Location and elimination of *Anisakis simplex* third stage larvae in Atlantic herring *Clupea harengus* L

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ABSTRACT

We here describe the location of anisakid third stage larvae in Atlantic herring *Clupea harengus* L. caught in the North Sea in August 2023. We further demonstrate how industrial processing (mechanical gutting, removal of entrails, head, tail, hypaxial anterior musculature and vertebral column) reduces the overall infection and worm load in the musculature. The isolated anisakid larvae were identified as *Anisakis simplex* sensu stricto by a combination of morphometrics and molecular methods (PCR of rDNA and mtDNA, sequencing, BLAST analysis). As a baseline we examined a total of 75 specimens of freshly caught and ungutted herring and showed a positive correlation between host size (fish length and weight) and infection level. The overall prevalence of infection was 84 %, the mean intensity 11.3 (range 1–38 parasites per fish) and the abundance 9.52. The main part of the overall worm population was associated with stomach and pyloric caeca in the body cavity (77 %) and only 5 % was found in the musculature. Larvae occurred in the hypaxial part of the musculature (21), the epaxial part (7 worms) and the caudal part (5 worms). The prevalence of muscle infection was 28 % and the mean intensity 1.6 (range 1–5) parasites per fish and abundance 0.44 parasites per fish. In order to assess the effect of industrial processing on worm occurrence in the fish we examined a total of 67 specimens of herring, from exactly the same batch, but following processing. This included removal of organs in the body cavity, cutting the lower part of the hypaxial segment but leaving the right and left musculature connected by dorsal connective tissue. Five out of these fish carried one larva (prevalence 7.5%, mean intensity 1, abundance 0.07 larvae per fish), and these worms were located in the ventral part of the anterior musculature (2), in the central part of the anterior musculature (2) and one larva in the central part of the caudal musculature. The industrial processing reduced the overall occurrence (abundance) of worms in the fish from 9.52 to 0.07 (136 times reduction) and the occurrence in the musculature from 0.44 to 0.07 (6.28 times reduction). The overall prevalence was reduced from 84 % to 7.5 % (11.2 times reduction). Muscle infection prevalence fell from 28 % to 7.5 % (3.7 times reduction). We then followed another batch of herring following a marinating process (11% NaCl for 24 h and subsequent incubation in acetic acid and vinegar) by artificially digesting the flaps during week 1–8. Although a total of 31 larvae were recovered from 144 fish examined no live nematode larvae were isolated. The importance of fish handling, processing and marination for consumer safety is discussed.

1. Introduction

The nematode genus *Anisakis* comprises a range of species such as *A. simplex, A. berlandi, A. pegreffi, A. physeteris, A. typica, A. ziphidarum, A. nascetti, A. brevispicula* and *A. paggiae* (Mattiucci et al., 2014). Some of these species are considered food hygienic problems associated with fish products due to their documented zoonotic potential (Adroher-Auroux and Benitez-Rodriguez, 2020). The life cycle of these nematodes comprises a cetacean as the definitive host (Lakemeyer et al., 2020), marine crustaceans (copepods, euphausids) as the first paratenic host and a number of marine fish species as the second paratenic host (Køie et al., 1995). Species within the genus occur from the Arctic to the Antarctic (Klimpel et al., 2010; Severin et al., 2020) and a wide range of fish species are susceptible to infection. The larvae may be abundant in wild fish carrying these parasites in various parts of the body including the musculature. Well characterized infections occur in gadids such as hake (Ceballos-Mendiola et al., 2010; Cipriani et al., 2015; Pascual et al., 2018; Santos et al., 2022); blue whiting (Gomez-Mateos et al., 2016;

Molina-Fernandez et al., 2018) and Atlantic cod (Yang et al., 2013; Mehrdana et al., 2014; Severin et al., 2020; Mercken et al., 2021; Karami et al., 2022), scombrids such as mackerel (Gutierrez-Galindo et al., 2010; Abattouy et al., 2011; Levsen et al., 2018), scorpaenids such as redfish (Klapper et al., 2015), clupeids such as Atlantic herring (Khalil, 1969, Smith and Wootten, 1975; Tolonen and Karlsbakk, 2003; Campbell et al., 2007; Bao et al., 2017; Mattiucci et al., 2018; Guardone et al., 2019), anchovies (Mladineo et al., 2012; Cipriani et al., 2016, 2018) and sardines (Buselic et al., 2018), salmonids such as Atlantic and Pacific salmon (Karl et al., 2011; Kent et al., 2020) and moronids such as seabass (Bernardi et al., 2011). Aquacultured fish kept in captivity, isolated from the environment and fed by artificial and heated fish feed, devoid of infective larvae, are generally found non-infected (Levsen and Maage, 2016; Penalver et al., 2010; Skov et al., 2009, 2014; Gonzalez et al., 2020; Castiglione et al., 2021; Fioravanti et al., 2021; Karami et al., 2022). One of the marine fish species known to carry significant burdens of *Anisakis simplex* is the Atlantic herring, Clupea harengus. The vernacular name of *A. simplex* is "herring worm" due to the frequent finding of the species in this host fish. Thus, different populations of herring have been subjected to parasitological investigations over time, and these studies have in most cases confirmed a significant worm load in the fish (Khalil, 1969; Smith and Wootten, 1975; McGladdery, 1986; Szostakowska et al., 2005; Campbell et al., 2007; Unger et al., 2014; Mattiucci et al., 2018). These parasites are zoonotic and may infect consumers following ingestion of fish meat containing live *Anisakis* larvae. The worm may penetrate the gastric or intestinal wall of the consumer and induce severe inflammatory reactions, which elicits the disease termed anisakiasis (Mattiucci et al., 2013; Buchmann and Mehrdana, 2016; Adroher-Auroux and Benitez-Rodriguez, 2020). Killing or inactivation of worms in fish products including herring has received special attention (Franssen et al., 2019a,b), and EU-regulation 853(2004) specifies that fish products intended for human consumption should be pre-treated with heat or freezing in order to kill live nematode larvae. However, active infections can sensitize the consumer and cause allergy. Several allergens released from even dead worms may therefore represent a problem for allergic consumers (Vidacek et al., 2011; Nieuwenhuizen and Lopata, 2013; Carballeda-Sangiao et al., 2014; 2016; Tejada et al., 2015; Ivanovic et al., 2017). This calls for further focus on occurrence of these nematode larvae in the products and methods for their detection of proteins from the worms are available (Werner et al., 2011; Fæste et al., 2015). The present study describes the occurrence of *Anisakis simplex* third stage larvae in Atlantic herring captured in the North Sea in August 2023. We followed the occurrence and site selection (microhabitat) of nematodes in the fish before and following industrial processing (mechanical removal of entrails, head and tail) in order to evaluate the extent of worm removal by the process. We further examined how incubation in a solution of acetic acid and salt affected survival of the remaining worms. Based on these results we discuss the importance of mechanical processing and post-treatment for improvement of consumer safety.

2. Materials and methods

We have performed a comparative parasitological examination of Atlantic herring before and after industrial processing in order to elucidate the location of parasites in different body compartments and the extent of process induced worm elimination of anisakid nematode larvae.

2.1. Fish

1) A shoal of Atlantic herring was caught by a commercial trawler in the North Sea (ICES area A4) August 8, 2023. The catch was immediately placed in refrigerated seawater (temperature −3 °C) on board. When reaching shore August 9, 2023, less than 24 h after capture, the fish were pumped into an industrial processing unit (Skagen, Denmark)

and subjected to the slaughter process (size grading, gutting, filletting and trimming). A total of 75 Atlantic herring specimens were sampled directly from the catch before entering the slaughter process in the factory. Their total body weight and total body length were recorded, whereafter they were examined for the presence of anisakid nematode larvae.
2) Following processing in the factory of this specific batch of herring an additional 67 herring specimens, which at this time point were only musculature and skin (right and left side of the fish were still connected dorsally by connective tissue), were examined for presence and location of nematode larvae.
3) We evaluated the effect, on the survival of nematode larvae, of industrial marination (24 h incubation in 11 % NaCl and subsequent storage in 200 L barrels containing a standard industrial acetic acid/NaCl solution at 3–4 °C). Over a period of 8 weeks we sampled and examined the musculature (right and left side connected) from a total of 144 herring from three 200 L barrels (18 fish weekly).

2.2. Parasitological examination

2.2.1. Examination of unprocessed fresh herring. An incision into the ventral part of the body cavity was performed in order to expose the body cavity for visual inspection. We recorded the number of fully or partly encapsulated nematode larvae and their precise location within the body cavity. Thus, their association with the mesenteries and surfaces of all visceral organs (stomach, pyloric caeca, intestine, liver and gonads) was recorded. Each individual herring was then filleted, skinned and separated into left and right sides. These were then divided into three compartments, the epaxial, hypaxial and caudal muscle segments, in order to reveal any site preference of parasites (Fig. 1). The fillets were then examined by the UV-press method (Karl and Leinemann, 1993). In brief, the musculature sections were placed individually in plastic bags (400 × 200 mm) and compressed to a 1–2 mm thickness. The bag was frozen at minus 20 °for 24 h and then exposed to UV irradiation (302 nm UV light using Macro Vue™ UV-20 Transilluminator, Hoefer©) in a dark room, which induced a white-bluish fluorescence of the *Anisakis* larvae, which facilitated fast detection. Worms were counted, isolated and identified using light microscopy and PCR with subsequent sequencing according to Mehrdana et al. (2014) and Zuo et al. (2018) as described below.

2.2.2. Examination of industrially processed herring. From the same herring batch a total of 67 fish were examined after industrial processing (mechanical and automatic gutting and filleting). These fish, consisting of the right and left side still connected dorsally (termed double fillet in the industry), were skinned and then subjected to a similar examination (left and right side, epaxial, hypaxial and caudal location of worms) using the UV-press method described above.

2.2.3. Examination of marinated herring. A total of 144 specimens of marinated herring (right and left side musculature of the herring connected dorsally), originating from another North Sea herring batch than 1) and 2), were subjected to artificial digestion using a solution of
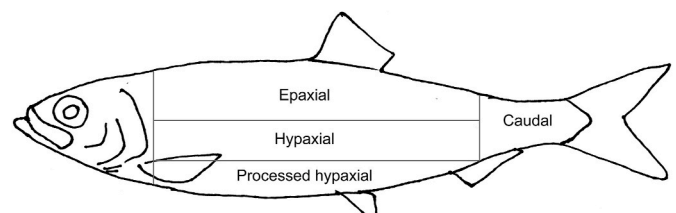

Fig. 1. Left side of a herring showing division of the musculature into epaxial, hypaxial and caudal segments. The lower part of the hypaxial part (processed hypaxial) is removed during industrial processing. The right side is segmented similarly. Following processing the right and left musculature segments are still kept connected by dorsal connective tissue.

pepsin, HCl and saline (Mehrdana et al., 2014) at 37 °C until full digestion. The lysate was then filtered and nematode larvae (if any) were recovered and placed in saline in glass Petri dishes for evaluation of viability (motility, vital staining by malachite green).

2.3. Morphological identification of nematode larvae to genus level

Subsamples of isolated nematode larvae were conserved in 96 % ethanol (CCS Health Care, Denmark) for one week, whereafter the anterior and caudal parts of each worm were mounted on a microscope slide with mounting medium Aquatec (Merck, Germany). These were then studied in the light microscope (Leica DM5000B, Germany) at 50, 200× and 400× magnification aiming at identifying diagnostic features (excretory pore location in relation to nerve ring, ventricle morphology, absence or presence of intestinal caecum and ventricular appendage, anterior and caudal morphology (with presence/absence of a larval boring tooth and mucron, respectively).

2.4. Molecular identification

Subsamples of the recovered larval worms preserved in 96% ethanol were also used for molecular identification. The middle part of the worm larva was removed aseptically for DNA extraction, whereas the anterior and caudal parts were mounted on slides for morphological analysis and genus determination as described above. The worm tissue was incubated in 100 μl lysis buffer (0.45%, Tween 20, 60 μl ml^{-1}, Proteinase K, 10 mM Tris and 1 mM EDTA) at 56 °C, 450 rpm, in the Eppendorf Thermomixer Comfort (Eppendorf AG). Incubation time continued until complete digestion as confirmed under the dissection microscope (magnification 8–100 x) (Leica MZ125, Germany). Proteinase K was then deactivated at 70 °C for 10 min, and the lysate was used for PCR amplification. For the rDNA we targeted the 18S, ITS1, 5.8S, ITS2 and 28S. For the mt DNA we targeted the cox2 gene. For the former rDNA region PCR was performed using the forward primer NC5 (5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and the reverse primer NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) (Zhu et al., 2007). For the latter cox2 region we used the primers 211F (5′-TTT TCT A TTA TAT AGA TTG RTT YAT-3′) and 210R (5′-CAC CAA CTC TTA AAA TTATC-3′) (Nadler and Hudspeth, 2000). Reactions were run in a T100™ Thermal Cycler (BioRad, Denmark) in a total reaction volume of 60 μL containing: 1 μM of each primer (Sigma-Aldrich, Denmark), 1 mM of dNTP-mix (Life Technologies, Denmark), 1.25 units of BIOTAQ DNA polymerase (DNA Technology A/S, Denmark), 1.5 mM MgCl2, and 6 μL 10 × PCR buffer and 2 μL DNA template was added. Finally, DNase and RNase free water (Invitrogen™, Life Technologies, Denmark) was added to a final reaction volume of 60 μL. Sterile water was used as negative control. The following PCR protocol for ITS was used: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s (s), annealing at 53 °C for 30 s, and elongation at 72 °C for 75 s. After cycling, a final elongation step at 72 °C for 7 min was performed. PCR conditions for cox2 were 2 min of pre-denaturation at 94 °C, followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 46 °C for 1 min and elongation at 72 °C for 1 min 30 s. A final step was performed for cox2 at 72 °C for 10 min. The PCR products were visualized by 2 % agarose gel electrophoresis and subsequently purified using Illustra™ GFX™PCR and Gel band purification kit (VWR, Denmark). Fragments were sequenced by Macrogen Inc. (Amsterdam, The Netherlands) using the PCR primers used for the amplification process. Complete sequences were submitted to GenBank.

2.5. Statistics and calculations

The prevalence of infection, the mean intensity and abundance was calculated according to Bush et al. (1997). The correlations between host total body length and total body weight and the number of *Anisakis* larvae was calculated using the non-parametric Spearman Rank test of correlation. The difference between worm counts in different groups

were tested by the non-parametric Mann-Whitney *U* test for differences of means. A probability level of 5 % was applied for all tests. All the statistical analyses were performed using Graph Pad Prism (USA).

3. Results

Fish. The Atlantic herring sampled for the analysis had a body length ranging from 25 cm to 33 cm, and a body weight from 130 g to 340 g. A significant correlation (r = 0.88) between body length and weight was found, but no significant size differences between the two sexes in the sample was seen (data not shown).

3.1. Species identification

The morphological analysis showed that all examined larvae belonged to the genus *Anisakis*, characterized by a ventriculus without appendage, no presence of an intestinal caecum, and the presence of a caudal mucron. The excretory pore was located anteriorly to the nerve ring surrounding the anterior oesophagus. The molecular analysis of the rDNA sequences and the mtDNA, obtained for all the tested nematode larvae in the subsample, showed that the worms in the herring were *Anisakis simplex* sensu stricto. Sequences for the rDNA and mt DNA were submitted to GenBank and obtained the accession numbers for the ITS region PP189864-PP189873 and for cox2 PP202994-PP203003. Comparison to other isolates revealed for ITS 100 % identity and for cox2 the identity ranged from 99.83 to 100 % (Supplementary file 1, Table of identities). The phylogenetic analysis confirmed a close affiliation with *A. simplex* s.s. (Supplementary file 2, Cladogram).

3.2. Infection level of un-processed herring

As a baseline we examined a total of 75 specimens of freshly caught and unprocessed herring and showed a positive correlation between host size (fish length and weight) and infection level (Fig. 2 AB). The overall prevalence of infection was 84 %, the mean intensity 11.3 (range 1–38 parasites per fish) and the abundance 9.52. No significant difference of infection level in the two host sexes was found (Table 1). The main part of the overall worm population (partially or fully encapsulated in mesenteries) was associated with stomach and pyloric caeca in the body cavity (77 %) and only 5 % was found in the musculature (Table 2, Fig. 3). The worm load in each of these microhabitats (organs and musculature) was positively correlated to fish size (Table 3). The musculature was further subdivided into compartments (Fig. 1) to detect differential internal niche selection of worms. This showed that although larvae mainly occurred in the hypaxial part of the musculature (21) they were also found in the epaxial part (7 worms) and the caudal part (5 worms) (Table 4). The prevalence of muscle infection was 28 % and the mean intensity 1.6 (range 1–5) parasites per fish and abundance 0.44 parasites per fish. No significant difference with regard to the number of worms in left side musculature (mean 1.40, SEM 0.07) when compared to the right side musculature (mean 1.33, 0.07) was found (Mann-Whitney *U* test p = 0.70).

3.3. Infection level of processed herring musculature

In order to assess the effect of industrial processing on worm occurrence in the fish we examined a total of 67 specimens of herring, from exactly the same batch, but following fish processing. Five out of 67 fish (in the form of left and right side musculature connected dorsally) carried one larva (prevalence 7.5%, mean intensity 1, abundance 0.07 larvae per fish), and these worms were located in the ventral part of the anterior musculature (2), in the central part of the anterior musculature (2) and one larva in the central part of the caudal segment of the musculature. The reduction of overall infection level decreased drastically by gutting and was further reduced by industrial processing. The overall infection fell to 4.6 % of the fresh herring level following
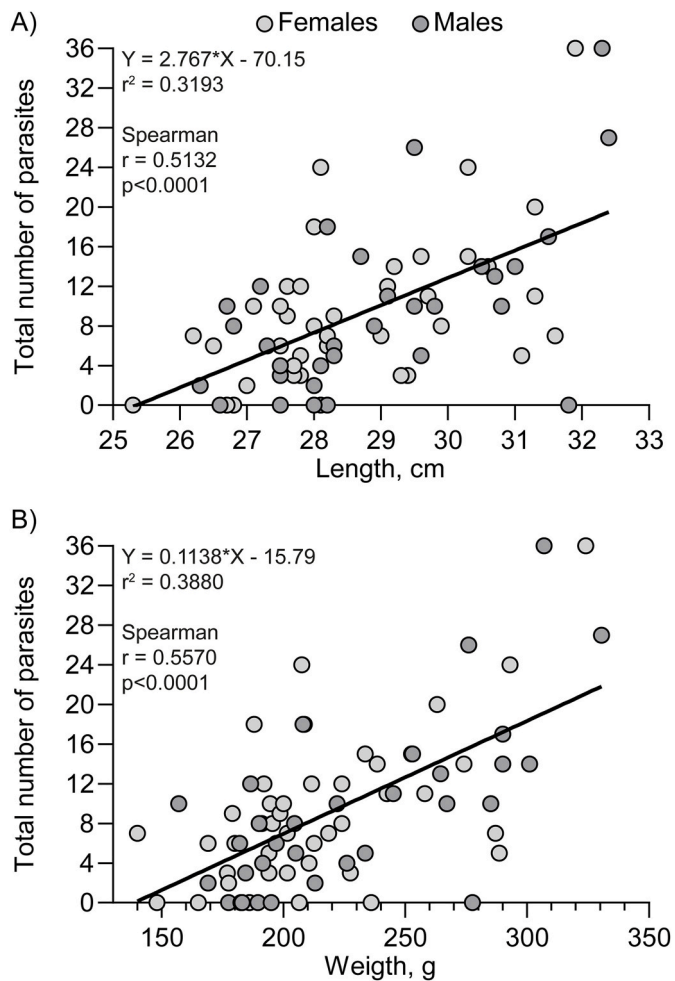
Fig. 2. A. The correlation between total body length of herring (unprocessed) and the number of nematode larvae in each fish.
B. The correlation between the total body weight of herring (unprocessed) and the number of nematode larvae in each fish.

Table 1
Infection level of *Anisakis simplex* in females and male herring. No significant difference between the two sexes was evident (Mann Whitney *U* test: p = 0.1836).

|	Females	Males
Abundance	9.45	9.61
Mean intensity	10.73	11.74
Range [min; max]	[0; 36]	[0; 38]
Prevalence	88%	82%

gutting, and by industrial processing the muscle infection fell to 15.6 % of the original level (Table 5).

3.4. Infection level of marinated herring musculature

Artificial digestion using pepsin and hydrochloric acid was used to isolate nematode larvae from herring musculature (left and right side of the herring connected dorsally, double fillets) following marination (salt and acetic acid incubation) over 8 weeks. From the 144 examined marinated herring examined we isolated a total of 31 nematode larvae, corresponding to an abundance of 0.22. The viability tests including microscopical evaluation of motility and vital staining showed no sign of viability in any of these worms, which by isolation all had a whitish and non-transparent appearance.

4. Discussion

The present study aimed primarily at determining the exact number and organ location of *A. simplex* third stage larvae in a commercial batch of Atlantic herring captured in the North Sea in 2023. Secondarily we wished to evaluate the effects on worm occurrence of industrial processing and post-processing with acetic acid and salt. We document a high overall infection level in fresh and unprocessed herring. This is in line with previous investigations of herring caught in the Atlantic and adjacent waters (Khalil, 1969; Smith and Wootten, 1975; Levsen and Lunestad, 2010; Mattiucci et al., 2018). We confirmed that only a minor part of the parasite population was located in the musculature, a picture which was reported previously (Levsen and Lunestad, 2010; Guardone et al., 2019). Nevertheless, these nematode larvae are of special concern due to their documented zoonotic potential and special care should be taken to reduce or inactivate the worm burden (Mattiucci et al., 2013; Franssen et al., 2019). We showed that the industrial processing reduced the occurrence of nematode larvae in the musculature. It is likely due to the partial removal of the lower ventral hypaxial musculature of the herring during the industrial processing by the automatic gutting and cutting machines. This part of the musculature carries a large part of the nematodes and the removal of the segment was previously suggested to remove a part of the parasites (Levsen and Lunestad, 2010). The remaining nematode larvae in the herring musculature are few, but their presence needs attention. Various methods are applicable for reduction of the presence of both live and dead worms. Mechanical removal of worm larvae from the body cavity of the fish may reduce migration of larvae post-capture, but additional measures taken to inactivate the
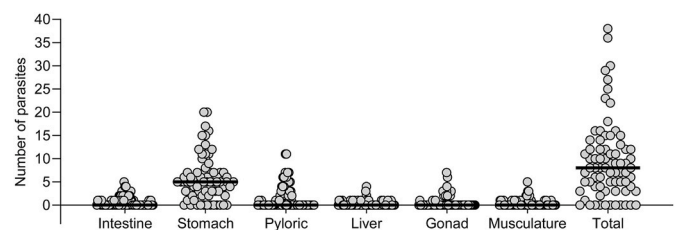
Fig. 3. The association of nematode larvae with musculature and body cavity organs in herring (unprocessed).

Table 2
Distribution of larval nematodes (*Anisakis simplex*) in a total of 75 specimens of unprocessed herring.

	Intestine	Stomach	Pyloric	Liver	Gonad	Musculature	All
No. of parasites	56	424	130	28	43	33	714
Percentage of all parasites	8%	59%	18%	4%	6%	5%	100%
Mean abundance	0.75	5.65	1.73	0.37	0.57	0.44	9.52
Mean intensity	1.81	6.73	4.06	1.33	2.69	1.57	11.16
Range [min; max]	[0; 5]	[0; 20]	[0; 11]	[0; 4]	[0; 7]	[0; 5]	[0; 38]
Prevalence	41%	84%	43%	28%	21%	28%	85%

Table 3

Spearman rank correlation coefficients for the association between the number parasites in a specific organ and the total length and the total weight of herring. *: p < 0.05.

	Intestine	Stomach	Pyloric	Liver	Gonad	Musculature	All
Organ location vs Weight							
Spearman r	0.4648	0.4301	0.6993	0.0289	0.2878	0.2037	0.5389
Spearman p	<0.0001*	0.0001*	<0.0001*	0.8059	0.0123*	0.0826	<0.0001*
Organ location vs Length							
Spearman r	0.3696	0.3714	0.6628	0.0222	0.2371	0.2614	0.4829
Spearman p	0.0011*	0.0010*	<0.0001*	0.8503	0.0405*	0.0235*	<0.0001*

Table 4

Distribution of larval nematodes *(Anisakis simplex)* in different parts of the musculature of fresh un-processed herring. Left and right side musculature of the fish were pooled, as there was no significant difference between them (Mann Whitney U- test p = 0.0918).

	Epaxial	Hypaxial	Caudal
No. of parasites	7	21	5
Percentage of total[a]	21%	64%	15%
Mean abundance	0.09	0.28	0.07
Mean intensity	1.40	1.24	1.67
Range [min, max]	[0; 3]	[0; 3]	[0; 3]
Prevalence	7%	23%	4%

parasites can secure consumer safety further. Apart from heat and freeze treatment (Lanfranchi and Sardella, 2010; Morsy et al., 2017) various physico-chemical treatments have been tested. These comprise pulsed-field-electric application (Onitsuka et al., 2022; Abad et al., 2023), high-pressure exposure (Lee et al., 2016), salt incubation (Anastasio et al., 2016) or incubation with vinegar in combination with sodium chloride (marination), a method which inactivates the nematode larvae within some weeks (Karl et al., 1995). Marinated (salt and acid treated) herring is a major commodity worldwide and a continuous focus on consumer health and safety rely on documentation of parasite elimination or inactivation from the raw materials. The present study confirmed that it was still possible to isolate larvae from marinated herring flaps when sampled weekly (week 1–8) after incubation in a marinating solution of acetic acid and NaCl, but that all the isolated larvae were dead. The main aim is to prevent that infected products reach the consumer and the present study has shown that a combination of catch handling, fast gutting and processing may reduce the overall parasite load. Thus, the industrial processing reduced the overall occurrence (abundance) of worms in the fish from 9.52 to 0.07 (136 times reduction) and the occurrence in the fillets from 0.44 to 0.07 (6.28 times reduction). The overall prevalence was reduced from 84 % to 7.5 % (11.2 times reduction). Muscle infection prevalence fell from 28 % to 7.5 % (3.7 times reduction). This indicates that a complete prevention is not achieved readily, but on the other hand that the processing has a significant effect on worm occurrence. In addition, subsequent incubation in high salt solution (11%) and exposure to acetic acid and NaCl was in this study associated with rapid loss of viability of the larvae in the musculature. Previous unpublished observations by the authors have

shown that *A. simplex* larvae recovered from the body cavity of Atlantic herring resist this treatment for several weeks, which suggests that a differential sensitivity of worm from different niches may exist. It has not been documented that live larvae isolated from the body cavity of herring are more resistant to chemical treatments when compared to larvae occurring in herring muscles. We know that fish react immunologically to *Anisakis* simplex larvae during their migration in the host (Bahlool et al., 2013), and it could be speculated that the larvae penetrating the musculature are exposed to the fish immune system to a higher extent than the encapsulated body cavity larvae. This raises the hypothesis that the larvae in the herring musculature are more sensitive to chemical treatments than the larvae in the body cavity, but further experimental studies should test this hypothesis.

5. Conclusion

The present study documented t*hat North Sea herring are infected with a significant load of Anisakis simplex s.s., but that the main part of the worm population is localized in the body cavity* along with the internal organs. The larvae in the musculature are mainly found in the hypaxial ventral muscular segment. Industrial processing (gutting) removes these larvae from the body cavity and the cutting of the musculature (left and right side connected dorsally) eliminates a part of the few nematodes still present in the musculature. The overall abundance in the herring was reduced 136 times by the industrial processing. The incubation of herring musculature in a standard solution of acetic acid and Sodium Chloride following an initial 24 h incubation in an 11 % salt solution left no live larvae in the musculature as judged from weekly examinations conducted over 8 weeks.

CRediT authorship contribution statement

Kaan Kumas: Investigation, Methodology, Writing – review & editing. **Azmi Al-Jubury:** Investigation, Methodology, Writing – review & editing. **Per W. Kania:** Investigation, Methodology, Software, Writing

Table 5

Infection data for 1) fresh ungutted herring, 2) fresh herring following manual gutting and 3) herring after industrial processing. Prevalence (P), mean intensity (MI, (range)) and abundance (A) shown for the whole fish, the body cavity with organs and the musculature. NA: not applicable.

	Whole fish	Percent of nematodes in the fish	Body cavity with organs	Musculature	Percent of nematodes in musculature
Fresh ungutted fish	P: 84 % MI: 11.3 (1–38) A: 9.52	100 %	P: 84 % MI: 9.7 (1–38) A: 8.2	P: 28 % MI: 1.6 (1–5) A: 0.44	100 %
Fresh gutted fish	P: 28 % MI: 1.6 (1–5) A: 0.44	4.6 %	NA (all organs removed)	P: 28 % MI: (1.6 (1–5) A: 0.44	100 %
Processed fish	P: 7.5 % MI: 1 A: 0.07	0.74 %	NA (all organs removed)	P: 7.5 % MI: 1 A: 0.07	15.6 %

– original draft, Writing – review & editing. **Taghrid Abusharkh:** Investigation, Methodology, Writing – review & editing. **Kurt Buchmann:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors all declare that they have no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.100937.

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