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

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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.

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/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). 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

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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 epigastic 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 °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°15′N 15°30′E) in October 2004 and in the outer basin of Vestfjorden (67°40′N 13°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 ±5 mm) and weighed (±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 ±5 mm and thickness ±0.1 mm) and weighed (±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 °C) for at least 12 h prior to visual inspection under a 366 nm UV-light source. Additionally, 200 IPF from the