to be less exposed to *C. osculatum* B larvae. The inconsistency of the results obtained in the five-year period may result from the dynamics of environmental conditions, especially those governed by the Gulf Stream (Sato et al. 2014). It also supports the hypothesis that the acquisition of *C. osculatum* B depends on the distribution of intermediate hosts as well. This explains why the abundance of *C. osculatum* B remains on the low level but consequently increases with the length of Greenland halibut.

The distribution of parasites in different hosts depends on the diet composition determined by the depth zone and the spectrum of preferred paratenic hosts occurring at each geographical location.

Conclusions

Greenland halibut is a convenient model for analyzing the distribution and abundance of ascaridoids due to the wide depth distribution and the wide spectrum of consumed food items. The differences between the groups of fish caught at different depths indicate that their feeding behavior depends on individual preferences of fish.

The location of parasites in organs, which varied depending on the species, indicates that the infected site is not accidental and is related to the optimal condition inside the organism of paratenic and/or intermediate hosts. The Greenland halibut from fishing grounds of the Barents Sea may be a serious source of human infections with ascaridoid parasites. The immediate evisceration of fish may reduce the zoonotic potential for humans by preventing the migration of larvae to the muscles of the host fish (Smith 1984). On the other hand, the viscera are generally thrown into the sea (as we have observed during the cruise) and are eaten by other fish and birds, which may increase the prevalence of parasites in the marine ecosystem.

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