A Survey of Anisakid Nematodes in Scottish Farmed Salmon

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Introduction

Anisakiasis is a potentially fatal condition associated with the accidental ingestion of larval nematodes in fish or squid, the incidence of which is increasing with the growing trend for consumption of raw, under-cooked or cured seafood (Rosales *et al*, 1999). Most anisakiasis is associated with parasitic worms from *Anisakis* spp., with the remainder caused by the related *Pseudoterranova* spp.

Although man is an "accidental" host, ingested larvae may attempt to penetrate the gastro-intestinal wall causing acute gastric or abdominal symptoms. A number of authors (e.g. Audicana *et al*, 2002) have reported a range of allergic reactions in humans exposed to anisakine allergens in seafood. Products which have been frozen or cooked to kill worms may retain antigens capable of eliciting an allergic response.

Until quite recently the nematode species present in the flesh of fish from Scottish waters were thought to be *Anisakis simplex* and *Pseudoterranova decipiens*. Research has established that each of these nominal nematode species is a complex of sibling species, morphologically indistinguishable and identifiable only by molecular techniques (Valentini *et al*, 2006; Paggi *et al*, 1991).

However, the biology of each of these species groups is likely to be broadly similar. The life cycle of marine ascaridoid nematodes involves a number of stages and hosts (Fig. 1) (Smith & Wootten 1978; McClelland 2002). Adult worms are found in the stomach of cetaceans in the case of *Anisakis* and pinnipeds for *Pseudoterranova*. Larvae derived from eggs shed in the faeces of the mammalian host are ingested by crustacean first hosts. For *Anisakis* these are primarily euphausiid crustaceans and for *Pseudoterranova* they are benthic species. Fish become infected by feeding on crustaceans. The larval parasites migrate into the viscera and musculature and become encysted. Worms may also pass from fish to fish as a result of predation. The life-cycle is completed by the predation of infected fish by the final hosts. *Anisakis* therefore has an essentially pelagic life-cycle, given the types of hosts involved, whereas *Pseudoterranova* has a more benthic habit.

Wild salmon, both Atlantic and Pacific, are known to be commonly infected with *Anisakis* with substantial numbers of worms in the muscle (see for example Deardorff & Kent 1989), which presumably reflects their pelagic feeding habits. On the other hand, wild salmon are not recorded as hosts of *Pseudoterranova*.

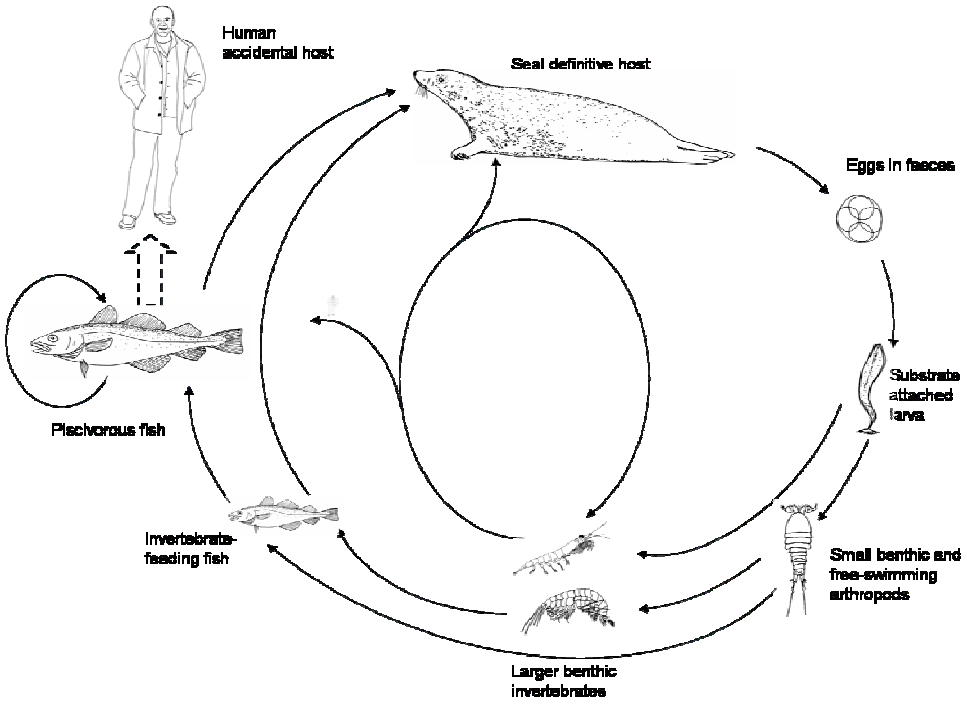

Figure 1 Diagrammatic representation of the life-cycle of the seal worm *Pseudoterranova decipiens*. In *Anisakis simplex* the definitive hosts are cetaceans and the invertebrate hosts are often euphausids.

There have been several studies of farmed salmon worldwide but there are no records of larval anisakids in their flesh (see, for example Lunestad 2003; Angot & Brasseur 1993). This has been assumed to reflect the fact that they are fed on processed diets and do not consume potentially infected hosts.

Objective

Under current legislation (Regulation (EC) No 853/2004) all fish to be consumed raw or almost raw are to be subjected to freezing to kill parasites, which includes farmed salmon. For several years prior to the implementation of these regulations in 2006 most food businesses were not freezing farmed salmon products that were to be consumed raw or almost raw as they did not perceive them to be at risk of parasites due to the use of controlled pelleted feed. Under the current legislation it is stated that the freezing treatment need not be carried out if epidemiological data indicates that the fishing ground of origin do not represent a health hazard with regards to the presence of parasites and the competent authority so authorises.

The objective of the present study was to determine if salmon farmed in Scotland contained larval anisakids within their muscle tissue. If parasites were not found then this would potentially provide epidemiological evidence that farmed salmon fed controlled pelleted feed were not at risk from anisakid parasites.

Materials and Methods

A total of 720 farmed salmon were examined during the course of this study. Fish originated from 12 cage sites situated in Shetland, Western Isles and the west coast mainland of Scotland. Sampling data are shown in Table 1 and the location of sites is illustrated in Fig. 2. Sites were selected to provide a geographical spread throughout salmon farming areas in Scotland. Sampling was facilitated by the Scottish Salmon Producers Organisation (SSPO).

All fish samples were of market size (Table 1) and had been in the sea, and thus potentially exposed to larval anisakids, for at least 12 months. Fish were sampled at random from cages or from processing plants and all fish sampled had been fed on commercial extruded pellets throughout their marine phase. The heat and pressure involved in production of such pellets ensures their freedom from pathogens.

Table 1. Sampling locations, site identities and morphometric details for sampled farmed salmon.

	Sample Location	**Sampling Date**	**Mean Length±S.D.**	**Sample Number**
1	Shetland	02/10/2007	70.2 ± 10.9cm	60
2	Shetland	03/10/2007	70.6 ± 4.1cm	60
3	NW Highlands	22/11/2007	72.8 ± 8.5cm	60
4	NW Highlands	25/11/2007	67.8 ± 3.8cm	60
5	N Argyll	06/02/2008	70.4 ± 2.5cm	60
6	N Argyll	12/02/2008	69.7 ± 3.0cm	60
7	W Isles	14/04/2008	67.8± 2.7cm	60
8	W Isles	10/07/2008	73.1 ± 2.3cm	60
9	W Isles	22/07/2008	74.8 ± 2.0cm	60
10	W Isles	01/08/2008	74.0 ± 2.6cm	60
11	W Highlands	18/08/2008	73.8 ± 2.1cm	60
12	W Highlands	20/08/2008	76.0 ± 3.0cm	60

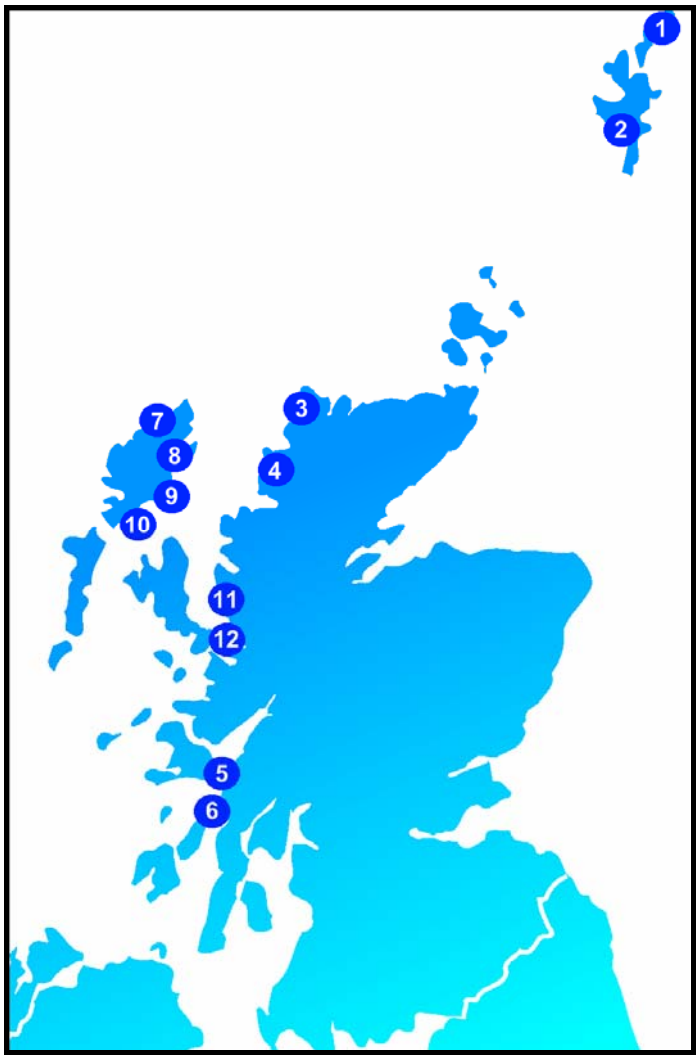

Figure 2. Map of sampling locations in Scotland. Site identities follow numbering in Table 1.

At the Stirling laboratory the total length of fish was measured. They were then filleted and the belly flaps or hypaxial muscles separated from the fillet or epaxial muscles. Samples were labelled for later identification and mostly frozen before examination. Whilst freezing is known to kill anisakid worms, they are minimally damaged by the process and therefore remain detectable to observers. For this reason the freezing of samples employed in this study will not have affected the ability to detect worms where present.

Fillets and belly flaps were examined for larval nematodes by slicing the flesh at approximately 2mm thickness over a light box (Fig. 3). However, the belly flaps were then digested separately in beakers in a pepsin hydrochloric acid mixture at 36.5º C. The contents of each beaker were examined under a dissecting microscope. The total weight of fillets from sampled fish meant that it was impractical to subject them to digestions.

Figure 3. Researcher slicing and candling farmed salmon fillets.

Results and Discussion

No larval anisakid nematodes were found in any of the salmon examined. Given that a large number of fish were examined in detail it would appear that the chances of Scottish farmed salmon containing larval anisakids in the flesh are negligible. There have been a number of previous studies on the occurrence of anisakids in farmed salmon from various countries including Norway (Lunestad 2003; Bristow & Berland 1991; Angot and Brasseur 1993), USA (Deardorff & Kent 1989), Canada (Marty 2008), Chile (Sepulveda, Marin & Carvajal 2004) and Japan (Inoue, Oshima, Hirata & Kimura 2000). Angot & Brasseur (1993) also examined several hundred of fillets of farmed salmon from Scotland. With the exception of Inoue *et al.* (2000) all these authors examined Atlantic salmon as at least part of their studies. Although these authors have used different methods of examination (slicing, digestion, dissection, histology) and sampling strategies (examination of whole muscle, sub-samples of muscle, viscera only), and thus studies are not strictly comparable, they have between them examined several thousand fish and found no anisakids within the muscle.

While larval anisakids that pose a health risk to humans do not appear to be found in the flesh of farmed salmon, they are known to be very abundant in both wild Atlantic and Pacific salmon and several of the studies cited above examined wild salmonids from the same general area as the farmed fish. Thus, Deardorff & Kent (1989) reported a 100% prevalence rate of *Anisakis* in wild Pacific salmon from Puget Sound, Washington State, with 87% of worms in the musculature , whereas no farmed fish were infected. Similarly Bristow & Berland (1991) reported that over 60% of wild salmon from Norwegian waters were parasitised by *Anisakis* within the viscera but no farmed fish were infected. Preliminary results of a FSAS-funded project at this laboratory indicate that 100% of wild salmon caught in Scottish coastal waters contain *Anisakis* larvae within the musculature, with some fish containing more than 100 parasites. The presence of anisakine nematodes in high numbers in wild fish further supports the suggestion that the absence of larval anisakids in Scottish farmed salmon is a consequence of them being fed to satiation on a pelleted diet, such that they do not ingest infective larvae of anisakids through feeding on small fish or invertebrates. There is still a risk that salmonids fed fresh, trash marine fish may become infected (Wootten & Smith 1975). This practice is illegal in Scotland and therefore fish fed on wet feed should not be available in Scotland.

Fish may become infected with anisakids by feeding on other infected fish or crustacean hosts. For *Anisakis* the major crustacean host would appear to be euphausiids and these are more abundant in offshore waters, together with cetacean final hosts. Thus, in Scottish waters *Anisakis* is generally more abundant in the offshore northern North Sea (see Smith & Wootten 1978) than in coastal areas where salmon farming is practiced.

The other anisakid commonly found in the flesh of fish in Scottish coastal waters is *Pseudoterranova decipiens* (see Wootten & Waddell 1978). This parasite has a benthic habit (McClelland 2002) and is not commonly recorded from wild salmonids. Since sea cages are raised well above the sea bed it would seem very unlikely that farmed fish will come into contact with infective stages of this parasite, and indeed it was not detected during the course of this study.

Conclusions

Scottish farmed salmon were not found to be parasitised by larval anisakids within the muscle. This suggests that salmon farmed in Scotland and fed controlled, pelleted feed do not present a significant risk to the consumer of the ingestion of these parasites.

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