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Microhabitat preference of *Anisakis simplex* in three salmonid species: Immunological implications

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ABSTRACT

Three salmonid fish species, *Oncorhynchus mykiss*, *Salmo salar* and *Salmo trutta*, were infected experimentally with the parasitic nematode *Anisakis simplex* (*A. simplex*) and the difference between *in vivo* behaviour of the nematode in the three fish species was investigated. Infection success rate differed between species. *S. salar* (Baltic salmon) showed the highest number of successfully established nematodes, whereas *S. trutta* (brown trout) and *O. mykiss* (rainbow trout) had a higher natural resistance. Microhabitat selection of nematodes differed according to fish species. In brown trout, *A. simplex* larvae were attached to the digestive tract (stomach, pyloric caeca, intestine), while the majority of larvae found in rainbow trout were located between the pyloric caeca. In Baltic salmon, nematodes were dispersed in and on spleen, head kidney, liver, swim bladder and musculature. Encapsulation and inflammatory cellular reactions differed accordingly. Histopathological and immunohistochemical studies using monoclonal antibodies raised against salmonid IgM, CD8 and MHCII were performed to detect the presence of immune cells around the infecting nematodes. None of the three fish species showed positive reactions for IgM-bearing cells in the inflammatory tissue connected with nematodes. CD8+ cells were detected in all three species and MHCII-bearing cells were found associated with encapsulated *A. simplex* in rainbow trout and brown trout, but not in Baltic salmon. Physiological, immunological and pathological implications of microhabitat differences are discussed.

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1. Introduction

Third stage larvae of *Anisakis simplex* (Nematoda: Anisakidae) are capable of infecting a wide range of fish species, which serves as intermediate/paratenic hosts for the nematode (Podolska and Morozinska, 1994; Larsen et al., 2002; Strømnes and Andersen, 2003; Murphy et al., 2010; Karl et al., 2011; Hermida et al., 2012). However, various host species may show different susceptibility to infection. Furthermore, the physiological and immunological status of the fish host may affect the fate of the

nematode following infection but the exact host elements confers resistance are unknown. *A. simplex* may cause inflammation of the host digestive tract, epithelial necrosis and endogenous protein and fat loss (Hiscox and Brocksen, 1973; Mladineo et al., 2009). Furthermore, *A. simplex* larvae have the ability to penetrate the gut wall and encapsulate in different fish organs. This can lead to modification of structure and function of local tissues in the fish during penetration and causes haemorrhages and local immune reactions that differ in severity (Abollo et al., 2001; Dezfili et al., 2008).

Various immune cells have been described to be involved during *Anisakis* infection in fish. Neutrophils followed by monocytes/macrophages in a biphasic wave was suggested to be the first reaction of nematode-infected teleosts (Reite and Evensen, 2006). Rodlet cells and

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Table 1

Average length and weight $\pm$ standard deviation of rainbow trout, brown trout and Baltic salmon used during the experiment.

	Average total length (cm)	Average weight (g)
Rainbow trout ($n = 40$)	14.2 ± 2.1	34 ± 14.8
Brown trout ($n = 40$)	13.7 ± 1.5	29.8 ± 10.6
Baltic salmon ($n = 40$)	10.4 ± 1.0	11.6 ± 2.9

n , number of fish.

eosinophilic granule cells may also play a role in immunity against nematode infection (Reite, 2005; Dezfuli et al., 2007). Immunohistochemistry may improve specific *in situ* recognition of immune cells involved in the immune reactions against parasites (Olsen et al., 2011). The present study aimed to detect the difference in the predilection site of *A. simplex* following infection of three different host species with this nematode. Immunohistochemical characterization of the response using monoclonal antibodies against cell markers such as CD8, MHCII and IgM was performed to detect possible differences in cellular reactions whereby specific immune elements affecting resistance/susceptibility may be identified.

2. Materials and methods

2.1. Fish

Pathogen-free rainbow trout (*Oncorhynchus mykiss*) (Fousing trout farm, Jutland, Denmark) and Baltic salmon (*Salmo salar*) (Lule River, Sweden) were hatched and reared at the Bornholm salmon hatchery (Nexø, Denmark) under controlled, pathogen-free conditions and brought to our fish keeping facility in Copenhagen for infection. Likewise, brown trout (*Salmo trutta*) were transported from Esrum trout hatchery (Esrums River, Northern Zealand, Denmark) to the fish facility. Random samples were picked from brown trout upon arrival and absence of gross pathology, external and internal parasitic infections was confirmed by standard methods. A total of 40 fish of each species were used in our experiment. Length and weight of experimental fish (Table 1) were measured.

2.2. Parasites

A. simplex third stage larvae were recovered from the body cavity of freshly caught herring (*Clupea harengus*) purchased from a local fishmonger in Copenhagen. Sub-samples of larvae recovered were examined using light microscopy for morphological identification to the genus level. Further, molecular diagnosis to species level was performed (see Section 2.4). Nematodes were incubated in a phosphate buffered saline (PBS) solution containing ampicillin (200 μ g/ml) and 400 μ g/ml kanamycin (Sigma Aldrich, Denmark) for 2 h. The nematodes were then washed in sterile physiological saline and kept for about 1 h at room temperature until the experimental infection procedure was initiated. *Anisakis* larvae selected for infection of fish were of approximately similar length (14–18 mm). Only non-encapsulated *A. simplex* from the abdominal cavity of herring were used in the present

experiment, which comprised almost 65% of all nematodes found inside the used herrings.

2.3. Fish aquaria and rearing conditions

Fish were acclimatized in 40 cm $\times$ 40 cm $\times$ 80 cm glass aquaria (40 fish per tank) for at least one week before the experiment started. Each aquarium contained internal biofilters (Eheim, Germany) and the water was changed every second day. Continuous aeration secured high oxygen saturation (100%). Water temperature was kept between 14 and 15 °C during the whole period. Light/dark cycle was set to 12/12 h and controlled automatically. Ammonia (NH₃), nitrate (NO₃[−]) and nitrite (NO₂[−]) concentrations were regularly tested and registered (NH₃ < 1.0 ppm, NO₃[−] < 5 ppm and NO₂[−] < 0.3 ppm) (Merckoquant®, Merck Chemicals, Germany).

2.4. Molecular identification of *A. simplex*

The middle parts of five *Anisakis* larvae were lysed by protease treatment. Briefly, the individual nematodes preserved in 96% ethanol were lysed in 200 μ l lysis buffer (Tween 20 (0.45%), Proteinase K (60 μ l ml^{−1}), 10 mM Tris and 1 mM EDTA) at 65 °C until complete digestion of tissue as confirmed by microscopy at 50 $\times$ magnification. Thereafter the protease was inactivated at 95 °C for 10 min.

PCR was conducted in 60 μ l reaction volumes using 6 μ l lysate as template and 1 unit of a Taq polymerase (Bioline no. BIO21040), 1 μ M dNTPs, 1 μ M of each primer and 1.5 mM MgCl₂. Forward primer was AnITS.F2 (CGG ATA AAC AAG TTC GGA) and reverse primer was AnITS.R1 (CGC TGA ATT TAA GCA TAT AATC). PCR conditions were 2 min of pre-denaturation at 94 °C followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, elongation at 72 °C for 1 min 15 s. Finally a post-elongation step was performed at 72 °C for 7 min. All PCRs were performed in a Biometra T3 Thermocycler. Products were analysed by 2% ethidium bromide stained agarose gels.

The PCR products were purified by Illustra™ GFX™ (GE Healthcare, cat. no. 28-9034-71) prior to sequencing at Macrogen Inc. in South Korea.

2.5. Experimental design and infection method

The experiment was conducted in duplicate for each of the three fish species. Six aquaria, each containing 22 fish were used. Fishes were anesthetized with 40 mg/l tricaine methanesulfonate (MS 222, Sigma–Aldrich, Denmark) and four *Anisakis* larvae were placed in the stomach of each fish with the aid of forceps. Anaesthetized fishes were then transferred to a small observation aquarium until they regained consciousness. Sampling was done at 7, 14, 21 and 28 days after experimental infection. For each species, 2 $\times$ 5 fish were investigated at each time point.

2.6. Parasite recovery

Fish were euthanized using MS 222 (80 mg/l) prior to examination. Necropsy was performed and the abdominal cavity in addition to the digestive tract lumen was

thoroughly investigated. Recovery and quantification of nematodes was done by opening and inspecting the abdominal cavity for the presence of parasites. Opening the stomach and intestine was done after investigating the pyloric caeca carefully and after removing and inspecting liver, spleen, kidney and head kidney. Content of stomach and intestine was also inspected in separate petri dishes for presence of *Anisakis* nematodes. Fillets were de-skinned, compressed between two glass plates and then scrutinized under the dissection microscope for presence of *Anisakis* larvae (Buchmann, 2007). Infected tissue with parasites were excised from infected fish and fixed in 4% neutral formalin for 24 h at room temperature. Preserved tissue samples were then transferred to 70% ethanol at 4 °C until processing for histology and immunohistochemistry.

2.7. Histology and immunohistochemistry (IHC)

Tissue samples were dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin blocks, which were sectioned using a Leica RM2135 microtome (Leica Microsystems, Germany) at 4 µm thicknesses. Sections were placed on SuperFrost® positively charged glass slides (Menzel-Gläser, Germany) and dried for 24 h at 40 °C.

Slides were deparaffinised in xylene and rehydrated using graded ethanol series. To quench endogenous peroxidase activity, IHC slides were incubated in 1.5% H₂O₂ in Tris-buffered saline (TBS) for 10 min. Antigen retrieval was performed by heat-induced epitope retrieval (HIER) by boiling slides in Tris-EDTA buffer (10 mM Tris Base, 1 mM EDTA, pH 9.0) in a microwave oven for 15 min. Slides were then cooled for 30 min at room temperature and transferred to TBS. Non-specific binding of antibodies was blocked incubating the slides in 2% BSA in TBS at room temperature for 10 min.

Antibodies were diluted in 1% BSA in TBS and all slides were incubated overnight with primary antibody at 4 °C. Primary antibodies used were raised against IgM (Pettersen et al., 2005), MHCII and CD8 cells (Hetland et al., 2010) and used as described by Olsen et al. (2011).

After incubation with primary antibody, slides were incubated for 30 min in anti-mouse EnVision+System HRP (K4001, Dako, Denmark) secondary antibody. Finally, bound antibody was visualized by incubating slides in carbazole solution for 20 min (50 mM acetic acid buffer, pH 5.0 with 4 mg 3-amino-9-ethyl carbazole and 0.015% H₂O₂). All sections were counterstained with Mayer's haematoxylin (Dako, Denmark) for 30 s and subsequently mounted in a water soluble mounting medium (Aquamount, Merck, UK). The slides were examined by light microscopy (Leica DMLB microscope, Leica Microsystems, Germany) and microphotographs captured with a Leica CD 300 digital camera (Leica Microsystems, Germany).

For tissue histology, staining directly after deparaffinisation and rehydration was done. Samples were stained with Meyer's haematoxylin for 6 min, decolorized in acid alcohol only for 1–3 s and counter-stained with Eosin (Sigma-Aldrich, Germany) for 15 s. Samples were then gradually dehydrated in ethanol and cleared in xylene. Mounting and examination was done as mentioned above.

2.8. Quantification of positive cells

Abundance of immune-reactive cells in the vicinity of parasites was determined in infected fish by counting positive cells in up to ten cell layers surrounding *A. simplex* cross sections. Cell layers were divided into 3 areas, close area that include the first two proximal cell layers, middle area between three and five layers and distant area, which is between six to ten cell layers surrounding the nematode. Counting was done on two fish in each group and three *A. simplex* cross sections investigated per fish.

2.9. Statistics

Kruskal–Wallis one way analysis of variance by ranks was performed to compare *A. simplex* abundances and densities of immunohistochemical reactive cells between fish species at each sampling time point. Pair-wise comparison between fish species was done using Mann–Whitney U test when the *P* value of Kruskal–Wallis test was lower than 0.05.

3. Results

3.1. Molecular diagnosis of *A. simplex*

PCR using lysates from five nematodes were performed and products of 1334 bp length were obtained. These products were subjected to sequencing and revealed the 3' end of the 18S rRNA gene, the ribosomal internal transcribed spacers 1 (ITS1), the 5.8S rRNA gene, ITS2 and the 5' end of the 28S rRNA gene. The sequences have been submitted to GenBank (GenBank accession numbers JX237370–JX237374).

All five sequences were identical, and they exhibited 100% identity to GenBank accession numbers GQ472924–GQ472930 (Skov et al., 2009) all originating from *A. simplex* from Denmark.

3.2. Site selection of nematode larvae

Overall prevalence of *A. simplex* in rainbow trout and brown trout was low (35% and 22.5%, respectively). Baltic salmon exhibited a high prevalence, reaching 72.5% (Table 2). By the end of the experiment, the mean intensity of *A. simplex* in rainbow trout, brown trout and Baltic salmon was 1.7, 1.3 and 1.8 respectively. A higher mean abundance of infection was obvious in Baltic salmon (1.3) in comparison to both rainbow trout and brown trout (0.5 and 0.3, respectively).

A. simplex showed different microhabitat preferences in the three different host species (Table 3). They were all concentrated in one area of the rainbow trout, 15 out of 21 nematodes discovered were found partially or totally inserted between the pyloric caeca. The remaining nematodes were found free in the abdominal cavity or were loosely connected to the external surface of the stomach with a serosal encapsulating layer. Nematodes found in brown trout were located along the digestive tract, mainly around the stomach, but also between the pyloric caeca and

Table 2

Prevalence, mean intensity and abundance of *Anisakis* sp. infection in rainbow trout, brown trout and Baltic salmon at each sampling time point. Difference in the abundance of *Anisakis* sp. in fish species was done using Kruskal–Wallis test. Ten fish of each species examined at each time point.

Sampling time	Rainbow trout			Brown trout			Baltic salmon		
	Prevalence %	Intensity $\pm$ SE	Abundance $\pm$ SE	Prevalence %	Intensity $\pm$ SE	Abundance $\pm$ SE	Prevalence %	Intensity $\pm$ SE	Abundance $\pm$ SE
Day 7	40	1.3 $\pm$ 0.3	0.5 $\pm$ 0.2	20	2	0.4 $\pm$ 0.3	70	1.7 $\pm$ 0.3	1.2 $\pm$ 0.3
Day 14	30	1.7 $\pm$ 0.7	0.5 $\pm$ 0.3	40	1 $\pm$ 0	0.4 $\pm$ 0.2	70	2.3 $\pm$ 0.4	1.6 $\pm$ 0.5
Day 21*	20	2.5 $\pm$ 0.5	0.5 $\pm$ 0.3	10	1	0.1 $\pm$ 0.1	70	1.7 $\pm$ 0.3	1.2 $\pm$ 0.3
Day 28**	50	1.2 $\pm$ 0.2	0.7 $\pm$ 0.2	20	1	0.2 $\pm$ 0.1	80	1.4 $\pm$ 0.2	1.1 $\pm$ 0.2

SE: standard error. In both 21 and 28 days post-infection the difference was significant between Baltic salmon and brown trout at $P=0.007$.

* $P=0.016$.

** $P=0.019$.

Table 3

Place and number of *Anisakis* sp. recovered from three salmonid species following experimental infection with 4 nematodes per fish.

Rainbow trout		Brown trout		Baltic salmon	
Organ (inside/attached to)	Number found	Organ (inside/attached to)	Number found	Organ (inside/attached to)	Number found
Stomach	3	Stomach	1	Stomach	5
Pyloric caeca	15	Pyloric caeca	5	Pyloric caeca	17
Spleen	1	Intestine	4	Spleen	4
Muscle	1 ^a	Liver	1	Intestine	13
Free	2			Muscle	4 ^a
Total	22		11		43 + 8 ^b = 51

^a *Anisakis* found partially penetrating the organ specified.

^b The remaining eight *Anisakis* sp. recovered from Baltic salmon; 2 were partially penetrating the liver, 2 connected to the swim bladder, 1 was partially penetrating the head kidney and 3 were free in the abdominal cavity.

around the intestine. Nematodes found in Baltic salmon were more scattered around different organs in the abdominal cavity. Four *Anisakis* larvae were partially penetrating the hypaxial muscles of three Baltic salmon at 21 and 28 days post-infection (Fig. 1A). Some nematodes were also partially penetrating liver parenchyma of two Baltic salmon (Fig. 1B) and the head kidney of a third one. In addition, *A. simplex* larvae were encapsulated on the external surface of the spleen, swim bladder, stomach, intestine and pyloric caeca. Only *Anisakis* specimen was found free in the abdominal cavity. *A. simplex* found in rainbow trout and brown trout were not fully encapsulated until day 28 post infection, but merely partially encapsulated at day 21 post-infection. In contrast, all nematode larvae retrieved from Baltic salmon were fully encapsulated already at day 21 post-infection.

3.3. Microscopic findings

Eosin and haematoxylin (H&E) stained sections showed different degrees of granulomatous reaction surrounding the nematodes. Severity of reactions and accumulation of immune cells were dependent on the infection period and nematode location. Thin capsules were demonstrated around nematodes recovered from the pyloric caeca (Fig. 2). All three fish species reacted with the presence of CD8+ cells at day 28 post-infection, but not at 21 days post-infection. CD8+ cells possessed the same morphological and staining lymphocyte-like characteristics as described by Hetland et al. (2010) and Olsen et al. (2011). The number of CD8+ cells surrounding nematodes in infected Baltic salmon was lower than the number of positive CD8+ cells in infected brown trout or rainbow trout (Table 4).

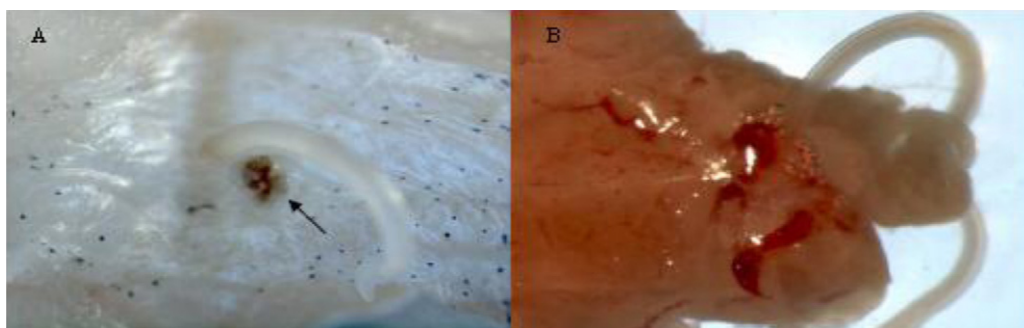


Fig. 1. *Anisakis simplex* infection in Baltic salmon at 21 days post-infection. (A) penetration of hypaxial muscle and formation of necrosis (arrow). (B) penetration of liver parenchyma leading to cellular disruption and local haemorrhages.

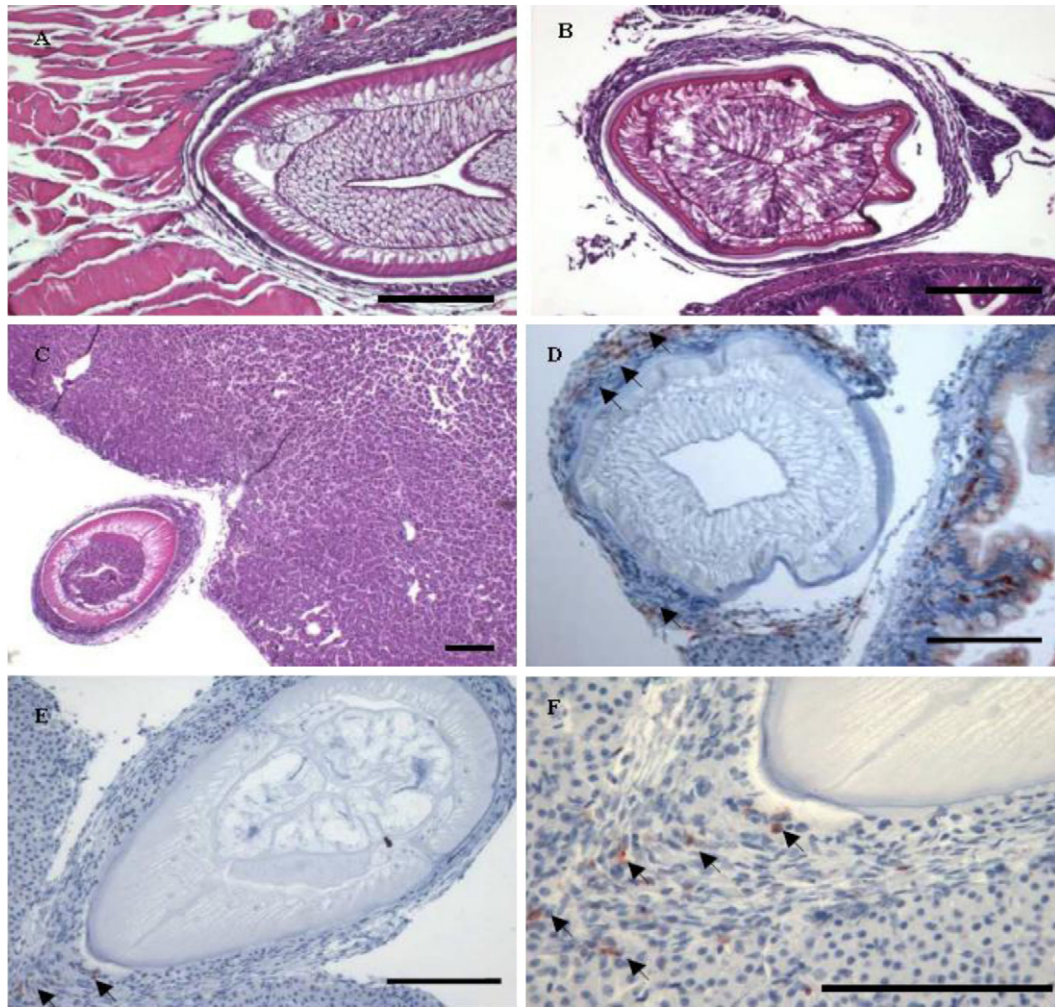


Fig. 2. (A–C) H&E staining of encapsulated *Anisakis* sp. larvae in different tissues. (A) hypaxial muscle of Baltic salmon at 28 days post-infection (dpi). (B) Pyloric caeca of rainbow trout at 28 dpi. (C) Liver of Baltic salmon at 21 dpi. (D–F) Immunohistochemical detection of CD8+ cells (arrows) surrounding *Anisakis simplex* larvae. (D) Pyloric caeca of rainbow trout at 28 dpi. (E and F) Liver of Baltic salmon at 28 dpi. Scale bar = 50 µm.

Occurrence of CD8+ cells differed between tissues targeted by *A. simplex* larvae. In Baltic salmon, no positive cells were detected around nematodes that had penetrated the muscle tissue, while CD8+ cells were clearly present around the *Anisakis* larvae extracted from pyloric caeca. Positive cells were to a lesser extent present in the capsule surrounding a nematode found in intestinal adipose tissue of one Baltic salmon, and around a nematode partially penetrating the liver of another salmon at 28 days post-infection (Fig. 2).

At day 28 post-infection MHCII+ cells were found around nematodes infecting only rainbow trout and brown trout, but not in infected Baltic salmon. In both rainbow trout and brown trout, positive cells were numerous and scattered around nematodes. No accumulations of MHCII+ cells were detected at 21 days post-infection in any of the fish species. No IgM positive cells in any of the infected fishes at 21 and 28 days post-infection.

Table 4

Immune response following *Anisakis* sp. infection in three salmonids. Number of cell layers (capsule layers) surrounding nematodes. Mean number of IHC positive cells (CD8 and MHCII) around *Anisakis* larvae infecting rainbow trout, brown trout and Baltic salmon at 28 days post-infection. Cells in ten layers around two parasites cross sections were enumerated per fish. No IgM positive cells were located in this area.

	Capsule layers (range)		Mean number of CD8+ cells			Mean number of MHCII+ cells		
	Min.	Max.	Within 0–2 layers	Within 3–5 layers	Within 6–10 layers	Within 0–2 layers	Within 3–5 layers	Within 6–10 layers
Rainbow trout	2.3 ± 0.6	16 ± 6.3	1.7 ± 0.6	1.3 ± 1.5	5.7 ± 3.1	4.5 ± 2.9	10.3 ± 4.3	14.7 ± 2.9
Brown trout	4	12	0	2 ± 1	6.3 ± 1.5	7 ± 1.7	12.3 ± 1.5	9.3 ± 2.1
Baltic salmon	4 ± 1.8	14 ± 5.9	1	1.7 ± 0.6	3 ± 1	0	0	0

4. Discussion

Larvae recovered from Atlantic herring and used for experimental infections were identified as third-stage nematode larvae belonging to the genus *Anisakis* by morphometric methods (Smith, 1983). Our molecular analysis of a subsample of five nematodes showed 100% identity to *A. simplex*, which corresponds to the findings of Skov et al. (2009) showing that local Danish fish stocks are infected exclusively with *A. simplex*.

The results demonstrate differences in the prevalence and abundance of *A. simplex* infection in three closely related fish species, *O. mykiss*, *S. trutta* and *S. salar*, following experimental infection, which indicates susceptibility differences among these species. The number of infected rainbow trout was higher compared to the number of infected brown trout. This differed slightly from studies by Wootten and Smith (1975) in which the prevalence of experimentally infected rainbow trout was only 27% and the prevalence of infected brown trout was 50.6%. In the present study, Baltic salmon showed the highest infection prevalence and mean abundance. In addition, nematodes in salmon were more diffusely distributed in the abdominal cavity and elicited more pathologic reactions. It should be noted that Baltic salmon used in this experiment were smaller than the trout used. It cannot be excluded that this may have a minor influence on the infection rate results. However, in nature smaller fish have lower infection rates whereas we found a higher infection rate in those relatively small-sized salmon. This could be interpreted as evidence of a high susceptibility of salmon.

Previous infection studies on rainbow trout, Atlantic herring, olive flounder and Alaska pollock did also indicate differential site specificity of *A. simplex* larvae according to the host species (Smith and Wootten, 1975; Arthur et al., 1982; Santamarina et al., 1994; Quiazon et al., 2011b). Additional studies by Quiazon et al. (2011a) recorded the distribution of *A. simplex* and *A. pegreffii* in seven marine fish species and showed that the Japanese mackerel (*Scomber australasicus*) was the only host infected with two *Anisakis* spp.

While investigating the migration of *A. simplex* to the musculature of three fish species post mortem, Smith (1984) suggested that *A. simplex* migrate into the flesh in 'fatty fish' as in herring and mackerel but not the 'non-fatty fish', such as blue whiting (*Micromesistius poutassou*) or whiting (*Merlangius merghus*). The presence of high lipid content in the muscle of 'fatty fish' could indicate the presence of chemical factors that trigger this type of migration to musculature. There is no difference in fat content amongst fish species used in our experiment, yet the site specificity differences of *Anisakis* infection could be due to differences in some chemoattractants. However, the encapsulation process differed between fishes, indicating a variation in immune reaction among these species. After 21 days post-infection, most larvae were fully encapsulated in Baltic Salmon, but not in rainbow trout nor in brown trout. Previous studies in oral and intraperitoneal infection of *A. simplex* in rainbow trout showed that the encapsulation process occurred between 31 and 36 days post-infection (Santamarina et al., 1994) while in our study all *A. simplex*

larvae were encapsulated between 21 and 28 days post-infection in rainbow trout. The temperature used by these authors was the same as ours and possibly rainbow trout strain differences could explain this deviation.

No IgM positive cells were found in any of the three species (Table 4), suggesting that local antibody responses play a less prominent role in immune defence against encapsulated nematodes. The presence of CD8+ and MHCII+ cells around the infective nematodes confirms the role of these cells in fish immune reaction against *A. simplex* larvae. Macrophages, which are MHCII+ cells, in brown trout were previously shown to interact with *A. simplex* in the peritoneal cavity (Larsen et al., 2002). The differences of CD8+ and MHCII+ cells count found in several tissues of the same fish species indicate a variation in immune response of each tissue against *A. simplex* infection. Moreover, enumerating cells in histological sections revealed that MHCII+ cells generally outnumbered CD8+ cells of the same nematode sections. It is thus noteworthy that no macrophage-like MHCII cells were found in salmon reactions around the worm, indicating that this cell type partly play a role in differential susceptibility of different salmonids against nematodes.

CD8+ receptors are associated with cytotoxic T cells in mammals and found to be involved in cellular immunity against the nematode *Trichinella spiralis* infection in humans (Morales et al., 2002). However, antiparasitic immune reactions in fish may show much larger variation due to the large variability of immune system in different teleost groups (Buchmann, in press).

The full life cycle of *A. simplex* does not occur in the Baltic Sea. However, some populations of herring, such as the Rügen herring that migrates for long distances, obtain the infection in the North Sea and introduce the parasite into the Baltic during the spawning migration (Grabda, 1974). The infected herring may transfer the parasite to predatory fish (Grabda, 1976) and the local salmon population can probably get infected by this route. *A. simplex* infection in Atlantic salmon was previously reported in different parts of the Atlantic (Beverley-Burton and Pippy, 1977; Deardorff and Kent, 1989; Bristow and Berland, 1991; Noguera et al., 2009) but not in the Baltic Sea. To our knowledge, this work is the first to experimentally demonstrate the ability of *A. simplex* larvae to penetrate musculature in Baltic salmon. Presence of *A. simplex* nematodes in the muscles of salmon is considered to be a risk factor for raw fish consumers (Audicana et al., 2002), and the present work indicates that at least part of the Baltic salmon population may be at risk of infection.

Differential susceptibility of various salmonids towards helminth infection has previously been investigated. Thus, Baltic salmon have shown much higher resistance compared to East-Atlantic salmon against infections with *Gyrodactylus salaris* (Dalgaard et al., 2003; Lindström et al., 2006). The immunological implications have not been finally resolved but reaction patterns involving inflammatory cytokines interleukin 1 (IL-1 β), interferon gamma (IFN γ), complement factors and acute phase proteins such as SAA have been suggested to cause the susceptibility difference in the infection rate between infected Baltic and Atlantic salmon (Kania et al., 2010). The difference in

infection rate and site selections in our experiment could also be related to the difference in the number and types of immune cells (MHCII+ and CD8+ cells) surrounding encapsulated nematodes found in experimentally infected fish.

In conclusion, our findings support the distinctive site predilection of *A. simplex* larvae for each fish species and indicate the influence of cellular reaction in the process. Based on these first observations on characteristic cellular reactions associated with differential behaviour of worm in different host fish, it is suggested to further dissect the intricate interactions between nematode's genes/molecules and host reactions.

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