



Contents lists available at ScienceDirect

## Veterinary Parasitology

journal homepage: [www.elsevier.com/locate/vetpar](http://www.elsevier.com/locate/vetpar)



# *Anisakis simplex* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh

Arne Levsen\*, Bjørn Tore Lunestad

National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, 5817 Bergen, Norway

### ARTICLE INFO

#### Article history:

Received 24 November 2009

Received in revised form 25 March 2010

Accepted 29 March 2010

#### Keywords:

*Anisakis simplex*

Nematoda

*Clupea*

Norwegian spring spawning herring

Abundance

Human health risk

### ABSTRACT

The third stage larvae of the parasitic nematode *Anisakis simplex* commonly occur in most commercially important fish species of the North Atlantic, including Norwegian spring spawning herring (*Clupea harengus* L.). The presence of nematode larvae in the flesh of fish may significantly lower the aesthetical quality of the product, or even pose a consumer health risk, especially with regard to the possible allergenic nature of the larvae or molecular traces thereof. In this study, the occurrence and spatial distribution of *A. simplex* larvae in comparable size groups of Norwegian spring spawning herring caught in the north-eastern Norwegian Sea in October 2004 and in the outer basin of Vestfjorden, northern Norway, in November 2007, was investigated. Emphasis was put on manually- and industrially produced, i.e. automatically trimmed and skinned fillets of herring. The overall larval prevalence was 98–100% in the herring of all size groups and the abundance increased with increasing body weight in both sampling years. On an average 3.5% of the larvae were found in the belly flaps, i.e. the ventral portion of the body musculature covering the visceral cavity on both sides, while 0.5% occurred in the dorsal part of the fillets. The larval prevalence varied from 42 to 70% and 8 to 10% in the manually- and industrially produced fillets, respectively. Thus, any product that is based on industrially produced fillets of Norwegian spring spawning herring may still carry nematode larvae when put on the market. However, compared to the manually produced ones, especially those untrimmed, the probability of *A. simplex* larvae to be present in industrially produced fillets appears to be approximately 5–8 times lower.

© 2010 Elsevier B.V. All rights reserved.

## 1. Introduction

The Norwegian spring spawning (NSS) herring (*Clupea harengus* L.) comprises one of the largest and most valuable pelagic fish stocks in the North Atlantic. The stock is currently distributed from off south-west Norway to the Barents Sea and across the Norwegian Sea to the eastern coast of Iceland ([www.fisheries.no/marine.stocks/](http://www.fisheries.no/marine.stocks/)

[fish.stocks/herring](http://www.fish.stocks/herring)). The total catching volume of herring (NSS herring and North Sea herring combined) from the Norwegian economic zone reached 616,220 and 1,025,500 metric tons in 2004 and 2008, respectively ([www.fiskeridir.no/fiskeridir/english/statistics](http://www.fiskeridir.no/fiskeridir/english/statistics)). Most Norwegian herring catches are bound for various international markets and often shipped in a deep-frozen whole state, i.e. the fish is neither gutted nor filleted prior to export. However, a minor proportion of the catches are filleted automatically before packing, deep-freezing and shipping.

As with most marine fish species of the north-east Atlantic, NSS herring usually carry third stage larvae of the

\* Corresponding author. Tel.: +47 97740545; fax: +47 55905299.  
E-mail addresses: [Arne.Levsen@nifes.no](mailto:Arne.Levsen@nifes.no), [ale@nifes.no](mailto:ale@nifes.no) (A. Levsen), [blu@nifes.no](mailto:blu@nifes.no) (B.T. Lunestad).

parasitic nematode *Anisakis simplex* (Levsen et al., 2005; Tolonen and Karlsbakk, 2002). In north-east Atlantic waters including the current distribution area of NSS herring, *A. simplex sensu stricto* appears to be the only *Anisakis* species present (Mattiucci and Nascetti, 2006; Skov et al., 2009). The life cycle of the parasite involves various whale species as definitive host, apparently krill (Crustacea, Euphausiacea) as main intermediate host, and numerous fish species including herring as transport host, transferring the larvae from krill to the whales. In fish, the majority of *A. simplex* larvae are typically encapsulated as flat and tight coils, measuring 4–5 mm across, on the visceral organs, mesenteries and peritoneum (Berland, 1989; Davey, 1972). However, a smaller number of larvae may migrate from the abdominal cavity into the flesh, sometimes penetrating deeply into the epaxial musculature of the fish host (Davey, 1972; Smith, 1984). This behaviour eventually results in the presence of worms in the fish fillets, which again may draw attention from consumers and food safety authorities.

Besides the considerable quality reducing effect inferred from the presence of anisakid nematode larvae in fish, the parasites are zoonotically significant as well. Thus, anisakiasis, i.e. human infection with live *Anisakis* spp. larvae, is most frequently reported from East-Asia and some southern European countries where raw or lightly salted or marinated fish is part of the everyday diet (Adams et al., 1997; Chai et al., 2005; Higashi, 1985; Maggi et al., 2000). Resulting from an immediate immune reaction, the clinical manifestations of acute anisakiasis include epigastric pain, nausea, vomiting and diarrhoea (Bouree et al., 1995; Sakanari and McKerrow, 1989). Moreover, the results of various studies indicate that *A. simplex* larvae, both dead and alive, may cause allergic reactions after consumption of infected seafood (Audicana et al., 1995, 2002; Baeza et al., 2001; Pozo et al., 1996; Valls et al., 2005), or even at indirect contact with the parasite during occupational activities such as filleting or cooking (Nieuwenhuizen et al., 2006; Scala et al., 2001).

The potential consumer health hazard associated with the presence of anisakid larvae in fish is reflected in several national and international regulations, e.g. the EC-regulation 853/2004 laying down specific hygiene rules for food of animal origin (Anon., 2004). The main preventive measures to minimize the nematode-related consumer health risk include deep-freezing of the fish or fishery product at a core temperature below  $-20^{\circ}\text{C}$  for at least 24 h. However, the rules do neither cover the aesthetical, i.e. quality reducing aspect, nor the potential allergenic property of even dead worms or molecular traces thereof. Moreover, the use of fresh, unfrozen fillets of marine fish, e.g. lightly salted or marinated herring, produced by local enterprises or private households may represent a significant pathway for *Anisakis*-related disorders as well.

The present study aims to investigate the occurrence and spatial distribution of *A. simplex* third stage larvae in NSS herring, with emphasis on manually- (MPF) and industrially (IPF) produced fillets in two sampling years. Additionally, possible differences or correlations in larval abundance between comparable herring size groups, fillet types and sampling year were examined.

## 2. Materials and methods

Various NSS herring were obtained from commercial catches in the north-eastern Norwegian Sea ( $70^{\circ}15'N$   $15^{\circ}30'E$ ) in October 2004 and in the outer basin of Vestfjorden ( $67^{\circ}40'N$   $13^{\circ}45'E$ ) in November 2007. Immediately after landing of the catches at a pelagic fish production plant in Lofoten Islands, northern Norway, whole herring were randomly collected from the assembly line, measured (fork length  $\pm 5$  mm) and weighed ( $\pm 1$  g), and subsequently attributed to the following size groups (SG): SG I, small  $< 200$  g, SG II, medium 200–400 g, and SG III, large  $> 400$  g. Additionally, various IPF ( $n = 50$  and  $n = 200$  in 2004 and 2007, respectively), were randomly collected immediately after emergence from the filleting machine and subsequently measured (length  $\pm 5$  mm and thickness  $\pm 0.1$  mm) and weighed ( $\pm 1$  g). In either year, the fillets were produced from herring belonging to the same catches from which the whole fish were obtained. In the catches of 2004, only a few SG I herring were present which, after automatic sorting, were all discarded. Thus, fish belonging to this size group were not included that year. Length, weight, fillet thickness and sample size of both whole herring and IPF per sampling year are shown in Table 1.

In 2004, whole herring ( $n = 50$ ) were gutted and manually filleted prior to placing the visceral organs including the mesenteries in Petri dishes before examination for nematodes under a dissecting microscope. Additionally, the visceral cavity and the peritoneal linings of each fish were macroscopically examined for nematode larvae. The fillets including the belly flaps and the backbone carrying epaxial muscle remains of every herring were then subjected to artificial digestion in an aqueous HCl-Pepsin solution as described by Lunestad (2003). The procedure was conducted by using 2.5 L glass flasks each containing the skin, flesh and backbone of a single herring. After complete degradation of the soft tissue, the hydrolysate of every flask was visually examined following Levsen et al. (2005). The same method and set-up was used to artificially digest 50 IPF produced from the same catch as the manually processed whole herring. Thus, the trial comprised 100 untrimmed MPF and 50 trimmed, industrially produced fillets without belly flaps.

In 2007, another approach was applied in order to facilitate work speed, thus allowing the processing of a larger sample number per time unit compared to the former procedure. The method utilises the fluorescence of frozen nematodes (Pippy, 1970) and is based on visual inspection of flattened/pressed and deep-frozen fish fillets or viscera under UV-light (Karl and Leinemann, 1993). A total of 150 whole herring were gutted, manually filleted and skinned before placing the visceral organs including the heart and swim bladder, and both left and right flesh side (fillets incl. belly flaps) of each fish into separate clear plastic bags. The position of the fillets in the bags was indicated using a fibre tip permanent marker. The fillets and viscera were then pressed to 1–2 mm thickness in a commercially available pressing device. The bags containing the flattened fillets or viscera were then deep-frozen ( $-18^{\circ}\text{C}$ ) for at least 12 h prior to visual inspection under a 366 nm UV-light source. Additionally, 200 IPF from the

**Table 1**

Length, weight and thickness of whole fish and industrially produced fillets of Norwegian spring spawning (NSS) herring in 2004 and 2007. The data are given as mean  $\pm$  SD (range).

|                                    | Length (mm)            | Weight (g)             | Thickness <sup>a</sup> (mm) |
|------------------------------------|------------------------|------------------------|-----------------------------|
| <b>NSS herring, Oct. 2004</b>      |                        |                        |                             |
| SG I: <200 g ( <i>n</i> = 30)      | 301 $\pm$ 11 (285–320) | 322 $\pm$ 39 (265–388) |                             |
| SG II: 200–400 g ( <i>n</i> = 20)  | 326 $\pm$ 7 (315–345)  | 446 $\pm$ 34 (402–528) |                             |
| All size groups ( <i>n</i> = 50)   | 311 $\pm$ 16 (285–345) | 371 $\pm$ 71 (265–528) |                             |
| IPF <sup>b</sup> ( <i>n</i> = 50)  | 175 $\pm$ 15 (148–203) | 58 $\pm$ 8 (40–71)     | 12.1 $\pm$ 1.3 (10.3–14.1)  |
| <b>NSS herring, Nov. 2007</b>      |                        |                        |                             |
| SG I: <200 g ( <i>n</i> = 49)      | 250 $\pm$ 14 (200–270) | 147 $\pm$ 26 (72–193)  |                             |
| SG II: 200–400 g ( <i>n</i> = 52)  | 302 $\pm$ 16 (265–330) | 314 $\pm$ 57 (203–399) |                             |
| SG III: >400 g ( <i>n</i> = 49)    | 337 $\pm$ 10 (315–365) | 448 $\pm$ 32 (403–530) |                             |
| All size groups ( <i>n</i> = 150)  | 296 $\pm$ 38 (200–365) | 303 $\pm$ 129 (72–530) |                             |
| IPF <sup>b</sup> ( <i>n</i> = 200) | 165 $\pm$ 16 (102–212) | 56 $\pm$ 12 (24–81)    | 11.8 $\pm$ 1.1 (9.5–13.0)   |

Abbreviations: SD, standard deviation; IPF, industrially produced fillets; SG, size group.

<sup>a</sup> Measured for the IPFs only.

<sup>b</sup> Not separated into size groups

same catches as the whole fish were similarly processed prior to examination under UV-light. Any *Anisakis* larvae present appear as more or less brightly fluorescent spots in the samples (Fig. 1). Additionally, the method allows the approximate determination of the larval infection site in the fillets, i.e. whether they are situated in the dorsal (upper) or ventral (lower) portion of the fish flesh.

In both sampling years, the nematode larvae of the visceral cavity were macroscopically separated at genus level, i.e. either *Anisakis* or *Hysterothylacium*, due to their different appearance *in situ*. At microscopic investigations of various *Anisakis* subsamples from both the visceral organs and the flesh each year, the larvae were identified as *A. simplex*, larval type I (Berland, 1961), which in north-east Atlantic waters has been shown to correspond genetically to *A. simplex* s.s. (Mattiucci and Nascetti, 2006; Skov et al., 2009).

### 2.1. Data analyses

*t*-Tests were run to check for differences in fish body weight between comparable size groups (SG II and SG III),

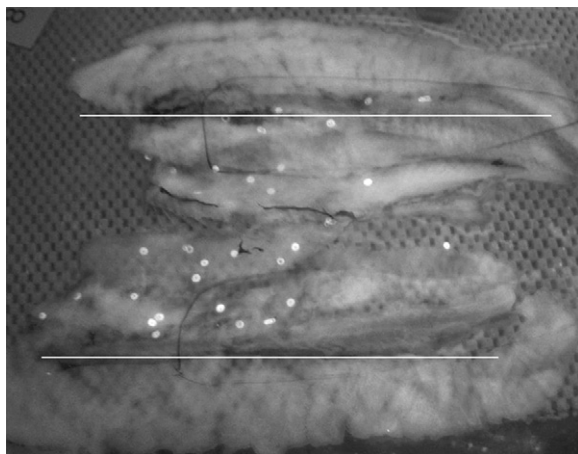


**Fig. 1.** Pressed and subsequently frozen untrimmed fish fillets seen under UV-light. The *Anisakis* larvae present emerge as brightly fluorescent spots. The straight lines mark the approximate boundary between the belly flaps and the dorsal musculature of each fish side.

and the thickness of the IPFs, of both sampling years. To analyse the effects of fish size (SG II and SG III) and sampling year on overall larval abundance (equals intensity at 100% prevalence), a generalised linear model (GLM) procedure was run, with fish size (weight) and year defined as continuous and categorical predictors, respectively. The abundance data were log-transformed ( $\ln x + 1$ ) in order to fit a normal distribution. The goodness-of-fit of the model was checked by the ratio of deviance to degree of freedom (deviance/df < 1). The contributions of each predictor and their significance were assessed with a likelihood type III test. The relationships between fish host size and larval abundance in the flesh and between abundance in the viscera and flesh each year were analysed by Spearman rank tests. Fisher's exact test was applied to test for differences between comparable herring size groups regarding the prevalence of *A. simplex* larvae in the MPFs – based on number of larvae per fish – and the IPFs in both sampling years. A two-sided bootstrap *t*-test was run (N bootstrap replications = 2000) to test for possible differences in larval abundance means between herring size groups II and III, and to compare the abundance means in the MPFs, of both sampling years. A bootstrap *t*-test was also used to compare the mean abundance in the left and right flesh side, and the ventral and dorsal muscle part, of the MPFs in 2007. The significance level was set at 0.05. The definition of terms (prevalence, abundance, mean abundance) to quantitatively describe the parasite infection data, are following Bush et al. (1997).

### 3. Results

The overall prevalence of *A. simplex* larvae was virtually total, i.e. 98–100%, in the herring of all size groups in both sampling years. Since there was no significant difference between the body weight of comparable fish size groups (SG II and SG III) of the 2004 and 2007 samples ( $p < 0.58$ ), the infection data of the two sampling years may be statistically compared. Additionally, there was no significant difference between the IPF thickness of both sampling years when correcting for the absence of small fish (SG I) in the catches of 2004 ( $p < 0.09$ ). Both fish host size (SG II and SG III) and sampling year had significant effects on total lar-

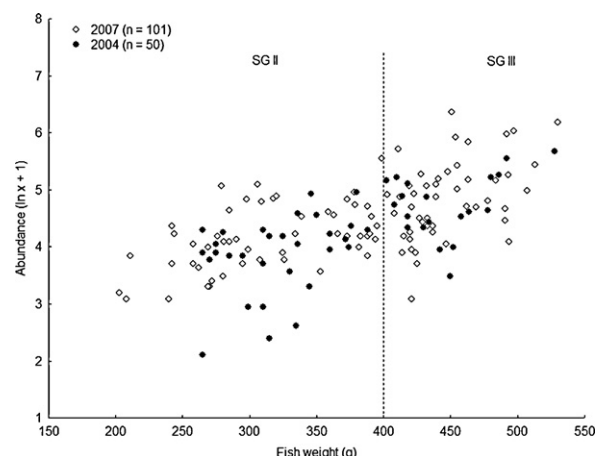


**Fig. 2.** Abundance ( $\ln x + 1$ ) of *Anisakis simplex* larvae in Norwegian spring spawning herring per fish size group (SG) and sampling year.

val abundance (df 1,  $\chi^2 = 72.1$ ,  $p < 0.00001$  and df 1,  $\chi^2 = 4.1$ ,  $p < 0.04$ , respectively). However, while there was a highly significant effect of fish host size on total larval abundance, sampling year appeared to have only secondary influence on the probability of infection. Fig. 2 illustrates the relationship between fish host weight of comparable size groups and total larval abundance per sampling year. The infection data including prevalence, abundance and maximum number of *A. simplex* third stage larvae in the MPFs, viscera and IPFs pr fish size group and sampling year are shown in Table 2.

In 2004, no significant correlation between fish weight and larval abundance in the flesh was found. This applied also for the relationship between larval abundance in the visceral organs and the flesh that year. In 2007, however, the larval abundance in the flesh was positively correlated with fish weight ( $r = 0.27$ ). Contrary to 2004, there was also a positive correlation in larval abundance between the viscera and flesh ( $r = 0.32$ ). The *A. simplex* abundance in the left and right flesh side (MPF) of the 2007 samples did not differ significantly (bootstrap  $t$ -test,  $p < 0.72$ ). However, there

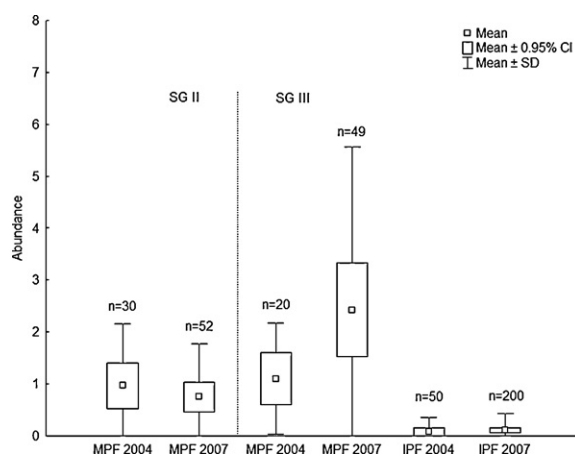


**Fig. 3.** Mean abundance of manually (MPF) and industrially (IPF) produced fillets of Norwegian spring spawning herring per fish size group (SG, for MPF only) and sampling year.

was a highly significant difference between larval abundance of the ventral and dorsal parts of the MPFs (bootstrap  $t$ -test,  $p < 0.0001$ ).

Comparison of the infection data regarding the MPFs of comparable size groups (SG II and SG III combined) in 2004 ( $n = 50$ ) and 2007 ( $n = 101$ ) showed no significant differences as to both prevalence (Fisher's exact test,  $p < 0.39$ ) and abundance (bootstrap  $t$ -test,  $p < 0.07$ ). However, the larval abundance in the MPFs of the largest size group (SG III) differed significantly between the two sampling years (bootstrap  $t$ -test,  $p < 0.02$ ), i.e. there was more than a twofold increase in this size group from 2004 to 2007 (Fig. 3). This apparently coincided with an increase in total abundance over the 3-year period between the two samplings (bootstrap  $t$ -test,  $p < 0.03$ ). The relative proportion of larvae residing in the dorsal part of the flesh was more than twice as high in the smallest herring size group (SG I) compared to size groups II and III. For all size groups combined, 0.5% and 3.5% of the larvae were found in the dorsal muscle part and the belly flaps, respectively (Fig. 4). The IPF sam-

**Table 2**

Prevalence, abundance and maximum number of *Anisakis simplex* larvae in whole fish and industrially produced fillets of Norwegian spring spawning (NSS) herring in 2004 and 2007.

|                               | Prevalence (%) |     |       | Abundance (mean ± SD; CI) |                   |                   | Abundance range |        | Max. no. of larvae |
|-------------------------------|----------------|-----|-------|---------------------------|-------------------|-------------------|-----------------|--------|--------------------|
|                               | MPF*           | VO  | Total | MPF*                      | VO                | Total             | MPF*            | VO     |                    |
| <b>NSS herring, Oct. 2004</b> |                |     |       |                           |                   |                   |                 |        |                    |
| SG II: 200–400 g (n = 30)     | 57             | 100 | 100   | 1.0 ± 1.2; 0.4            | 20.3 ± 11.6; 4.2  | 21.2 ± 11.9; 4.3  | 0–5             | 2–52   | 52                 |
| SG III: >400 g (n = 20)       | 70             | 100 | 100   | 1.1 ± 1.1; 0.5            | 46.6 ± 25.5; 11.2 | 47.7 ± 25.1; 11.0 | 0–4             | 11–107 | 107                |
| All size groups (n = 50)      | 62             | 100 | 100   | 1.0 ± 1.1; 0.3            | 30.8 ± 22.4; 6.4  | 31.8 ± 22.3; 6.4  | 0–5             | 2–107  | 107                |
| IPF (n = 50)                  |                |     | 8     |                           |                   | 0.1 ± 0.3; 0.08   |                 |        | 1                  |
| <b>NSS herring, Nov. 2007</b> |                |     |       |                           |                   |                   |                 |        |                    |
| SG I: <200 g (n = 49)         | 43             | 98  | 98    | 0.7 ± 1.1; 0.3            | 9.5 ± 9.4; 2.6    | 10.2 ± 9.7; 2.7   | 0–5             | 0–52   | 52                 |
| SG II: 200–400 g (n = 52)     | 42             | 100 | 100   | 0.8 ± 1.0; 0.3            | 26.7 ± 16.1; 4.4  | 27.5 ± 16.4; 4.5  | 0–3             | 8–92   | 95                 |
| SG III: >400 g (n = 49)       | 67             | 100 | 100   | 2.4 ± 3.1; 0.9            | 55.3 ± 43.8; 12.5 | 57.7 ± 44.7; 12.7 | 0–13            | 6–202  | 213                |
| All size groups (n = 150)     | 51             | 99  | 99    | 1.3 ± 2.1; 0.3            | 30.4 ± 33.0; 5.3  | 31.7 ± 33.9; 5.5  | 0–13            | 0–202  | 213                |
| IPF (n = 200)                 |                |     | 10    |                           |                   | 0.1 ± 0.3; 0.05   |                 |        | 2                  |

Abbreviations: CI, 95% confidence interval; IPF, industrially produced fillets; MPF, manually produced fillets; SG, size group; VO, visceral organs including mesenteries.

\* Data given for larval infection in the flesh per fish, i.e. both MPFs of each fish combined.

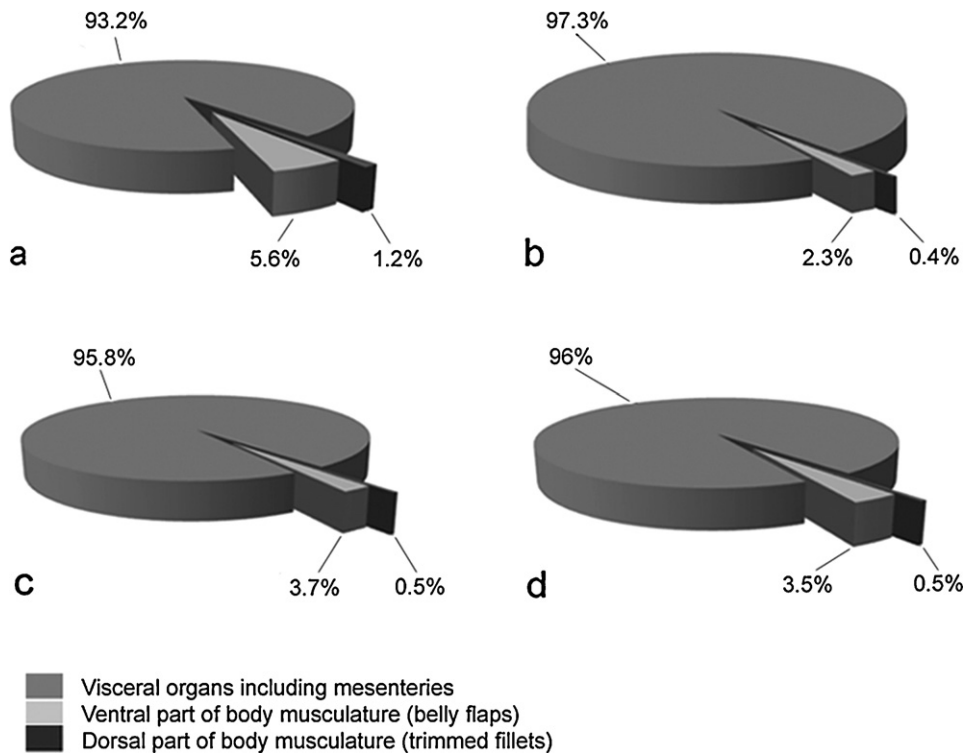


**Fig. 4.** Relative distribution of *Anisakis simplex* larvae in various body parts of Norwegian spring spawning herring. (a) Size group I, (b) size group II, (c) size group III, and (d) size groups I–III combined.

ples of 2004 and 2007 did not differ significantly, neither with regard to prevalence (Fisher's exact test,  $p < 0.79$ ) nor abundance (bootstrap  $t$ -test,  $p < 0.59$ ) (Fig. 3). The ratio in average larval prevalence between the MPFs and IPFs of the 2004 and 2007 samples was 7.8:1 and 5.1:1, respectively (Table 2).

#### 4. Discussion

Consumers expect parasite-free fish and fishery products. However, large-scale harvested and processed wild fish appears to be the only industrially produced food which is at risk to carry parasites when put on the market. As shown in the present study, this applies also for the NSS herring stock. The overall prevalence is almost total in all size groups of both sampling years. In both years, there was an increase in total larval abundance with increasing herring body size. The present findings of the 2004 samples are in close correspondence with those of Levsen et al. (2005) who, among other things, investigated the prevalence and abundance of *A. simplex* larvae in various body parts including the flesh of NSS herring from the southern spawning grounds (62°N 5°E) in February of 2003 and 2004 ( $n = 58$ ), and the wintering area (67°N 14°E) in October 2003 ( $n = 20$ ). However, the overall abundance and maximum number of larvae recorded in 2007 appear to be higher than the findings of Levsen et al. (2005) who used basically similar detection methods and size group categories. Although the overall sample size was considerably lower compared to the present study, the authors also recorded

a significantly positive relation between larval abundance and herring body size, which apparently was independent of locality and date of sampling.

Tolonen and Karlsbakk (2002) studied the parasite fauna including *A. simplex* larvae of young and mature NSS herring from the feeding grounds in the Norwegian Sea and the wintering areas in northern Norwegian fjords during summer 1993, December 1994 and April 1995. Their sampling stations correspond roughly to the sampling areas of the present study. When attributing the herring body length given by the authors to the different size groups of the current investigation, assuming that body length is fairly proportional to body weight, both prevalence and abundance appeared to be generally lower in 1993–1995 compared to the present findings. For example, the *A. simplex* infection data of December 1994, apparently covering all herring size groups as defined in the current study, ranged from 68–97% to 2.1–17.3 in prevalence and mean abundance, respectively ( $n = 68$ ). However, Tolonen and Karlsbakk (2002) based their investigation on plain visual inspection which may have lowered the detection efficiency considerably, especially regarding the detection of nematode larvae in the flesh.

In North Sea herring (*Clupea harengus*), another large and commercially important herring stock in the NE Atlantic, comparatively large variations in *Anisakis* infection parameters seem to exist as well. For example, Smith and Wootten (1975) who studied the occurrence and post-mortem flesh migrating behaviour of *Anisakis* sp. larvae in North Sea herring off East Shetland, UK, during summer

of 1971, recorded similar prevalence and abundances in the flesh of roughly corresponding herring size groups (SG I and SG II) compared to the present findings. Karl (2008), on the other hand, reported considerably lower *Anisakis* sp. abundances in the flesh of herring from the northern and central North Sea between 1996 and 2002. However, prior to analysis, he trimmed the fillets by removing most of the belly flaps in order to follow the commercial practice for mechanical filleting of herring (Karl, 2008). Additionally, the size of the fish investigated by the latter author, ranging 230–278 mm in average length, seemed to be smaller compared to the fish examined by Smith and Wootten (1975) as well as the current NSS herring samples. Smith and Wootten (1975) applied the artificial digestion method to detect nematode larvae in the flesh, while Karl (2008) used the pressing technique followed by visual inspection under a UV-light source. Hence, the results of both studies are likely to reflect the actual infection level in the flesh of North Sea herring at the time of sampling.

The results of previous studies (Banning and Becker, 1978; Davey, 1972; Karl, 2008; Khalil, 1969; Levsen et al., 2005; Smith and Wootten, 1975; Tolonen and Karlsbakk, 2002), along with the current findings, imply that considerable temporal and spatial variation in *Anisakis* sp. larval occurrence exists, both within and between the North Sea herring and the NSS herring stock. This variation may be due to ecological and/or behavioural differences, e.g. feeding habit, between the two herring stocks, but may also reflect differences in larval infection level in herring samples with only small or limited range in body size. Additionally, the use of plain visual inspection may significantly lower the detection efficiency, especially in the fish flesh, which again may, at least in part, account for the comparatively lower *A. simplex* prevalence and abundance recorded in NSS herring by Tolonen and Karlsbakk (2002). In the current study, the larval abundance is positively correlated with fish size. The same trend was found by Tolonen and Karlsbakk (2002) and Levsen et al. (2005), and appears to apply for North Sea herring as well (Banning and Becker, 1978; Davey, 1972; Khalil, 1969; Roepstorff et al., 1993).

The present findings as to the larval distribution in the MPFs show that most of the larvae are situated in the belly flaps, i.e. the ventral portion of the body musculature, regardless of body size. This is in general agreement with Karl (2008) who found, on an average, 1.8% of all larvae in the belly flaps while 0.6% of the larvae resided in the dorsal part of the flesh of North Sea herring. Thus, for herring of both stocks, manual trimming of the fillets by removing the belly flaps may contribute considerably to reduce the potential consumer health risk inflicted from the possible presence of *Anisakis* sp. larvae in actual products.

In the 2007 samples of the present study there are several 'extremes', especially in the upper range of both overall *A. simplex* abundance and abundance in the MPFs compared to the findings of 2004 (Figs. 2 and 3). Although statistically significant, the apparent difference in larval abundance between the 2004 and 2007 samples may imply that a sample size  $\leq 30$  is too low in order to cover the actual variance in *A. simplex* abundance per size group of NSS herring. This aspect may also be reflected by the significantly positive relationship between fish weight and larval abundance in

the flesh, and between the visceral organs and the flesh in 2007. In 2004, where the overall sample size was three-times lower compared to 2007, none of these parameters were significantly correlated. Thus, the apparent increase from 2004 to 2007 of both total larval abundance and larval abundance in the flesh (SG III) may as well reflect the difference in sample size rather than a true change of infection level. Moreover, due to the complexity of the north-east Atlantic pelagic ecosystem in general and the *A. simplex* life cycle in particular, it seems unlikely that a true increase in *A. simplex* abundance in any pelagic fish host species would manifest itself over just a 3-year period.

As far as we know, the present study provides the first occurrence data of *A. simplex* larvae in industrially produced, i.e. automatically trimmed and skinned fillets of herring. Since the thickness of the IPFs did not differ significantly between the 2004 and 2007 samples, the findings of the two years may be statistically compared. Both prevalence and abundance of larvae in the IPFs were low in both sampling years and did not differ significantly. The findings show, however, that consumers may still encounter, or become exposed to *A. simplex* larvae when preparing and consuming products based on industrially produced fillets of NSS herring. Although the IPFs are usually deep-frozen prior to shipping, a certain possibility of allergic reactions in sensitized consumers still exists. However, compared to the manually produced fillets, especially in untrimmed ones, the probability that *A. simplex* larvae are present in the IPFs appears to be about 5–8 times lower.

## Acknowledgements

We wish to thank the direction and staff of Lofoten Pelagiske AS for providing fish and work facilities during the field work of this study in 2004 and 2007.

## References

- Adams, A.M., Murrell, K.D., Cross, J.H., 1997. Parasites of fish and risk to public health. Rev. Sci. Tech. Off. Int. Epizoot. 16, 652–660.
- Anonymous, 2004. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.
- Audicana, M.T., De Corres, L.F., Muñoz, D., Fernández, E., Navarro, J.A., del Pozo, M.D., 1995. Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing fish. J. Allergy Clin. Immunol. 96, 558–560.
- Audicana, M.T., Ansotegui, I.J., Corres, D.E., Kennedy, M.W., 2002. *Anisakis simplex*: dangerous—dead and alive? Trends Parasitol. 18, 20–25.
- Baeza, M.L., Zubeldia, J.M., Rubio, M., 2001. *Anisakis simplex* allergy. J. Allergy Clin. Immunol. 13, 242–249.
- Banning, P., van, Becker, H.B., 1978. Long-term survey (1965–1972) on the occurrence of *Anisakis* larvae (Nematoda: Ascaridida) in herring, *Clupea harengus* L., from the North Sea. J. Fish Biol. 12, 25–33.
- Berland, B., 1961. Nematodes from some Norwegian marine fishes. Sarsia 2, 1–50.
- Berland, B., 1989. Identification of larval nematodes from fish. In: Möller, H. (Ed.), Nematode Problems in North Atlantic Fish. Workshop Report, Kiel, Germany, 3–4 April 1989. Int. Counc. Explor. Sea CM/F:6, pp. 16–22.
- Bouree, P., Paugam, A., Petithory, J.-C., 1995. Anisakidosis: report of 25 cases and review of the literature. Comp. Immun. Microbiol. Infect. Dis. 18, 75–84.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.
- Chai, J.-Y., Murell, K.D., Lymbery, A.J., 2005. Fish-borne parasitic zoonoses: status and issues. Int. J. Parasitol. 35, 1233–1254.

- Davey, J.T., 1972. The incidence of *Anisakis* sp. larvae (Nematoda: Ascaridata) in commercially exploited stocks of herring (*Clupea harengus* L., 1758) (Pisces: Clupeidae) in British and adjacent waters. *J. Fish Biol.* 4, 535–554.
- Higashi, G.I., 1985. Foodborne parasites transmitted to man from fish and other aquatic foods. *Food Technol.* 39, 69–74.
- Karl, H., 2008. Nematode larvae in fish on the German market: 20 years of consumer related research. *Arch. Lebensmittelhyg.* 59, 107–116.
- Karl, H., Leinemann, M., 1993. A fast and quantitative detection method for nematodes in fish fillets and fishery products. *Arch. Lebensmittelhyg.* 44, 105–128.
- Khalil, L.F., 1969. Larval nematodes in the herring (*Clupea harengus*) from British coastal waters and adjacent territories. *J. Mar. Biol. Assoc. U.K.* 49, 641–659.
- Levsen, A., Lunestad, B.T., Berland, B., 2005. Low detection efficiency of candling as a commonly recommended inspection method for nematode larvae in the flesh of pelagic fish. *J. Food Prot.* 68, 828–832.
- Lunestad, B.T., 2003. Absence of nematodes in farmed Atlantic salmon (*Salmo salar* L.) in Norway. *J. Food Prot.* 66, 122–124.
- Maggi, P., Caputi-Iambrenghi, O., Scardigno, A., Scoppetta, L., Saracino, A., Valente, M., Pastore, G., Angarano, G., 2000. Gastrointestinal infection due to *Anisakis simplex* in southern Italy. *Eur. J. Epidemiol.* 16, 75–78.
- Mattiucci, S., Nascetti, G., 2006. Molecular systematic, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. *Parasite* 13, 99–113.
- Nieuwenhuizen, N., Lopata, A.L., Jeebhay, M.F., Herbert, D.R., Robins, T.G., 2006. Exposure to fish parasite *Anisakis* causes allergic airway hyperactivity and dermatitis. *J. Allergy Clin. Immunol.* 117, 1098–1105.
- Pippy, J.H., 1970. Use of ultraviolet light to find parasitic nematodes *in situ*. *J. Fish. Res. Board Canada* 27, 963–965.
- Pozo, M.D. del, Moneo, I., De Corres, L.F., Audicana, M.T., Muñoz, D., Fernández, E., Navarro, J.A., García, M., 1996. Laboratory determinations in *Anisakis simplex* allergy. *J. Allergy Clin. Immunol.* 97, 997–984.
- Roepstorff, A., Karl, H., Bloemsma, B., Huss, H.H., 1993. Catch handling and the possible migration of *Anisakis* larvae in herring, *Clupea harengus*. *J. Food Protect.* 56, 783–787.
- Sakanari, J., McKerrow, J.H., 1989. Anisakiasis. *Clin. Microb. Rev.* 2, 278–284.
- Scala, E., Giani, M., Pirrotta, L., Guerra, E.C., Cadoni, S., Girardelli, C.R., De Pità, O., Puddu, P., 2001. Occupational generalised urticaria and allergic airborne asthma due to *Anisakis simplex*. *Eur. J. Dermatol.* 11, 249–250.
- Skov, J., Kania, P.W., Olsen, M.M., Lauridsen, J.H., Buchmann, K., 2009. Nematode infections of maricultured and wild fishes in Danish waters: a comparative study. *Aquaculture* 298, 24–28.
- Smith, J.W., 1984. The abundance of *Anisakis simplex* L3 in the body cavity and flesh of marine teleosts. *Int. J. Parasitol.* 14, 491–495.
- Smith, J.W., Wootten, R., 1975. Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridia) into the flesh of herring, *Clupea harengus* L. *Int. J. Parasitol.* 5, 133–136.
- Tolonen, A., Karlsbakk, E., 2002. The parasite fauna of the Norwegian spring spawning herring (*Clupea harengus* L.). *ICES J. Mar. Sci.* 59, 138–145.
- Valls, A., Pascual, C.Y., Martín Esteban, M., 2005. *Anisakis* allergy: an update. *Rev. Fran. Allerg. Immunol. Clin.* 45, 108–113.