A Survey of Anisakid Nematodes in Scottish Wild Atlantic Salmon

FSAS Project S14008

Final Report 3rd February 2010

R Wootten, G.-H. Yoon, J. E. Bron

Institute of Aquaculture, University of Stirling, FK9 4LA

Table of Contents

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 belonging to *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 also 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, Bristow & Berland, 1991), which presumably reflects their pelagic feeding habits. On the other hand, wild salmon are not recorded as hosts of *Pseudoterranova*.

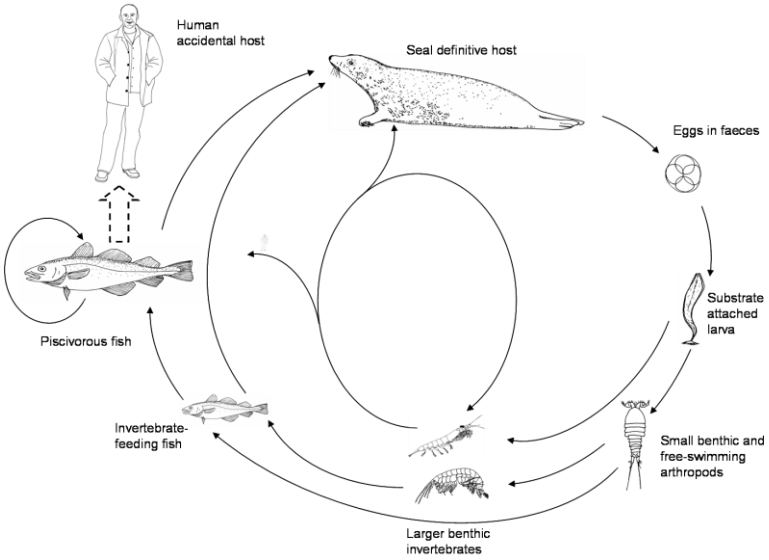

Figure 1. Diagrammatic representation of the life-cycle of the seal worm *Pseudoterranova decipiens*. In *Anisakis simplex* the definitive hosts are cetaceans and the invertebrate hosts are often euphausids.

Wild salmon have historically been known to be infected with *Anisakis* sp. worms. Heavy infection with these parasites has been suggested to be associated with the development of a disease syndrome known as Red Vent Syndrome (RVS), but there is currently no scientific evidence confirming this. A recent study on RVS in Atlantic salmon caught in rivers in England and Wales has indicated that the condition is associated with large numbers of *Anisakis* larvae in the musculature surrounding the anus (Beck *et al*. 2008). These findings have recently been confirmed for wild Atlantic salmon in Scotland (Noguera *et al*. 2009), but it is still not known if infection is the definitive cause of the syndrome.

Anisakid worms are killed by thorough cooking. The risk of anisakids to human health comes from the consumption of raw or undercooked fish. Salmon are traditionally "cold-smoked", during which process the flesh does not reach the temperature of 60ºC required to kill anisakid larvae. In addition salmon may be consumed raw as "sushi". In either of these cases the presence of live, or even dead, larvae might constitute a risk to consumers. Current EU legislation requires food businesses to freeze any fish that are to be consumed raw or almost raw, as freezing is also an effective method of killing the parasite.

Despite the presumed common occurrence of anisakid worms in wild salmon, these worms are not associated with farmed salmon. A study of 720 farmed salmon from 12 sites around Scotland did not find any anisakid larvae within the muscle (Wootten *et al*. 2009). This was attributed to the fact that farmed fish are fed an artificial diet and are held in inshore waters in cages raised off the bottom. The study concluded that the risk of the presence of anisakids in farmed salmon was likely to be minimal.

Objectives

The objective of this study was to determine the level of infection, and species ,of *Anisakis* and *Pseudoterranova* in the flesh of migrating wild Atlantic salmon *Salmo salar* L. returning to freshwater river systems in Scotland. This will confirm whether wild Atlantic salmon in Scotland are indeed at risk of infection and provide information on the species of anisakid worms found in Scottish wild salmon. The results will allow the FSA in Scotland to determine whether information on anisakid worms should be provided to members of the public consuming own-catch or wild caught salmon.

Materials & Methods

A total of 55 wild Atlantic salmon, *Salmo salar* L., and 5 sea trout, *Salmo trutta* L., were examined during this study. Fish originated from 3 locations around Scotland. Salmon sampling information is shown in Table 1. and the location of sampling sites is illustrated in Fig. 2. Sites 1 and 2 are commercial bag-net fisheries located in fully marine onshore waters. Site 3 (sample 2) is located on the River Spey and holds fish for artificial spawning for stock enhancement. Thus fish from the site had been resident in fresh water for up to several months before examination.

Table 1. Summary of sampling regime for Atlantic salmon sampled 2008-2009

Sample Source	Date	No. Salmon Sampled
1. Montrose	Sep-08	10
2. Spey	Nov-08	12
3. Armadale	May-09	10
4. Montrose	Jun-09	10
5. Montrose	Jul-09	12

Figure 2. Map of Scotland showing wild Atlantic salmon sampling sites. 1 = Montrose, 2 = Bridgend of Glenlivet (Spey), 3 = Armadale.

At the Stirling laboratory each fish was weighed and the total lengths measured. They were then filleted and the belly flaps or hypaxial muscles separated from the fillet or epaxial muscles. The muscle from both sides of the fish was then subdivided into 14 regions as shown in Fig. 3. Samples were labelled for future identification and mostly frozen before examination. Whilst freezing is known to kill anisakid worms, they retain their morphological integrity and therefore remain easily detectable and identifiable. Thus, freezing of samples does not affect the ability to detect worms when present. Fish were frozen or processed within 24 h of capture. It was not possible to determine whether ther had been any migration of *Anisakis* from the viscera into the musculature after death of the host as reported in some other fish species (e.g. Smith & Wootten 1975)

Each portion of musculature was digested in a separate glass beaker using a pepsin hydrochloric acid mixture at 36.5ºC (Smith & Wootten, 1975). The contents of each beaker were then examined under a dissecting microscope and any anisakids identified and counted.

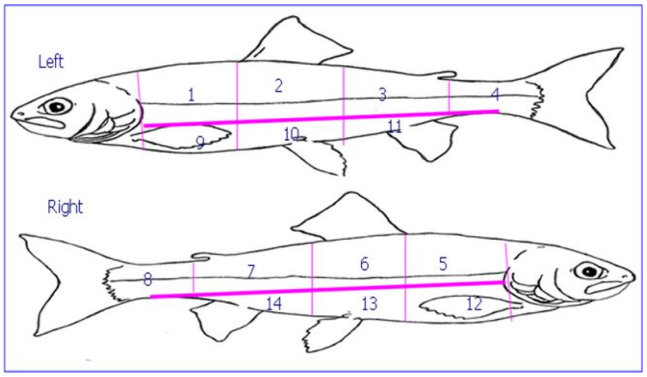

Figure 3. Diagram illustrating the muscle regions sampled for left and right sides of fish respectively. Key: 1) Back L 2) Dorsal L 3) Adipose L 4) Tail L 5) Back R 6) Dorsal R 7) Adipose R 8) Tail R 9) Pectoral L 10) Pelvic L 11) Anal L 12) Pectoral R 13) Pelvic R 14) Anal R.

Worms recovered from wild salmon and sea trout were identified using a combination of standard morphological and molecular analyses. Molecular analysis was undertaken by Dr Catherine Collins at Marine Scotland, Aberdeen. For molecular analyses a sub-sample of worms (n=5) was taken from 2 discrete body locations (right pelvic region and right anal region where possible) from a single fish captured at each sample location (Table 2.). Molecular analyses carried out initially involved PCR amplification of the ribosomal DNA ITS1 and ITS2 regions (Zhu *et al*. 1998). The resulting amplicons were then examined using a restriction fragment length polymorphism (RFLP) protocol involving digestion using HinfI and HhaI restriction endonucleases (D'Amelio *et al*. 2000, Abollo *et al*. 2003, Pontes *et al*. 2005, Umehara *et al*. 2007). The rDNA ITS regions were then subjected to direct sequencing to confirm identity. This protocol allows discrimination of the genera and species of *Anisakis* and *Pseudoterranova*.

Results

The techniques used to recover worms in previous studies proved to be highly successful in recovering worms from wild fish. All of the fish sampled were found to have worms present. A summary of the data for each sample location is presented in Table 2. Full tables of data for recovery and body musculature distribution of worms from each sample are given in Appendix 1.

Table 2. Summary data for wild Atlantic salmon sampled 2008-2009

Sample Source	Date	Salmon Sampled	Mean Length ± 1 S.D. (cm)	Mean Anisakis per Fish ± 1.S.D.
1. Montrose	Sep-08	10	64.6 ± 8.5	33.4 ± 36.6
2. Spey	Nov-08	12	67.1 ± 54.5*	41.0 ± 19.4
3. Armadale	May-09	10	62.0 ± 3.9	44.0 ± 51.2
4. Montrose	Jun-09	10	66.1 ± 4.2	17.5 ± 12.5
5. Montrose	Jul-09	12	54.8 ± 2.7	57.1 ± 33.2

. *Length of Spey fish estimated from weights in Kg (3.25 ± 1.68) using formula for length-weight relationship (±5%) derived from previous Scottish Atlantic salmon captures (http://www.letsflyfish.com/weight.htm).

All wild salmon examined carried three or more *Anisakis* worms in the musculature. The distribution of worms across all fish was found, in line with most parasites, to be significantly non-normal (Lilliefors test $p<0.01$) with a highly right-skewed distribution (Figure 4.).

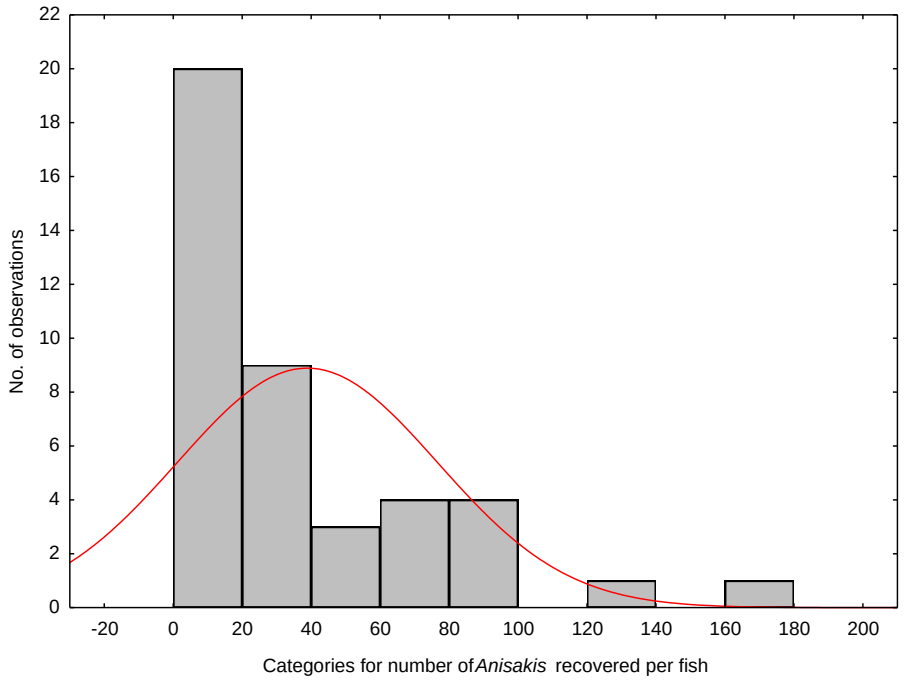

Figure 4. Frequency plot for number of *Anisakis* recovered per fish for wild Atlantic salmon sampled in Scotland showing departure from normal frequency distribution (appended curve).

Data for parametric analyses were therefore transformed using an $x^{0.25}$ transformation, which appropriately normalised the data (Lilliefors, $p>0.2$). Homogeneity of variance for transformed data was assured by use of a Levene's test ($p=0.1516$).

A GLM analysis, carried out to examine the effects of capture origin and length upon *Anisakis* infection of wild salmon, demonstrated that site of capture (which necessarily subsumes a date of capture component according to the run of fish involved) had a significant effect on *Anisakis* numbers ($p=0.0246$) but that fish length had no significant effect when considered across all sites ($p=0.3847$) (Table 3., Figure 5.). Fish captured at a single site (Montrose) showed significant ($p=0.0072$) differences in numbers of anisakids present between capture dates, possibly indicative of different marine histories for captured fish (Table 4., Figure 5.). A *post-hoc* test carried out on this data showed a significant difference in *Anisakis* numbers between fish captured at the Montrose site in second and third samplings, both carried out in 2009 (Tukey HSD Test, $p=0.0051$).

Table 3. GLM analysis of effects of capture origin and length on *Anisakis* infection of wild salmon

	SS	DF	MS	F	p
Intercept	0.206353	1	0.206353	0.773799	0.384722
Length	0.534112	1	0.534112	2.002852	0.165366
Origin	2.806468	3	0.935489	3.507969	0.024611*
Error	9.866994	37	0.266676		

Spey fish exempted from analysis due to availability of weights only. * Denotes significance at p<0.05 level.

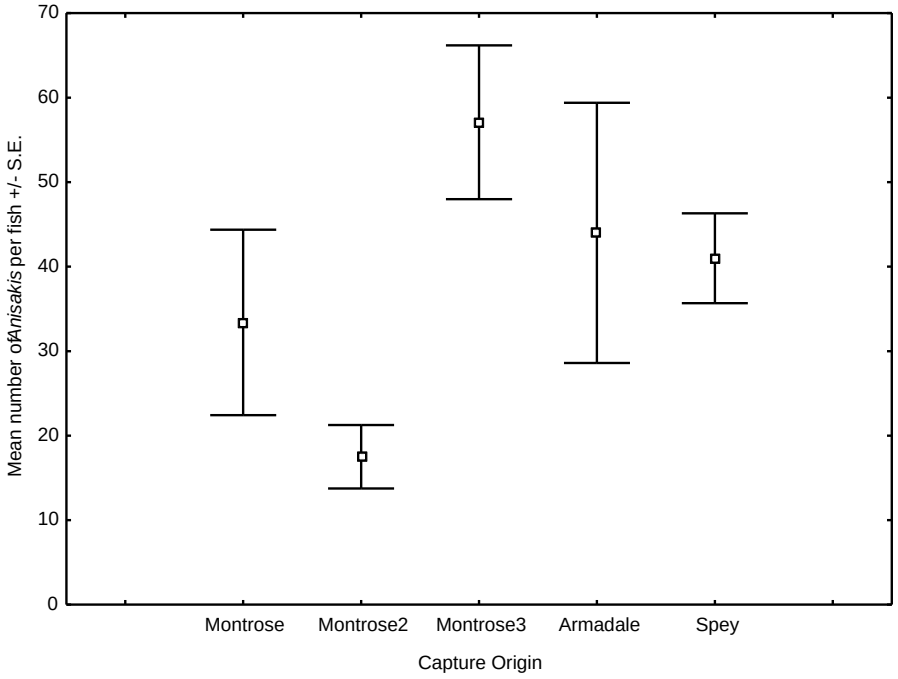

Figure 5. Plot of effect of site of fish capture on mean *Anisakis* numbers.

Table 4. GLM analysis of the effect of time of capture (Montrose fish only) on *Anisakis* numbers in wild salmon from a single site.

	DF	SS	MS	F	p
Intercept	1	165.3344	165.3344	780.2032	p<0.0001*
Time	2	2.488	1.244	5.8704	0.007234*
Error	29	6.1454	0.2119		
Total	31	8.6334			

* denotes a significant effect at p<0.05 level.

The numbers of *Anisakis* found in either the left or right side of sampled fish were compared. A paired t-test indicated no significant difference (p=0.459) in *Anisakis* numbers (t = -0.7669, Figure 6.).

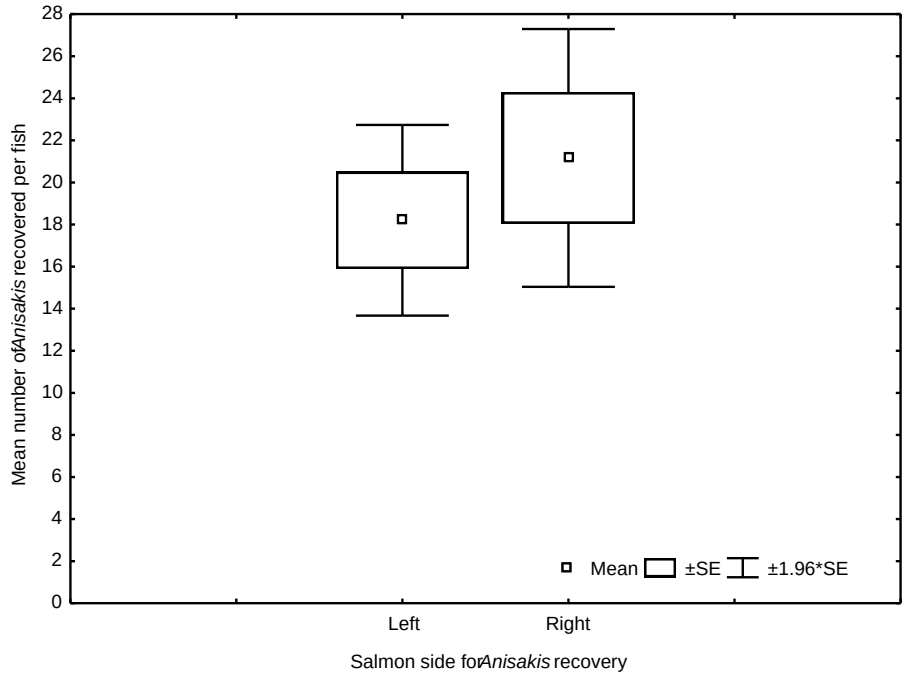

Figure 6. Box and whisker plot of mean number of *Anisakis* recovered from fish according to side of fish.

Fish were sexed from only two samples but no significant difference (unpaired t-test, t=-1.77, p=0.093) was observed in terms of differing *Anisakis* burdens between male and female salmon (Figure 7).

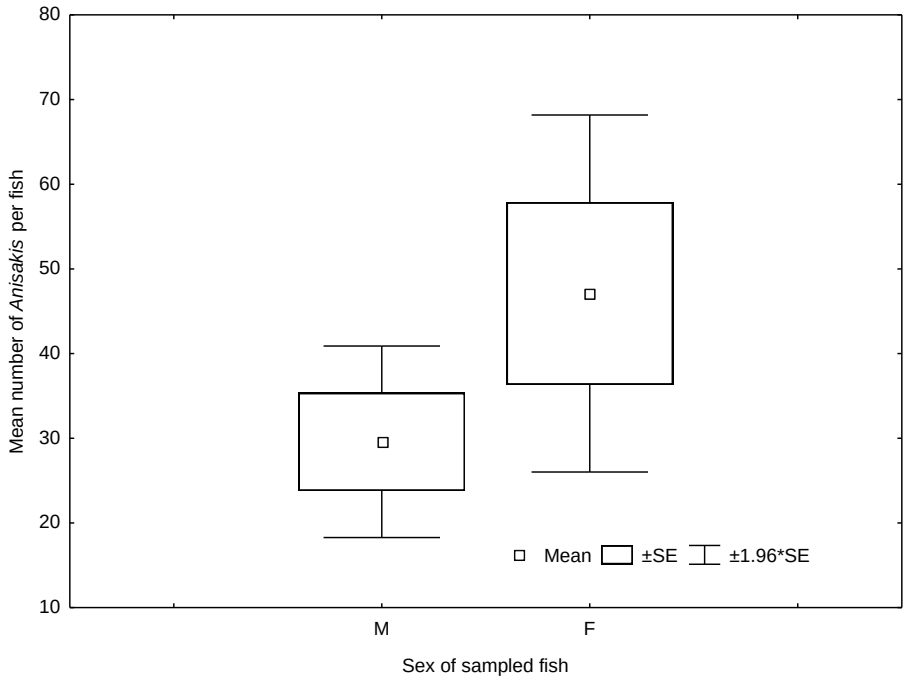

Figure 7. Box and whisker plot of mean number of *Anisakis* recovered from fish according to sex of fish.

Individual linear regressions of fish length against total *Anisakis* recovered are presented in Figure 8. For all the fish samples, save for sample 2 from Montrose, there was no significant relationship of *Anisakis* numbers with length of fish (correlations for transformed data: Montrose, r = 0.2515, p = 0.4834, Montrose2, r = 0.8327, p = 0.0028, Montrose3, r = 0.1626, p = 0.6137, Armadale, r = 0.0105, p = 0.9771). This is likely to reflect both the small sample numbers obtained and the fact that individuals within a group of returning fish caught on a given date and at a single location are likely to have similar sizes.

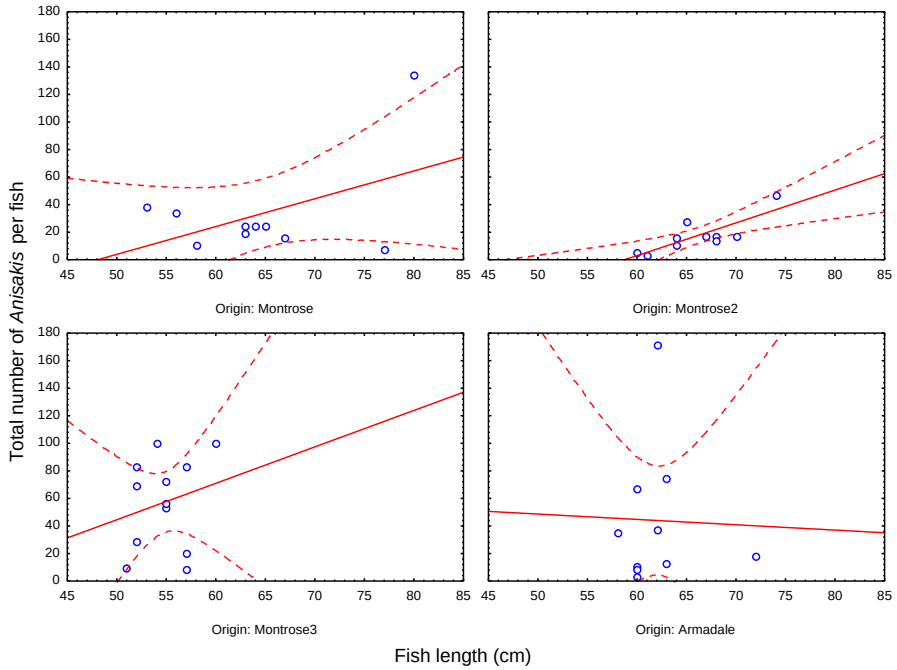

Figure 8. Linear regressions of fish length against number of *Anisakis* recovered per fish. Solid line represents fitted linear regression, dotted line represents 95% confidence limits for prediction. Note that x-axis is truncated at 45 cm to improve visualisation of data and that Spey data is missing due to lack of length data.

Because only weights were available for Spey fish, weight was used as a proxy for length. In common with the majority of fish length : *Anisakis* regressions, the weight:*Anisakis* relationship was not significant (for transformed Spey data: $r = 0.4091$, $p = 0.1866$).

A Kruskal-Wallis test conducted to compare numbers of *Anisakis* recovered from the 14 different body regions surveyed indicated that there was a significant difference in numbers of worms recovered between body regions ($H = 372.67$, $p<0.001$). A non-parametric multiple comparisons *post-hoc* test carried out following the Kruskal-Wallis analysis (Table5.) indicated that the ventral regions in proximity to the viscera (pectoral, pelvic and anal regions) all had significantly higher numbers of worms than dorsal, head and tail regions but were not significantly different from one another (Figure 9.). Though not significant, the numbers in the anal region were consistently highest and showed the greatest maximum levels of infection for a single region (maximum = 81 worms).

Table5. A non-parametric multiple comparisons *post-hoc* test comparing *Anisakis* numbers recovered from different host body regions. Bolded, italicised text indicates significant differences ($p<0.05$). Values are absolute differences between mean ranks.

	BackL	**DorsL**	**AdipoL**	**TailL**	**BackR**	**DorsR**	**AdipoR**	**TailR**	**PectL**	**PelvL**	**AnalL**	**PectR**	**PelvR**
DorsL	4.435												
AdipoL	9.852	5.417											
TailL	46.94	42.5	37.08										
BackR	14.53	10.09	4.676	32.41									
DorsR	21.93	26.36	31.78	68.86	36.45								
AdipoR	39.58	35.15	29.73	7.352	25.06	61.51							
TailR	46.94	42.5	37.08	0	32.41	68.86	7.352						
PectL	***224.8***	***229.2***	***234.6***	***271.7***	***239.3***	***202.8***	***264.3***	***271.7***					
PelvL	***200.4***	***204.8***	***210.2***	***247.3***	***214.9***	***178.4***	***239.9***	***247.3***	24.39				
AnalL	***307.3***	***311.7***	***317.1***	***354.2***	***321.8***	***285.3***	***346.8***	***354.2***	82.51	106.9			
PectR	***225.7***	***230.1***	***235.5***	***272.6***	***240.2***	***203.7***	***265.2***	***272.6***	0.907	25.3	81.6		
PelvR	***192.4***	***196.8***	***202.2***	***239.3***	***206.9***	***170.5***	***232***	***239.3***	32.36	7.972	114.9	33.27	
AnalR	***299.8***	***304.3***	***309.7***	***346.8***	***314.4***	***277.9***	***339.4***	***346.8***	75.08	99.47	7.426	74.18	107.4

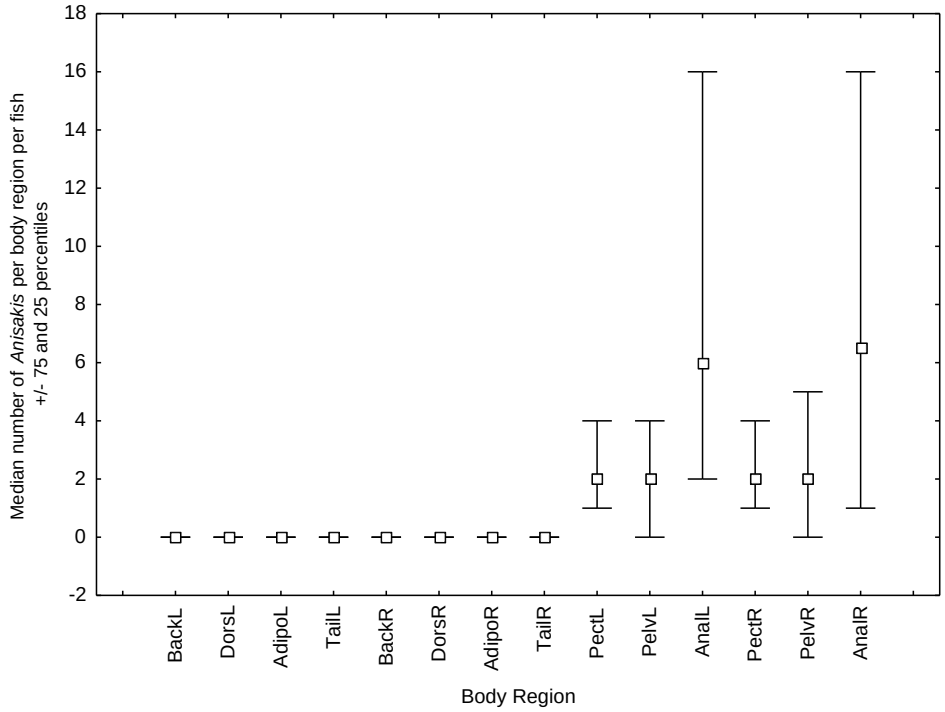

Figure 9. Median number of *Anisakis* per body region per fish showing higher abundance of worms in ventral regions.

Red vent fish were only observed from the Montrose sample captured in 2008. Although sample numbers are too small for representative conclusions to be drawn, an unpaired t-test shows no significant differences in worm burden between red vent and "normal" fish ($t=0.623$, $p=0.551$) although the range of values is greater in red vent fish (Figure 10.). Red vent fish were not seen in 2009 samples.

Both morphological and molecular techniques employed demonstrated that worms from all capture locations, salmonid species and body locations sampled belonged to the species *Anisakis simplex sensu stricto (s.s.)* (Table 6.).

Of the 33 specimens initially provided for molecular analysis, 29 specimens gave PCR products of which 22 were of sufficient strength to perform RFLP (*Hinf*I and *HhaI*). The ITS rDNA region was sequenced from 8 of these 22. RFLP analysis showed banding patterns identifying the species as *Anisakis simplex s.s.* and this was confirmed by direct sequencing of the ITS region.

Table 6. Summary data for *Anisakis* samples subjected to molecular species identification.

Tube Number	No. of Specimens	Sample Site	Species	Fish Specimen / Body Location	Sample Date	Specimens for ITS/RFLP	Species ID
1	5	Montrose	Salmon	4-12	Jul 09	5	*A. simplex s.s.*
2	5	Montrose	Salmon	4-14	Jul 09	5	*A. simplex s.s.*
3	5	Armadale	Salmon	A6-12	May 09	2*	*A. simplex s.s.*
4	5	Armadale	Salmon	A6-14	May 09	5	*A. simplex s.s.*
5	5	Spey	Salmon	17-12	Nov 08	5	*A. simplex s.s.*
6	5	Spey	Salmon	17-14	Nov 08	5	*A. simplex s.s.*
7	1	Montrose	SeaTrout	T3-1	Jun 09	3	
8	2	Montrose	SeaTrout	T3-12	Jun 09	2	*A. simplex s.s.*
Total	**33**					**32**	

*Only 2 specimens retrieved.

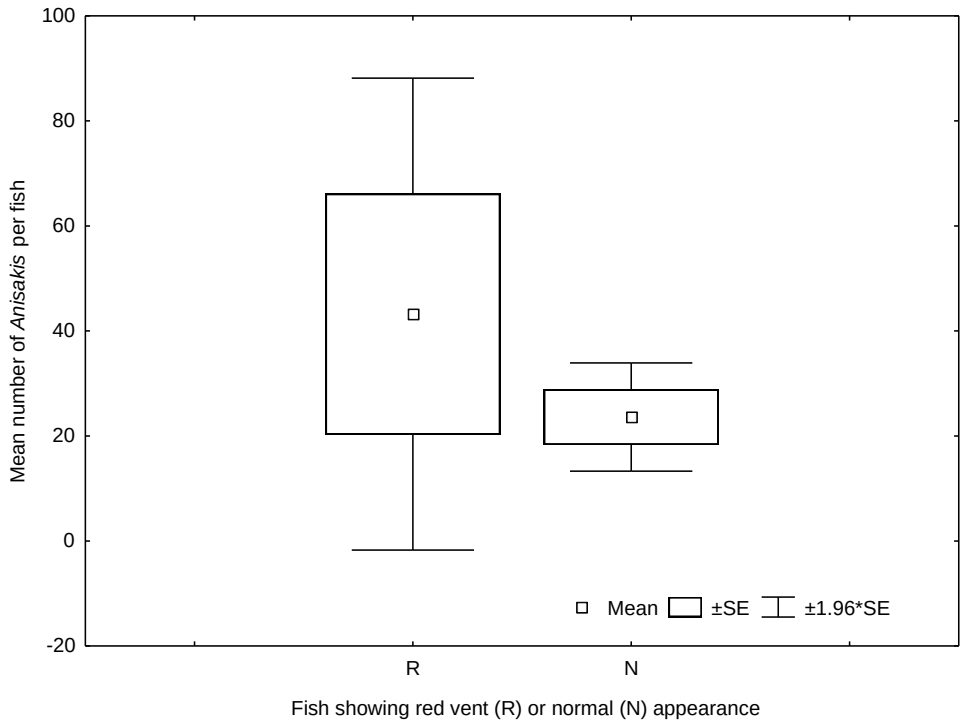

Figure 10. Plot showing mean number of *Anisakis* recovered from the first Montrose sample and displaying red vent or normal appearances.

Anisakis in sea trout

All five sea trout examined from Montrose contained larval worms morphologically identifiable as *Anisakis simplex* in the muscle. The identity of these worms was confirmed as *Anisakis simplex s.s.* by molecular analysis (see Table 6 above). Numbers of worms ranged from 2-10 per fish ($\bar{x}$ 5.8 ± 3.03 (1 S.D.)) Larvae were found in all muscle regions but unlike salmon, there was no obvious preference for any particular region(s). However, the numbers of larvae involved were small and no firm conclusions could be drawn.

Discussion

The results of this study suggest that wild Atlantic salmon caught in Scottish waters are susceptible to parasitisation by larvae of *Anisakis simplex sensu stricto* in the musculature. The prevalence of infection was 100% while the numbers of larvae found were variable but often exceeded 100. These results are in general agreement with previous studies on wild salmonids. For example, 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. Similarly, Bristow & Berland (1991) found that over 60% of wild Atlantic salmon from Norwegian waters were parasitised by *Anisakis* larvae in the viscera. A more recent study on the association of *A. simplex* with "red vent" syndrome (RVS) in rivers in England and Wales (Beck *et al*. 2008) showed that the condition was associated with large numbers of larvae in the musculature surrounding the anus. Similarly, a recent study of RVS in wild Atlantic salmon in Scotland by Noguera *et al*., found large numbers of *A. simplex s.s.* to be associated with the anal region, both in fish displaying RVS and in those with no visible lesions (Noguera *et al*., 2009). All worms recovered from both anal region and body cavity in the latter study were similarly found to be *A. simplex s.s*.

The abundance of *Anisakis* in wild salmon is perhaps not surprising in view of their essentially piscivorous and pelagic mode of life in the marine environment. It is likely that a large part, if not the great majority of their *Anisakis* burden is acquired through predation of infected fish such as capelin (Reddin & Friedland, 1993).

No *Pseudoterranova decipiens* were found in the salmon examined. This is believed to reflect the essentially pelagic nature of salmon during the marine phase, which makes it unlikely that they will come into contact with benthic invertebrates or fish that harbour infective larvae of *P. decipiens*.

There was no relationship of *Anisakis* numbers with length of fish, which is perhaps unexpected given the tendency for *Anisakis* to accumulate in fish with age (*e.g.* Podolska & Horbowy, 2003). However, it must be noted that in most of the samples the length of fish was quite uniform, and this, combined with small sample size, may have hidden any length effects.

An interesting result was the lower abundance of *Anisakis* in the second sample from Montrose, taken in June 2009, compared with the first Montrose sample taken in September 2008 and the final Montrose sample captured in July 2009. Given the time of year in which they were captured and their relatively larger size the June 2009 fish were likely to have been at least predominantly 2 sea-winter fish, whilst the other Montrose samples were more likely to have been grilse. As mentioned above, it might have been expected that older and larger fish would have been more heavily infected. Grilse and two sea-winter fish from Scottish waters are known to feed in different areas during their marine phase (Shearer, 1992) and the difference in *Anisakis* numbers may reflect the relative abundance of the parasite within these areas or differences in the feeding of the fish. However, small sample sizes prevent any firm conclusions being made.

The great majority of *Anisakis* found in salmon were located in the hypaxial musculature or belly flaps. This corresponds with results from other fish species such as cod and whiting (Wootten & Waddell, 1977). The concentration of worms in this region presumably reflects

the proximity of the hypaxials to the intestinal tract. Unlike in some other species of fish *e.g.* cod and whiting (Wootten & Waddell, 1977) there was no preference in salmon for the left or right sides of the fish. The reasons for this are unknown.

It was noteworthy that within the hypaxial muscle, the greatest number of *Anisakis* was found around the anus, as recorded in "red vent syndrome" fish (Beck *et al*. 2008; Noguera *et al* 2009). Again, the reasons for this are unknown. It is possible that this is a function of the site of penetration of the gut (*i.e.* the rectum), or alternatively it could be that the worms migrate to this region after penetration through more anterior parts of the gut.

Although very few fish that presented with symptoms of red vent syndrome were observed in this study, there was no significant difference in the numbers of *Anisakis* between those and apparently healthy fish. It would appear that salmon may contain large numbers of worms around the vent without visible signs of red vent syndrome.

Fish from the River Spey contained significant numbers of viable *Anisakis* larvae, comparable with those from marine caught fish. Wootten & Smith (1975) found *Anisakis* in trout in freshwater that had apparently become infected by being fed on unpasteurised marine fish. It would appear therefore that *Anisakis* will survive the freshwater migration of Atlantic salmon.

Sea trout were also all infected by *Anisakis* in the musculature, however numbers were much lower than in salmon. Whilst sample size was very small, the lower *Anisakis* burdens observed are consistent with the inshore habitat of sea trout in their marine phase, where *Anisakis* is not so abundant as in offshore areas. The apparently rather even distribution of *Anisakis* larvae throughout the musculature, rather than being concentrated in the hypaxial muscle is interesting, but the reasons for this are unknown.

Summary & Conclusions

- Wild Atlantic salmon sampled from the East and North Coasts of Scotland during 2008 and 2009 had a 100% prevalence of *Anisakis simplex s.s.* larvae within the musculature with up to 172 worms per fish. No *Pseudoterranova decipiens* larvae were found in the fish examined.

- There was significant variation in parasite numbers between fish samples, including between grilse and two-winter salmon caught at Montrose.

- There were no significant differences in parasite infection with respect to length, weight or sex of salmon.

- There was no significant difference in the number of parasites in salmon with "red vent syndrome" and apparently healthy fish.

- There were significant differences in the distribution of *Anisakis* between the different parts of the body musculature. The great majority of larvae were found the hypaxial muscle or belly flaps, and within these muscles most larvae were concentrated in the posterior region.

- Viable *Anisakis* larvae were found in Atlantic salmon which had been resident for at least some months in fresh water, indicating that the parasite will survive the freshwater migration of the fish.

- A small sample of sea trout from the East coast of Scotland were 100% infected with *Anisakis* larvae in the musculature, although at a lower abundance than salmon.

- Wild Atlantic salmon and sea trout thus present a real risk of infection with *Anisakis* if consumed raw or cold smoked.

Acknowledgements

The authors wish to thank the Food Standards Agency Scotland for financial support for this study. We are also grateful to Usan Salmon Fisheries, Armadale Salmon Fishery and the Spey District Salmon Fishery Board for providing fish samples. Molecular identification of parasites was carried out by Dr Catherine Collins, Marine Scotland, Aberdeen.

References

Abollo E., Paggi L., Pascual S. and D'Amelio S. (2003) Occurrence of recombinant genotypes of *Anisakis simplex s.s*. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. Infect. Genet. Evol. **3,** 175–181.

Audicana, M.T., Ansotegui I.J., de Corres, L.F. and Kennedy, M.W. (2002). *Anisakis simplex*: dangerous-dead and alive? Trends in Parasitology **18(1)**, 20-5.

Beck, M., Evans, R., Feist, S.W., Stebbing, P., Longshaw, M. and Harris, E. (2008). *Anisakis simplex sensu lato* associated with red vent syndrome in wild adult Atlantic salmon *Salmo salar* in England and Wales. Diseases of Aquatic Organisms 82: 62-65.

Bristow, G.A. and Berland B. (1991). A report on some metazoan parasites of wild marine salmon (*Salmo salar* L.) from the west coast of Norway with comments on their actual or potential interactions with farmed salmon. Aquaculture **98**:311-318.

D'Amelio S., Mathiopoulus K.D., Santos C., Pugachev O.N., Webb S.C., Picanço M. and Paggi L. (2000). Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. Int. J. Parasitol. **30**, 223–226.

Deardorff T.L. and Kent M.L. (1989). Prevalence of larval *Anisakis simplex* in pen-reared and wild-caught salmon (Salmonidae) from Puget Sound, Washington. Journal of Wildlife Diseases, **25(3)**, 416-419.

Podolska, M. & Horbowy, J. (2003). Infection of Baltic herring (*Clupea harengus membras*) with *Anisakis simplex* larvae, 1992–1999: a statistical analysis using generalized linear models. ICES Journal of Marine Science, **60(1)**, 85-93.

McClelland G. (2002). The trouble with sealworms (*Pseudoterranova decipiens*) species complex, (Nematoda): a review. Parasitology. **124** Suppl:S183-203.

Noguera, P., Collins, C., Bruno, D., Pert, C., Turnbull, A., McIntosh, A., Lester, K., Bricknell, I., Wallace, S. and Cook, P. (2009). Red vent syndrome in wild Atlantic salmon *Salmo salar* in Scotland is associated with *Anisakis simplex sensu stricto* (Nematoda: Anisakidae). Dis. Aquat. Org. **87(3)**, 199-215.

Paggi, L., Nascetti, G., Cianchi, R., Orecchia, P., Mattiucci, S., D'Amelio, S., Berland, B., Brattey, J., Smith, J.W. and Bullini, L. (1991). Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents Seas. International Journal for Parasitology **21**, 195–212.

Pontes T., D'Amelio S., Costa G. and Paggi L. (2005). Molecular characterisation of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. J. Parasitol. **91**,1430–1434.

Reddin D.G. and Friedland K.D. (1993) Marine environmental factors influencing the movement and survival of Atlantic salmon. In Mills D. (Ed.). Salmon in the Sea and New Enhancement Strategies(Fishing News Books, Blackwell, Oxford, UK) pp. 79–103.

Rosales M, Mascaro C., Fernandez C., Luque F., Sanchez Moreno M., Parras L., Cosano A. and Munoz J.R. (1999). Acute intestinal anisakiasis in Spain: a fourth-stage *Anisakis* simplex larva. Memórias do Instituto Oswaldo Cruz. **94(6)**, 823-6.

Shearer, W.M. (1992). *The Atlantic Salmon: natural history, exploitation and future management*. Oxford: Fishing News Books, xvii+244pp.

Smith J.W, and Wootten R. (1975). Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) into the flesh of herring, *Clupea harengus* L. International Journal for Parasitology, 5(2), 133-6.

Smith, J. W. and Wootten, R. (1978). *Anisakis* and anisakiasis. Advances in Parasitology **16**, 92-163.

Umehara A., Kawakami Y., Araki J. and Uchida A. (2007). Molecular identification of the etiological agent of the human anisakiasis in Japan. Parasitol. Int. **56**, 211–215.

Valentini A., Mattiucci S., Bondanelli P., Webb S.C., Mignucci-Giannone A.A., Colom-Llavina, M.M. and Nascetti G (2006).Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial cox2 sequences, and comparison with allozyme data. J. Parasitology, **92(1)**, 156-66.

Wootten, R., and Smith, J. W. (1975). Observational and experimental studies on the acquisition of *Anisakis* sp. larvae (Nematoda: Ascaridida) by trout in fresh water. Int. J. Parasitol. **5,** 373-378.

Wootten, R. and Waddell I. F. (1977). Studies on the biology of larval nematodes from the musculature of cod and whiting in Scottish waters: Journal du Conseil International pour l'Exploration de la Mer, **37(3)**, 266-273.

Wootten, R., Yoon, G.H. and Bron, J.E. (2009). Survey of anisakid nematodes in Scottish farmed salmon. Food Standards Agency Scotland report for project S14008.

Zhu X., Gasser R.B., Podolska M., Chilton N.B. (1998). Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. Int. J. Parasitol. **28**, 1911–1921.

Formatted: French (France)

APPENDIX 1 – Worm recovery and body musculature distribution data

Table showing numbers and distribution in body regions of *Anisakis* retrieved from Montrose-caught Atlantic salmon, (Sample 1., September 2008).

Fish / Region	BackL	DorsL	AdipoL	TailL	BackR	DorsR	AdipoR	TailR	PectL	PelvL	AnalL	PectR	PelvR	AnalR	Total
1	0	0	0	0	1	0	0	0	3	2	13	0	0	6	**25**
2	0	2	0	0	0	0	0	0	0	5	16	0	4	11	**38**
3	0	2	0	0	2	0	0	0	1	2	12	1	4	10	**34**
4	0	1	0	0	0	0	0	0	3	1	11	0	0	0	**16**
5	0	0	0	0	0	0	0	0	2	4	0	1	2	2	**11**
6	0	0	0	0	0	0	0	0	1	1	10	5	0	2	**19**
7	1	0	0	0	0	0	0	0	4	3	3	3	9	1	**24**
8	0	0	0	0	1	1	0	0	0	1	0	0	1	21	**25**
9	0	0	0	0	0	0	0	0	1	0	7	0	0	0	**8**
10	0	0	2	0	0	1	0	0	7	9	2	55	3	55	**134**
Total	**1**	**5**	**2**	**0**	**4**	**2**	**0**	**0**	**22**	**28**	**74**	**65**	**23**	**108**	**334**

Table showing numbers and distribution in body regions of *Anisakis* retrieved from Spey-caught Atlantic salmon, (Sample 2., November 2008

Fish/Region	BackL	DorsL	AdipoL	TailL	BackR	DorsR	AdipoR	TailR	PectL	PelvL	AnalL	PectR	PelvR	AnalR	Total
1	0	0	3	0	0	0	0	0	11	5	19	1	5	3	**47**
2	0	0	0	0	0	0	0	0	4	3	2	2	3	0	**14**
3	0	0	0	0	0	0	0	0	5	2	3	10	7	14	**41**
4	2	0	0	0	0	0	0	0	2	0	6	3	0	15	**28**
5	0	0	0	0	0	0	0	0	3	3	4	7	6	3	**26**
6	0	0	0	0	0	0	0	0	13	17	3	8	7	9	**57**
7	0	1	0	0	0	1	0	0	0	2	16	1	3	29	**53**
8	0	0	0	0	0	0	0	0	0	3	3	1	2	15	**24**
9	1	0	0	0	0	0	0	0	14	2	3	19	1	3	**43**
10	0	0	0	0	0	1	0	0	2	11	2	2	0	0	**18**
11	0	0	0	0	0	0	0	0	5	7	19	3	10	29	**73**
12	1	0	2	0	0	0	0	0	3	3	2	2	44	11	**68**
Total	**4**	**1**	**5**	**0**	**0**	**2**	**0**	**0**	**62**	**58**	**82**	**59**	**88**	**131**	**492**

Table showing numbers and distribution in body regions of *Anisakis* retrieved from Armadale-caught Atlantic salmon. (Sample 3., May 2009).

Fish / Region	BackL	DorsL	AdipoL	TailL	BackR	DorsR	AdipoR	TailR	PectL	PelvL	AnalL	PectR	PelvR	AnalR	Total
1	0	0	0	0	0	0	0	0	2	7	12	7	0	9	**37**
2	0	0	0	0	0	0	0	0	0	0	8	3	2	5	**18**
3	0	0	0	0	0	0	0	0	1	5	3	4	0	0	**13**
4	0	0	0	0	0	1	0	0	1	0	1	0	0	0	**3**
5	1	0	2	0	2	1	0	0	1	1	0	1	1	1	**11**
6	0	0	0	0	0	7	0	0	9	0	81	1	7	67	**172**
7	0	0	0	0	0	22	0	0	4	1	7	0	0	1	**35**
8	2	1	0	0	0	0	0	0	2	4	21	2	0	35	**67**
9	0	0	0	0	0	0	0	0	0	0	4	2	0	3	**9**
10	0	0	0	0	0	0	0	0	1	1	47	2	5	19	**75**
Total	**3**	**1**	**2**	**0**	**2**	**31**	**0**	**0**	**21**	**19**	**184**	**22**	**15**	**140**	**440**

Table showing numbers and distribution in body regions of *Anisakis* retrieved from Montrose-caught Atlantic salmon. (Sample 4., June 2009).

Fish / Region	BackL	DorsL	AdipoL	TailL	BackR	DorsR	AdipoR	TailR	PectL	PelvL	AnalL	PectR	PelvR	AnalR	Total
1	0	0	0	0	0	0	0	0	5	11	15	8	0	8	**47**
2	0	0	0	0	0	0	0	0	6	0	0	0	4	6	**16**
3	0	0	0	0	0	1	0	0	4	0	1	4	1	0	**11**
4	0	0	0	0	1	0	0	0	0	0	1	1	0	0	**3**
5	0	1	0	0	0	1	0	0	3	0	0	0	0	0	**5**
6	0	0	0	0	0	0	0	0	1	1	2	1	0	12	**17**
7	0	0	0	0	0	0	4	0	0	0	7	2	2	2	**17**
8	0	0	0	0	0	0	0	0	1	0	13	3	0	0	**17**
9	0	0	1	0	0	0	0	0	0	9	6	2	0	10	**28**
10	0	0	0	0	0	0	0	0	5	0	0	5	0	4	**14**
Total	**0**	**1**	**1**	**0**	**1**	**2**	**4**	**0**	**25**	**21**	**45**	**26**	**7**	**42**	**175**

Table showing numbers and distribution in body regions of *Anisakis* retrieved from Montrose-caught Atlantic salmon. (Sample 5., July 2009).

Fish / Region	BackL	DorsL	AdipoL	TailL	BackR	DorsR	AdipoR	TailR	PectL	PelvL	AnalL	PectR	PelvR	AnalR	Total
1	0	0	0	0	0	0	0	0	1	0	1	1	2	15	**20**
2	5	1	0	0	0	0	0	0	1	0	0	2	1	0	**10**
3	0	0	0	0	0	0	0	0	5	0	0	1	2	1	**9**
4	0	0	0	0	0	1	0	0	3	3	42	7	7	20	**83**
5	0	0	0	0	0	0	0	0	1	0	30	0	4	65	**100**
6	0	0	3	0	1	0	0	0	11	15	18	3	11	7	**69**
7	0	0	0	0	0	0	0	0	0	2	24	7	9	58	**100**
8	0	0	0	0	0	0	0	0	0	3	10	0	0	16	**29**
9	0	0	0	0	0	0	0	0	0	0	47	0	1	5	**53**
10	0	0	0	0	0	0	0	0	4	4	37	0	5	22	**72**
11	0	1	0	0	0	0	0	0	0	18	21	2	4	37	**83**
12	3	0	0	0	0	2	0	0	1	1	0	13	10	27	**57**
Total	**8**	**2**	**3**	**0**	**1**	**3**	**0**	**0**	**27**	**46**	**230**	**36**	**56**	**273**	**685**

Table showing numbers and distribution in body regions of *Anisakis* retrieved from Montrose-caught sea trout. (June 2009).

Fish / Region	BackL	DorsL	AdipoL	TailL	BackR	DorsR	AdipoR	TailR	PectL	PelvL	AnalL	PectR	PelvR	AnalR	Total
1	2	0	0	0	0	0	0	0	1	0	0	0	0	1	**4**
2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	**2**
3	2	0	0	0	1	0	0	0	3	1	0	2	1	0	**10**
4	0	0	0	0	1	1	0	1	0	0	1	0	1	1	**6**
5	1	3	1	0	0	0	0	1	0	0	0	0	0	1	**7**
Total	**5**	**3**	**1**	**0**	**2**	**1**	**0**	**2**	**4**	**1**	**1**	**3**	**2**	**4**	**29**