FINAL REPORT

A survey of parasitic nematodes in maricultured finfish in Scotland

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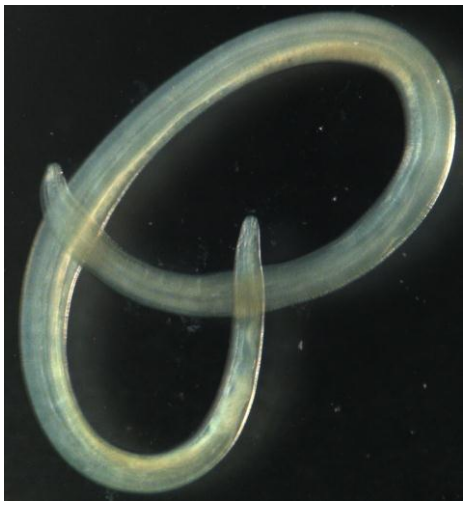

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Executive Summary

Nematodes, also known as roundworms, are found in a wide range of aquatic and terrestrial habitats, with over half of the known species being parasitic. Anisakid nematodes, which are commonly found in sea fish, are of particular importance as they are able to infect humans. The natural hosts of anisakids are whales and seals, but humans can become infected when raw or undercooked fish (*e.g.* cured or smoked) is eaten. Symptoms of anisakid infection (anisakiasis) often include nausea, stomach pain and vomiting. On rare occasions anisakid infection of humans can be fatal. With growing trends in the consumption of raw and undercooked fish, anisakiasis currently affects over 2000 people per annum worldwide, with 95% of cases located in Japan. In order to kill any anisakids present in fish, it must be either frozen or sufficiently cooked. However, even if there are no live anisakids, chemicals that they produce can still be present in fish flesh, which can cause allergic reactions in some people.

An amendment in December 2011 of Annex III to Regulation (EC) No 853/2004, which concerns “treatment to kill viable parasites in fishery products for human consumption” permits that farmed fish do not need to be frozen to kill anisakids when intended to be marketed in a raw state (or is not intended to undergo a treatment that will kill viable parasites), where it can be proven that fish have been reared in an environment free of infection, or that adequate monitoring programmes are in place to verify that fishery products do not represent a health hazard with regards to anisakid worms.

Although it is not possible to reduce anisakid infections in wild fish, in aquaculture, there is greater control over fish stocks so it is possible to prevent exposure to sources of nematode infection and to monitor infection levels to ensure that the fish are free from parasites. Previous research has shown that farmed Atlantic salmon in Scotland are free of anisakid infections. This study aims to analyse current farming practices for other Scottish mariculture species (Atlantic halibut, rainbow trout and sea trout) in order to identify potential sources / risks of anisakid infection, and to

examine samples of farmed fish to provide evidence concerning the presence / absence of anisakid worms in cultured fish.

An analysis of the current farm cycles for halibut, rainbow trout and sea trout in Scotland indicated that the risk of infection with anisakids is extremely low. To become infected a fish must consume an infected prey item. As commercial aquaculture in Scotland relies on processed pelleted feed, the risk of wild infected prey being consumed is very low, particularly since fish are generally fed to satiation. The only exception is the early stage of halibut rearing, where live planktonic organisms are employed for early feeding of juveniles. However, as long as cultured plankton species are employed rather than wild plankton, the risk of infection with nematodes is eliminated in this phase. Where fish are raised throughout their entire growth cycle in tanks that use treated / filtered water only, the risk of infection with anisakids is eliminated. The risk of infection, while remaining extremely low, is nominally increased where farms are located close to anisakid host populations *e.g.* seal colonies. An additional factor is the length of time the fish are raised at sea and therefore exposed to the risk of parasitisation.

A sample of 225 rainbow trout and 150 Atlantic halibut were obtained from 4 and 2 farms on the West coast of Scotland, respectively. Twelve further Atlantic halibut were obtained from wild fisheries. Fish flesh examined for nematode larvae using standard techniques, provided no evidence for the presence of anisakid nematodes in any farmed fish sampled. In addition, no food items, other than pelleted feed, were found in the stomach and intestines of any farmed fish. Sampled wild halibut showed a prevalence of 75% and intensity of 12.75 ± 25.81 of *Anisakis* spp., although no *Pseudoterranova decipiens* were found. Therefore, it can be assumed that, under current farming practices, Scottish farmed halibut and rainbow trout are wholly free from anisakid infection or that such infection is extremely rare and therefore that these do not pose a significant risk to consumers in terms of the ingestion of these parasites.

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1.0 Introduction

In 2010, following a request from the European Commission, the Panel on Biological Hazards of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on food safety related to parasites in fishery products. EFSA concluded that human fishery product-borne parasitic diseases primarily include those caused by cestodes, trematodes and nematodes. These diseases are either caused by an infection following ingestion of viable parasites, or as an allergic (hypersensitivity) reaction against parasite antigens.

Of the major parasitic pathogens of marine fish and shellfish, ascaridoid nematodes, most notably those from the family *Anisakidae*, are of particular importance due to the ability of some species to cause pathology in humans. The natural final hosts of anisakid nematodes are marine mammals, but humans can become infected accidentally when raw or undercooked infected fish or squid is ingested, resulting in a condition known as anisakiasis. Symptoms of anisakiasis often include nausea, severe epigastric pain, vomiting and other abdominal discomfort, as the parasite penetrates the gut wall (Margolis, 1977; Smith & Wootten, 1978; Rosales, 1999). Severe cases resulting from lesions of the stomach and intestine can occasionally be fatal.

The incidence of infection in humans is increasing with growing trends in the consumption of raw or undercooked fish. The most recent data indicates that anisakiasis infects over 2000 people per annum world-wide, with 95% of these cases located in Japan (Rosales, 1999). Of the remaining cases, 3.5% have been reported from Europe, with 95% of these from the Netherlands, Germany, France and Spain (Audicana, *et al.*, 2002). The incidence of infection is also linked to cultural traditions in preparation, such as smoking, salting and pickling of fish.

Four recognised species of anisakid nematodes commonly infect British marine teleosts, with larval stages occurring in the flesh and viscera. Of these, two species are known to infect humans: *Anisakis simplex* and *Pseudoterranova decipiens*. *A. simplex* is one of the most widespread parasites of marine teleost fish (MacKenzie,

1979) and *P. decipiens* has also been reported from a number of marine teleosts (Smith and Wootten, 1978; McClelland, 2002). Research has shown that each of these nominal worm species actually comprises a complex of sibling species, morphologically indistinguishable and identifiable only by molecular techniques (Valentini *et al*. 2006, Paggi *et al*. 1991).

The only parasite in fishery products that has been implicated in allergic reaction is *Anisakis simplex.* It is for this reason that *A. simplex* is considered the greatest risk to human health from fishery borne parasites. Antigens of *A. simplex* can persist in seafood even when there are no live nematodes remaining and are still able to cause allergic reactions in some people (Purello-D'Ambrosio *et al.*, 2000; Audicana *et al.*, 2002). The exact aetiology of the allergic reaction to this parasite is unknown, but the current general consensus is that individuals may require to be sensitised initially through ingestion of live worms. Once sensitisation has occurred, response to nematode allergens can be highly aggressive and generate severe allergic disease (EFSA, 2010).

The life-cycle of marine anisakid nematodes is indirect, involving intermediate or paratenic hosts in their transmission (Figure 1) (Smith & Wootten, 1978). The life-cycle begins with the release of eggs by mature female worms in the digestive tracts of aquatic mammalian hosts. These are incorporated into faeces that then pass into the marine environment (Podolska, 2003). The eggs undergo an incubation period in seawater and one or two moults occur within the egg, with second or third stage larvae hatching as the free-living stage (Smith, 1983; McClelland, 2002). The larvae are ingested by intermediate invertebrate hosts, such as decapods, copepods and amphipods that are in turn eaten by fish and cephalopods (Chai *et al.*, 2005). For *P. decipiens*, benthic invertebrates are the more common initial hosts while for *A. simplex*, free-swimming invertebrates are more usual.

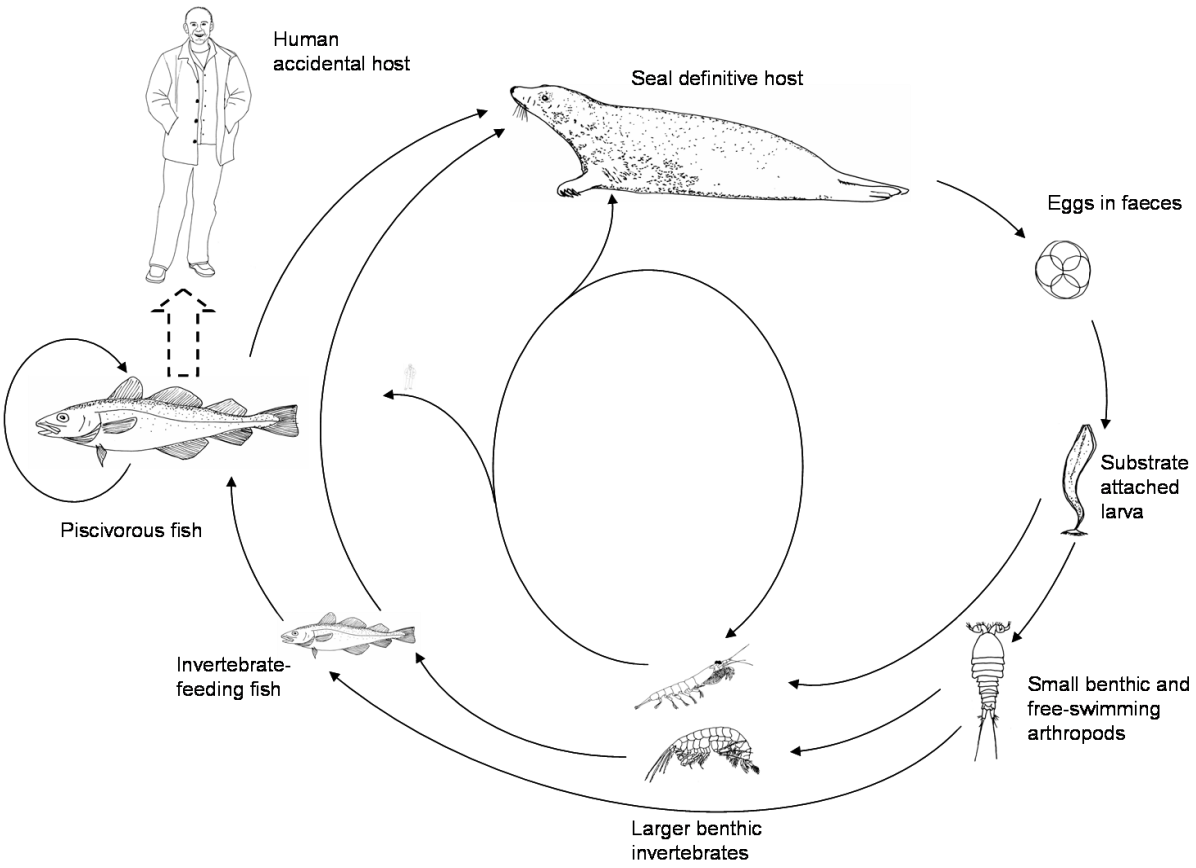

Figure 1. Diagrammatic representation of the life-cycle of the seal worm *Pseudoterranova decipiens*. For *Anisakis simplex* the definitive hosts are cetaceans and the invertebrate hosts are commonly euphausids.

Larvae are released from the prey during digestion, penetrate the digestive tract and migrate to various organs in the body cavity or to the musculature, where they are usually encapsulated (Smith & Wootten, 1978). Larvae of *A. simplex* are common in pelagic species of fish as a result of their feeding on free-swimming invertebrates, such as copepods and euphausiids (*e.g.* Podolska, 2003). *P. decipiens* is more frequently found in demersal species of fish, which feed on benthic invertebrate hosts (Køie *et al.*, 1995; Martell & McClelland, 1995), and infections of *P. decipiens* are common in inshore waters (McClelland, 2002). Piscivorous fish and cephalopods may also be infected by ingestion of infected prey fish (Nagasawa *et al.*, 1995; Klimpel *et al.*, 2004). Once ingested by suitable mammalian final hosts (commonly cetaceans for *A. simplex* and pinnipeds for *P. decipiens* (Young, 1972; Wootten and Waddell, 1977)), the infected prey fish are digested, releasing the larvae, which

remain in the alimentary tract of the mammalian host and moult through a pre-adult fourth stage to an adult stage.

Until recently the nematode species found in the flesh of fish from Scottish waters were thought to be *A. simplex* and *P. decipiens*. Recent research has established that each of these nominal worm species actually comprises a complex of sibling species, morphologically indistinguishable and identifiable only by molecular techniques. *Anisakis simplex sensu stricto* is now regarded as one of a complex of 6 related species (Valentini *et al*. 2006). *Pseudoterranova* consists of a similar group of species of which at least 3 are found in the N. Atlantic (Paggi *et al*. 1991). Work by Japanese researchers indicates that some of these sibling species have higher associations with human pathology than others (Umehara *et al*. 2007); suggesting that identification of species by molecular marker may be important in assessing risk. Of the two species studied by these workers, *A. simplex s. str.* and *A. pegreffi*, it was found that the former was responsible for more human infections in Japan. To date the most abundant species found in Scottish waters correspond to *A. simplex sensu stricto* and *P. decipiens sensu stricto.*

Anisakid nematodes in fish may be killed by exposure to temperatures of -35°C in a blast freezer for >15hrs, or at -20°C for no less than 24h or by cooking thoroughly at ≥60°C (at the core of the product for 1 minute). If fish are to be eaten without such measures, and as it is not possible to reduce nematode infections in wild fish, monitoring the prevalence of infection in fish destined for human consumption and rejecting heavily infected fish is the best way to reduce the incidence of nematodes entering the human food chain. This is commonly achieved by the “candling” technique (*i.e.* viewing on a light box) (Hafsteinsson & Rizvi, 1987). However, the candling technique can be inefficient (Levsen *et al.*, 2005), so it is not possible to completely eliminate nematodes from wild caught fish for human consumption, and therefore it is necessary to take appropriate steps to kill any worms that are present by freezing or cooking fish. The persistence of antigens in seafood originating from nematodes, which can cause allergic reactions in humans, represents another risk to consumers. A method for detecting anisakid proteins in seafood using ELISA has recently been developed (Werner *et al.*, 2010), although this is not currently being used for routine monitoring.

In aquaculture, where there is greater control over the life-history of fish stocks, it is possible to prevent exposure to sources of nematode infection to ensure that the fish are free from the parasite. However, this involves identifying the potential sources of infection throughout the farm culture cycle and implementing new measures or altering current practices to ensure that the risk of infection is eliminated. In Scotland, Atlantic salmon is the dominant cultured fish species, and a survey of nematode infections in farmed salmon carried out by Wootten *et al.* (2010a) for the Food Standards Agency in Scotland (FSAS) confirmed that Scottish farmed salmon are free of anisakids unlike their wild counterparts.

Following the EFSA review, the European Commission and member states reviewed the hygiene legislation. At the time this study commenced, the amendments were only in the initial stages of development. However, in December 2011 amendments were agreed that allow a risk-based approach to be taken when applying the freezing requirements for fishery products. The new requirements amending Annex III to Regulation (EC) No. 853/2004 (see Appendix I) outline that *"…certain fishery products, including those to be consumed raw or almost raw, undergo a freezing treatment to kill viable parasites that may represent a risk to the health of the consumer*" (L 327/39, statement 2), this including "*marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite*" (L 327/41, requirement 1b). Certain farmed fishery products can, however, be exempted from the freezing requirement. Such fish must be "*cultured from embryos and fed their whole life on a diet that cannot contain viable parasites*" and either (1) "*have been exclusively reared in an environment that is free from parasites*" or (2) have a status whereby "*the food business operator verifies through procedures, approved by the competent authority, that the fishery products do not represent a health hazard with regard to the presence of viable parasites*" (L 327/41, requirement 3d).

The EFSA opinion concerning risk assessment of parasites in fishery products (2010) identified that farmed Atlantic salmon farmed in a specific way represents a negligible risk with regards to parasites, but also concluded that "*Apart from farmed Atlantic salmon, sufficient monitoring data are not available for any other farmed fish*

therefore it is not possible to identify which farmed fish species do not present a health hazard with respect to the presence of parasites if these products are to be eaten raw or almost raw" (pg 65, criterion 7).

In anticipation of the new regulatory requirements, the aims of the current study were to:

1. Identify potential sources / risks of nematode infection by analysing the current farming practices for relevant Scottish mariculture species.
2. Provide evidence of the prevalence and intensity of anisakids in relevant Scottish maricultured species, by sampling fish from selected farms and examining them for anisakids.

It was anticipated that the results of this study would provide epidemiological evidence regarding the risk of anisakid infection in farmed halibut and rainbow trout, to assist in determining whether certain fishery products from these farmed species would meet the criteria for an exemption from the freezing requirements that were being proposed at the time the study commenced, and which have now been agreed.

2.0 Sources and risks of nematode infection in Scottish mariculture

2.1 Cultured marine species in Scotland

Other than Atlantic salmon, two species of marine fish are currently being cultured at significant commercial levels in the UK and these farms are solely located in Scotland – Atlantic halibut (*Hippoglossus hippoglossus*) and rainbow trout (*Oncorhynchus mykiss*). In addition, there is a small production of sea trout (*Salmo trutta*) taking place in Scotland. As a result this review will focus only on these three species. However, due to the small scale of the farmed sea trout production in Scotland, only halibut and rainbow trout were sampled and examined for nematodes. Throughout the rest of the UK, there is some farming of sea bass. However, this is only on a small scale and is carried out in a fully re-circulated on-shore tank system

with no route for parasite infection. For this reason sea bass was not reviewed as part of this study and only species farmed in Scotland, and associated farming practices, were included.

Atlantic halibut are currently being farmed in Scotland, Norway and Iceland. The latest figures show that worldwide production in 2010 was 1,821 Tonnes at a value of $22,149 and that production has been relatively stable since 2005 (FAO, 2011). The majority of this production was Norwegian, with Scotland producing 189 T in 2009 (*ibid.*). As halibut are sometimes used for producing cold smoked products (smoking temperatures usually <38°C) and are also eaten raw as sushi, any nematode infections carried in the flesh could potentially be transmitted to humans.

While rainbow trout are not an endemic species in the U.K. and there are no sea-running populations, they are nevertheless an established marine cultured species in Scotland. Although they are initially cultured in freshwater, they are grown out in cages in a marine or brackish water environment, and therefore they could potentially become infected by marine nematodes and consequently will be subject to investigation in this study.

The latest production figures show that worldwide aquaculture freshwater rainbow trout production was 441,128 T in 2010 with 61 countries producing fish at a total value of $1,580,655 thousand and UK producing 11,988 T at a value of $44,482 thousand (FAO, 2011). Worldwide marine and brackish water aquaculture production of rainbow trout in 2010 was 287,319 T with 18 countries producing fish at a value of $1,835,892 thousand and with 1,606 T being produced in the UK at a value of $5,090 thousand. As with halibut, rainbow trout may be cold smoked or prepared as sushi or gravadlax and as such represents a potential risk to consumers.

UK production of sea trout was 580 T in 2010 at a value of $4,481 (*ibid.*), although this figure is likely to have been lower in subsequent years, due to the closure of one of the few farms producing sea trout.

2.2 Prevalence of nematode infections in wild fish

In order to understand the sources and risks of nematode infection in cultured fish, it is necessary to consider the distribution and incidence of infection by anisakid nematodes in the marine environments that farms are located in. For both *A. simplex* and *P. decipiens,* risk of infection is dependent on the geographical ranges of their preferred hosts. *A. simplex* has a wide distribution due to its broad range of intermediate and definitive hosts (Smith & Wootten, 1978). Its range extends throughout temperate and polar waters worldwide and it has been recorded in 23 cetaceans (whales, dolphins and porpoises) and 11 pinniped (seals and their relatives) species (Davey, 1971). Euphausiid shrimps are a major intermediate host for *A. simplex* (Smith, 1983). These are predated upon by shoaling pelagic fish, such as herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) (Smith, 1984; Podolska, 2003) and as a result *A. simplex* is frequently found in these species.

Although previous records of *A. simplex* in wild Atlantic halibut are lacking, they have been found in wild Pacific halibut (*Hippoglossus stenolepsis*). Blaylock *et al.*, 1998 reported a prevalence of *A. simplex* of 43% and intensity of 7.1 in juvenile Pacific halibut (10-55cm) and a prevalence of 99% and intensity of 106.7 in adult fish (55-102cm), suggesting that halibut accumulate infections as they grow older. Other demersal species from the North Atlantic, such as monkfish (*Lophius piscatorius*) (Petrie, *et al.*, 2007), grey gurnard (*Eutrigla gurnardus* L.) (Karl & Levsen, 2011), megrim (*Lepidorhombus boscii*) and scorpionfish (*Scorpaena scrofa*) (Abollo *et al.*, 2001) have also shown to be infected with *A. simplex*. Therefore, it is likely that wild Atlantic halibut will also be infected, especially as they are high in the food chain and so will more readily accumulate anisakid infections from fish prey items.

While there are no records of wild freshwater rainbow trout infected with *A. simplex*, Shaw (1947) found infections in sea-running, migratory rainbow trout (known as steelhead trout) off the Oregon coast and Urquhart *et al.* (2009) reported a prevalence of up to 100% in wild sea trout in Scotland. In addition, *A. simplex* is a common parasite of Atlantic and Pacific salmon (Deardorff & Kent, 1989; Bristow & Berland, 1991; Wootten *et al.*, 2010b), which exhibit similar migratory and feeding behaviour to steelhead trout. High prevalences (90-100%) of *A. simplex* have been

found in chum salmon (*Oncorhynchus keta*) from the Bering Sea, Gulf of Alaska and Hokkaido, Japan (Sugawara *et al.*, 2004; Urawa & Fujisaki, 2006). As a result of their opportunistic feeding habits it is likely that infection in rainbow trout is not uncommon where infected invertebrate hosts are found.

P. decipiens is not as widely distributed as *A. simplex*, but is more common in benthic hosts, which feed on infected benthic invertebrates (Køie *et al.*, 1995; Martell & McClelland, 1995). Grey seals are thought to be the primary definitive host in temperate regions of the North Atlantic and populations of *P. decipiens* are generally restricted to the geographical areas of grey seal populations (Bowden, 1990; Desportes & McClelland, 2001). Juvenile wild halibut can be a common host for *P. decipiens* since they consume infected intermediate invertebrate hosts. A prevalence of 11% and mean intensity of infection of 2.0 for *P. decipiens* has been found in juvenile (10-55cm) Pacific halibut (*Hippoglossus stenolepsis*) (Blaylock *et al.*, 1998). Similarly, high densities of *P. decipiens* can be found in large adult demersal fish, including halibut, as they become increasing piscivorous and accumulate infections from their fish prey (McClelland & Martell, 2001).

Although Smith *et al.* (1990) showed experimentally that infection of rainbow trout with *P. decipiens* is possible, they have not been recorded in wild rainbow or steelhead trout. However, Urquhart *et al.* (2009) found a prevalence of up to 13% in sea trout from Scotland. Migratory salmonids are generally pelagic and feed in surface waters, which may explain the low incidence of infection with *P. decipiens* since these are primarily found in benthic species (Wootten *et al.*, 2010b). The fact that steelhead trout undertake open ocean migrations, while sea trout often follow coastlines (where the definitive hosts for *P. decipiens* are found) may explain why *P. decipiens* is absent from steelhead trout, but is often found in sea trout.

2.3 Potential key risks arising from current farming practices

By identifying the potential infection routes throughout the culture cycle it is possible to establish the risk of infection. Nematodes are not able to infect fish directly and infection of fish with *A. simplex* and *P. decipiens* relies on the ingestion of wild infected intermediate hosts. Therefore, in assessing the risk of infection of farmed

fish the possibility of ingesting infected material must be considered. This section provides details of potential infection routes for both halibut and trout farming cycles, estimates the level of risk, and suggests methods for reducing the risk, where possible.

Halibut farming

As outlined in Figure 2, the larval rearing stages of halibut culture occur in onshore tank systems supplied with sterilised salt water until the first feeding stage. There is no risk of parasite infestation at these stages since the water supply is controlled and the fish larvae are not feeding, living instead of the nutrient supplies of their own yolk-sacs. During the subsequent larval phase of halibut rearing, live feed must be presented, which has the possibility of being infected with nematode larvae. Reports of infection of cultured cod larvae fed natural plankton have been outlined by Karlsbakk *et al.* (2001). First feeding of halibut is initiated by the transfer of the larvae to containers supplied with live prey and micro-algae (Figure 2) (Næss *et al.*, 1996). In the past, halibut larvae have been produced using semi-intensive outdoor bag systems, supplied with natural zooplankton, supplemented with brine shrimp nauplii (*Artemia*) when zooplankton numbers were limited (Berg, 1997). However, use of natural zooplankton has a number of drawbacks, including often limited access to sufficient quantities of wild zooplankton and the possibility for infection of the cultured fish larvae with nematodes and other parasites (Figure 2).

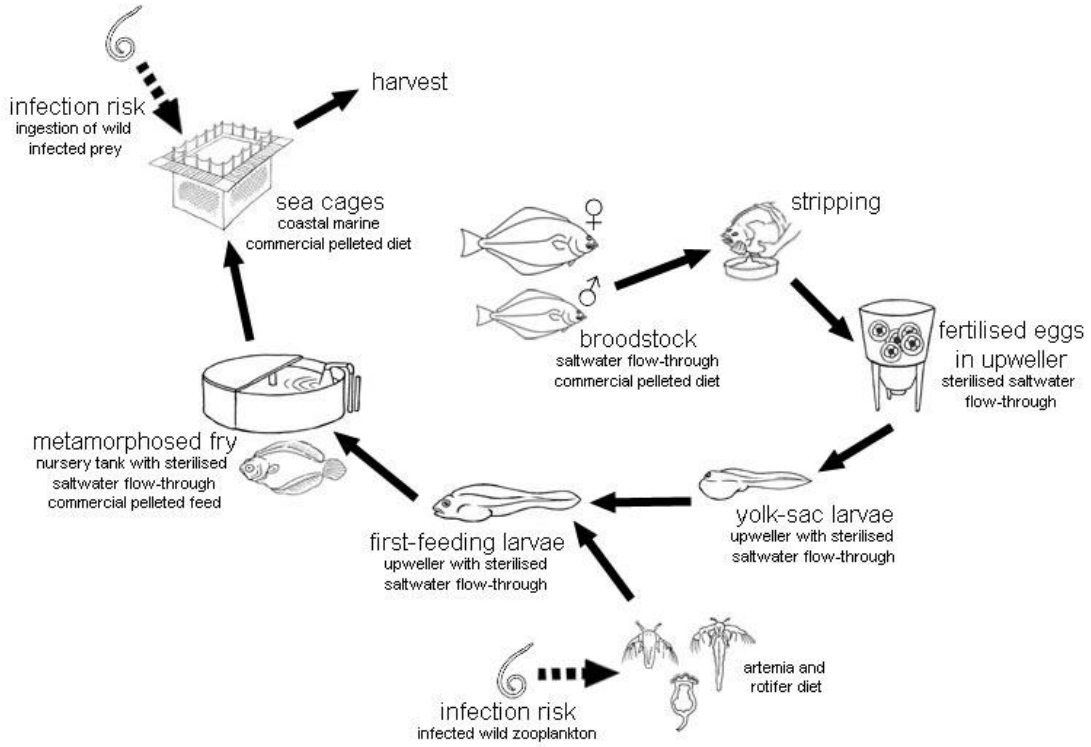

Figure 2. The production cycle for halibut, with rearing habitats and diet types specified throughout. Stages of production where there is a potential risk of infection by nematodes are indicated (images adapted from www.fao.org).

Modern hatcheries often utilise an intensive, indoor 'green water' system at the first feeding fry stage, which is where waters containing particulate matter and wastes from the culture system are re-circulated and the wastes decomposed by natural populations of bacteria and algae, which thrive in the reservoirs. The micro-algae present in the water promote higher survival and growth rates of the feeding larvae, due to the rapid development of intestinal micro-flora (Naas *et al.*, 1992)

Halibut larvae diets have been the focus of a large number of studies. The majority of this research has been concerned with intensively cultured live zooplanktonic diets (*e.g.* rotifers and *Artemia*), as a result of the limited availability of sufficient quantities of wild zooplankton for intensive culture. Studies have consistently shown that natural zooplankton is nutritionally superior to *Artemia* and rotifer diets and results in greater survival and higher growth rates (*e.g.* Shields *et al.*, 1999a). Van der Meeren (1995) showed that halibut larvae fed a natural zooplankton diet have the opportunity to feed on increasing larger zooplankton as they grow and their dietary needs change. When fed exclusively on *Artemia,* the halibut larvae can be expected to

ingest large numbers (~2500) of *Artemia* per day (Van der Meeren, 1995). Subsequent trials have shown that high survival and growth rates can be achieved with larvae fed solely on *Artemia*, provided that prey density is sufficiently high (Harboe *et al.*, 1998; Gara *et al.*, 1998). However, halibut larvae fed exclusively on *Artemia* diets are susceptible to pigmentation and eye abnormalities (Gara *et al.*, 1998; Pittman *et al.*, 1998). One approach that has been used successfully in commercial culture is the feeding of natural zooplankton for a short period, to supplement the cultured *Artemia*, resulting in normally metamorphosed fry (Næs *et al.*, 1995).

Modern culture primarily uses cultured *Artemia*, as zooplankton cannot be collected in the quantities required for intensive halibut rearing (Mangor-Jensen *et al.*, 1998b), eliminating the risk of infection by nematodes via infected larval feed. However, as wild zooplankton are known to be nutritionally superior to cultured *Artemia* (*e.g.* Shields *et al.*, 1999a) they may still be used in some instances, or for short periods during the larval phase, although this does not occur in Scotland. This practice carries a risk of infection by nematodes as a result of larvae ingesting zooplankton infected with nematode larvae. Discussions with Scottish halibut farmers have revealed that cultured artemia are currently the sole source of live feed for halibut larvae, which removes the risk of anisakid infection via this route during the early stages of culture.

Therefore, during the nursery stages, where the young fish are normally maintained in on-shore tanks supplied with sterilised sea water, the risks of parasite infestation are minimal. However, when the fish are transferred to sea cages for the final stages of on-growing there is a risk of exposure to parasites through the ingestion of infected wild prey.

Although it is impossible to prevent fish in a cage system from eating wild prey, the risk of infection with nematodes is influenced by several factors. The mesh size of the cages may limit the size of wild prey that is permitted to pass through the cages. Halibut cages require a rigid bottom to prevent the net from sagging (Midling *et al.*, 1998) and are often modified circular cages designed for salmon culture. A fine mesh bottom net is held taught by a lead ring that surrounds the bottom of the cage

(Stuart *et al.*, 2010). The size of the mesh must be carefully selected to prevent feed pellets from falling through the bottom of the cage, but large enough avoid the built up of faeces (Brown, 2002). A predator net is essential as halibut spend most of their time lying on the netting and therefore are prone to predation (*ibid.*). Where adult halibut are grown in onshore tank systems, water is passed through a coarse screen to remove large debris, which limits the size of wild prey that may enter the system.

As a proportion of the nematodes that are ingested by fish remain within the musculature or viscera throughout the life of the fish, fish that are reared in sea cages for a longer period and from a smaller size are more likely to become infected and will be more likely to consume a broader range of infected prey. Halibut are transferred to sea cages or grow-out tanks when they are between 100 – 800g and 1.5 – 2.5 years old (Figure 2). Although halibut can be transferred when they are as small as 100g, this results in lower survival rates due to aggression in cages, and so a larger transfer size is preferred (Brown, 2002; Power, 2009), reducing the length of time the fish will be held in seawater. Rearing of halibut in sea-cages usually takes 2 – 2.5 years to produce a market size of 3 – 5kg (Bromage *et al.*, 2000), although fish over 5kg are preferable (Glover *et al.*, 2006). However, females grow faster and reach a larger size than males (Stuart *et al.*, 2010) and techniques are available to produce all-female stocks (Tvedt *et al.*, 2006; Hendry *et al.*, 2003).

The size of fish at stocking into cages and the mesh size of the cages may also influence the risk of fish becoming infected by consuming wild infected prey. Smaller fish are more likely to consume smaller wild prey, including copepods and amphipods from the zooplankton, which may be infected with nematodes. As fish grow they will ignore smaller prey and are more likely to consume larger prey, such as euphausiids and small fish.

The primary control factor in the latter growing stages for halibut is the use of commercially prepared feed. This ensures that the feed is of a consistent nutritional quality and it is heat processed to ensure it is free of pathogens. Observations suggest that fish fed to satiation on pelleted food are extremely unlikely to ingest wild prey items, limiting the risk of infection. However, at certain periods during the growth cycle, typically transport, grading, net changing and harvest, fish are usually

starved for a period between 24 hours and 4 days. During these times there is an increased risk of fish ingesting wild prey when they are not satiated. Compared to the well-established feeding regimes for salmon, feeding takes longer in halibut and they are slower to reach satiation (Brown, 2002). Automatic feeders that feed a small amount of food over a longer period are more appropriate for halibut as they tend to feed slowly and continuously (Stuart *et al.*, 2010). Halibut can be aggressive at the start of feeding, but this can be avoided by combining hand feeding and automatic feeding when the fish are most hungry (Greaves & Tuene, 2001).

Rainbow trout and sea trout farming

In trout culture, the hatchery phase is carried out entirely in freshwater and therefore there is no risk of infection with marine nematodes during this stage of the growth cycle (Figure 3). In addition, where fish are reared in on-shore tank systems supplied with fully re-circulated water, or where the water is sufficiently treated or filtered before entering the culture system, the risk of infection is eliminated. However, where fish are reared in marine cage systems, it is possible that fish may ingest infected prey, either invertebrates or fish, as they are carried through the cages by currents.

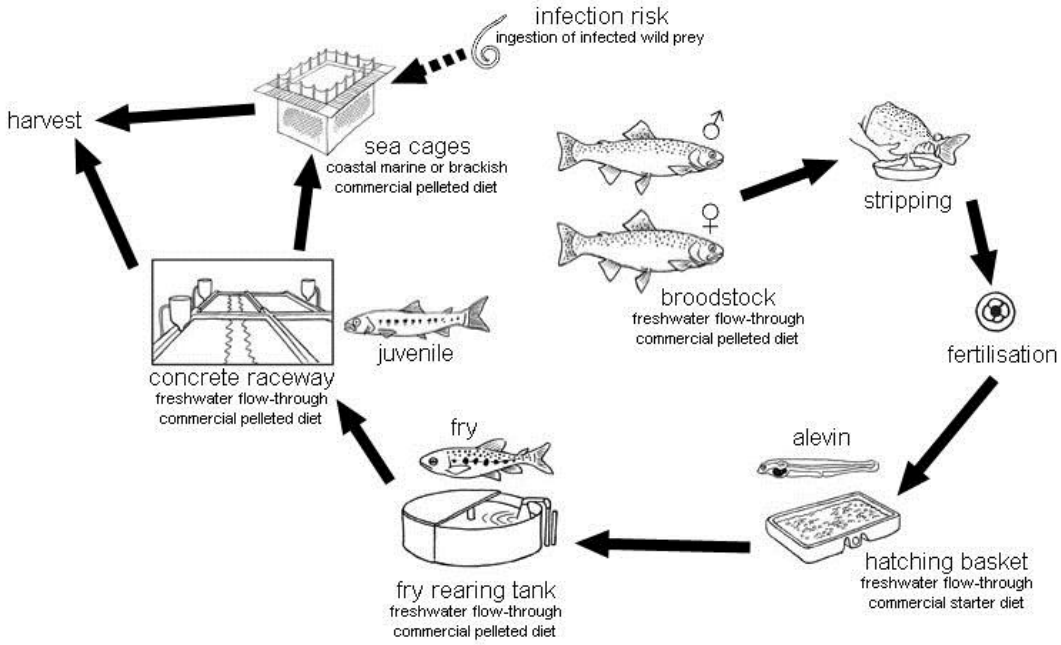

Figure 3. The production cycle of rainbow and sea trout, with rearing habitats and diet types specified throughout. Stages of production where there is a potential risk of infection by nematodes are indicated. (images adapted from www.fao.org)

As outlined for halibut there are several practices employed during the seawater stages of rainbow trout (if carried out) and sea trout culture. The cages used for trout are similar to those used for Atlantic salmon and either square steel platform or polar circle type cages (60-120m circumference) are preferred, with nets typically being 5-20m in depth. Mesh sizes are typically 18mm square initially, with larger meshes of around 22mm being used towards the end of the growth cycle. The harvest period for rainbow trout is determined by growth to the desired market size (usually 2 – 4kg for marine rainbow trout) and the onset of maturation (Purser & Forteath, 2003). For sea trout, harvesting can begin after one year when the fish are around 1kg and continues until fish are around 2kg. Trout are reared in seawater for a similar period of time to salmon so it can be concluded that the risks of infection from this route are comparable to that for salmon.

At all stages in trout production the feed used is commercially produced pellets, which are very similar to those used for farmed salmon. At no point in the production is live feed used. Farmers take great care to ensure that the fish are fed to satiation, which not only ensures a good feed conversion ration and growth rates, but during marine stages of the production cycle the practice also reduces the risk that the fish will take any wild prey that manage to enter the sea cage. For trout, feed is distributed using hand feeding, automated feeders, or a combination of both. Using both methods allows the farmer to observe the fish, ensuring that the fish are fully satiated while avoiding overfeeding. Providing the bulk of feed automatically reduces labour costs.

While pelleted feed is used for the majority of trout and halibut culture, untreated fish offal may still be used on occasion in some countries, particularly on small independent farms. Due to the paratenic nature of nematode larvae, this practice has a high risk of infecting farmed fish if the feed is infected. This was demonstrated by Wootten & Smith (1975), who showed that feeding untreated fish offal to freshwater rainbow trout in England led to infection with *Anisakis*. However, in the UK this practice is illegal for fish that are destined for human consumption, due to the high risk of disease that it confers.

In the wild the distribution of anisakid nematodes is determined by the distribution of their host populations and therefore the risk of infection is increased where fish cages are located in close proximity to host populations. For *P. decipiens*, where the primary definitive hosts are seals, the risk of infection may be reduced by locating sites away from known seal populations. As seals are a major problem for aquaculture in Scotland, due to preying upon farmed fish, proximity to local seal populations is already taken into consideration. However, it is not always possible to locate farms away from seal populations as other factors may determine the site location. For *A. simplex*, where the primary definitive hosts are cetaceans, locating farms away from known populations may not be possible, as cetaceans are primarily nomadic species. Interviews with fish farmers have revealed that harbour porpoise (*Phocoena phocoena*) are commonly seen in close proximity to cage sites, with occasional visits by bottlenose dolphin (*Tursiops truncatus*) and minke whale (*Balaenoptera acutorostrata*).

2.4 Amelioration measures

Bearing these risks in mind, the possibility of farmed halibut and trout acquiring a nematode infection appears to be extremely low. However, from analysis of the farm cycles for halibut and trout in Scotland and consideration of the potential infection routes for nematodes, the following practices are likely to reduce or remove risks of infection:

a) Only cultured zooplankton species and *Artemia* should be used as live feed for halibut larvae. This is current practice in Scotland.
b) Other than live feed for halibut larvae, commercial pelleted feed should be the only source of feed for farmed fish. This is current practice in Scotland.
c) Rearing fish wholly in a tank system using either treated / filtered recirculated water or sufficiently treated (mechanical filtration, UV or ozone treated) water eliminates the possibility of infection with nematodes. No cultured marine finfish in Scotland are currently reared in this manner so far as the authors are aware, although sea bass farmed in Wales are protected from nematode infection by such measures.

d) Stocking of larger fish into marine cages reduces risk of feeding on infected invertebrates and shortening of marine time to harvest reduces the risk of acquiring nematode infections through ingestion of wild infected prey. Shortest commercial cycles are also in the interest of the industry and are therefore likely to be current practice in Scotland.
e) Where possible, cages should be located away from known definitive host populations *e.g.* seal colonies. As well as providing a source for anisakid infection, seals are also predators of cultured marine finfish so that farm siting in Scotland already takes this into account.

3.0 Survey of Scottish marine finfish for nematode infections

In order to assess the prevalence and intensity of anisakid nematode infections in farmed halibut and rainbow trout in Scotland, a programme of sampling was undertaken through liaison with individual aquaculture companies. In addition, wild halibut were sampled through liaison with landing ports and fish merchants to allow a comparison of nematode infections in wild and farmed fish.

3.1 Methodology

Whole specimens of cultured rainbow trout and halibut were collected from distribution depots in central Scotland after delivery by specific aquaculture companies. Due to the small size of the farmed halibut and marine rainbow trout fisheries in Scotland, it was possible to take samples from all known companies that culture these fish. Five farm sites were sampled in total, with 75 fish being sampled from each site (Table 1). For each species of fish studied this provides the targeted minimum of 150 sampled specimens. This is sufficient to detect a parasite present in the population at 2% prevalence (suggested by OIE) with 95% confidence given detection techniques with 100% efficacy of detection. The sensitivity of visual detection of *Pseudoterranova* is considered to be ~100% due it its large size and colouring. *Anisakis* sp. is likely to have a lower sensitivity of detection. CEFAS and other UK government departments currently use a sample of 30 fish to determine nominal freedom from disease in a population, this being capable of detecting a

prevalence of 10% at 95% confidence. Fish were sampled from commercial harvests and were of marketable size, to ensure that they had received the same potential nematode exposure period as fish destined for market. In addition, 12 wild halibut caught in ICES region IVa (Northern North Sea) by trawling, were obtained from a fish merchant in Peterhead for the purposes of assessing anisakid detection capabilities in halibut flesh and to permit a comparison to be made between wild and farmed fish. No wild marine rainbow trout exist in U.K waters, allowing an assessment of the detection efficiency in rainbow trout flesh, however, the similarity in colour and texture of this flesh to that of Atlantic salmon supports the assumption that detection efficiency will be the same as that established for the latter species (Wootten et al. 2010b). All samples were transported to the Institute of Aquaculture, University of Stirling on ice, processed and frozen within two days. Individual fish were weighed (ungutted where possible and gutted), measured (fork length to the nearest 0.5 cm) and sexed before being filleted and individually bagged with a unique identification number. Viscera were also retained and marked with the same number. Fillets and viscera were frozen at -20°C in a domestic chest freezer until required, when they were defrosted for 24 hours prior to examination.

Samples were assessed for the presence and distribution of nematodes within the musculature alone, since only nematodes in muscle tissue can reach consumers in the final product. As a consequence of the asymmetric disposition of the viscera, it is known that nematodes follow an asymmetric distribution in the musculature. Therefore, the distribution of nematodes in each fillet was recorded separately. In rainbow trout, fillets (epaxial musculature) and flaps (hypaxial musculature - surrounding the body cavity) were examined together as “Left Fillet and flaps” and “Right Fillet and flaps”. For halibut, fillets were examined as “Dorsal Left Fillet”, “Dorsal Right Fillet”, “Ventral Left Fillet” and “Ventral Right Fillet”. The numbers and species of nematode in each fillet (left / right / dorsal / ventral) and the area of occurrence (*e.g*. fillet or flap) were plotted on a pre-drawn body map for the given host species in order to provide guidance on areas of the target fish species that are most likely to be infected. Well-established protocols were employed for the detection and removal of nematodes from the flesh as follows:

a) Naked eye

Nematodes present on the surface of the fillets or flaps and detectable with the naked eye were removed. Whilst *P. decipiens* can often be detected by eye due to their large size and prominent colouring, *A. simplex*, being relatively small in size, and of a similar colour to that of the muscle tissue, are often difficult to observe. Therefore, once nematodes on the surface of the musculature had been removed, the fillets and flaps were further examined using method b).

b) Slicing and candling

The principal method used for non-destructive detection of nematodes in fillets is candling, although the efficacy of this technique is limited, in particular, by the thickness of the candled fillet (Hafsteinsson and Rivzi 1987). Therefore, fillets and flaps from defrosted fish were sliced transversely, using a professional sashimi knife, into thin sections along the length of the fillet, giving thin slices, approximately 7-10 mm in thickness, which were then placed over a cold light source (light box) to allow nematodes to be detected within the tissue (see Figure 4.) (Valdimarsson *et al*., 1985). The slices were then teased apart using forceps and carefully examined for nematodes, which were removed. This method allows detection of nematodes buried deep within the tissue, which might otherwise have gone undetected. Once examination using candling was complete all musculature overlaying the viscera of the wild halibut and any sub-samples of fish tissue from farmed fish showing the presence of nematodes were subjected to method c).

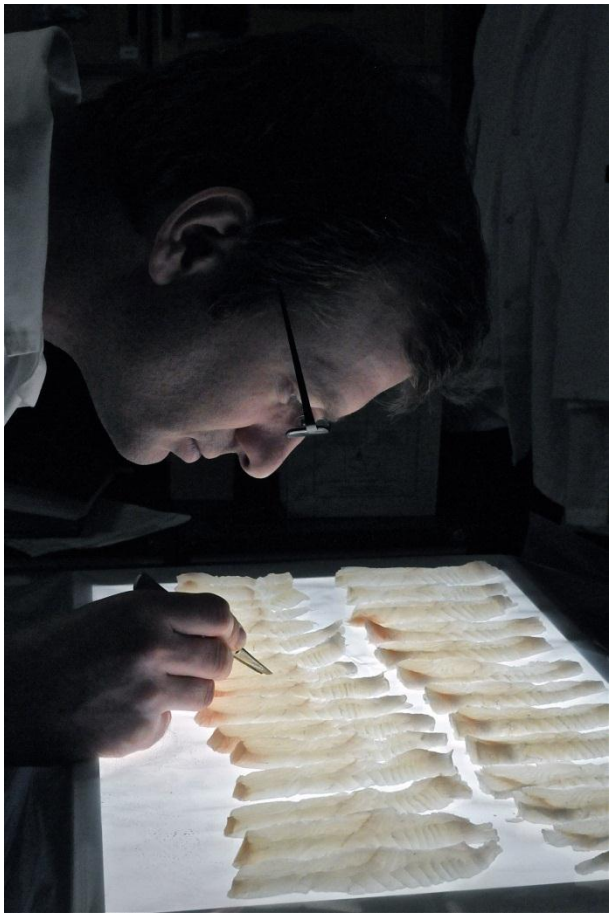

Figure 4. Researcher examining sliced halibut fillets for anisakids by candling

c) Peptic digestion

First described by Smith and Wootten (1975), this method involves the preparation of a digestion solution, which digests surrounding fish flesh, leaving any nematodes intact. To prepare the digestion solution, 20 g of pepsin powder was dissolved in 1 litre of 0.85% NaCl solution. Each flesh sample was placed into a 1L conical flask and digestion solution was added to cover the sample. 37% hydrochloric acid was added to lower the pH of the solution to 2, which was measured using a hand-held pH meter. The sample was then heated at 40°C and agitated lightly in a shaking water bath for at least 12 hours. After cooling for 15 minutes, each sample was then sieved and examined in a 15cm Petri dish for the presence of nematodes, which were removed using forceps. This method was only used when nematodes were detected in a sampled population by eye or candling/slicing in order to estimate detection error/technique sensitivity from the presence of previously undetected worms.

Recovered nematodes were removed from their capsules (if present), fixed in 80% alcohol and cleared in a solution of 50:50 100% ethanol: 80% glycerol in distilled water. Identification and staging of nematodes recovered from fish was carried out using a combination of gross appearance and microscopic examination of key morphological characters *e.g*. the digestive and excretory systems, the tail and the boring tooth (Smith, 1983). Where nematodes could not be identified by eye, nematodes were examined at high power under a light microscope and identified by looking at the position of the excretory pore, the structure of the digestive system and the structure of the tail.

Once samples had been examined for nematodes in the musculature, selected sub-samples of viscera were examined for foreign food items (*i.e.* not pelleted feed). Ten viscera were randomly selected from each batch of farmed fish for examination. As the wild halibut were supplied as gutted fish it was not possible to examine their viscera. In addition, the protocol dictated that for any farmed fish where nematodes were found in the musculature, these were also to be examined for foreign food items. For each examined sample, the stomach and intestines were dissected and the contents were washed out into a Petri dish, which was then visually scanned both by naked eye and using a stereo microscope. Any foreign food items were identified and fixed in 80% ethanol.

For each sample the total number of *Anisakis* spp. and *Pseudoterranova decipiens* detected using each of the three methods (eye, candling / slicing and peptic digestion) were calculated and these were combined to give a total for each species. The prevalence and intensity of infection for each species was then calculated from these totals. Statistical analyses were carried out using Microsoft Excel 2002 SP3.

3.2 Results

Farmed Fish

A summary of metadata for each fish sample is shown in Table 2. In total 375 farmed fish and 12 wild fish were examined for nematodes. No anisakid nematodes were found in any of the farmed fish using methods (a) naked eye and (b) candling and slicing. Method (c), pepsin digestion, was not undertaken for farmed fish in this study as no nematodes were detected either by eye or candling / slicing (see discussion).

Examination of the farmed fish viscera did not reveal any foreign food items. For halibut from Loch Melfort all stomachs and intestines were empty and in trout from Bonawe, Loch Etive, 90% of stomach and intestines were empty, suggesting that the fish were starved prior to harvest.

Wild Atlantic halibut

A total of 153 *Anisakis* spp. were found in the wild halibut sample, with 75% of fish being infected (Figure 5). No *Pseudoterranova decipiens* were found in any of the fish. Detection by eye proved to be the most effective detection method as the majority of the worms were encapsulated immediately adjacent to the visceral cavity. Consequently 90 worms (55.82%) were detected by eye and 30 worms (19.61%) were detected using the candling and slicing method. Following peptic digestion a further 33 worms (21.57%) were found, giving an overall detection efficiency of 78.43% using a combination of eye / candling detection methods. The mean intensity of infection was 12.75 ± 25.81 worms per fish. Of the 33 worms found by digestion, 21 were from a single highly infected fish.

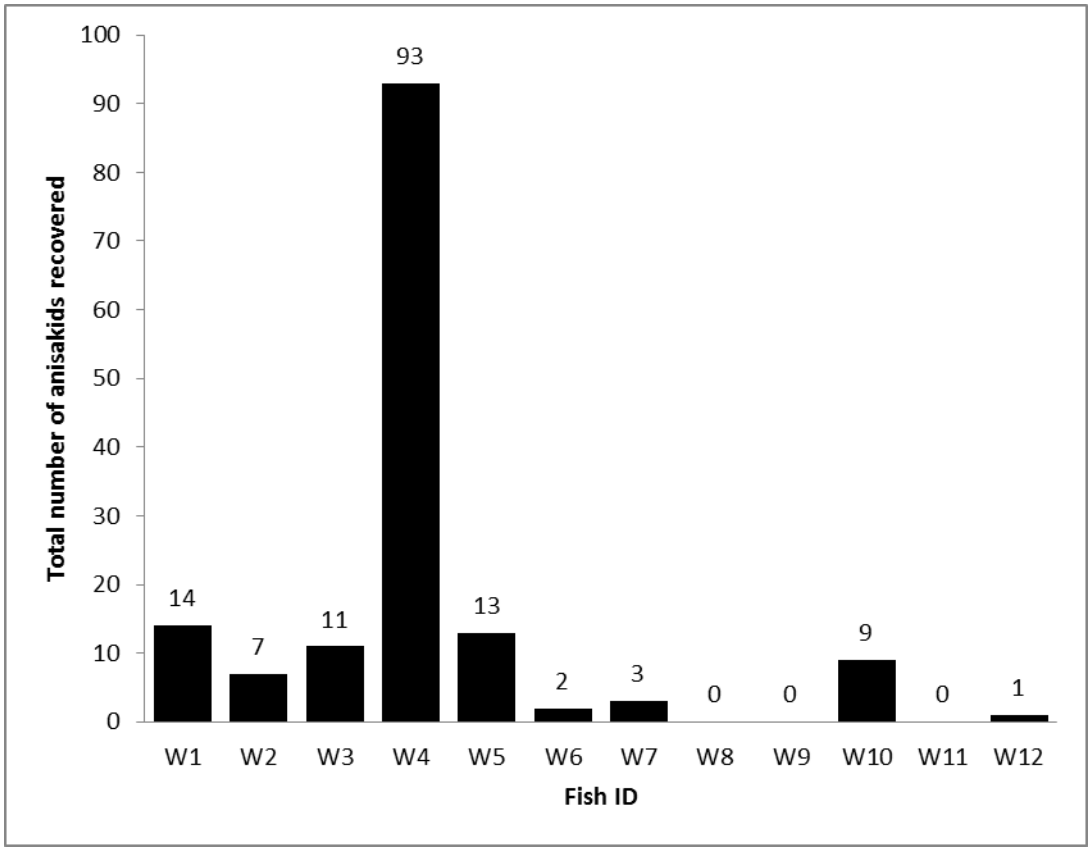

Figure 5. Numbers of *Anisakis* spp. recovered from wild-caught halibut (n = 12) detected by naked eye, candling and digestion techniques.

The majority of anisakids within the fillet from those recovered by eye / candling were in the ventral right fillet overlying the visceral cavity, with 90 worms (74.38%) being found in this region (Figure 6.). Twenty-five worms (20.66%) were found within the dorsal right fillet overlying the visceral cavity, and the number of worms found decreased significantly with increasing distance from the visceral cavity, with only 1 worm (0.83%) being found in the tail section of the fillets.

Morphological examination of worms from each wild halibut found them to be morphologically identical to *Anisakis simplex sensu stricto*, although this morphology is also expected for 6 other morphologically identical *Anisakis* species that may only be discriminated using molecular techniques (Valentini *et al*. 2006). A previous study (Wootten et al. 2010b) examining *Anisakis* spp. retrieved from wild Scottish Atlantic salmon found them all to be *Anisakis simplex sensu stricto* using molecular discrimination techniques.

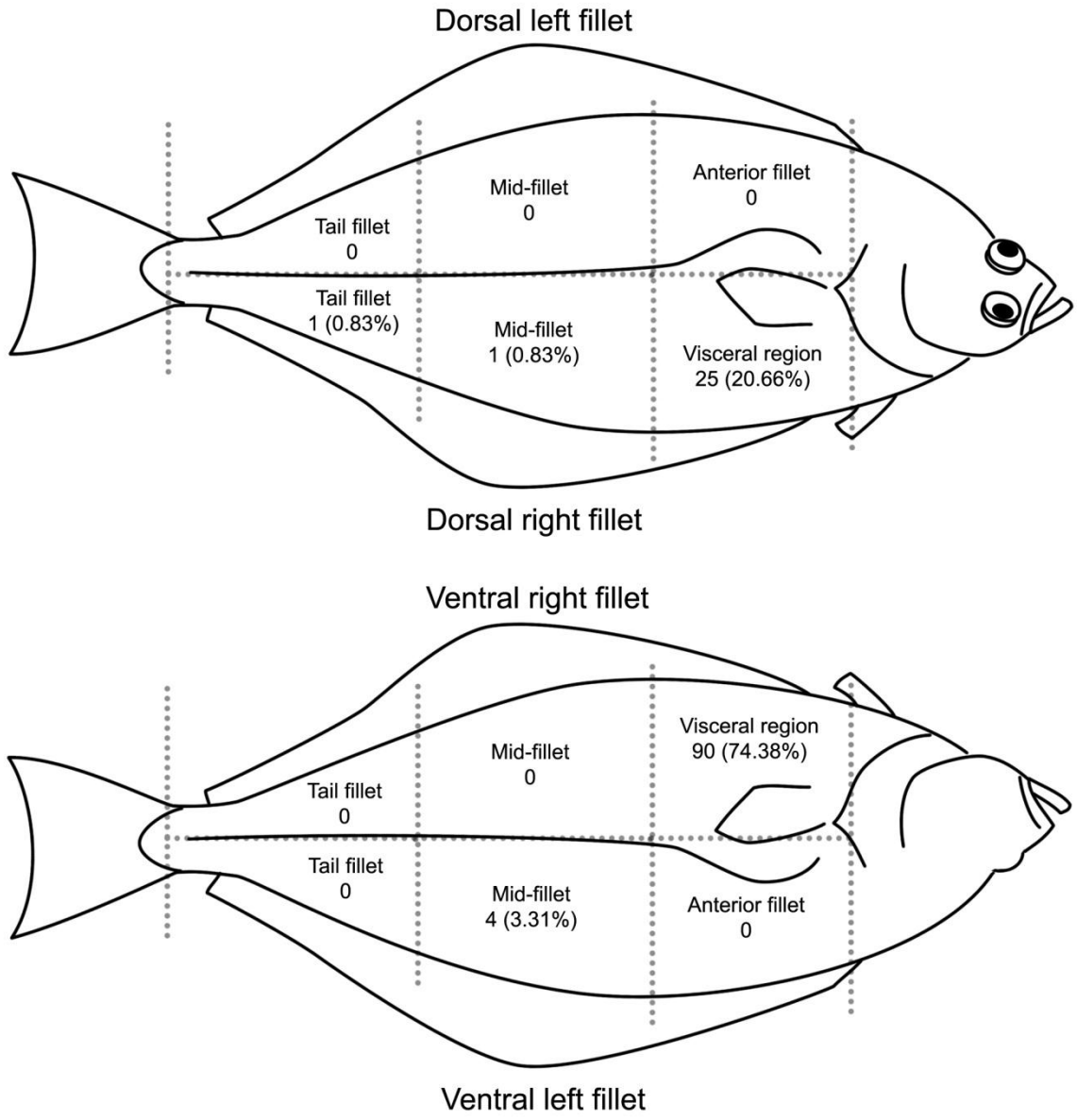

Figure 6. Distribution of *Anisakis* spp. in wild-caught halibut (n = 12) detected by naked eye and candling techniques.

Table 1. Source of fish samples and collection dates

Harvest date	Collection date	Species	No.	Company	Sample location	Site salinity
07/11/11	08/11/12	Rainbow trout	75	Dawnfresh Seafoods Ltd	Bonawe, Loch Etive	10 PSU
13/11/11	15/11/11	Halibut	75	Otterferry Seafish Ltd	Gigha, Kintyre	35 PSU
15/01/12	16/01/12	Rainbow trout	75	Dawnfresh Seafoods Ltd	Ardchatten, Loch Etive	17 PSU
30/01/12	31/01/12	Rainbow trout	75	Kames Fish Farming Ltd	Kames Bay, Loch Melfort	35 PSU
30/01/12	31/01/12	Halibut	75	Kames Fish Farming Ltd	Kames Bay, Loch Melfort	35 PSU
02/06/12	12/06/12	Halibut	12	Wild caught	ICES region IVa (Northern North Sea)	35 PSU (est.)

Table 2. Summary statistics of fish samples

Species	Company	Total weight (kg)	Mean weight (ungutted) (kg)	Mean weight (gutted) (kg)	Mean fork length (cm)	Genetic / maturity status
Rainbow trout	Dawnfresh Seafoods Ltd	308.84	4.11	3.43	60.09	Diploid
Halibut	Otterferry Seafish Ltd	167.64	-	2.24	57.2	Immature
Rainbow trout	Dawnfresh Seafoods Ltd	351.26	4.59	3.82	61.81	Triploid
Rainbow trout	Kames Fish Farming Ltd	226.56	3.00	2.50	57.9	All female
Halibut	Kames Fish Farming Ltd	205.88	2.78	2.62	61.1	Immature
Halibut	Wild caught	25.51	-	2.13	61.04	-

4.0 Assessment of risk to consumers

As no anisakid larvae were found in any farmed fish in this study, it can be assumed that the chance of Scottish farmed trout and halibut being infected by anisakids is negligible. This result ties in with a previous study that indicated that Scottish farmed Atlantic salmon are free of anisakids, unlike their wild counterparts (Wootten *et al.*, 2010a). The present study also shows that wild halibut are commonly infected with *Anisakis simplex* and consequently it can be assumed that, while anisakid infections are common in wild fish, current farming practices avoid their farmed equivalents from becoming infected. In addition, no foreign food items were found in any farmed fish, suggesting that fish fed to satiation on a commercial pelleted feed rarely ingest wild prey that may be infected with anisakid larvae. As fish are often starved for up to 3 days before harvest, they are more likely to ingest wild prey during this period. As no foreign food items were found in the gut of the fish sampled as part of the current study, it suggest that these were collected during this period of highest risk and it is very unlikely that substantial numbers of wild prey will be ingested at other times.

While this study is the first to examine farmed halibut for anisakids, previous studies show similar results for rainbow trout. Skov *et al.*, (2009) examined 166 rainbow trout from a Danish marine cage farm, but found no infections, despite wild fish from the same area being commonly infected with both parasites. Similarly, Inoue *et al.* (2000) failed to find any nematodes from a sample of 40 rainbow trout from a marine cage farm in Japan and in addition, no evidence was found of candidate intermediate hosts in the alimentary canals of the trout. However, although no nematodes were found in the studies noted above, sampling effort and analysis techniques may influence the detection of nematodes in a population. For instance, Marty (2008) examined a total of 894 farmed Atlantic salmon from British Columbia, Canada and found a single anisakid larva, although the species was not identified. Skov *et al.* (2009) used the pepsin digestion technique to detect nematodes in rainbow trout muscle, while Inoue *et al.* (2000) used the candling only technique, which has been shown to have a lower detection efficiency (only 7-10% of nematodes present in fillets are detected this way commercially) (Levsen *et al.*, 2005). Therefore, it is possible that the number of samples and / or detection methods used in these

studies may have been insufficient to detect a low prevalence of infection, which may still be a risk to human health. While the sample sizes used in the present study were adequate to detect 2% prevalence for any given species with 95% confidence assuming 100% detection efficacy, fillets were not subjected to peptic digestion in the current study so that a proportion of worms could theoretically have been missed. Using candling and slicing alone ~100% of any *Pseudoterranova decipiens* present were likely have been detected (Petrie *et al*. 2007) so risk estimates for this species should be highly accurate. Calculating the prevalence that would be detected by use of the stated methods for *P. decipiens* and using a detection sensitivity of 1 (100%) and given the number of fish sampled indicates that a prevalence of 1.3 % at 95% confidence would have been detected in rainbow trout (225 fish sampled) and 1.9% in halibut (150 fish sampled). Previous authors provide different estimates for the detection of additional *Anisakis* by peptic digest over slicing / candling. In cod (*Gadus morhua*), Brattey and Bishop (1992) found that an additional ~68% of *Anisakis* could be recovered by digestion, while in another *Oncorhynchus* species more closely related to rainbow trout, Stern *et al*. (1958) found that peptic digestion recovered 21.9% more *Anisakis* larvae from the flesh of chum salmon (*Oncorhynchus keta*) than dissection. Petrie *et al*. (2007) found no significant difference between number of worms recovered from herring by visual examination of pressed fillets versus peptic digestion.Calculating the prevalence that would be detected by use of the stated methods for this worm species and using a lowered detection sensitivity of 0.78 (78%), reflecting results for the wild halibut in this study and the number of fish sampled indicates that a prevalence of 1.7 % at 95% confidence would have been detected in rainbow trout (225 fish sampled) and 2.6% in halibut (150 fish sampled).A previous study, (Wootten *et al*. 2010a), found no worms in 720 Scottish farmed Atlantic salmon examined by slicing \ candling *and* peptic digestion, which supports the findings of the current study as salmon are reared in the same environment and under identical conditions of exposure to anisakids as rainbow trout. This contrasts to findings for wild Scottish Atlantic salmon (Wootten et al. 2010b) where 100% of fish examined were infected, suggesting that risks of infection of farmed fish are substantially lower. Although sensitivity of detection of anisakids in wild halibut by slicing and candling was lower than for digestion, this probably being associated with the very dark peritoneal lining, visual inspection by eye and slicing / candling nevertheless detected the majority of worms. From the above and despite

the lack of peptic digestion in farmed rainbow trout and halibut, the lack of detection of any anisakids in any Scottish farmed fish sample indicate that the risk posed to consumers is negligible and that consumption of unprocessed farmed rainbow trout and halibut fish flesh does not pose a significant risk to human health from ingestion of anisakids under Scottish current farm practices. In addition, the fact that no anisakids were found in the current study indicates that the risk of anisakid antigens (which elicit an allergic response in some people) being present in farmed halibut and rainbow trout is similarly negligible.

Wild halibut samples showed that infection with anisakids was common, as might be expected for a highly piscivorous species. The vast majority of worms (95.04%) were isolated from muscle areas adjoining the viscera (*i.e.* those lying at the shortest distance from the gut). Removal or specific processing of these areas could therefore be used to reduce risks to consumers for wild fish, this being more relevant to presence of intact antigens in frozen fish as freezing or cooking are normally employed to ensure that worms are killed prior to consumption in the U.K.

In order to increase the number of total fish sampled and to ensure that the current findings of lack of infection of farmed fish are maintained under conditions of changing climate and future developments in farming practices, it may be helpful to implement monitoring schemes that sample harvested fish on a repeated basis *e.g.* annually / every five years.

5.0 Conclusions

Under current farming practices, Scottish farmed halibut and trout appear to be, on the basis of the present study, wholly free from anisakid infection. The lack of infection in fish musculature suggests that these fish do not present a significant risk to consumers of the ingestion of these parasites, and can be considered safe for inclusion in raw or lightly cooked preparations without a requirement for prior processing of the fish to remove infection.

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APPENDIX I

COMMISSION REGULATION (EU) No 1276/2011

REGULATIONS

COMMISSION REGULATION

(EU) No 1276/2011 of 8 December 2011

amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the treatment to kill viable parasites in fishery products for human consumption

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to 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 [1], and in particular Article 10(1) thereof,

Whereas:

(1) Regulation (EC) No 853/2004 lays down specific rules on the hygiene of food of animal origin for food business operators. It provides, inter alia, that food business operators are to place products of animal origin on the market in the European Union, only if they have been prepared and handled exclusively in establishments that meet the relevant requirements of Annex III to that Regulation.

(2) Part D of Chapter III of Section VIII of Annex III to Regulation (EC) No 853/2004 provides that food business operators must ensure that certain fishery products, including those to be consumed raw or almost raw, undergo a freezing treatment to kill viable parasites that may represent a risk to the health of the consumer.

(3) In April 2010, the European Food Safety Authority adopted a scientific opinion on risk assessment of parasites in fishery products [2] (the EFSA Opinion). That Opinion includes information regarding the cases where fishery products may present a health hazard with regard to the presence of viable parasites. The EFSA Opinion also analyses the effects of various treatments for killing such parasites in fishery products.

[1] OJ L 139, 30.4.2004, p. 55. [2] *EFSA Journal* 2010; 8(4):1543.

(4) Though the EFSA Opinion indicates that all wild caught seawater and freshwater fish must be considered at risk of containing viable parasites of human health hazard if these products are to be eaten raw or almost raw, in the case that epidemiological data show that the fishing grounds do not represent a health hazard with regard to the presence of parasites, the competent authority may adopt national measures which authorise an exemption from the required freezing treatment on fishery products derived from wild catches. These national measures should be notified to the Commission.

(5) The EFSA Opinion concludes that where farmed Atlantic salmon is reared in floating cages or onshore tanks, and fed compound feedstuffs, which are unlikely to contain live parasites, the risk of infection with larval anisakids is negligible unless changes in farming practices occur. Though the Opinion concludes that sufficient monitoring data are not available for any other farmed fish EFSA has set up criteria for considering when fishery products from aquaculture do not present a health hazard with regard to the presence of parasites.

(6) Therefore, if the same rearing procedures based on these criteria are followed, farmed fishery products other than Atlantic salmon may be considered to present a negligible risk for parasites that may be a risk to the health of the consumer. Consequently, such farmed fishery products may also be exempted from the freezing requirements while the high level of health protection is still ensured.

(7) It is therefore appropriate to amend the requirements set out in Part D of Chapter III of Section VIII of Annex III to Regulation (EC) No 853/2004 in order to take account of certain points of the new scientific advice included in the EFSA Opinion and practical experience gained.

(8) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

Article 1

Annex III to Regulation (EC) No 853/2004 is amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the 20th day following its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 8 December 2011.

For the Commission
The President
José Manuel BARROSO

ANNEX

In Annex III, Section VIII, Chapter III to Regulation (EC) No 853/2004, Part D is replaced by the following:

'D. REQUIREMENTS CONCERNING PARASITES

1. Food business operators placing on the market the following fishery products derived from finfish or cephalopod molluscs:

   (a) fishery products intended to be consumed raw; or

   (b) marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite;

   must ensure that the raw material or finished product undergo a freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer.

2. For parasites other than trematodes the freezing treatment must consist of lowering the temperature in all parts of the product to at least:

   (a) – 20 °C for not less than 24 hours; or

   (b) – 35 °C for not less than 15 hours.

3. Food business operators need not carry out the freezing treatment set out in point 1

   for fishery products: (a) that have undergone, or are intended to undergo before

   consumption a heat treatment that kills the viable parasite. In the case of parasites other than trematodes the product is heated to a core temperature of 60 °C or more for at least one minute;

   (b) that have been preserved as frozen fishery products for a sufficiently long period to kill the viable parasites;

   (c) from wild catches, provided that:

      (i) there are epidemiological data available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites; and

      (ii) the competent authority so authorises;

   (d) derived from fish farming, cultured from embryos and have been fed exclusively on a diet that cannot contain viable parasites that present a health hazard, and one of the following requirements is complied with:

      (i) have been exclusively reared in an environment that is free from viable parasites; or

      (ii) the food business operator verifies through procedures, approved by the competent authority, that the fishery products do not represent a health hazard with regard to the presence of viable parasites.

4. (a) When placing on the market, except when supplied to the final consumer, fishery products referred to in point 1 must be accompanied by a document issued by the food business operator performing the freezing treatment, stating the type of freezing treatment that the products have undergone.

   (b) Before placing on the market fishery products referred to in points 3(c) and (d) which have not undergone the freezing treatment or which are not intended to undergo before consumption a treatment that kills viable parasites that present a health hazard, a food business operator must ensure that the fishery products originate

from a fishing ground or fish farming which complies with the specific conditions referred to in one of those points. This provision may be met by information in the commercial document or by any other information accompanying the fishery products.'