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Anisakis simplex (s.s.) larvae in wild Alaska salmon: no indication of post-mortem migration from viscera into flesh

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ABSTRACT: The prevalence, mean intensity and distribution of *Anisakis* nematode third-stage larvae (L3) in the muscle and viscera of wild-caught chum salmon *Oncorhynchus keta*, pink salmon *O. gorbuscha* and sockeye salmon *O. nerka* were compared immediately after catch. Salmon were collected during the fishing season in July 2007 in Bristol Bay and Prince William Sound close to Cordova, Alaska (USA). All fish were infected, and more than 90% of the nematode larvae were found in the edible muscle meat. The isolated anisakid L3 were genetically identified as *A. simplex* (s.s.). The distribution of nematodes in the muscle meat of fresh-caught salmon was examined in 49 *O. keta*, 50 *O. nerka* and 12 *O. gorbuscha* from Cordova. Most of the larvae were detected in the muscle parts around the body cavity, but nematodes were also found in the tail meat and epaxial muscle (loins). The mean intensity of *Anisakis* larvae in the edible part was 21 individuals for *O. gorbuscha*, 62 individuals for *O. keta* and 63 individuals for *O. nerka*. No difference in the intensity of *Anisakis* larvae in the hypaxial muscle was found between fresh-caught and immediately gutted salmon and individuals stored ungutted for 24 h either on ice or in refrigerated sea water.

KEY WORDS: Pacific salmon · *Anisakis simplex* (s.s.) · Muscle meat · Refrigerated sea water (RSW) storage · *Onchorhynchus keta* · *O. nerka* · *O. gorbuscha*

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INTRODUCTION

The presence of *Anisakis* spp. and other nematode larvae of health significance in fish as seafood has been recognised for a long time (Agersborg 1918, Kahl 1936). Live nematodes, when ingested in raw, not adequately processed or undercooked fish can cause anisakidosis.

Species of the nematode genus *Anisakis* are globally distributed fish parasites and typically infect cetaceans or pinnipeds as final hosts. This genus previously included only 3 species (Davey 1971). Beside readily described morphospecies (*A. physeteris, A. simplex, A. typica*), the analyses of allozymes, PCR-RFLPs of rDNA and mtDNA cox-2 data have revealed that this genus currently includes 9 distinct species: *A. simplex* (s.s.), *A. pegreffii* and *A. simplex* C (representing the *A. simplex*-complex), together with *A. typica*, *A. nascettii* and *A. ziphidarum*; and *A. paggiae*, *A. brevispiculata*, and *A. physeteri*s representing the *A. physeteris*-complex (e.g. Valentini et al. 2006, Klimpel et al. 2008, 2010, Mattiucci & Nascetti 2008, Mattiucci et al. 2009). All of these species are morphologically very similar but genetically distinct and have specific host preferences, life cycles and zoogeographical distributions (Valentini et al. 2006, Kellermanns et al. 2007, Klimpel et al. 2007, Mattiucci et al. 2007, Mattiucci & Nascetti 2008).

In general, the life cycle of *Anisakis* proceeds in several steps and has been studied and described in detail by several authors (e.g. Grabda 1991, Klimpel et al.

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2004, 2008). *Anisakis* is taken up by marine fish via food. After ingestion of infested intermediate hosts by the live fish, *Anisakis* larvae penetrate the intestinal wall. Most larvae coil up on the surface of the internal organs but some migrate into the flesh. Several investigations have shown that *Anisakis* larvae are regularly present in the flesh of marine teleost fish immediately after death (Davey 1972, Smith 1984, Højgaard 1995, Karl & Leinemann 1995). However, there is some controversy as to whether migration takes place from the viscera into the flesh during the post-mortem storage of fish.

Roepstorff et al. (1993) studied the effect of modern handling practices such as storage in refrigerated seawater (RSW) of fat and lean herring *Clupea harengus*. They did not find any indication of *Anisakis* larvae migrating from the belly cavity into the surrounding flesh. This concurred with the results of earlier studies in lean fish species stored on ice such as whiting *Merlangius merlangus* and blue whiting *Micromesistius poutassou* (Smith 1984), and Alaska pollock *Theragra chalcogramma* (Arthur et al. 1982). In mackerel *Scomber scombrus*, Smith (1984) showed a migration into the flesh from the viscera. These studies and recent investigations (Karl 2008) on North Atlantic marine fish species showed that between 90 and 98% of the *Anisakis* larvae are situated in the belly cavity and the visceral organs at the time of catch and only few are embedded in the surrounding tissue of the peritoneal cavity (belly flaps). One exception seems to be maturing Pacific salmon. Sugawara et al. (2004) found a higher abundance of *A. simplex* s.l. in the muscle of chum salmon *Oncorhynchus keta*, and Deardorff & Kent (1989) recovered 87% of *A. simplex* s.l. from the edible musculature of wild caught sockeye salmon *O. nerka*.

A high increase of parasite infection was observed by Urawa & Fujisaki (2006) in adult *Oncorhynchus keta* between 2002 and 2006. The abundance was less than 20 parasites fish^{-1} in 2002 and reached 160 parasites fish^{-1} in 2006. Similar findings were observed by Etzel & Ramdohr (2006), who investigated cold smoked Pacific salmon fillets and slices on the German market. Aware of the increasing abundance of *Anisakis* larvae in German wild salmon products, a comprehensive study was initiated by the German fish industry in close cooperation with the Max Rubner-Institut to monitor the actual situation in wild salmon. The study focused on wild salmon from Alaska (USA), because most of the raw wild salmon of interest for the German fish industry is delivered as headed and gutted deep-frozen fish from various fishing areas of the state of Alaska, the Russian Federation and Canada.

Our objective was to analyse the prevalence, abundance and distribution of *Anisakis* larvae in the muscle and viscera of wild-caught *Oncorhynchus keta*, *O. gorbuscha* and *O. nerka*, collected from different fishing areas of Alaska immediately after catch. We also discuss the possible influence of delayed gutting under typical handling practices such as storage of whole salmon for 24 h in RSW as well as the alternative storage on wet ice.

MATERIALS AND METHODS

Sample collection. Salmon samples were collected during their migratory spawning run within 1 wk in July 2007 at 2 locations (Nushagak and Naknek district) of the Bristol Bay area and at the Prince William Sound close to Cordova in Alaska. Fresh-caught sockeye *Oncorhynchus nerka*, chum *O. keta* and pink salmon *O. gorbuscha* from Cordova were measured for total length and immediately gutted after catch on board the respective trawler. The viscera (intestines and all visceral organs) and the gutted fish were separately packed into plastic bags and deep frozen. Detailed data on the samples are compiled in Table 1. To study the effect of delayed gutting, salmon of the same catch were stored ungutted as whole fish on board tenders either on flake ice in covered ice boxes or in RSW tanks (capacity 20 to 25 t) under commercial conditions. After 24 h, all stored samples were gutted, the fish were cut with a sharp knife along the lateral line into 2 halves, the hypaxial muscle parts (belly flaps) and viscera were packed into separate plastic bags and deep frozen. If migration of *Anisakis* larvae from the viscera occurs, they will preferably move into the surrounding muscle tissue of the body cavity resulting in a higher abundance of *Anisakis* in the belly flaps. Taking this and also the high transport costs to Europe into account, it was decided for this part of the study to collect only the hypaxial muscle below the lateral line and the corresponding visceral organs. The same procedure was applied to fresh-caught samples from the Nushagak and the Naknek district. All samples were deep frozen and shipped by container to Europe and reached the laboratory deep frozen in December 2007. Samples were stored at –24°C until analysis.

Sample preparation. Fish or hypaxial musculature and the corresponding viscera were thawed. The gonads were separated from the viscera and deep frozen again. The remaining organs were digested by means of pepsin/HCl (see next subsection). Gutted whole fish were washed, drained, weighed and filleted by hand, taking care to preserve the complete belly flaps and the costal bones intact. Approximately 1 cm of the lower part of the belly flaps (fat edges) were removed, and the trimmed fillets were skinned. Each fillet was weighed and divided into several parts:

Table 1. *Oncorhynchus* spp. Origin, description of samples and sample sizes of the 3 species of Pacific salmon studied. Length and weight data given as mean (range). Fillet weight is that of a single fillet, including belly flap and without skin; weight of belly flaps represents both flaps without skin. RSW: refrigerated sea water; nd: not determined

Fishing ground	Storage	Sample	No. of samples	Length (cm)	Weight (g)	Fillet weight (g)	Weight of belly flaps (g)
Chum salmon *O. keta*							
Cordova	Fresh-caught	Whole fish gutted	49	63 (58–70)	2253 (1600–3110)	595 (398–793)	357 (230–515)
Cordova	24 h ice	Hypaxial muscle	23	nd	nd	nd	430 (298–600)
Cordova	24h RSW	Hypaxial muscle	50	nd	nd	nd	426 (300–605)
Nushagak district	Fresh-caught	Hypaxial muscle	26	nd	nd	nd	432 (225–591)
Pink salmon *O. gorbuscha*							
Cordova	Fresh-caught	Whole fish gutted	12	54 (49–64)	1711 (1120–2670)	450 (281–660)	268 (178–408)
Cordova	24 h ice	Hypaxial muscle	14	nd	nd	nd	302 (221–410)
Sockeye salmon *O. nerka*							
Cordova	Fresh-caught	Whole fish gutted	50	60 (52–67)	2219 (1530–3300)	641 (414–986)	376 (246–618)
Cordova	24 h ice	Hypaxial muscle	49	nd	nd	nd	502 (322–897)
Cordova	24 h RSW	Hypaxial muscle	50	nd	nd	nd	465 (261–686)
Nushagak district	Fresh-caught	Hypaxial muscle	25	nd	nd	nd	390 (226–689)
Naknek district	Fresh-caught	Hypaxial muscle	25	nd	nd	nd	420 (187–663)

upper and lower epaxial muscle, tail muscle and belly flap. The belly flap was further divided into upper part, lower part and tail part. A detailed drawing is given in Fig. 1. All parts were separately analysed for nematodes applying the UV-press method (Karl & Leinemann 1993) as described below. The viscera samples of the storage experiments were prepared and analysed as described above. The hypaxial muscle sample was split into left and right part, skinned and trimmed in a way to obtain belly flaps comparable to those of the whole gutted fish. Further treatment followed the same protocol as described above.

Detection of nematodes. Nematodes were detected by using the digestion- and the UV-press method. For the digestion method, the viscera were digested by the pepsin-HCl process as described in detail in the CODEX standard for salted Atlantic herring and salted sprat in Annex Ia (CODEX STAN 244 2004). The solution was decanted through a sieve, and the contents of the sieve were examined for nematode larvae.

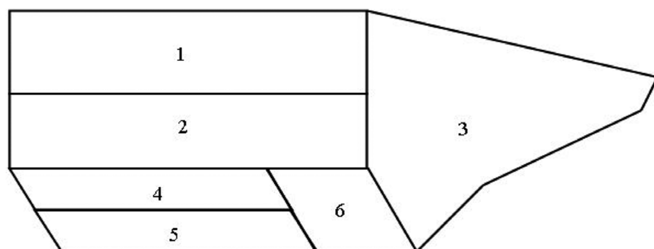

Fig. 1. *Oncorhynchus* spp. Subdivision of trimmed fresh-caught Alaska salmon fillets to study the distribution of *Anisakis* larvae. 1: upper epaxial muscle; 2: lower epaxial muscle; 3: tail muscle; 4: upper belly flap; 5: lower belly flap; 6: belly flap tail part

The UV-press method was described in detail by Karl & Leinemann (1993). In brief, the fillet parts were pressed in plastic bags to thin layers of 1 to 2 mm by means of an automatic press (holding time 20 s at 8 bar) and examined under UV light. Deep-frozen nematode larvae show fluorescence under UV light (366 nm) and can be counted visually. The method also allows a differentiation of *Anisakis*, *Pseudoterranova* and *Hysterothylacium* larvae at genus level due to their different appearance under UV-light (Levsen & Lunestad 2010).

To ensure that the data from the digestion and UV-press procedure were suitable for comparison, various samples of *Oncorhynchus gorbuscha*, *O. keta* and *O. nerka* fillet parts were first examined by the UV-press method and later digested. The results showed excellent agreement in the number of nematodes detected.

Nematode species identification. *Anisakis* larvae were macroscopically identified by their more or less bright light blue fluorescence and typical appearance under UV light. Various subsamples of anisakid larvae were isolated from the flesh and identified by light microscopy following the morphological criteria proposed by Berland (2003).

Genetic identification was performed on 45 individual *Anisakis* larvae, which could be isolated undamaged from the pressed fillets. Genomic DNA was extracted and purified from each of 10 *Anisakis* larvae randomly taken from the meat of *Oncorhynchus nerka* and *O. keta* of both sampling areas (Bristol Bay and Cordova) as well as 5 individuals from the flesh of *O. gorbuscha* (Cordova) using the PeqGOLD genomic DNA extraction kit (Peqlab Biotechnology) according

to the manufacturer's instructions. The rDNA region comprising the ITS-1, 5.8S, ITS-2 and flanking sequences (=ITS+) was amplified using the primers NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) and TK1 (5′-GGC AAA AGT CGT AAC AAG GT-3′; Zhu et al. 2000, Kuhn 2010). The PCR-reaction (50 µl) included 25 µl of Master-Mix (Peqlab) containing dNTPs, $MgCl_2$, buffer and *Taq* polymerase, 3 µl of each primer, 14 µl double distilled H_2O and 5 µl genomic DNA. Each PCR reaction was performed in a thermocycler (Peqlab) under the following conditions: initial denaturation at 95°C for 1 min, 40 cycles of 94°C for 45 s (denaturation), 55°C for 45 s (annealing) and 72°C for 45 s (extension), followed by a final extension at 72°C for 10 min. Samples without DNA were included in each PCR run. PCR products were examined on 1% agarose gels. A 100 bp ladder marker (Peqlab) was used to estimate the size of the PCR products. To identify the anisakid nematodes, the PCR products were purified with PeqGOLD Cycle-Pure Kit (Peqlab). Afterwards a total volume of 7 µl, including 2 µl primer (individually) and 5 µl of the PCR product (250 ng µl^{-1}) were sequenced by Seqlab GmbH (Goettingen, Germany).

Data analysis. A Mann-Whitney rank sum test was used to test possible differences in larvae intensities in the hypaxial muscle of sockeye and chum salmon from different fishing grounds and to compare the intensity in fresh and chilled stored Pacific salmon. To describe the parasite infection data, the terms prevalence, intensity and abundance are used following Bush et al. (1997) and Rózsa et al. (2000).

RESULTS AND DISCUSSION

Anisakis larvae in different fresh-caught salmon species from Alaska

During their spawning migration in the Prince William Sound close to Cordova, 50 *Oncorhynchus nerka* and 49 *O. keta* were randomly taken from commercial catches on board trawlers. Only 12 individuals of *O. gorbuscha* could be collected. Size, weight and appearance were typical for the raw material used by the German fish industry for wild salmon products (Table 1). The fish were gutted directly after catch to prevent a possible post-mortem migration of nematodes. The infection levels, summarised in Table 2, reflect the situation in live salmon from this area at capture. All salmon of each species from the Cordova area were infected with anisakid larvae (prevalence = 100%), and more than 90% of the larvae were found in the muscle meat (edible part). Virtually all larvae were macroscopically and morphologically identified as *Anisakis simplex*. The flesh of *O. nerka* and *O. keta* was heavily infected, with a mean intensity of 62 and 63 larvae, respectively. *O. gorbuscha* was less infected, with a mean intensity of 21 individuals in the flesh.

Fig. 2 shows the frequency distribution of *Anisakis* larvae in the edible muscle meat of the 3 Pacific salmon species and demonstrates the large variation of the infection levels observed between the individuals of each host salmon species. The number of nematode larvae varied between a minimum of 5 and a maximum

Table 2. *Anisakis* sp. infecting *Oncorhynchus* spp. Infection parameters in 3 species of fresh-caught wild salmon from Cordova, Alaska (USA). Data of the intensity, abundance and the number of nematodes kg^{-1} are given as mean (range). Fillet indicates a single fillet without belly flap; belly flap represents a single belly flap. na: not applicable

Infection parameter	Whole fish	Flesh	Viscera	Fillet	Belly flap
***O. keta* (n = 49)**					
Prevalence (%)	100	100	74	93	100
Intensity	65.3 (9–301)	63.2 (8–297)	2.8 (1–16)	6.2 (1–36)	25.9 (2–131)
Abundance	65.3 (9–301)	63.2 (8–297)	2.1 (0–16)	5.7 (0–36)	25.9 (2–131)
Nematodes kg^{-1}	na	56 (4–328)	na	15 (0–107)	152 (11–936)
Relative frequency (%)	na	95	5	na	na
***O. gorbuscha* (n = 12)**					
Prevalence (%)	100	100	67	67	100
Intensity	23.5 (5–43)	21.4 (5–41)	3.1 (1–5)	2.6 (1–7)	8.5 (2–20)
Abundance	23.5 (5–43)	21.4 (5–41)	2.1 (0–5)	1.7 (0–7)	8.5 (2–20)
Nematodes kg^{-1}	na	23 (3–59)	na	5 (0–18)	65 (9–177)
Relative frequency (%)	na	91	9	na	na
***O. nerka* (n = 50)**					
Prevalence (%)	100	100	72	95	100
Intensity	63.7 (13–175)	61.5 (12–165)	3.1 (1–32)	4.6 (1–17)	26.4 (3–88)
Abundance	63.7 (13–175)	61.5 (12–165)	2.2 (0–32)	4.3 (0–17)	26.4 (3–88)
Nematodes kg^{-1}	na	48 (5–179)	na	9 (0–34)	142 (13–528)
Relative frequency (%)	na	96	4	na	na

of 41 nematodes in the flesh of *Oncorhynchus gorbuscha*, between 8 and 297 nematodes in *O. keta* and between 12 and 165 nematodes in *O. nerka*.

The results are in accordance with the very high infection levels reported by Urawa & Fujisaki (2006) for the muscle meat of *Oncorhynchus keta* returning to the Chitose River, Japan.

Sugawara et al. (2004) reported that immature *Oncorhynchus keta* caught in the open ocean had the same distribution pattern, with *Anisakis simplex* larvae being most abundant in the muscles. Our data show that the high intensity of nematodes in the muscle seems to be an attribute related not only to *O. keta* but also to other Pacific salmon species. This is in contrast to most other marine fish species, where parasites infect mainly the body cavity and the visceral organs (Strømnes & Andersen 1998).

Infection sites

To study the distribution of nematodes in the flesh of fresh-caught Pacific salmon, the fillets, including belly flaps, of each fish were divided into several parts according to Fig. 1 and analysed. Table 3 gives the average percentage of the *Anisakis* larvae in each compartment. All 3 fish species showed a comparable distribution pattern of the larvae. Approximately 80% of the *Anisakis* larvae were found in the belly flap area (positions 4, 5, 6; Fig. 1), and the larvae were more or less equally distributed within the belly flap. A slightly different picture was observed in the epaxial muscle. The upper parts (position 1) of the *Oncorhynchus nerka* and *O. keta* fillets were less infected than the lower epaxial muscle (position 2) and the tail parts (position 3). In the case of *O. gorbuscha*, the small sample size did not allow a reliable comparison. Our data are in excellent agreement with the distribution pattern in fillets of adult *O. keta* returning to the Chitose River, as reported by Sugawara et al. (2004).

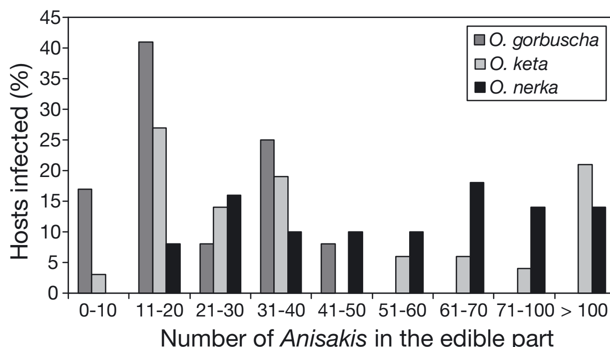

Fig. 2. *Anisakis* sp. infecting *Oncorhynchus* spp. Frequency distribution of *Anisakis* larvae in the edible part of 3 Pacific salmon species from Cordova, Alaska (USA)

Reduction of *Anisakis* infestation in fillets

Removal of the belly flaps

The high levels of *Anisakis* larvae in wild salmon fillets led to a discussion of technological measures to reduce the number of nematodes. The removal of the complete belly flap in a 'jumbo cut' (Priebe 2007) resulted in a considerable reduction of *Anisakis* larvae in the Pacific salmon fillets studied (Table 2). The mean intensity of nematodes in the remaining *Oncorhynchus gorbuscha*, *O. keta* and *O. nerka* fillets dropped to 2.6, 4.6 and 6.2, respectively, but the infection levels of single fillets without belly flaps were still high due to the large variation of the infection intensity, e.g. ranging between 1 and 36 larvae per chum salmon fillet. The removal of the belly flap also led to a considerable weight loss of approximately 30%.

Etzel et al. (2007) proposed to establish an allowable limit of 20 nematodes kg^{-1} wild salmon fillet product in Germany. In Table 2, the infection levels are also calculated as number of nematodes kg^{-1}. The data show that the average number of nematodes kg^{-1} in the fillets remained below the proposed limit after complete removal of the belly flaps, but according to our data, more than 20% of the chum salmon and approximately 8% of the sockeye salmon fillets from Cordova still exceeded the proposed limit (Fig. 3).

Selection of sizes

Within the length range of 52 to 67 cm for *Oncorhynchus nerka* and 58 to 70 cm for *O. keta*, no relationship was found between the number of *Anisakis* larvae and fish body size (*O. nerka*: r = 0.245; *O. keta*: r = 0.07; Fig. 4). The results correspond to the results of Sugawara et al. (2004), who found no correlation between length and infection intensity for adult *O. keta* salmon returning to the Chitose River. Thus the

Table 3. *Anisakis* sp. infecting *Oncorhynchus* spp. Average distribution (%) of larvae in the flesh of fresh-caught wild Alaska salmon species. Positions 1 to 6 correspond to those shown in Fig. 1

Position	*O. keta*	*O. gorbuscha*	*O. nerka*
Epaxial muscle			
1. Upper part	3.1	7.7	3.2
2. Lower part	6.2	7.7	6.5
3. Tail	9.4	7.7	6.5
Belly flap			
4. Upper part	28.1	30.8	29.0
5. Lower part	28.1	23.0	35.5
6. Tail part	25.1	23.0	19.4

selection of certain size classes cannot be used as a measure to reduce nematode abundance in the edible part.

Genetic anisakid nematode species identification

The ITS-1, 5.8S and ITS-2 sequences were determined for 45 *Anisakis* nematodes isolated from the 3 studied *Oncorhynchus* species. The sequences obtained were compared with those previously deposited for the same marker in GenBank using BLASTX. The length of the PCR product including the 3 regions ITS-1, 5.8S and ITS-2 was 857 bp, while the length of the ITS-1 and ITS-2 sequences of *A. simplex* (s.s.) was 392 and 308 bp, respectively. The 5.8S sequences were all 157 bp long. The G+C contents of all sequences were 46.68% (ITS-1), 51.59% (5.8S) and 42.21% (ITS-2). The obtained sequences of *A. simplex* (s.s.) from the 3 fish species *O. gorbuscha*, *O. keta* and *O. nerka* (GenBank accession numbers GU735486, GU735487, GU735488, GU735489 and GU735490, respectively) were identical to a sequence already isolated from the sibling species *A. simplex* (s.s.) from the macrourid *Trachyrincus scabrus* (EU718471.1). *A. simplex* (s.s.) is a typical parasite of cetaceans of the families Delphinidae, Monodontidae, Phocoenidae and Balaenopteridae and has been genetically identified mainly in the North Atlantic and Pacific Oceans (e.g. Mattiucci & Nascetti 2008). Species of these cetacean families constitute the migratory final hosts, occurring in waters around the Pacific coast of Alaska. They play an important role in the transport of the north Pacific population of *A. simplex* (s.s.) into other regions of the Pacific and North Atlantic Ocean.

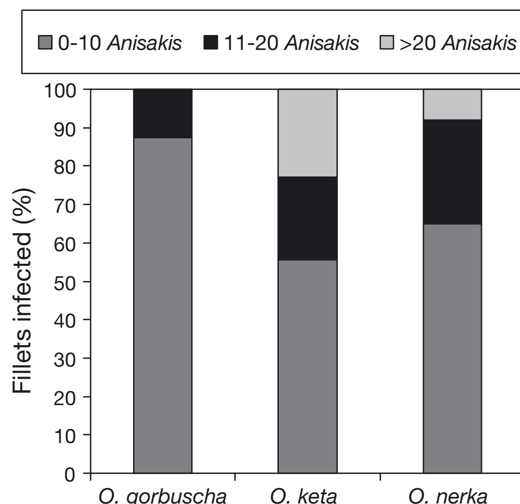

Fig. 3. *Anisakis* sp. infecting *Oncorhynchus* spp. Percentage of Pacific salmon fillets without belly flaps infected with 0–10, 11–20 and >20 nematodes kg^{-1} flesh

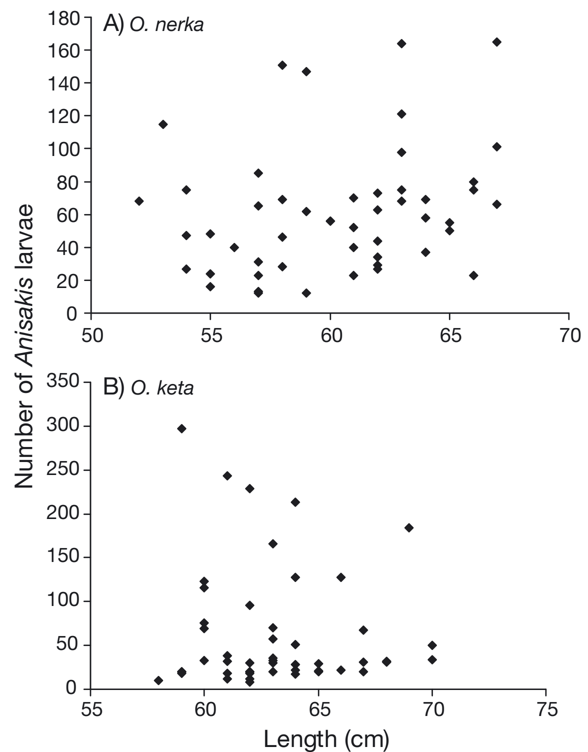

Fig. 4. *Anisakis* sp. infecting *Oncorhynchus* spp. Relationship between number of *Anisakis* larvae and fish total length in the edible part of (A) sockeye *O. nerka* and (B) chum salmon *O. keta* from Cordova, Alaska (USA)

Comparison of different fishing grounds

The intensity of *Anisakis* larvae was compared in fresh-caught *Oncorhynchus nerka* and *O. keta* salmon of commercial catches from the Bristol Bay area and from Cordova, respectively. In the Bristol Bay area, *O. nerka* samples were taken from the Nushagak and Naknek district and *O. keta* samples from the Nushagak district.

As most of the nematodes in Pacific salmon products on the German market have been detected in the belly flap area, only the hypaxial muscle was collected from salmon of Bristol Bay in this part of the investigation. It is known from other species that the distribution of *Anisakis* larvae within the same species does not vary much between different fishing grounds (Karl 2008). A comparison of the intensity in the belly flaps was therefore considered to be sufficient to estimate possible differences in infection levels in relation to the fishing ground.

The mean intensity of *Anisakis* larvae in the hypaxial muscle of *Oncorhynchus nerka* salmon was 3 times higher in the Bristol Bay area than in Prince William Sound close to Cordova (Table 4). On the other hand, there were no significant differences in the intensity of

Table 4. *Anisakis* sp. infecting *Oncorhynchus* spp. Comparison of larvae in the hypaxial muscle (belly flaps) of sockeye (*O. nerka*) and chum salmon (*O. keta*) from different fishing grounds of Alaska (PWS: Prince William Sound). Intensity data are given as mean (range). Intensity values with different superscripts within a row are significantly different (Mann-Whitney rank sum test, $p \leq 0.01$)

Infection parameter	Cordova, PWS	Bristol Bay area: Naknek	Bristol Bay area: Nushagak
O. nerka			
Number of fish	50	25	25
Prevalence (%)	100	100	100
Intensity	53[a] (7–150)	164[b] (43–311)	157[b] (70–279)
O. keta			
Number of fish	49		26
Prevalence (%)	100		100
Intensity	52[a] (7–244)		59[a] (5–285)

nematode larvae in *O. keta* salmon from both areas. The data indicate that the infection levels may vary between fishing grounds depending on the salmon species, but further studies are necessary to confirm these preliminary results.

Post-mortem migration studies

Previous studies on other fish species have shown rather controversial results concerning possible migration, and a hypothesis has been proposed that *Anisakis* larvae are only stimulated to migrate in 'fatty' fish species (Wharton et al. 1999). Alaskan salmon can be considered as more fatty than lean, and the purpose of this part of the study was to obtain more knowledge about possible post-mortem migration of *Anisakis* larvae from the viscera into the surrounding flesh of Alaskan salmon under commercial storage conditions either on ice or in RSW.

The storage of fresh-caught salmon in RSW for up to 24 h is normal practice which is applied widely on board the tenders receiving fish from small fishing boats during the salmon run seasons. Storage on wet ice was also tested, which is currently not viable due to the lack of ice manufacturing capacity in the US salmon industry. No tendency of post-mortem migration was observed from the viscera into the flesh, irrespective of the fish species and the storage conditions (Table 5). The results are not unexpected, considering the low abundance and intensity of *Anisakis* larvae in the viscera. The high number of nematodes in the muscle meat of fresh-caught Pacific salmon indicates that a migration into the flesh must occur either directly after intake of the nematodes via feeding or during earlier stages of the host life cycle.

CONCLUSION

One of the most important conclusions which can be drawn from this investigation is the fact that in maturing *Oncorhynchus keta*, *O. keta* and *O. gorbuscha* salmon from Alaska, *Anisakis* larvae (genetically identified as *A. simplex* s.s.) are more abundant in the musculature than in the viscera when returning from the open sea. More than 90% of the nematodes were found in the flesh, mainly in the hypaxial muscle. It appears that the infection level of the edible part depends on the fishing ground and the species, but further studies are required to get a more detailed picture of the infection intensities of Pacific salmon from the different commercial fishing areas of Alaska and

Table 5. *Anisakis* sp. infecting *Oncorhynchus* spp. Prevalence, intensity and abundance of larvae in the hypaxial muscle (belly flaps) and the viscera (without gonads) of Pacific salmon species in relation to the chilled storage conditions. Intensity and abundance data are given as mean (range). For the hypaxial muscle, no significant differences were found between storage methods within each species (Mann-Whitney rank sum test performed for each species, $p \leq 0.01$). RSW: refrigerated sea water

Storage conditions	n	Hypaxial muscle: Prevalence (%)	Hypaxial muscle: Intensity	Viscera: Prevalence (%)	Viscera: Intensity	Viscera: Abundance
O. keta						
At capture	49	100	52 (7–244)	74	2.8 (1–16)	2.1 (0–16)
24 h ice	23	100	49 (6–267)	91.4	5.2 (0–25)	5.7 (1–25)
24 h RSW	50	100	43 (7–328)	88	2.6 (1–7)	2.3 (0–7)
O. nerka						
At capture	50	100	52 (7–150)	72	3.0 (1–32)	2.2 (0–32)
24 h ice	50	100	55 (1–221)	68	3.6 (1–14)	2.5 (0–14)
24 h RSW	50	100	56 (8–160)	80	2.9 (1–14)	2.3 (0–14)
O. gorbuscha						
At capture	12	100	18 (4–38)	67	3.1 (1–5)	2.1 (0–5)
24 h ice	14	100	13 (2–25)	64	2.0 (1–5)	1.3 (0–5)

other countries. *O. keta* and *O. nerka* were more infected than *O. gorbuscha.* No migration of *Anisakis* larvae was observed from the viscera into the flesh during storage on ice or commercially in RSW storage for 24 h.

A reduction of the nematode number in the flesh is possible by complete removal of the belly flaps, but this cannot guarantee fillets with an abundance of <20 nematode larvae kg^{-1}. According to our data, more than 20% of *Oncorhynchus keta* and more than 8% of *O. nerka* fillets from the Prince William Sound close to Cordova exceeded the proposed limit.

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